

THE UNIVERSITY of ADELAIDE

Bridging the Gap Between Parkinson's Disease and Traumatic Brain Injury: The Role of Biological Assessments for Early Detection of Neurodegenerative Disease Risk and Prognosis of Clinical Outcomes

Angus McNamara

A thesis submitted in fulfilment for the degree of

DOCTOR OF PHILOSOPHY

in

The Discipline of Anatomy and Pathology

School of Biomedicine

The University of Adelaide

[Dec 2024]

Copyright © 2024 – Angus McNamara

All rights reserved

Table of Contents

Chapter 1 - Towards a Biologically Defined Diagnosis: Incorporating Pathophysiological Measures into Parkinson's Disease Clinical Criteria 19

1.1 Parkinson's Disease Prevalence & Burden 19

1.2 Symptomatic Profile of PD 20

1.3 Evolution of the Diagnostic Criteria for PD 24

1.3.1 United Kingdom Parkinson's Disease Society Brain Bank Criteria 24
1.3.2 Gelb's Criteria 25
1.3.3 Criticisms and Addressing Prodromal PD 27
1.3.4 MDS-PD Criteria 28
1.3.5 Overall Commentary on Current Diagnostic Criteria 33
1.3.6 Moving Forward – A Biologically Defined Diagnosis 34

1.4 The Role of Alpha Synuclein (α-syn) in PD 36

1.4.1 Oligomerisation 37
1.4.2 Fibrillation 38
1.4.3 Lewy Body Formation 39
1.4.4 Braak's Staging of Lewy body pathology 41
1.4.5 Tools to measure α-syn as a biomarker in PD 42

1.5 Co-pathology: Other Pathological Proteins Implicated in PD 45

1.5.1 Tools to measure p-tau and Aβ as biomarkers in PD 46

1.6 The Role of Dopaminergic Neuron Loss in PD 49

1.6.1 Tools to measure dopamine alterations as a biomarker in PD 50

1.7 The role of loss of other neurotransmitters in PD 57

1.7.1 Tools to measure norepinephrine alterations as a biomarker in PD 57

1.8 The role of neuroinflammation in PD 59

1.8.1 Tools to measure neuroinflammation as a biomarker in PD 60

1.9 The role of iron accumulation in PD 63

1.9.1 Tools to measure iron accumulation as a biomarker in PD 63

1.10 Conclusion 66

Chapter 2 - Utility of Baseline Pathological, Neuroimaging and Clinical Markers for Prognosis in Early Parkinson's Disease 72

2.1 ABSTRACT 73

2.2 BACKGROUND 74

2.3 METHODS 77

2.3.1 Hierarchical Clustering 79
2.3.2 Principal Component Analysis 80
2.3.3 MRI ROI Delineation 81
2.3.4 Statistical Analyses 82
2.3.5 Machine Learning 83

2.4 RESULTS 84

2.4.1 Baseline Comparisons 84
2.4.2 Year-5 Follow-up Characteristics 87

2.4.3 Rate of Progression 88
2.4.4 Cluster Membership Prediction 91
2.4.5 Machine Learning 92

2.5 DISCUSSION 93

2.6 CONCLUSION 99

2.7 DATA AVAILABILITY 100

2.8 ACKNOWLEDGEMENTS 100

2.9 FUNDING 100

2.10 AUTHOR INFORMATION 101
2.10.1 Author Affiliation 101
2.10.2 Contributions 102

2.11 CORRESPONDING AUTHOR 102

2.12 ETHICS DECLARATIONS 102
2.12.1 Ethics approval and consent to participate 102
2.12.2 Competing interests 102

2.13 SUPPLEMENTARY MATERIAL ..... Error! Bookmark not defined.

Chapter 3 - Risk Factors of Parkinson's Disease: Importance of Considering At-Risk Populations for Earlier Diagnosis and Therapeutic Intervention 111

3.1 Demographic Factors 111
3.1.1 Age 111
3.1.2 Biological Sex 112

3.2 Environmental Risk Factors 115
3.2.1 Traumatic Brain Injury 115
3.2.2 Pesticide Exposure 117

3.3 Genetic Risk Factors 120

Chapter 4 - History of Traumatic Brain Injury Does Not Influence Rate of Progression of Clinical or Pathological Outcomes in Early Parkinson's Disease 127

4.1 ABSTRACT 128

4.2 BACKGROUND 129

4.3 METHOD 131
4.3.1 PPMI Cohort 131
4.3.2 PostCEPT Cohort 133
4.3.3 TBI Classification 135
4.3.4 Principal Component Analysis 135
4.3.5 Statistical Analyses 136

4.4 RESULTS 137
4.4.1 History of TBI 137
4.4.2 Injury Severity 144

4.5 DISCUSSION 149

4.6 CONCLUSION 156

4.7 FUNDING 156

4.8 AUTHOR INFORMATION 157
4.8.1 Author Affiliation 157

4.8.2 Contributions 157
4.9 CORRESPONDING AUTHOR 158
4.10 ETHICS DECLARATIONS 158
4.10.1 Ethics approval and consent to participate 158
4.10.2 Competing interests 158
4.11 ACKNOWLEDGEMENTS 158
4.12 CONFLICTS OF INTEREST 159
Chapter 5 - Utility of Biofluid Markers of Cell Injury for Prognosis of Behavioural Outcomes Following Traumatic Brain Injury in Adults: A Systematic Review & Multilevel Meta Analysis 163
5.1 ABSTRACT 164
5.2 BACKGROUND 165
5.3 METHODS 171
5.3.1 Eligibility Criteria 172
5.3.2 Search Strategy 174
5.3.3 Study Records 174
5.3.4 Study Risk of Bias Assessment 176
5.3.5 Data Synthesis 177
5.3.6 Data Coding 178
5.3.7 Statistical Analyses 180
5.3.8 Publication Bias & Outlier Testing 183
5.3.9 Confidence in Body of Evidence 184
5.4 RESULTS 184
5.4.1 Global Outcome 188
5.4.2 Cognition 218
5.4.3 Mood 227
5.4.4 Motor 227
5.4.5 Risk of Bias 229
5.4.6 Assessing Confidence in Body of Literature 233
5.5 DISCUSSION 236
5.5.1 Global Outcome 236
5.5.2 Cognition 244
5.5.3 Mood 247
5.5.4 Motor 249
5.5.5 Review strengths/limitations and directions for future research 249
5.6 CONCLUSION 251
5.7 SOURCES OF FUNDING 252
5.8 AUTHOR INFORMATION 253
5.8.1 Author Affiliation 253
5.8.2 Contributions 253
5.9 CORRESPONDING AUTHOR 253
5.10 DECLARATION OF COMPETING INTEREST 253
5.11 ACKNOWLEDGEMENTS 254
5.12 SUPPLEMENTARY MATERIAL 255

Chapter 6 - Assessing Nigrosome-1 Voxelwise Differences Between Traumatic Brain Injury and Parkinson's Disease as an Indicator of Disease Development and Predictor of Cognition: A FIND-TBI Preliminary Study *283*

6.1 ABSTRACT **284**

6.2 BACKGROUND **285**

6.3 METHODS **288**

6.3.1 Participants and Cohorts 289
6.3.2 Deriving a Global Cognitive Ability Score 291
6.3.3 Image Acquisition and Pre-Processing 292
6.3.4 Statistical Analyses 293

6.4 RESULTS **294**

6.4.1 Clinical Variable Comparisons 294
6.4.2 Voxelwise Group Comparisons 296
6.4.3 Clinical Variable Comparison After Stratifying by Age 297
6.4.4 Voxelwise Comparisons Across Age-Stratified Groups 300
6.4.5 Utility of the Nigrosome to Predict Cognitive Ability 302

6.5 DISCUSSION **303**

6.6 CONCLUSION **308**

6.7 FUNDING **308**

6.8 AUTHOR INFORMATION **309**

6.8.1 Author Affiliation 309
6.8.2 Contributions 309

6.9 ACKNOWLEDGEMENTS **310**

6.10 CONFLICTS OF INTEREST **310**

6.11 ETHICS DECLARATIONS **310**

Chapter 7 - Thesis Discussion, Future Considerations and Conclusions *311*

7.1 Research summary **311**

7.2 Fluid Pathophysiological Markers Demonstrate Promise in Forecasting Impairment in PD and TBI **316**

7.3 Critical Need to Assess Specific Outcomes in PD & TBI **321**

7.4 Neuroimaging Demonstrated Little Utility as a Prognostic Factor Across Conditions **323**

7.5 Inconsistencies in TBI and Outcome Assessment Demand Harmonisation Across Studies **327**

7.6 Thesis strengths and limitations **330**

Chapter 8 - References *334*

Thesis Abstract

Parkinson's Disease (PD) is currently the second most common neurodegenerative disease and concerningly, the most rapidly growing neurological disorder in the world. Once considered a hypokinetic motor disorder, it is now well-established that PD corresponds to various non-motor impairments. While recent reforms of diagnostic guidelines have been made to reflect this, diagnosis still places heavy emphasis on motor presentation. This corresponds to various shortcomings, such as high rates of misdiagnosis, as well as relatively late diagnosis given that PD pathophysiology is known to develop several years prior to clinical onset, greatly diminishing efficacy of therapeutic interventions. Although, several strides have recently been made towards biologically redefining PD, offering an avenue for earlier and more effective diagnosis. This would heavily rely on earlier diagnosis requires identification of individuals within groups presenting high risk of future disease development, with one of research prominence being traumatic brain injury (TBI). As such, this thesis investigated the link between PD and TBI, particularly the utility of biological assessment for improved characterisation of outcomes in both neurological populations.

Chapter 1 reviews literature outlining reform in PD diagnostic criteria, and how advancements in biological assessments could enable biological redefinition. Chapter 2 assesses the utility of biological assessment, such as biofluid biomarkers and neuroimaging, as well as comprehensive clinical assessment for prognosis in early PD. It was determined that of the predictors assessed, baseline concentrations of cerebrospinal fluid alpha synuclein and phosphorylated tau, as well as prodromal features such as hyposmia and autonomic dysfunction, significantly improved prognosis of more marked impairment well above clinical assessments currently utilised at time of diagnosis. Chapter 3 briefly outlines the importance of considering at-risk populations given the recent push towards biologically redefining PD for

earlier diagnosis. Chapter 4 explored the influence TBI, as well as the potential moderating effects of injury severity, has on rate of progression of clinical and pathophysiological outcomes in two early PD cohorts. Chapters 5 and 6 assessed the potential prognostic utility of biological assessments in a TBI cohort. Given Chapter 2 identified biomarker concentrations collected acutely following diagnosis demonstrated prognostic utility up to several years, Chapter 5 employed a multilevel meta-analytic strategy to explore the associations between markers of cell injury collected within a week of injury with behavioural outcomes at any-time point following injury. It was found that regardless of moderators explored, biomarkers were significantly associated with global outcome following injury, however, literature assessing more specific outcomes such as cognition, mood and motor were much more limited. Chapter 6 explored the potential diagnostic of nigrosome-1 assessment using susceptibility weighted imaging across a TBI cohort, encompassing several injury severities and times since injury, relative to healthy controls and PD. Further, potential associations between nigrosome-1 voxel wise features and cognition across cohorts was explored.

Overall, this thesis highlights that PD and TBI are complex disorders, reflected by large heterogeneity in the way they present clinically. Further, while the implementation of biological assessments acutely following diagnosis or time of injury for PD and TBI, respectively, shows promise for better characterisation of clinical course, further research must be conducted to determine how biological assessments correspond to more specific outcomes. Finally, this thesis brings attention to several gaps and limitations in current PD and TBI literature, advocating for further assessment of biomarkers in both cohorts to allow for earlier diagnosis and improved prognosis.

Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I also acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Angus McNamara

Acknowledgements

First and foremost, a massive thank you to my principal supervisor Lyndsey. I think the phrase “the bones were there” was the single largest motivator to dedicate myself to everything PhD has to offer and hone my skills as a researcher, just so I never had to receive that comment again. In all seriousness though, thank you for your unwavering investment and support over the years. Your passion inspired me to look beyond the gruelling hours and often tedious work and remember that I am very privileged to be involved in research that could make a world of difference for many people. I am very fortunate to be on a project, and inevitably a career, I find fulfilling so I will always be grateful for providing me the opportunities you have. To Irina and Mark, at several points you stepped up to help me when I needed it, offering your time, counsel and expertise, all of which have been invaluable for me. I am grateful to all of you, and I hope you find this thesis is as much a reflection of your hard work as it is mine.

While PhD was stressful and at times, unbearable, I count myself lucky to have been in the company of many wonderful people that never failed to make me laugh or put a smile on my face. S518 was many things, but boring was never one of them. Ben, you have earned that title “work husband”. Thank you for your limitless patience whenever I lost focus, and always supporting me in any way I needed, whether it was offering to help format my thesis, to suggesting we play video games to unwind. Out of everyone I could have been paired with, I am so fortunate that I was paired with you. Laura, you are undoubtedly one of the funniest people I know and sitting next to you every day made a world of difference. We honestly spent more time together or spoke to one another more than our partners. Be it professional or personal, you were always willing to help, so thank you. From honours to now, I cherish my friendship with both of you.

Liv, you were seriously an anchor for all of us, and your dry sense of humour never failed to make me smile. Also thank you for dealing with my absolute stupidity when it came to Endnote, I honestly don't think I would have finished this thesis if you didn't teach me. Isaac, I wish you started a year earlier because you are genuinely one of the loveliest people I have met. Beyond our banter, I consider you one of the most capable people I know, and I truly think you are an amazing person and researcher. Justin, sucked in for being stuck with me, dating your best friend would do that. While we banter, just know I think you are one of the most capable people I have met, and I truly value your opinion and friendship. To the rest of CANDL, TNL and a special few from the Bardy lab, I'm grateful to you all.

Outside of the office, I would like to thank all my friends that offered a shoulder to lean on, or a much-needed mistake when I needed a break. Adele, Annabelle, Emily, Katie, Andy, Elle, Elli, Ira, Josh, Ellie, Tilly, Elisha, Maddi and Jodi. All of you are incredible and all your support meant the world to me. To my wonderful family: Mum, Dad and the Warming clan: Jenny, John, Ash, Britt and Alex; even if you had no idea what my PhD was about, you always showed interest and made life easier when I needed help.

Last but not least, my wonderful partner Jake. I couldn't have gone through this journey without you. I could never thank you enough for your patience, kindness and willingness to always be there whenever I needed you. You constantly challenged me to remember that PhD is not the be all and end all, and to prioritise my wellbeing. You always spoiled me, and you honestly deserve a medal for dealing with me sometimes (more than sometimes) being neurotic or frantic in moments of stress. You are the most incredible person I have met, and I will forever count myself lucky that you are my partner. I love you lots.

Contribution Made by Co-Authors

The following people have contributed to authorship of the manuscripts enclosed in this thesis (in alphabetical order): Irina Baetu, Lyndsey Collins-Praino, Benjamin Ellul, Mark Jenkinson, and Stephan Lau. The individual contributions of each author can be summarised as:

Conceptualisation of the work: AM, LCP, with advice from IB, MJ, SL

Realisation of the work: AM, LCP, with experimental assistance from IB, MJ, SL & BE

Documentation of the work: AM, IB, LCP, MJ, SL, BE

Editing and review of the work: IB, LCP, MJ, SL

Presentations Arising from Thesis

Poster Presentations

2022

McNamara A, Ellul B, Baetu I, Jenkinson M, Lau S & Collins-Praino LE. *Utility of Baseline Pathological, Neuroimaging and Clinical Markers for Prognosis in Early Parkinson's Disease***.** Presented at the meeting of the Australasian Neuroscience Society Annual Conference. Melbourne, Australia.

2023

McNamara A, Ellul B, Baetu I, Jenkinson M, Lau S & Collins-Praino LE. *Utility of Baseline Pathological, Neuroimaging and Clinical Markers for Prognosis in Early Parkinson's Disease.* Presented at the 6th World Parkinson's Congress. Barcelona, Spain

- Abstract was awarded a travel award by the World Parkinson Congress and Shake It Up Australia

Oral Presentations

2023

McNamara A, Ellul B, Baetu I, Jenkinson M, Lau S & Collins-Praino L Utility of Baseline Pathological, Neuroimaging and Clinical Markers for Progression Prognosis and Subtype Classification in Early Parkinson's Disease: Implications for Cognitive Impairment. Presented at the Alzheimer's Association International Conference. Amsterdam, Netherlands

- Abstract was awarded a travel award by the Alzheimer's Association

2024

McNamara A, Baetu I & Collins-Praino L**.** More than a One-Hit Wonder: Exploring Synergistic Effects of Genetic and Environmental Risk Factors in the Progression of Parkinson's Disease. Presented at the Australasian Winter Conference for Brain Research. Queenstown, New Zealand

- Abstract was awarded a travel award by the School of Biomedicine, University of Adelaide

List of Figures

Figure 1-1 Clinical time course and symptomatic progression in PD, adapted from Kalia and Lang 2015 (Kalia and Lang, 2015). 21

Figure 1-2: Progression of Lewy pathology, highlighting α-syn conformational changes and the eventual formation of Lewy Bodies, as well as additional proteins that constitute them. 40

Figure 1-3 Braak's proposed staging of Lewy Pathology: Stage 1 – Peripheral and enteric nervous system; Stage 2 – Medulla Oblongata; Stage 3 – Pontine Tegmentum; Stage 4: Basal, mid and forebrain, hypothalamus and thalamus; Stage 5 – Mesocortex and allocortex; and Stage 6 – Neocortex 41

Figure 1-4 Dopamine-dependant pathways and key projections, including the nigrostriatal, mesolimbic and mesocortical pathways 49

Figure 2-1 (A) Cropped midbrain region in an axial T2-weighted MRI, along with manual masks (B) for the SN-related T2 hypointensity (blue) and RN (red). 82

Figure 2-2 A 5-fold cross-validation pipeline was developed to create models using identical predictor inputs as used to develop the logistic regression models. 84

Figure 2-3 Median & IQR values at each timepoint displayed for variables with significant interaction effects in the non-parametric mixed ANOVA's. Differences in slopes between clusters were assessed via Mann-Whitney U tests 90

Figure 3-1 Summary of demographic, environmental (TBI and pesticide exposure) and genetic risk factors and their association with PD development 124

Figure 4-1Rate of progression of clinical and pathophysiological outcomes variables in PD with and without a history of TBI within the PPMI cohort. Median & IQR values are displayed at each timepoint for extracted variables of interest. No significant interaction effect was present when comparing rate of progression in PD-Controls compared to PD-TBI 141

Figure 4-2 Rate of progression of clinical and pathophysiological outcomes variables in PD with and without a history of TBI within the PostCEPT cohort. Median & IQR values are displayed at each timepoint for extracted variables of interest. No significant interaction effect was present when comparing rate of progression in PD-Controls compared to PD-TBI 142

Figure 4-3 Rate of progression of clinical and pathophysiological outcomes variables in PD without TBI, as well as PD with TBI encompassing several injury severities were assessed within the PPMI cohort. Median & IQR values are displayed at each timepoint for extracted variables of interest. While there was a significant interaction between timepoint and TBI severity for CSF α-syn (pg/mL)(3B), there was no significant difference in progression as determined via Dunn tests conducted on slopes between injury severity groups. Significant main effects for injury severity and follow-up timepoint were present for striatal DaT binding (3A), however, no other significant main or interaction effect was present for any other variable of interest. 147

Figure 4-4 Mixed ANOVA's determined significant main effects for (4A) TBI severity and (4B) follow-up timepoint for striatal DaT binding. An increase in injury severity and a longer follow-up timepoint were associated with significantly lower striatal DaT binding. 148

Figure 5-1 PRISMA flow diagram 186

Figure 5-2 Orchard plots visualising moderator analyses with significant differences in moderator effect sizes. NF-L demonstrated significantly lower effect sizes relative to S100B (acting as the reference category) for both (A) global outcome, whereby continuous and categorical assessment were collapsed and (B) when assessed on a continuous scale. Further, moderator analysis assessing the time of sample extraction per individual biomarker determined that (C) tau extracted within 1-6 hours of injury (acting as the reference category) had a significantly higher effect size compared to tau extracted seven days following injury, indicating tau extracted at earlier timepoints demonstrate higher associations with global outcome. 215

Figure 5-3 Outlier tests for effect sizes included in Global Outcome multilevel meta-analysis. (A) For residual assessment, effect sizes with a residual z-score > 1.5 or < -1.5 were classified as outliers. (B) Effect sizes with a Cook's distance value of > 1.5 multiplied by the mean Cook's distance score (0.0014) were classified as outliers. 217

Figure 5-4 Sunset (power-enhanced) funnel plot of multilevel meta-analysis exploring association between biomarker of cell injury concentration and global outcome. Each circle represents a unique effect size, with the shading corresponding to power. 217

Figure 5-5 Outlier tests for effect sizes included in Cognition multilevel meta-analysis. (A) For residual assessment, effect sizes with a residual z-score > 1.5 or < -1.5 were classified as outliers. (B) Effect sizes with a Cook's distance value of > 1.5 multiplied by the mean Cook's distance score (0.0013) were classified as outliers. 226

Figure 5-6 Sunset (power-enhanced) funnel plot of multilevel meta-analysis exploring association between biomarker of cell injury concentration and cognition. Each circle represents a unique effect size, with the shading corresponding to power. 226

Figure 5-7 (A) Risk of bias summary plot and (B) Risk of bias traffic plot detailing risk of bias per domain per individual study 232

Figure 6-1 Kruskal-Wallis tests determined significant variance in age and cognitive ability between groups. Post-hoc Dunn tests determined that, relative to healthy control and TBI cohorts, the PD cohort was (A) significantly older, as well as having (B) significantly lower cognitive ability. 296

Figure 6-2 Voxelwise differences in the midbrain were detected between the PD cohort and both the (A) healthy control and (B) TBI cohorts, with significant voxels presented in colour. The scale represents the p-value from the GLM after multiple comparison correction, with yellow-white voxels indicative of voxels that were most significantly different (p-value near 0). 297

Figure 6-3 Kruskal-Wallis tests determined significant differences in age and cognitive ability between groups testing while controlling for age. Post-hoc Dunn tests determined that: (1) relative to the young healthy control and TBI cohorts, the aged cohorts, as well as PD, were significantly older, with the PD cohort also being significantly older than the aged healthy control cohort; (2) a negative relationship was present between age and cognitive ability, with the aged TBI and PD cohorts demonstrating significantly lower cognitive ability compared to both the young healthy control and TBI cohorts. 299

Figure 6-4 Voxelwise differences in the midbrain were detected between the PD cohort and the (A) young healthy control, (B) young TBI, (C) aged healthy control and (D) aged TBI cohorts, with significant voxels presented in colour. The scale represents the p-value from the GLM after multiple comparison correction, with yellow-white voxels indicative of voxels that were most significantly different (p-value near 0). 301

List of Tables

Table 1-1 Clinical phenotypes of PD and their presentation 22

Table 1-2 Comparison of several features across UKPDSBB, Gelb's and MDS-PD diagnostic criterion ..30

Table 1-3 Additional proteins that contribute to the development of Lewy Bodies in PD 39

Table 1-4 Radioligands that bind to TSPO receptor sites as a marker of microglial activation and neuroinflammation.....60

Table 2-1 Data extracted from the PPMI database and their corresponding assessments.....77

Table 2-2 Differences between clusters in all extracted assessments were assessed retrospectively at baseline. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via T-tests. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Mann-Whitney U tests. Count data are presented as percentages (%) and differences were assessed via a Chi-square test. A Bonferroni correction was applied to account for multiple comparisons, setting the threshold to $p < 0.003$. Statistically significant differences are denoted by bolded p-values. 85

Table 2-3 Baseline characteristics of participants included in the final dataset, who all had MDS-UPDRS scores up to year-5 follow-up, compared against participants lost to attrition. Overall, participants lost to follow-up demonstrated significantly worse outcomes in multiple domains at baseline. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via independent samples t-test. Non-parametric variables are displayed as median (IQR), with differences determined via Mann-Whitney U. Count data are presented as percentages (%) and differences were assessed via a Chi-square test. A Bonferroni correction was applied to account for multiple comparisons, setting the threshold to $p < 0.003$. Statistically significant differences are denoted by bolded P-values. 86

Table 2-4 Differences between clusters in assessments with follow-up data were assessed at year-5. Further, clinical characteristics, such as Hoehn Yahr staging and cognition-related diagnoses, were compared. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Mann-Whitney U tests. Count data are presented as percentages (%) and differences were assessed via a Chi-square test. A Bonferroni correction was applied to account for multiple comparisons, setting the threshold to $p < 0.006$. Statistically significant differences are denoted by bolded P-values. 88

Table 2-5 Two logistic regression models were created with cluster membership as the outcome variable. The first model only included baseline values of the MDS-UPDRS scores (the year-5 values for the same measures were used in the hierarchal clustering protocol) as predictors, whereas the second model included additional baseline demographic, neuroimaging, biofluid marker and prodromal assessment scores. Significant predictors are denoted by bold P values, with threshold set to 0.05 as R computes internal corrections for multiple corrections. 91

Table 3-1 Pesticides commonly researched within PD literature, as well as evidence of associated risk and pathophysiological changes resulting from exposure. 119

Table 3-2 Most common genetic risk factors of PD and supporting evidence highlighting their association with the pathophysiological hallmarks of PD and increased risk of disease development.....121

Table 4-1 Data extracted from the PPMI database and their corresponding assessments.....132

Table 4-2 Data extracted from the PostCEPT database and their corresponding assessments.....134

Table 4-3 Differences in variables of interest between PD-Controls and PD-TBI were assessed at baseline. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via T-tests. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Mann-Whitney U tests. Statistically significant differences are denoted by bolded p-values 139

Table 4-4 Multivariate linear regression was conducted to determine whether a history of TBI in the (A) PPMI or (B) PostCEPT cohort was a significant predictor of follow-up outcomes. Outcomes included neuropsychiatric (cognitive ability and mood dysfunction) or motor (rigidity and tremor scores) at follow-up. In addition, striatal DaT binding and CSF α-syn was assessed as an outcome in the PPMI cohort at year-4 and year-3, respectively. All models controlled for age, education years, and sex of the participants. Significant predictors are denoted by bold p-values. 143

Table 4-5 Differences in variables of interest between PD-Controls and different severities of PD-TBI (PD-mTBI, PD-rmTBI, PD-M/sTBI) were assessed at baseline in the PPMI cohort. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via one-way ANOVA's. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Kruskal-Wallis tests. Statistically significant differences are denoted by bolded p-values 145

Table 4-6 Multivariate linear regression was conducted to in the PPMI cohort to determine whether injury was a significant predictor of outcomes at follow-up. Outcomes included neuropsychiatric (cognitive ability and mood dysfunction) or motor (rigidity and tremor scores) at year-5 follow-up, as well as striatal DaT binding and CSF α-syn at year-4 and year-3 respectively. 149

Table 5-1 Biomarkers of cell injury and studies highlighting their up-regulation following TBI 169

Table 5-2 Magnitude of effect size strength across various statistical techniques 177

Table 5-3 Formulae used to convert various statistical tests into correlation coefficients, to ensure standardised effect sizes across studies 178

Table 5-4 Characteristics and descriptive statistics of studies assessing relationship between biomarker of cell injury and global outcome 189

Table 5-5 Multilevel meta-analysis assessing the relationship between biomarkers of cell injury extracted within a week of TBI and global outcome, as assessed via GOS and GOSE, at any timepoint post-injury 205

Table 5-6 Results of global outcome moderator analysis 207

Table 5-7 Characteristics and descriptive statistics of studies assessing relationship between biomarker of cell injury and cognition 219

Table 5-8 Multilevel meta-analysis assessing the relationship between biomarkers of cell injury extracted within a week of TBI and cognition assessed at any timepoint post-injury 223

Table 5-9 Results of cognition moderator analysis 224

Table 5-10 Characteristics and descriptive statistics of study assessing relationship between biomarkers of cell injury and mood 228

Table 5-11 Characteristics and descriptive statistics of study assessing relationship between biomarker of cell injury and motor outcome 228

Table 5-12 Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Summary of Findings table for primary outcomes 235

Table 6-1 Cohort specific inclusion criteria 289

Table 6-2 Cognitive measures used to derive a composite cognitive ability score, including the domains they assess and the way they were scored 291
Table 6-3 PCA outputs for cognitive ability scores 292
Table 6-4 Differences in variables of interest between healthy controls, TBI and PD. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via one-way ANOVAs. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Kruskal-Wallis tests. Statistically significant differences are denoted by bolded p-values. 295
Table 6-5 Group differences while controlling for potentially confounding effects of age. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via one-way ANOVAs. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Kruskal-Wallis. Statistically significant differences are denoted by bolded p-values 298
Table 6-6 Multivariate linear regressions were conducted to determine whether confounders present within the voxelwise GLM were significant predictors of cognitive ability. Regressions determined that both older age and membership in the PD cohort relative to healthy controls yielded significant negative associations with cognitive ability, with a higher number of years of education demonstrating a significant positive association**.** 302

Style Conventions

The abbreviations, punctuations and reference style used in this thesis conform with the *Oxford style manual* and *Harvard referencing style*. The spelling is British English and conforms with the *Oxford English Dictionary*.

Financial Support

My doctoral studies were supported by funding from the Government of Australia (Research Training Program Scholarship).

The research carried out throughout the course of this thesis was supported by funding from the Medical Research Future Fund and James and Diana Ramsay Foundation.

Chapter 1 - Towards a Biologically Defined Diagnosis: Incorporating Pathophysiological Measures into Parkinson's Disease Clinical Criteria

1.1 Parkinson's Disease Prevalence & Burden

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide (Pang et al., 2019). From 1990 to 2015, the number of individuals diagnosed with PD increased by 118%, with a worldwide prevalence of 6.2 million (Feigin et al., 2017). Notably, as of 2019, this increased again, rising ~37% to approximately 8.5 million confirmed PD cases worldwide within just a four-year period (Organization, 2022b). Concerningly, prevalence of PD is expected to exceed 12 million cases by 2040 (Dorsey and Bloem, 2018), making it the most rapidly growing neurological disorder in the world (Feigin et al., 2017). This is largely thought to be a result of the rapid increase in the ageing population, with the World Health Organisation (WHO) reporting in 2019 that the average life expectancy was 9.8% higher than in 2000, a figure that is projected to increase even further in years to come (WHO, 2020). For example, in Sweden, of 50,000 babies born in 2007, more than 50% are projected to become centenarians, compared to just ~750 who reached age 100 in 2007 (Vaupel, 2010). This is significant, as age has been identified as the single most significant risk factor for PD (Tanner and Goldman, 1996).

This dramatic increase in PD is of particular concern, given that PD corresponds to considerable burden not only for those diagnosed and their families, but also for society at large. In line with this, as of 2019, the number of years of healthy life lost to disability (YLDs) caused by PD increased by 154.73% compared to 1990 (Ou et al., 2021). Further, PD resulted in 5.8 million disability adjusted life years (DALYs), an increase of 81% compared to 2000, and resulted in 329,000 deaths, a 100% increase since 2000 (Organization, 2022a). Such disability corresponds to reduced quality of life (QoL) for those with PD, with a recent meta-analysis of 27 studies reporting that people with PD (PwP) reported lower QoL in multiple

domains, particularly physical functioning and mental health (Zhao et al., 2021). Further, reduced QoL extends beyond PwP, as disease burden also has profound consequences on familial caregivers, particularly spouses (Lee et al., 2019a). This also has implications for economic burden, with a recent study conducted in the United States reporting that PD corresponds to an economic burden of $51.9 billion annually (Yang et al., 2020). Given the projected increased incidence of PD over the coming years, this is expected to rise to $79 billion by 2037 (Yang et al., 2020).

Due to the considerable burden of PD, now and in the future, early identification of the disease is essential. Currently, diagnosis of PD relies primarily on its symptomatic presentation, but there is increasing pressure in the field to move towards a more biological-based definition (Simuni et al., 2024). This review will discuss current challenges surrounding PD diagnosis, due to primary reliance on clinical criterion, summarise recent efforts to re-define diagnosis based on PD biological presentation and propose how recent advancements in assessing PD pathophysiology *in vivo* may help to make such a biological definition feasible.

1.2 Symptomatic Profile of PD

While often considered a hypokinetic motor disorder, PD is characterised by a range of both motor and non-motor symptoms that advance over time due to progressive cell loss and neurotransmitter imbalances in several regions (for review, see (Kouli et al., 2018)). The cardinal motor features include tremor at rest, bradykinesia, muscle rigidity, postural instability and gait disturbance (Moustafa et al., 2016). In the early stages of PD, said motor symptoms are often unilateral and mild; however, as PD advances, symptoms generally become bilateral and increasingly severe, with postural instability and freezing of gait becoming more pronounced (Kalia and Lang, 2015). Motor features are of particular relevance to PD, considering that their clinical presentation aligns with current diagnostic criteria (Kalia and

Lang, 2015). Nevertheless, PD is also associated with a variety of non-motor symptoms, referred to as prodromal symptoms, that begin to emerge years, or even decades, prior to motor features. Prodromal features are multi-faceted and involve various body systems, including autonomic dysfunction, sleep disturbances, such as REM sleep behaviour disorder (RBD), hyposmia, sexual dysfunction and mood changes (Kumaresan and Khan, 2021). Beyond the prodromal period, PD is also associated with the emergence of other non-motor symptoms, including, of particular note, cognitive decline (Kumaresan and Khan, 2021). Over time, cognitive decline can evolve into mild cognitive impairment (PD-MCI) and, eventually, PD Dementia (PD-D) (Kalia and Lang, 2015). In fact, 50% of PwP will develop PD-D within 10-years of diagnosis (Williams-Gray et al., 2013), increasing to 80% within 20-years (Aarsland and Kurz, 2010). A visual representation of PD features and their progression over time is detailed in Figure 1-1:

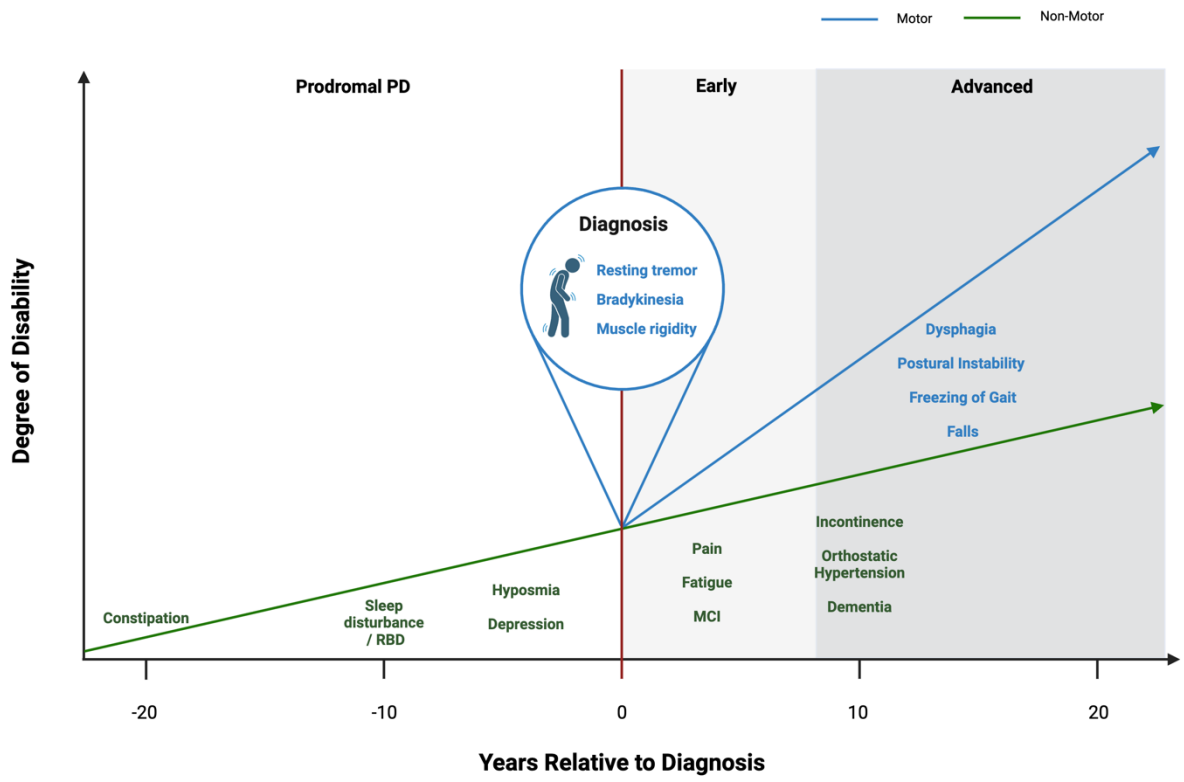


Figure 1-1 ***Clinical time course and symptomatic progression in PD****, adapted from Kalia and Lang 2015 (Kalia and Lang, 2015).*

It is important to note that clinical presentation and progression across PwP is heterogeneous (Greenland et al., 2019). This has translated to substantial efforts in recent years to better characterise disease course and outcomes across PD, to improve disease management. This is reflected by the development of clinical phenotypes, briefly outline in Table 1-1:

***Table 1-1** Clinical phenotypes of PD and their presentation*

Subtype	Age of Onset	Motor Features	Cognitive Decline	Prodromal Profile	Progression
		Motor Phenotypes (Ren et al., 2020, Rajput et al., 2009)			
Tremor Dominant	Younger	Primary symptom: Resting tremor	Slow	Milder	Slow
Postural Instability Gait Disturbance	Older	Primary symptoms: Postural instability & freezing of gait	Rapid	More profound	Rapid
Akinetic-Rigid		Primary Symptom: Axial and appendicular akinesia & muscle rigidity			
		Subtypes Determined via Comprehensive Assessment of Motor, Cognitive and Autonomic Function, Disease Severity & Medication Responsiveness (Fereshtehnejad et al., 2017)			
Mild-Motor	Younger	*Resting Tremor:* Mild or intermittent *Bradykinesia:* Present, but less severe *Rigidity:* Mild to moderate stiffness Gait difficulties: Mild *Postural Instability:* Mild	Slow	Milder	Slow
Diffuse Malignant	Older	*Resting Tremor:* Severe and persistent	Rapid	More profound	Rapid

	Bradykinesia: Challenges with executing movement *Rigidity:* Marked muscle stiffness *Gait difficulties:* Severe *Postural Instability:* Pronounced			

Age of Onset Phenotypes (Pagano et al., 2016)

Early-Onset	Younger (< 50)	Slow	Milder	Slow
Late-Onset	Older (≥ 50)	Rapid	More profound	Rapid

However, subtyping based on clinical features does not adequately capture all possible disease presentations, with many PwP not neatly falling into a single category. This is reflected by phenotypes based on both motor features and more comprehensive subtyping having an indeterminate and intermediate category, respectively. In fact, motor phenotype classification within the BioFIND (n = 42) and Parkinson Progression Markers Initiative (PPMI) (n = 423) resulted in approximately 12% of individuals being classified as indeterminate in both cohorts (Luo et al., 2019). Likewise, in the Personalised Parkinson Project cohort (n = 431), when following Fereshtehnejad and colleagues subtyping guidelines, approximately 32% were classified as intermediate (Johansson et al., 2023). Therefore, while subtyping has been crucial in disentangling differential outcomes in PD, it is evident that, rather than clear distinctions, PD progression and clinical presentation is a spectrum and should be considered as such. In fact**,** a recent meta-analysis of 38 studies highlights the shortcomings of subtyping, recommending that continued efforts must be made to address heterogeneity at the individual level (Mestre et al., 2021). Recognising diversity in PD presentation, several reforms in diagnostic criteria and clinical rating scales have been made over the years to better reflect this.

1.3 Evolution of the Diagnostic Criteria for PD

1.3.1 United Kingdom Parkinson's Disease Society Brain Bank Criteria

The United Kingdom Parkinson's Disease Society Brain Bank (UKPDSBB) criteria were developed in 1988 and acted as the first official diagnostic criteria for PD. Prior to this, diagnosis was reliant on clinical judgement of cardinal symptoms and their progression, rather than a standardised diagnostic procedure (Gibb and Lees, 1988). These criteria consist of three classes (Gibb and Lees, 1988):

1) *Inclusion Criteria*: Presence of bradykinesia and at least one of the following: resting tremor (4-6 Hz), muscular rigidity or postural instability
2) *Exclusion Criteria*: Symptoms attributed to other disorders (i.e. repeated strokes or head injury, encephalitis, early severe autonomic dysfunction or dementia) exclude PD diagnosis. Additionally, a negative response to levodopa treatment is a contraindication of PD.
3) *Supporting Criteria*: At least three supporting criteria required from the following: unilateral onset, resting tremor, progressive disease course, persistent asymmetry, profound response to dopaminergic therapy, levodopa-induced dyskinesia or a positive levodopa response for five years or more.

Such criteria advanced diagnosis considerably, particularly highlighting bradykinesia as a core feature of PD (Berardelli et al., 2001). Further, incorporation of levodopa-responsiveness showed promise in distinguishing PD from other parkinsonisms, such as progressive supranuclear palsy (PSP), which show minimal benefit from dopaminergic therapies (Kompoliti et al., 1998). Despite such advancements, however, the UKPDSBB criteria present several diagnostic pitfalls. In line with this, Hughes and colleagues demonstrated that the UKPDSBB criteria displayed only a 76% autopsy-confirmed diagnostic accuracy (Hughes et al., 1992b). The remaining 24% of cases were diagnosed with other disorders post-mortem,

such as PSP, multiple system atrophy (MSA), Alzheimer's disease (AD) and basal ganglia (BG) vascular pathology, highlighting challenges with differential diagnosis based on symptom presentation alone (Hughes et al., 1992b).

Of note, adjusting criteria to include asymmetric onset, the presence of no atypical features and no potential cause for other forms of parkinsonism increased diagnostic accuracy of true-positive cases to 93% (Hughes et al., 1992a). This, however, came at the expense of 32% of pathologically confirmed cases of PD no longer being identified clinically pre-mortem, highlighting that stricter application of the criteria with additional features reduces misdiagnosis, but at the expense of excluding genuine PD cases (Hughes et al., 1992a).

1.3.2 Gelb's Criteria

Following implementation of the UKPDSBB criteria, Gelb and colleagues proposed a new set of clinical criteria in 1999 (Gelb et al., 1999). Like the UKPDSBB criteria, Gelb's clinical criteria were primarily based on the cardinal motor features, including an individual presenting with at least two of the following features: resting tremor, bradykinesia, unilateral onset or muscle rigidity, as well as a positive levodopa response (Gelb et al., 1999). Similarly, Gelb's criteria also provided exclusion criteria, although offering a more comprehensive list that the UKPDSBB criteria, such as the early presence of postural instability (< 3 years), hallucinations not resulting from medications, dementia prior to motor symptoms in the first year and several more (Gelb et al., 1999).

A key distinction between UKPDSBB criteria and Gelb's criteria was the introduction of three levels of diagnostic confidence: 1) clinically possible 2) clinically probable and 3) clinically definite, adapted from previous attempts to redefine diagnostic criteria, albeit with limited traction (Calne et al., 1992, Larsen et al., 1994). Assignment to diagnostic confidence levels was as follows (Gelb et al., 1999):

1) *Clinically possible*: Presence of bradykinesia and at least one of the following: muscular rigidity, resting tremor or postural instability, as well as the absence of atypical features (early onset i.e. < 40, severe autonomic dysfunction, rapid disease progression, substantial cognitive impairment, profound early postural instability and other neurological signs i.e. cerebellar-related difficulties)
2) *Clinically probable*: Clinically possible criteria are met, along with at least three supporting criteria (responsive to levodopa treatment, resting tremor, asymmetrical onset, good initial response to dopamine agonists and the absence of red flags i.e. atypical features)
3) *Clinically definite*: Confirmation based on post-mortem histological assessment

Consequently, only the first two levels are based on clinical assessment, with a confirmed diagnosis only being possible following autopsy.

Direct comparison between UKPDSBB and Gelb's criteria for diagnostic validity is limited, with, to our knowledge, only one study directly comparing the two (Hughes et al., 2001). This study determined that both criteria performed comparably for possible and probable PD (UKPDSBB: 92% positive predictive value, 25% negative predictive value; Gelb's: 93% positive predictive value and 14% negative predictive value) (Hughes et al., 2001). However, sensitivity was higher using UKPDSBB criteria (90%), compared to both Gelb's clinically possible PD (87%) and probable PD (72%), with both sets of criteria demonstrating low specificity (30-40%) (Hughes et al., 2001). Low sensitivity may reflect broad-spectrum features seen commonly across parkinsonisms, rather than features specific to PD, thus resulting in a potentially high rate of false positive diagnoses.

1.3.3 Criticisms and Addressing Prodromal PD

A major critique of both the UKPDSBB and Gelb diagnostic criteria is the heavy focus on motor features to provide a PD diagnosis. In support of this, Alder and colleagues determined that using such motor symptom-based criteria, clinical diagnosis of PD yielded only an 88% sensitivity and 68% specificity for pathologically confirmed PD. (Adler et al., 2014) This highlights the need to incorporate other features of PD into diagnosis, in order to better distinguish PD from other forms of parkinsonism. Of note, as discussed above, the prodromal phase of PD, which occurs years or even decades prior to clinical diagnosis, is characterised by the presence of several non-motor symptoms, many of which predate the onset of cardinal motor features, including hyposmia, sleep disturbance, autonomic dysfunction and mood changes (see review, (Postuma and Berg, 2019)). In fact, a retrospective study of 93 PD participants reported that 98.8% experienced an average of 7.6 different prodromal symptoms prior to clinical diagnosis, with an average time span of 10.2 years prior (Gaenslen et al., 2011). Further, assessing prodromal features shows benefits in distinguishing PD from other parkinsonisms, a challenge with earlier clinical criteria. For example, Müller and colleagues found that olfactory tests were effective in discriminating PD from both MSA and PSP (Müller et al., 2002), with another study highlighting that impaired olfaction is present in 82% of nondemented PwP (Double et al., 2003). Additionally, rates of RBD are disproportionately high in PD compared to other disorders (see review, (Mahmood et al., 2020)), with a study assessing 457 sleep-disturbed PwP reporting a frequency of 46% (Sixel-Döring et al., 2011). Overall, this highlights that incorporating additional clinical features within diagnostic criteria may correspond to a more robust strategy for identifying and diagnosing PD, even prior to motor symptom onset.

1.3.4 MDS-PD Criteria

Considering this, the Movement Disorders Society (MDS) task force proposed a revised set of clinical criteria, known as the MDS-PD criteria (Postuma et al., 2015a). While designed for use in clinical research, its standardisation and consequent reproducibility across sites and centres make it suitable to guide clinical diagnosis (Postuma et al., 2015a). Like past criteria, motor features are still a central feature of PD diagnosis, however, several non-motor features are now considered for both PD diagnosis (Postuma et al., 2015a), as well as prodromal PD assessment (Berg et al., 2015).

To this end, it is recommended that examination of cardinal PD features should be conducted as described in the MDS-Unified Parkinson Disease Rating scale (MDS-UPDRS), a clinical rating scale including self-report of motor and non-motor aspects of daily living, as well as clinician assessment of motor features and motor complications (Goetz et al., 2008). Clinician assessment in the MDS-PD criteria still heavily relies on traditional motor features, with the essential criteria required to establish parkinsonism being the presence of bradykinesia, as well as either resting tremor (4-6 Hz) or muscle rigidity. Once parkinsonism is established, MDS-PD criteria build upon previous diagnostic criteria through the inclusion of several key features including establishing certainty level (i.e. clinically established vs. clinically probable PD), including both negative features (i.e. absolute exclusions and red flags) and positive features (i.e. supportive criteria) that argue against/for PD diagnosis, and assigning weightings (e.g. absolute exclusion criteria vs red flags). Specifically, MDS-PD criteria are applied to determine the following:

1) Diagnosis of clinically established PD
    - Absence of absolute exclusion criteria indicative of other disorders that could mimic PD, such as vascular parkinsonism, drug-induced parkinsonism, etc.

- At least two of the following supportive criteria: clear and substantial beneficial response to dopaminergic therapy; presence of levodopa-induced dyskinesia; resting tremor of a limb; positive results from at least one ancillary diagnostic test reflective of prodromal symptoms (e.g., olfactory loss or meta-iodo-benzyl-guanidine (MIBG) scintigraphy, indicative of cardiac sympathetic denervation).
- No red flags, including: rapid progression of gait impairment; absence of motor symptom progression or over five-years; early bulbar dysfunction; inspiratory respiratory dysfunction; severe autonomic dysfunction within five-years of diagnosis; recurrent (> once a year) falls due to impaired balance within three years of onset; disproportionate anterocollis (i.e. cervical dystonia that causes the neck muscles to contract in a repetitive, patterned way); absence of any common non-motor features within five-years of onset (e.g. sleep dysfunction, autonomic dysfunction, hyposmia or psychiatric dysfunction); unexplained pyramidal tract signs and bilateral symmetric parkinsonism throughout disease course.

2) Diagnosis of clinically probable PD
   - Absence of absolute exclusion criteria
   - Presence of red flags, but which are counterbalanced by supportive criteria. Further, a maximum of two red flags can be present before a diagnosis of PD is excluded

Comparison of UKPDSBB, Gelb's and MDS-PD diagnostic criteria is presented in Table 1-2:

Table 1-2 Comparison of several features across UKPDSBB, Gelb's and MDS-PD diagnostic criterion

	UKPDSBB Criteria (1988)	Gelb's Criteria (1999)	MDS-PD Criteria (2015)
Year Established	1988	1999	2015
Core Motor Features Required for Diagnosis	Presence of bradykinesia and at least one of the following: resting tremor (4-6 Hz), muscular rigidity or postural instability	Two of the following: resting tremor (4-6 Hz), bradykinesia, unilateral onset or muscle rigidity	Presence of bradykinesia, as well as either resting tremor (4-6 Hz) or muscle rigidity
Exclusion Criteria	Other disorders (i.e. stroke, head injury, encephalitis, early severe autonomic dysfunction, dementia); poor or limited response dopamine therapy	Early postural instability (<3 years), hallucinations not resulting from medications, dementia prior to motor symptom onset, rapid progression, other neurological signs (e.g., cerebellar difficulties)	Conditions and corresponding features that mimic PD (i.e., vascular parkinsonism, drug-induced parkinsonism)
Establishment of Parkinsonism	Not explicitly present, but bradykinesia is emphasised as a central feature	Not explicitly present, but bradykinesia is emphasised as a central feature	Key initial step in MDS-PD criteria: Parkinsonism is determined by the presence of core motor features (bradykinesia + resting tremor or rigidity). Once parkinsonism is established, additional

			diagnostic procedures are applied to determine PD
Supporting Criteria	Unilateral onset, resting tremor, progressive course, asymmetry, response to dopaminergic therapy, levodopa-induced dyskinesia	Unilateral onset, resting tremor, progressive course, asymmetry, response to dopaminergic therapy, levodopa-induced dyskinesia, progressive disease course	Benefit to dopaminergic therapy; presence of levodopa-induced dyskinesia; resting tremor of a limb; positive results from at least one ancillary diagnostic test reflective of prodromal symptoms (i.e. olfactory loss, sleep disturbance or MIBG scintigraphy, indicative of cardiac sympathetic denervation)
Diagnostic Levels	No distinctions in diagnostic confidence are present. Diagnosis requires core motor features and three supporting criteria.	Three levels 1) **Clinically possible**: Bradykinesia + one additional feature, no atypical features 2) **Clinically probable**: Clinically possible + three supporting criteria like levodopa response, asymmetry, absence of red flags 3) **Clinically definite**: Post-mortem confirmation	Two levels: 1) **Clinically established PD**: Absence of exclusion criteria, at least two supportive criteria, no red flags 2) **Clinically probable PD**: Absence of exclusion criteria, presence of up to two red flags counterbalanced by supportive criteria

Red Flags	Not present in criterion	Not present in criterion	Rapid gait progression, absence of motor progression >5 years, early bulbar dysfunction, severe autonomic dysfunction, recurrent falls, unexplained pyramidal tract signs, bilateral symmetric parkinsonism
Emphasis on Non-Motor Features	Not present in criterion	Limited: Early cognitive decline and hallucinations are included as atypical features	Stronger focus on non-motor features like olfactory loss, MIBG scintigraphy, sleep dysfunction, autonomic dysfunction in both diagnosis and prodromal assessment
Autopsy Confirmation	Post-mortem assessment required for diagnostic confirmation	Required for clinically definite PD diagnosis	Required for clinically definite PD diagnosis (not synonymous with clinically established PD)

Overall, MDS-PD criteria demonstrate high utility for PD diagnosis, particularly when compared to other clinical criteria. Postuma and colleagues highlighted this in a cohort of 434 PwP, MDS-PD diagnosis compared against the gold standard, a neurologist with > 10 years' experience in PD diagnosis. Overall, 94.5% of PwP met criteria for probable PD, along with 192 (88.5%) non-PD participants being correctly identified as non-PD, according to MDS-PD criteria. Further, MDS-PD criteria displayed significantly higher accuracy than UKPDSBB criteria (92% vs. 86% respectively). this demonstrates the advancements made in PD diagnosis in recent years, although even revised criteria are not without limitations.

1.3.5 Overall Commentary on Current Diagnostic Criteria

In particular, MDS-PD criteria are reliant on the MDS-UPDRS rating scale, which is not routinely applied in clinical settings, as it is both time consuming and can be subjective, due to its reliance on patient self-report (Goetz et al., 2008), as well as clinical judgement, which heavily relies on rater expertise, with MDS-UPDRS ratings displaying large variability across practitioners (Post et al., 2005). This subjectivity may influence high rates of misdiagnosis, with an estimated 20% of PD cases being misdiagnosed and, subsequently, mistreated (Holroyd et al., 2008). This is further corroborated by a recent case study from China, highlighting significant misdiagnosis rates of approximately 25%, which, interestingly, were more prevalent in individuals who presented with rigidity or gait abnormalities, compared to resting tremor (Yingying, 2015). Rates of misdiagnosis may also be influenced by the heavy priority still placed on motor features to identify PD, with many of the classic motor symptoms of PD overlapping with those seen in other forms of parkinsonism, such as PSP and MSA (MartÌ et al., 2003).

1.3.6 Moving Forward – A Biologically Defined Diagnosis

As outlined previously, clinical onset of PD, denoted by motor symptom manifestation, does not truly reflect point of disease onset, as evident by the extensive prodromal phase prior to motor symptom presentation (Postuma et al., 2012). This is of critical importance, as continued reliance nearly solely on motor features will correspond to late diagnosis and, consequently, reduced efficacy of treatment strategies (Armstrong and Okun, 2020). Moving forward, rather than relying on the presentation of motor features, which do not manifest until >60% of the dopamine neurons of the substantia nigra are lost (Dauer and Przedborski, 2003), it would be of great benefit to incorporate changes seen in the prodromal period into diagnostic criteria. While prodromal clinical assessment displays promise, however, it is also recognised that such symptoms are non-specific (Postuma et al., 2012). Instead, targeting pathological changes, which are present years, or even decades, prior to clinical symptom onset (Wu et al., 2011), may have implications for refining diagnostic criteria. In line with this, Rizzo et al have suggested that incorporating objective assessments of underlying pathological markers, measured either within biofluids or using neuroimaging, into diagnostic criteria may help to address current shortcomings (Rizzo et al., 2016).

In line with this, in recent years, several efforts have been made to integrate biomarkers and neuroimaging into PD diagnosis. To date, two types of nuclear imaging have been approved by the FDA for use in clinical settings; namely, dopamine transporter (DaT) single photon emission computed tomography (SPECT) and fluorodopa (F-Dopa) positron emission tomography (PET) scans in 2011 and 2019, respectively (de la Fuente-Fernández, 2012, Jiang et al., 2021a). Both imaging techniques assess the nigrostriatal system, with DaT SPECT assessing dopaminergic terminal density (Akdemir et al., 2021), whereas F-Dopa works by assessing uptake of a radioactively labelled form of levodopa (Berti et al., 2011). Both techniques have demonstrated similar utility in clinical settings, with both able to distinguish

parkinsonism from healthy controls (Eshuis et al., 2009), as well as advanced PD from *de novo* cases (Eshuis et al., 2006a), although DaT SPECT appears to demonstrate higher promise, on account of higher sensitivity and is considered to be the current gold standard (Wallert et al., 2022). Beyond nuclear imaging, research has also been conducted using structural magnetic resonance imaging (MRI) sequences, particularly susceptibility weighted imaging (SWI) and neuromelanin-sensitive MRI. Both sequences are optimised to assess PD-relevant structures, such as nigrosome-1, and display high diagnostic utility for differentiating PD from healthy controls (Chau et al., 2020, Cho et al., 2021a). Of note, these imaging modalities also display utility in differentiating PD from similar neurological conditions that present substantial clinical overlap, including other parkinsonisms, such as PSP and MSA (Peralta et al., 2022). Most recently, strides have also been made in the detection of alpha-synuclein (α-syn), with the recently developed seed amplification assay (SAA) being of particular promise. In fact, the SAA demonstrates not only high sensitivity in distinguishing PD from healthy controls (Siderowf et al., 2023), but can even identify prodromal PD prior to the emergence of cardinal motor features (Yan et al., 2024), suggesting that it may be a critical tool for earlier diagnosis. Additionally, a recent cross-sectional study exploring the efficacy of skin biopsies to assess phosphorylated α-syn, as opposed to total, determined that such a marker was highly present in PD (as well as other synucleinopathies), relative to healthy controls (Gibbons et al., 2024).

Despite the potential such objective biomarker assessments present for diagnosis, however, this has not yet translated to changes in diagnostic guidelines that formally incorporate these measures. In fact, cardiac sympathetic denervation on MIGB scintigraphy is the only laboratory test currently included as a diagnostic criterion within MDS-PD criteria (Postuma et al., 2015a). Notably, however, the Neuronal α-syn Disease (NSD) research framework, was proposed in 2024, which aims to assess underlying biology preceding clinical presentation as a staging system of PD (Simuni et al., 2024). While very much in its infancy,

and not yet recommended to be applied within a clinical setting, such a framework sets the stage for major advancements in the field. Using such a framework, it might be possible to routinely explore markers known to be relevant to PD pathology within cohorts with a high risk of future PD development (e.g. those with a particular genetic mutation or a history of exposure to a known risk factor, such as prior history of head injury or pesticide/herbicide exposure), in order to track changes in such markers over time and aid in earlier diagnosis of PD. The pathological basis of PD, however, is highly complex, and therefore it is important to consider what aspects demonstrate the highest current promise, in order to focus future biomarker development.

1.4 The Role of Alpha Synuclein (α-syn) in PD

PD pathology is complex (Zaman et al., 2021), involving interplay between several different markers of interest; however, Lewy body pathology is recognised as the hallmark pathological landmark of PD. The primary protein implicated in Lewy pathology is α-syn, which is comprised of 140 amino acids and possesses three distinct structural features: 1) an N-terminal region (residues 1-60) 2) a central region, with a non-amyloid compartment prone to beta-sheet formation (residues 61-95) and 3) a C-terminal region (residues 96-140) (Skaanning et al., 2020). While its function under normal physiological conditions is still a matter of debate, α-syn is hypothesised to play a role in neurotransmitter release due to its presynaptic location and interactions with synaptic membranes (Calabresi et al., 2023). Of note, α-syn in its monomeric form is natively unfolded and without a defined structure (Weinreb et al., 1996). Consequently, monomeric α-syn is relatively unstable, primarily within the C-terminal region (Pieri et al., 2016). When exposed to post-transitional modifications, such as serine 129 phosphorylation, ubiquitination and oxidation (for review, see (Zhang et al., 2019a)), its inherently flexible structure demonstrates a propensity to undergo structural modifications,

such as misfolding and aggregation (see review, (Gallegos et al., 2015)). Consequently, in the pathophysiology of PD, α-syn monomers undergo several structural modifications, including oligomerisation, fibrillation and, eventually, Lewy body formation.

1.4.1 Oligomerisation

Following post-translational modifications, as well as an imbalanced rate of production and clearance, resulting in an overproduction of α-syn, monomeric α-syn misfolds and begins to aggregate, forming intracellular soluble oligomers (Stefanis, 2012). Oligomers vary in composition and size and are key hallmarks of PD (Stefanis, 2012), with the presence of soluble α-syn aggregates demonstrated both in midbrain neurons in α-syn transgenic mouse brains and in both PD and DLB post-mortem tissue (Sharon et al., 2003).

Many converging lines of evidence suggest that α-syn oligomers are the toxic species within Lewy pathology that drive neurodegenerative processes. For example, in *in vitro* models of mesencephalic dopaminergic neurons, exposure to extracellular oligomeric α-syn promotes cell dysfunction and death via tubulin polymerisation and mitochondrial dysfunction (Chen et al., 2007). Interestingly, Emin and colleagues also found that, *in vitro,* aggregates of wild-type α-syn, smaller than 200 nm in length, promote neurotoxicity via inflammation and permeabilization (Emin et al., 2022). Not only does this suggest that oligomeric α-syn induces a cascade of neurotoxic events, but that the size of α-syn aggregates contributes to their toxicity. Similar findings have also been reported *in vivo*, with lentivirus and adeno-associated virus viral vector rat models of PD demonstrating that, following injection of α-syn variants into the substantia nigra (SN), t oligomeric species are the key driver of SN *pars compacta* (SNc) degeneration via membrane disruption (Winner et al., 2011), resulting in dystrophic neurites and gliosis within the striatum (Dimant et al., 2013).

Not only do α-syn oligomers display high levels of toxicity, but, importantly, they also demonstrate seeding capacity, which is critical for the spread of Lewy pathology throughout the brain. It has been shown that α-syn oligomers can be released from diseased neurons into the extracellular space, and consequently taken up by previously healthy neurons (Volpicelli-Daley et al., 2011, Hoban et al., 2020, Kordower et al., 2011, Hansen et al., 2011). Oligomers then act as templates, promoting endogenous monomers to undergo misfolding and aggregation, perpetuating increased oligomer formation and contributing to the development of Lewy body pathology.

1.4.2 Fibrillation

During the nucleation and growth phase of oligomerisation, oligomers increase in size and elongate, due to the incorporation of β-sheets which stack together, resulting in the formation of protofibrils (see review, (Lashuel et al., 2013)). Compared to the flexible monomeric and oligomeric form of α-syn, protofibrils display a more organised structure (Lashuel et al., 2013). Protofibrils can undergo further structural changes, such as alignment with other protofibrils and increased β-sheet content, corresponding to highly organised, insoluble mature fibril formation (Crowther et al., 2000, Celej et al., 2012).

Overall, fibrillar forms of α-syn are regarded as a less toxic species than the oligomeric structure, with inhibition of fibrillation leading to the persistent presence of oligomeric species, with subsequently increased oxidative damage and cell death (Qin et al., 2007). Similarly, α-syn variants with a propensity for fibril formation display less toxicity than those that form oligomers (Winner et al., 2011). In fact, it has been suggested that mature fibrils may yield neuroprotective benefits by encapsulating toxic oligomers (Cremades et al., 2012). Nevertheless, evidence suggests that fibrils may still contribute to neurodegeneration, at least

to some degree, through various mechanisms, including the promotion of neuroinflammation (Gustot et al., 2015) and impaired lysosomal function upon uptake (Guiney et al., 2020).

1.4.3 Lewy Body Formation

Once mature fibrils are formed, they accumulate and aggregate with surrounding fibril structures, eventually leading to the formation of intracellular inclusions called Lewy bodies. While a major component, it is important to note that Lewy Bodies are not purely comprised of α-syn, with several other constituent proteins also present (Table 1-3):

Table 1-3 Additional proteins that contribute to the development of Lewy Bodies in PD

Protein	Function	Role in Lewy Body Formation
Ubiquitin	Tags proteins, such as α-syn, for degradation by proteasomes (Lim and Tan, 2007)	Presence of ubiquitin may indicate failure of the ubiquitin-proteasome system to degrade toxic α-syn aggregates (Lim and Tan, 2007)
Alpha B Crystallin	Present in pathological conditions (Iwaki et al., 1992), a molecular chaperon that binds to misfolded proteins to stabilise them via preventing misfolding and aggregation (Hayashi and Carver, 2020)	Chaperone activity is proposed to be either ineffective or overwhelmed in PD (Cox et al., 2014)
Neurofilament Light	A key component of the neurofilament network, it provides structural support to neurons, as well as axonal transport (Gentil et al., 2015)	An emerging marker of neurodegeneration, its elevated presence indicates potential axonal damage or neuronal injury (Goldman et al., 1983)
Tau	Microtubule-associated protein, involved in stabilising neuronal microtubules (Avila et al., 2004)	Literature suggests that hyperphosphorylated tau co-aggregates with α-syn, suggesting overlapping

pathologies between neurodegenerative disorders (Moussaud et al., 2014)

An emerging theory is that, similar to fibrils, Lewy bodies may, in fact, be a neuroprotective mechanism- a strategy to sequester the more toxic oligomeric forms of α-syn (Wakabayashi et al., 2013). In line with this, Olanow and colleagues have proposed that Lewy bodies are a dysfunctional aggresome, forming in response to cellular stress to facilitate clearance of toxic α-syn species (Olanow et al., 2004). A summary of α-syn conformational changes and Lewy body formation is outlined in Figure 1-2:

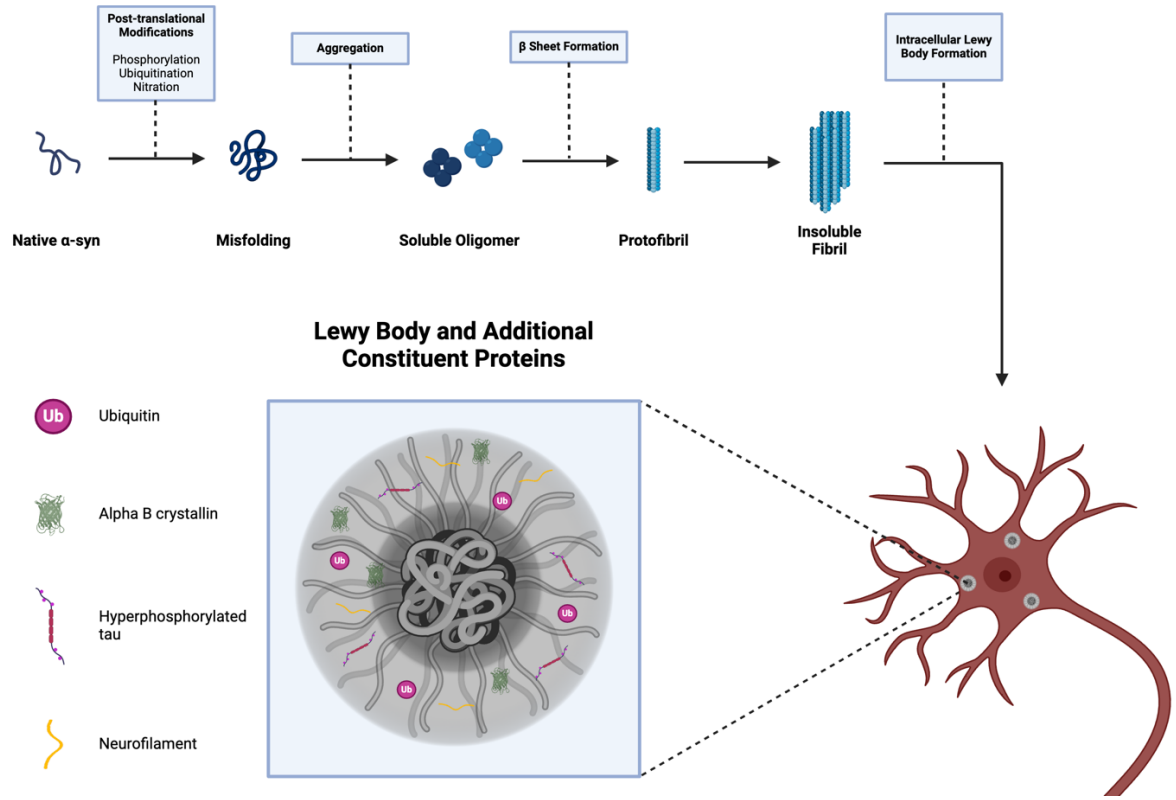


Figure 1-2: ***Progression of Lewy pathology****, highlighting α-syn conformational changes and the eventual formation of Lewy Bodies, as well as additional proteins that constitute them.*

1.4.4 Braak's Staging of Lewy body pathology

In 2003, Heiko Braak notably proposed a staging of brain pathology in idiopathic PD based on the stereotypical spread of Lewy neurite and Lewy body pathology throughout the brain (Braak et al., 2003). This was based on post-mortem analysis of PD brains, which highlighted a topographical spread of pathology, starting in the anterior olfactory nucleus and dorsal motor nuclei of the glossopharyngeal and vagal nerves and gradually spreading to less vulnerable cortical areas in later stages of the disease (Braak et al., 2003). Importantly, this spread mirrors the clinical presentation of the disease. A representation of Braak staging for PD is outlined in Figure 1-3.

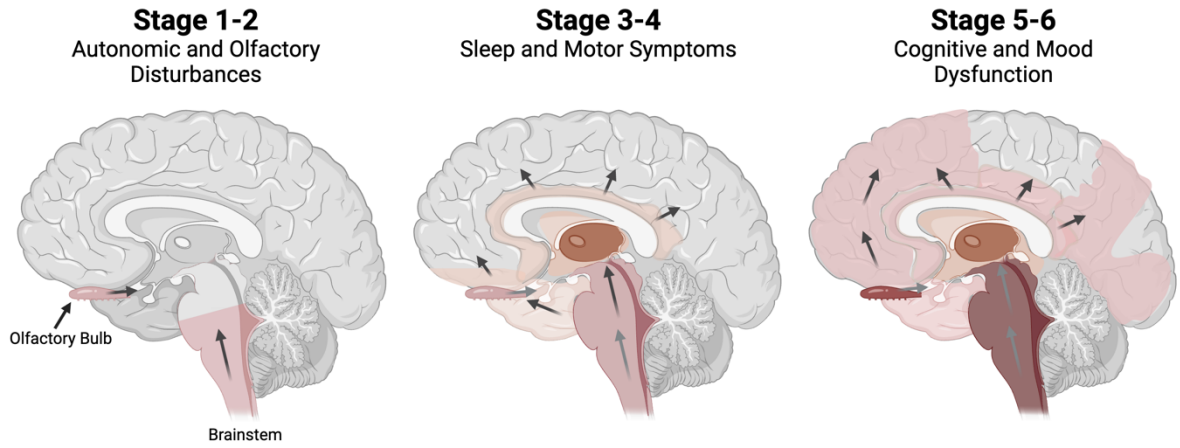


Figure 1-3 Braak's proposed staging of Lewy Pathology*: Stage 1 – Peripheral and enteric nervous system; Stage 2 – Medulla Oblongata; Stage 3 – Pontine Tegmentum; Stage 4: Basal, mid and forebrain, hypothalamus and thalamus; Stage 5 – Mesocortex and allocortex; and Stage 6 – Neocortex*

While extremely influential within the field, however, the Braak staging model is not without contention for several reasons ranging from the current lack of a marker to investigate Lewy body progression *in vivo*, to limitations inherent with *post mortem* investigation, such their retrospective nature and lack of information on clinical presentation (Halliday et al., 2012), to concerns that it does not fully capture the development of PD in all cases (for review, (Rietdijk et al., 2017)). This has led to calls to ensure that other pathological markers, such as neuronal loss and neuroinflammation, are considered, in addition to Lewy body pathology,

when considering PD progression (Greffard et al., 2006, Lema Tomé et al., 2013) (for review, see (Rietdijk et al., 2017)).

1.4.5 Tools to measure α-syn as a biomarker in PD

To date, one of the major limitations in detecting α-syn in PD is that there is no current PET tracer capable of measuring α-syn pathology that is validated for clinical use (for review, see (Korat et al., 2021)). This is in large part because of the low density of α-syn aggregates in PD, compared to the higher density of amyloid beta (Aβ) or hyperphosphorylated tau (p-tau) in Alzheimer's Disease, makes developing a specific tracer challenging (Xiang et al., 2023). This limitation may be overcome by the recent development of ^{18}F ACI-12589, which has shown promising *in vitro* target engagement and specificity for pathological α-syn in post-mortem tissue from both PD and MSA cases, as well as ability to distinguish individuals with MSA from healthy controls and other neurodegenerative diseases (including other synucleinopathies) (Smith et al., 2023). Similarly, other potential PET tracer candidates have recently been put forward, including ^{18}F-F0502B, which shows selective binding with α-syn fibrils *in vitro*, as well as good brain permeability, fast brain washout and recognition of α-syn aggregates in non-human primate models of PD (Xiang et al., 2023). To date, however, research into such compounds is still in the relatively early stages, and significant further validation is required prior to such compounds being ready for translation into the clinic and routine diagnostic use.

Nevertheless, measuring α-syn in CSF has provided critical insight into the presentation and progression of pathophysiology in PD. Across several studies, there is consensus showing that CSF concentrations of α-syn are significantly lower in PD compared to healthy controls (Mollenhauer et al., 2019b, Mollenhauer et al., 2013, Hong et al., 2010, Mollenhauer et al., 2011) . Further, longitudinal assessment within the PPMI cohort demonstrated that, compared

to baseline, CSF α-syn was significantly reduced at both 24- and 36-month follow-ups, suggesting that CSF α-syn levels decrease as the disease advances. Nevertheless, while decreases were seen over time, these did not correlate with either longitudinal motor scores, as measured by the MDS-UPDRS, or striatal DaT scans, suggesting that levels of CSF α-syn may not be an accurate reflection of underlying dopamine neurodegeneration, and hence, disease progression (Mollenhauer et al., 2019a). Further, a meta-analysis of 34 studies highlighted that levels of CSF α-syn did not significantly differ between PD and other parkinsonisms, as well as only yielding a sensitivity and specificity of 72% and 65%, respectively, when distinguishing PD from healthy controls (Eusebi et al., 2017). This limits the diagnostic utility of CSF α-syn in clinical practice, highlighting the need to explore alternative measures of α-syn with higher sensitivity and specificity to PD.

The recent development of the α-syn SAA provides one such promising tool that can be used to measure α-syn in clinical populations. In support of this, a cross-sectional study of 1,123 individuals in the Parkinson's Progression Markers Initiative database reported that α-syn SAA was able to distinguish PD from healthy controls with high sensitivity (98·6%) (Siderowf et al., 2023). In fact, this sensitivity extends beyond clinically recognised PD, as SAA has also shown promise in detecting prodromal PD, with SAA being detected in 86% of individuals with REM sleep behaviour disorder or olfactory impairment, two common prodromal symptoms of PD, showing a positive α-syn SAA (Siderowf et al., 2023). Therefore, this assay shows great potential in not only providing higher diagnostic confidence than can be achieved using current clinical criteria but could also translate to earlier diagnosis and indeed enhanced recognition of risk within the prodromal period. Likewise, neuronally derived extracellular vesicle α-syn (i.e. L1EV-associated α-syn) has also been proposed as a promising biomarker of PD (Yan et al., 2024). In a recent cross-sectional study of 576 individuals, L1EV α-syn differentiated high-risk participants (>80% probability of developing PD) from those

with low-risk, as well as controls. Further, in a cohort of 40 individuals who later developed PD and related dementia, blood tests were positive for L1EV α-syn in more than 80% (Yan et al., 2024).

Recent developments also raise the possibility of detecting α-syn in other accessible biological samples, including measurement of oligomeric α-syn in plasma (El-Agnaf et al., 2006), saliva (Vivacqua et al., 2016, Vivacqua et al., 2019, De Bartolo et al., 2023) and phosphorylated α-syn deposits in the skin (Donadio et al., 2014, Donadio et al., 2017, Donadio et al., 2018). An observational study in the Systemic Synuclein Sampling Study measured α-syn within multiple tissues and biofluids in the same individuals (59 individuals with PD and 21 healthy controls). Interestingly, while CSF levels of total α-syn showed low specificity (63.2%) for distinguishing PD from healthy controls within that study, the specificity for α-syn immunoreactivity in the skin and submandibular gland were much higher (92.9 and 100%, respectively), suggesting that this method may have enhanced diagnostic utility (Chahine et al., 2020). Nevertheless, however, the sensitivity of such measurements was very low (only 56.1 and 24.1%, respectively), suggesting that alternative measurement methods or different α-syn targets (other than total α-syn) may be needed (Chahine et al., 2020). In support of this, a recent cross-sectional study of 428 participants found that the proportion of individuals with PD with a skin biopsy positive for phosphorylated, as opposed to total, α-syn was 92.7%, compared to just 3.3% in healthy controls (although it should also be noted that positive biopsies were found in 98.2% of individuals with MSA, 96% of individuals with dementia Lewy bodies and 100% of individuals with pure autonomic failure, highlighting that additional methods are necessary to distinguish between synucleinopathies) (Gibbons et al., 2024). Combining measures of α-syn may also be necessary to enhance diagnostic accuracy. For example, a recent study in 38 individuals with PD and 24 healthy controls showed that combining measurements of soluble α-syn oligomers within the saliva with skin biopsies for

phosphorylated (P-S129) aggregates distinguished PD from healthy controls with an AUC of 0.91 (Costanzo et al., 2024). Taken together, continued advancements into the measurement of α-syn levels *in vivo* provide hope for the development of a diagnostic/prognostic biomarker for PD, but further work must be done to validate such assays prior to their routine adoption into clinical practice. Further, such work should incorporate recent advances in the field of α-syn biology, including findings that different strains of α-syn target distinct brain regions and cell types (Lau et al., 2020), to further enhance sensitivity and specificity.

1.5 Co-pathology: Other Pathological Proteins Implicated in PD

A number of other proteins, namely p-tau, as opposed to its native form, as well as Aβ, demonstrate interactions with α-syn, suggesting synergistic effects between these proteins as drivers of PD pathology and emphasising the important of co-pathology in the disease (see review, (Sengupta and Kayed, 2022)). For example, reduced cerebrospinal fluid (CSF) concentrations of $A\beta^{42}$ are associated with more profound cognitive decline in PD, being a significant predictor of reduced delayed memory recall over a two-year period (Hall et al., 2015), as well general cognitive ability over a one-year period, even when adjusting for age, disease duration and baseline cognitive status (Siderowf et al., 2010). Further, assessment of CSF concentrations of α-syn and $A\beta^{42}$ in individuals with polygenic risk scores for PD found that increased risk scores were associated with reduced concentrations of both markers, with interactions between markers associated with cortical atrophy and reduced cognitive ability (Lee et al., 2021b). Beyond Aβ, a recent study by Chu and colleagues demonstrated that tau, rather than α-syn aggregation, may mediate nigrostriatal DA neuron degeneration (Chu et al., 2024). Despite this, however, few studies have examined the relationship between p-tau and PD progression. One study reported that increased CSF levels of p-tau significantly predicted more marked motor impairment over a two-year period (Hall et al., 2015); however, evidence

supporting its role in cognitive decline throughout PD is conflicting. For example, while a prospective cohort study of 45 PwP reported no associations between CSF p-tau concentration and cognitive decline over at least one-year follow-up (Siderowf et al., 2010), more recent work, assessing plasma extracellular vesicles from 103 individuals with PD, showed that elevated tau levels within plasma extracellular vesicles at baseline corresponded to significantly greater decline in cognition over one-year follow-up, as well as motor function (Chan et al., 2023).

1.5.1 Tools to measure p-tau and Aβ as biomarkers in PD

As highlighted above, several studies have measured CSF concentrations of Aβ and p-tau in PD. In recent years, however, other less invasive techniques have been utilised to assess PD co-pathologies *in vivo,* including PET tracers.

1.5.1.1 Aβ Ligands

In 2002, the ligand Pittsburgh Compound-B (PiB) was developed, capable of detecting Aβ aggregates by binding to $A\beta^{42}$ fibrils and protofibrils (Yamin and Teplow, 2017), with several other ligands developed since (see review, (Chapleau et al., 2022)). Further, PET-measured Aβ burden may be an important predictor of cognitive decline in PD. For example, a subset of idiopathic PD participants in the PPMI cohort (n = 25) underwent [^{18}F]Florbetaben imaging, reporting that Aβ burden was significantly higher in PD compared to healthy controls (n = 30) (Mihaescu et al., 2022). Further, in a model predicting global cognitive change over a one-year period, Aβ burden in the left gyrus rectus, the left anterior cingulate cortex and the right parietal cortex were significant predictors, explaining 49.5% of the total variance (Mihaescu et al., 2022). Additionally, region-specific Aβ burden may contribute to changes in certain cognitive domains, with ^{18}F-florbetapir assessment in 61 non-demented PwP finding

that Aβ levels in the posterior cingulate gyrus negatively correlated with verbal memory performance, whereas Aβ in the frontal cortex, precuneus, and anterior cingulate gyrus negatively correlated with naming performance (Akhtar et al., 2017).

PET studies have also highlighted the potential utility of Aβ for predicting conversion to PD-MCI and PD-D. For example, of 25 PD-MCI individuals receiving [^{18}F]flutemetamol scans, 32% demonstrated Aβ positivity and had significantly higher deposition in the right caudal and rostral middle frontal cortex, as well as the putamen bilaterally (Garon et al., 2021). Further, higher cortical Aβ burden was significantly associated with poorer executive performance, although not global cognition or motor function (Garon et al., 2021). Likewise, a study assessing 23 individuals diagnosed with PD-D found that 17.5% of participants reported significant Aβ in the cortex, as well as significantly poorer performance in executive outcomes (Na et al., 2020). Therefore, Aβ tracers may provide insight into individuals who are most susceptible to developing executive dysfunction throughout PD course. While not all PwP present with positive Aβ scans, in a recent study utilising [^{18}F]FDDNP, it was found that individuals with PD-D had significantly higher binding in lateral temporal regions compared to non-demented PD controls, which highly correlated with CSF Aβ concentrations (Buongiorno et al., 2017). Further, higher Aβ deposition in the lateral temporal cortex was associated with more rapid cognitive decline over an 18-month period (Buongiorno et al., 2017), highlighting [^{18}F]FDDNP as a potential biomarker of dementia risk or conversion in PD.

1.5.1.2 Tau Ligands

While PD is not considered to be a tauopathy, as outlined above, several lines of evidence implicate synergisms between α-syn and tau pathology as drivers of PD development (Moussaud et al., 2014). Tau PET imaging has demonstrated efficacy in enhancing diagnosis

in AD, with the tracer ^{18}F-flortaucipir approved for clinical use for AD diagnosis in 2020 (Barthel, 2020). Similarly, tau imaging may provide a promising avenue to enhance PD diagnostics. In line with this, studies exploring tau ligands have demonstrated potential utility for distinguishing PD from other disorders. In a multicentre study assessing PwP (n = 26) or PSP (n = 33), individuals with PD demonstrated lower bilateral ^{18} F-Flortaucipir uptake in the GPi, putamen, subthalamic nucleus, midbrain and dentate nucleus, relative to PSP. Of note, GPi binding demonstrated the highest utility for distinguishing PSP from PD (AUC = 0.893) (Schonhaut et al., 2017). Similarly, a systematic review of 15 studies reported reduced tracer uptake in the same regions compared to PSP, as well as reduction in the frontal and occipital lobes relative to AD and the infratemporal and occipital lobe compared to DLB (Zhang et al., 2023). Therefore, regional-specific binding patterns could aid in differential diagnosis of PD from other neurodegenerative disorders.

Beyond diagnosis, tau imaging may also be a critical indicator of disease course, particularly for cognitive impairment and eventual dementia diagnosis. In line with this, a study assessing [^{18}]F-florzolotau in PD-D (n = 10), PD (n = 9) and age-matched healthy controls (n = 9) found that cortical uptake was significantly higher in PD-D compared to both other groups (Tang et al., 2023). Further, uptake within the occipital lobe in PD-D significantly correlated with cognitive impairment, as measured by Mini Mental State Examination scores ($R = -0.635$, $P = 0.0486$) (Tang et al., 2023). Therefore, assessing tau pathology may not only enhance differential diagnosis, but could also be used to better track disease course in PD, as well as provide a more objective strategy for forecasting risk of potential cognitive impairment or dementia.

1.6 The Role of Dopaminergic Neuron Loss in PD

In addition to pathological protein aggregation, the other pathological hallmark of PD is the loss of dopaminergic neurons, primarily those within the SNc (see review, (Dauer and Przedborski, 2003)). When 60-70% of dopaminergic neurons within the SNc are lost, the cardinal motor features of PD arise, leading to clinical diagnosis (Fearnley and Lees, 1991). Dopaminergic neurons also comprise a significant portion of the VTA, an area which also experiences neuronal death throughout PD, albeit to a lesser extent than the SNc (Alberico et al., 2015). In fact, the VTA is the primary dopaminergic input for the mesocortical (Floresco and Magyar, 2006) and mesolimbic (Alcaro et al., 2007) pathways, responsible for cognitive functions, working memory and executive function (mesocortical), as well as reward processing, motivation and reinforcement learning (mesolimbic). These dopamine-dependant pathways are outlined in Figure 1-4.

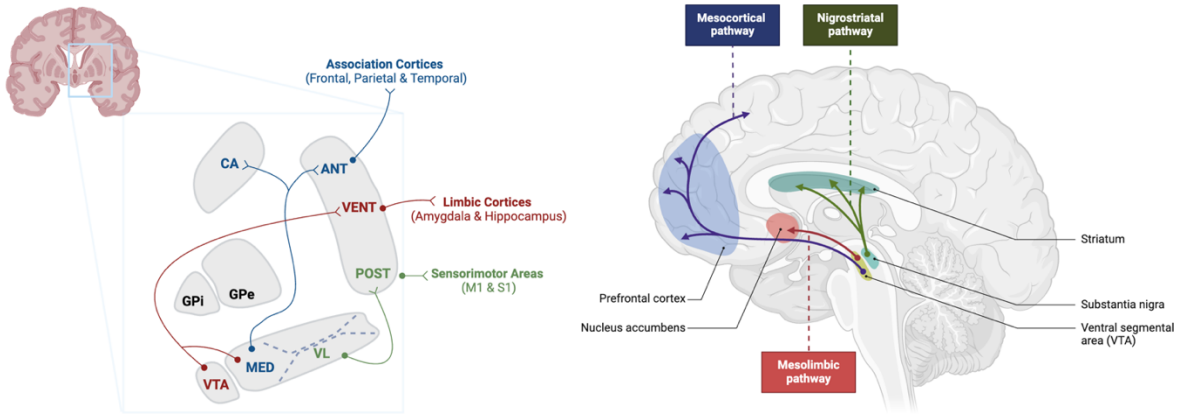


Figure 1-4 Dopamine-dependant pathways and key projections*, including the nigrostriatal, mesolimbic and mesocortical pathways*

As a consequence of progressive degeneration of the SNc and VTA throughout PD, dopaminergic input to such pathways is substantially reduced, leading to alterations in BG circuitry (Lanciego et al., 2012). Resulting from this is widespread impairment in functions associated with each of the affected pathways, including motor function (nigrostriatal pathway), cognitive and executive impairments (mesocortical) and impaired reward processing

and motivation (mesolimbic) (Lanciego et al., 2012). Further, it should be noted that there is overlap between pathways and functional domains, such as nigrostriatal deficits also being associated with changes to reward processing in PD (Wise, 2009). Therefore, it is evident that dopamine plays a central role in the clinical presentation of PD, making effective assessment and tracking of dopaminergic changes in PD critical.

1.6.1 Tools to measure dopamine alterations as a biomarker in PD

1.6.1.1 DaT SPECT

In 2011, the FDA approved the use of DaT SPECT imaging, specifically Ioflupane or [^{123}I]-FP-CIT, as a tool to assist in PD diagnosis (de la Fuente-Fernández, 2012). DaT SPECT works by assessing dopaminergic terminal density (Akdemir et al., 2021), which is substantially reduced in PD due to significant neuronal loss. Overall, DaT SPECT imaging possesses high sensitivity in identifying dopaminergic deficiency, with a systematic review of eight studies reporting that nigrostriatal cell loss was detected with a sensitivity and specificity of 98% (Suwijn et al., 2015). Further, use of DaT SPECT has been shown to correspond to improved diagnostic confidence, with a study of 57 individuals presenting with movement disorders determining that, in clinically diagnosed PD (n = 24), DaT imaging had a positive predictive value of 92%, as well as high interobserver agreement between scans (91%), supporting its replicability within clinical settings (Gayed et al., 2015).

Additionally, studies exploring DaT SPECT have reported associations between imaging findings and symptom presentation and progression in PD. In line with this, in 41 PwP, 123I-FP-CIT uptake in the caudate, putamen and overall striatum was negatively associated with disease severity, as measured by the UPDRS ($r = -0.54$, $p = 0.0003$), as well as disease duration ($r = -0.67$, $p < 0.0001$) (Benamer et al., 2000b). Similarly, DaT also has implications for tracking non-motor symptoms, with a study of 76 PwP showing that not only was PD striatal

uptake significantly lower in PD compared to 46 age-matched healthy controls, but reduced DaT binding in the putamen corresponded to more severe anxiety, depression and overall affective outcomes (Sakakibara et al., 2020). Such effects may be regionally specific, with individuals in the PPMI cohort presenting with bilateral caudate impairment (22.5%) at baseline presenting with higher rates of cognitive impairment and depression compared to individuals with normal caudate DaT levels (51.6%) (Pasquini et al., 2019). Further, in longitudinal studies, DaT binding in striatal regions has been shown to reduce over time (~4-15% annually) throughout disease course, highlighting its sensitivity for capturing progressive dopaminergic loss in PD (Simuni et al., 2018, Sakakibara et al., 2020). In line with this, a previous postmortem study showed a significant correlation between striatal DaT imaging and neuron count within the SN (Kraemmer et al., 2014). Although, not all studies have supported such an association, with a more recent study proposing that reduced striatal DaT binding in PD may instead reflect axonal damage or changes in DaT expression, rather than nigrostriatal neuron loss (Saari et al., 2017).

Importantly, DaT SPECT imaging demonstrates some potential for differentially diagnosing PD from other conditions with overlap in their clinical presentation, but which are not associated with degeneration of the dopamine system. For example, in individuals with essential tremor, while mild reductions were found in striatal DaT when compared to healthy controls, this was not as profound as what is reported in PD (Isaias et al., 2008). This is reflected by DaT SPECT allowing for differential diagnosis between essential tremor and PD with a specificity ranging from 97 to 100% (Benamer et al., 2000a). Similarly, DaT scans have been shown to have utility for distinguishing between PD and drug-induced (Diaz-Corrales et al., 2010), psychogenic parkinsonism (Umeh et al., 2013) and, in at least a subset of cases, vascular parkinsonism (Benítez-Rivero et al., 2013). Beyond this, however, DaT is unable to reliably differentiate between PD and other neurodegenerative parkinsonisms associated with

presynaptic loss of dopamine terminals, such as MSA, PSP or corticobulbar degeneration (for review, see (Ogawa et al., 2018)). Further, a subset of individuals with a clinical diagnosis of PD, including responsiveness to levodopa therapy, show normal DaT SPECT (i.e. Scans without evidence of dopaminergic deficit 0 or SWEDDs) (Erro et al., 2016, Palermo et al., 2022). Thus, DaT on its own cannot be recommended to either rule out or confirm PD diagnosis.

1.6.1.2 F-DOPA-PET

In 2019, the FDA approved the use of [^{18}F]-DOPA positron emission tomography (F-Dopa PET) imaging to aid in early diagnosis of PD (Jiang et al., 2021b). F-Dopa PET is used to evaluate the nigrostriatal system by binding to dopaminergic neuron terminals (Berti et al., 2011). Overall, F-Dopa PET demonstrates high sensitivity in identifying dopaminergic deficiency in PD, with a systematic review of 13 studies reporting that all studies found that a diagnosis of PD corresponded to significant reductions in striatal F-Dopa uptake, with prospective studies also showing that F-Dopa PET can capture reductions in uptake with disease progression (Puñal-Riobóo et al., 2009). Compared to DaT SPECT, the reductions in striatal activity found using F-Dopa PET are consistently smaller (Kaasinen and Vahlberg, 2017), although it is important to note that studies comparing the two methods within the same individuals are quite limited (Eshuis et al., 2009, Huang et al., 2003, Ishikawa et al., 1996, Wallert et al., 2022).

Furthermore, altered F-Dopa uptake of the nigrostriatal system is reportedly topographical, with specific regions demonstrating less F-Dopa uptake, indicative of more profound degeneration (Puñal-Riobóo et al., 2009). For example, it has been reported that the posterior portion of the putamen is one of the most severely affected in PD, as many of its projections come from the ventrolateral SNc, showing only 30-40% uptake compared to

healthy controls (Brooks et al., 1990). However, caudate nucleus decline is reportedly smaller, showing 60-70% F-Dopa uptake in PD compared to healthy controls (Brooks et al., 1990).This is in line with the results of another study, which reported that, compared to controls, F-Dopa uptake was reduced in early PD (symptom duration: 1.6 ± 1.1 years) in the posterior putamen, as well as in the caudate nucleus, but to a lesser extent (Rinne et al., 2001). This reflects regional vulnerability within the SNc, whereby the ventrolateral SNc deteriorates earlier and more rapidly compared to the medial portion (Fearnley and Lees, 1991, German et al., 1989), which project to the putamen and caudate, respectively (Parent et al., 1983) .

Of note, F-Dopa also may have utility for predicting symptom severity and progression (see review, (Brooks, 2000)). In line with this, decreases in F-Dopa uptake have been shown to have a high negative correlation with patient rigidity and bradykinesia scores, but not degree of tremor (Otsuka et al., 1996). Similarly, PD MDS-UPDRS motor ratings have been shown to be moderately correlated with striato-occipital ratios (SOR) measured using F-Dopa imaging in those with a mild motor symptom presentation (Ishikawa et al., 1996), as well as with both motor UPDRS scores (R^2 = -0.38) and disease duration (R^2 = -0.49) in comparisons between *de novo* PD and advanced PD (Eshuis et al., 2006b). Interestingly, regional differences in F-Dopa uptake may have utility for predicting specific functional changes. In line with this, while both putamen and caudate uptake correlated with PD severity, the former was more implicated in locomotor disability (Broussolle et al., 1999). Similarly, in a study in an advanced PD cohort (disease duration = 11.8 ± 4.5 years), regional F-Dopa uptake was associated with differential cognitive impairments (van Beilen et al., 2008). Specifically, reduced F-Dopa uptake in the putamen was most associated with executive function (R = 0.44), but less so with memory (R = 0.26) and verbal fluency (R = 0.32), whereas the inverse was reported for the caudate (executive function: R = 0.37; memory: R = 0.41 and fluency: R = 0.44) (van Beilen et al., 2008).

Despite its promise, however, F-Dopa PET is not without its limitations. Most notably, as seen with DaT SPECT, F-Dopa PET is unable to distinguish idiopathic PD from other parkinsonian disorders that present with progressive dopaminergic neuron loss (Rinne, 2009). For example, familial PSP patients exhibit significantly reduced F-Dopa uptake in the striatum bilaterally, orbitofrontal cortex bilaterally and right amygdala, making it impossible to distinguish idiopathic PD from PSP based on F-Dopa uptake alone (Tai et al., 2007). Thus, it may be necessary to employ a multi-tracer approach (Rinne, 2009). Further, several limitations with nuclear imaging limit clinical efficacy, such as high costs, limited availability and challenges surrounding the production of tracers. It may therefore be beneficial to explore the incorporation of more cost-effective and readily available imaging modalities, including recent advances in magnetic resonance imaging (MRI).

1.6.1.3 Neuromelanin-Sensitive T1 MRI

Historically, conventional T2-weighted MRI has been used to detect degeneration in regions of interest known to be affected during PD, such as the SNc. For example, early clinical studies found significantly higher SNc iron content in PD compared to healthy controls (Gorell et al., 1995, Martin, 2009). Such increased iron content was found to be proportional to disease severity, supporting the use of iron as measured by MRI as a potential biomarker of PD (Martin, 2009). Despite this, however, conventional MRI is limited in its ability to image key brainstem nuclei and other changes to BG circuitry. Conventional T1 and T2 MRI images are not sufficiently detailed in such deep regions, due to unspecific contrast and poor spatial resolution between structures (Heim et al., 2017). Additionally, SNc imaging with standard magnetic resonance sequences usually appears normal in early PD (Heim et al., 2017). In recent years however, several advances in MRI, particularly the development of neuromelanin-sensitive T1 MRI, have made this an increasingly valuable tool in the diagnosis of PD.

Neuromelanin (NM) is a by-product of iron-modulated oxidation of cytosolic dopamine not accumulated in synaptic vesicles within the midbrain (Sulzer et al., 2000). Progressive loss of dopaminergic neurons throughout PD results in significant reductions in overall dopamine content and, consequently, less NM (Cassidy et al., 2019, Haining and Achat-Mendes, 2017). To this end, Sasaki and colleagues developed a T1-weighted protocol using a 3 Tesla magnet, aimed at enhancing the contrast between NM signal and background tissue (Sasaki et al., 2006). They reported that not only were the SN and LC visible within the brainstem using a NM sensitive sequence, but that signal in said regions was significantly reduced in PD compared to healthy controls (Sasaki et al., 2006). Further, a post-mortem analysis of PD and DLB formalin-fixed midbrains found than NM-MRI intensity in the SNc was highly correlated with the number of dopaminergic neurons present (Kitao et al., 2013). To this end, NM-MRI was considered a promising proxy marker of dopaminergic neuron concentration.

Following this, several subsequent studies demonstrated that NM-MRI has utility for characterising SNc degeneration in PD (Schwarz et al., 2017, Ohtsuka et al., 2013), with a consensus that NM-MRI signal in the SNc is significantly reduced in PwP compared to healthy controls (see, for example, a systematic review of 12 studies, (Cho et al., 2021b)). In fact, NM-MRI may play a key role in improving diagnosis, with *de novo* PD patients showing reduced signal in the lateral, medial and central SNc compared to controls (Wang et al., 2018a). Of note, previous work investigating maximal SN length has reported significant differences between *de novo* PD and controls with a specificity and sensitivity of 70% and 83.3%, respectively (Reimão et al., 2015a). This suggests that NM-MRI can act as a marker of neuromelanin content within the SNc, even at early stages of PD. Furthermore, NM-MRI can characterise progressive deterioration of the SNc. In line with this, longitudinal studies have reported that NM-MRI is sensitive enough to capture progressive signal loss in the SNc over time, with this

loss correlated with both disease duration and disease severity (Matsuura et al., 2016, Gaurav et al., 2021).

One major limitation in early NM-MRI studies, as well as conventional MRI sequences, is that SNc reductions in PD are comparable to those seen in other parkinsonian disorders, namely PSP, MSA and corticobasal degeneration (CBD). This highlighted challenges in providing differential diagnosis between disorders, similar to those seen for DaT SPECT and F-Dopa PET. Nevertheless, advances in NM-MRI acquisition in recent years may allow these limitations to be overcome. In line with this, a study comparing MSA (n = 30), and PD (n = 10) determined that, using simple visual MRI analysis, decreased unilateral SN and LC NM-MRI signal was suggestive of PD (sensitivity = 90%, specificity = 81% and accuracy = 83%) (Simões et al., 2020). Likewise, more profound NM-MRI signal loss in the SNc and locus coeruleus (LC) has demonstrated promise in distinguishing PD tremor from essential tremor (Wang et al., 2023c, Reimão et al., 2015b). Increased sensitivity has also translated to the ability to better characterise differential outcomes and progression across subtypes of PD. In one such study assessing 54 PwP, while both tremor-dominant and rigid-dominant PD had significantly lower SNc signal compared to controls, those classified as PIGD also showed significantly lower contrast to noise ratios in the lateral SNc compared to those classified as TD, suggesting more advanced pathology or rapid rate of progression in the PIGD group (Wang et al., 2021). Further, SNc signal was able to distinguish between subtypes with a sensitivity and specificity of 76.5% and 78.6%, respectively (Wang et al., 2021). Similarly, in a study of 32 PwP exploring freezing of gait (FOG), while SNc deterioration was present in individuals with and without FOG, LC degeneration was more profound in individuals with FOG (Wang et al., 2023b). Overall, not only does NM-MRI show utility in differentiating PD from other parkinsonian disorders, but it may also provide an opportunity for improved characterisation of more individualised outcomes, translating to improved tracking and management.

1.7 The role of loss of other neurotransmitters in PD

While dopamine loss has remained a core focus of PD research, given its central role in the emergence of motor symptoms, it is important to acknowledge that neurotransmission changes are far more widespread. In fact, multiple neurotransmitter systems are impacted in PD, including, but not limited to, glutamate, serotonin, acetylcholine and norepinephrine (Barone, 2010). Of particular importance is norepinephrine, given its implications in the development of several non-motor impairments in PD, such as autonomic dysfunction and cognitive complaints, as well as the neuroprotective benefits it confers to the dopaminergic system, which are lost throughout PD (Paredes-Rodriguez et al., 2020).

In support of this, a critical structure affected by PD pathology early on in disease course is the locus coeruleus (LC), the primary source of norepinephrine for the CNS (Weinshenker, 2018). Neuronal loss within the LC of PwP is even more profound than that seen in the SNc (Zarow et al., 2003). Of note, Lewy pathology disrupts the LC noradrenergic system prior to the dopaminergic nigrostriatal system, resulting in the emergence of various non-motor symptoms, such as cognitive impairment, depression and sleep disorders (Mahul-Mellier et al., 2020). Therefore, *in vivo* assessment of the LC and the noradrenergic system may prove critical in earlier diagnosis, as well as improved characterisation of non-motor outcomes in PD.

1.7.1 Tools to measure norepinephrine alterations as a biomarker in PD

1.7.1.1 Neuromelanin-Sensitive T1 MRI

In addition to investigating alterations within the SNc, as discussed above, NM-MRI has also been explored in the LC. In individuals with PD, significantly lower neuromelanin content has been found in this region compared to healthy controls (Sasaki et al., 2006, Wang et al., 2018a, Hwang et al., 2023). Further, degeneration of the LC is heavily implicated in the development of several non-motor symptoms of PD, with relationships demonstrated between

such outcomes and NM-MRI signal of the LC. For example, *de novo* PD participants with depressive symptoms display significantly lower signal in the left LC compared to both controls and PwP without depressive symptoms (Wang et al., 2018a), suggesting that depression in PD may be related to dysfunction within these pathways. Likewise, the LC may directly relate to early executive dysfunction and cognitive impairment in PD, with a study exploring *de novo* PD with MCI (n = 23) and without (n = 48) reporting that PD-MCI demonstrated lower contrast-to-noise ratios in the LC which were negatively associated with Trail Making B scores (Li et al., 2019). Additionally, a comparative clinical study of PD and those with RBD demonstrated that there was decreased NM-MRI intensity in the LC, as well as widespread reductions in noradrenergic PET ligand uptake, which correlated with the amount of REM sleep (Sommerauer et al., 2018). Despite promising findings, several limitations and gaps are present in current literature. For instance, many studies assessing NM-MRI to characterise degeneration of the SNc and LC are cross-sectional in nature, making it difficult to determine how neurodegeneration in said regions corresponds to changes in clinical presentation. Further, comparisons that are currently being made in NM-MRI PD research would benefit from larger cohorts, corresponding to more reliable and consequently, translatable findings.

1.7.1.2 Noradrenergic Transporter Ligand

Several PET ligands have been developed to evaluate the noradrenergic system, with the most prominent of these being [^{11}C]MRB , which targets the norepinephrine transporter (NET) (Ding et al., 2006). NET is a protein found primarily on the membranes of noradrenergic neurons and is essential for the re-uptake of norepinephrine following neurotransmission (Ding et al., 2003). Consequently, in disease states characterised by loss of noradrenergic neurons, NET expression decreases. [^{11}C]MRB was developed and optimised within primate models,

with early studies demonstrating that the regional signal seen post injection is consistent with the known distribution of NET in the brain (Ding et al., 2003). Further, in a study in baboons comparing [^{11}C]MRB to other NET ligands, including [^{18}F]fluororeboxetine and (*R*)-[^{11}C]nisoxetine, as well as the serotonin transporter tracers [^{11}C]oxaprotiline and [^{11}C]lortalamine, [^{11}C]MRB demonstrated superior signal-to-noise ratio and binding specificity (Ding et al., 2005). Therefore, [^{11}C]MRB represents a promising *in-vivo* tool to evaluate the integrity of the noradrenergic system, and may even allow for earlier diagnosis of PD, given that degeneration of the LC occurs earlier than that seen in the nigrostriatal dopamine pathways in PD (Espay et al., 2014).

Despite this, however, PD-specific [^{11}C]MRB studies are scarce and represent an important avenue for future research. A recent study aimed to reduce PET acquisition time for [^{11}C]MRB in idiopathic PD patients, demonstrating that a 30-minute acquisition was achievable (Brumberg et al., 2019). This reduced acquisition time would significantly improve [^{11}C]MRB studies in PD cohorts, reducing overall discomfort, particularly if participants are asked to be off medication during the scan time. Therefore, [^{11}C]MRB could be a useful tool that should be further validated in PD studies to probe beyond the nigrostriatal system.

1.8 The role of neuroinflammation in PD

Neuroinflammation is the innate inflammatory response of the central nervous system (CNS). It is a multi-system reaction, involving both activation of resident glial cells, primarily microglia and astrocytes (see review, (Kwon and Koh, 2020)), and the recruitment of peripheral immune cells, with the overarching objective of restoring brain tissue to homeostatic levels following insult (DiSabato et al., 2016). While neuroinflammation is vital, sometimes it becomes maladaptive and persists chronically (DiSabato et al., 2016). Sustained

neuroinflammation is highly detrimental to the CNS, as activated glial cells produce free radicals and neurotoxic substances (DiSabato et al., 2016). Such chronic elevations of neuroinflammation have been widely reported in PD, with concentrations of several pro-inflammatory cytokines shown to be significantly up regulated in the CSF (Mogi et al., 1996) and blood (Qin et al., 2016) of individuals with PD compared to healthy controls. In fact, it is hypothesised that oxidative stress and neurotoxicity due to chronic inflammation act as key players in brainstem nuclei degeneration in PD (Varçin et al., 2012). In line with this, targeting neuroinflammation via selective inhibition of NF-kappaB (Ghosh et al., 2007) or knockout of toll-like receptor four (Noelker et al., 2013) corresponds to a reduced inflammatory response following MPTP administration, as well as preservation of the nigrostriatal system. Given this, methods of accurately assessing inflammation may provide a novel diagnostic tool to diagnose and track progression in PD.

1.8.1 Tools to measure neuroinflammation as a biomarker in PD

1.8.1.1 TSPO Ligands

PET can be used to measure neuroinflammation via 18kDa translocator protein (TSPO) ligands (Alam et al., 2017). TSPO is a mitochondrial protein located on outer mitochondrial membranes and is primarily found in activated microglia (Alam et al., 2017), along with reactive astrocytes (Lavisse et al., 2012). Under normal conditions, TSPO is not highly present in the brain; however, during neuroinflammation, it is highly up regulated (Alam et al., 2017). Several TSPO ligands have been developed to date, as highlighted in Table 1-4.

Table 1-4 ***Radioligands that bind to TSPO receptor sites*** *as a marker of microglial activation and neuroinflammation*

Generation	Radioligand	Reference

1st	[^{11}C]PK11195	(Folkersma et al., 2011, Gerhard et al., 2006, Ramlackhansingh et al., 2011)
	[^{11}C]SSR180575	(Chauveau et al., 2011)
	[^{11}C]-DPA-713	(Doorduin et al., 2009)
2nd	[^{11}C]DAA1106	(Yasuno et al., 2008)
	[^{18}F]FM-PBR28	(Owen et al., 2012)
	[^{18}F]PBR06	(Liu et al., 2022b)
3rd	[^{18}F]GE-180	(Boutin et al., 2015)

[^{11}C]PK11195 was first radiolabelled in 1984 and subsequently integrated into many clinical and animal studies as a TSPO ligand used to measure neuroinflammation *in vivo* (Folkersma et al., 2011). Of particular note, clinical imaging studies have shown significantly increased mean binding of [^{11}C]PK11195 in the pons, BG and frontal regions in PD patients, compared to healthy controls (Gerhard et al., 2006). Furthermore, [^{11}C]PK11195 has been shown to be able to successfully detect microglial activation in individuals after a single moderate/severe TBI at varying time points, ranging from 11 months to 17 years, highlighting its potential to detect chronic levels of neuroinflammation (Ramlackhansingh et al., 2011). However, [^{11}C]PK11195 displays a low signal-to-noise ratio on account of non-specific binding, with one study in baboons determining that, without correction for peripheral clearance, only 50% of tracer uptake was specific (Petit-Taboué et al., 1991). This corresponded to substantial challenges with interpretability and confidence in research findings.

To address these limitations, extensive research allowed for the development of 2nd generation radioligands, with improved viability for measuring neuroinflammation. These 2nd generation ligands have heightened binding affinity and specificity to TSPO, on account of their indole-ring structure (Alam et al., 2017). A study using an acute neuroinflammation rat

model, induced via 0.5µl of (*R*,*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic stereotaxically injected into the right striatum, showed increased [^{11}C]SSR180575 binding in the ipsilateral striatum, and an improved image contrast, compared to [^{11}C]PK11195 (Chauveau et al., 2011). In a study using one such 2nd generation ligand, [^{18}F]PBR06, to assess neuroinflammation in early PD (average disease duration = 2.5 years), it was reported that standard uptake value ratio (regional uptake normalised against global brain uptake) in the putamen was significantly increased in PD compared to healthy controls, particularly on the ipsilateral side of motor onset (Liu et al., 2022b). Further, [^{18}F]PBR06 uptake was positively associated with the extent of dopaminergic denervation within the brainstem, measured using [^{18}F]-FP-DTBZ PET (Liu et al., 2022b).

While 2nd generation ligands show improvement in binding affinity and specificity compared to 1st generation ligands, it must be noted that binding affinity is significantly lower in a subset of individuals, due to a single nucleotide polymorphism in the TSPO gene present in 30% of the Caucasian population, but less so in Native American, African, Han Chinese and Japanese populations, resulting in the production of A147t TSPO, which destabilises the protein and ligand-binding pocket, corresponding to reduced binding affinity (Milenkovic et al., 2018). To resolve this difficulty, [^{18}F]GE-180, a 3rd generation TSPO radioligand, has recently been developed. In addition to showing improved binding affinity for A147t TSPO, ^{18}[F]GE-180 is valued for its higher binding potential overall, allowing for increased signal to noise ratios (Werry et al., 2019). Excitingly, preliminary data suggests that this ligand may improve assessment of neuroinflammation compared to previous generations. In line with this, a focal cerebral ischaemia rat model found that, compared to [^{11}C]PK11195, [^{18}F]GE-180 uptake was 24% higher in the lesion and 18% lower in the contralateral healthy tissue, translating to a 0.2-fold increase in signal-to-noise ratio (Boutin et al., 2015). To date however, research evaluating [^{18}F]GE-180 in PD is limited. Therefore, future research needs to

investigate [^{18}F]GE-180 in PD, reflected by clinical trials doing so currently being conducted. This highlights that TSPO tracers are promising tools for investigating the role of neuroinflammation in the context of dopaminergic neurodegeneration, warranting future research.

1.9 The role of iron accumulation in PD

Ferroptosis is a form of cell death, distinct from other forms such as apoptosis and necrosis, arising from iron-dependant lipid peroxidation, which results in oxidative damage and eventual cell death (see review, (Li et al., 2020)). Further, ferroptosis has been identified as a major driver of neuronal loss in PD (Zeng et al., 2024). This is supported by the fact that substantial iron accumulation is reported in PD, with Sofic and colleagues reporting that, in post-mortem analysis of eight PD brains, total iron and ferric content were 176% and 225% higher in the PD SNc compared to age-matched healthy controls (Sofic et al., 1988). Excitingly, advancements in imaging techniques allow for *in vivo* quantification of regional iron content, which may show utility as a biomarker of PD.

1.9.1 Tools to measure iron accumulation as a biomarker in PD

1.9.1.1 Susceptibility-Weighted Imaging and Quantitative Susceptibility Mapping

Susceptibility-weighted imaging (SWI) is an MRI modality developed to enhance visibility of certain types of tissue or abnormalities within the brain. This results from SWI being sensitive to different magnetic properties of various tissues, which are influenced by factors such as calcium, blood products and, most importantly for PD, iron (Halefoglu and Yousem, 2018). It is widely recognised that, in PD, regional iron content is significantly higher

within the SNc (Mochizuki and Yasuda, 2012, Zecca et al., 2004), as well as other important nuclei, such as the LC (Zecca et al., 2004). Further, a study of untreated PwP reported significantly higher R2* values (a metric used in MRI research to quantify transverse relaxation rates, with higher values corresponding to increased iron content (Gelman et al., 1999)) in the lateral SNc in PD compared to healthy controls, which significantly predicted lateralized motor scores, a promising marker of disease severity (Martin et al., 2008).

Of note, regional iron accumulation in PD shows a progressive pattern, depending on the stage of PD, with iron deposition in the SNc predominately occurring in early stages (Guan et al., 2017). Thus, SWI could be a useful tool in assessing disease progression. Importantly, however, SWI is unable to provide quantitative measures of magnetic susceptibility. This limitation has recently been addressed by the development of quantitative susceptibility mapping (QSM). QSM has shown promise in identifying areas of high iron accumulation (de Rochefort et al., 2008, Shmueli et al., 2009), such as the SNc, with one study in individuals with multiple sclerosis (MS) demonstrating that QSM yielded superior sensitivity compared to R2* mapping, obtained via conventional MRI techniques (Langkammer et al., 2013). This was reflected by higher susceptibility in the caudate and BG in individuals with MS compared to either clinically isolated syndrome (CIS) or healthy control comparator groups, with R2* mapping not identifying differences between MS and CIS (Langkammer et al., 2013).

In PD, several studies have now assessed QSM, with distinguishable iron accumulation noted in key brain regions of interest, namely subcortical BG structures, in individuals with PD (Guan et al., 2017, Langkammer et al., 2016, Murakami et al., 2015, Barbosa et al., 2015, Tan et al., 2021, Cheng et al., 2020). Of note, this pattern of accumulation can differentiate between those with PD and healthy controls, with significantly higher iron accumulation detected by QSM in the SNc in PD (Langkammer et al., 2016, Murakami et al., 2015, Barbosa et al., 2015). Furthermore, QSM displayed higher discriminatory capacity (AUC = 0.77) compared to either

MRI transverse relaxation rate R2 (AUC = 0.72) or R2* (AUC = 0.65) values (Barbosa et al., 2015), suggesting that QSM may be more suitable for use in the diagnosis/prognosis of PD, compared to conventional MRI techniques. In line with this, QSM was able to detect abnormal SNc iron accumulation even in early PD (Tan et al., 2021), a major limitation of conventional T2 MRI sequences (Gelman et al., 1999). Further, while many studies utilise MRI to visualise degeneration of the SNc in its entirety, a key focus in PD neuroimaging research involves delineating the SNc into nigrosomes to better characterise degeneration (see review, (Pavese and Tai, 2018)). Of particular interest is nigrosome-1, located within the dorsal region of the SN (Damier et al., 1999a). As Damier and colleagues established, the dopaminergic neurons that comprise nigrosome-1 are particularly vulnerable and are amongst the earliest to degenerate in PD (Damier et al., 1999b). Thus, the absence of nigrosome-1 could be a potentially early marker of PD. To date, however, there is a lack of standard protocols to visualise this territory (Cheng et al., 2020). Despite this limitation, an optimisation study demonstrated bilateral reductions in QSM-derived values in the nigrosome-1 of atypical parkinsonism and PD when compared to healthy controls (Cheng et al., 2020).

QSM may also have utility for predicting presentation and progression of disease symptoms in PD. Regional iron accumulation within the SNc highly correlated to Hoehn-Yarr stage and UPDRS-measured motor outcomes, with late PD demonstrating increased QSM values within the SNr, red nucleus (RN) and the GP, particularly the GPi, suggesting that these structures become involved during advanced stages of the disease (Guan et al., 2017). In fact, a longitudinal study assessing 59 PwP over a 3-year period found that baseline susceptibility of the SN, BG, RN, insular cortex and dentate gyrus predicted greater motor severity at 3-year follow-up (Thomas et al., 2024). Beyond motor symptoms, QSM also demonstrated promise in predicting cognitive decline in this cohort, with baseline susceptibility of the right temporal

cortex, nucleus basalis of Meynert, and putamen predicting poorer cognitive performance at follow-up (Thomas et al., 2024). This is in line with the results of another study, reporting that individuals with PD exhibited higher iron accumulation in their thalamic and hippocampal regions compared to healthy controls, which correlated negatively with their scores on the Mini-Mental State Examination (Thomas et al., 2020). Interestingly, this susceptibility was significantly higher in Lewy-body dementia compared to PD and healthy controls (Thomas et al., 2020), with different iron deposition patterns also reported between multiple system atrophy and PD (Wang et al., 2012b), suggesting that QSM-derived metrics are capable of distinguishing PD from other parkinsonian disorders, even those involving degeneration of dopaminergic systems, a limitation of techniques such as DaT SPECT and F-Dopa PET.

As highlighted by Du and colleagues however, QSM is not without its own limitations, such as not being able to isolate the source of iron driving signal within the scan, nor can it isolate the where iron is located on a cellular level (neurons and glial cells, primarily microglia) within regions of interest (Du et al., 2016). Further, there is lack of standardisation relating to image and magnetic susceptibility acquisition, such as variation in the reference regions considered for relative measurement of magnetic susceptibility, limiting translatability of findings across studies and overall (Ravanfar et al., 2021). Therefore, future work should focus on assessing QSM and its correlation with histopathological features in PD, as well as harmonisation of imaging and analytic protocols.

1.10 Conclusion

While several strides have been made in advancing PD diagnosis and management in recent years, substantial gaps remain. This is, at least in part, due to heavy reliance on clinical signs, particularly of the cardinal motor features of the disease, for diagnosis. This may contribute to the high rates of misdiagnosis seen in PD, and consequent mistreatment. This has

led to a push to redefine PD based on its biological presentation (Simuni et al., 2024, Chahine et al., 2023), aided in large part by recent advances in neuroimaging. While a field still in its infancy, PET ligands show great promise in advancing the integration of neuroimaging into clinical settings for PD diagnosis. However, in isolation, such ligands lack specificity for PD and, therefore, need to be used in conjunction with additional markers to provide more confidence in differential diagnosis. These challenges may be mitigated by the development of a PET ligand specific for measuring α-syn pathology, currently a major area of need for the field. Encouragingly, one such ligand, ^{18}F ACI-12589, is currently in development, but is not yet validated for clinical use (Smith et al., 2023). Additionally, further research, particularly longitudinal studies tracking changes in signalling profiles over time, are critically needed before widespread use of these radioligands in PD diagnosis and prognosis can be recommended. Another significant limitation of PET imaging is its considerable cost, and the fact that it is not widely available. Thus, less expensive and more readily available imaging techniques, such as MRI, may offer a better alternative.

In recent years, specialised structural MRI sequences, including NM-MRI and QSM, have delivered many promising results. Nevertheless, they still present shortcomings that limit their clinical utility if used in isolation. Currently, there is a lack of automatic methods to segment PD regions of interest such as the SNc (Visser et al., 2016, Basukala et al., 2021). Therefore, manual segmentation is still the primary method used to delineate ROIs, which gives rise to subjectivity and variability between studies. Additionally, analysing subcortical structures in the inferior parts of the brain with structural sequences poses issues, due to pulsatile artefacts that are present in these deeper brain structures, due to their proximity to major blood vessels (Herbst et al., 2014).

In addition to neuroimaging, direct measurement of biomarkers within biologic fluids, such as CSF or blood, offers exciting potential for revolutionising the diagnosis/prognosis of

PD. Unfortunately, the measurement of such biomarkers in PD has traditionally only been available through CSF collection via highly invasive lumbar puncture. This limits its clinical utility, particularly for measuring disease progression over time. Importantly, recent advancements, such as the α-syn seed amplification assay (SAA) and skin biopsies, offer a new way forward. Early data has supported the utility of SAA and α-syn skin biopsies for distinguishing between PD and healthy controls (Siderowf et al., 2023, Gibbons et al., 2024), as well as the SAA showing promise for identifying which individuals with prodromal symptom presentation are likely to go on to develop PD (Yan et al., 2024), paving the way for earlier diagnosis (Chahine et al., 2023). While promising, however, significant additional work is needed to validate these tools prior to adoption into routine clinical practice.

Taken together, given that PD is a multi-faceted disorder, it is likely that no one assessment used in isolation will be sufficient for diagnosis, or for tracking disease progression over time. Rather, the development and validation of a multi-modal panel of assessments, incorporating several of the key pathological markers known to be associated with the disease, may be better suited, leading to improved diagnostic confidence and advances in the understanding of the relationship between neuropathological changes and how they co-contribute to disease progression and outcome in PD.

Chapter 2: Statement of Authorship

Title of Paper	Utility of Baseline Pathological, Neuroimaging and Clinical Markers for Prognosis in Early Parkinson's Disease
Publication Status	☐ Published ☐ Accepted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style ■ Submitted for Publication
Publication Details	Submitted to the European Journal of Neurology on September 21st 2024

Principal Author

Name of Principal Author (Candidate)	Angus McNamara		
Contribution to the Paper	Conceptualised, performed and interpreted statistical analyses and wrote the initial manuscript		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	16/09/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Benjamin Ellul		
Contribution to the Paper	Assisted in data analysis and MRI related segmentations and approval of the submitted manuscript		
Signature		Date	16/09/2024

Name of Co-Author	Stephan Lau		
Contribution to the Paper	Contributed to the organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	17/09/2024

Name of Co-Author	Mark Jenkinson		
Contribution to the Paper	Contributed to the organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	17/09/2024

Name of Co-Author	Irina Baetu		
Contribution to the Paper	Contributed to the organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	18/09/2024

Name of Co-Author	Lyndsey Collins-Praino		
Contribution to the Paper	Contributed to the conceptualisation of the manuscript, organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	16/09/2024

Chapter 2 - Utility of Baseline Pathological, Neuroimaging and Clinical Markers for Prognosis in Early Parkinson's Disease

Angus McNamara[1], Benjamin Paul Ellul[1], Irina Baetu[2], Mark Jenkinson[3,4], Stephan Lau[3,4] & Lyndsey Collins-Praino[1]

[1] School of Biomedicine, University of Adelaide, SA 5000, Australia
[2] School of Psychology, University of Adelaide, SA 5000, Australia
[3] Australian Institute for Machine Learning, School of Computer and Mathematical Sciences, University of Adelaide, SA 5000, Australia
[4] South Australian Health and Medical Research Institute, SA 5000, Australia

2.1 ABSTRACT

Background: Currently, prognostication of Parkinson's Disease (PD) progression is limited. Emerging literature highlights potential of multi-modal biomarkers and neuroimaging to provide insight into clinical progression, potentially improving prediction of long-term outcomes.

Methods: Data were extracted from the Parkinson's Progression Markers Initiative (PPMI). Hierarchical clustering was applied to Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) scores at year-5 follow-up, identifying two clusters. Differences in progression, as well as retrospective assessment of baseline differences, between clusters were explored for pathological biomarkers, neuroimaging, and prodromal measures. Additionally, logistic regression and machine learning were employed to determine utility of variables at baseline as predictors of cluster membership.

Results: The more impaired cluster demonstrated worse motor and non-motor outcomes, including higher rates of dementia and cognitive complaints at year-5, as well as more profound rigidity than cluster one. Further, retrospective comparisons showed cluster two performing worse in all prodromal measures and demonstrated lower striatal dopamine transporter binding and cognitive ability. Logistic regression determined that membership in this cluster was predicted by higher autonomic dysfunction and p-tau, along with reduced smell and alpha-syn, predicting 49.1% of variance. This was significantly higher (p <0.001) than the model including MDS-UPDRS scores alone, which only accounted for 27.4% of variance. Findings were corroborated by machine learning, whereby multi-modal assessment corresponded to 74% classification accuracy, compared to 60% with MDS-UPDRS alone.

Conclusion: Prediction of more marked impairment at year-5 follow-up was substantially improved via multi-modal assessment, specifically, pathological biomarkers, suggesting that incorporating pathophysiological assessments into clinical criteria could enhance the ability to provide long-term prognosis.

2.2 BACKGROUND

The official Movement Disorder Society (MDS) Clinical Diagnostic Criteria for Parkinson's disease (MDS-PD criteria) were proposed in 2015 (Postuma et al., 2015a), presenting high sensitivity and specificity (Postuma et al., 2018). Although these criteria incorporate non-motor symptoms, motor symptoms remain the core feature of MDS-PD diagnosis, specifically bradykinesia and either resting tremor or rigidity (Postuma et al., 2015a). A widely implemented clinical rating scale for assessing PD features, including motor impairment, is the Movement Disorders Society Unified Parkinson Disease Rating Scale (MDS-UPDRS) (Martínez-Martín et al., 1994). The MDS-UPDRS is also widely utilised to identify subtypes of PD, a critical consideration for personalised treatment and management, as PD subtypes display differential outcomes and rates of progression (see review, (Fereshtehnejad and Postuma, 2017)). Despite this, however, Kohat and colleagues found that subtype classification based solely on MDS-UPDRS assessment demonstrated poor stability over a three-year period, highlighting that clinical assessment alone may be a poor indicator of long-term outcomes and progression (Kohat et al., 2021).

To mitigate such challenges, the incorporation of biomarkers, as is currently employed for Alzheimer's Disease (AD), may offer a path forward (McKhann et al., 2011). Like PD, AD is a neurodegenerative disorder, with diagnosis originally focused on clinical symptoms, namely cognitive impairment (Chaves et al., 2011, Di Pucchio et al., 2018). Later research established

AD pathology present in the brain several years prior to clinical onset (Kumar et al., 2023). Accordingly, the A/T/N framework ("A" - β-amyloid (Aβ); "T" – phospho or total tau and "N" – biomarkers of neurodegeneration and neuronal injury) was developed as a biomarker-focused framework assessing pathological hallmarks of AD and their utility for diagnosis (McKhann et al., 2011, Jack Jr et al., 2011). Preliminary assessment of these criteria indicate higher discriminative accuracy of AD versus cognitively unimpaired adults and other neurodegenerative diseases (Pascoal et al., 2023), along with high utility for predicting cognitive decline (Xiong et al., 2023).

Success seen with the A/T/N framework suggests incorporating multi-modal markers into PD criterion may be similarly beneficial. Currently, however, PD diagnostic criteria is based almost entirely on clinical judgment, with cardiac sympathetic denervation on meta-iodo-benzyl-guanidine (MIBG) scintigraphy being the only laboratory test included as a diagnostic criterion (Postuma et al., 2015a). However, recent developments, such as the alpha-synuclein (α-syn) seed amplification assay (SAA), proposed to have utility in differentiating those with PD from controls (see review, (Bellomo et al., 2022)), challenge this notion. In support of this, a cross-sectional study of 1,123 participants in the Parkinson's Progression Markers Initiative (PPMI) cohort by Siderowf and colleagues (2023) demonstrated that α-syn SAA could classify PD with high sensitivity and specificity (Siderowf et al., 2023). Importantly, α-syn SAA could detect prodromal individuals prior to diagnosis (Siderowf et al., 2023), with 86% of individuals with REM sleep behaviour disorder or olfactory impairment, two common prodromal symptoms, showing a positive α-syn SAA. Similarly, it has been proposed that neuronally-derived extracellular vesicle α-syn (i.e. L1EV-associated α-syn) may be a potential biomarker for PD (Yan et al., 2024). In a recent cross-sectional study of 576 individuals, Yan and colleagues reported that L1EV α-syn differentiated high-risk participants (>80% probability of developing

PD) from those with low-risk or matched controls. Further, in a cohort of 40 who later developed PD and related dementia, blood tests were positive for L1EV α-syn in more than 80% (Yan et al., 2024), further supporting the use of biomarkers to advance PD diagnosis and prognosis. Such findings are reflected in the recent development of the Neuronal α-syn Disease (NSD) framework, exploring underlying biology as a staging system of PD (Simuni et al., 2024).

In addition to biological fluid-based biomarkers, neuroimaging may also help to improve diagnosis and prognosis in PD. While a positron emission tomography (PET) tracer for detecting α-syn pathology is in development (^{18}F ACI-12589), it is not yet validated for clinical use (Smith et al., 2023). However, other PET tracers have shown promise, such as Flourodopa F18 (F-Dopa), recently approved by the Food and Drug Administration to aid in earlier diagnosis of PD (Jiang et al., 2021a). Encouragingly, F-Dopa imaging yields high correlations with PD motor outcomes and severity (Otsuka et al., 1996). Additionally, conventional T2-weighted MRI has been used to detect degeneration of regions known to be affected during PD. For example, early clinical studies have reported significantly higher substantia nigra *pars compacta* (SNc) iron content in PD compared to healthy controls (Gorell et al., 1995, Martin, 2009). Such elevations in iron content are proportional to disease severity, highlighting the potential prognostic utility of MRI (Martin, 2009).

Given these findings, this study aimed to determine whether a comprehensive multi-modal approach, incorporating neuroimaging and fluid-based markers, possessed utility in improving prognosis in PD beyond baseline MDS-UPDRS assessment alone. This study utilised data from PPMI to conduct data-driven subtyping on MDS-UPDRS scores 5-years after baseline assessment in an early PD cohort. The predictive utility of neuropathological measures of PD,

including biological-fluid and neuroimaging-based biomarkers, in conjunction with prodromal assessments and current clinical criteria, to predict cluster membership was explored.

2.3 METHODS

Participants & Materials

Analysis used data openly available from the Parkinson's Progression Markers Initiative (PPMI) (Marek et al., 2011), with the cohort of interest consisting of recently diagnosed PD participants (n = 422). A description of PPMI participant selection criteria can be found in Table S1. An overview of extracted data can be found in Table 2-1.

***Table 2-1** Data extracted from the PPMI database and their corresponding assessments.*

Category	Measures
Baseline Demographics	Age Sex Education
Neuroimaging-Based Markers	**T2-weighted MRI** Obtained via a Siemens 3T TIM trio scanner model at baseline. Manual masking protocol (further information below) was conducted on scans to delineate regions of interest, acting as a proxy marker of SN volume. **DaT SPECT** Participants were injected with radionuclide ligand DaTScan at baseline. Specific binding ratio of mean striatum (average of left and right caudate + putamen) was the measure of interest for this analysis
Fluid-Based Pathological Markers	**Cerebrospinal fluid (CSF) (pg/mL)** Obtained via standard lumbar puncture and processing protocols at baseline. Measured using standard ELISA protocols. - Unphosphorylated alpha synuclein (α-syn) Measured using Elecsys electrochemiluminescence (ECL) immunoassays: - Phosphorylated tau (phosphorylation site: threonine 181) (p-tau) - β-amyloid$^{1–42}$ (Aβ) **Serum (ng/mL)** Obtained via standard venepuncture and processing protocols at baseline. Measured using standard ELISA protocols: - Insulin Growth Factor (IGF-1)

Autonomic Function	**Scales for Outcomes in PD-Autonomic (SCOPA-AUT)** The SCOPA-AUT is a self-reported questionnaire with 25 questions, used to assess autonomic function in the following domains (Bostantjopoulou et al., 2016): 1) Gastrointestinal 2) Urinary 3) Cardiovascular 4) Thermoregulation 5) Pupillomotor 6) Sexual Collected at baseline
Sleep	**REM Sleep Behaviour Disorder Questionnaire (RSBDQ)** A 10-item, self-report questionnaire covering the clinical features of REM sleep behaviour disorder (RBD) (Stiasny-Kolster et al., 2007) Collected at baseline
Smell	**University of Pennsylvania Smell Identification Test (UPSIT)** An odour recognition test with 40-items, it is routinely used across a variety of olfactory disorders on account of its sensitivity and replicability (Doty et al., 1984) Collected at baseline
Motor Symptoms & Disease Severity	**Movement Disorder Society-Unified Parkinson's Rating Scale (MDS-UPDRS)** A comprehensive questionnaire containing 50 items pertaining to motor and non-motor symptoms of Parkinson's and is a combination of clinical assessment and self-report (Marsili et al., 2018). This was extracted at baseline and at annual follow-ups up to 5 years while participants were on medication. The following components were used: <u>Part 1: Non-Motor Experiences of Daily Living</u> - Total score <u>Part 2: Motor Experiences of Daily Living</u> - Total score <u>Part 3: Motor Examination</u> - Rigidity score (sum of MDS-UPDRS subitems of item 3.3) - Tremor score (sum of MDS-UPDRS subitems of items 3.15, 3.16, 3.17 & 3.18) Hoehn and Yahr staging scores at year-5 was also extracted
Cognitive Ability	Various aspects of cognition were assessed at baseline at annual follow-ups up to 5 years, as changes in cognition are widely recognised within PD. **Hopkins Verbal Learning Test (HVLT)** - Verbal recognition and recall (learning and memory) **Semantic Fluency (SFT)** - Executive function and semantic memory

	Letter Number Sequencing (LNS) - Working memory **Benton Judgement of Line Orientation (BJLOT)** - Visuospatial ability **Symbol Digit Modalities (SDM)** - Psychomotor/Processing Speed Cognitive assessment, along with PPMI investigator rating and information from external sources (family or friends), also acted as the basis for determination of cognitive disorders (i.e. subjective complaint, PD-MCI, PD-D). This was primarily based on MDS guidelines for diagnosing cognitive impairment (Litvan et al., 2012). Categorisation was extracted at year-5 follow-up
Mood Dysfunction	Various aspects of mood were assessed at baseline at annual follow-ups up to 5 years, as changes in mood are widely recognised within PD. **State-Trait Anxiety Inventory (STAI)** - Anxiety **Geriatric Depression Score (GDS-15)** - Depression
Medical History	Medical history for each participant was extracted, specifically to explore the prevalence of cognitive disorders at year-5 follow-up.

2.3.1 Hierarchical Clustering

Improved characterisation of PD outcomes is critically needed, with a proposed strategy being data-driven subtyping (Chen et al., 2023). Showing promise is hierarchical clustering, which displays several advantages over other methods, such as K-means, (see review, (Hendricks and Khasawneh, 2021)). Therefore, this study utilised enhanced agglomerative hierarchical clustering using Euclidean distance, conducted on year-5 MDS-UPDRS scores, a non-biased approach to identify PD subtypes. Per recommendations to incorporate both motor and non-motor features (Mu et al., 2017), clustering inputs included MDS-UPDRS part 1 and part 2, as well as clinically-rated motor features, specifically rigidity and tremor scores, at year-5 follow-up.

While other motor symptoms, namely gait and postural stability, were considered for inclusion, a recent review by Bloem and colleagues reported that clinical rating scales such as the MDS-UPDRS perform sub-optimally when assessing such features (Bloem et al., 2016). Additionally, MDS-UPDRS derived postural instability – gait disturbance (PIGD) scores lack sensitivity to change, and may not be suitable for mild PD, of particular relevance given this study assessed *de novo* participants (Bloem et al., 2016). Therefore, gait and postural stability were omitted to minimise biasing clustering outputs. A recent study demonstrated that a score based solely on tremor and rigidity features was sufficient to identify a clinically relevant subtype (Adams et al., 2023). Additionally, the same rigidity score has already been used within the PPMI dataset, demonstrating sensitivity to change over time (Pasquini et al., 2023), supporting its use within the current study.

Clustering was performed using the *hclust* function within *factoextra* (Kassambara, 2016), and a phylogenetic tree was generated using the *fviz_dend* function. Prior to clustering, MDS-UPDRS data were converted into z-scores, and the number of clusters was determined via the *NbClust* package (Charrad et al., 2014), with the optimal number of clusters identified in this case being two, illustrated in dendrogram format (Figure S2).

2.3.2 Principal Component Analysis

Principal component analysis (PCA) was conducted on cognitive assessments to develop a single composite score of cognitive ability, and separately on STAI and GDS as a measure of mood dysfunction. This was used to develop baseline cognition and mood scores, as well as a composite score reflecting the change in cognitive ability and mood dysfunction over time, by conducting PCA on the slopes of each neuropsychiatric assessment. Loadings confirmed that

assessments were highly correlated, making it appropriate to convert into a single factor (Table S3).

2.3.3 MRI ROI Delineation

Axial T2-weighted MRI scans were used to assess the structural quality of regions in proximity to the SN. Scans (n = 135) were acquired using a Siemens 3T TIM trio scanner, with a 12-channel matrix head coil and total acquisition time of 5 minutes and 8 seconds. Phase encoding direction was L/R (TE = 11 ms; TR = 3000 ms), with an acquired matrix size of 228 x 256 x 48 and voxel dimensions being 0.93 x 0.93 x 3 mm^3.

The SN was evaluated using a 2D proxy marker, the hypointense region comprised of crural fibres and anterosuperior portions of the SN (Oikawa et al., 2002). Accordingly, we have adopted the term "SN-related T2 hypointensity" to describe this marker. The red nucleus (RN) was chosen as a control region, due to possessing A10 dopaminergic neurons, which, despite being adjacent to the SN, are spared during PD (Grealish et al., 2010). FSLeyes, an image viewing programme available in the FSL package (Jenkinson et al., 2012), was used to create manual delineations of the ROI. Spatial masks were obtained bilaterally from two independent raters (Figure 1) and were averaged. SN-related T2 hypointensity and RN volume values were converted into ratios, which then accounts for the global effect of individual head size. Larger ratios corresponded to a larger SN-related T2 hypointensity and, consequently, more advanced disease.

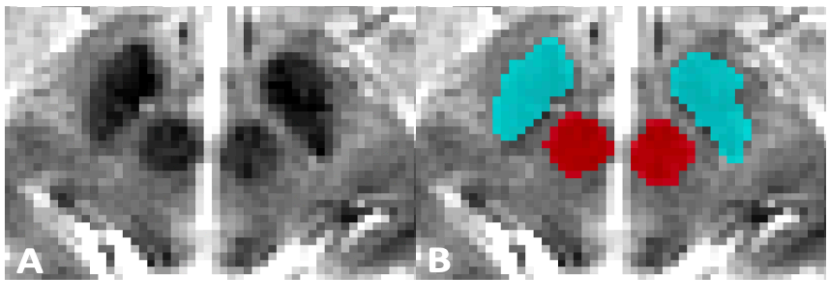


***Figure 2-1** (A) Cropped midbrain region in an axial T2-weighted MRI, along with manual masks (B) for the SN-related T2 hypointensity (blue) and RN (red).*

2.3.4 Statistical Analyses

All data were analysed via R (version 1.4.1717) (Team, 2010). A Shapiro-Wilk test determined several variables deviated significantly from normality, with only striatal DaT binding and SN-related T2 hypointensity being normally distributed. Normally distributed variables are presented as mean ± standard deviation, whereas non-normally distributed variables are reported as median (interquartile range), along with categorical outcomes displayed as counts (percentage).

As cluster membership was determined via MDS-UPDRS scores at year-5 follow-up, retrospective assessment was conducted to determine whether differences between clusters were present at baseline using Mann-Whitney U tests, or T-tests for normally distributed data. To account for the effect of attrition, group differences for all baseline variables were assessed between participants included in the cluster analysis and those lost to follow-up. Further, differences in assessment scores at year-5, as well as clinical features such as Hoehn and Yahr staging and clinician determined cognitive outcomes, were compared between clusters. If data included baseline and corresponding follow-up, a non-parametric equivalent of a mixed ANOVA was conducted via the *nparLD* package (Noguchi et al., 2012), with a between-subjects factor (cluster membership) and a within-subjects factor (year). If a significant interaction effect was present, differences between clusters in motor, mood, and cognition

slopes (indicating the extent of degeneration in both motor and non-motor outcomes over the follow-up period) were assessed via Mann-Whitney U tests.

Logistic regression was conducted to determine if baseline variables of interest predicted cluster membership, displayed using the *sjPLot* package (Lüdecke, 2021). Two models were compared: 1) baseline MDS-UPDRS scores (the same measures whose year-5 values were used as inputs for the clustering algorithms) and 2) baseline MDS-UPDRS scores, as well as demographic data, biomarkers and prodromal autonomic outcomes measured at baseline. An ANOVA was run between models to determine whether the inclusion of these additional variables significantly improved predictive accuracy beyond MDS-UPDRS alone. Both models only included participants with complete data (n = 111). The relative importance of each predictor was calculated via the *Caret* package (Kuhn, 2015).

2.3.5 Machine Learning

A stratified cross-validation pipeline was developed in Python (Figure 2-2) using the *scikit-learn* package (Pedregosa et al., 2011). Models with identical predictor inputs to the logistic regression models were developed, incorporating three classifiers: support vector machines (SVM) (kernel = radial basis function), k-nearest neighbours (neighbours = 40, range = 2-60), and random forests (estimators = 100, range = 2-60). Balanced accuracy scores were compared to chance performance (50%), and between each classifier to determine the best classifier per predictor input to use on the test dataset. The final test result was compared to chance performance to determine the degree that the chosen machine learning classifier improved predictiveness. Overall accuracy in predicting cluster membership was compared to the corresponding logistic regression models, to determine whether the non-linearity feature of the machine learning models improved classification confidence.

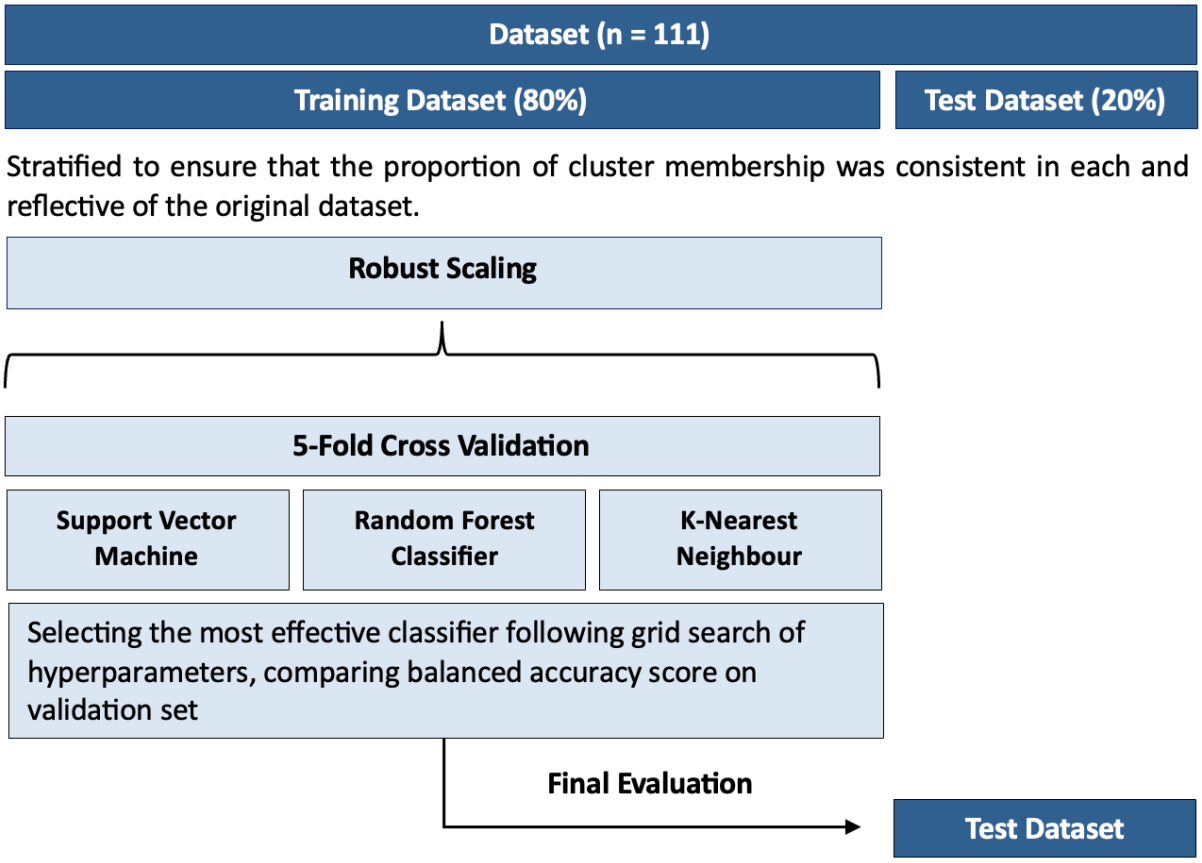


***Figure 2-2** A 5-fold cross-validation pipeline was developed to create models using identical predictor inputs as used to develop the logistic regression models.*

2.4 RESULTS

2.4.1 Baseline Comparisons

Cluster 2 demonstrates a significantly higher (p = 0.02) age (median = 63.5) compared to cluster 1 (median = 61.2), with both clusters reporting a higher proportion of males than females (cluster 1 = 63%; cluster 2 = 76%). In brief, cluster 2 displayed significantly worse performance in multiple MDS-UPDRS domains, including higher part 1 and 2 scores, as well as higher mood dysfunction (Table 2-2). Further, cluster 2 performed significantly worse in all prodromal assessments, along with significantly lower striatal DaT binding and cognitive ability.

Table 2-2 ***Differences between clusters in all extracted assessments were assessed retrospectively at baseline****. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via T-tests. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Mann-Whitney U tests. Count data are presented as percentages (%) and differences were assessed via a Chi-square test. A Bonferroni correction was applied to account for multiple comparisons, setting the threshold to p < 0.003. Statistically significant differences are denoted by bolded p-values.*

	Cluster 1 n = 221		Cluster 2 n = 79		
	n	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**p-value**
Age	221	61.2 (54.7 – 67.9)	79	63.5 (66.9 – 70.9)	0.02
Education	221	16 (14 – 18)	79	16 (14 – 18)	0.39
Sex					
Female		81 (37)		19 (24)	0.06
Male		140 (63)		60 (76)	
MDS-UPDRS Part 1	221	4 (2 – 6)	79	7 (5 – 10)	**< 0.0001**
MDS-UPDRS Part 2	221	4 (2 – 6)	79	9 (5 – 12.75)	**< 0.0001**
Rigidity Score	221	3 (2 – 5)	79	4 (2.25 – 6)	0.03
Tremor Score	221	4 (2 – 6)	79	3 (2 – 5)	0.09
Cognitive Ability	220	0.27 (-0.42 – 0.88)	79	-0.03 (-0.81 – 0.37)	**0.001**
Mood Dysfunction	221	-0.35 (-0.77 – 0.27)	79	0.16 (-0.58 – 0.84)	0.004
CSF α-syn	216	1375.5 (1062.9 – 1854.2)	79	1322.6 (1036 – 1794)	0.17
CSF p-tau	197	13.19 (11 – 17.1)	71	13.48 (11.8 – 17.1)	0.46
CSF Aβ	214	848.6 (650.2 – 1195.8)	78	906 (671.30 – 1157.3)	0.73
Serum IGF-1	215	137.70 (103.3 – 166.2)	76	132.2 (99.3 – 176)	0.78
UPSIT	221	23 (17 – 29)	79	19 (12 – 26)	**0.002**
RBDSQ	221	3 (2 – 5)	79	5 (2 – 7)	**0.0008**
SCOPA-AUT	218	7 (4 – 10)	78	13 (10 – 17)	**< 0.0001**
SN Proxy Ratio	101	1.27 ± 0.22	34	1.30 ± 0.21	0.42
Striatal DaT Binding	219	1.45 ± 0.35	78	1.25 ± 0.41	**0.0002**

Analysis was conducted to determine if participants lost to attrition, and therefore not included in cluster analysis, presented differences at baseline. In brief, participants lost to attrition performed worse in several domains, including significantly higher scores in MDS-UPDRS part 2, along with lower cognitive ability and serum IGF-1 concentrations (Table 2-3). While not significant following correction for multiple comparisons, participants without follow-up also trended towards having higher MDS-UPDRS part 1 and SCOPA-AUT scores and higher age, along with lower UPSIT scores.

Table 2-3 ***Baseline characteristics of participants included in the final dataset, who all had MDS-UPDRS scores up to year-5 follow-up, compared against participants lost to attrition****. Overall, participants lost to follow-up demonstrated significantly worse outcomes in multiple domains at baseline. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via independent samples t-test. Non-parametric variables are displayed as median (IQR), with differences determined via Mann-Whitney U. Count data are presented as percentages (%) and differences were assessed via a Chi-square test. A Bonferroni correction was applied to account for multiple comparisons, setting the threshold to p < 0.003. Statistically significant differences are denoted by bolded P-values.*

	Participants with Follow-up n = 300 **Mean ± SD / Median (IQR)**	**Participants w/o Follow-Up** n = 122 **Mean ± SD / Median (IQR)**	**p-value**
Age	61.82 (54.3 – 68.5)	64.05 (58.5 – 70.1)	0.013
Education	16 (14 – 18)	16 (14 – 17)	0.12
Sex			
Male	200 (66.7%)	77 (66%)	
Female	100 (33.3%)	45 (34%)	0.56
MDS-UPDRS Part 1	5 (2 – 7)	6 (3 – 8)	0.023
MDS-UPDRS Part 2	4 (2 – 8)	6 (4 – 9)	**0.002**
Rigidity Score	3 (2 – 5)	3 (2 – 6)	0.99
Tremor Score	4 (2 – 6)	4 (2 – 7)	0.18
Cognitive Ability	0.17 (-0.47 – 0.80)	-0.19 (-0.97 – 0.37)	**< 0.001**
Mood Dysfunction	-0.28 (-0.73 – 0.44)	-0.26 (-0.65 – 0.45)	0.58

CSF α-syn	1374.1 (1034.4 – 1792.2)	1422.5 (1127 – 1758.3)	0.33
CSF p-tau	13.2 (11.13 – 17.12)	14.3 (11.6 – 18.3)	0.08
CSF Aβ	867.2 (642.1 – 1150.3)	831.7 (590.6 – 1053.3)	0.32
Serum IGF-1	135 (103.1 – 166.9)	109.8 (85.2 – 152.1)	**0.001**
UPSIT	22 (15.75 – 28)	23 (16 – 30)	0.30
RBDSQ	3 (2 – 5)	4 (2 – 6.5)	0.009
SCOPA-AUT	8 (5 – 12)	10 (6.3 – 13.8)	0.006
Striatal DaT Binding	1.40 ± 0.38	1.45 ± 0.45	0.29

2.4.2 Year-5 Follow-up Characteristics

At year-5 follow-up, cluster 2 consistently performed worse across all assessments, demonstrating a significantly higher MDS-UPDRS part 1, MDS-UPDRS part 2 and rigidity score compared to cluster 1, as well as higher prevalence of mood dysfunction. Additionally, cluster 2 demonstrated more profound postural instability, indicated by significantly higher Hoehn and Yahr scores, as well as a larger proportion of individuals with postural instability (score ≥ 3), albeit not significant following correction for multiple comparisons. Cluster 2 also demonstrated significantly lower cognitive ability, further corroborated by a much larger proportion of participants within cluster 2 reporting mild cognitive impairment (MCI) and dementia compared to cluster 1. Year-5 follow-up comparisons between clusters are presented in Table 2-4.

Table 2-4 ***Differences between clusters in assessments with follow-up data were assessed at year-5.*** *Further, clinical characteristics, such as Hoehn Yahr staging and cognition-related diagnoses, were compared. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Mann-Whitney U tests. Count data are presented as percentages (%) and differences were assessed via a Chi-square test. A Bonferroni correction was applied to account for multiple comparisons, setting the threshold to p < 0.006. Statistically significant differences are denoted by bolded P-values.*

	Cluster 1 n = 221		**Cluster 2** n = 79		
	n	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**p-value**
MDS-UPDRS Part 1	221	7 (4 – 10)	79	15 (11.5 – 20)	**<0.0001**
MDS-UPDRS Part 2	221	7 (4 – 11)	79	17 (13 – 19)	**<0.0001**
Rigidity Score	221	4 (2 – 6)	79	6 (3 – 8.5)	**<0.0001**
Tremor Score	221	4 (0 – 7)	79	2 (0 – 3)	**<0.0001**
Cognitive Ability	220	0.26 (-0.41 – 0.79)	79	-0.22 (-1.03 – 0.49)	**0.0003**
Mood Dysfunction	221	-0.45 (-0.85 – -0.01)	79	0.41 (-0.17 – 1.50)	**<0.0001**
Hoehn and Yahr		2 (2 – 2)		2 (2 – 3)	**0.0008**
≤ 2		211 (96%)		64 (81%)	0.007
≥ 3		8 (4%)		15 (19%)	
Cognitive Outcome					
Normal		150 (69%)		32 (41%)	
Indeterminate		25 (11%)		12 (15%)	
Cognitive Complaint		28 (13%)		18 (23%)	**<0.0001**
MCI		11 (5%)		4 (5%)	
Dementia		4 (2%)		9 (11%)	

2.4.3 Rate of Progression

For variables with significant interaction effects (Table S4), slopes were calculated using all timepoints and compared across clusters via Mann-Whitney U tests (Figure 2-3). Cluster 2

demonstrated significantly higher rates of progression in various assessments, including MDS-UPDRS part 1 ($p < 0.0001$) and part 2 scores ($p < 0.0001$), as well as mood dysfunction ($p < 0.0001$). Further, cluster 2 demonstrated a significantly lower rate of progression in tremor scores ($p = 0.0007$). While cluster 2 displayed a trend towards more rapid progression in rigidity scores ($p = 0.02$), this did not reach significance after applying a correction for multiple comparisons (threshold: $p < 0.01$).

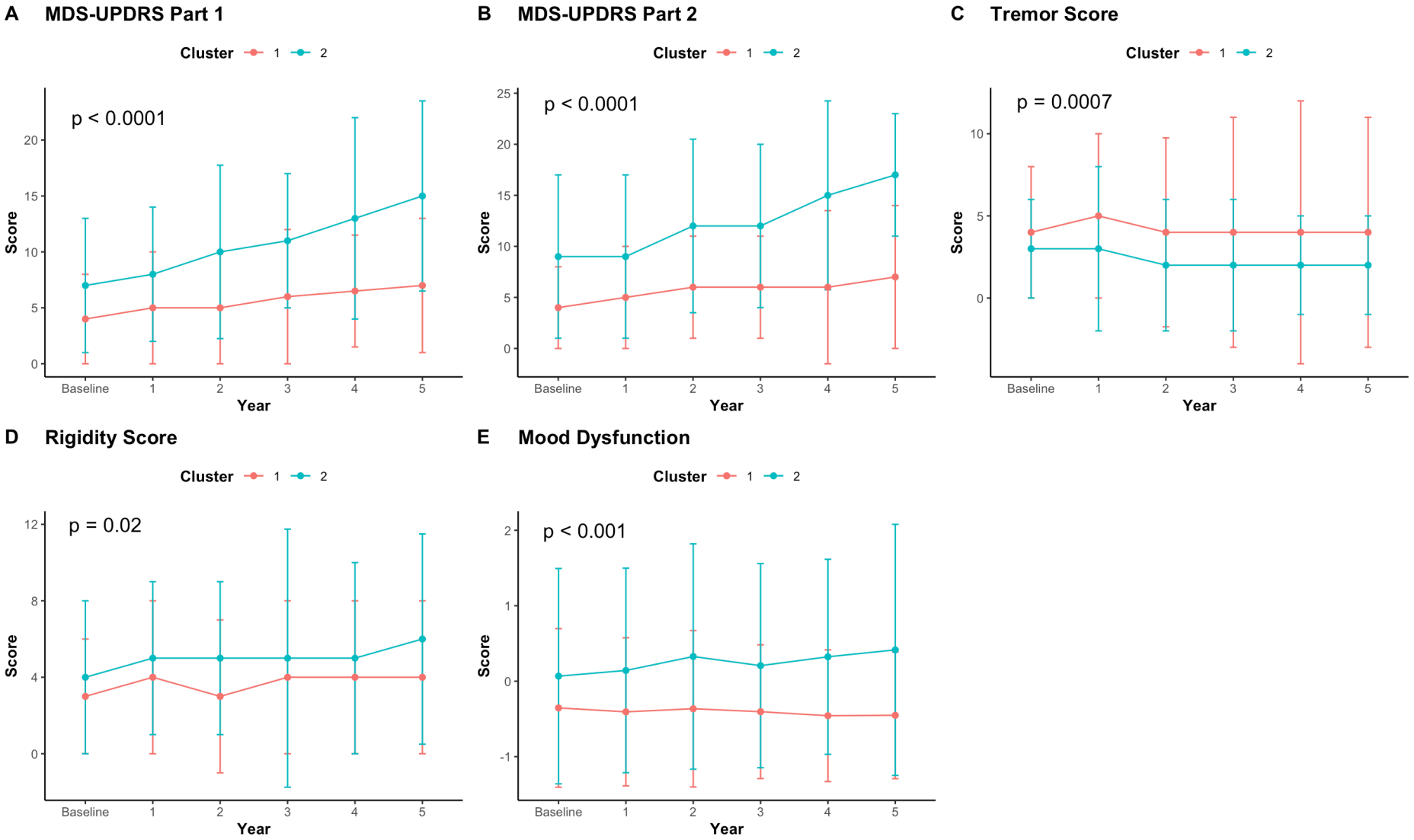


Figure 2-3 Median & IQR values at each timepoint displayed for variables with significant interaction effects in the non-parametric mixed ANOVA's. *Differences in slopes between clusters were assessed via Mann-Whitney U tests*

2.4.4 Cluster Membership Prediction

Logistic regression was conducted on participants with no missing data across all baseline predictor inputs (n=111; cluster 1 = 85 & cluster 2 = 26) (Table 2-5). Demographic information on this subset is reported in Table S5.

Table 2-5 Two logistic regression models were created with cluster membership as the outcome variable. *The first model only included baseline values of the MDS-UPDRS scores (the year-5 values for the same measures were used in the hierarchal clustering protocol) as predictors, whereas the second model included additional baseline demographic, neuroimaging, biofluid marker and prodromal assessment scores. Significant predictors are denoted by bold P values, with threshold set to 0.05 as R computes internal corrections for multiple corrections.*

Cluster Membership

	MDS-UPDRS Assessment			**Multi-Modal Assessment**		
Predictors	*Odds Ratios*	*std. Beta*	*p*	*Odds Ratios*	*std. Beta*	*p*
Intercept	0.04	0.23	**<0.001**	0.38	0.10	0.849
MDS-UPDRS Part 1 Score	1.10	1.38	0.250	1.03	1.10	0.838
MDS-UPDRS Part 2 Score	1.32	2.76	**<0.001**	1.29	2.54	**0.018**
Rigidity Score	1.03	1.07	0.799	0.93	0.83	0.680
Tremor Score	0.96	0.89	0.661	0.99	0.97	0.940
Age				0.96	0.67	0.378
Female Sex				0.18	0.43	0.072
Education				1.16	1.51	0.309
SN Proxy Marker				7.28	1.54	0.221
Striatal DaT Binding				0.15	0.51	0.093
CSF α-syn				1.00	0.09	**0.038**
CSF p-tau				1.49	6.45	**0.023**
CSF Aβ				1.00	1.16	0.741
Serum IGF-1				1.00	1.07	0.845
RBDSQ				1.10	1.26	0.501
UPSIT				0.92	0.47	**0.040**

SCOPA-AUT		1.25	3.64	**0.026**
Observations	111	111		
R^2 Tjur	0.274	0.491		

When only including baseline MDS-UPDRS assessments, the only significant predictor of cluster membership was MDS-UPDRS part 2 score, with an overall predictive value of 27.4% ($R^2 = 0.274$). Similarly, MDS-UPDRS part 2 score remained a significant predictor in the multi-modal assessment model, along with CSF levels of both α-syn and p-tau, UPSIT score and SCOPA-AUT score. The additional predictors included in model 2 increased the proportion of variance explained by 21.7% relative to model 1, and this difference was significant ($F(12, 94) = 29.25$, $p = 0.004$). Relative importance of predictors for both models are presented in Figures S6 & S7.

2.4.5 Machine Learning

Validation scores determined that the best classifier for MDS-UPDRS assessment, in isolation was SVM (0.75) compared to KNN (0.67) and RFC (0.7), whereas the classifier with the highest score for the multi-modal assessment was RFC (0.72), with SVM and KNN being 0.66 and 0.57, respectively.

Balanced accuracy scores were obtained from the selected classifier in each case, for each dataset partition (training, validation, test). While both assessments showed higher classification accuracy compared to chance performance, the multi-modal assessment (validation score = 0.67 ± 0.04) had a higher test result of 0.74, compared to a score of 0.60 for the assessment using MDS-UPDRS alone (validation score = 0.75 ± 0.05).

2.5 DISCUSSION

Currently, PD diagnosis and prognosis are largely reliant on clinical criteria, such as the MDS-UPDRS, corresponding to several shortcomings, such as high rates of misdiagnosis. A possible strategy to mitigate this and advance understanding of PD outcomes is to utilise a multi-modal combination of markers, incorporating not only the MDS-UPDRS, but also biofluid and neuroimaging-based biomarkers, as well as more comprehensive clinical evaluations, such as prodromal assessments. In this study, the combination of such markers resulted in better prediction of membership in the more impaired cluster at year-5 follow-up, and improved classification accuracy in utilised machine learning techniques.

Hierarchal clustering on MDS-UPDRS scores at year-5 follow-up identified two clusters. Retrospective assessment determined cluster 2 demonstrated significantly higher rigidity at year-5 follow-up, as well as a faster rate of progression in rigidity over time (albeit not statistically significant after applying multiple comparison correction), along with a significantly slower progression in tremor scores. Additional features reported across the motor subtyping literature were also identified, including older age of onset (Kang et al., 2005, Pagano et al., 2016), albeit not statistically significant following multiple comparison correction, and poorer functional outcomes, measured by MDS-UPDRS part 1 and 2 scores (Jankovic and Kapadia, 2001), in the more-rigid cluster at both baseline and year-5 follow-up. Additionally, cluster 2 demonstrated significantly higher scores on the Hoehn and Yahr scale. Overall, this suggests the clusters identified are similar to clinically recognised subtypes of PD, with cluster 1 reflecting tremor dominant (TD), whereas cluster 2, displaying the aforementioned features, is more similar to clinically relevant motor phenotypes, namely akinetic-rigid- (AR) or PIGD-PD (Eggers et al., 2012). However, it should be noted that there was no significant difference in either tremor and rigidity scores between clusters at baseline,

and neither measure acted as a significant predictor in the logistic regression models predicting cluster membership. This further corroborates past assertions in the literature that MDS-UPDRS derived motor scores at time of diagnosis are not reflective of future assessment outcomes (Kohat et al., 2021), and highlights the need to extend prediction of disease progression beyond the MDS-UPDRS alone.

Differences in the progression of cognition and mood were also explored between clusters. Consistent with the literature, the more-rigid cluster had significantly lower cognitive ability scores, both at baseline and year-5 follow-up (Kelly et al., 2015). The more-rigid cluster also demonstrated higher rates of both dementia diagnosis and subjective cognitive complaints at year-5 follow-up compared to the more TD-dominant cluster (although not higher rates of MCI diagnosis). Disparate risk for dementia conversion between motor subtypes is widely recognised in the PD literature (Burn et al., 2006), with a possible explanation being distinct pathophysiology's. Interestingly, however, there was no difference in rate of cognitive decline between the two clusters. This could be attributed to difficulties in assessing cognition, particularly in early PD. This is highlighted in a meta-analysis exploring PD conversion into PD-mild cognitively impaired and PD-dementia, where high reversion rates were seen within the first 3 years of diagnosis (Saredakis et al., 2019). Contrastingly, the more-rigid cluster demonstrated significantly higher mood dysfunction at baseline and year-5 follow-up and a faster rate of progression over the 5-year period following diagnosis. This aligns well with previous literature, where mood dysfunction, such as depression and apathy, is more profound in non-TD subtypes (Reijnders et al., 2009).

Interestingly, despite significant differences in baseline clinical assessments, no baseline differences in biomarker concentrations were found between clusters. This is inconsistent with

previous literature, in which different subtypes have been shown to display distinct biomarker profiles. For example, compared to TD-subtypes, plasma levels of α-syn and Aβ have been shown to be significantly higher and lower, respectively (Ding et al., 2017), with CSF concentrations of CSF p-tau shown to be higher (Zuo et al., 2017), in PIGD-PD. This inconsistency may be because the PPMI dataset at baseline is highly homogenous (Kang et al., 2016). Although previous work in this dataset has shown baseline differences in biomarkers, such as CSF α-syn, between individuals with PD and healthy controls (Mollenhauer et al., 2019a), it may be more difficult to detect differences between subtypes. Alternatively, this may be due to the restricted sample size of the current cohort, as only cases that had data for all variables of interest could be included in the analysis.

Nevertheless, a lack of significant differences in these biomarkers at baseline does not necessarily equate to poor prognostic utility. In support of this, lower levels of CSF α-syn and higher levels of CSF p-tau (but not CSF levels of Aβ or serum levels of IGF-1) were significant predictors of cluster membership within the multi-modal logistic regression model, both indicative of more advanced pathology (Katayama et al., 2020, Baek et al., 2021). This is similar to previous literature, where reductions in CSF α-syn over time were associated with worsening motor function, particularly in those of the AR-subtype, in the DATATOP cohort (Majbour et al., 2016) (although see (Hong et al., 2010, Mollenhauer et al., 2011, van Dijk et al., 2014)). Similarly, CSF α-syn also displayed predictive value for change in cognitive function (although, interestingly, not of motor symptoms) in another study analysing data from the DATATOP cohort (Stewart et al., 2014). Of note, higher CSF α-syn was associated with a faster rate of cognitive decline, contrary to what might be expected and inconsistent with results of the current work, where no difference in progression of cognitive symptoms was noted between clusters. Regarding p-tau, emerging evidence suggests that p-tau plays a critical role

in PD pathophysiology and may interact with α-syn to influence its pathology (for review, see (Pan et al., 2021)). Indeed, a recent study by Chu and colleagues (2024) demonstrated that tau, rather than α-syn aggregation, may mediate nigrostriatal DA neuron degeneration (Chu et al., 2024). Despite this, few studies to date have examined the relationship between PD progression and p-tau. One study showed no association between CSF p-tau levels and cognitive decline over time in a prospective cohort study (n = 45) with at least one yearly follow-up (Siderowf et al., 2010). More recent work, however, assessing plasma extracellular vesicles from 103 individuals with PD showed that elevated baseline plasma extracellular vesicle tau levels corresponded to significantly greater decline in both motor function and cognition (Chan et al., 2023), suggesting further work in this area is warranted, particularly to understand pathological distinctions between disease subtypes.

While biological fluid-based biomarkers showed potential for predicting disease progression in this study, the use of neuroimaging measures appeared less promising. While the more-rigid cluster displayed significantly lower striatal DaT binding at baseline compared to the tremor cluster, no significant difference in the SN-related hypointensity measure between clusters was noted. Further, neither neuroimaging modality were significant predictors of cluster membership in the multi-modal model. Despite studies reporting significant correlations between baseline DaT and disease staging (Nissen et al., 2014) and rigidity severity (Ramani et al., 2017), our findings reflect those reported by Chahine et al., whereby model performance to predict MDS-UPDRS change was noticeably low with baseline measures of DaT imaging (Chahine et al., 2019). This was ameliorated by incorporating a short-term change to DaT binding as a predictor, a potential avenue moving forward. Regarding the MRI marker, a potential issue with the delineation protocol is assessing ROIs in 2D rather than 3D, leading to a potentially inaccurate representation of structural changes. Additionally, T2-weighted MRI

may lack the sensitivity required to distinguish SN-related differences between clusters (see review, (Santos García et al., 2021)). In support of this, the SN-related hypointensity did not correlate with motor outcomes or disease severity in a previous study (Langley et al., 2017). Instead, alternative imaging sequences may prove more beneficial for capturing differences between PD phenotypes. For example, AR-PD demonstrates more distinct pathological features in the SN compared to TD-PD, including increased regional iron deposition, as measured by quantitative susceptibility mapping (Xiong et al., 2020), and more profound decline of neuromelanin, as measured by T1 neuromelanin-sensitive MRI (Lakhani et al., 2024). Thus, to probe further whether changes in neuroimaging markers are predictive of disease progression in PD, future studies should employ sequences sensitive to such pathological changes in PD, which were not available in the current work.

Outside of pathophysiological assessments, clinical assessments encompassing a wide array of prodromal symptoms show high promise for predicting risk of PD development (Ross et al., 2012), and therefore may show promise for prognosis of clinical progression. Here, the results of the multi-modal logistic regression highlight worse UPSIT and SCOPA-AUT scores significantly predicted membership in the more-rigid cluster. Supporting this, He et al. reported patients presenting hyposmia display a worse clinical course (He et al., 2020) and are more likely to be classified as PIGD-dominant (He et al., 2020, Stern et al., 1994). Similarly, autonomic dysfunction, as measured by SCOPA-AUT, has consistently been reported to be higher in PIGD-PD compared to TD-PD (van der Heeden et al., 2016, Arnao et al., 2021) and even intermediate subtypes (Arnao et al., 2021). However, interestingly, the RBDSQ was not a significant predictor in this model, consistent with other studies reporting a lack of association between RBD or sleep disturbance and PIGD (Suzuki et al., 2017, Benninger et al., 2010). Despite promise, it should be noted that prodromal symptoms are still largely underutilised

clinically, due to non-specificity (see review (Postuma et al., 2012)). Therefore, their utility may be highest when used in conjunction with more validated assessments, as seen in this study.

Overall, the findings of this study suggest that incorporating a multi-modal panel including biological measures, prodromal assessments, and current clinical criteria displays high prognostic utility for disease progression, beyond MDS-UPDRS in isolation. In regression models, baseline MDS-UPDRS assessment alone could only account for 27.4% of variance, whereas the incorporation of neuroimaging, biofluid pathological markers, prodromal symptom presentation and key demographic variables increased the proportion of the variance explained to 49.1%. This was corroborated by machine learning, where MDS-UPDRS assessment alone had a classification accuracy close to chance (60%), increasing to 74% when utilising multi-modal assessment. Further, while the multimodal assessment demonstrated lower validation scores, its higher test result suggests it performs more robustly on unseen data, indicative of higher generalisation ability, which is crucial for clinical translatability. However, for the machine learning, the test and validation scores were substantially different and inconsistent, suggesting a considerable amount of variance, requiring future testing in new data to assess this.

While promising, several limitations must be considered when interpreting findings from current work. Firstly, while data-driven clustering shows high promise, a systematic review has highlighted the difficulty in comparing across studies and raised concerns that true replications of clinical phenotypes are not reproducible (Hendricks and Khasawneh, 2021). Therefore, harmonisation of clustering protocols is essential to make effective comparisons. Secondly, lack of available follow-up data for variables, such as biomarkers and prodromal features, made

their incorporation into the data-driven subtyping unfeasible, as it would have greatly truncated sample size. Finally, between baseline and year-5, approximately 30% of participants were lost to follow-up and therefore excluded. Interestingly, compared to those included in the current analysis, participants lost to follow-up demonstrated considerably worse outcomes in various domains at baseline, including MDS-UPDRS scores, cognitive ability and prodromal assessments. This is an important consideration, as it is likely that effects of prognostic utility reported within this study may be an underestimation of the wider dataset. Finally, while limitations of the MDS-UPDRS have been referenced, Evers and colleagues also note that the MDS-UPDRS presents with a substantial amount of error variance, particularly when assessing change overtime (Evers et al., 2019), as well as potential limitations surrounding only having a single rater at each timepoint potentially introducing bias. Therefore, while the current gold standard, more stable and reliable instruments should be utilised in future.

2.6 CONCLUSION

Current diagnostic criteria for PD rely heavily on clinical judgment, with misdiagnosis occurring in a significant percentage of individuals. Further, the use of the MDS-UPDRS alone for long-term prognosis in early PD cohorts has limitations, perhaps because the MDS-UPDRS is not comprehensive enough to capture the heterogeneity of PD progression. In the current study, incorporating additional assessments, primarily pathological biomarkers, substantially improved sub-type classification and may provide key insight into differential outcomes across PD. Continued exploration into underlying pathophysiology of PD will advance development of prognostic models, which could greatly enhance more personalised management of PD.

2.7 DATA AVAILABILITY

All data analysed within the current study are available within the PPMI database, https://www.ppmi-info.org/access-data-specimens/download-data

2.8 ACKNOWLEDGEMENTS

Data used in the preparation of this article were obtained [on August, 01, 2021] from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/access-data-specimens/download-data), RRID:SCR_006431. For up-to-date information on the study, visit www.ppmi-info.org"

2.9 FUNDING

Funding sources and conflict of interest: This work was funded by grants to LCP and IB from the James and Diana Ramsay Foundation and the NeuroSurgical Research Foundation. AM and BE are supported by Australian government research training programme scholarships.

The authors declare that there are no conflicts of interest relevant to this work.

PPMI – a public-private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including 4D Pharma, Abbvie, AcureX, Allergan, Amathus Therapeutics, Aligning Science Across Parkinson's, AskBio, Avid Radiopharmaceuticals, BIAL, Biogen, Biohaven, BioLegend, BlueRock Therapeutics, Bristol-Myers Squibb, Calico Labs, Celgene, Cerevel Therapeutics, Coave Therapeutics, DaCapo Brainscience, Denali, Edmond J. Safra Foundation, Eli Lilly, Gain Therapeutics, GE

HealthCare, Genentech, GSK, Golub Capital, Handl Therapeutics, Insitro, Janssen Neuroscience, Lundbeck, Merck, Meso Scale Discovery, Mission Therapeutics, Neurocrine Biosciences, Pfizer, Piramal, Prevail Therapeutics, Roche, Sanofi, Servier, Sun Pharma Advanced Research Company, Takeda, Teva, UCB, Vanqua Bio, Verily, Voyager Therapeutics, the Weston Family Foundation and Yumanity Therapeutics."

Full financial disclosures for the previous twelve months: AM and BE are supported by an Australian government research training programme scholarship. MJ receives royalties from Oxford University Innovations for licensing of the FSL software for commercial, non-academic use.

2.10 AUTHOR INFORMATION

2.10.1 Author Affiliation

School of Biomedicine, University of Adelaide, SA 5000, Australia

Angus McNamara, Benjamin Ellul & Lyndsey Collins-Praino

School of Psychology, University of Adelaide, SA 5000, Australia

Irina Baetu

Australian Institute for Machine Learning, School of Computer and Mathematical Sciences, University of Adelaide, SA 5000, Australia

Mark Jenkinson & Stephan Lau

South Australian Health and Medical Research Institute, SA 5000, Australia

Mark Jenkinson & Stephan Lau

2.10.2 Contributions

AM, conceptualized, analyzed data, and wrote the original manuscript. BE assisted in data analysis and MRI related segmentations. LCP, IB & MJ contributed to experimental concept and design, interpretation of results, as well as LCP playing a key role in manuscript conceptualisation. SL contributed to interpretation of results and specific assistance in interpreting MRI related findings. All authors were involved in editing the original manuscript. All authors read and approved the submitted manuscript.

2.11 CORRESPONDING AUTHOR

Address all correspondence to Lyndsey Collins-Praino

2.12 ETHICS DECLARATIONS

2.12.1 Ethics approval and consent to participate

The PPMI study was approved by the local Institutional Review Boards of respective institutions (a full list is available at the following link https://www.ppmi-info.org/about-ppmi/ppmi-clinical-sites). Written informed consent were obtained from each participant at enrolment, in accordance with the Declaration of Helsinki. All methods were performed in accordance with the relevant guidelines and regulations

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines

2.12.2 Competing interests

The authors declare that they have no competing interests.

2.13 SUPPLEMENTARY MATERIAL

Table S1: *Inclusion and exclusion criteria that PPMI have developed to categorise participants into the early PD cohort.*

Inclusion Criteria	Exclusion Criteria
1) Presence of 2 or more cardinal motor symptoms: 1. Resting tremor 2. Bradykinesia 3. Rigidity OR a) Asymmetric resting tremor OR b) Asymmetric bradykinesia & 2) Dopamine transporter deficit on SPECT imaging & 3) Diagnosis of PD < 2 years before baseline visit	First-degree relative of a PD patient (Genetic cohort) Patients with scans without evidence of dopaminergic deficit (SWEDD cohort) < 30 years old

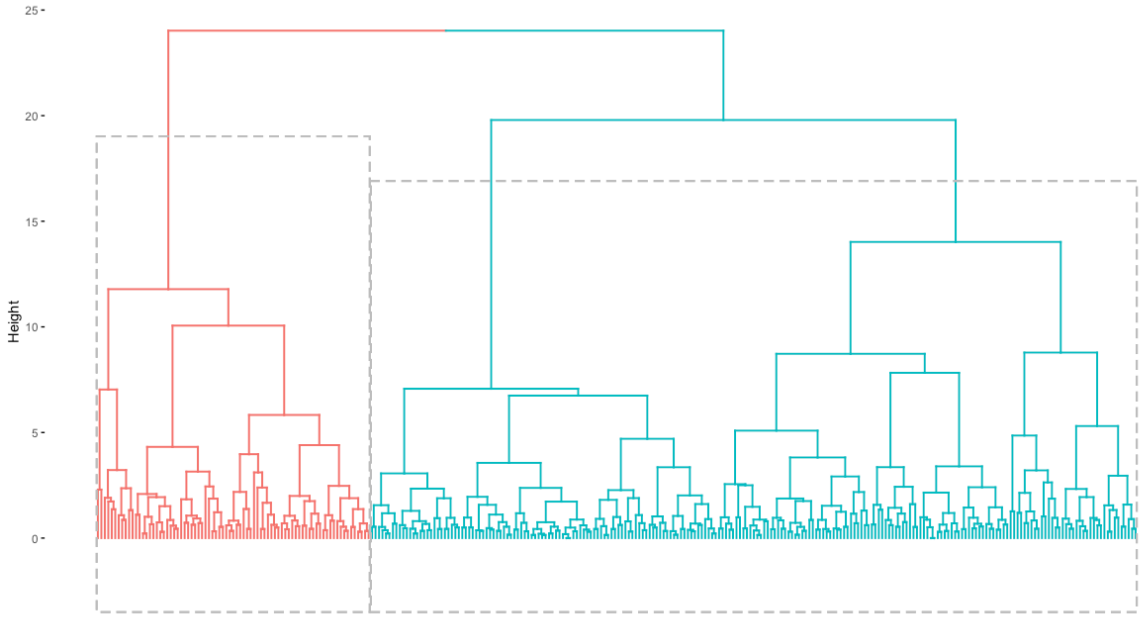


Figure S2: *Dendrogram detailing cluster delineation using MDS-UPDRS assessments at year-5 follow-up.*

Table S3: *PCA was conducted on cognitive assessments and mood-related assessments to provide composite scores based on the first unrotated factor. This was conducted on baseline values as well as on beta coefficients that estimated the slope of change in mood or cognition over the follow-up period.*

	Loadings	Eigenvalue	Variance Accounted by Factor
Baseline Cognitive Ability PCA		2.27	45%
HVLT	.69		
LNS	.72		
SFT	.71		
BJLOT	.48		
SDM	.74		
Baseline Mood Dysfunction PCA		1.65	83%
STAI	.91		
GDS	.91		
Cognitive Ability Beta Coefficient PCA		2.17	77%
HVLT slope	.74		
LNS slope	.66		
SFT slope	.61		
BJLOT slope	.58		
SDM slope	.69		
Mood Dysfunction Beta Coefficient PCA		1.62	91%
STAI slope	.9		
GDS slope	.9		

Table S4: *Non-parametric equivalent of Mixed ANOVAs with a between-subjects factor (cluster membership) and a within-subjects factor (year) were conducted. Of primary interest was whether significant interaction effects were present to determine whether clusters differed in rates of progression*

	Cluster	**Year**	**Interaction Effect**
MDS-UPDRS Part 1	F(1, 298) = 138.18 **P < 0.001**	F(1, 298) = 254.72 **P < 0.001**	F(1, 298) = 21.45 **P < 0.001**
MDS-UPDRS Part 2	F(1, 298) = 193.46 **P < 0.001**	F(1, 298) = 216.28 **P < 0.001**	F(1, 298) = 11.61 **P < 0.001**
Rigidity Score	F(1, 298) = 22.42 **P < 0.001**	F(1, 298) = 22.77 **P < 0.001**	F(1, 298) = 5.58 **P = 0.02**
Tremor Score	F(1, 298) = 21.13 **P < 0.001**	F(1, 298) = 23.69 **P < 0.001**	F(1, 298) = 12.10 **P < 0.001**
Cognitive Ability	F(1, 292) = 12.23 **P < 0.001**	F(1, 292) = 2.71 P = 0.1	F(1, 292) = 0.34 P = 0.56
Mood Dysfunction	F(1, 296) = 40.01 **P < 0.001**	F(1, 296) = 4.85 **P = 0.02**	F(1, 296) = 24.05 **P < 0.001**

Table S5: *Demographic information for the subset of participants part of the final logistic regression models, based on data completeness. Data are displayed as median (IQR), with differences determined via Mann-Whitney U. Qualitative data were presented as counts (%) and differences were assessed via a Chi-square test. Statistically significant differences are denoted by bolded P-values.*

	Cluster 1 n = 85	**Cluster 2** n = 26	**P-Value**
Age	61.16 (53.8 – 67.7)	63.51 (55.86 – 70.9)	0.21
Education	16.00 (14 – 18)	16.00 (14 – 18)	0.99
Sex			
Females	36 (42%)	5 (19%)	0.06
Males	49 (58%)	21 (81%)	

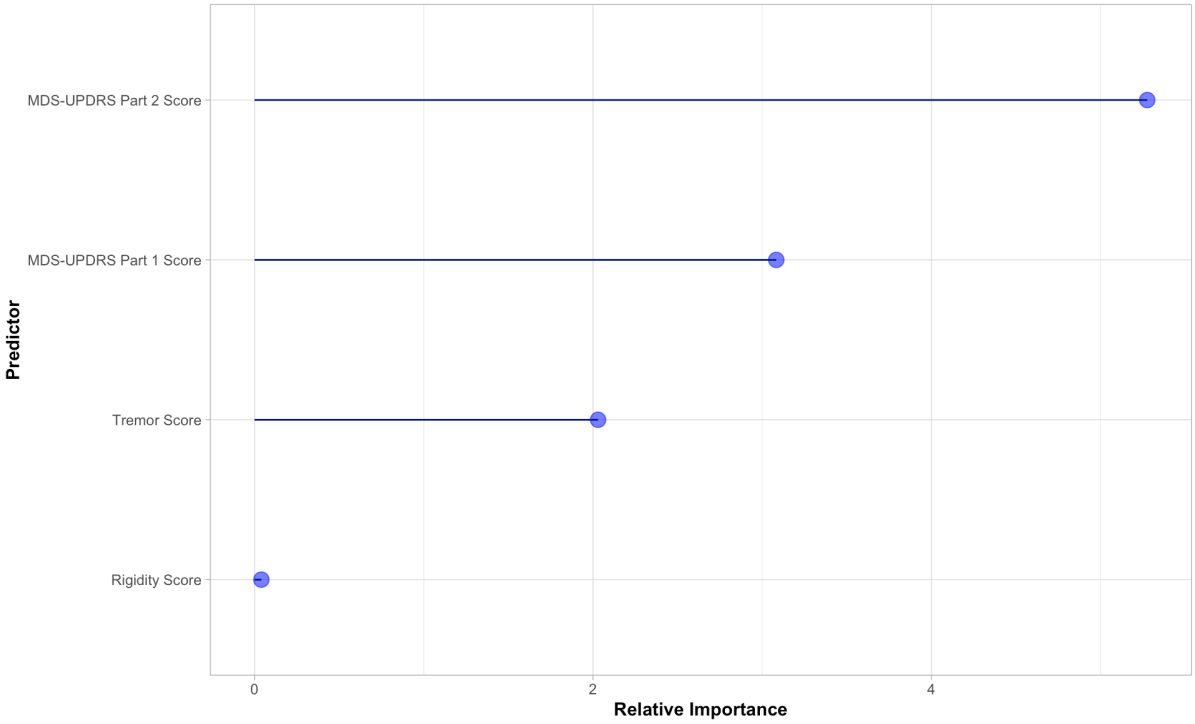


Figure S6: *Relative importance of predictors in the logistic regression model exploring cluster membership as the outcome, which only included baseline MDS-UPDRS assessments. Values on the x-axis should be interpreted as a representation of the overall variance explained by predictors in reference to one another.*

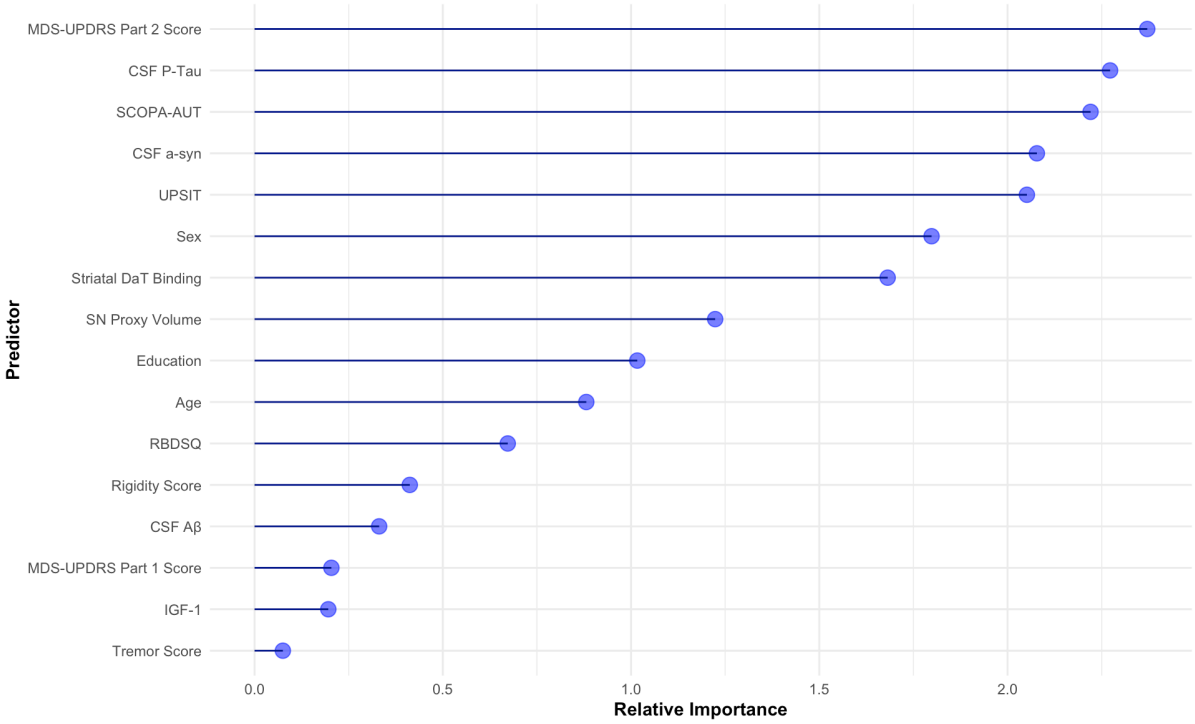


Figure S7: *Relative importance of predictors in the multi-modal logistic regression model exploring cluster membership as the outcome, including baseline MDS-UPDRS scores and additional predictors, including various biomarkers, neuroimaging modalities and clinical assessments of prodromal symptoms. Values on the x-axis should be interpreted as a representation of the overall variance explained by predictors in reference to one another.*

Contextual Statement for Chapters 3 and 4

Chapter 2 identified pathophysiological markers and prodromal assessments as important predictors of more profound impairment at five-year follow-up in early PD, far outweighing the utility of current clinical rating scales. This highlights that despite major strides in diagnostic reform and characterisation of differential progression in PD via subtyping, both are still heavily reliant on cardinal motor features, which demonstrate limited value at time of diagnosis for forecasting impairment or divergence in disease course. However, like pathophysiological and prodromal changes, several other features relevant to PD predate clinical presentation of motor symptoms. In fact, several risk factors of PD have been identified and despite extensive literature establishing their link with consequent development, a relative scarcity of literature pertaining to risk factors influencing disease course exists. Therefore, Chapter 3 will briefly outline a selection of risk factors and their association with PD development and pathophysiology, as well as Chapter 4 assessing whether traumatic brain injury in particular influence on disease course.

Chapter 3 - Risk Factors of Parkinson's Disease: Importance of Considering At-Risk Populations for Earlier Diagnosis and Therapeutic Intervention

While approximately 5-10% of PD cases are linked to genetic factors, the majority are idiopathic, resulting from demographic factors, such as age and sex, and exposure to various environmental stressors, including traumatic brain injury (TBI) and pesticide exposure (Pang et al., 2019). To this end, assessing PD-related pathophysiology in at-risk populations may provide valuable insight into who is most likely to develop PD prior to clinical onset.

3.1 Demographic Factors

3.1.1 Age

Ageing is commonly recognised as the greatest risk factor of PD, with increasing age corresponding to greater risk of developing disease (Tanner and Goldman, 1996). This has been well-established for several years, with a 1990 census study on ~7000 participants in Rotterdam finding that the prevalence of PD increased with age, with diagnoses being 0.3% in the 55 to 65-year cohort and substantially increasing to 4.3% for those aged 85 to 94 (de Rijk et al., 1995). This was corroborated by a meta-analysis of 47 studies, reporting a profound positive association between increasing age and PD prevalence (Pringsheim et al., 2014).

The link between ageing and increased risk of PD development may be explained, at least in part, by age-related vulnerability of the dopamine system. Overall, ageing corresponds to declined function of the dopamine system, resulting from changes to multiple regions. Dopaminergic neuronal populations demonstrate progressive age-related degeneration, with hematoxylin and eosin staining of the caudal substantia nigra (SN) *pars compacta* across 36 healthy controls demonstrating a 4.7% decrease per decade (Fearnley and Lees, 1991).

However, a study applying the dissector method on post-mortem 26 brain samples, ranging from 17 to 90 years of age, suggests this rate is potentially higher, revealing a significant decrease of pigmented neurons up to 9.8% per decade (Ma et al., 1999).

Similar trends are observable in dopamine transporter (DaT) protein. In a positron emission tomography (PET) study assessing 23 healthy volunteers (age range 20 – 74), (11^{C})d-threo-methylphenidate binding, a tracer used to assess DaT, in subjects >40 years of age was significantly lower compared to subjects <40, with a 6.6% transporter reduction per decade of life (Volkow et al., 1996). Further, significant negative correlations between age and transporter availability were present in both the caudate nucleus ($r = -0.74$) and putamen ($r = -0.72$). Of note, age-related decline in DaT availability is associated with impairment in both motor (Volkow et al., 1998) and cognitive function (Erixon-Lindroth et al., 2005, Mozley et al., 2001) in healthy individuals, clinical features substantially affected in PD. Therefore, age-related changes in the dopaminergic system may set the stage for the emergence of PD, particularly if exacerbated by underlying vulnerabilities resulting from other risk factors.

3.1.2 Biological Sex

Several sex-related differences are present in PD, with men two times more likely to develop the disorder (Cerri et al., 2019). This was highlighted in a meta-analysis of 27 studies, whereby the overall incidence of PD in males over 40 was 61.21 per 100,000 person-years, compared to only 37.55 per 100,000 person-years for females (Hirsch et al., 2016). Further, men demonstrated a greater increase in incidence with age compared to females, demonstrating significantly higher rates between the ages of 60 and 69 (58.22 per 100,000 person-years, $p = 0.0012$) compared to 30.32 per 100,000 person-years for females, as well as between the ages

of 70 and 79 (162.58 per 100,000 person-years, p = 0.023) compared to 93.32 per 100,000 person-years for females (Hirsch et al., 2016).

This may be due, at least in part, to different vulnerability of the dopaminergic system in males and females. Even under normal physiological conditions, females demonstrate several differences in their dopaminergic systems compared to males. For example, a rat study exploring dopamine release and uptake via cyclic voltammetry found that electrical stimulation of the medial forebrain (60 Hz) provoked significantly higher extracellular dopamine within the caudate nucleus of female rates compared to males (Walker et al., 2000). Further, kinetic analyses confirmed that dopamine uptake was also more profound in females, although no differences in DaT efficacy were reported (Walker et al., 2000). Taken together, such differences suggest that females are better able to sustain dopaminergic function over time, with more efficient uptake as well as enhanced sensitivity and responsiveness to dopamine corresponding to greater functional capacity of the dopaminergic system.

Additionally, dopaminergic neurons in females demonstrated higher resistance to neurodegeneration relative to males. Bispo and colleagues reported that in male and female Wistar rates with gonads intact, as well as ovariectomised female rates, receiving 0.1 mg/kg of reserpine daily for 15 days found that female rats overall were more resistant to repeated injections, as well as showing reduced immunoreactivity in the substantia nigra *pars compacta* (SNc) (Bispo et al., 2019). Likewise, in a mouse model of PD, sex differences were present in adult (three-months) wild type and Ras homolog enriched in striatum (RHES) knockout mice in response to 4×20 mg/kg intraperitoneal injections of 3,4-methylenedioxymethamphetamine (Costa et al., 2019). Overall, astrocyte activation was present in the striatum and SNc of male rats, although not in the SNc of female rats (Costa et al., 2019). Beyond this, differences in

oestrogen levels in males and females may also play a role. Oestrogen has been shown to be neuroprotective to the dopaminergic system, with the presence of oestrogen reducing methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity of dopaminergic neurons in several pre-clinical mouse studies (Dluzen et al., 1996, Callier et al., 2000, Grandbois et al., 2000). Such findings have also been reported clinically within a PD cohort, with a low-oestrogen exposure group demonstrating significantly reduced striatal dopamine DaT binding in the posterior and ventral putamen compared to the high-oestrogen exposure group (Lee et al., 2019b). Further, the low-exposure group demonstrated significantly worse motor symptoms and higher levodopa dose equivalent changes (Lee et al., 2019b), suggesting that sex-related differences are a critical predictor of PD progression. Beyond dopamine, males also demonstrate higher expression of alpha-synuclein (α-syn) and PINK1 genes, heightened microglial sensitivity and increased susceptibility to iron accumulation, all important pathophysiological features of PD as outlined by Cerri and colleagues (Cerri et al., 2019).

Further, it is important to consider sex in the context of other risk factors due to phenotype distinctions between males and females. While biological differences between sexes are clear, men also demonstrate an increased likelihood of being exposed to environmental risk factors associated with increased risk of PD development. For example, in a meta-analysis of 15 studies exploring the incidence of TBI in a general adult population, males were 2.22 times more likely to sustain a TBI than women (Frost et al., 2013). Similarly, in a cross-sectional study exploring pesticide exposure in farmers in Thailand reported a male-to-female ratio of 2:1, with men also having a larger average duration of pesticide exposure (14.1 ± 10.8 years) compared to women (9.1 ± 8.3 years) (Bunsri et al., 2023). A similar study conducted in China found that not only were men more likely to use pesticides, but they were also less likely to apply proper protective measures against pesticide exposure (Wang et al., 2017). Taken

together, it is evident that the reasons for increase susceptibility to PD in males are multi-faceted, with a range of different biological and behavioural distinctions implicated (for review, see the seminal 2019 review by Cerri and colleagues (Cerri et al., 2019)).

3.2 Environmental Risk Factors

Environmental risk factors of PD encompass several external exposures that may contribute to disease development. Such factors include history of TBI (Delic et al., 2020) and exposure to toxic chemicals, such as pesticides (Islam et al., 2021) and heavy metals (Pyatha et al., 2022), as well as lifestyle factors, including diet (Rees et al., 2024), stress (van der Heide et al., 2021) and physical activity (LaHue et al., 2016), smoking (Rose et al., 2024) and medical risk factors such as type two diabetes (Bantounou et al., 2024). Improved characterisation of such risk factors will play a critical role in identifying who is most at risk of developing PD. This section will primarily focus on the established link between TBI and pesticide exposure with consequent PD development.

3.2.1 Traumatic Brain Injury

A history of TBI corresponds to an elevated risk of developing PD (Crane et al., 2016), corroborated by a meta-analysis of 22 studies reporting a pooled odds ratio of 1.57 (Jafari et al., 2013a). Of note, across three large prospective studies, it was found that a history of TBI with loss of consciousness greater than one hour was associated with significantly elevated risk for both Lewy body pathology and consequent PD (Crane et al., 2016). Similarly, Gardner et al. reported that a single case of mild TBI (mTBI) is associated with a 56% higher risk of developing PD, increasing to 83% for moderate to severe injuries, demonstrating that injury severity may increase risk in a dose-dependent manner (Gardner et al., 2018).

Furthermore, several studies report pathophysiological changes akin to PD following TBI, including disruption of the dopaminergic system and abnormal alpha synuclein deposits. Compared to controls, a history of TBI (6 – 366 months post-injury) was associated with reduced DaT binding in the caudate (12.2%) and putamen (9.0%) (Jenkins et al., 2020). Similarly, α-syn levels have been shown to be several times higher in the cerebrospinal fluid of both adult (Mondello et al., 2013) and paediatric (Su et al., 2010) patients following severe TBI, with α-syn accumulation having been found in swollen axons post-injury in post-mortem investigations of cases of severe TBI, ranging from four hours to five weeks post-injury (Uryu et al., 2007). In contrast however, Peltz and colleagues reported that in a cohort of older veterans, history of TBI (average time since most recent injury = 37 years) did not result in altered levels of exosomal α-syn relative to controls (Peltz et al., 2020), highlighting that literature is mixed as well as poorly explored long-term post-injury.

Several pre-clinical models have also reported such pathophysiological changes, with Acosta and colleagues reporting increased ipsilateral SN *pars compacta* α-syn accumulation, accompanied by dopaminergic neuron loss and increased inflammation, eight-weeks after TBI inducement via a controlled cortical impact model (Acosta et al., 2015). Additionally, in a separate study by the same group increased α-syn oligomerisation and disruption of tyrosine hydroxylase, an enzyme critical for dopamine synthesis, has been reported within both the SN and striatum at two and seven days following mild blast injury (Acosta et al., 2019). Other groups have also observed similar findings, with reduced DaT levels, accompanied by concomitant increases in neuroinflammation, reported in the SN at 30-days post-injury following a CCI-induced moderate TBI in mice (Impellizzeri et al., 2016) and a 15% ipsilateral neuronal loss within this region reported in a lateral fluid percussion model of moderate TBI in adult rats (Hutson et al., 2011). Such findings suggest that TBI is associated with key

pathophysiological characteristics of PD and might explain why having such a history is a critical predictor of future disease development. However, it is currently largely unknown who following TBI is most susceptible to consequent development, as well as scarcity of literature determining whether a history of TBI influences disease course throughout PD, warranting further research.

3.2.2 Pesticide Exposure

Pesticide exposure increases risk of future PD development by inducing several pathophysiological features of PD, such as mitochondrial dysfunction, α-syn aggregation and oxidative stress (Afsheen et al., 2024). In line with this, participants in the Cancer Prevention Study II Nutrition Cohort were prospectively assessed to determine whether pesticide exposure increased risk of PD, finding that of the 143,325 participants, 7,864 (5.7%) reported pesticide exposure in their lifetime (Ascherio et al., 2006). Notably, participants exposed to pesticides demonstrated a 70% increased incidence of PD development compared to participants with no exposure (Ascherio et al., 2006). It is also important to note that exposure does not need to be direct, highlighted by Gorell and colleagues in a population-based case control study of PD participants (n = 144), finding that associations between PD and farming lifestyle maintained even when adjusting for occupational herbicide exposure (Gorell et al., 1998).

The association of occupational exposure to herbicides or insecticides with PD remained after adjustment for farming. The association of farming with PD was maintained after adjustment for occupational herbicide exposure and was of borderline significance after adjustment for occupational insecticide exposure. These results suggest that PD is associated with occupational exposure to herbicides and insecticides and to farming and that the risk of farming cannot be accounted for by pesticide exposure alone

Pesticide exposure, however, is a broad term, with many factors relevant to exposure that moderate consequent risk of PD development. Several case-control studies demonstrate that duration of exposure moderates the association between pesticide exposure and PD risk, with increasing exposure duration corresponding to enhanced risk (Chan et al., 1998, Gorell et al., 1998, Liou et al., 1997). Similarly, a dose-dependent response has also been observed. In line with this, in a case-control study of 368 PD participants exposed to paraquat or maneb, risk was substantially higher in individuals exposed to both chemicals (odds ratio = 4.17), compared to exposure to only one (odds ratio = 2.27) (Costello et al., 2009).

Further, some specific pesticides have demonstrated higher associations with PD development than others (Paul et al., 2023, Anirudhan et al., 2023). In particular, a study by Tanner and colleagues found that in 110 individuals with PD within the Agricultural Healthy Study, rotenone and paraquat were strongly associated with PD, as well as mitochondrial dysfunction and oxidative stress. Likewise, a common technique to induce PD within pre-clinical rodent models is utilising a combination of paraquat and maneb. In fact, synergism between the two pesticides results in selective nigrostriatal dopaminergic cell death and overall reductions in striatal dopamine in young mice models (Thiruchelvam et al., 2000b, Thiruchelvam et al., 2000a), which has also shown to be exacerbated by age (Thiruchelvam et al., 2003), at doses that if administered individually yield minimal effects. Table 3-1 briefly summarises pesticides commonly utilised in pre-clinical models of PD, and provides evidence supporting their association with the development of relevant PD-related pathophysiological changes:

Table 3-1 Pesticides commonly researched within PD literature, as well as evidence of associated risk and pathophysiological changes resulting from exposure.

Pesticide	Class	Evidence
Paraquat	Herbicide	Repeated intraperitoneal paraquat injections in mice demonstrated age- and dose-dependent death of dopaminergic neurons within the SN (McCormack et al., 2002) Dose-dependent acceleration of alpha synuclein fibril formation *in vitro* (Manning-Bog et al., 2002) 8-week-old mice with weekly intraperitoneal paraquat injections for 3-weeks demonstrate up-regulated α-syn deposits and aggregate formation in the SNc compared to saline-injected controls (Manning-Bog et al., 2002) 2–3 month-old mice receiving intraperitoneal paraquat injections, twice-weekly for six weeks, demonstrated up-regulation of α-syn and phosphorylated-tau in the striatum (Wills et al., 2012)
Rotenone	Insecticide	Male Lewis rats, aged 7-9 months, receiving once daily intraperitoneal injections of rotenone for five consecutive days display the following at 9-months post-treatment (Van Laar et al., 2023): • Significantly higher accumulation of endogenous α-syn compared to vehicle cohort • Significant increase in pSer129 α-syn in SN dopaminergic neurons

		Male Lewis and Sprague-Dawley rats, aged 8 weeks, were administered rotenone via osmotic mini pumps for 28 days, reporting selective death of dopaminergic neurons, as well as motor features of PD, namely rigidity (Betarbet et al., 2000).
Maneb	Fungicide	Male Swiss albino mice administered intraperitoneal injections twice weekly for 9 weeks displayed impaired dopaminergic neuron viability associated with mitochondrial dysfunction and oxidative stress (Liu et al., 2022a) Male Sprague-Dawley rats given intraventricular injections of maneb daily for 14 days displayed substantial striatal dopamine efflux via mitochondrial dysfunction, suggesting abnormal neurotransmission post-injury (Zhang et al., 2003) *In vitro* human neuroblastoma model demonstrated that maneb induces α-syn overexpression and increased production of reactive oxygen species in control neurons (compared to human wild type α-syn expressing neurons) exposed to maneb (Conde et al., 2023)

3.3 Genetic Risk Factors

While PD is generally not considered a genetic disorder, with only 10-15% of cases resulting from genetic causes, many genetic risk factors have been identified, contributing to higher susceptibility to PD pathophysiology, as well as increased likelihood of disease development (Billingsley et al., 2018). This has been determined via conducting several genome-wide associated studies (Kia et al., 2021, Pitz et al., 2024) and meta-analyses to corroborate such findings (Nalls et al., 2019, Kim et al., 2024). Of the several identified genes, there are

particular genes of research prominence, highlighted in Table 3-2 as well as evidence supporting their association with PD pathophysiology and development.

Table 3-2 Most common genetic risk factors of* PD and *supporting evidence highlighting their association with the pathophysiological hallmarks of PD and increased risk of disease development.

Gene	Encoded Protein	Evidence
LRRK2	Leucine-rich repeat kinase 2	Most frequent cause of autosomal dominant PD, with the G2019S mutation, the most common LRRK2 mutation, accounting for 1–6% of sporadic and 3–19% of familial cases (Artzi et al., 2017). Risk of PD development dependant on mutation: • G2019S: 28% 59 years of age, 51% at 69, and 74% at 79 years (Healy et al., 2008) • G2385R: 10% at 80 years of age (Trinh et al., 2014) • R1441G: 80% at 80 years of age (Ruiz-Martínez et al., 2010) LRRK2 PD has a distinct clinical presentations relative to idiopathic PD, such as higher likelihood of gait difficulties and postural instability (Alcalay et al., 2015), lower age of onset (Healy et al., 2008) and an absence of increased male prevalence (Gan-Or et al., 2015).
PRKN	Parkin	Mutations in the PRKN gene are the most common cause of autosomal recessive PD (Kitada et al., 1998). Denoted by an early age of onset (≤50 years), with one study of 73 families with at least one member diagnosed with early onset PD finding that 49% presented with PRKN mutations (Lücking et al., 2000).

		Compared to idiopathic PD, clinical presentation of PRKN-PD is a pure motor disorder, with substantial response to dopaminergic therapy, slower progression and minimal signs of cognitive decline (Alcalay et al., 2014, Kasten et al., 2018, Khan et al., 2003). Lack of cognitive challenges throughout PRKN-PD may result from pathological sparing of nucleus basalis of Meynert and cerebral cortex (Doherty et al., 2013)
SNCA	α-syn	Missense mutations implicated in both idiopathic (Hoffman-Zacharska et al., 2013) and familial (Polymeropoulos et al., 1997, Krüger et al., 1998) forms of PD Several mutations have been identified, with a meta-analysis of 36 studies, including 24,075 PD cases and 22,877 controls, reporting that of 16 SNCA variants explored, seven significantly increased the risk of PD development (ORs of 1.22 – 1.38). Further, exploratory analyses determined the important of considering ethnicity when determining risk, such as rs2736990 and rs11931074 corresponding to heightened risk (ORs of 1.22 and 1.34, respectively) in the East Asian group (Zhang et al., 2018). Different SNCA mutations exhibit differential effects on α-syn pathology, with a study exploring mutations H50Q and G51D identifying that while H50Q accelerated rate of α-syn aggregation, G51D demonstrated opposite effects, despite corresponding to earlier disease onset (Rutherford et al., 2014).
GBA	Glucocerebrosidase	Approximately 10-15% of European PD cases are carriers of a GBA variant (Skrahina et al., 2021).

An *in vitro* model utilising stable human dopaminergic cell lines found that cell lines expressing GBA mutations demonstrated lysosomal dysfunction and impaired lipid metabolism, offering a mechanistic link between GBA mutations and abnormal accumulation of toxic α-syn species in PD (Navarro-Romero et al., 2022). Similarly, single-cell transcriptional analysis and proteomics of GBA+ PD brains reported reduced chaperon-mediated-autophagy, corresponding to α-syn accumulation (Kuo et al., 2022).

While clinical presentation is similar to idiopathic PD, GBA-PD demonstrates a more rapid rate of progression (Brockmann et al., 2015), as well as more severe non-motor symptoms such as anosmia, sleep disturbances and cognitive impairments (Brockmann et al., 2011).

A summary of risk factors outlined within this chapter can be found in Figure 3-1:

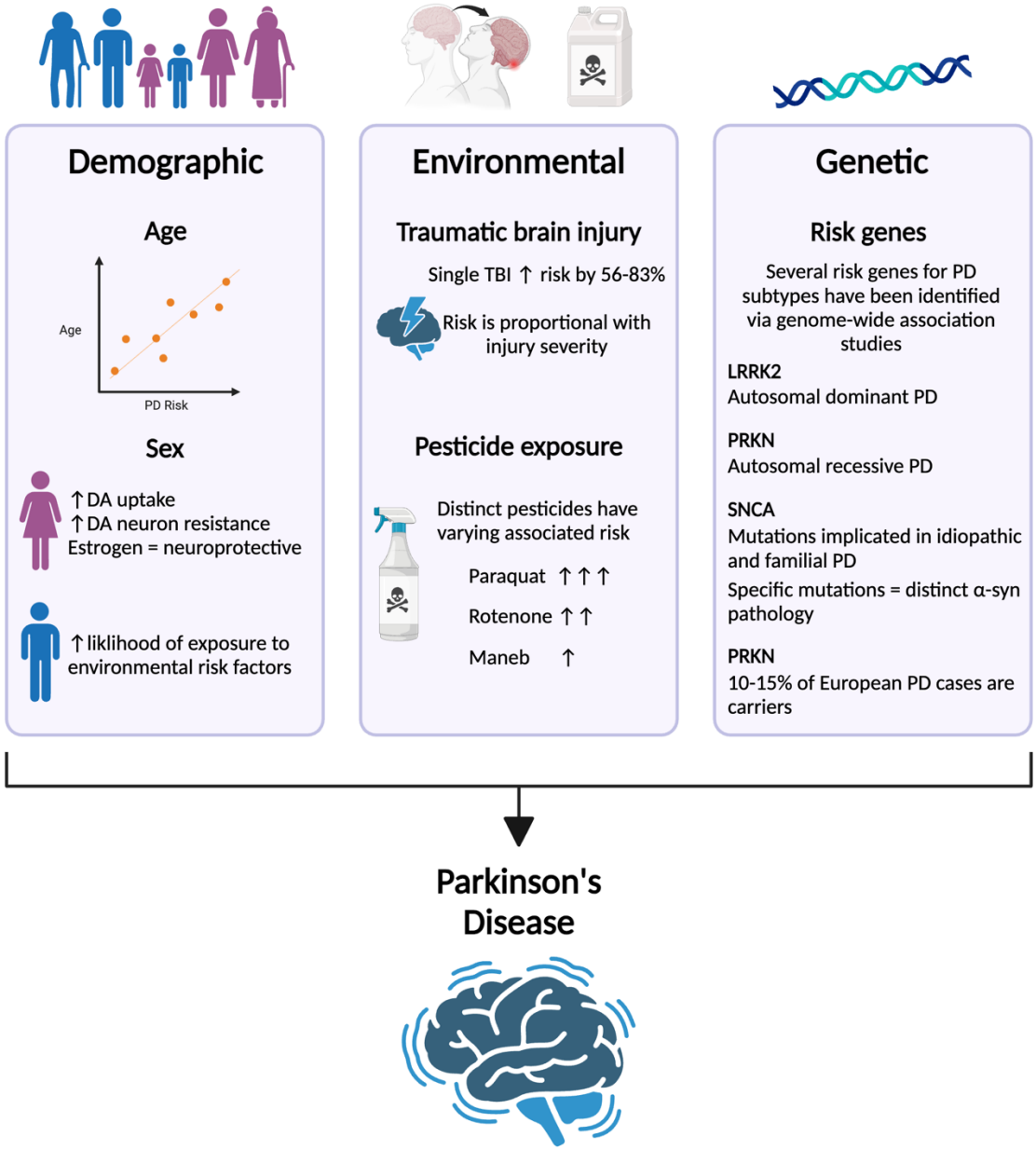


Figure 3-1 Summary of demographic, environmental (TBI and pesticide exposure) and genetic risk factors and their association with PD development

Taken together, it is evident that PD is a multifaceted disorder, influenced by various non-modifiable and environmental risk factors described above. Understanding how PD pathophysiology presents within at-risk populations will not only aid in identifying individuals at higher risk of PD development but will also allow for advancements in targeted prevention and intervention strategies.

Chapter 4: Statement of Authorship

Title of Paper	History of Traumatic Brain Injury Does Not Influence Rate of Progression of Clinical or Pathological Outcomes in Early Parkinson's Disease
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ■ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	Angus McNamara		
Contribution to the Paper	Conceptualised, performed and interpreted statistical analyses and wrote the initial manuscript		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/12/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Irina Baetu		
Contribution to the Paper	Contributed to the organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Name of Co-Author	Lyndsey Collins-Praino		
Contribution to the Paper	Contributed to the conceptualisation of the manuscript, organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Chapter 4 - History of Traumatic Brain Injury Does Not Influence Rate of Progression of Clinical or Pathological Outcomes in Early Parkinson's Disease

Angus McNamara[1], Irina Baetu[2] & Lyndsey Collins-Praino[1]

[1] School of Biomedicine, University of Adelaide, SA 5000, Australia

[2] School of Psychology, University of Adelaide, SA 5000, Australia

4.1 ABSTRACT

Background: A history of traumatic brain injury (TBI) is associated with increased risk of developing neurodegenerative disorders, including Parkinson's Disease (PD). However, TBI's influences disease progression remains underassessed. This study explored whether history of TBI influences progression of pathological and clinical outcomes up to five-years of follow up in individuals with early PD.

Methods: Longitudinal data were extracted from the Parkinson's Progression Markers Initiative (PPMI) and the PostCEPT observational study. Participants in PostCEPT had complete head injury data, while PPMI participants were eligible if they completed the head injury section of the PD Risk Factor Questionnaire (n = 208). Principal component analysis was used to derive composite scores of cognitive ability and mood dysfunction, with motor outcomes calculated using the Movement Disorders Society Unified Parkinson's Disease Rating Scale. Progression of clinical and pathological outcomes up to five years and four years following study entry were compared, including subset analyses in PPMI examining injury severity.

Results: Individuals with a history of TBI in the PPMI dataset exhibited a younger age of onset, however, a history of TBI did not affect progression rates of any assessed variables across both cohorts. Exploratory analysis determined that injury severity significantly predicted mood dysfunction and striatal dopamine transporter binding but accounted for only a small portion of outcome variance.

Conclusion: While a prior history of TBI was associated with earlier PD onset, it did not correspond to differential disease course within established disease. However, given

differences in TBI characterisation between cohorts, additional research must be conducted to validate these findings.

4.2 BACKGROUND

A history of sustaining a traumatic brain injury (TBI) corresponds to an elevated risk of developing several different neurodegenerative disorders, including Parkinson's Disease (PD) (Crane et al., 2016). Extensive prior research has established the link between TBI and later PD development, with a meta-analysis of 22 studies reporting a pooled odds ratio of 1.57 (Jafari et al., 2013a). Of note, across three large prospective studies, it was found that a history of TBI with loss of consciousness (LoC) greater than one hour was associated with significantly elevated risk for both Lewy body pathology and incident PD (Crane et al., 2016). Similarly, Gardner and colleagues reported that even a single case of mild TBI (mTBI) is associated with a 56% higher risk of developing PD (Gardner et al., 2018). For moderate or severe injuries, this risk increases to 83%, demonstrating that injury severity may increase risk in a dose-dependent manner (Gardner et al., 2018). Further, age at time of injury may also be a contributing factor, with one study reporting a 44% increased risk of developing PD over a five–seven-year period in people sustaining a TBI after the age of 55, a finding thought unlikely to be due to reverse causation (Gardner et al., 2015).

In addition, several studies report pathophysiological changes akin to PD following TBI, including both abnormal alpha synuclein (α-syn) deposits and disruption of the dopaminergic system (for a review, see (Padmakumar et al., 2022)). α-syn levels are several times higher in the cerebrospinal fluid of both adult (Mondello et al., 2013) and paediatric (Su et al., 2010) patients following severe TBI, and α-syn accumulation has been found to be present in swollen axons post-injury in post-mortem investigations of cases of severe TBI (Uryu et al., 2007).

Similarly, compared to controls, a history of TBI (ranging from 6 – 366 months post-injury) is associated with reduced dopamine transporter (DaT) binding in both the caudate (12.2%) and putamen (9.0%), two prominent regions affected in PD (Jenkins et al., 2020). These findings have also been corroborated by investigations in pre-clinical TBI models. Acosta and colleagues reported increased ipsilateral substantia nigra (SN) *pars compacta* α-syn accumulation, accompanied by dopaminergic neuron loss and increased inflammation, in the controlled cortical impact (CCI) model (Acosta et al., 2015), as well as increased α-syn oligomerisation and disruption of tyrosine hydroxylase, a key enzyme for dopamine synthesis, within both the SN and striatum following mild blast injury (Acosta et al., 2019). In line with this, reduced DaT levels, with concomitant increases in neuroinflammation, have been reported in the SN at 30-days following a CCI-induced moderate TBI in mice (Impellizzeri et al., 2016), with Hutson and colleagues (2011) reporting a 15% ipsilateral neuronal loss within this region using a lateral fluid percussion model of moderate TBI in adult rats (Hutson et al., 2011). Such findings suggest that TBI is associated with key pathophysiological characteristics of PD and might explain why having such a history is a critical predictor of future disease development.

Despite the well-established link between TBI and future PD development, however, the degree to which TBI influences disease progression in people with PD (PwP) has been understudied. A longitudinal study assessing PwP with and without a history (n = 25 per group) of TBI determined that individuals with a history of mild-moderate TBI demonstrated more profound cognitive decline compared to PD without TBI over a two-year follow-up (Schiehser et al., 2016). No differences were found between groups when assessing motor and mood profiles (Schiehser et al., 2016). However, this study has a noticeably small sample size, as well only assessing a minimal follow-up period and therefore may not effectively capture heterogeneity in disease course over-time (Schiehser et al., 2016). This is a significant gap, as a longitudinal

study across an 11-year follow-up period in Alzheimer's disease, another neurodegenerative disease with a past history of TBI as a recognised risk factor (Mielke et al., 2022), suggested that a history of TBI may be a vital predictor of functional progression in the disease (Gilbert et al., 2014). Therefore, a more expansive longitudinal study assessing history of TBI in PwP, including larger sample sizes and longer follow-up is critically needed. To address this, the current study utilised data extracted from two different cohorts, the Parkinson's Progression Markers Initiative (PPMI) (Marek et al., 2011) and the Parkinson Research Examination of CEP-1247 trial (PRECEPT) follow-up PostCEPT observational study (PostCEPT) (Ravina et al., 2009), to determine if a prior history of TBI was associated with differences in either pathological markers or clinical presentation in PwP at baseline, and whether such a history affected disease progression up to a 5-year follow-up period. Further, we conducted exploratory analyses within the PPMI cohort to assess whether injury severity influenced these outcomes.

4.3 METHOD

This analysis used data from two distinct cohorts: PPMI and PostCEPT, with the PostCEPT cohort acting as the validation cohort.

4.3.1 PPMI Cohort

The cohort of interest for this analysis were recently diagnosed (diagnosis < two years before baseline visit) PwP (n = 422), with details on selection criteria detailed in Table S1. From this pool of participants, only participants from the University of California San Francisco (UCSF) site who had completed the Parkinson's Disease Risk Factor Questionnaire (PDRFQ), which provided information on past history of head injury, were eligible for this study (n = 208). These participants were part of the Follow-Up of Persons with Neurologic Disease' (FOUND) project (Tanner, 2006).

The following data were extracted from the PPMI dataset:

Table 4-1 Data extracted from the PPMI database and their corresponding assessments

Category	Measures
Baseline Demographics	Age at time of study entry Sex Education (years)
Neuroimaging	**Dopamine Transporter – Single Photon Emission Computed Tomography (DaT-SPECT)** Participants were injected with radionuclide ligand DaTScan. Scans were recorded at baseline and annually up to 4-years follow-up, excluding at 3-year follow-up. Specific binding ratio of mean striatum (average of left and right caudate + putamen) was the measure of interest for this analysis.
Biofluid Pathological Markers	**Cerebrospinal fluid (CSF) biomarkers (pg/mL)** Obtained via standard lumbar puncture at baseline and annually up to 5-years follow-up. Measured using standard ELISA protocols: - Unphosphorylated alpha synuclein (α-syn) Measured using Elecsys electrochemiluminescence immunoassays: - Total Tau (T-Tau) - Phosphorylated tau (phosphorylation site: threonine 181) (P-Tau) - β-amyloid$^{1–42}$ (Aβ)
Motor Symptoms	**Movement Disorder Society-Unified Parkinson's Rating Scale (MDS-UPDRS)** A comprehensive questionnaire containing 50 items pertaining to motor and non-motor symptoms of Parkinson's; combination of clinical assessment and self-report (Marsili et al., 2018). This was extracted at baseline and at annual follow-ups up to 5-years while participants were on medication. The following parts were used: <u>Part 3: Motor Examination</u> - Rigidity score (sum of MDS-UPDRS subitems of item 3.3) - Tremor score (sum of MDS-UPDRS subitems of items 3.15, 3.16, 3.17 & 3.18)

	Various aspects of cognition were assessed at baseline and at annual follow-up up to 5 years.
Cognition	**Hopkins Verbal Learning Test (HVLT)** - Verbal recognition and recall (learning and memory) **Sematic Fluency (SFT)** - Executive function and semantic memory **Letter Number Sequencing (LNS)** - Working memory **Benton Judgement of Line Orientation (JLO)** - Visuospatial ability **Symbol Digit Modalities (SDM)** - Psychomotor/Processing Speed
	Various aspects of mood were assessed at baseline and at annual follow-up up to 5 years.
Mood	**State-Trait Anxiety Inventory (STAI)** - Anxiety (the total STAI scores were analysed) **Geriatric Depression Score (GDS)** - Depression
Parkinson's Disease Risk Factor Questionnaire (PDRFQ)	The PDRFQ is used to evaluate risk factors of PD by compiling information on lifetime experiences pertaining to health and lifestyle. Of particular interest for this study was the head injury portion of this survey. Questions that comprise this questionnaire are reported in Table S3

4.3.2 PostCEPT Cohort

The cohort of interest for this analysis included participants enrolled in both the PRECEPT and follow-up PostCEPT study (n = 635), with a detailed outline of inclusion criteria detailed in Table S2 (Ravina et al., 2009). Further, an adapted version of the FOUND protocol utilised

within PPMI was applied to acquire relevant TBI information, making said cohort an appropriate validation cohort for the current work. This cohort was primarily used to validate whether the rate of progression of clinical symptoms differed in those with and without a history of head injury, with extracted data outlined in Table 4-2:

***Table 4-2** Data extracted from the PostCEPT database and their corresponding assessments*

Category	Measures
Baseline Demographics	Age at time of study entry Sex Education (coded as highest level of education ranges 1 = < 9th grade and 7 = Graduate or professional degree)
Motor Symptoms	**MDS-UPDRS** This was extracted at baseline and at annual follow-ups up to 4-years while participants were on medication. The following parts were used: <u>Part 3: Motor Examination</u> - Rigidity score (sum of MDS-UPDRS subitems of item 3.3) - Tremor score (sum of MDS-UPDRS subitems of items 3.15, 3.16 & 3.17)
Cognition	Various aspects of cognition were assessed at year-1 and at annual follow-up up to 4 years. **Hopkins Verbal Learning Test (HVLT)** - Verbal recognition and recall (learning and memory) **Sematic Fluency (SFT)** - Executive function and semantic memory **Letter Number Sequencing (LNS)** - Working memory
Depression	The Geriatric Depression Scale (GDS) was assessed at baseline and at annual follow-up, up to 4-years.
History of TBI	An adapted version of the FOUND protocol was used within the PostCEPT study, whereby participants were asked if they had sustained a TBI resulting in LoC in their lifetime. Information on injury severity or other injury characteristics were not collected.

4.3.3 TBI Classification

Based on PDRFQ responses, PPMI participants were first categorised as either PwP who had not sustained a TBI throughout their lifetime (n = 128; PD-Control) or PD participants who had previously experienced a TBI (n = 80; PD-TBI). For the PostCEPT study, participants who answered yes to sustaining a head injury resulting in LoC were classified as PD-TBI (n = 139), while those who did not report such a history were classified as PD-Control (n = 496).

An exploratory analysis was conducted within the PPMI cohort, where the PD-TBI cohort was then further sub-categorised according to injury severity, a potential moderator of the effect that TBI has on long-term outcome progression (Cappa et al., 2011). Injury severity was based on LoC data available in the PDRFQ, as follows:

1) Mild TBI (n = 31; PD-mTBI) – Reported a single case of TBI and LoC < 60 minutes.
2) Moderate-to-severe TBI (n = 9; PD-M/sTBI) – Reported a single case of TBI and LoC ≥ 60 minutes.
3) Repeated mTBI (n = 40; PD-rmTBI) – Reported > 1 case of mTBI.

Three cases were excluded from this classification, due to participants experiencing more than one case of M/sTBI (n = 2) or providing an inadequate amount of LoC information to properly classify injury severity (n = 1).

4.3.4 Principal Component Analysis

Principal component analysis (PCA) was utilised to develop composite scores of cognitive ability and mood dysfunction in the PPMI cohort, using corresponding assessments detailed in Table 1. PCA was also conducted to develop a cognitive ability score for the PostCEPT cohort, albeit with less component assessments (Table 2). Composite scores were created for baseline cognitive ability and mood dysfunction scores, as well as for changes in cognitive ability and mood dysfunction over time by conducing PCA on the slopes (estimated via linear regression)

of the assessments across all timepoints. Assessments in each category (mood or cognition) were highly correlated, as confirmed by loading values, justifying their conversion to a single factor (Table S4).

4.3.5 Statistical Analyses

All data were analysed using R (version 1.4.1717) (Team, 2010). Given distinctions in the way TBI history was ascertained in the PPMI and PostCEPT cohorts, as well as differences in the way clinical variables were measured, the datasets were not pooled together and instead treated as separate cohorts in subsequent analyses. A Shapiro-Wilk test was conducted and determined that the distribution of several variables deviated significantly from normality, with only age and baseline cognitive ability in the PPMI cohort being normally distributed. Therefore, normally distributed variables are displayed as mean ± standard deviation, whereas non-normally distributed variables are reported as median (interquartile range). In addition, frequency data were summarised as counts (percentage). For group level comparisons, independent T-tests and Mann-Whitney U tests were conducted when comparing history of TBI, however, when exploring injury severity, one-way ANOVA's and Kruskal-Wallis tests were conducted, and if significance was detected, post-hoc Tukey or Dunn tests were conducted for normal and non-normally distributed data respectively, with a Bonferroni multiple comparison correction applied.

When assessing rates of progression over time, given significant deviations from normality when accounting for follow-up timepoints, a non-parametric equivalent of a mixed ANOVA was conducted via the *nparLD* package (Noguchi et al., 2012), with history of TBI or TBI severity as a between-subjects factor and follow-up period as a within-subjects factor. If significant interaction effects were present, slopes were calculated using all timepoints and

compared between groups via either Mann-Whitney U tests, or Dunn tests, if more than two groups were present.

Finally, simple linear regression models were conducted to assess both history of TBI and injury severity as predictors of progression in clinical outcomes including cognitive, mood and motor outcomes. Further, if either a significant interaction effect or a significant main effect of history of TBI or injury severity were present in the mixed ANOVA for a specific pathological biomarker assessed, an additional linear regression model was created to assess predictors of the progression of that marker. For these models, injury severity was re-converted into an ordinal format, ranked from least severe (1 = PD without TBI (PD-Control)) to most severe (4 = PD-M/sTBI). Age and sex were controlled for in the models. All models were displayed via the *sjPlot* package (Lüdecke, 2021). The significance level for all analyses was set at $p < 0.05$.

4.4 RESULTS

4.4.1 History of TBI

Of the 208 participants extracted from the PPMI cohort, approximately 40% (n = 80) reported a TBI in their lifetime. Prevalence of TBI within the PostCEPT cohort was markedly lower, at approximately 22% (n = 139).

4.4.1.1 Baseline Comparisons

To determine whether having a history of TBI corresponded to differences in variables of interest at time of diagnosis, statistical comparisons of demographics, striatal DaT binding, biomarker concentrations and clinical outcomes were conducted on baseline values (Table 4-3). PD-TBI participants were not statistically different to PD-Control in any of our comparisons, except for age in the PPMI cohort (Table 3). Overall, PD-TBI were significantly

(t(158) = 2.04, p = 0.04) younger (59.85 ± 10.31) compared to PD-Control (62.80 ± 9.69). Further, within the PostCEPT cohort, the only significant difference was found in GDS assessment, with PD-TBI displaying significantly higher scores (2 (1 – 4); U = 30264, p = 0.04) compared to PD-Control (2 (0.5 – 3)).

4.4.1.2 Rate of Progression

Overall, no significant interaction effects were present in PwP with (PD-TBI) versus without a history of TBI (PD-Control) in either the PPMI (Figure 4-1) or PostCEPT cohort (Figure 4-2).

4.4.1.3 Linear Regression

Across all models, a history of TBI was not a significant predictor of any neuropsychiatric or motor outcome at follow-up in either the PPMI (Table 4-4A) or PostCEPT cohorts (Table 4-4B). In the PPMI models including a history of TBI as a predictor, sex was a significant predictor of year-5 cognitive ability (β = -0.51, p = 0.007) and education was a significant predictor of year-5 tremor score (β = -0.18, p = 0.034), with no other predictor reaching statistical significance. Similarly, within the PostCEPT cohort, male sex and age were significant predictors of both year-4 cognitive ability (sex: (β = -0.34, p = 0.026; age: (β = -0.04, p < 0.001) and rigidity score (sex: β = 1.32 , p = 0.018; age: β = 0.08 , p = 0.003), with educational level attained also acting as a significant predictor of cognitive ability (β = 0.18 , p < 0.001). It is important to note, however, that the included predictors explained only a small amount of the variance across all models (range = 0.008 – 0.265).

Table 4-3 ***Differences in variables of interest between PD-Controls and PD-TBI were assessed at baseline****. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via T-tests. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Mann-Whitney U tests. Statistically significant differences are denoted by bolded p-values*

	PPMI Cohort					PostCEPT Cohort				
	PD-Control		PD-TBI			PD-Control		PD-TBI		
	n	Mean ± SD / Median (IQR)	n	Mean ± SD / Median (IQR)	p-value	n	Mean ± SD / Median (IQR)	n	Mean ± SD / Median (IQR)	p-value
Age	128	62.80 ± 9.69	80	59.85 ± 10.31	**0.04**	496	63.64 ± 9.63	139	63.26 ± 9.85	0.69
Education *	124	16 (16-18)	75	16 (15-18)	0.39	496	6 (4-7)	139	6 (4-7)	0.57
Sex										
Male		75 (59%)		53 (60%)	0.92		315 (64%)		98 (70%)	0.14
Female		53 (41%)		35 (40%)			179 (36%)		40 (30%)	
Rigidity Score	128	3 (2-6)	80	3 (2-6)	0.19	496	4 (2-7)	139	4 (2-7)	0.70
Tremor Score **	128	4 (2-7)	80	4 (2-6)	0.96	496	3 (1-4)	139	3 (1-5)	0.20
Cognitive Ability †	123	0.08 ± 0.91	75	-0.17 ± 1.13	0.12	371	0.07 (-0.56 - 0.73)	107	0.01 (-0.52 - 0.58)	0.57
Mood Dysfunction ‡	124	-0.29 (-0.73-0.39)	75	-0.13 (-0.54-0.54)	0.10	495	2 (0.5 – 3)	138	2 (1 – 4)	**0.04**
Striatal DaT Binding	122	1.34 (1.13-1.61)	77	1.32 (1.02-1.52)	0.17					

***CSF α*-syn**	86	1356.1 (1034.6-1760.1)	55	1390.7 (1097.7-1711.9)	0.68
CSF P-Tau	110	13.51 (10.93-17.26)	72	13.57 (11.15-15.55)	0.71
CSF Aβ	114	876.96 (684.63-1217.25)	72	863.75 (678.63-1204.23)	0.85

***** Education was graded differently between cohorts: PPMI = Years; PostCEPT = Highest level of education (1 = < 9th grade and 7 = Graduate or professional degree) (Tables 1 & 2)

****** PostCEPT did not have available data on tremor constancy, therefore this was not included in the PostCEPT tremor score (Tables 1 & 2)

† Assessments used to derive cognitive ability composites differed between cohorts (Tables 1 & 2)

‡ PostCEPT cohort did not have available data on mood measures outside of GDS, therefore GDS alone acted as the mood dysfunction measure in that cohort

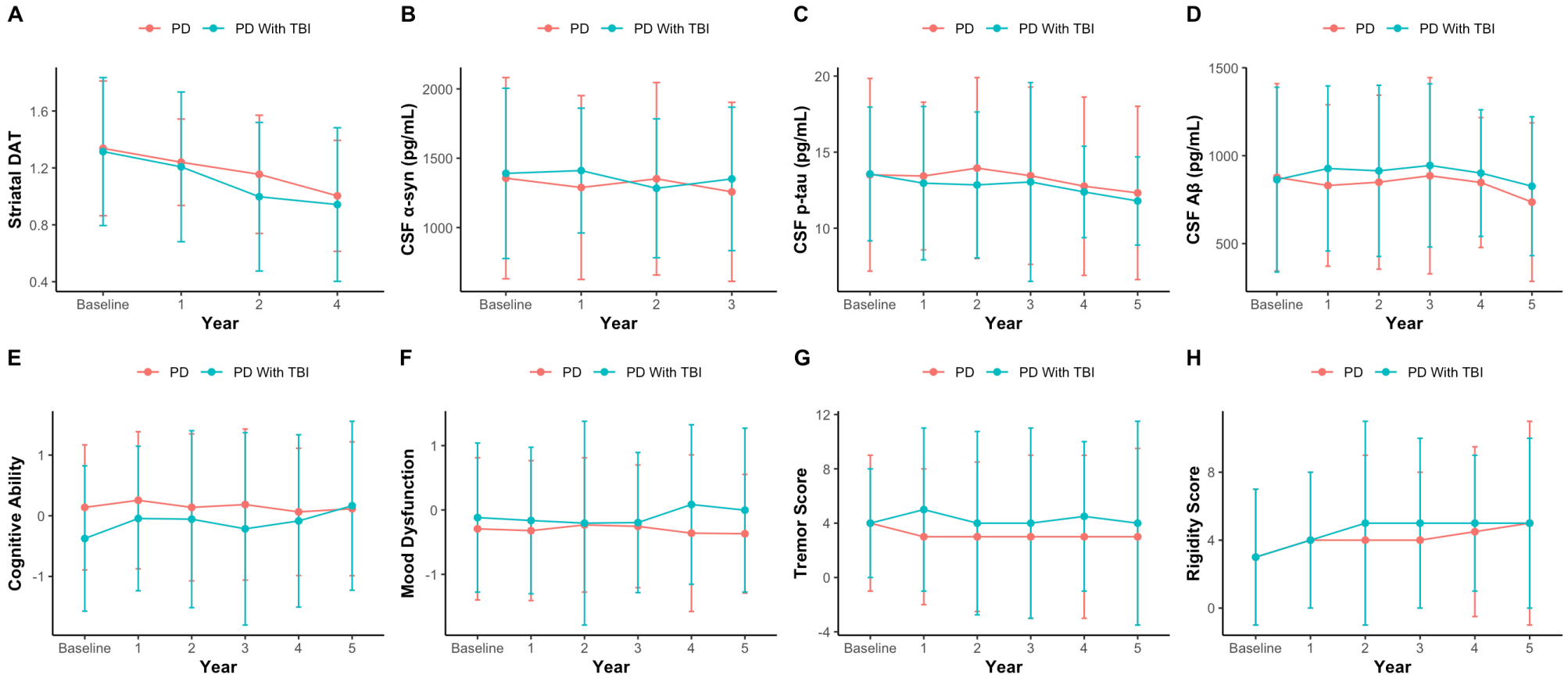


Figure 4-1Rate of progression of clinical and pathophysiological outcomes variables in PD with and without a history of TBI within the PPMI cohort. *Median & IQR values are displayed at each timepoint for extracted variables of interest. No significant interaction effect was present when comparing rate of progression in PD-Controls compared to PD-TBI*

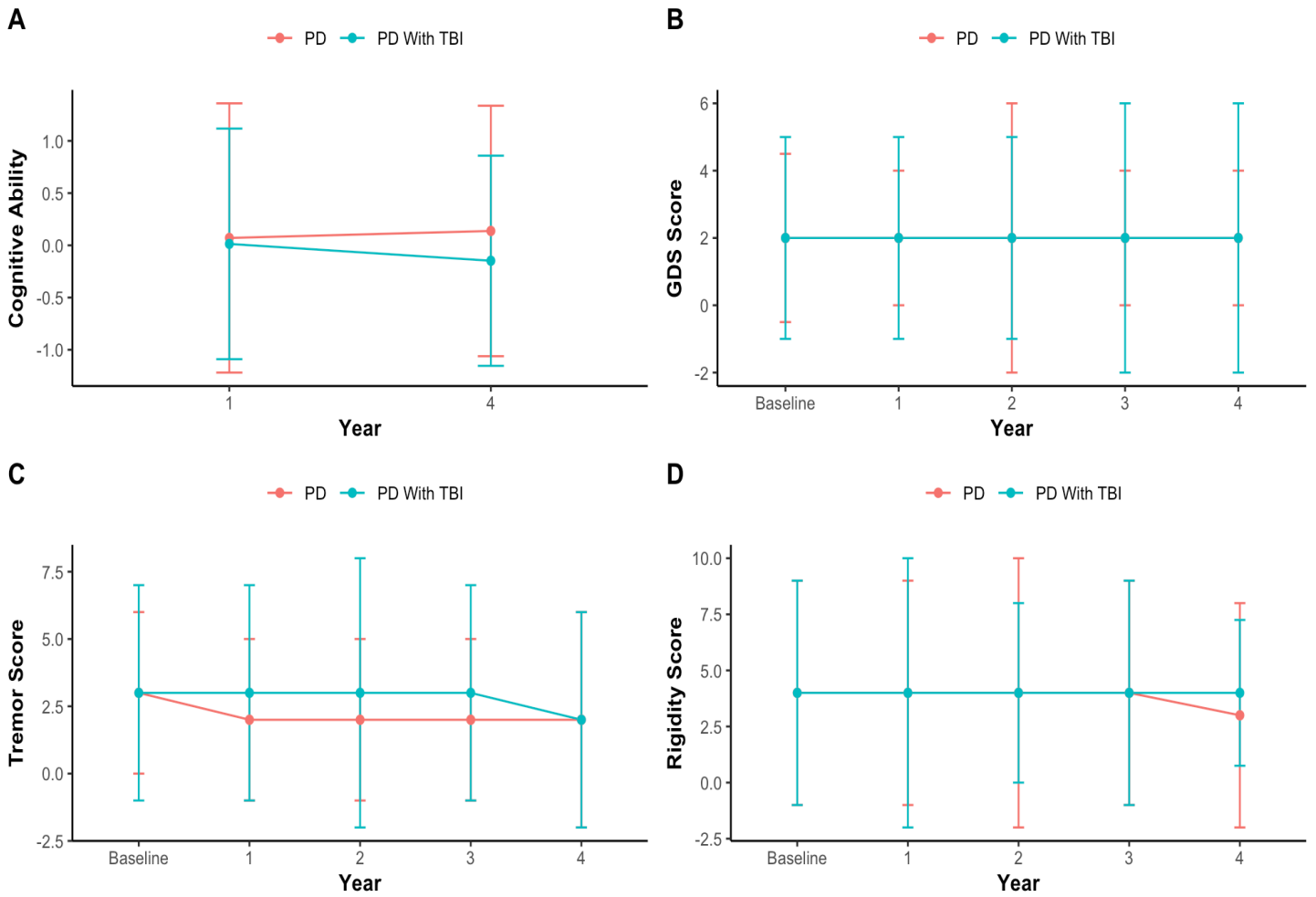


***Figure 4-2* Rate of progression of clinical and pathophysiological outcomes variables in PD with and without a history of TBI within the PostCEPT cohort.** *Median & IQR values are displayed at each timepoint for extracted variables of interest. No significant interaction effect was present when comparing rate of progression in PD-Controls compared to PD-TBI*

***Table 4-4 Multivariate linear regression was conducted to determine whether a history of TBI in the (A) PPMI or (B) PostCEPT cohort was a significant predictor* of follow-up outcomes**. Outcomes included *neuropsychiatric (cognitive ability and mood dysfunction) or motor (rigidity and tremor scores) at follow-up. In addition, striatal DaT binding and CSF α-syn was assessed as an outcome in the PPMI cohort at year-4 and year-3, respectively. All models controlled for age, education years, and sex of the participants. Significant predictors are denoted by bold p-values.*

(A) PPMI Cohort

	Year 5 Cognitive Ability		**Year 5 Mood Dysfunction**		**Year 5 Rigidity Score**		**Year 5 Tremor Score**		**Year 3 CSF α-syn**		**Year 4 Striatal DaT**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Intercept	0.25	0.693	-0.06	0.165	-0.06	0.077	-0.03	**<0.001**	-0.09	**<0.001**	0.15	**<0.001**
Age	-0.08	0.421	-0.09	0.280	0.04	0.609	-0.10	0.218	-0.05	0.662	0.01	0.887
Male Sex	-0.51	**0.007**	-0.08	0.625	0.02	0.911	0.06	0.709	0.19	0.425	-0.13	0.480
Education	0.17	0.070	-0.08	0.353	-0.04	0.612	-0.18	**0.034**	-0.13	0.260	-0.01	0.873
History of TBI	0.10	0.591	0.26	0.124	0.12	0.504	-0.01	0.944	-0.07	0.779	-0.18	0.303
Observations	114		156		143		147		84		143	
R^2 / R^2 adjusted	0.098 / 0.065		0.038 / 0.012		0.008 / -0.021		0.043 / 0.017		0.026 / -0.023		0.013 / -0.016	

(B) PostCEPT Cohort

	Year 4 Cognitive Ability		**Year 4 GDS**		**Year 4 Rigidity Score**		**Year 4 Tremor Score**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Intercept	1.12	0.053	2.75	0.120	-1.01	0.621	1.86	0.337
Age	-0.04	**<0.001**	0.01	0.605	0.08	**0.003**	0.01	0.588
Male Sex	-0.34	**0.026**	0.25	0.600	1.32	**0.018**	0.41	0.436
Education	0.18	**<0.001**	-0.17	0.203	0.07	0.658	0.02	0.873
History of TBI	-0.14	0.419	0.45	0.379	0.43	0.469	0.44	0.435
Observations	154		162		162		162	
R^2 / R^2 adjusted	0.265 / 0.246		0.021 / -0.004		0.110 / 0.087		0.013 / -0.012	

4.4.2 Injury Severity

When accounting for injury severity within the PPMI cohort, PD-TBI subjects were predominantly classified as rmTBI (50%; n = 40), followed by mTBI (37.5%; n = 31) and M/sTBI (12.5%; n = 9).

4.4.2.1 Baseline Comparisons

While it was expected that accounting for injury severity would be associated with more profound differences between groups, there were no statistically significant differences between TBI severity groups, likely due to the small number of participants across groups, particularly for m/STBI (Table 4-5).

4.4.2.2 Rate of Progression

In the TBI severity analyses, there was only a significant interaction between TBI severity and follow-up time for CSF α-syn ($F(2,89) = 3.25$, $p = 0.02$; Figure 4-3B). However, a post-hoc analysis of the slopes found no significant differences between severity groups ($p > 0.05$). Of note, while for striatal DaT binding there was no significant interaction between TBI severity and follow-up period ($F(6,186) = 1.56$, $p = 0.20$) (Figure 4-3A), there was a significant main effect of TBI severity ($F(3, 87) = 3.8$, $p = 0.02$), as well as follow-up period ($F(2, 186) = 28.6$, $p < 0.0001$). Post-hoc Dunn tests revealed a negative relationship between injury severity and striatal DaT binding was present (Figure 4-4A), with PD-M/sTBI (0.90(0.74-1.14) having significantly lower binding compared to PD-Controls (1.21(0.99-1.42); $Z(4, 405) = -3.56$, $p = 0.002$) and PD-mTBI (1.20(0.96-1.54); $Z(4, 123) = -3.29$, $p = 0.005$). Additionally, PD-rmTBI (1.06(0.87-1.33) also demonstrated significantly lower binding compared to PD-Controls ($Z(4, 494) = -3.06$, $p = 0.009$). Further, striatal DaT binding progressively deteriorated at each follow-up timepoint (Figure 4-4B).

Table 4-5 Differences in variables of interest between PD-Controls and different severities of PD-TBI (PD-mTBI, PD-rmTBI, PD-M/sTBI) were assessed at baseline in the PPMI cohort*. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via one-way ANOVA's. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Kruskal-Wallis tests. Statistically significant differences are denoted by bolded p-values*

	PD-Control		PD-mTBI		PD-rmTBI		PD-M/sTBI		
	n	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**p-value**
Age	128	62.8 ± 9.69	31	61.11 ± 10.42	40	59.42 ± 10.03	9	57.38 ± 11.75	0.15
Education	124	16 (16-18)	29	16 (14-17)	37	16 (16-18)	9	18 (15-19)	0.35
Sex									
Male		75 (59%)		20 (65%)		23 (57.5%)		6 (66.6%)	
Female		53 (41%)		11 (35%)		17 (42.5%)		3 (33.3%)	0.9
Striatal DaT Binding	122	1.34 (1.13-1.61)	30	1.34 (1.11-1.56)	39	1.33 (1.11-1.62)	8	1.34 (1.11-1.59)	0.33
***CSF α*-syn**	86	1356.1 (1034.60-1760.08)	24	1362.7 (1040.9-1716.5)	26	1380.2 (1036.7-1780.9)	5	1382.15 (1095.95-1757.03)	0.40
CSF P-Tau	110	13.51 (10.93-17.26)	29	13.52 (11.0-16.60)	35	13.62 (11-17.21)	8	13.43 (10.97-16.58)	0.68
CSF Aβ	114	876.96 (684.63-1217.25)	29	873.6 (661.3-1202)	36	878.35 (702.45-1224.25)	7	879.5 (682.8-1215.5)	0.35
Rigidity Score	128	3 (2-6)	31	4 (3-6)	40	3 (1-6)	9	2 (2-3)	0.24
Tremor Score	128	4 (2-7)	31	4 (2-6)	40	4 (2-6)	9	5 (2-7)	0.83
Cognitive Ability	123	0.08 ± 0.91	29	-0.22 ± 1.29	9	-0.05 ± 0.64	37	-0.15 ± 1.11	0.40

Mood Dysfunction	124	-0.29 (-0.73-0.39)	29	-0.32 (-0.76-0.18)	9	-0.07 (-0.41-0.65)	37	-0.19 (-0.45-0.15)	0.19

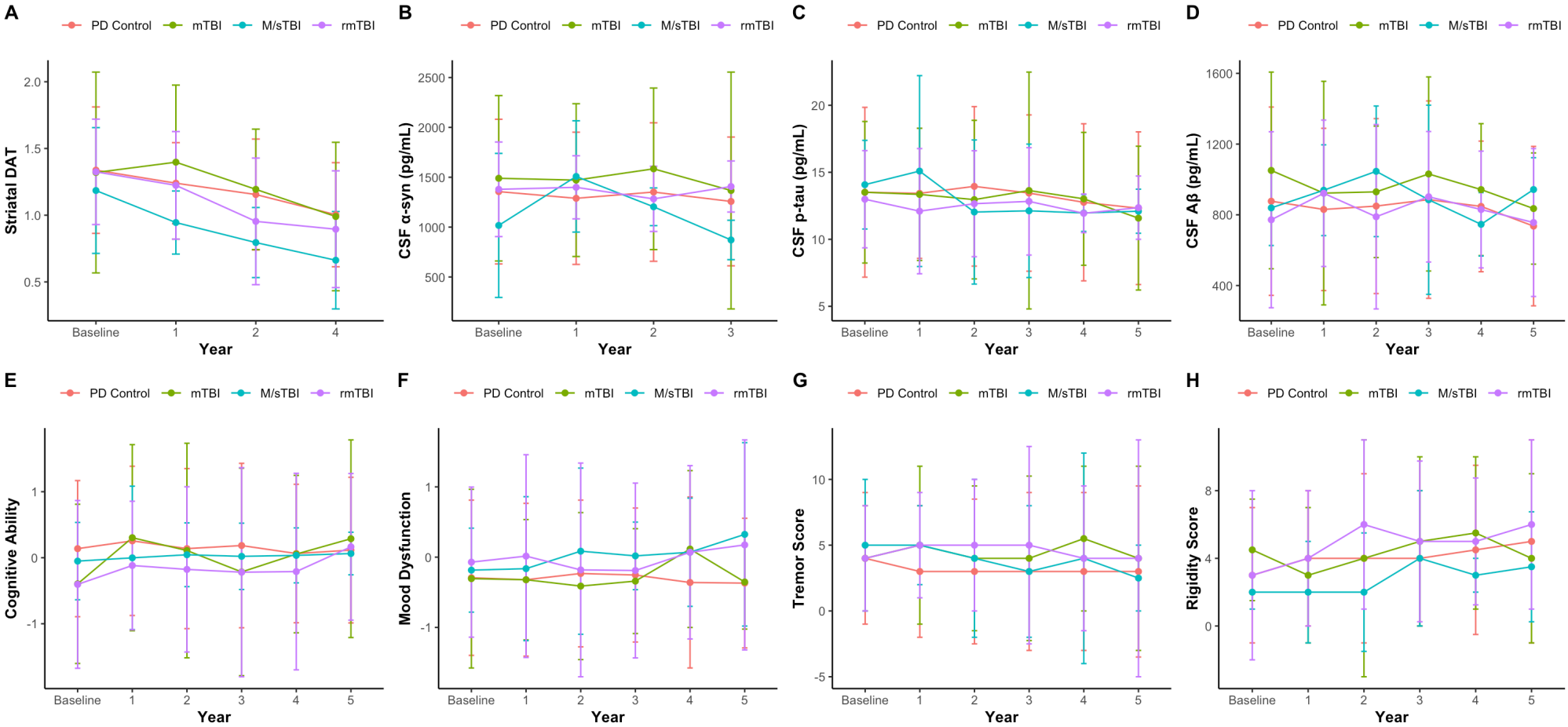


***Figure 4-3* Rate of progression of clinical and pathophysiological outcomes variables in PD without TBI, as well as PD with TBI encompassing several injury severities were assessed within the PPMI cohort.** *Median & IQR values are displayed at each timepoint for extracted variables of interest. While there was a significant interaction between timepoint and TBI severity for CSF α-syn (pg/mL)(3B), there was no significant difference in progression as determined via Dunn tests conducted on slopes between injury severity groups. Significant main effects for injury severity and follow-up timepoint were present for striatal DaT binding (3A), however, no other significant main or interaction effect was present for any other variable of interest.*

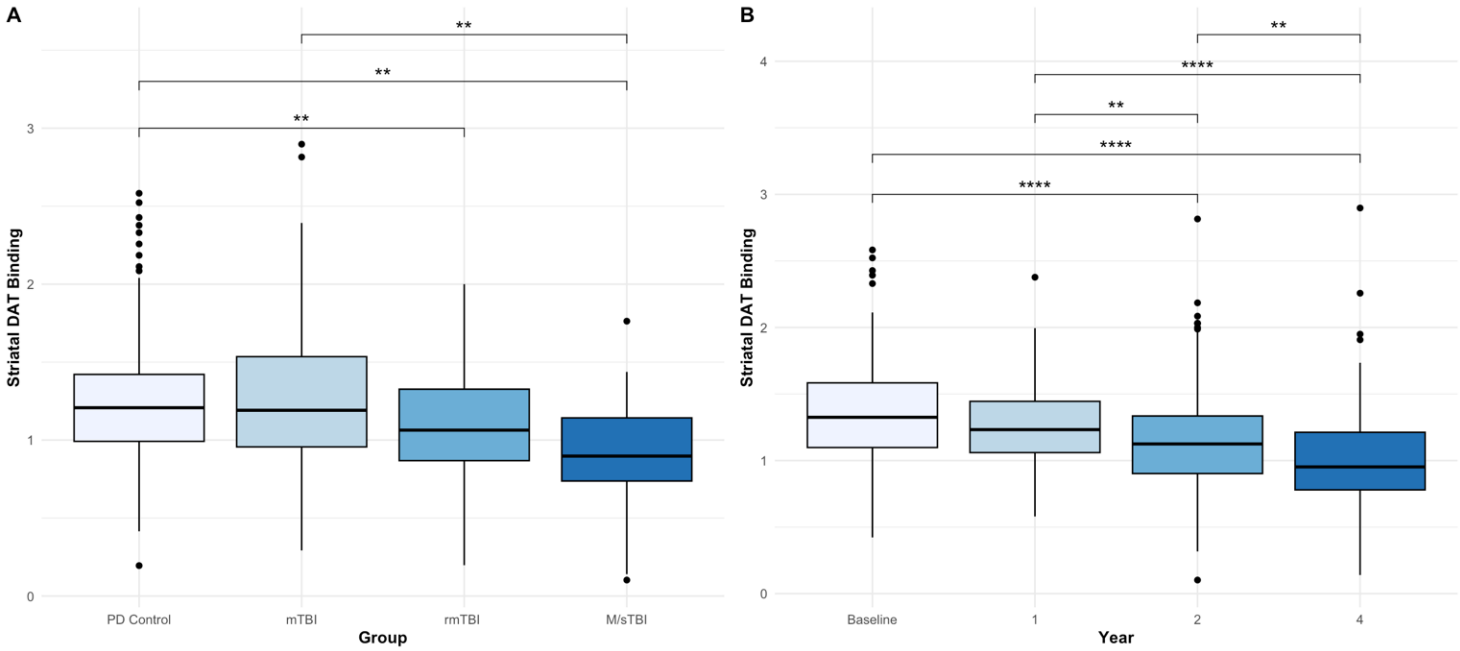


Figure 4-4 Mixed ANOVA's determined significant main effects for (4A) TBI severity and (4B) follow-up timepoint for striatal DaT binding*. An increase in injury severity and a longer follow-up timepoint were associated with significantly lower striatal DaT binding.*

4.4.2.3 Linear Regression

When considering injury severity (Table 4-6), TBI severity was a significant predictor of both year-5 mood dysfunction (β= 0.17, p = 0.041) and year-4 striatal DaT binding (β= -0.18, p = 0.039). Other significant predictors in the models included sex as a significant predictor of year-5 cognitive ability (β= -0.51, p = 0.007) and education as a significant predictor of year-5 tremor score (β= -0.18, p = 0.032). Similar to the models with history of TBI as a predictor, the proportion of the variance explained by the included predictors, however, was very low (R^2 range = 0.007 – 0.098).

Table 4-6 Multivariate linear regression was conducted to in the PPMI cohort to determine whether injury was a significant predictor of outcomes at follow-up. *Outcomes included neuropsychiatric (cognitive ability and mood dysfunction) or motor (rigidity and tremor scores) at year-5 follow-up, as well as striatal DaT binding and CSF α-syn at year-4 and year-3 respectively.*

PPMI: Injury Severity

	Year 5 Cognitive Ability		**Year 5 Mood Dysfunction**		**Year 5 Rigidity Score**		**Year 5 Tremor Score**		**Year 3 CSF α-syn**		**Year 4 Striatal DaT**	
Predictors	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*	*Estimates*	*p*
Intercept	0.29	0.680	0.05	0.326	-0.01	0.100	-0.04	**<0.001**	-0.12	**<0.001**	0.06	**<0.001**
Age	-0.07	0.430	-0.08	0.344	0.05	0.603	-0.11	0.209	-0.07	0.546	-0.01	0.913
Male Sex	-0.51	**0.007**	-0.09	0.591	0.02	0.903	0.07	0.699	0.20	0.412	-0.11	0.534
Education	0.17	0.073	-0.08	0.347	-0.05	0.594	-0.18	**0.032**	-0.12	0.276	-0.01	0.902
TBI Severity	0.04	0.654	0.17	**0.041**	0.06	0.526	-0.02	0.810	-0.10	0.395	-0.18	**0.039**
Observations	114		156		143		147		84		143	
R^2 / R^2 adjusted	0.098 / 0.065		0.049 / 0.024		0.007 / -0.021		0.044 / 0.017		0.034 / -0.015		0.036 / 0.008	

4.5 DISCUSSION

This study was among the first to look beyond whether TBI increases risk for PD development, and instead consider how either a history of TBI or the severity of injury may influence progression of motor and non-motor symptoms, as well as pathological markers, in established disease. Overall, across both the PPMI and PostCEPT early PD cohorts, aside from PD-TBI demonstrating a significantly younger age of onset compared to PD-Controls in the PPMI dataset, no significant differences were found in any other domain either at baseline or for the rate of symptom progression over a 5-year follow-up period for any variable of interest. Similarly, a history of TBI was not a significant predictor of motor, cognitive or mood function at follow-up timepoints in either cohort. Although severity of injury was a significant predictor of both mood dysfunction and striatal DaT binding at year-5 and year-4 follow-up, respectively,

within the PPMI cohort, it accounted for only a very small proportion of the total variance. Thus, while this could have potential important implications for the clinical management of PwP with a prior history of TBI, suggesting that those with a history of more severe injury may be particularly vulnerable to the mood-related symptoms of PD, as well as potentially greater degradation in the dopaminergic system, this finding should be interpreted with caution, and future research is warranted.

To date, the previous literature exploring whether a history of TBI affects disease progression in PD is limited and not entirely consistent. Paul Crane and colleagues (2016) reported that a history of TBI with LoC greater than one hour was associated with faster progression of parkinsonian symptoms in two prospective cohorts (the Religious Orders Study and Memory and Aging Project) (Crane et al., 2016). This previous study, however, did not consider both motor and non-motor domains, or treat these symptoms as continuous variables, as we have done here. Instead, they derived a summary global parkinsonian sign score based on performance on a 26-item assessment from a modified version of the motor section of the UPDRS, conducted by a trained nurse, which was re-coded into an ordinal format as a measure of progression (Crane et al., 2016). Thus, it may be that the operationalisation of the rate of progression outcome variable influenced the differences observed between groups. For example, the sign score incorporated other aspects of motor performance not assessed in the current work, such as bradykinesia and gait disturbance. Additionally, as a multi-cohort study, the prior study by Crane and colleagues included a substantially larger sample size (n = 7130) compared to the present study (n = 208 in the PPMI cohort; n = 635 in the PostCEPT cohort), and therefore possessed greater statistical power to detect differences in rates of progression between groups. Conversely, in a much smaller cross-sectional cohort study of 69 PwP, a history of TBI was not associated with worse motor scores on the UPDRS part three (Joyce et

al., 2020). However, within that study, the number of TBIs sustained was associated with poorer performance across several measures of cognition, including global cognition, executive function, memory and language, as well as increased depressive symptoms, compared to those without such a history, an effect not seen in the current work (Joyce et al., 2020). It is important to note, however, that this study did not consider progression over time in the PD cohort, as we have done here. Additionally, the sample of PD participants in that study was more heterogeneous, being a community dwelling sample with larger variability in time since diagnosis (6.52 ± 4.69 years), while the present study consisted only of individuals within two to five years of PD diagnosis (for PPMI and the PostCEPT cohort, respectively). Given that cognitive decline tends to worsen over time in PD (Roheger et al., 2018), it may be that our relatively limited follow-up period was too short to accurately assess the effect of TBI on progression of cognitive change.

It should be noted that divergence within early PD populations has been reported within such timeframes (Johansson et al., 2023, Dadu et al., 2022, Jeong et al., 2023), meaning rather than an insufficient follow-up period, lack of differences may be due to the nature of the PD-TBI cohort available for analysis in the current study. While currently the gold standard PD database, PPMI was not designed at its outset to assess the effect of TBI on disease progression, with head injury questions instead included as part of one questionnaire given at a single site. This reduced the pool of participants available for analysis for the current work, with inconsistent collection and reporting of variables, further contributing to the relatively small sample size. This also limited the power of the subgroup analyses investigating injury severity and made it impossible to assess the effect of other variables of interest, such as number of injuries sustained or age at time of injury. Further, substantial differences between the rates of TBI were present between cohorts explored in the current work, with PPMI reporting a 40%

incidence rate, whereas PostCEPT incidence was noticeably lower, at approximately 20%. Incidence within the PPMI cohort is considered almost two-fold higher than what has been found across other cohorts, with a meta-analysis of 14 studies reporting an incidence rate of 18% (Balabandian et al., 2023). However, disparity in rates, particularly across PPMI and PostCEPT, could be attributed to differences in head injury characterisation. PostCEPT classified TBI as any head injury resulting in LoC; however, only approximately 10% of concussions result in LoC (Delaney et al., 2002, Guskiewicz et al., 2003). Therefore, it is likely that the true incidence of TBI reported within the PostCEPT dataset is underrepresented. Further, such categorisation did not allow for exploration of additional variables of interest related to the injury itself, such as injury severity, an important factor to consider, as illustrated by our subgroup analyses in the PPMI cohort in the current study. To address these limitations, a more purposeful, prospective study design should be employed, specifically targeted at recruiting PwP with and without a history of head injury, with consistent characterisation of head injury and collection of additional variables of interest related to the injury.

Interestingly, when evaluating progression of disease-related pathology over five-year follow-up in the PPMI cohort, a significant interaction between injury severity and follow-up time was reported for CSF levels of α-syn, although post-hoc analysis did not reveal any significant differences in slopes between injury severities. This may be due to a lack of sufficient follow-up time, with CSF levels of α-syn only measured up to 3-years follow-up. It may be that, because the PPMI includes only those with newly diagnosed PD (i.e. within two years of diagnosis), this timeframe was not sufficient to fully evaluate the potential effect of TBI on pathological α-syn progression, with longer follow-up times needed to see more marked differences between groups. This is supported by Førland et al, who reported that, over a four-year period, no substantial change in CSF α-syn was present in a PD cohort, although it should

be noted that the sample was small (Førland et al., 2018). Conversely, Spiegel et al have suggested that, while TBI promotes earlier clinical onset of PD, it does so in the absence of more advanced pathology (Spiegel et al., 2021). Rather, it lowers the threshold required for pathology to manifest itself as visible symptoms. In line with this, while those with a history of TBI in the PPMI cohort demonstrated a significantly younger age of onset of disease than those without such a history (an effect that was not seen in the PostCEPT cohort), there were no significant baseline differences between groups for any of the pathological markers assessed.

Of note, despite there being no difference in rate of progression between groups, TBI severity acted as a significant predictor of increased mood dysfunction at five-year follow-up in the PPMI cohort. It is widely recognised that having a history of TBI is associated with an increased likelihood of developing a mood disorder, such as anxiety and depression (see review, (Howlett et al., 2022)). For example, one study reported that individuals hospitalised due to TBI had substantially higher rates of mood dysfunction at one-year post-injury compared to the general population (Deb et al., 1999b). Depressive symptoms were present in 13.9% of TBI participants, compared to only 2.1% in the general population, with 9.0% experiencing panic disorders, compared to only 0.8% in the general population (Deb et al., 1999b). Injury severity may be an important predictor of this risk. In line with this, a recent longitudinal study in South Korea, assessing incidence of post-TBI depression rates in 2.2 million adults, found that those with a history of M/sTBI or skull fracture demonstrated significantly higher risk of developing depression (HR 1.47, CI 1.44-1.4) compared to those with a history of concussion (HR 1.21, CI 1.20-1.22 (Choi et al., 2022). This is further corroborated by a 2014 meta-analysis of 99 studies, which found that while mTBI was associated with a prevalence of 16% for major depressive disorder or dysthymia, this increased two-fold in a mixed sample of mild, moderate,

and severe TBI (30%) (Osborn et al., 2014). Similarly, a meta-analysis across 41 studies reported that the prevalence of generalised anxiety disorder also increased slightly with injury severity (mild = 11%; severe = 15%), although the difference was not significant (Osborn et al., 2016). Thus, while the effect of a previous TBI on mood dysfunction in PD has not been previously assessed, to the best of our knowledge, it is not surprising that more severe injury would be a predictor of greater decline in mood function over the follow-up period in PwP.

TBI severity was also a significant predictor of greater reductions in striatal DaT binding at four-year follow-up, again despite there being no significant difference in rate of progression between groups. This is in line with previous literature in TBI cohorts, which has shown that TBI severity may be an important contributor to the reductions in dopaminergic levels observed on SPECT DAT imaging in TBI survivors. For example, using the I-β-CIT tracer, Donnemiller et al reported greater than 50% reduction in striatal DaT binding at an average of 141 days following head trauma leaving participants in a vegetative state (Donnemiller et al., 2000). In studies assessing less extreme cohorts, but still M/sTBI, reductions have also been noted in striatal DaT binding, albeit to a smaller degree, ranging from 15 – 20% (Jenkins et al., 2018, Wagner et al., 2014). To date, however, there is very limited literature assessing concussion-induced changes in striatal DaT binding, making it difficult to fully assess the effect of TBI severity on dopaminergic integrity. In the current study, however, there was a main effect of TBI severity reported in the mixed ANOVA, with more severe injury associated with lower striatal DaT binding. This suggests that assessing TBI severity may be an important consideration for future research aiming to characterise disease progression in PwP.

It is important to note, however, that the regression models exploring the predictive value of TBI-related factors yielded a markedly low proportion of variance explained in all outcome

variables, and a history of TBI was not a significant predictor in any model. This suggests that TBI in isolation is not the sole driver of PD development and progression and that it must be considered in the wider context of other known risk factors for PD. For example, in addition to a history of TBI, PD is also known to be associated with multiple other genetic and environmental variables and PD pathogenesis is thought to involve complex interactions between these factors (for a review, see (Tsalenchuk et al., 2023)). One such factor is pesticide exposure, with a meta-analysis reporting that exposure substantially increases risk of PD development (Ahmed et al., 2017). Interestingly, TBI has been reported to exacerbate the effects of pesticide exposure, with pesticide-related PD being substantially higher in individuals who also have a history of TBI (Shrestha et al., 2020). In line with this, Lee et al reported that while TBI and pesticide exposure in isolation were each associated with only a moderately increased risk of PD development, dual exposure increased risk three-fold (Lee et al., 2012). This was further corroborated by the results of a rodent study, where a moderate TBI induced by lateral fluid percussion in adult rats resulted in 15% dopaminergic neuron loss ipsilaterally, an effect which was exacerbated by additional exposure to pesticide (30% dopaminergic neuron loss bilaterally) (Hutson et al., 2011). Thus, pesticide exposure may be a critical environmental risk factor to consider in conjunction with TBI history when investigating PD progression. Similarly, genetic factors may also interact synergistically with a history of TBI to influence the development or progression of PD. In support of this, a randomised controlled trial reported significant interactions between a history of TBI and polymorphisms in the REP1 gene, with a combination of these factors corresponding to a significantly younger age of disease onset compared to individuals with no such history (Goldman et al., 2012). Interestingly, however, head injury alone was not significantly associated with PD in that study (Goldman et al., 2012), further highlighting that TBI in isolation may not be sufficient to influence PD progression. To date, however, literature

exploring such synergistic effects between TBI and other known risk factors for PD are scarce, indicating an important avenue for future research.

4.6 CONCLUSION

While a known risk factor for the later development of PD, present findings do not support that a history of TBI changes progression of either disease-related pathology or clinical presentation in those with established PD in two independent cohorts. Future research should also aim to address limitations within the current study. Further research aiming to evaluate the effects of TBI itself, as well as several important associated characteristics, such as injury severity, number of injuries and age at time of injury, on PD progression should employ a purposely designed study, rather than the convenience samples utilised here, to adequately assess these questions. Further, given the small amount of variance explained in our regression models incorporating only a history of TBI and key demographic variables, it suggests that other factors, or combinations of factors, may contribute to predicting disease progression. Therefore, future work should investigate other known genetic and environmental risk factors for PD, as well as any potential synergistic effects, on PD progression.

4.7 FUNDING

Funding sources and conflict of interest: This work was funded by grants to LCP and IB by the Medical Research Future Fund (2020/MRF1202188), James and Diana Ramsay Foundation and NeuroSurgical Research Foundation. AM was supported by an Australian government Research Training Program scholarship. The authors declare that there are no conflicts of interest relevant to this work.

PPMI – a public-private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners, including 4D Pharma, Abbvie, AcureX, Allergan,

Amathus Therapeutics, Aligning Science Across Parkinson's, AskBio, Avid Radiopharmaceuticals, BIAL, Biogen, Biohaven, BioLegend, BlueRock Therapeutics, Bristol-Myers Squibb, Calico Labs, Celgene, Cerevel Therapeutics, Coave Therapeutics, DaCapo Brainscience, Denali, Edmond J. Safra Foundation, Eli Lilly, Gain Therapeutics, GE HealthCare, Genentech, GSK, Golub Capital, Handl Therapeutics, Insitro, Janssen Neuroscience, Lundbeck, Merck, Meso Scale Discovery, Mission Therapeutics, Neurocrine Biosciences, Pfizer, Piramal, Prevail Therapeutics, Roche, Sanofi, Servier, Sun Pharma Advanced Research Company, Takeda, Teva, UCB, Vanqua Bio, Verily, Voyager Therapeutics, the Weston Family Foundation and Yumanity Therapeutics."

Full financial disclosures for the previous twelve months: AM is supported by an Australian government research training programme scholarship.

4.8 AUTHOR INFORMATION

4.8.1 Author Affiliation

School of Biomedicine, University of Adelaide, SA 5000, Australia

Angus McNamara & Lyndsey Collins-Praino

School of Psychology, University of Adelaide, SA 5000, Australia

Irina Baetu

4.8.2 Contributions

AM, conceptualized, analysed data, and wrote the original manuscript. LCP & IB contributed to experimental concept and design, interpretation of results, as well as LCP playing a key role

in manuscript conceptualisation. All authors were involved in editing the original manuscript. All authors read and approved the submitted manuscript.

4.9 CORRESPONDING AUTHOR

Address all correspondence to A/Professor Lyndsey Collins-Praino

4.10 ETHICS DECLARATIONS

4.10.1 Ethics approval and consent to participate

The PPMI study was approved by the local Institutional Review Boards of respective institutions (a full list is available at the following link https://www.ppmi-info.org/about-ppmi/ppmi-clinical-sites). Written informed consent were obtained from each participant at enrolment, in accordance with the Declaration of Helsinki. All methods were performed in accordance with the relevant guidelines and regulations

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this work is consistent with those guidelines

4.10.2 Competing interests

The authors declare that they have no competing interests.

4.11 ACKNOWLEDGEMENTS

Data used in the preparation of this article were obtained on July 6th, 2023, from the PPMI database (www.ppmi-info.org/access-dataspecimens/download-data), RRID:SCR 006431. For up-to-date information on the study, visit www.ppmi-info.org.

PPMI – a public-private partnership – is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners. The list with full names of all PPMI funding partners found at www.ppmi-info.org/fundingpartners

The authors would also like to acknowledge Professor Caroline Tanner for her contributions, particularly pertaining their pivotal role in designing the FOUND protocol, which has been applied to both PPMI and PostCEPT, as well as assistance in gaining access to PostCEPT data for analysis.

4.12 CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Contextual Statement for Chapters 5 and 6

Chapter 4 provided limited evidence that a history of TBI, or the effects of injury severity, drove differential progression in clinical or pathophysiological features of PD. While several limitations regarding inconsistent outcome operationalisation and TBI assessment were highlighted, it is also possible that the effects of TBI were minimal in comparison to that of established PD. Therefore, it is possible that rather than exploring TBI's potential influence on disease course, that pathophysiological assessment within a pure TBI cohort may provide a strategy towards identifying individuals at-risk of persistent impairment and potential disease development following injury. Given the success of pathophysiological markers at time of diagnosis in PD to forecast impairment, a similar research question was developed to identify biomarkers extracted at time of injury and determine their relation to behavioural outcomes following injury. Biomarkers of cell injury have garnered research interest in TBI and therefore, Chapter 5 utilised a meta-analytic strategy to determine the association between biomarkers of cell injury extracted a week within TBI and behavioural outcomes. Additionally, certain markers of PD, such as nigrosome-1 assessment via specialised imaging techniques, demonstrate promise for objective and earlier diagnosis. Therefore, Chapter 6 utilised susceptibility weighted imaging to assess nigrosome-1 across a TBI cohort of varying times since injury and injury severities, relative to both healthy controls and PD. Further, given both TBI and PD report cognitive impairments resulting from progressive dopamine depletion, the potential prognostic value of nigrosome-1 for cognitive ability was also assessed.

Chapter 5: Statement of Authorship

Title of Paper	Utility of Biofluid Markers of Cell Injury for Prognosis of Behavioural Outcomes Following Traumatic Brain Injury in Adults: A Systematic Review & Multilevel Meta Analysis
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ■ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	Angus McNamara		
Contribution to the Paper	Conceptualised, performed and interpreted statistical analyses and wrote the initial manuscript		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/12/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Benjamin Ellul		
Contribution to the Paper	Assisted in data collection, including abstract and full-text screening, quality assessment and risk of bias determination, in addition to supporting with analytical interpretation		
Signature		Date	01/12/2024

Name of Co-Author	Irina Baetu		
Contribution to the Paper	Contributed to the organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Name of Co-Author	Lyndsey Collins-Praino		
Contribution to the Paper	Contributed to the conceptualisation of the manuscript, organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Chapter 5 - Utility of Biofluid Markers of Cell Injury for Prognosis of Behavioural Outcomes Following Traumatic Brain Injury in Adults: A Systematic Review & Multilevel Meta Analysis

Angus McNamara[1], Benjamin Ellul[1], Irina Baetu[2]& Lyndsey Collins-Praino[1]

[1] School of Biomedical Science, University of Adelaide, SA 5000, Australia

[2] School of Psychology, University of Adelaide, SA 5000. Australia

5.1 ABSTRACT

Introduction

Traumatic Brain Injury (TBI) is now recognised as a chronic condition and is associated with several functional impairments across cognitive, mood and motor functions. In recent years, biomarkers of cell injury have garnered research interest, demonstrating utility to streamline TBI diagnostic protocols, reflected by their recent integration into clinic. However, there is a lack of consensus on how markers of cell injury are associated with functional impairments following injury. The purpose of this review, therefore, was to determine the association between markers of cell injury and functional outcomes in adults following TBI.

Methods

PubMed, Embase, Web of Science and Cochrane databases were searched with a search strategy containing key search times for TBI, markers of cell injury and outcome. Original research articles reporting associations between biomarkers of cell injury and functional outcomes at any timepoint following injury were included.

Results

A total of 64 studies met the inclusion criteria, encompassing 58 studies assessing global outcome, seven assessing cognition and a single study each assessing mood and motor function. Due to the high number of effect sizes reported within studies, multilevel meta-analyses were conducted for global outcome and cognition, finding moderate and weak associations with each outcome, respectively. Further, substantial heterogeneity was present within models, justifying moderator analyses to identify drivers of differential effect sizes. However, assessed moderators across global outcome and cognition did result in significance, as effect sizes were

similar when comparing specific biomarkers, or time between sample extraction and outcome assessment relative to injury for global outcome, or cognitive domain for cognition.

Conclusion

Overall, biomarkers of cell injury, regardless of moderating factors, demonstrated moderate associations with global outcome following injury, with noticeably weaker associations being present for cognition, mood or motor outcomes. However, the vast majority of studies only assessed global outcomes, highlighting the need for further research to establish associations between biomarkers and more specific outcomes post-injury.

5.2 BACKGROUND

Traumatic Brain Injury (TBI), resulting from external force to the head and often resulting in altered brain structure or function, has an estimated global prevalence of 69 million people per annum (Dewan et al., 2019). Further, it is a broad term encompassing several severities, ranging from mild TBI (mTBI) with a Glasgow coma scale (GCS) score of 13-15, moderate TBI (MTBI; GCS 9 – 12) and severe TBI (sTBI; GCS 3-8) (Teasdale and Jennett, 1974). Following more severe injuries, up to 40% of adults hospitalised post-TBI with neurobehavioral symptoms still exhibited three or more symptoms 12 months later (Deb et al., 1999a), with up to 51% of TBI survivors still experiencing substantial disability, as determined by a score ≤ 4 on the Glasgow outcome scale-extended (GOSE), up to 14 years post-injury (McMillan et al., 2012). Conversely, individuals sustaining a mTBI typically exhibit functional impairments acutely following injury, with most cases recovering to pre-injury functioning within two-weeks (Schretlen and Shapiro, 2003). In recent years, however, it is recognised that a subset of individuals exhibits more prolonged functional impairments following mTBI. In line with this, within the TRACK-TBI cohort, 53% of the 1453 mTBI participants assessed reported

substantial functional impairments, as measured by the Glasgow outcome scale (GOS) at 12-months post-injury (Nelson et al., 2019). Similarly, in an analysis of 61 cases of mTBI presenting at a UK Major Trauma Centre, 28% reported incomplete recovery two years post-injury, determined using GOS (Carroll et al., 2020). When functional impairments persist for >3 months post-injury, this is referred to as persistent post-concussion syndrome (PPCS), which is estimated to affect ~15% of individuals following mTBI (Pavlovic et al., 2019, Arciniegas et al., 2005).

Of note, PPCS corresponds to considerable reductions in quality of life (QoL) and difficulties re-engaging with pre-injury activities. A study assessing 731 mTBI cases found that, following injury, individuals presenting with PPCS had significantly lower physical and mental health scores, as measured by the Short-Form Health Survey, as well as lower mean QoL (Voormolen et al., 2019). This may result from challenges surrounding re-engaging with pre-injury responsibilities, with previous literature finding that individuals with a history of TBI reported worse general health, elevated depression rates, social isolation and lower re-integration into the work force compared to age- and gender-matched controls (Hawthorne et al., 2009). In fact, TRACK-TBI investigators determined that 17% of individuals sustaining a mTBI were unable to return to work, a finding which has been corroborated in other cohorts (Kristman et al., 2010). Further, 20% of individuals reported a decline in annual income (Kristman et al., 2010). Beyond PPCS, it is established that disability is injury-dependent, with a TRACK-TBI study determining that relative to mTBI, sTBI survivors reported lower rates of functional independence on the GOSE one-year post-injury, as well as MTBI and sTBI corresponding to higher rates of mortality (5.5%) than mTBI (1.5%) between one to five years post-injury (Nelson et al., 2023). Taken together, such evidence highlights the challenges surrounding chronic post-injury outcomes the negative impact they have on QoL, making it critical to

accurately identify individuals most at-risk of persistent impairments in order to provide tailored assistance and intervention.

Several risk factors of PPCS have been identified, such as older age, female gender, greater injury severity and abnormalities detected by computed tomography (CT) imaging following injury (Lubbers et al., 2024). This is reflected by their incorporation into current prediction tools, namely the International Mission for Prognosis and Clinical Trials in TBI (IMPACT)(Perel et al., 2008) and Corticosteroid Randomisation after Significant Head Injury (CRASH) (Steyerberg et al., 2008) prognostic calculators. Both of these prognostic calculators were developed using large cohorts and report high predictive validity (Gao and Zheng, 2015). In line with this, a comparative study assessing moderate-to-severe TBI outcomes in an elderly population reported that both calculators showed high accuracy in predicting mortality at 6-months post-injury, with IMPACT (area under the curve (AUC) = 0.976, sensitivity = 88% & specificity = 93%) slightly outperforming CRASH (AUC = 0.959, sensitivity = 88% & specificity = 88%) (Moorthy et al., 2021). In fact, a meta-analysis assessing the prognostic utility of IMPACT and CRASH determined near identical performance in predicting mortality and unfavourable outcomes as determined by GOS or GOSE (Zarei et al., 2023).

Despite the promise of these tools, however, they have inherent limitations which constrain their clinical utility. A major limitation is that favourable vs unfavourable as an outcome is quite broad, with neither calculator providing much insight into specific impairments that a person may experience, such as cognitive deficits, mood changes and motor outcomes. Additionally, both IMPACT and CRASH have reportedly overestimated mortality and unfavourable outcomes in severe TBI cohorts (Olivecrona and Olivecrona, 2013, Olivecrona and Koskinen, 2012). Additionally, both calculators have only been validated for use up to six-

months following injury, a critical limitation given the emerging evidence that persistent impairment can occur for several years (or potentially even beyond) following injury (Nelson et al., 2019). Furthermore, most studies have explored the utility of IMPACT and CRASH in moderate-to-severe TBI cohorts. Consequently, the validity of such calculators in less severe cohorts, namely mTBI, is relatively unknown, a major oversight given that, as discussed above, mTBI can also be associated with persistent complications and represents the vast majority of TBIs, comprising approximately 70 – 90% of all TBI cases (Cassidy et al., 2004). Thus, an emerging area of research is focused on improving current prognostic models in TBI, with a key recommendation being to incorporate biomarkers of TBI neuropathology that may underlie the development and maintenance of behavioural impairments (Czeiter et al., 2012a, Gao and Zheng, 2015). This is highlighted by Czeiter and colleagues, with the core IMPACT model (Nagelkerke $R^2 = 0.214$) being substantially enhanced with the inclusion of cerebrospinal fluid (CSF) glial fibrillary acidic protein (GFAP) (R^2=0.476). Further, integration of several markers (namely CSF and serum concentrations of GFAP, as well as CSF levels of αII-spectrin breakdown product (SBDP) corresponded to more robust model performance than GFAP in isolation ($R^2 = 0.7$) [13].

Of particular interest are biomarkers of cellular injury, considered for their potential utility in TBI management, including advancing diagnostics and prognostics, as well as acting as a theragnostic tool to facilitate treatment advancements (see review, (Wang et al., 2018b)). Several such markers have been identified, including markers of neuronal injury, axonal injury, glial cell damage and beyond (see review, (Shahim and Zetterberg, 2022)). sGenerally, biomarkers of cell injury are highly up-regulated following injury, with specific changes within markers summarised in Table 5-1.

Table 5-1 Biomarkers of cell injury and studies highlighting their up-regulation following TBI

Marker	Location within Cell	Evidence Demonstrating Marker Change in TBI
NSE	Neuronal cytoplasm	Serum NSE following mTBI or M/sTBI are significantly elevated compared to healthy controls, with AUC values significantly correlating ($r = -0.59$, $p < 0.001$) with GCS scores (Herrmann et al., 2000). Ratio between serum NSE extracted within six hours of injury and admission GSE were significantly higher in CT-confirmed diffuse axonal injury (DAI) compared to non-DAI TBI controls (Chen et al., 2022)
UCH-L1	Neuronal cytoplasm	Higher concentration of serum UCH-L1 extracted within four hours of injury discriminated mTBI (GCS 9-15) from healthy controls (AUC = 0.87) (Papa et al., 2012) Mean levels of serum UCH-L1 extracted within 24 hours of injury were significantly higher in M/sTBI (GCS 3-12) and mTBI (GCS 13-15) ($p < 0.0001$), as well as complicated mTBI with abnormal cranial CT and uncomplicated mTBI ($p = 0.012$) (Diaz-Arrastia et al., 2014)
S100B	Astrocyte cytoplasm	Serum S100B levels extracted within six hours of injury were significantly higher in mTBI (GCS 13-15) compared to healthy controls ($d= 0.26$ µg/l; 95% CI: 0.21–0.31) and maintained after controlling for age and sex ($d=0.23$ µg/l; 95% CI: 0.18–0.29) (De Kruijk et al., 2001) Median serum concentrations of S100B extracted within six hours of injury were significantly elevated in mTBI (GCS 13-15) with CT confirmed cranial abnormalities compared to mTBI without ($p = 0.032$) (Kotlyar et al., 2011)
GFAP	Astrocyte cytoplasm	Plasma concentrations of GFAP extracted within 24 hours of injury were significantly elevated in TBI compared to controls ($p < 0.0001$) (Bogoslovsky et al., 2017) Serum GFAP extracted within 24 hours of injury were significantly elevated in severe TBI (Traumatic Coma Data Bank (TCDB) classification of ≥ 3) compared to more mild TBI ($p <0.0001$) (Nakamura et al., 2022)
NF-L	Axonal cytoplasm	Plasma levels of NF-L extracted within 24 hours of hospital admission were significantly correlated with diffusion magnetic resonance imaging determined DAI 3-months following TBI (Hossain et al., 2023)

pNFH	Axonal cytoplasm	GCS scores were negatively correlated with blood concentrations of pNFH extracted at admission (r = -0.66 with a $p < 0.005$) and 7-days post-injury (r = -0.78, p < 0.0005) (Ghonemi et al., 2013) Compared to healthy controls, individuals with an mTBI (GCS 13-15) demonstrated significant elevations in serum PNFH levels at 24-hours (p <0 .0001) and 72 hours (p < 0.0001) following injury (Gatson et al., 2014)
Tau	Primarily axonal cytoplasm	Total tau levels in plasma were significantly increased in TBI compared to healthy controls, with increasing levels associated with increasing severity of initial brain injury, number of TBIs and severity of post-concussive symptoms (Olivera et al., 2015)
SBDP	Neuronal cytoplasm	CSF levels of SBDP extracted within six hours of admission, and every six-hours following for up to seven days, were significantly higher at all timepoints in severe TBI (GCS ≤8) compared to controls (Mondello et al., 2010)
MBP	Axonal (myelin sheath)	Serum levels of MBP extracted within 24 hours of injury did not differ between injury severity groups (GCS ≥ 8 ~ < 8) (Yamazaki et al., 1995) Recent review highlights that MBP is critically underassessed in mTBI (Shahim and Zetterberg, 2022)

NSE – Neuron-specific enolase, UCH-L1 – Ubiquitin C-terminal hydrolase L1, S100B – S100 calcium binding protein B, GFAP – Glial fibrillary acidic protein, NF-L – Neurofilament light, pNFH – Phosphorylated neurofilament heavy chain, SBDP – Spectrin breakdown products, MBP – Myelin basic protein

In fact, the association between markers of cell injury and CT-detected intracranial abnormalities has resulted in a push to integrate acute measurement of these markers into clinical settings as a screening tool following injury. For example, a study exploring diagnostic efficacy of S100B reported that CT abnormalities of any kind 24 hours after injury were most significantly associated with venous S100B collected within 6-hours of injury (AUC = 0.73, sensitivity = 93.7% and specificity = 30.8%), outperforming S100B collected within 24-hours (AUC = 0.65, sensitivity = 82.9% and sensitivity = 30.8%) (Rogan et al., 2023). Additionally, in a TRACK-TBI cohort study, UCH-L1 collected within the first eight hours of injury showed the highest diagnostic ability when assessing CT-positive scans, encompassing any intracranial

abnormality, within 24 hours of injury (AUC = 0.78), outperforming NSE (AUC = 0.69) (Wang et al., 2023a). A recent study corroborated this in a cohort of 349 mTBI patients, whereby the combination of UCH-L1 and GFAP yielded 100% sensitivity in detecting CT lesions (Rogan et al., 2023). In fact, following findings from a multi-centre observational study of 1959 individuals sustaining a TBI (GCS 9 – 15) (Bazarian et al., 2018), serum concentrations of GFAP and UCH-L1 collected within 12 hours of injury have been approved by the Food and Drug Administration (FDA) to aid in screening for intracranial abnormalities in emergency room settings (Food and Administration, 2018).

Despite advances in diagnostics, however, currently, no biomarker has been clinically validated for predicting prognosis of TBI-related behavioural changes, which will require consolidation of findings across multiple studies. To date, several studies assessing the relationship between levels of biomarkers of cell injury collected acutely following injury (i.e., within 7 days post-injury) and behavioural outcomes at several time points post-injury have been conducted. Thus, this review aimed to investigate the associations between acute levels of biofluid markers of cell injury and behavioural outcomes at any timepoint following injury. To probe this further, factors such as time of sampling and clinical assessment(s) conducted were considered. Ultimately, the identification of specific markers of cell injury that predict functional outcomes following TBI could play a key role in better identification of individuals most susceptible to persistent impairments following TBI.

5.3 METHODS

This review was completed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analysis Protocols (PRISMA-P) (Moher et al., 2015) and was pre-registered with PROSPERO (CRD42022368622).

5.3.1 Eligibility Criteria

5.3.1.1 Outcome Measurement

Studies deemed eligible for inclusion had to assess a behavioural outcome, including motor, cognition or mood function, at any timepoint following injury. Motor function had to be assessed either using a corroborated clinical measure, such as the Balance Error Scoring System (BESS) to measure postural stability (Riemann and Guskiewicz, 2000), or biomechanical measures. Likewise, cognitive function had to be measured using a validated cognitive assessment that is either automated or administered by a trained individual, such as the Montreal Cognitive Assessment (MoCA) as a measure of global cognitive function (Nasreddine et al., 2005). While a validated screening tool had to be utilised if a study was assessing mood, it is widely recognised that self-report of symptom-based outcomes is the current gold-standard when collecting mood data (Davis et al., 2019) and therefore self-report was acceptable for mood measures. Additionally, widely utilised scales of global outcome following TBI, such as the GOS (Jennett and Bond, 1975) and GOSE (Wilson et al., 2021) were eligible, as they are regarded as the current "gold standard" for post-injury behavioural assessments (Jennett and Bond, 1975). While other scales, such as the Rivermead Post-Concussion Questionnaire (RPQ), are also widely utilised, the latter is self-report; therefore, given the availability of a more objectively assessed global outcome measures (i.e. GOS or GOSE), the RPQ was excluded.

5.3.1.2 Prognostic Factor Measurement

In addition to including an assessment of functional outcome, studies must also have measured at least one biomarker of cell injury within a defined biofluid, such as blood, saliva or CSF Furthermore, markers had to be objectively quantified, with the method of assessment used (e.g. ELISA, SIMOA, etc.) explicitly stated. Additionally, studies were only considered eligible

if biofluids were collected from participants acutely post-injury (≤ 7 days). Only studies with available data assessing the relationship between the marker(s) of cell injury and a behavioural outcome were eligible for inclusion in this review.

5.3.1.3 Participants

Only studies that included participants with a clinically diagnosed TBI, obtained ≥15 years of age, were considered. Studies assessing paediatric TBI (<15 years of age), or other forms of brain injury other than a TBI, such as stroke or encephalopathy, were excluded. Furthermore, studies only including self-reported TBI, without clinical assessment, were also excluded. Comparator cohorts, such as healthy or orthopaedic controls, were not required. While detailed description of injury characteristics was preferred, underreporting of factors, such as injury severity, were not grounds for exclusion from this review.

5.3.1.4 Study Types

Peer-reviewed and published cross-sectional, cohort and longitudinal prospective studies were eligible for inclusion. Prior reviews or meta-analyses, grey literature, theses, case reports, papers written in a language other than English and studies where full text was unavailable were excluded. Studies that assessed data from large, international online databases, such as TRACK-TBI and CENTER-TBI, were excluded, as studies utilising said data would be drawing from the same sample. Authors aimed to conduct analysis on the original data from these databases but were not able to obtain access to either dataset as requested data was not publically available.

5.3.2 Search Strategy

An extensive search was conducted to identify eligible peer-reviewed studies, including Medline via PubMed, EMBASE via Ovid, Cochrane and Web of Science. Three key terms guided development of search terms for four relevant databases: “markers of cell injury”, “TBI” and “behavioural outcomes”. Search terms were combined using Boolean operators, as well as exploding MeSH terms and filtering clinical studies written in English. Searches were not limited by year of publication. Initial searches of databases were performed on the 11th of January 2023 and repeated on the 21st of November 2023 (Supplementary Material 1).

5.3.3 Study Records

5.3.3.1 Data Management

Studies identified using search strategies were exported to EndNote v.20 and imported into Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia). Covidence is an online platform developed to streamline title/abstract and full text screening in a collaborative environment, as well as to automatically remove duplicate studies found across database searches. From the initial database search, 2,262 studies were found, with 800 duplicates being identified and removed, leaving 1,462 for screening (Figure 1). Each remaining study was screened for eligibility based on title and abstract, following predetermined inclusion criteria agreed upon by two independent reviewers (AM and BE). Agreement had to be unanimous for a study to be considered eligible or excluded and when disagreements arose, they were resolved via discussion and settled by consensus. At this stage, agreement between raters was high, as determined by a proportionate agreement of 93% and a substantial Cohen’s Kappa (0.83). 1,022 studies were excluded during title and abstract screening, leaving 440 studies subject to full-text screening.

Full-text and, when required, supplementary materials, were retrieved and uploaded to Covidence. AM and BE critically determined study eligibility for inclusion based on pre-established criteria. If studies were excluded at this stage, each reviewer had to specify a reason for exclusion from a set of predetermined reasons (Figure 1). Of note, a total of 90 corresponding authors were contacted for eligible studies that required additional information, such as studies that reported collecting biomarker concentrations and outcome measures but not explicitly assessing their association or needing additional data for effect size conversion (outlined in the "Data coding" section of the methods). One month was provided for authors to reply to the initial email, after which a reminder email was sent, and an additional week was provided to allow for a response. Of these studies, 65 were excluded as authors did not respond or were unable to provide data (outlined in exclusion reasons in Figure 1).

Discrepancies were resolved through discussion and consensus between reviewers. Agreement between raters was also high during full-text screening, corresponding to a proportionate agreement of 96% and a Cohen's Kappa of 0.87. During this stage, 376 studies were deemed ineligible for review inclusion, leaving 64 studies for final data extraction.

5.3.3.2 Data Items and Collection Process

A data extraction template consisting of 83 items was developed to extract relevant study information (Supplementary Material 2). The primary reviewer (AM) conducted data extraction for all studies that were deemed eligible after full-text screening, while the secondary reviewer (BE) extracted data for a subset of 16 studies (25%), with a proportionate agreement of 89%.

5.3.4 Study Risk of Bias Assessment

Quality assessment was conducted for every study included in the final review by the primary reviewer, while the secondary reviewer completed a subset of 16 studies (25%), ensuring consistent appraisal between reviewers. The Quality in Prognostic Studies (QUIPS) tool was chosen, as it is one of the most universally used quality assessments for evaluating prognostic studies (Grooten et al., 2019a, Riley et al., 2019), and it is used to assess six bias domains. Furthermore, the QUIPS is highly adaptable, and its authors recommend adjusting the tool to be more review specific (Hayden et al., 2013). For the purposes of this study, only 4 domains from the original QUIPS were retained: 1) key study variables and recruitment 2) prognostic factor measurement 3) outcome measurement and 4) statistical analysis and reporting. The adapted version of the QUIPS tailored to this study can be found in Supplementary Material 3. As recommended by Grooten et al (Grooten et al., 2019b) and Hayden et al (Hayden et al., 2013), rather than using a tallied score to approximate overall risk of bias per study, the following classifications were used for each domain: low, moderate, or high risk of bias, allowing for determination of an overall risk of bias rating. Evaluations with domain-based approaches mitigate challenges in validating such subjective assessment methods, improving validity of the final appraisal (Grooten et al., 2019a, Hayden et al., 2013).

Interrater agreement was deemed sufficient if the calculated Kappa statistic was ≥ 0.6, based on Cohen's guidelines to indicate substantial agreement. Overall, interrater agreement for quality assessment was high (Kappa = 0.80). When considering domain-specific agreement, a perfect agreement (Kappa = 1) was reported in "Key Study Variables and Recruitment," as well as a high value for "Statistical Analysis and Reporting" (Kappa = 0.84). When assessing "Prognostic Factor Measurement" and "Outcome Measurement", more conflicts arose, corresponding to lower Kappa values of 0.70 and 0.66, respectively.

5.3.5 Data Synthesis

Studies initially underwent descriptive analyses, with several categorisations being applied to effect sizes to improve interpretability. Firstly, due to the variation in effect sizes encompassed within this review, the magnitude of the effect sizes was determined using the following conversion scenarios:

Table 5-2 Magnitude of effect size strength across various statistical techniques

p-value	**Pearson R / Spearman Rho** (Cohen, 2013, Cohen, 2016)	**AUC** (De Hond et al., 2022)	**Strength of Effect Size**
> 0.05	0 – 0.1	0.5 – 0.6	Very low (ns)
< 0.05	0.1 – 0.3	0.6 – 0.7	Low (+)
< 0.01	0.3 – 0.5	0.7-0.8	Moderate (++)
< 0.001	> 0.5	> 0.8	High (+++)

In instances where several effect sizes were provided, such as a correlation coefficient and p-value, magnitude was determined using the p-value. Further, given the scope of behavioural outcomes assessed within this review, effect sizes were first categorised according to the broader domain that they were assessing: 1) global outcome 2) cognition 3) mood and 4) motor function. For effect sizes derived from cognitive measures, each cognitive assessment was categorised into a specific cognitive domain, based on consensus across studies included within this review, a recent literature review (Harvey, 2019) and expert opinion (Supplementary Material 4). Further, studies assessing cognition may have explored several cognitive assessments that target a single cognitive domain, such as IMPACT verbal and visual memory also being measures of working memory. Therefore, for descriptive analysis, effect sizes within the same cognitive domain were collapsed and an average effect size was approximated.

5.3.6 Data Coding

Effect sizes extracted from studies assessing global outcome or cognition as an outcome variable were coded for inclusion in a random-effects meta-analysis and moderator analyses. Effect sizes were not coded for studies of either mood or motor outcomes, as only a single study was identified for each outcome, making meta-analysis unfeasible. Further, when assessing global outcome, if dichotomised into unfavourable and favourable outcome, only studies where GOS and GOSE were classed as unfavourable and favourable with a score of 1-3 and 4-5 or 1-4 and 5-8, respectively, were coded for inclusion into a meta-analysis. This was due to such dichotomies representing the most commonly utilised grouping strategies, reflected by preliminary search of studies within this review. Additionally, preliminary searches determined that several studies explored associations between biomarker concentration and continuous outcome scores via correlation analysis, with several others assessing global outcome as categorical variables via t-tests and ROC curve analysis. For uniformity, and to effectively pool categorical and continuous effect sizes, it was decided that correlation coefficients would be analysed as the common effect size of interest. If studies reported correlation coefficients, these were coded directly, while other measures were converted into correlation coefficients, in order to standardise pooling of effect sizes (Harrer et al., 2021). Below are the conversion formulae for different statistical tests:

Table 5-3 ***Formulae used to convert various statistical tests into correlation coefficients, to ensure standardised effect sizes across studies***

Statistical Method	Conversion Scenario
Logistic Regression	Odds ratios were converted into Cohen's d ($\frac{Log\ OR\ x\ 3}{\sqrt{\pi}}$) and then into a correlation coefficient ($\frac{d}{\sqrt{d^2+4}}$) (Borenstein et al., 2010)

Linear Regression	Values for sample size, number of predictors, overall variance explained by the model, and beta coefficient for the predictor of interest were used to estimate a semi-partial correlation coefficient (Aloe and Becker, 2012)
T-Test	If studies reported mean, standard deviation and sample size per group, the t-value and degrees of freedom were calculated using a test statistic calculator (Graphpad, (n.d)). The T-value was then inputted into the following formula to approximate a correlation coefficient: $(\sqrt{\frac{t^2}{t^2+df}})$ (Rosenthal et al., 1994)
ROC Curve Analysis	If studies conducted receiver operating characteristic (ROC) curve analysis and reported the AUC value, it was converted into a Cohen's d = $\sqrt{(2\ x\ z\ (A))}$, and then into a correlation coefficient ($\frac{d}{\sqrt{d^2+4}}$) (Salgado, 2018)

OR = Odds ratio, df = degrees of freedom, t = t-statistic, d = Cohen's d, A = AUC

Several factors should be considered regarding the standardisation of effect sizes within this study. Firstly, some assessments within this review are scored in a manner where higher scores are indicative of worse performance, such as reaction-timing or speed-based tasks. Therefore, for uniformity and per recommendation from other studies (Gucciardi et al., 2022, Assink and Wibbelink, 2016), said effect sizes were inversely coded. This allowed for consistent directionality across effect sizes, whereby negative correlations corresponded to poorer outcome, with the opposite being true for positive correlations. Additionally, some studies assessed non-normally distributed data and therefore employed non-parametric statistical techniques, e.g., Spearman Rho. For the purposes of this study, parametric and non-parametric coefficients were pooled together. Secondly, all coded effect sizes were included in this study, regardless of within-study dependencies using multilevel meta-analysis (see below). Several studies explored multiple effect sizes of interest within the same sample, particularly in relation to the association of different biomarkers with outcome. While all assessed markers fall under

the umbrella category of markers of cell injury, they are still distinct enough to warrant consideration in isolation. For example, NSE and UCH-L1 are considered neuronal proteins, whereas S100β and GFAP are reflective of glial injury, with each biomarker displaying distinct profiles of time course of release (Azizi et al., 2021, Barbu et al., 2022).

Therefore, in instances where multiple biomarkers were assessed within a study, rather than collapsing effect sizes into a singular score, each effect size has been analysed separately. However, dependencies within effect sizes were controlled for by estimating variance with the following formula: $\left(\frac{(1-r^2)^2}{n-1}\right)$ (Cheung et al., 2012). Finally, per recommendation from other studies, Assink & Wibbelink (2016) (Assink and Wibbelink, 2016) and Gucciardi et al. (2022) (Gucciardi et al., 2022), factors considered as potential moderators, namely biomarker type, biofluid source, time of sample, timepoint of assessment and cognitive domain, were coded categorically and analysed subsequently.

5.3.7 Statistical Analyses

All analyses were conducted in R (version 1.4.1717), with the following packages being utilised: *metafor* (Viechtbauer and Viechtbauer, 2015), *metaviz* (Kossmeier et al., 2020), *dmetar* (Harrer et al., 2019), *clubSandwich* (Pustejovsky, 2023), *orchard* (Nakagawa et al., 2023) and *robvis* (McGuinness and Higgins, 2021).

5.3.7.1 Multilevel Meta-Analysis

Multilevel models are utilised when studies include several non-independent, within-study effect sizes, which are unable to be assessed via traditional random-effect meta-analytic techniques. By nature, traditional random-effects meta-analyses rely on a multilevel structure, with effect sizes differing due to sampling error of individual studies and between-study

heterogeneity. Therefore, random effects are allocated to the study level, corresponding to participants being nested within each study, creating a multilevel structure. However, to account for several non-independent effect sizes, another random-effect level within the model is required. Adding an extra intermediate level converts traditional two-level models into a three-level model, accounting for variation amongst effect sizes within-studies, and allowing the inclusion of several effect sizes per study Within-study effect size dependency is accounted for by converting individual studies into a random factor, while within-study effects are coded as fixed factors (Van den Noortgate et al., 2013). Variance distribution across all levels within models (sampling, within-study, and between-study) were calculated manually (Cheung, 2014). Within this study, rather than only assigning random-effects at the study level, the three-level structure involves adding random effects to both between (study level) and within-study effects (individual effect size level). This accounts for dependency in study nested effects in the within-study level.

5.3.7.2 Moderator Analyses

Per recommendation from Hunter and Schmidt (Hunter, 2004), if less than 75% of a multilevel model's variance can be attributed to sampling variance, there is likely a high degree of heterogeneity that warrants moderator analysis. When assessing global outcome, moderators included demographics, such as age and sex, as well as individual biomarker influence on effect size. Further, models were conducted per individual marker to determine whether time of sampling or timepoint post-injury assessment was administered were significant moderators. However, given the differences in blood-based and CSF-derived biomarker kinetics (see review, (Agoston et al., 2017)), it would be inappropriate to collapse such effect sizes together and consequently, time of sampling was independently assessed in each fluid source. When

assessing cognition, the potential influence of demographics, namely age and sex, and cognitive domains on effect sizes were assessed.

In moderator analyses comparing biomarker associations, biofluid source and cognitive domains, the grouping with the largest number of effect sizes was used as the reference category. However, when assessing time of sampling and timepoint post-injury assessment was administered as moderators, the earliest time-point acted as the reference category. Inclusion of reference categories allowed us to compare whether effects between categories significantly differed from the reference. Comparison to the reference category was possible via removal of the model intercept, providing differences in mean effect size between the reference category and the other moderators, and confidence intervals and the p-values were determined from an omnibus test of moderators. For each multilevel moderator analysis, variance-covariance matrices were imputed to control for effect size dependency. In addition, all models' random effects were tested via two techniques: 1) The inner | outer formula and 2) specifying random effects at the unique effect size level (Kevin et al., 2010). This formula has several underlying assumptions, namely that unique studies (outer factor) are independent, while effects within studies (inner factor) are assumed to share correlated random effects, with a level of variance being attributed to the moderator type. Multilevel moderator models used a heteroscedastic compound symmetric structure, which fixes heterogeneity for the levels of the inner factor to the corresponding inner | outer formula, which was suitable for the current review as it optimises balance between theoretical application and feasibility within the extracted data. Further, a minimum of 2 unique effect sizes were required for a moderator to be included in the model. Results of multilevel moderator models are presented in an orchard plot format, allowing for plotting of a large pool of effect sizes and visual comparison of different moderators (Nakagawa et al., 2023).

5.3.8 Publication Bias & Outlier Testing

To ensure the validity of our analyses and consequent findings, sensitivity analyses were conducted, including testing for publication bias, and ensuring effect size estimates were not driven by the presence of outliers. It is recommended that a minimum of two techniques should be conducted per sensitivity analysis to be confident in consequent findings (Noble et al., 2017). To assess potential publication bias, a multilevel version of an Egger's test was utilised and presented in a sunset (power-enhanced) funnel plot format to review the relationship between individual effect sizes and standard errors (Kossmeier et al., 2020). The multilevel version of the Egger's regression reflects the multilevel nature of the models, by accounting for between- and within-study variability by allowing each level of variance to have an added residual component (Fernández-Castilla et al., 2021). Presence of publication bias can be determined statistically via a significant standard error coefficient ($p < 0.05$). This is further validated by results from the sunset funnel plot, whereby the presence of publication bias can be inferred if asymmetry is present, as well as providing the R-index as a measure of estimated replicability across findings (Kossmeier et al., 2020).

Outlier identification was conducted via two methods: Cook's distance and a residual approach (Gucciardi et al., 2022). Cook's distance was used to detect the presence of outliers by setting a threshold to 1.5 multiplied by the average Cook's distance value across individual effect sizes for the multilevel model of interest, with all effect sizes falling outside of the threshold being considered outliers. The residual approach consisted of calculating residuals for each individual effect size and converting them into z-scores, with z-scores greater than 1.5 and less than -1.5 being deemed as outliers. If outliers were detected, multilevel models with outliers removed using each method were conducted for global outcome and cognition. If the model retained significance, it was deemed that outliers were not driving significance in the initial model.

5.3.9 Confidence in Body of Evidence

Evidence across all studies was assessed via an adapted version of the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework (Guyatt et al., 2011). An adapted version of GRADE developed to assess the quality of prognostic studies was utilised (Huguet et al., 2013). GRADE quality of evidence scores can be classified into three domains: 1) high 2) moderate and 3) low. Initially, quality of evidence for outcomes were graded as high. While it is recommended that more exploratory, earlier phase studies (phase 1) should be graded lower before adjusting ratings, given markers of cell injury and their association with TBI is well-established, all studies were considered phase 2 or 3 (Huguet et al., 2013). Quality of evidence was then downgraded by one level for the following reasons: risk of bias (overall high/moderate rating according to QUIPS), inconsistency (pertaining to how outcomes were assessed), indirectness (limited outcome representation, heterogeneity across assessments within outcome, variability across prognostic factor measures), imprecision (variability across effect sizes) and publication bias (noticeable absence of follow-up analysis for non-significant data, corroborated by significant multilevel Egger's test for multilevel meta-analysis.) Quality of evidence was also upgraded by one level if a moderate/large effect size (≥ 0.30) or significant differences if statistical comparison was categorical) or an exposure-gradient response was present. Given that the outcome data was further categorised as global outcome (determined by GOS & GOSE), cognition, mood and motor, it was decided that GRADE would be conducted on each outcome individually.

5.4 RESULTS

Two identical search strategies were conducted for each database in January 2023 and November 2023, yielding a total of 2262 records. Eight hundred records were identified as duplicates and removed prior to screening. Following title and abstract screening, 1022 of the

remaining 1462 records were excluded, leaving 440 studies assessed for eligibility in full-text screening. Following screening, 64 full-text records were deemed eligible for inclusion. Of the 376 records that were excluded, 65 studies required additional data from authors to be included, but were excluded either because the authors were unable to provide data due to legislation (n = 3) or did not respond (n = 62). Additional exclusion criteria are presented in a PRISMA flow diagram (Figure 5-1).

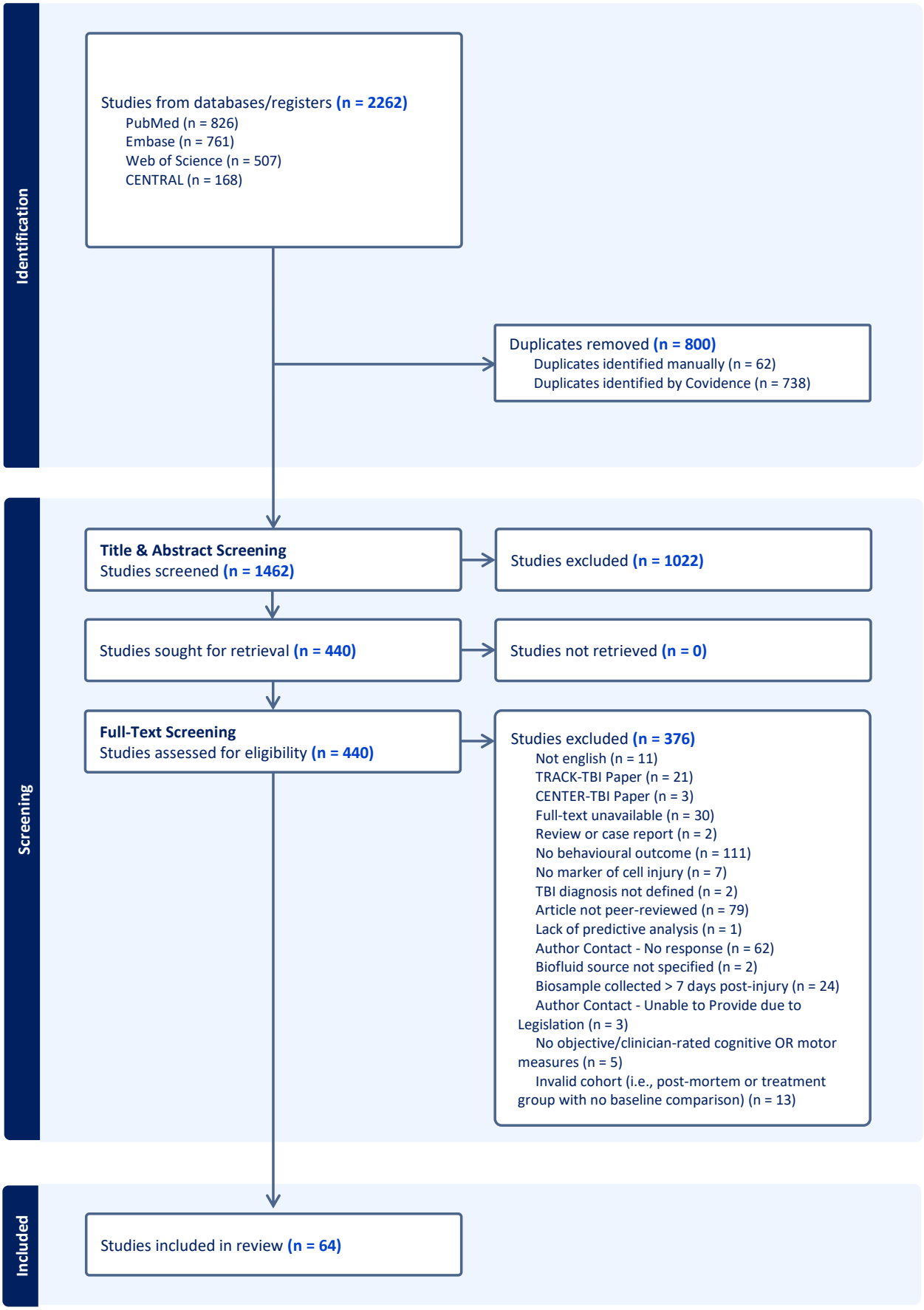


Figure 5-1 PRISMA flow diagram

As summarised in Table 2, a total of 5,686 TBI participants were assessed across 64 studies, with a sample size ranging from 9 to 566 and an average sample size of 89. Studies from China (11 studies), Sweden (9 studies), the United States of America (8 studies), Germany (6 studies), India (5 studies), UK (4 studies) and Netherlands (4 studies) were the most prevalent, accounting for approximately 75% of included studies. Publication dates for included studies ranged between 1997 and 2023, with more than 50% of studies being published between 2014 and 2023. Across all studies, the mean age of participants was 38.5 years, with a standard deviation of 8.5 years, with 4,040 participants being male (71%; reported in 60 studies).

In terms of TBI severities explored in the included studies, the most prevalent was severe (41 studies), followed by mild (23 studies) and moderate (21 studies), with 14 studies pooling severities rather than considering them as distinct groups. Several different biomarkers of cell injury were explored across studies, including S100B (43 studies), NSE (22 studies), GFAP (18 studies), Tau (12 studies), UCH-L1 (11 studies), NF-L (7 studies), pNF-H (3 studies) MBP (3 studies), C-Tau (3 studies) and HMGB1 (1 study). Of the behavioural outcomes assessed, there was a substantial bias towards GOS (44 studies), followed by GOSE (15 studies), with measures related to cognition (7 studies), mood (1 study) and motor outcomes (1 study) explored in a much smaller number of studies. Of the seven studies assessing cognition, the most common cognitive domain assessed was working memory (n = 4), followed by global cognition and attention / executive function (n = 3), with language, visual processing, visuospatial ability and learning / long-term memory being assessed the least (n = 2 per domain). For the single studies assessing mood and motor outcomes, the sub-domain assessed was depression and postural stability, respectively.

5.4.1 Global Outcome

A total of 58 studies explored global outcome and, of these, 44 assessed GOS (76%) and 15 assessed GOSE (26%) (Table 5-4). In general, a negative relationship was detected across studies assessing global outcome, whereby higher biomarker concentration was associated with lower GOS and GOSE scores, and therefore poorer outcome, albeit with substantial variability in effect size strength both between and within-studies. Of these studies, a minority explored the association between biomarkers and global outcome as a continuous variable (n = 18; 31%), while majority explored dichotomised global outcome (n = 52; 90%). The most common dichotomy was classifying unfavourable outcome as GOS 1-3 (n = 34; 59%) or GOSE 1-4 (n = 12; 21%). However, variation in outcome categorisation were present, such as GOS 1 ~ 2-3 ~ 4-5 (n = 2; 3%), 1-2 (n = 1; 1.5%) or 2-3 (n = 1; 1.5%), as well as GOSE 1-3 (n = 1; 1.5%). Nevertheless, trends within these studies reflected the more commonly utilised dichotomies, as when assessing death (GOS = 1), unfavourable outcome (GOS 2-3) and favourable outcome (4-5) categories, significant differences were detected, with Pelinka et al. reporting favourable outcomes being associated with significantly lower GFAP concentrations compared to both unfavourable outcomes and death. Similarly, Castaño-Leon et al found synonymous findings with their chosen dichotomy (unfavourable = GOSE 1-3), with all biomarkers displaying a moderate to high AUC. However, in two studies exploring optimal compared to suboptimal recovery (GOSE 1-7 ~ 8; 3%), only weak associations between biomarkers and global outcome were reported, likely due to the broad nature of sub-optimal recovery in the utilised dichotomy. Further, 15 (26%) studies were ineligible for inclusion in the meta-analysis, either due to lack of available statistics required for conversion into standardised correlation coefficients (n = 9; 16%) or only assessing dichotomies derived from measures other than GOS 1-3 ~ 4-5 or GOSE 1-4 ~ 5-8 (n = 6; 10%).

Table 5-4 Characteristics and descriptive statistics of studies assessing relationship between biomarker of cell injury and global outcome

Study Author (Year) & Country	N	Age; Sex (% male)	TBI Severities & GCS Score	Global Outcome Measure(s)	Grouping	Timepoint(s) post-injury Outcome was Assessed	Time of Sample Extraction	Biofluid Source	Biomarker(s)	Magnitude of Effect
(Al Nimer et al., 2015)* Sweden	182	55†; 75%	Mild; 14-15 Moderate; 9-13 Severe; 3-8	GOS	1-3 ~ 4-5	6-12 months	24 Hours	Serum	NF-L S100B NSE	ns +++ ns
							48 Hours		NF-L S100B NSE	ns + +
							≥72 Hours		NF-L S100B NSE	+++ +++ ++
					Continuous scale		24 Hours		NF-L S100B NSE	ns +++ ns
							48 Hours		NF-L S100B NSE	ns +++ +
							≥72 Hours		NF-L S100B	+++ +++

				NSE	ns
		24 Hours		NF-L	++
				S100B	+
				NSE	ns
	1-3 ~ 4-5	48 Hours		NF-L	ns
				S100B	ns
				NSE	+
		≥72 Hours		NF-L	+++
				S100B	+++
				NSE	+
			CSF		
		24 Hours		NF-L	++
				S100B	++
				NSE	ns
	Continuous scale	48 Hours		NF-L	+
				S100B	ns
				NSE	+
		≥72 Hours		NF-L	+++
				S100B	++
				NSE	+++

					1-3 ~ 4-5				S100B NSE MBP	ns ns ns
(Baker et al., 2009)* Canada	64	41.4; 64.1%	Severe; ≤ 8	GOS	Continuous scale	≤ 1 month	Peak (12 – 48 hours)	Serum	S100B NSE MBP	+ + ns
					1-4 ~ 5-8				S100B NSE MBP	ns ns ns
				GOSE	Continuous scale				S100B NSE MBP	+ + +
(Castaño-Leon et al., 2022) Spain	87	38†; 77%	Mild; 13-15 Moderate; 9-12 Severe; 3-8	GOSE	1-3 ~ 4-8	6 months	< 24 hours	Serum	GFAP NF-L UCH-L1 Tau	++ +++ ++ +++
(Chabok et al., 2012)* Iran	28	31.32; 93%	Severe; ≤ 8	GOS	1-3 ~ 4-5	3 months	< 24 hours	Serum	S100B	ns
							24 hours		S100B	+
							48 hours		S100B	++

							≥72 hours		S100B NSE	++ +
							< 24 hours		S100B	ns
					1-3 ~ 4-5		24 hours		S100B	+
							48 hours		S100B	++
						24 months	≥72 hours		S100B	++
							24 hours		S100B	+
					Continuous scale		48 hours		S100B	++
							≥72 hours		S100B	++
(Chatfield et al., 2002) UK	20	29; -	Severe; ≤ 9	GOS	1-3 ~ 4-5	6 months	24 hours	Plasma	S100B Tau	+++ ns
							48 hours		S100B Tau	+ ns

(Chen et al., 2021a)*	80	- ; 68	Moderate-to-Severe; 3-12	GOS	Continuous scale	1 month	< 24 hours	Serum	S100B	++
							24 hours		S100B	++
							≥72 hours		S100B	++
					Continuous scale	3 months	< 24 hours		S100B	++
							24 hours		S100B	++
							≥72 hours		S100B	++
(Chen et al., 2021b) China	74	34.2; 54%	Moderate; 9-12	GOS	1-3 ~ 4-5	3-6 Months	24 hours	Serum	S100B NSE	+++ +++
			Severe; 3-8						S100B NSE	ns ns
(Chen et al., 2022)*	115	57†; 70%	Moderate-to-Severe; ≤ 12	GOSE	1-4 ~ 5-8	6 months	< 24 hours	Serum	NSE	+++
					Continuous scale				NSE	+++
(Czeiter et al., 2012b)* Hungary	45	49.78; 73.3%	Severe; ≤ 8	GOS	1-3 ~ 4-5	1 week	< 24 hours	Serum	GFAP UCH-L1	+ +
								CSF	GFAP UCH-L1	ns +

						1 month	Serum	GFAP UCH-L1	++ ++
							CSF	GFAP UCH-L1	ns ns
						6 months	Serum	GFAP UCH-L1	++ ns
							CSF	GFAP UCH-L1	ns ns
(Di Battista et al., 2015)* Canada	85	45.8; 77.6%	Moderate; 9-12 Severe; 3-8	GOSE	1-4 ~ 5-8	6 months	< 24 hours	Plasma	GFAP S100B NSE	+ + ns
							24 hours		GFAP S100B NSE	+ + ns
(Du et al., 2018)* China	106	35†; 61%	Severe; 3-8	GOS	1-3 ~ 4-5	6 months	< 24 hours	Serum	GFAP UCH-L1 S100B NSE Tau MBP pNF-H	++ +++ +++ +++ ++ ++ ++

(Frankel et al., 2019)* United States	566	34†; 75.3%	Moderate-to-Severe; 4-12	GOSE	1-4 ~ 5-8	6 months	< 24 hours	Serum	GFAP UCH-L1 S100B	+++ +++ +++
(Gonzalez-Ortiz et al., 2023)* Sweden	39	36†; 66.6%	Severe; ≤ 8	GOS	1-3 ~ 4-5	> 12 months	48 hours ≥72 hours	Serum	NF-L Tau NF-L Tau	ns ns ns ns
(Goyal et al., 2013) United States	138	35.6; 79.7%	Severe; ≤ 8	GOS	1 ~ 2-3 ~ 4-5	6 months	0-5 days	Serum CSF	S100B S100B	ns ++
(Huebschmann et al., 2020) United States	121	75.1; 51.2%	Mild; 14-15	GOSE	1-6 ~ 7-8	1 week	< 24 hours	Plasma Serum	GFAP GFAP	+ ++
(John et al., 2022)* India	23	29.4; -	Severe; ≤ 8	GOS	1-3 ~ 4-5 Continuous scale	6 months	48 hours	Serum	S100B S100B	+ +
(Lagerstedt et al., 2020) Finland	88	- ; 69%	Mild; 13-15 Moderate; 9-12 Severe; 3-8	GOSE	1- 4 ~ 5-8	6 – 12 months	< 24 hours	Serum	GFAP NF-L S100B	++ ns ns

(Lee et al., 2015)* Korea	45	58.5†; 73%	Mild; 13-15 Severe; < 6	GOS	1-3 ~ 4-5	3 months	< 24 hours	Serum	GFAP UCH-L1 S100B	+++ +++ +++
(Lei et al., 2015)* China	67	37.2; 76%	Severe; 3-8	GOS	1-3 ~ 4-5	6 months	< 24 hours	Serum	GFAP	+++
							24 hours		GFAP	++
							48 hours		GFAP	+
							≥72 hours		GFAP	++
(Lesko et al., 2014)* United Kingdom	100	31†; 81%	Mild-to-Moderate; ≥ 9 Severe; ≤ 8	GOS	1-3 ~ 4-5	3 months	24 hours	Serum	S100B	+++
(Liliang et al., 2010)* Taiwan	34	40.7; 79%	Severe; ≤ 8	GOS	1-3 ~ 4-5	6 months	< 24 hours	Serum	Tau	+++
(Ljungqvist et al., 2017)* Sweden	9	40.4; 78%		GOSE	1-4 ~ 5-8	12 months	Within 6 days	Serum	NF-L S100B	ns ns
									NF-L S100B	ns ns

(Lu et al., 2020)* China	52	39†; 58%	Severe; 3-8	GOSE	1-4 ~ 5-8 Continuous scale	6 months		Serum CSF Serum CSF	NSE NSE NSE NSE	+ + +++ +++
(Meric et al., 2010)* Turkey	80	31†; 31%	Mild; 14-15 Moderate; 9-13 Severe; ≤ 8	GOS	1-3 ~ 4-5	1 month	< 24 hours	Serum	NSE	+++
(Metting et al., 2012) Netherlands	94	34.3; -	Mild; 13-15	GOSE	1-7 ~ 8 Continuous scale	6 months	"Admission"	Serum	GFAP S100B GFAP S100B	ns ns + ns
(Naeimi et al., 2006) Austria	45	52; 66%	Mild; 13-15 Moderate; 9-12 Severe; ≤ 8	GOS	Continuous scale	3-12 months	< 24 hours	Serum	S100B NSE	ns ns
(Ni et al., 2020b)* China	229	41.3; 67%	Mild; 13-15 Moderate; 9-12 Severe; ≤ 8	GOS	1-3 ~ 4-5	6 months	24 hours ≥72 hours	Serum	Tau Tau	+ +

							24 hours		Tau	+++
							≥72 hours		Tau	++
(Niyonkuru et al., 2013) United States	138	35.6; 80%	Severe; ≤ 8	GOS	1 ~ 2-3 ~ 4-5	6 months	"First week of injury"	CSF	S100B	ns
(Olivecrona et al., 2009)* Sweden	48	35.5; 65%	Severe; ≤ 8	GOS	1-3 ~ 4-5	3 months	Within 48 hours	Serum	S100B NSE	ns ns
						12 months			S100B NSE	ns ns
(Olivecrona et al., 2015)* Sweden	48	35.5; 65%	Severe; ≤ 8	GOS	Continuous scale	3 months	< 24 hours	Serum	S100B NSE	+ ns
							≥72 hours		S100B NSE	+ ++
(Ost et al., 2006)* Sweden	39	49†; 77%	Severe; ≤ 8	GOSE	1-4 ~ 5-8	12 months	24 hours			ns
							48 – 72 hours	CSF	Tau	++
							≥72 hours			ns

(Pandey et al., 2017b)* India	40	38.8; 70%	Severe; 3-8	GOS	1-3 ~ 4-5	Discharge	< 24 hours	Serum	C-Tau	+++
(Pandey et al., 2017a)* India	40	39.5; 77.5%	Mild; 13-15	GOS	1-3 ~ 4-5	Emergency room setting	< 24 hours	Serum	C-Tau	+++
(Papa et al., 2010)* United States	41	38; 80%	Severe; ≤ 8	GOS	1-3 ~ 4-5	6 months	≥72 hours	CSF	UCH-L1	ns
(Pelinka et al., 2004) Austria	101	25†; 75%	Moderate-to-severe; 3-12	GOS	2-3 ~ 4-5	3 months	< 24 hours	Serum	GFAP	+
(Raabe and Seifert, 2000) Germany	25	47†; -	Severe; ≤ 8	GOS	1-3 ~ 4-5	6 months		Serum	S100B NSE	++ ns
(Raheja et al., 2016)* India	86	32.3; 85%	Severe; 4-8	GOS	Continuous scale	6 months	< 24 hours	Serum	GFAP S100B NSE	ns ns +
							≥72 hours		GFAP S100B NSE	+++ ns ns

						12 months	< 24 hours		GFAP S100B NSE	ns ns +
							≥72 hours		GFAP S100B NSE	+++ ns ns
(Rainey et al., 2009)* United Kingdom	100	31†; 81%	Severe; -	GOS	1-3 ~ 4-5 Continuous scale	3 months	24 hours	Serum	S100B S100B	+ ++
(Stefanovic et al., 2017)* Serbia	130	40.2; 77%	Mild; 13-15 Moderate; 9-12 Severe; ≤ 8	GOS	1-3 ~ 4-5	2 weeks	< 24 Hours	Serum	S100B NSE	+++ +++
							24 Hours		S100B NSE	+++ +++
							48 Hours		S100B NSE	+++ +++
							≥72 Hours		S100B NSE	+++ +++

(Shahim et al., 2016)* Sweden	72	36†; -	Severe; ≤ 8	GOS	Continuous scale	12 months	< 24 Hours	Serum	NF-L S100B NF-L S100B	++ ns ++ ns
(Svingos et al., 2022)* United States	59	40.27; 75%	Severe; ≤ 8	GOS	1-3 ~ 4-5	7-10 years	< 24 hours	CSF	UCH-L1	+
(Thelin et al., 2016) Sweden	417	52†; 80%	Mild; 14-15 Moderate; 9-13 Severe; ≤ 8	GOS	1-3 ~ 4-5	3-6 months	Within 48 Hours	Serum	S100B NSE	+++ +++
(Thelin et al., 2013) Sweden	265	50†; 80%	Mild; 14-15 Moderate; 9-13 Severe; ≤ 8	GOS	1-3 ~ 4-5	6-12 months	Within 48 Hours	Serum	S100B	+++
(Tomita et al., 2019) Japan	40	- ; 60%	Severe-to-Mild; 3-15	GOS	1-3 ~ 4-5	Discharge	< 24 Hours	Serum	Tau	ns
(Trnka et al., 2023)* Czech Republic	124	61.2†; 71%	-	GOS	Continuous scale	1 month	< 24 Hours 24 Hours	Serum	S100B S100B	+++ +++

							72 hours		S100B	+++
(Vos et al., 2004)* Netherlands	83	32†; 72%	Severe; ≤ 8	GOS	1-3 ~ 4-5	6 months	Within 36 hours	Serum	GFAP S100B NSE	+++ +++ +++
(Vos et al., 2010)* Netherlands	79	47†; 72%	Moderate; 9-12 Severe; 3-8	GOSE	1-4 ~ 5-8	6 months	< 24 hours	Serum	GFAP S100B	++ +++
(Walder et al., 2013)* Switzerland	49	39†; 77.5%	Severe; -	GOSE	1-4 ~ 5-8	3 months	< 24 hours	Plasma	S100B	ns
							24 Hours		S100B	ns
							48 Hours		S100B	ns
							< 24 hours		S100B	++
					Continuous scale		24 Hours		S100B	+++
							48 Hours		S100B	++
(Wang et al., 2012a)* China	106	45.4; 72%	Severe; ≤ 8	GOS	1-3 ~ 4-5	12 months	< 24 hours	Plasma	HMGB1	+++

(Wang et al., 2016)* China	56	30.4; 62.5%	Mild; 13-15 Moderate; 9-12 Severe; 3-8	GOSE	1-4 ~ 5-8	12 months	48 Hours	Serum	Tau	+++
(Wiesmann et al., 2010)* Germany	60	43.8†; 75%	Mild; 13-15 Moderate; 9-12 Severe; 3-8	GOS	1-3 ~ 4-5	6 months	< 24 Hours 24 Hours < 24 Hours 24 Hours	Serum Plasma	GFAP GFAP S100B S100B	+++ ns +++ +++
(Woertgen et al., 1997) Germany	30	32; 77%	Severe; 3-8	GOS	1-2 ~ 3-5	Discharge	N/A	Serum	S100B NSE	+ ns
(Woertgen et al., 1999)* Germany	44	35; 73%	Severe; ≤ 8	GOS	1-3 ~ 4-5 -	11 months (average)	< 24 hours	Serum	S100B S100B	+++ +++
(Woertgen et al., 2002)* Germany	51	34.5; 75%	Severe; ≤ 8	GOS	1-3 ~ 4-5 Continuous scale	5-24 months	< 24 hours	Serum	S100B S100B	+++ +++

(Wunderlich et al., 2003)* UK	119	49†; 60%	Mild; 13-15	GOSE	1-4 ~ 5-8 -	1 month	< 24 Hours	Serum	S100B S100B	+++ +++
(Zemlan et al., 2002)* Germany	28	35.1; 79%	Severe; 3-8	GOS	1-3 ~ 4-5 Continuous scale	Discharge	24 Hours 48 Hours 72 Hours 24 Hours	CSF	C-Tau C-Tau C-Tau C-Tau	+++ ++ + ++
(Zhang et al., 2014)* United States	102	40.5; 67%	Severe; 3-8	GOS	1-3 ~ 4-5	6 months	< 24 Hours	Plasma	GFAP UCH-L1 S100B NSE Tau pNF-H MBP	+++ +++ +++ +++ +++ +++ +++
(Zhang et al., 2019b)* China	102	36†; 63%	Severe; ≤ 8	GOS	1-3 ~ 4-5	6 months	< 24 Hours	Serum	GFAP UCH-L1 S100B NSE Tau pNF-H MBP	+++ +++ +++ +++ +++ +++ +++

† = Age reported as median; * = Included in meta-analysis

5.4.1.1 Multilevel Meta-Analysis of Global Outcome

A multilevel meta-analysis of 243 biomarker effect sizes across 43 studies demonstrated that higher biomarker concentration in the 7-day period following injury was moderately associated (as determined by conversion formulas in Table 2) with lower GOS and GOSE scores, indicative of worse global functional outcome at any time point post-injury. Sensitivity analyses were conducted by separating GOS and GOSE effect sizes; however, as the overall coefficient and model performance did not substantially differ, GOS and GOSE effect sizes were pooled together to improve statistical power (Table 5). The mixed-effect model presented high heterogeneity, determined by an I^2 value of 84% and high *Q* statistic (1582.95, $p < 0.0001$), representing high variance in effects within and between studies. Using formulae described by Cheung et al (Cheung, 2014), variance was calculated for each level of the model, with little variance attributed to sampling (16.5%) and most being attributed to within-studies (40.8%) and between-studies effects (42.7%).

Table 5-5 Multilevel meta-analysis assessing the relationship between biomarkers of cell injury extracted within a week of TBI and global outcome, as assessed via GOS and GOSE, at any timepoint post-injury

Outcome	*k*	*n*	Mean CC (95% CI)	*p*	I^2	*t-statistic*	*Q* (*p*)
Glasgow Outcome Scale	33	214	-0.47 (-0.52, -0.42)	**< 0.0001**	78%	-20.28	1217.03 **(<0.0001)**
Glasgow Outcome Scale-Extended	11	29	-0.47 (-0.62, -0.32)	**< 0.0001**	93%	-6.41	355.82 **(<0.0001)**
Combined	43	243	-0.48 (-0.53, -0.43)	**< 0.0001**	84%	-19.45	1562.97 **(<0.0001)**

k = number of unique studies. n = number of effect sizes. CC = correlation coefficient. CI = confidence interval. β = regression coefficient. Q = Q statistic test for residual heterogeneity. t = t-statistic

p-value column indicates whether the models were significant

5.4.1.2 Moderator Analyses

Significant heterogeneity in the global outcome multilevel model justified exploration of potential sources of variation through moderator analyses. Results from these moderator analyses are summarised in Table 5-6, displaying the mean correlation for each category within a model and their corresponding beta coefficient, reflective of a moderator's deviation relative to a reference category. The reference category p-value reflects whether the coefficient significantly deviates from 0, whereas the p-values in other categories reflect whether they deviate significantly from the reference category. Significant differences between moderators and the reference category, where present, are denoted by bolded p-values and t-statistics. Further, in moderator analyses not explicitly separating continuous and categorically assessed effect sizes, if a biomarker within a single study was assessed both continuously and categorically, the continuous assessment was retained, while the categorical variable was filtered out, to minimise skewing the model findings. Additionally, when assessing time-point post-injury sample was extracted, due to the limited number of effect sizes reflective of CSF-derived biomarkers, moderator analyses exploring individual biomarkers could not be conducted.

Table 5-6 ***Results of global outcome moderator analysis***

Predictor	*k*	*n*	Mean CC (95% CI)	*β* (95% CI)	*p*	*t*	*Q* (*p*)	*ToM* (*p*)
Demographics							1105.93	
Age	-	-	-	-0.003 (-0.01, 0.01)	0.65	0.46	(**<0.0001**)	
Proportion of Males	-	-	-	0.003 (-0.03, 0.01)	0.95	0.06	1120.21 (**<0.0001**)	
Biofluid Source							1046.97	0.05
Blood †	39	154	-0.48 (-0.53, -0.43)	*Reference*	**<0.0001**	-17.83	(**<0.0001**)	(0.83)
CSF	7	40	-0.50 (-0.65, -0.34)	-0.02 (-0.17, 0.14)	0.83	-0.22		
Global Outcome Operationalisation								
Continuous							623.37	2.07
S100β †	11	38	-0.50 (-0.56, -0.43)	*Reference*	**<0.0001**	-15.56	(**<0.0001**)	(0.09)
GFAP	1	2	-0.32 (-0.62, -0.03)	0.18 (-0.12, 0.46)	0.25	1.17		
NF-L	3	14	-0.35 (-0.46, -0.25)	0.15 (0.04, 0.25)	**0.01**	2.63		
NSE	6	20	-0.48 (-0.67, -0.29)	0.02 (-0.18, 0.21)	0.88	0.15		
Tau	1	4	-0.41 (-0.62, 0.21)	0.09 (-0.13, 0.29)	0.45	0.75		
Dichotomised							775.28	0.64
S100β †	20	46	-0.48 (-0.54, -0.41)	*Reference*	**<0.0001**	15.44	(**<0.0001**)	(0.77)
C-Tau	3	5	-0.47 (-0.66, -0.27)	0.01 (-0.19, 0.21)	0.922	0.08		
GFAP	11	27	-0.51 (-0.59, -0.44)	-0.03 (-0.12, 0.04)	0.35	-0.92		
MBP	3	3	-0.49 (-0.63, -0.35)	-0.01 (-0.16, 0.13)	0.80	-0.25		
NF-L	2	13	-0.44 (-0.53, -0.36)	0.04 (-0.05, 0.12)	0.44	0.77		
NSE	10	24	-0.43 (-0.52, -0.33)	0.05 (-0.03, 0.13)	0.22	1.23		
pNFH	3	3	-0.46 (-0.60, -0.32)	0.02 (-0.13, 0.16)	0.84	0.20		

SBDP	2	7	-0.43 (-0.56, -0.30)	0.05 (-0.08, 0.18)	0.47	0.70		
Tau	8	12	-0.47 (-0.61, -0.33)	0.01 (-0.13, 0.14)	0.97	0.04		
UCH-L1	7	20	-0.45 (-0.56, -0.33)	0.03 (-0.08, 0.14)	0.60	0.53		
Overall							1133.36	1.43
S100β †	24	64	-0.49 (-0.54, -0.45)	*Reference*	**<0.0001**	-20.06	(**<0.0001**)	(0.18)
C-Tau	3	5	-0.49 (-0.66, -0.31)	0.00 (-0.17, 0.19)	0.92	0.10		
GFAP	13	31	-0.48 (-0.55, -0.40)	0.01 (-0.06, 0.1)	0.65	0.46		
MBP	4	5	-0.35 (-0.56, -0.14)	0.14 (-0.06, 0.35)	0.18	1.36		
NF-L	4	15	-0.36 (-0.45, -0.27)	0.13 (-0.04, 0.23)	**0.005**	2.88		
NSE	13	31	-0.44 (-0.54, -0.34)	0.05 (-0.04, 0.15)	0.22	1.24		
pNFH	3	3	-0.44 (-0.61, -0.28)	0.05 (-0.11, 0.22)	0.54	0.62		
SBDP	2	7	-0.40 (-0.54, -0.27)	0.09 (-0.05, 0.23)	0.20	1.3		
Tau	8	12	-0.50 (-0.63, -0.38)	-0.01 (-0.13, 0.12)	0.90	-0.13		
UCH-L1	8	21	-0.42 (-0.54, -0.31)	0.07 (-0.05, 0.19)	0.23	1.20		
Time Post-Injury Sample								
was Extracted							559.46	1.3
Blood-Derived Biomarkers							(**<0.0001**)	(0.26)
1-6 Hours	17	51	-0.46 (-0.52, -0.39)	*Reference*	**<0.0001**	-14.11		
6-12 Hours	2	2	-0.47 (-0.57, -0.43)	-0.01 (-0.19, 0.17)	0.93	-0.09		
12-24 Hours	13	27	-0.50 (-0.57, -0.43)	-0.04 (-0.12, 0.03)	0.22	-1.24		
Day 2	6	10	-0.50 (-0.60, -0.39)	-0.04 (-0.15, 0.07)	0.46	-0.74		
Day 3	8	14	-0.52 (-0.60, -0.43)	-0.06 (-0.15, -0.03)	0.20	-1.30		
Day 4	2	4	-0.64 (-0.89, -0.39)	-0.18 (-0.43, 0.06)	0.13	-1.51		
Day 5	4	7	-0.47 (-0.57, -0.37)	-0.01 (-0.12, 0.10)	0.85	-0.19		
Day 6	1	3	-0.31 (-0.45, -0.18)	0.15 (0.003, 0.29)	**0.04**	1.98		
Day 7	5	10	-0.50 (-0.68, -0.33)	-0.04 (-0.22, 0.12)	0.58	-0.56	122.69	0.33
Blood-Derived S100B							(**<0.0001**)	(0.90)

1-6 Hours †	13	14	-0.44 (-0.52, -0.36)	*Reference*	**<0.0001**	-11.48		
6-12 Hours	2	2	-0.44 (-0.58, -0.30)	0.0 (-0.16, 0.16)	0.99	0.02		
12-24 Hours	11	13	-0.51 (-0.60, -0.42)	-0.07 (-0.20, 0.06)	0.27	-1.11		
Day 2	4	5	-0.49 (-0.57, -0.40)	-0.05 (-0.16, 0.07)	0.42	-0.83		
Day 3	6	8	-0.47 (-0.55, -040)	-0.03 (-0.14, 0.08)	0.57	-0.53		
Day 5	3	4	-0.45 (-0.54, -0.36)	-0.01 (-0.13, 0.11)	0.86	-0.15		
Day 7	2	3	-0.40 (-0.55, -0.26)	0.04 (-0.12, 0.19)	0.63	0.61	91.27	0.17
Blood-Derived GFAP							(**<0.0001**)	(0.85)
1-6 Hours †	8	10	-0.50 (-0.58, -0.41)	*Reference*	**<0.0001**	-12.71		
12-24 Hours	5	8	-0.45 (-0.64, -0.26)	0.05 (-0.212, 0.21)	0.57	0.58		
Day 7	1	2	-0.50 (-0.70, -0.31)	0.0 (-0.22, 0.20)	0.92	-0.11	47.29	0.50
Blood-Derived NSE							(**<0.0001**)	(0.69)
1-6 Hours †	7	8	-0.40 (-0.51, -0.28)	*Reference*	**<0.0001**	-7.82		
12-24 Hours	2	3	-0.37 (-0.51, -0.22)	0.02 (-0.11, 0.17)	0.64	0.48		
Day 2	2	3	-0.33 (-0.45, -0.22)	0.07 (-0.07, 0.20)	0.31	1.07		
Day 3	3	3	-0.45 (-0.76, -0.14)	-0.05 (-0.33, 0.22)	0.67	-0.44	3.58	7.65
Blood-Derived NF-L							(0.17)	(0.12)
12-24 Hours †	2	2	-0.27 (-0.58, -0.04)	*Reference*	**<0.0001**	-0.37		
Day 7	2	2	-0.16 (-0.64, 0.33)	0.11 (-0.21, 0.84)	0.34	1.25		
Blood-Derived Tau							6.01	12.49
1-6 Hours †	4	4	-0.59 (-0.73, -0.46)	*Reference*	**<0.0001**	-12.32	(0.20)	(**0.02**)
Day 7	2	2	-0.34 (-0.49, 0.19)	0.25 (0.06, 0.46)	**0.02**	5.53		
Blood-Derived UCH-L1							84.53	2.28
1-6 Hours †	5	7	-0.44 (-0.67, -0.22)	*Reference*	**<0.0001**	-4.41	(**<0.0001**)	(0.17)
12-24 Hours	2	4	-0.62 (-0.75, -0.48)	-0.18 (-0.43, 0.09)	0.17	-1.51		
CSF-Derived Biomarkers							113.01	1.57
1-6 Hours †	2	10	-0.32 (-0.49, -0.16)	*Reference*	**<0.0001**	-3.98	(**<0.0001**)	(0.20)
12-24 Hours	3	13	-0.48 (-0.64, -0.33)	-0.16 (-0.35, 0.03)	0.09	-1.76		
Day 2	3	5	-0.47 (-0.64, -0.31)	-0.15 (-0.37, 0.07)	0.18	-1.39		
Day 3	2	2	-0.21 (-0.47, -0.05)	0.11 (-0.19, 0.41)	0.46	0.74		

Day 5	2	4	-0.38 (-0.67, -0.09)	-0.06 (-0.37, 0.26)	0.72	-0.36		
Day 6	1	3	-0.36 (-0.46, -0.15)	-0.04 (-0.21, -0.22)	0.79	-0.27		
Time Post-Injury								
Outcome was Assessed								
All Biomarkers							1145.27	0.37
< 1-Month †	11	51	-0.47 (-0.54, -0.39)	*Reference*	**<0.0001**	-13.17	(**<0.0001**)	(0.78)
3-Months	7	23	-0.51 (-0.63, -0.41)	-0.04 (-0.16, 0.07)	0.44	-0.78		
6-Months	18	67	-0.47 (-0.53, -0.40)	0.0 (-0.08, 0.08)	0.99	0.01		
> 6-Months	12	53	-0.45 (-0.55, -0.35)	0.02 (-0.10, 0.14)	0.73	0.24		
S100B							196.10	0.47
< 1-Month †	4	14	-0.45 (-0.53, -0.36)	*Reference*	**<0.0001**	-12.11	(**<0.0001**)	(0.71)
3-Months	7	19	-0.53 (-0.64, -0.42)	-0.08 (-0.20, 0.05)	0.24	-1.18		
6-Months	9	10	-0.47 (-0.58, -0.36)	-0.02 (-0.15, 0.11)	0.81	-0.24		
> 6-Months	5	19	-0.48 (-0.57, -0.39	-0.01 (-0.14, 0.09)	0.65	-0.46		
GFAP							90.92	1.18
< 1-Month †	2	10	-0.41 (-0.60, -0.22)	*Reference*	**<0.0001**	-4.40	(**<0.0001**)	(0.34)
6-Months	12	19	-0.47 (-0.54, -0.39)	-0.06 (-0.26, 0.14)	0.55	-0.61		
NSE							281.06	1.18
< 1-Month †	3	8	-0.43 (-0.60, -0.26)	*Reference*	**<0.0001**	-5.20	(**<0.0001**)	(0.34)
3-Months	1	2	-0.31 (-0.67, 0.06)	0.12 (-0.28, 0.52)	0.54	1.33		
6-Months	8	9	-0.48 (-0.67, -0.30)	-0.05 (-0.31, 0.20)	0.65	0.62		
> 6-Months	2	12	-0.27 (-0.45, -0.09)	0.16 (-0.09, 0.41)	0.19	-0.46		
Tau							43.98	0.001
6-Months †	6	8	-0.55 (-0.67, -0.43)	*Reference*	**<0.0001**	-9.91	(**<0.0001**)	(0.98)
> 6-Months	3	4	-0.55 (-0.93, -0.16)	0 (-0.40, 0.41)	0.98	0.03		

k = number of unique studies. n = number of effect sizes. CC = correlation coefficient. CI = confidence interval.
β = regression coefficient. Q = Q statistic test for residual heterogeneity. ToM = Test of Moderators

† Reference category for model. Effect sizes and 95% CI for each specific predictor are obtained from separate models that used that predictor as the reference category.

t = t-statistic relative to the reference category

The p-value column and associated β-values indicate whether individual predictors significantly differ (≤ 0.05) from the reference category. Significance is denoted by bolded values.

Demographics

A random effects meta-regression was conducted to determine if age and the proportion of males influenced biomarker associations with global outcome between studies, with models finding no significant influence of these factors. Further, substantial heterogeneity was still present in the models ($p < 0.0001$), indicating that other moderators, rather than age and sex, drive variations in biomarker associations with global outcome.

Fluid Source

A multilevel model was conducted assessing the potential effect size differences between blood- and CSF-derived biomarkers, with blood-based biomarkers acting as the reference category. Both moderators significantly deviated from 0 ($p < 0.0001$), indicating that both blood and CSF-derived biomarkers are significantly associated with global outcome following injury. However, no significant differences in effect sizes between biomarker and CSF-derived markers were present

Global Outcome Operationalisation

Due to variation in the way global outcome was operationalised, multilevel models separating effect sizes where global outcome was assessed as a continuous outcome versus dichotomised GOS (unfavourable = GOS 1-3) and GOSE (unfavourable = GOSE 1-4) outcome assessment were conducted. A multilevel model was also conducted collapsing the continuous and categorical assessments together, to improve statistical power Further, while all biomarkers

within this review are reflective of cell injury, markers may demonstrate differential prognostic utility. Therefore, individual biomarkers acted as moderators within these multilevel models, with S100B acting as a reference category. Across all models, all moderators significantly deviated from 0 ($p < 0.0001$), indicating all biomarkers of cell injury are significantly associated with global outcome following injury. While minimal differences between biomarkers relative to S100B as a reference category were present, NF-L demonstrated significantly weaker effect size in the model assessing global outcome as a continuous variable (0.15, $p = 0.01$), as well as the model collapsing continuous and dichotomised outcomes (0.13, $p = 0.005$), suggesting that it is not as prognostic for global outcome post-injury. Minimal differences between moderators were reflected by non-significant test of moderators across models ($p > 0.05$). Further, substantial variability was still present in the models, as determined by high Q-statistics ($p < 0.0001$), warranting further exploration into additional moderating variables at the individual biomarker level.

Time Post-Injury Sample was Extracted

A contributor to the large number of between and within-study effect sizes within the current review is variability in the time post-injury that the biofluid sample was extracted. Therefore, models were conducted to determine the potential moderating effects of sampling time on individual biomarkers, as well as all biomarkers collapsed together, with the earliest timepoint acting as a reference category. Further, given differences in biomarker and release patterns between blood- and CSF-derived biomarkers (Agoston et al., 2017), biofluid sources were assessed separately in these multilevel models. Across all models, the time of sampling moderating categories significantly deviated from 0 ($p < 0.05$) in most cases, indicating that biomarkers of cell injury collected within a week of injury generally yield significant associations with global outcome. In only a few cases was this non-significant, including blood-

derived NF-L and tau extracted seven days following injury (Table 6). In fact, minimal differences relative to a reference category were found across models, with two exceptions: in the overall blood-derived biomarker model, markers extracted six days post-injury, and in the blood-derived tau model, tau extracted seven days post-injury, resulted in significantly weaker effect sizes relative to the reference category (i.e. 1-6 hours post-injury). This was supported by the fact that within the blood-derived tau model, a significant test of moderators was found ($p = 0.02$), albeit not the blood-derived biomarker model ($p = 0.26$). Further, while several models still presented significant heterogeneity ($p < 0.0001$), save for blood-derived NF-L and tau, Q-statistics were shown to substantially decrease when compared to other models.

Time Post-Injury Outcome was Assessed

Given variability in the timepoints outcome were assessed, ranging from emergency room discharge up to 10-years post-injury, the moderating effects of timepoint following injury at which outcome assessment was administered were assessed. Like the models exploring time post-injury when the sample was extracted, models separating individual biomarkers, as well as a model collapsing all biomarkers together, were developed, with the earliest timepoint available acting as a reference category. Across all models, all moderators significantly deviated from 0 ($p < 0.05$), aside from the association between NSE and global outcome assessed at 3-months post-injury. Further, across all models, no significant differences between time-points assessed relative to the earliest timepoint as a reference category were found, which was further reflected by no model resulting in a significant test of moderators. Additionally, while all models reported substantial heterogeneity ($p < 0.0001$), the Q-statistic was shown to substantially decrease relative to previous multilevel models not assessing individual biomarker associations. This indicates that assessing biomarkers individually allows for the

model to better account for variation, as opposed to models collapsing markers of cell injury together corresponding to higher heterogeneity.

Analyses where significant differences in moderators relative to a reference category were found are presented in Figure 5-2:

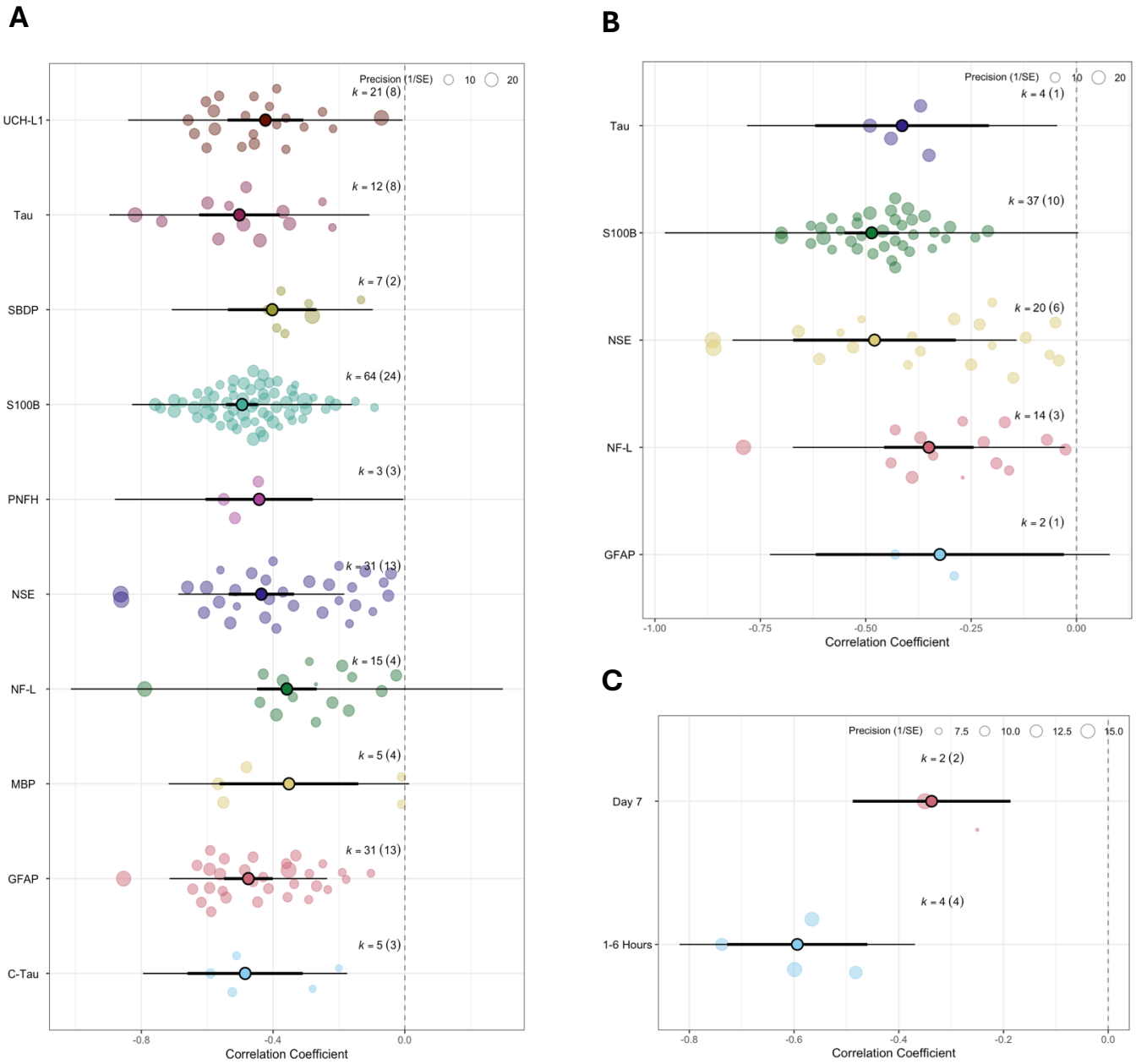


Figure 5-2 ***Orchard plots visualising moderator analyses with significant differences in moderator effect sizes.*** *NF-L demonstrated significantly lower effect sizes relative to S100B (acting as the reference category) for both (A) global outcome, whereby continuous and categorical assessment were collapsed and (B) when assessed on a continuous scale. Further, moderator analysis assessing the time of sample extraction per individual biomarker determined that (C) tau extracted within 1-6 hours of injury (acting as the reference category) had a significantly higher effect size compared to tau extracted seven days following injury, indicating tau extracted at earlier timepoints demonstrate higher associations with global outcome.*

5.4.1.3 Outlier and Publication Testing

Outlier effect sizes were determined via two methods: 1) Residual test and 2) Cook's distance approach. Sensitivity analysis was conducted, with outliers removed using both methods and compared to the initial overall model, to ensure outliers were not driving effects. In addition, Egger's test was conducted via meta-regression to assess publication bias via calculating standard errors from the variance of each effect size. Publication bias was assessed from visual inspection of a power-enhanced sunset funnel plot of symmetry.

5.4.1.4 Outlier Analysis

Out of the 243 unique effect sizes, 38 and 29 outliers were identified as outliers using the residuals analysis and Cook's distance, respectively (Figure 5-3). Outlier removal via the residual method resulted in no significant change ($r = 0.48$) in the association between biomarkers of cell injury and global outcome, and the model remained significant. While heterogeneity was still significant, as determined via the Q statistic, it was noticeably lower after the removal of outlier effect sizes ($Q(208) = 665.24$, $p < 0.0001$) compared to the initial multilevel model ($Q(247) = 1582.95$, $p < 0.0001$) . Likewise, while removal of outliers using Cook's distance slightly reduced the overall effect ($r = 0.45$) compared to the initial model ($r = 0.47$), the model remained significant and heterogeneity was still significant, albeit with a reduced Q statistic ($Q(220) = 1164.91$, $p < 0.0001$). Therefore, it can be inferred that outliers were not driving pooled effects for overall global outcome

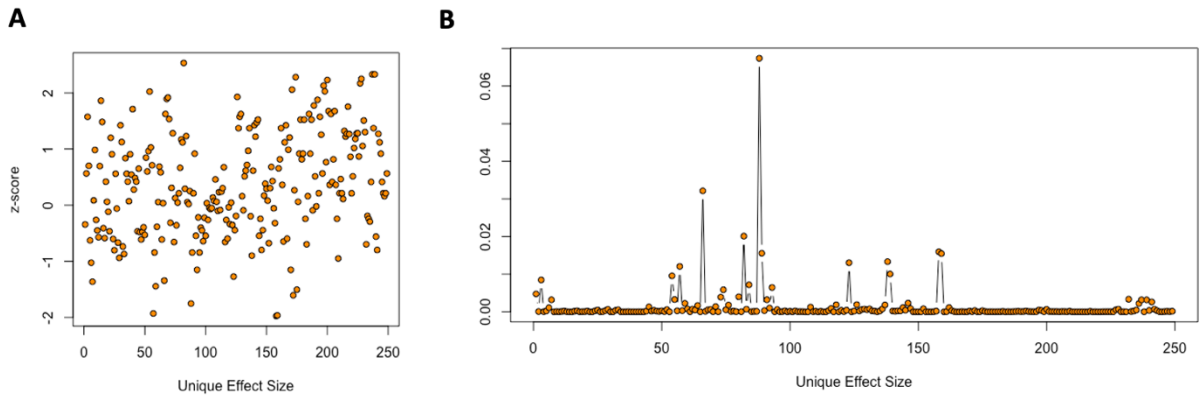


Figure 5-3 Outlier tests for effect sizes included in Global Outcome multilevel meta-analysis. *(A) For residual assessment, effect sizes with a residual z-score > 1.5 or < -1.5 were classified as outliers. (B) Effect sizes with a Cook's distance value of > 1.5 multiplied by the mean Cook's distance score (0.0014) were classified as outliers.*

5.4.1.5 Publication Bias

A sunset (power-enhanced) funnel plot of unique effect sizes included in the overall combined GOS and GOSE multilevel meta-analysis is presented in Figure 5-4. Visual inspection of the plot, and a significant multilevel Egger's regression test ($p < 0.0001$), indicated likely asymmetry, suggesting that publication bias impacted the association between concentrations of biomarkers of cell injury and global outcome. Additionally, the reported median power of unique effects was 99.7%, with an estimated likelihood of results replication of 100% probability.

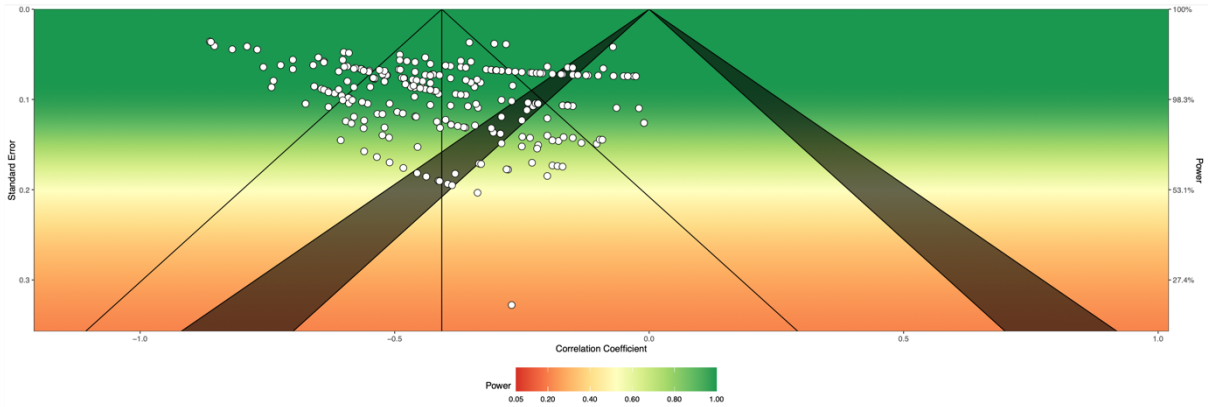


Figure 5-4 Sunset (power-enhanced) funnel plot of multilevel meta-analysis exploring association between biomarker of cell injury concentration and global outcome. *Each circle represents a unique effect size, with the shading corresponding to power.*

5.4.2 Cognition

Seven studies assessed the association between biomarkers of cell injury and cognitive outcome, encompassing several cognitive domains (Table 5-7). The most common cognitive domain assessed was working memory (n = 4), followed by global cognition and attention / executive function (n = 3), with language, visual processing, visuospatial ability and learning / long-term memory being assessed the least (n = 2 per domain). It was noted that high variability of effect size magnitude within cognitive domains was present. For example, in measures assessing global cognition, two studies reported moderate to high associations between biomarkers of cell injury and outcome (Ni et al., 2020a, Herrmann et al., 2001); however, Nygren de Boussard and colleagues reported no such associations (Nygren de Boussard et al., 2005). However, the latter study operationalised outcome and biomarkers as dichotomised variables, rather than continuous measures, which in part may explain discrepancies between effect sizes since analyses of continuous data have greater statistical power. Further, when assessing associations within specific cognitive domains, a noticeable lack of consensus was noted between studies, which may be in part due to the small number of studies assessing each individual domain, as well as the use of different assessments to assess said domains. Further, included studies reported relatively small sample sizes, another factor that could correspond to effect size variance across studies. Two studies were not eligible for inclusion in the multilevel meta-analysis, due to not assessing biomarkers as a continuous outcome (n = 1) (Nygren de Boussard et al., 2005) and not providing enough information required for conversion of statistics into a standardised correlation coefficient (n = 1) (Svingos et al., 2022), leaving a total of 5 studies and 98 effect sizes included in the meta-analysis.

Table 5-7 ***Characteristics and descriptive statistics of studies assessing relationship between biomarker of cell injury and cognition***

Study Author (Year) & Country	N	Age; Sex (% male)	TBI Severities & GCS Score	Cognitive Outcome Measure(s)	Timepoint(s) post-injury	Time of Sample Extraction	Biofluid Source	Cognitive Domain	Biomarker(s)	Magnitude Of Effect
(Asken et al., 2020)* United States	36	19†; 50%	Mild; -	ImPACT; King-Devick test	"Acutely following SRC"	≤ 48 hours	Serum	Working Memory	GFAP	++
									UCH-L1	++
									Tau	+
									NF-L	+
								Visuospatial	GFAP	ns
									UCH-L1	ns
									Tau	+
									NF-L	+
								Attention / Executive Function	GFAP	+
									UCH-L1	+
									Tau	+
									NF-L	ns
(Dey et al., 2017)* India	20	30.5; 90%	Mild; 14-15	Digit span; Spatial span; COWA; Stroop test; Trail Making Test A & B; AVLT learning; AVLT (verbal); CFT (visual)	3 months	< 24 hours	Serum	Working Memory	S100B	ns
									UCH-L1	ns
								Visuospatial	S100B	+
									UCH-L1	ns
								Language	S100B	+
									UCH-L1	+
								Attention / Executive Function	S100B	+
									UCH-L1	+
								Visual Processing	S100B	ns

								UCH-L1	ns
							Learning & Long-Term Memory	S100B UCH-L1	ns +
(Herrmann et al., 2001)* Germany	69	30†; 81%	Mild; 14-15 Moderate-to-Severe; 3-12	Composite score derived from the following assessments: MMSE; Frontal Lobe Score; Digit and Visual spans; Selective Reminding test; Block Design; Stroop test; Computerised Test Battery for Attentional Performance; finger tapping	2 weeks	Median = 27 hours	Serum	Global Cognition	S100B NSE	+++ ++
					6 months				S100B NSE	++ ++
(Ni et al., 2020b)* China	95	41.3; 67%	Mild; 13-15 Moderate; 9-12 Severe; ≤ 8	MoCA	6 months	24 hours	Serum	Global Cognition	Tau	+++
						≥ 72 hours			Tau	++
(Nygren de Boussard et al., 2005) Sweden	97	37.2; 60%	Mild; 14-15	Composite score derived from the following assessments: Finger tapping; K-test; single and 2-choice visual reaction time; O-	1-14 days	≤ 24 hours	Serum	Global Cognition	S100B	ns

				Test *Extended neuropsychological test at 3-months:* Digit span; Block span; Buschke Selective Reminding Test; Stroop test; Paced Auditory Serial Addition test; Trail Making Test A&B.						
(Svingos et al., 2022) United States	59	40.27; 75%	Severe; ≤ 8	Digit span	3-months	≤ 48 hours	CSF	Working Memory	S100B	ns
(Watt et al., 2006)* Australia	23	26.2†; 74%	Severe; ≤ 8	WMS-R; AVLT; Category Test; ELF; CalCAP	2 weeks	< 24 hours	Serum	Working Memory	S100B	+
								Attention / Executive Function	S100B	++
								Visual Processing	S100B	++
								Learning & Long-Term Memory	S100B	++
								Language	S100B	++
						48 hours		Working Memory	S100B	ns
								Attention / Executive Function	S100B	+

	Visual Processing	S100B	+
	Learning & Long-Term Memory	S100B	ns
	Language	S100B	++

WMS-R: Wechsler Memory Scale-Revised; AVLT: Auditory Verbal Learning Test; ELF: Excluded letter fluency: CalCAP: Attention and Processing: California Computerised Assessment Package; MoCA: Montreal Cognitive Assessment; COWA: Controlled oral word association; ImPACT: Immediate Post-concussion Assessment and Cognitive Testing; MMSE: Mini Mental State Examination

† = Age reported as median; * = Included in meta-analysis

5.4.2.1 Multilevel Meta-Analysis of Cognition

Multilevel meta-analysis demonstrated that higher biomarker concentration in the 7-day period following injury was weakly associated (as determined by conversion scenarios in Table 2) with lower cognitive function at any timepoint following injury (r = -0.25) (Table 5-8). The mixed-effect model presented high heterogeneity, as demonstrated by an I^2 value of 81% and high *Q* statistic (535.97, p = 0.025), representing a high degree of variance within and between study effects. Variance was calculated for each level of the model, with minimal variance attributed to sampling (19.2%) and most being attributed to between-study effects (49.7%), as well as within-study effects to a lesser degree (31.1%).

Table 5-8 Multilevel meta-analysis assessing the relationship between biomarkers of cell injury extracted within a week of TBI and cognition assessed at any timepoint post-injury

Outcome	*k*	*n*	Mean CC (95% CI)	*p*	I^2	*t-statistic*	*Q* (*p*)
Cognition	5	98	-0.25 (-0.46, -0.03)	**0.025**	81%	-2.28	535.98 **(<0.0001)**

k = number of unique studies. n = number of effect sizes. CC = correlation coefficient. CI = confidence interval. β = regression coefficient. Q = Q statistic test for residual heterogeneity. t = t-statistic
p-value column indicates whether the models were significant

5.4.2.2 Moderator Analyses

Significant heterogeneity in the cognitive function multilevel model justified exploration of potential sources of variation through moderator analyses. Results from these moderator analyses are summarised in Table 5-9, displaying the mean correlation for each category within a model and their corresponding beta coefficient, reflective of a moderator's deviation relative to a reference category. The reference category p-value reflects whether the coefficient significantly deviates from 0, whereas the p-values in other categories reflect whether they deviate significantly from the reference category. Significant differences between moderators and the reference category, where present, are denoted by bolded p-values and t-statistics.

Table 5-9 ***Results of cognition moderator analysis***

Predictor	*k*	*n*	Mean CC (95% CI)	*β* (95% CI)	*p*	*t*	*Q* (*p*)	*ToM* (*p*)
Demographics							523.07	
Age	-	-	-	0.003 (-0.03, 0.04)	0.88	0.15	(**<0.0001**)	
Proportion of Males	-	-	-	-0.003 (-0.01, 0.005)	0.39	-0.86	526.39 (**<0.0001**)	
Cognitive Domain							478.43	1.73
Working Memory	3	24	-0.27 (-0.45, -0.95)	*Reference*	**0.003**	-3.04	(**<0.0001**)	(0.12)
Global Cognition	2	8	-0.25 (-0.57, 0.07)	0.03 (-0.34, 0.39)	0.91	0.03		
Attention / Executive Function	3	24	-0.27 (-0.75, 0.22)	0.0 (-0.41, 0.42)	0.98	0.12		
Language	2	6	-0.42 (-0.84, -0.02)	-0.15 (-0.51, 0.22)	0.43	-0.79		
Visual Processing Speed	2	6	-0.08 (-0.75, 0.58)	0.19 (-0.41, 0.80)	0.52	1.12		
Visuospatial	2	12	-0.10 (-0.34, 0.13)	0.17 (-0.03, 0.37)	0.09	0.65		
Learning & Long-Term Memory	2	18	-0.14 (-0.44, 0.16)	0.13 (-0.11, 0.38)	0.27	1.73		

k = number of unique studies. n = number of effect sizes. CC = correlation coefficient. CI = confidence interval. β = regression coefficient. Q = Q statistic test for residual heterogeneity. ToM = Test of Moderators

† Reference category for model. Effect sizes and 95% CI for each specific predictor are obtained from separate models that used that predictor as the reference category.

t = t-statistic relative to the reference category

The p-value column and associated β-values indicate whether individual predictors significantly differ (≤ 0.05) from the reference category. Significance is denoted by bolded values.

Demographics

A random effects meta-regression was conducted to determine whether either age or the proportion of males between studies influenced biomarker associations with cognition.

Substantial heterogeneity was still present in the models ($p < 0.0001$), indicating that other moderators rather than age and sex are driving variations in biomarker associations with cognitive outcomes.

Cognitive Domain

With working memory as the reference category, the test of moderators did not reach significance (Table 9), and no comparisons between working memory and other cognitive domains yielded significantly different effect sizes.

5.4.2.3 Outlier Testing

Of the 98 unique effect sizes, 24 and 18 outliers were identified as outliers using the residuals analysis and Cook's distance, respectively (Figure 5-5). Outlier removal via the residual method resulted in no change ($r = 0.25$) to the association between biomarkers of cell injury and cognition; however, the model did not maintain significance ($p = 0.08$). While heterogeneity was still significant, as determined via the Q-statistic, it was noticeably lower after the removal of outlier effect sizes ($Q (73) = 403.1094$, $p < .0001$) compared to the initial model ($Q (97) = 535.98$, $p < 0.0001$). Additionally, the removal of outliers using Cook's distance had no appreciable influence on the overall effect ($r = 0.24$ compared to $r = 0.25$ in the initial model). The model-maintained significance and heterogeneity was still significant, albeit with a substantially reduced Q-statistic ($Q (79) = 198.73$, $p < 0.0001$) compared to the initial multilevel model ($Q (98) = 535.98$ (<0.0001). Taken together, it can be inferred that outliers are possibly driving effects reported within the meta-analysis and therefore, should be interpreted with caution.

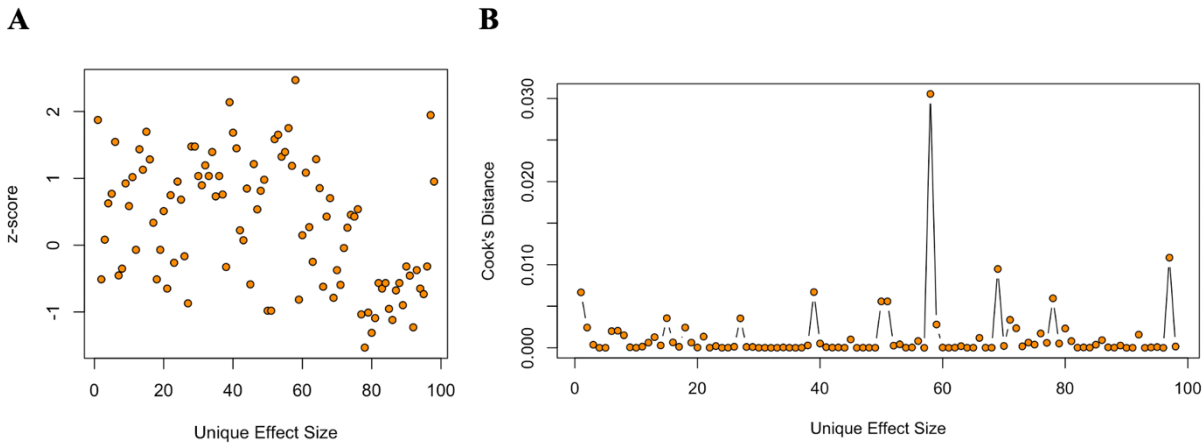


Figure 5-5 Outlier tests for effect sizes included in Cognition multilevel meta-analysis. *(A) For residual assessment, effect sizes with a residual z-score > 1.5 or < -1.5 were classified as outliers. (B) Effect sizes with a Cook's distance value of > 1.5 multiplied by the mean Cook's distance score (0.0013) were classified as outliers.*

5.4.2.4 Publication Bias

A sunset (power-enhanced) funnel plot of unique effect sizes included in the overall cognition multilevel meta-analysis is presented in Figure 5-6. Visual inspection of the plot, and a significant multilevel Egger's regression test ($p < 0.0001$), indicated probable asymmetry, suggesting that publication bias impacted the association between concentration of biomarkers of cell injury and cognition. Further, the reported median power of unique effect sizes was 13.8%, with an estimated likelihood of results replication of 0% probability.

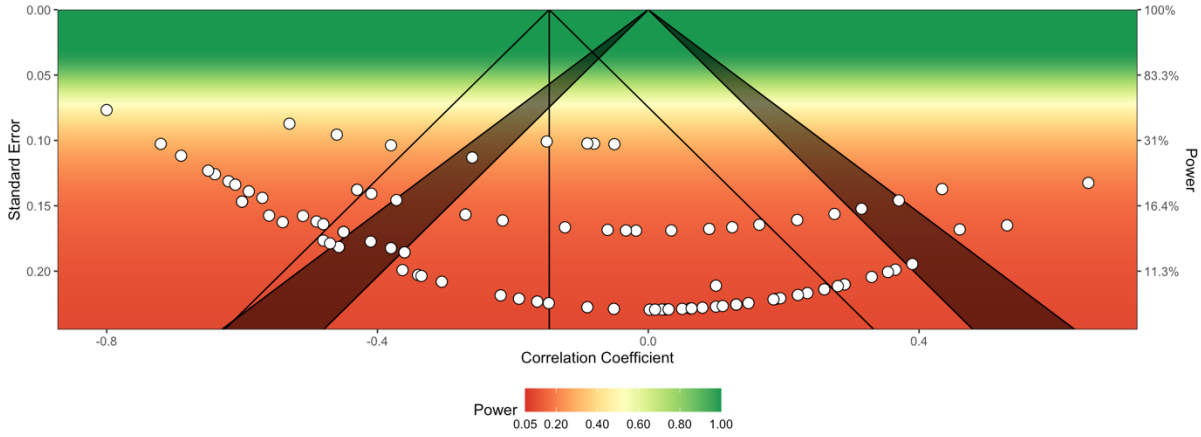


Figure 5-6 ***Sunset (power-enhanced) funnel plot of multilevel meta-analysis exploring association between biomarker of cell injury concentration and cognition.*** *Each circle represents a unique effect size, with the shading corresponding to power.*

5.4.3 Mood

One study assessed the relationship between levels of biomarkers of cell injury and depression, as measured by the dysthymic subscale of the Post-traumatic Complaints following TBI Questionnaire at 6-months post-injury (De Kruijk et al., 2002). In linear regression models adjusting for sex, age and advised bed rest, S100B and NSE both demonstrated positive, albeit non-significant, associations with depressive outcomes. Study details are reported in Table 5-10.

5.4.4 Motor

One study explored the relationship between levels of several biomarkers of cell injury and balance "acutely following sports-related concussion" using the balance error scoring system (BESS) assessment, as summarised in Table 8 (Asken et al., 2020). All biomarkers correlated negatively with balance, Tau demonstrating weak associations with postural stability (UCH-L1 = -0.044 & Tau = -0.178). However, associations between postural stability and GFAP and NF-L were non-significant. Study details are reported in Table 5-11.

Table 5-10 ***Characteristics and descriptive statistics of study assessing relationship between biomarkers of cell injury and mood***

Study Author (Year) & Country	N	Age; Sex (% male)	TBI Severities & GCS Score	Mood Outcome Measure(s)	Timepoint(s) post-injury	Time of Sample Extraction	Biofluid Source	Mood Domain	Biomarker(s)	Magnitude Of Effect
(De Kruijk et al., 2002) Netherlands	103	37.2; 57%	Mild; 14-15	Depression sub-scale of Post-traumatic Complaints Questionnaire, with the relevant subscale being: dysthymic	6-months	< 24 hours	Serum	Depression	S100B NSE	ns ns

Table 5-11 ***Characteristics and descriptive statistics of study assessing relationship between biomarker of cell injury and motor outcome***

Study Author (Year) & Country	N	Age; Sex (% male)	TBI Severities & GCS Score	Motor Outcome Measure(s)	Timepoint(s) post-injury	Time of Sample Extraction	Biofluid Source	Motor Domain	Biomarker(s)	Magnitude Of Effect
(Asken et al., 2020) United States	36	19†; 50%	Mild; -	BESS	Acutely following SRC	≤ 48 hours	Serum	Postural Stability	GFAP NF-L UCH-L1 Tau	ns ns + +

BESS: Balance error scoring system

5.4.5 Risk of Bias

Assessing risk of bias, measured using an adapted version of the QUIPS, of included studies yielded mixed ratings (Figure 5-7). A relatively equal distribution between low (n = 34 studies) and moderate (n = 28 studies) risk of bias was present, along with two studies being classified as having a high risk of bias. A greater overall risk of bias rating for individual studies was commonly due to weak statistical analysis and reporting, followed by poor outcome measurement, such as dichotomising outcome variables or reporting broad or inconsistent timeframes within which outcome was assessed following injury.

5.4.5.1 Key Study Variables and Recruitment

Many of the included studies (94%) had a low risk of bias for key study variables and recruitment. Only one study was classified as having a high risk of bias in this domain, resulting from inadequate reporting of key sample features, including both sample (i.e. gender and age) and injury characteristics, as well as recruitment strategy, whereby the source of the TBI sample was not explicitly stated. Of the studies that were classified as moderate risk of bias (5%), all were noted to not provide explicit inclusion criteria, with other factors also contributing to bias, including inadequate reporting of sample characteristics for two of the studies, as well as inadequate detail on injury characteristics for the other. Aside from a single study presenting high risk of bias for inadequate reporting of demographics, injury characteristics and inclusion criteria (Raabe and Seifert, 2000), included studies consistently reported the source of their target population, as well as details on TBI diagnosis.

5.4.5.2 Prognostic Factor Measurement

All studies were rated as having a low risk of bias for prognostic factor measurement. It was noted that most studies recruited their target population from hospitals, allowing routine and

uniform collection of biofluids from participants. Further, all methods adequately detailed the methods used to quantify biomarker levels, including unit of measurement, as well as all but one study reporting biomarkers as continuous variables (Nygren de Boussard et al., 2005). The item with the highest heterogeneity was the time of sampling post-injury, with some studies using broad ranges (> 24 hours; 14%) and some having a time of sampling that was inconsistent between participants (9.5%).

5.4.5.3 Outcome Measurement

Most studies (80%) were rated as having a low risk of bias for outcome measurement, while the remainder were rated as moderate (20%). All studies had a detailed description of the outcome explored and a reliable and valid method of assessing it. Additionally, studies generally had a high proportion of participants (≥ 80%) completing the outcome measurement, as well as information about the effect size (of any type) for the association between biomarker concentration and functional outcome. For studies rated as having a moderate risk of bias, items where issues were found included: broad or inconsistent timeframes post-injury during which the outcomes were assessed; dichotomising outcomes into categorical variables; and having a setting in which assessment was conducted that was either inconsistent between participants or not explicitly stated. No studies in this review were rated as having a high risk of bias for outcome measurement.

5.4.5.4 Statistical Analyses and Reporting

Most studies (64%) were rated as having a low risk of bias for statistical analysis and reporting, followed by a moderate risk of bias (34%), with only two yielding a high risk of bias. Moderate and high risk of bias studies were often classified as such due to: not assessing normality within their datasets; associative analysis involving categorical variables (dichotomised outcome

scores with clear cut-offs; namely, outcome being converted into a categorical outcome, such as unfavourable and favourable GOS or GOSE, or high and low cognition, rather than analysing the continuous scores); and potential publication bias, whereby only specific effect sizes showing significance were reported. One case of high risk of bias was identified due to 1) not explicitly reporting an associative analysis between biomarkers and outcome, with the analysis having to be conducted from data available within paper and 2) providing minimal information on key study characteristics and recruitment strategies.

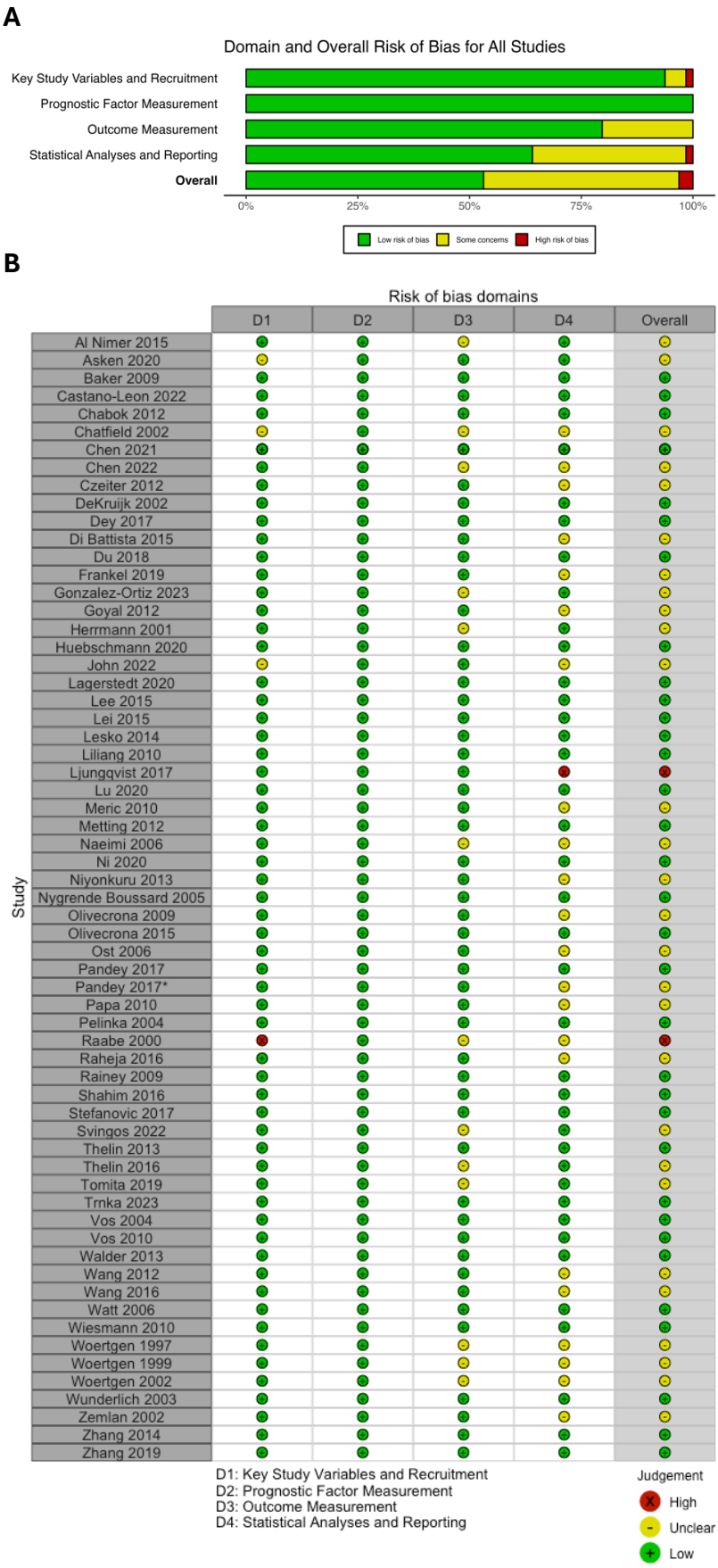


Figure 5-7 (A) Risk of bias summary plot and (B) Risk of bias traffic plot detailing risk of bias per domain per individual study

5.4.6 Assessing Confidence in Body of Literature

Table 5-12 displays a summary of the GRADE framework used to evaluate pooled effects. GRADE was conducted in two domains: 1) global functional outcome (pooled GOS and GOSE outcome) and 2) cognition. As only singular studies assessed the relationship between biomarkers of cell injury and mood or motor outcomes, this was not considered a body of literature and was therefore omitted from GRADE assessment.

5.4.6.1 Global Outcome

When assessing downgrading factors, a serious breach was noted in two categories: risk of bias and inconsistency. Risk of bias was determined via a combination of quality assessment (whereby statistical analysis and reporting was noted to have highest overall risk of bias compared to all other domains, in part due to underreporting of non-significant associations), as well as a significant Egger's test for studies eligible for the meta-analysis. Further, large heterogeneity was present across studies exploring global outcome, including different operationalisation of the GOS and GOSE (assessing associations between biomarkers and outcome as a continuous variable, or dichotomising outcome), supported by a substantial I^2 statistic in the multilevel meta-analysis. However, no serious breach was present for upgrading factors, with moderate/large-pooled effect sizes (as reported in the multilevel meta-analysis), as well as a dose-effect between higher marker of cell injury concentration corresponding to poorer global outcome contributing to an overall certainty of evidence to 'High'.

5.4.6.2 Cognition

Several breaches were noted across downgrading factors, namely risk of bias, inconsistency and imprecision. Risk of bias was primarily based on a significant Egger's test for studies included in the meta-analysis (71% of studies). Further, large variability in the way that

cognition was assessed was present, primarily inconsistency in the cognitive assessments utilised across studies. Further, the cognition literature was deemed imprecise due to the variability in effect sizes between studies, making comparability challenging. For example, the timepoint at which assessments were administered varied substantially and several studies had relatively low sample sizes. While not downgraded, as all studies explicitly explored biomarker associations with cognition, it should be noted that there was a substantial bias towards S100B as the marker of interest. When assessing upgrading factors, while biomarkers demonstrated only relatively weak associations with cognition across studies, the literature was upgraded on account of a general dose-dependent effect being found, whereby higher marker concentration corresponded to poorer cognitive performance. This upgrading factor resulted in the overall certainty of evidence being deemed 'Low'.

Overall, while confidence in biomarker associations with global outcome is high, confidence in the associations between biomarkers of cell injury and more specific functional outcomes, such as cognition, is far more tenuous.

Table 5-12 ***Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Summary of Findings table for primary outcomes***

Relationship between Biomarkers of Cellular Injury with Global Outcome and Cognition in TBI Cohorts

Population: Individuals with a clinically diagnosed TBI

Prognostic factor of interest: Biomarkers of cell injury

			GRADE Factors						
	Study Characteristics		Downgrading Factors				Upgrading Factors		
Outcomes	**Number of studies (number of participants)**	**Phase**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Moderate/large effect size**	**Dose effect**	**Overall quality of evidence**
Global Outcome	57 (5290)	2	✗	✗	✓	✓	✓	✓	++++
Cognition	7 (399)	2	✗	✗	✓	✗	✗	✓	++

Phase, phase of investigation. Dose effect, exposure-gradient response. ✗ = serious breach/limitation. ✓ = no serious breach/limitation.
Overall quality of evidence: +, very low; ++, low; +++, moderate; ++++, high.

5.5 DISCUSSION

This study conducted a comprehensive systematic review of the literature assessing the relationship between biomarkers of cell injury collected within seven days of TBI and behavioural outcomes assessed at any timepoint post-injury. Further, this study utilised a multilevel meta-analytic approach (albeit only for global outcome, as measured by either GOS or GOSE, and cognition, given limitations of the current literature), allowing for several within-study effects to be assessed. Diversity in within-study effects was probed by conducting moderator analyses, allowing for critical comparison of specific biomarkers, as well as assessing key variables that may influence clinical utility per biomarker, such as time of biomarker sampling and the timepoint at which functional assessment was administered following injury. This is particularly noteworthy, since studies included in this review measured multiple biomarkers or timepoints of interest, resulting in several effect sizes per study. Traditional meta-analytic approaches would only have allowed for a single effect size to be extracted per study, truncating the amount of available data and, consequently, limiting critical interpretation of meta-analytic findings. Thus, the use of multilevel meta-analysis is a significant strength of the current work.

5.5.1 Global Outcome

Overall, a clear dose-dependent effect was reported consistently across studies, whereby a higher biomarker of cell injury concentration acutely following injury (< 7 days) was associated with more profound impairment, as measured by either GOS or GOSE, at any timepoint following injury. This was made particularly evident in the multilevel meta-analysis, whereby the estimated effect size was not only significant but also fairly robust. Said findings are largely in line with previous literature, with a CENTER-TBI study assessing serum biomarkers of cell

injury in 2283 participants determining that elevated biomarker concentration within 24-hours of injury corresponded to more profound functional impairment, measured using GOSE, six-months post-injury (Helmrich et al., 2022). Further, such marker concentrations were shown to substantially develop upon preexisting prognostic models, resulting in a relative increase in R^2 for IMPACT and CRASH models by 48-65% and 30-34% respectively (Helmrich et al., 2022). Likewise, meta-analysis exploring similar research questions report synonymous findings (Pei et al., 2022, Zarei et al., 2024). However, the former studies utilised traditional meta-analytic techniques, involving pooling within-study effect sizes rather than treating them independently. This could somewhat limit interpretability of research findings, given that several factors may moderate the association seen between biomarker concentration and global outcome, which were explored in the present review.

First, it may be relevant to consider where the biomarkers of interest are derived from. In line with this, several biofluid sources were included within the studies encompassed in this review, namely serum, plasma and CSF. Of note, some of these studies suggested that markers derived from specific sources may provide differential utility, but this was not consistent between studies. For example, Goyal and colleagues found that when assessing outcome at 6-months, serum derived S100B did not have significant associations with outcome, whereas CSF-derived S100B demonstrated moderate associations (Goyal et al., 2013). In contrast, however, Czeiter et al. compared CSF- and serum-derived GFAP and UCH-L1 and their association with outcome at several time points, finding that at one-month following injury, while serum concentrations of GFAP and UCH-L1 yielded moderate associations with outcome, CSF-derived markers demonstrated little to no associations (Czeiter et al., 2012b). This discrepancy in findings was reflected in our moderator analyses comparing blood- and CSF-derived biomarkers of cell injury and their influence on effect sizes, showing that both blood and CSF-

derived markers had a significant moderate association with outcome, with no significant difference noted between the two. This suggests that blood-derived biomarkers have as much utility for predicting functional outcomes following injury as those derived from CSF and, thus, might provide a less-invasive strategy to assess biomarkers of cell injury within clinical settings.

Another potential factor of interest is the specific biomarker(s) assessed within each study, as markers reflective of changes in different cellular processes may have differential utility for predicting functional outcomes post-injury (Mafuika et al., 2022). For example, Mafuika and colleagues (2022) have posited that S100B and GFAP, markers of astrocyte damage or dysfunction, are brain-specific, robust and highly sensitive for intracranial abnormalities following TBI, suggesting that the combination of these two proteins may have utility for improving TBI diagnosis relative to other markers. Considering this, individual biomarkers were extracted to determine whether any specific biomarker demonstrated stronger associations with global outcome following injury compared to other markers. Overall, moderator analyses demonstrated that relative to S100B, which demonstrated significant moderate associations with outcome, only NF-L presented with a significantly lower effect size, although it was still a significant predictor of global outcome. Interestingly, it has previously been reported that serum NF-L extracted 30 days following TBI demonstrated significant associations ($B = 0.64$ log pg/mL, $p = 0.0002$) with GOSE assessed at 90-days post-injury (Shahim et al., 2016). Thus, it is possible that NF-L demonstrates higher prognostic utility when extracted beyond the seven-day period assessed within this review and may be an appropriate marker to measure more chronically following injury. Overall, however, the results of our analysis suggest that no specific biomarker appears to have greater utility for predicting functional outcome following injury than any other marker, making it impossible to advocate

for the preferential analysis of any specific biomarker when considering recovery trajectory post-injury. Encouragingly, however, all markers had a moderate association with later outcome, highlighting the overall utility of measuring biomarkers within the acute period following injury to help inform about expected outcomes following TBI.

Another key consideration is the potential moderating effect of individual biomarker kinetics. In line with this, Azizi et al developed a kinetic model of blood-based biomarkers of cell injury, including S100B, UCH-L1, NF-L, GFAP and tau, and demonstrated clear distinctions between markers in both rate of elevation and rate of return to baseline following mTBI (Azizi et al., 2021). This may have implications for diagnostic/prognostic cut-offs, with the potential to improve optimal sampling times within clinical settings. Therefore, the influence of time of sampling across all biomarkers, as well as per individual marker, was considered within the current work. Further, given the distinct kinetics seen in blood- versus CSF-derived biomarkers following injury (see review, (Agoston et al., 2017)), these were also considered separately. Contrary to our expectations, however, when considering time of biomarker sampling relative to injury, only very minimal differences were found, and only for blood-derived tau. Specifically, the ability of levels of tau measured within blood to predict global outcome post-injury decreased with increased time elapsed between sample collection and time of injury (i.e. from r = -0.59 at 1-6 hours-post injury to r = -0.34 at 7 days post-injury). Previous literature has suggested that tau generally peaks within the first day (specifically 12-24 hours) following injury, and while elevated concentrations may persist, they slowly taper off following this initial peak (Shahim et al., 2014, Rubenstein et al., 2017). This may help to explain why tau measured in samples collected a week following injury showed a weaker association with global outcome than those collected more acutely (i.e. within 1-6 hours) post-injury. To date, however, only one study has assessed tau levels within the CSF, showing that within a severe

TBI cohort, tau collected between two to three days post-injury, however, not within 24 hours or beyond 72 hours following injury, was significantly elevated in GOSE determined unfavourable outcome relative to favourable (Ost et al., 2006). Thus, it is not possible to comment on whether the pattern observed for blood-derived tau would also be reflected within the CSF.

Of note, the previous literature on the relationship between time of sampling and functional outcomes for the other biomarkers is mixed, with some, but not all, studies noting an effect of sampling time. In line with this, a previous study in 265 individuals reported that early sampling (1-12 hours) of serum S100B post-injury yielded minimal prognostic value, with samples extracted between 12-36 hours being the most optimal to inform prediction of GOS determined unfavourable outcome 6-12 months post-injury (Thelin et al., 2013). Conversely, a study of 80 individuals sustaining a M/sTBI (GCS 3-12) found that serum S100B extracted at various timepoints (< 24 hours, 24 hours and ≥ 72 hours) post-injury demonstrated comparable associations with global outcome at one- and three-months post-injury, irrespective of when the sample was collected following injury (Chen et al., 2021a). Within our own model for blood-derived S100B, while both the 12-24 hour and day two post-injury time points corresponded to the highest effect sizes (r= -0.51 and r = -0.49, respectively), this difference was negligible (with effect sizes at other time points ranging from r = -0.40 to -0.45), with no statistically significant differences noted. Likewise, despite GFAP and UCH-L1 demonstrating highest utility for detecting intracranial abnormalities within a 12-hour window following injury (Bazarian et al., 2018), this was not reflected by statistical differences at other timepoints, albeit later timepoints in both biomarkers demonstrated either likewise utility (GFAP at day seven compared to 1-6 hours post-injury) or even trending towards stronger effect sizes (UCH-L1 12-24 hours compared to 1-6 hours post-injury). Taken together, this suggests

that while time of sample collection may be an important consideration when tau is the biomarker of interest, for other injury-related biomarkers, sampling at any point up to one week following injury is unlikely to significantly affect the utility of the marker for forecasting overall functional outcome following injury.

Beyond biomarkers themselves, the timepoint post-injury at which functional outcome was assessed also has potential to moderate the effects seen. Interestingly, in our multilevel meta-analysis combining all biomarkers, the time when functional assessment was performed relative to time of injury did not appear to influence the association between biomarker level and global function, with similar moderate effects sizes noted at all timepoints. Further, when looking at individual biomarkers, a similar pattern was seen. Of note, the majority of individual biomarkers also demonstrated moderate associations (ranging from r = -0.41 to -0.55) with outcome, aside from NSE, which had weaker associations with outcome at both 3-months (r = -0.31) and greater than 6-months (r = -0.27) post-injury, albeit no significant differences in NSE moderator analysis was reported. It is important to note, however, that there were a limited number of studies assessing the relationship between NSE and functional outcome at these timepoints, so results should be interpreted with caution. Overall, our findings are in line with those of several previous studies, which have supported the utility of biomarkers for predicting outcomes even at long-term timepoints following injury. For example, it has been reported that HMGB1 derived from the plasma within 24-hours of severe injury was significantly elevated in GOS-determined unfavourable outcome was strongly associated with outcome at 12-months post-injury (Wang et al., 2012a). Similarly, S100B extracted at several timepoints within a week of injury demonstrated low to moderate associations with outcome even at 24-months post-injury (Chabok et al., 2012). Even longer term, at 7 – 10 years following injury, CSF-derived UCH-L1 collected within 24 hours of severe injury significantly elevated for those

presenting with unfavourable outcome (assessed via GOS) (Svingos et al., 2022). It should be noted, however, that not all previous studies have been consistent, with several failing to find a significant association between the biomarker of interest and later functional outcome. Of note is that the former studies assessed outcome following severe cases of injury, whereas several studies included in the present study assess mTBI or a range of injury severities, emphasising the need for further research in this area. Nevertheless, our findings provide at least preliminary support that the incorporation of biomarkers has potential to enhance prognostic models, such as IMPACT and CRASH, which are currently only informative up to a period of six-months post-injury (Nelson et al., 2019).

Given the importance of demographics, such as age and sex, in the clinical presentation of TBI, moderator analysis was conducted to determine that such factors were not influencing effect sizes. Overall, increased age is associated with worse outcomes following TBI, corroborated by a longitudinal study reporting that over a five-year period, older individuals (≥40 years of age) demonstrated a greater rate of decline in that period, as well as reporting less improvement in disability compared to the youngest cohort (Marquez de la Plata et al., 2008). Interestingly, age is also a critical predictor of the type of impairment one may present with following injury, with younger individuals more likely to report social isolation, anxiety and depression, whereas older individuals report difficulties regarding functional independence and employment (Downing et al., 2024b). Therefore, age is not only implicated in increased likelihood of behavioural impairment, but also the type, and should be considered in a context of long-term outcome, particularly in a therapeutic intervention context when developing tailored rehabilitation. Similarly, while men are more likely to experience TBI (see review, (Biegon, 2021)), a CENTER-TBI study reported that women are more likely to report worse outcomes at six-months post-injury compared to men, which was also in part driven by age (Mikolić et

al., 2021). However, moderator analysis determined that such factors did not influence associations between biomarkers of cell injury and global outcome within the present review.

While our findings overall support that various biomarkers of cell injury extracted acutely (< 7 days) following TBI demonstrate significant associations with global outcome measured even beyond six-months post-injury, several factors pertaining to GOS and GOSE as an outcome measurement should be considered when interpreting the clinical relevance of these findings. Importantly, both assessments possess psychometric limitations that may explain why the effect sizes generally trended towards being moderate-high, even when accounting for moderating factors. In line with this, a 2019 TRACK-TBI study assessing GOSE utility reported that the scale itself lacks clear distinctions between scale items, that there is a paucity of items capturing subtler levels of disability and that the assessment as a whole captures relatively vague, non-specific information on impairments (Ranson et al., 2019). Similarly, a workshop sponsored by the National Institute of Neurological Disorders and Stroke concluded that such an ordinal scale is only a crude reflection of TBI outcomes and is heavily skewed towards severe disability, corresponding to limited sensitivity to detect individual differences and milder functional changes (Narayan et al., 2002). In the current review, 41 of the 64 included studies (64%) focused on individuals following a severe injury, with a further 21 (33%) including individuals with moderate injury. This contrasts with the 23 included studies (36%) encompassing those with mild injury. Thus, it is possible that our results may have been skewed, contributing to the moderate-high effect sizes observed. While biomarkers of cell injury may show robust associations with more profound disability, the degree to which they are associated with more subtle changes in individuals following mild injury are not as well captured in our data, highlighting the need for future work investigating the value of biomarkers for predicting long-term functional outcome following mTBI.

5.5.2 Cognition

Compared to global outcome, a substantially lower number of studies assessed the relationship between markers of cell injury and cognition following TBI (n = 7). While, similar to global outcome, a dose-dependent response was noted for cognition, with higher concentrations of markers of cell injury corresponding to poorer cognitive performance, effect sizes were generally considerably weaker overall (ranging from r = -0.08 to -0.27 for the individual cognitive domains). This is supported by the multilevel model assessing cognition, where of the 98 included effect sizes across five studies, a small, but still significant, average effect size of -0.25 was reported, compared to the overall global outcome model effect size of -0.47. Interpretation of this finding, however, is limited by the paucity of studies investigating the research question of interest, as well as the significant variability between studies in terms of the individual measures, and indeed even the cognitive domains, assessed. This was further complicated by differences between studies in the specific biomarkers measured, as well as the sampling method/time associated with these biomarkers, making it difficult to draw meaningful conclusions across studies. To try to mitigate these challenges, effect sizes were collapsed according to cognitive domain, with each interpreted individually.

Three of the included studies looked at overall global cognition, with one studying measuring this using the Montreal Cognitive Assessment (MoCA) and the others using comprehensive neuropsychological batteries assessing multiple domains (with one also including the Mini-Mental State Exam and the other including the K-test). In the single study assessing global outcomes using the MoCA in a sample of mixed TBI severities, significant associations with serum levels of tau extracted at one (p = 0.001), three (p = 0.02) and five (p = 0.03) days post injury were reported, although not seven days following injury (p = 0.14) (Ni et al., 2020b). It should be noted, however, that the MoCA presents several psychometric limitations, with

Bruijnen and colleagues recommending caution when interpreting domain-specific changes using such global outcome measures (Bruijnen et al., 2020). Conversely, the two studies that assessed global cognition using comprehensive neuropsychiatric assessment batteries reported conflicting findings. While Herrmann and colleagues found that serum levels of S100B and NSE demonstrated moderate-to-high associations with global cognition assessed at both 2-weeks (AUC = 0.81 and 0.72 for S100B and NSE, respectively) and 6-months following injury AUC = 0.77 and 0.65 for S100B and NSE, respectively), Nygren de Boussard et al found no significant association between serum levels of S100B and global cognition up to 14 days post-injury (Nygren de Boussard et al., 2005). It should be noted, however, that the latter study only assessed a cohort of mTBI participants (GCS 14 – 15), whereas the former studies included individuals ranging from GCS 3 – 15, which may help to account for the discrepancies noted.

When considering specific cognitive domains, the only cognitive domains with a significant effect size in the current review were working memory (-0.27, CI = -0.45, -0.10) and language (-0.42, CI = -0.84, -0.002). In a meta-analysis of 21 studies determined that following severe TBI, working memory is commonly impaired and rarely recovers (Dunning et al., 2016). Further, working memory is largely dependent on proper functioning of the prefrontal cortex and associated circuits (Lara and Wallis, 2015), which has been reported to been impaired following TBI across both preclinical (Smith et al., 2015, Feng et al., 2021) and clinical studies (Witt et al., 2010, Kobori et al., 2006). Therefore, biomarkers of cell injury being associated with a function dependant on common site of damage and overall impairment is unsurprising. However, findings pertaining to associations between biomarkers of cell injury and language following injury were less expected. While less common, impaired language is common, particularly following M/sTBI, with MacDonald and colleagues reporting an approximate incidence of 75% (MacDonald, 2017). However, it is also considered that cognitive-

communication difficulties result from impairments to other cognitive domains rather than specific language disorders, such as attention, working memory and executive function (Coelho, 2007). In fact, executive function and attention are the most common cognitive domains affected following TBI, as highlighted by a study assessing an aged (≥ 50 years of age) subset of the PROTECT-TBI cohort (n = 15,764) (Lennon et al., 2023). This was reflected by attention / executive function resulting in a synonymous estimated effect to working memory in the present moderator analyses. Therefore, it is possible that alterations to the former domains are contributing to the large effect size reported in language. However, attention / executive function was not deemed a significant moderator, likely on account of large variability across studies that assessed it.

Further, other assessed domains reported appreciably lower effects sizes, including learning and long-term memory (r = -0.14), visuospatial function (r = -0.10) and visual processing speed (r = -0.08). However, it should be noted that all cognitive domains had a small number of studies assessing them, meaning drawing conclusions on their effect sizes challenging. This is reflected moderator analyses where, relative to working memory as a reference category, no significant differences in the effect sizes across cognitive domains was reported, which may reflect the limited number of studies assessing each cognitive domain and, subsequently, low statistical power. Further, like global outcome, moderator analysis determining exploring age and sex determined demographic factors were not driving effect sizes between biomarkers of cell injury and cognition.

Given the limited number of studies exploring the relationship between biomarkers of cell injury and cognition, it is difficult to draw meaningful conclusions with present findings. Further, substantial heterogeneity in what cognitive domains were assessed across studies, as

well as variability in utilised cognitive assessments, highlights a critical need to harmonise how cognition is explored post-injury. Further, future studies exploring associations between biomarkers of cell injury and cognition should conduct robust assessment of several biomarkers and potential moderating factors akin to the global outcome literature, such as time of sample extraction and timepoint cognition is assessed following injury. Doing so would be an important step forward towards generating reliable and generalisable findings regarding the relationship between biomarkers of cell injury and cognitive outcomes across studies.

5.5.3 Mood

Only a single study meeting inclusion criterion, exploring the association between depression and biomarkers of cell injury, specifically serum S100B and NSE within 24 hours following injury, finding no significant associations ($p > 0.05$). Assessing depression within 24 hours is potentially problematic, given that previous studies have found that mental health trajectories evolve over time, with Zarzaur and colleagues finding that 25% of TBI survivors demonstrating poor mental health trajectories over a 12-month period (Zarzaur et al., 2017). Further, limited representation of mood impairments is a significant gap in the literature, considering that mood impairments and disorders are frequent outcomes following TBI (see review, (Howlett et al., 2022)). In fact, rates of mood disorders are substantially higher in TBI relative to control cohorts, with a study assessing 92 individuals with TBI reporting that 51.6% developed a mood disorder of some sort within the first year of injury, compared to 22.2% of orthopaedic controls ($n = 27$) (Jorge et al., 2004). Of these, depression was the most common mood impairment, with the prevalence of major depressive disorder (MDD) and depression without major depressive features being 33% and 10% in this cohort, respectively. This also aligns with findings in two large cross-sectional cohorts, assessing outcome at 10 – 126 months (Seel et al., 2003) and 2.5 ± 3.5 years (Kreutzer et al., 2001) post-injury, respectively, which highlighted

the potential long-term persistence of mood impairment following TBI. It should be noted, however, that rates reported across studies are variable, with a recent meta-analysis assessing 43 studies encompassing 724,842 individuals with TBI reporting that lifetime incidence of depression in those with a history of TBI was 13%, and that risk relative to non-TBI controls was 2.10 (Dehbozorgi et al., 2024b). Similarly, a recent meta-analysis of 20 studies found that across 705,024 individuals with TBI, the incidence of anxiety was 17.5%, with a 1.9-fold increased risk of developing anxiety relative to non-TBI controls (Dehbozorgi et al., 2024a). While not as commonly assessed, other mood disorders may also arise from injury, such as mania (1.7-9%) (Silver et al., 2001, van Reekum et al., 2000) and aggression (11-34%) (Brooke et al., 1992, Tateno et al., 2003). Such mood disorders may also show high comorbidity with one another. In support of this, Jorge and colleagues reported that of the 33% of their TBI cohort reporting MDD, 76.7% demonstrated anxiety as a comorbid disorder, while 56.7% exhibited aggressive behaviours.

Interestingly, evidence suggests that pathological changes associated with TBI, such as elevated levels of pro-inflammatory cytokines, may be associated with increased risk of mood impairments following TBI (for review, see (Risbrough et al., 2022)). However, as highlighted in the current review, the association between acute levels of biomarkers of cell injury and mood impairments following injury is poorly characterised, warranting further research. Future studies should not only explicitly explore the associations between biomarkers and mood outcome, but also account for the high rates of comorbid mood disorders following injury by conducting a comprehensive assessment, encompassing several facets of mood function, rather than single measures used in isolation. Doing so will provide critically needed insight into the degree to which biomarkers may provide insight into who is most likely to develop mood impairments following injury.

5.5.4 Motor

Similar to mood, only one study meeting inclusion criteria explored the association between biomarkers of cell injury and motor outcome, specifically postural stability, reporting very weak to weak associations (GFAP = -0.060; UCH-L1 = -0.044, tau = -0.178 and NF-L = 0.154). Previous literature has supported that TBI can result in several motor deficits, with mTBI resulting in balance disturbance and impaired coordination (Lin et al., 2015, Gera et al., 2018), whereas sTBI can lead to more pronounced deficits, such as impaired motor coordination (i.e. postural instability and gait disturbances), as well as reduced fine motor control (Walker and Pickett, 2007). Such multi-faceted injury-dependant outcomes may help to explain the weak associations observed between various biomarkers of cell injury and motor function noted within the present review, as the sole study included BESS, a measure of postural stability, as the only motor measure and assessed outcome exclusively within an mTBI cohort (Asken et al., 2020). Further, this study only assessed BESS within 48 hours or less following injury. While it is true that the majority of motor impairments resulting from mTBI resolve within weeks of injury, more severe, or repeated, injury may be associated with persistent motor impairment, as highlighted by a recent systematic review of the literature by our group (Corrigan et al., 2023). Thus, investigating the relationship between markers of cell injury and motor outcomes following TBI is an important area for future research.

5.5.5 Review strengths/limitations and directions for future research

Unlike previous similar reviews assessing biomarkers of cell injury and their associations with outcome (Undén and Romner, 2010, Cheng et al., 2014, Behzadi et al., 2024), which often restrict their studies based on factors such as individual biomarker, sample source and injury severity, this review instead encompassed all such factors. This allowed for investigation into differences in both within and between-study effects using multilevel models, a technique not

typically utilised in meta-analytic research (Assink and Wibbelink, 2016), with effect size dependency often being avoided in conventional meta-analyses by only extracting single effect sizes or deriving an aggregated effect size within-studies (Assink and Wibbelink, 2016, Cheung, 2019). In doing so, this limits investigation into the underlying variability that may be important to consider, particularly in a clinical context. Instead, structuring meta-analytic models with three levels allowed for effect size dependencies to be accounted for, ensuring that dependencies did not inflate effect sizes, while also maintaining sufficient statistical power within the models. Utilising said methods allowed for the inclusion of 243 unique global outcome effect sizes across 43 studies, as well as 98 unique cognition outcome effect sizes across 5 studies. Of note, this approach not only improved statistical power, but also allowed for possible moderating effects to be examined. The comprehensive nature of the global outcome literature, as well as robust effect sizes reported overall, corresponded to a high overall quality of evidence as determined by the GRADE framework.

Despite these significant strengths, however, the findings of this review should be interpreted with several limitations in mind. First, while a substantial number of studies and effect sizes could be extracted when assessing associations between markers of cell injury and global outcome, effect sizes were much more limited when assessing specific functional outcomes following injury. While a multilevel meta-analysis was able to be conducted for cognition, given the limited number of studies, it was not possible to conduct several moderator analyses of interest for cognition, including individual biomarker utility, timepoint at which cognition was assessed following injury and time post-injury that the biofluid sample was extracted. While such moderators did not demonstrate substantial differences in the global outcome literature, it is possible that they could be of more relevance for specific outcomes following injury. Additionally, motor and mood outcomes were only assessed by a single study each,

making a meta-analysis for these outcomes impossible. This highlights the need for future studies explicitly exploring the association between specific markers of cell injury and cognition, mood and motor outcomes following injury, with potential moderating effects in mind. This future work could be further strengthened by harmonising assessment protocols, such as timing of sampling/assessment or measures used, between studies.

The limited representation of literature pertaining to specific behavioural outcomes within the current review was reflected by the overall quality of the cognition literature being deemed low according to the GRADE framework. Further, GRADE could not be applied to mood and motor outcomes due to not being considered a sufficient body of literature, highlighting that findings pertaining to such outcomes must be interpreted with caution. Additionally, a high risk of publication bias was determined in meta-analytic models assessing global outcome and cognition following injury, as determined by a significant Egger's test. Further, visual assessment of funnel plots determined a clear bias was present within the clear literature, albeit this pattern was largely absent within the global outcome funnel plot. While there is a lack of standardisation and agreement on what publication bias test is most appropriate when considering multi-level models, the adapted Egger's test is considered the most suitable approach (Kossmeier et al., 2020). Nevertheless, it has also been shown to correspond to overestimated type one and two errors, particularly in models with highly heterogeneous effect sizes, potentially limiting the validity of current results given the heterogeneous nature of both multilevel models for global outcome and cognition (Nakagawa et al., 2022).

5.6 CONCLUSION

This systematic review, and associated multilevel meta-analysis, demonstrated that biomarkers of cell injury collected within a week of injury demonstrate high associations with global

outcome following TBI. Further, moderator analyses determined that factors, such as individual biomarker, time of sample collection and timepoint at which functional outcome was assessed post-injury did not substantially influence the moderate effect size reported in the overall multilevel model. This suggests that biomarkers of cell injury overall, regardless of when they were extracted within a week of injury, demonstrate high promise for detecting global impairment following injury, regardless of timepoint. Despite this, global outcome presents with several limitations, such as not being informative on the specific impairments that an individual may present with following injury, as well as not effectively capturing subtle disability following injury, highlighting the need to assess specific behavioural outcomes. However, insight into the associations between biomarkers of cell injury and specific functional outcomes, including cognition, mood and motor outcomes, is extremely limited, a significant gap in the current literature given emerging literature implicating TBI as a factor contributing to persistent impairment across said domains. Thus, it is imperative that future work better characterises associations between biomarker concentration and more specific outcome, to determine their potential utility in highlighting specific impairments that may help inform on clinical decision making regarding personalised treatment strategies and interventions.

5.7 SOURCES OF FUNDING

Funding sources and conflict of interest: This work was funded by grants to LCP and IB by the Medical Research Future Fund (2020/MRF1202188), James and Diana Ramsay Foundation and NeuroSurgical Research Foundation. AM and BE were supported by an Australian government Research Training Program scholarship. The authors declare that there are no conflicts of interest relevant to this work.

Full financial disclosures for the previous twelve months: AM is supported by an Australian government Research Training Programme scholarship.

5.8 AUTHOR INFORMATION

5.8.1 Author Affiliation

School of Biomedicine, University of Adelaide, SA 5000, Australia

Angus McNamara & Lyndsey Collins-Praino

School of Psychology, University of Adelaide, SA 5000, Australia

Irina Baetu

5.8.2 Contributions

AM, conceptualized, analysed data, and wrote the original manuscript. BE acted as a secondary reviewer during all stages of data collection. LCP & IB contributed to review concept and design, interpretation of results, as well as LCP playing a key role in manuscript conceptualisation. All authors were involved in editing the original manuscript. All authors read and approved the submitted manuscript.

5.9 CORRESPONDING AUTHOR

Address all correspondence to A/Professor Lyndsey Collins-Praino

5.10 DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interests.

5.11 ACKNOWLEDGEMENTS

The authors would like to thank Vikki Langton, Liaison Librarian, for assistance with developing the systematic search strategy for each of the databases used.

5.12 SUPPLEMENTARY MATERIAL

Supplementary Material 1: Search strings and search strategies with the retrieved results at each step, presented for all 4 databases

Embase

Search	Query	Records Retrieved
1	Exp cell damage OR (Neurofilament light* OR Nf-L OR GFAP OR Glial fibrillary acid protein* OR *Ubiquitin carboxy-terminal hydrolase L1 OR UCH L1 OR UCHL1 OR UCH-L1 OR S100B OR S-100B OR S100 calcium binding protein B OR SNTF OR* Phosphorylated Neurofilament Heavy Chain OR PNfH OR Neuron-specific enolase* OR NSE OR High mobility group box 1 OR HMGB1 OR tau* OR T-tau OR *cell injury OR cellular injury OR cell damage OR nervous tissue damage OR nervous tissue injury).*ti,ab	1192712
2	Exp traumatic brain injury OR Exp Glasgow coma scale OR (TBI OR Head injur* OR Head trauma OR Concussion OR Neurotrauma OR Glasgow Coma Scale OR traumatic brain injury OR mTBI OR Posttrauma*).ti,ab	206813
3	Exp Glasgow outcome scale OR Exp Prognosis OR Exp neuropsychological test OR Exp behavior change OR exp attitude OR exp emotion OR exp functional behavior OR exp reward seeking behavior OR Exp memory OR Exp attention Exp Motor coordination OR Exp motor performance OR Exp motor control OR exp motor dysfunction OR exp depression OR (Glasgow Outcome Scale OR Mood OR Cognition OR Attention OR Neuropsychiatric OR psychiatric OR Behavio?r* OR Tremor* OR Bradykinesia OR Rigid* OR Dyskinesia* OR Balance OR Postur* OR Gait OR Walk* OR Akinesia OR Mobility OR Motor Impairment OR Motor Outcome OR movement disorder OR motor disorder OR neuromotor disorder* OR psychomotor disorder OR movement abnormalit* OR motor abnormalit* OR neuromotor abnormalit* OR psychomotor abnormalit* OR movement symptom* OR motor symptom*OR neuromotor symptom*OR psychomotor symptom* OR movement function* OR motor function* OR neuromotor function* OR psychomotor function* OR movement dysfunction* OR motor dysfunction* OR neuromotor dysfunction* OR psychomotor dysfunction* OR movement deficit* OR motor deficit* OR deficit* OR psychomotor deficit* OR movement disturbance* OR motor disturbance* OR neuromotor disturbance* OR psychomotor disturbance* OR Mild Cognitive Impairment OR MCI OR Memory OR Executive function OR diagnos* or prognos* OR learning OR attention OR working memory OR processing speed OR MMSE OR Mini Mental State Examination OR MoCA OR Montreal cognitive assessment).ti,ab	10510035
4	exp human or human experiment.sh **NOT** (Specialties, Surgical.sh OR Animals OR Preclinical OR * in vitro OR Infant* OR Child* OR Paediatric OR Youth OR Haemorrhage OR Hemorrhage OR Reperfusion OR epilepticus OR seizure OR epilepsy OR Review OR Spinal* OR COVID* OR Neuroimaging OR Intervention* OR self-report* OR Enceph* OR Mening* OR Cardiac) NOT nonhuman.sh	13981774
5	#1 & #2 & #3 & #4	725
Limited to English Language and Humans		

Medline via Pubmed

Search	Query	Records Retrieved
1	"Intermediate Filament Proteins" [MeSH Terms] OR "Ubiquitin Thiolesterase" [MeSH Terms] OR "S100 Calcium Binding Protein beta Subunit" [MeSH Terms] OR "tau Proteins" [MeSH Terms] OR (Cell damage *[Title/Abstract] OR* Cell Injur* *[Title/Abstract] OR* cellular injur* *[Title/Abstract] OR* Neurofilament light* *[Title/Abstract] OR Nf-L [Title/Abstract] OR GFAP [Title/Abstract] OR* Glial fibrillary acid protein* *[Title/Abstract] OR Ubiquitin carboxy-terminal hydrolase L1 [Title/Abstract] OR UCH L1 [Title/Abstract] OR UCHL1 [Title/Abstract] OR UCH-L1 [Title/Abstract] OR S100B [Title/Abstract] OR S-100B [Title/Abstract] OR S100 calcium binding protein B [Title/Abstract] OR SNTF [Title/Abstract] OR* Phosphorylated Neurofilament Heavy Chain *[Title/Abstract] OR PNfH [Title/Abstract] OR* Neuron-specific enolase* *[Title/Abstract]* OR NSE *[Title/Abstract] OR* High mobility group box 1 *[Title/Abstract] OR HMGB1 [Title/Abstract] OR tau [Title/Abstract] OR cell damage [Title/Abstract] OR cellular damage [Title/Abstract] OR nervous tissue damage [Title/Abstract] OR nervous tissue injury [Title/Abstract])*	201,418
2	"Brain Injuries, Traumatic" [MeSH Terms] OR "Glasgow Coma Scale" [MeSH Terms] OR ("Traumatic Brain Injur*" *[Title/Abstract]* OR "TBI" *[Title/Abstract]* OR "Head injur*" *[Title/Abstract]* OR "Head trauma" *[Title/Abstract]* OR "Concussion" *[Title/Abstract]* OR "Neurotrauma" *[Title/Abstract]* OR "Glasgow Coma Scale" *[Title/Abstract]* OR "GCS" *[Title/Abstract]* OR "traumatic brain injur*" *[Title/Abstract]* OR "mTBI "*[Title/Abstract]* OR "Posttraumatic" *[Title/Abstract]*)	181,433
3	"Glasgow Outcome Scale" [MeSH Terms] OR "Diagnosis" [MeSH Terms] OR "Prognosis" [MeSH Terms] OR "Behavior" [MeSH Terms] OR "Psychophysiology" [MeSH Terms] OR "Behavioral Symptoms" [MeSH Terms] OR "Cognitive Dysfunction" [MeSH Terms] OR "Emotions" [MeSH Terms] OR "psychomotor performance" [MeSH Terms] OR "Mental Processes" [MeSH Terms] OR "Depression" [MeSH Terms] OR ("Glasgow Outcome Scale" *[Title/Abstract]* OR "Mood" *[Title/Abstract]* OR "Cognition" *[Title/Abstract]* OR "Attention" *[Title/Abstract]* OR "Neuropsychiatric" *[Title/Abstract]* OR "psychiatric" *[Title/Abstract]*"Behavior*" OR *[Title/Abstract]* OR "Behaviour*" *[Title/Abstract]* OR "Tremor*" *[Title/Abstract]* OR "Bradykinesia" *[Title/Abstract]* OR "Rigid*" *[Title/Abstract]* OR "Dyskinesia*" *[Title/Abstract]* OR "Balance" *[Title/Abstract]* OR "Postur*" *[Title/Abstract]* OR "Gait" *[Title/Abstract]* OR "Walk*" *[Title/Abstract]* OR "Akinesia" *[Title/Abstract]* OR "Mobility" *[Title/Abstract]* OR "Motor Impairment" *[Title/Abstract]* OR "Motor Outcome" *[Title/Abstract]* OR "movement disorder" *[Title/Abstract]* OR "motor disorder" *[Title/Abstract]* OR "neuromotor disorder" *[Title/Abstract]* OR "psychomotor disorder" *[Title/Abstract]* OR "movement abnormalit*" *[Title/Abstract]* OR "motor abnormalit*" *[Title/Abstract]* OR "neuromotor abnormalit*" *[Title/Abstract]* OR "psychomotor abnormalit*" *[Title/Abstract]* OR "movement symptom*" *[Title/Abstract]* OR "motor symptom*" *[Title/Abstract]* OR "neuromotor symptom*" *[Title/Abstract]* OR "psychomotor symptom*" *[Title/Abstract]* OR "movement function*" *[Title/Abstract]* OR "motor function*" *[Title/Abstract]* OR "neuromotor function*" *[Title/Abstract]* OR "psychomotor function*" *[Title/Abstract]* OR "movement dysfunction*" *[Title/Abstract]* OR "motor dysfunction*" *[Title/Abstract]* OR "neuromotor dysfunction*" *[Title/Abstract]* OR "psychomotor dysfunction*" *[Title/Abstract]* OR "movement deficit*" *[Title/Abstract]* OR "motor deficit*" *[Title/Abstract]* OR "psychomotor deficit*" *[Title/Abstract]* OR "movement disturbance*" *[Title/Abstract]* OR "motor disturbance*" *[Title/Abstract]* OR "neuromotor disturbance*" *[Title/Abstract]* OR "psychomotor disturbance*" *[Title/Abstract]* OR "Mild Cognitive Impairment" *[Title/Abstract]* OR "MCI"	13,905,225

	[Title/Abstract] "Memory" *[Title/Abstract]* OR "Executive function" *[Title/Abstract]* OR "Depres*" *[Title/Abstract]* OR "Anxi*" *[Title/Abstract]* OR "prognos*" *[Title/Abstract]* OR "diagnos*" *[Title/Abstract]* OR "learning" *[Title/Abstract]* OR "attention" *[Title/Abstract]* OR "working memory" *[Title/Abstract]* OR "processing speed" *[Title/Abstract]* OR "MMSE" *[Title/Abstract]* OR "Mini Mental State Examination" *[Title/Abstract]* OR "MoCA" *[Title/Abstract]* OR "Montreal cognitive assessment" *[Title/Abstract]*)	
4	"Animals"[Mh] NOT ("Animals"[Mh] AND "Humans"[Mh])	5,073,063
5	"Neuroimaging" [MeSH Terms] OR ("Animal*" [Title/Abstract] OR "Preclinical" [Title/Abstract] OR "in-vitro" [Title/Abstract] OR "Infant*" [Title/Abstract] OR "Child*" [Title/Abstract] OR "Pediatric" [Title/Abstract] OR paediatric[tiab] OR "Youth" [Title/Abstract] OR "Haemorrhage" [Title/Abstract] OR "hemorrhage" [Title/Abstract] OR "Reperfusion" [Title/Abstract] OR "epilepticus" [Title/Abstract] OR "seizure" [Title/Abstract] OR "epilepsy" [Title/Abstract] OR "Spinal*" [Title/Abstract] OR "COVID*" [Title/Abstract] OR "Intervention*" [Title/Abstract] OR "self-report*" [Title/Abstract] OR "Enceph*" [Title/Abstract] OR "Mening*" [Title/Abstract] OR "Cardiac" [Title/Abstract])	7,441,736
6	1 AND 2 AND 3	2,269
7	6 NOT 4	1,575
8	7 NOT 5	749
9	English language filter	706

Web of Science

Search	Query	Records Retrieved
1	TI=("Cell damage" OR "Cell Injur*" OR "cellular injur*" OR "Neurofilament light*" OR Nf-L OR GFAP OR "Glial fibrillary acid protein*" *OR "Ubiquitin carboxy-terminal hydrolase L1" OR "UCH L1" OR UCHL1 OR UCH-L1 OR S100B OR "S100 calcium binding protein B" OR SNTF OR "*Phosphorylated Neurofilament Heavy Chain" OR PNfH OR "Neuron-specific enolase*" OR NSE OR "High mobility group box 1" OR HMGB1 OR tau OR T-tau OR "*nervous tissue damage" OR "nervous tissue injury") OR AB*=("Cell damage" OR "Cell Injur*" OR "cellular injur*" OR "Neurofilament light*" OR Nf-L OR GFAP OR "Glial fibrillary acid protein*" OR *"Ubiquitin carboxy-terminal hydrolase L1" OR "UCH L1" OR UCHL1 OR UCH-L1 OR S-100B S100B OR "S100 calcium binding protein B" OR SNTF OR "*Phosphorylated Neurofilament Heavy Chain" OR PNfH OR "Neuron-specific enolase*" OR NSE OR "High mobility group box 1" OR HMGB1 OR "tau" OR T-tau OR*"nervous tissue damage" OR "nervous tissue injury")*	212,754
2	TI=("Traumatic Brain Injur*" OR TBI OR "Head injur*" OR "Head trauma" OR Concussion OR Neurotrauma OR "Glasgow Coma Scale" OR GCS OR "traumatic brain injur*" OR mTBI OR Posttraumatic) OR AB=("Traumatic Brain Injur*" OR TBI OR "Head injur*" OR "Head trauma" OR Concussion OR Neurotrauma OR "Glasgow Coma Scale" OR GCS OR "traumatic brain injur*" OR mTBI OR Posttraumatic)	161,997
3	TI=("Glasgow Outcome Scale" OR "Mood" OR "Cognition" OR "Attention" OR "Neuropsychiatric" OR "psychiatric" OR "Behaviour*" OR "Behavior*" OR "Tremor*" OR "Bradykinesia" OR "Rigid*" OR "Dyskinesia*" OR "Balance" OR "Postur*" OR "Gait" OR "Walk*" OR "Akinesia" OR "Mobility" OR "Motor Impairment" OR "Motor Outcome" OR "movement disorder" OR "motor disorder" OR "neuromotor disorder" OR "psychomotor disorder" OR "movement abnormalit*" OR "motor abnormalit*" OR "neuromotor abnormalit*" OR "psychomotor abnormalit*" OR "movement symptom*" OR "motor symptom*" OR "neuromotor symptom*" OR "psychomotor symptom*" OR "movement function*" OR "motor function*" OR "neuromotor function*" OR "psychomotor function*" OR "movement dysfunction*" OR "motor dysfunction*" OR "neuromotor dysfunction*" OR "psychomotor dysfunction*" OR "movement deficit*" OR "motor deficit*" OR "psychomotor deficit*" OR "movement disturbance*" OR "motor disturbance*" OR "neuromotor disturbance*" OR "psychomotor disturbance*" OR "Mild Cognitive Impairment" OR MCI OR Memory* OR "Executive function" OR Depres* OR Anxi* OR prognos* OR diagnos* * OR "learning" OR "attention" OR "working memory" OR "processing speed" OR "MMSE" OR "Mini Mental State Examination" OR "MoCA" OR "Montreal cognitive assessment") OR AB=("Glasgow Outcome Scale" OR "Mood" OR "Cognition" OR "Attention" OR "Neuropsychiatric" OR "psychiatric" OR "Behaviour*" OR "Behavior*" OR "Behaviour*" OR "Behavior*" OR "Tremor*" OR "Bradykinesia" OR "Rigid*" OR "Dyskinesia*" OR "Balance" OR "Postur*" OR "Gait" OR "Walk*" OR "Akinesia" OR "Mobility" OR "Motor Impairment" OR "Motor Outcome" OR "movement disorder" OR "motor disorder" OR "neuromotor disorder" OR "psychomotor disorder" OR "movement abnormalit*" OR "motor abnormalit*" OR "neuromotor abnormalit*" OR "psychomotor abnormalit*" OR "movement symptom*" OR "motor symptom*" OR "neuromotor symptom*" OR "psychomotor symptom*" OR "movement function*" OR "motor function*" OR "neuromotor function*" OR "psychomotor function*" OR "movement dysfunction*" OR "motor dysfunction*"	11,748,155

	OR “neuromotor dysfunction*” OR “psychomotor dysfunction*” OR “movement deficit*” OR “motor deficit*” OR “psychomotor deficit*” OR “movement disturbance*” OR “motor disturbance*” OR “neuromotor disturbance*” OR “psychomotor disturbance*” OR “Mild Cognitive Impairment” OR MCI OR Memory* OR “Executive function” OR Depres* OR Anxi* OR prognos* OR diagnos* OR “learning” OR “attention” OR “working memory” OR “processing speed” OR “MMSE” OR “Mini Mental State Examination” OR “MoCA” OR “Montreal cognitive assessment”)	
4	#1 & #2 & #3	1,396
5	**NOT** TI=(Non-human OR nonhuman OR Animal* OR Rodent OR Murine OR Mouse OR Mice OR Rat OR Preclinical OR “in vitro” OR in-vitro OR Infant* OR Child* OR Paediatric OR Youth OR Haemorrhage OR Hemorrhage OR Reperfusion OR epilepticus OR seizure OR epilepsy OR Review OR Spinal* OR COVID* OR Neuroimaging OR Intervention* OR self-report* OR Enceph* OR Mening*) OR AB= (Non-human OR nonhuman OR Animal* OR Rodent OR Murine OR Mouse OR Mice OR Rat OR Preclinical OR “in vitro” OR in-vitro OR Infant* OR Child* OR Paediatric OR Youth OR Haemorrhage OR Hemorrhage OR Reperfusion OR epilepticus OR seizure OR epilepsy OR Review OR Spinal* OR COVID* OR Neuroimaging OR Intervention* OR self-report* OR Enceph* OR Mening*)	12,249,286
6	#4 & #5	422
Limited to English Language and Humans		

Cochrane

Search Name: Systematic Review

Comment: Cochrane search

ID	Search	Hits
#1	MeSH descriptor: [Glasgow Coma Scale] 1 tree(s) exploded	473
#2	MeSH descriptor: [Brain Injuries, Traumatic] explode all trees	1113
#3	(“Traumatic Brain Injur*” OR TBI OR “Head injur*” OR “Head trauma” OR Concussion OR Neurotrauma OR “Glasgow Coma Scale” OR GCS OR “traumatic brain injur*” OR mTBI OR Posttraumatic):ti,ab,kw (Word variations have been searched)	15584
#4	#1 OR #2 OR #3	15721
#5	MeSH descriptor: [Neurofilament Proteins] 1 tree(s) exploded	42
#6	MeSH descriptor: [Glial Fibrillary Acidic Protein] this term only	19
#7	MeSH descriptor: [Ubiquitin Thiolesterase] 1 tree(s) exploded	18
#8	MeSH descriptor: [S100 Calcium Binding Protein beta Subunit] 1 tree(s) exploded	141
#9	MeSH descriptor: [tau Proteins] 1 tree(s) exploded	94
#10	(Cell damage OR “Cell Injur*” OR “cellular injur*” OR “cellular damage” OR “Neurofilament light*” OR Nf-L OR GFAP OR “Glial fibrillary acid protein*” OR “Ubiquitin carboxy-terminal hydrolase L1” OR “UCH L1” OR UCHL1 OR UCH-L1 OR S-100B OR S100B OR “S100 calcium binding protein B” OR SNTF OR “Phosphorylated Neurofilament Heavy Chain” OR PNfH OR “Neuron-specific enolase*” OR NSE OR “High mobility group box 1” OR HMGB1 OR tau OR T-tau “nervous tissue damage” OR “nervous tissue injury”):ti,ab,kw (Word variations have been searched)	12455
#11	#5 OR #6 OR #7 OR #8 OR #9 OR #10	12506
#12	MeSH descriptor: [Prognosis] this term only	14770
#13	MeSH descriptor: [Early Diagnosis] explode all trees	2068
#14	MeSH descriptor: [Glasgow Outcome Scale] this term only	153
#15	MeSH descriptor: [Psychomotor Performance] this term only	5542
#16	MeSH descriptor: [Behavior and Behavior Mechanisms] explode all trees	136789
#17	MeSH descriptor: [Neurobehavioral Manifestations] explode all trees	9206

#18 (“Glasgow Outcome Scale” OR “Mood” OR “Cognition” OR “Attention” OR “Neuropsychiatric” OR “psychiatric” OR “Behaviour*” OR “Behavior*” OR “Tremor*” OR “Bradykinesia” OR “Rigid*” OR “Dyskinesia*” OR “Balance” OR “Postur*” OR “Gait” OR “Walk*” OR “Akinesia” OR“Mobility” OR “Motor Impairment” OR “Motor Outcome” OR “movement disorder” OR “motor disorder” OR “neuromotor disorder” OR “psychomotor disorder” OR “movement abnormalit*” OR “motor abnormalit*” OR “neuromotor abnormalit*” OR “psychomotor abnormalit*” OR “movement symptom*” OR “motor symptom*” OR “neuromotor symptom*” OR “psychomotor symptom*” OR “movement function*” OR “motor function*” OR “neuromotor function*” OR “psychomotor function*” OR “movement dysfunction*” OR “motor dysfunction*” OR “neuromotor dysfunction*” OR “psychomotor dysfunction*” OR “movement deficit*” OR “motor deficit*” OR “psychomotor deficit*” OR “movement disturbance*” OR “motor disturbance*” OR “neuromotor disturbance*” OR “psychomotor disturbance*” OR “Mild Cognitive Impairment” OR MCI OR Memory* OR “Executive function” OR Depres* OR Anxi* OR prognos* OR diagnos* OR learning OR attention OR “working memory” OR “processing speed” OR MMSE OR “Mini Mental State Examination” OR MoCA OR “Montreal cognitive assessment”):ti,ab,kw (Word variations have been searched) 0

#19 #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 1959777

#20 #4 AND #11 AND #19 529

#21 (“Non-human” OR “nonhuman” OR “Animal*” OR “Rodent” OR “Murine” OR “Mouse” OR “Mice” OR “Rat” OR “Preclinical” OR “in vitro” OR “in-vitro” OR “Infant*” OR “Child*” OR “Paediatric” OR “Youth” OR “Haemorrhage” OR “Hemorrhage” OR “Reperfusion” OR “epilepticus” OR “seizure” OR “epilepsy” OR “Review” OR “Spinal*” OR “COVID*” OR “Neuroimaging” OR “Intervention*” OR “self-report*” OR “Enceph*” OR “Mening*”):ti,ab,kw (Word variations have been searched) 822141

#22 #20 NOT #21 **157**

Supplementary Material 2: Extraction template developed for the research question of interest

Data to Extract	Description
Date of Extraction	Record date the data was extracted from the study in the format dd/mm/yy.
Person Extracting	Record name of person extracting data
Study ID	Record study ID in the format [first author surname, year of publication]. Where there are two papers with the same first author and year of publication, use letters (a, b, etc.) placed immediately after the year of publication to distinguish the papers from each other's, as per APA referencing style. The assignment of letters should be based on the alphabetical ordering of the references for the papers, based on the title of the paper.
Study Title	Title of paper / report that data are extracted from.
APA Reference	Record the full APA reference for the study.
Lead Author Contact Details	Record contact details of the corresponding author (if possible).
Country in which the study conducted.	Record country where the research was conducted. If multiple countries, list all countries. o United States o UK o Canada o Australia o Other
Study design	o Randomised controlled trial. o Non-randomised experimental study o Cohort study o Cross sectional study o Case control study o Prevalence study o Case series o Diagnostic test accuracy study o Clinical prediction rule o Economic evaluation o Other
Paper Objectives	Record the key aims and/or overall focus of the study.
Overall TBI Sample Size (N)	Record the total sample size (N).
Age - Mean (SD) OR Median (IQR/Range)	Record the average age for the sample (with standard deviation in parentheses) or if non-parametric, median age and IQR or Range
Gender Distribution	Record how many males and females the study has for all their samples (with percentages of these values in parentheses)
Ethnicity	Record any summary data (e.g., counts or proportions/percentages) pertaining to the race/ethnicity of the sample, if reported; otherwise, write 'Not reported'.
Recruitment Method	Record details relating to how contact was made for participants to be involved in the study.
Data Collection Timeframe	Record the timeframe in which data was collected from study participants in the format dd/mm/yy - dd/mm/yy
Sampling Method	Record the sampling method used (e.g., convenience sampling, consecutive sampling, random sampling).
Inclusion & Exclusion Criteria	Record any inclusion/exclusion criteria used. For example, if the sample was restricted to mTBI only.
Population Description	Provide a brief description of the cohort
TBI Diagnosis Method and Criteria	Record the diagnostic method and criteria used to classify TBI participants for the study. For example, report if they determined severity via the Glasgow Coma Scale. If self-reported, write 'self-report'. If not reported, write 'Not reported'.

TBI Severities Explored	Record the TBI severities explored within this study i.e. mild TBI, moderate TBI, severe TBI etc. If several, please check all boxes that are applicable: o Concussion / Mild TBI o Moderate TBI o Severe TBI o Moderate/Severe TBI
How was TBI Severity Classified	Record method to distinguish injury severities i.e., if they used the Glasgow Coma Scale or other injury features such as loss of consciousness, post-traumatic amnesia etc. If a combination of variables is used, please check all boxes applicable. o GCS o LoC o PTA o Imaging i.e., CT o Other
Provide Specific Details Per Variable	For each variable, provide thresholds or details on how they were used to define injury severity i.e., mTBI was classified as a GCS greater than 12 etc.
If Other, Record Method of Classifying Injury Severity	Record method
Time Since Injury	Record the timeframe following injury that is being explored i.e. acute (outcome assessed < 1 year), chronic (outcome assessed > 1 year) or both
Other Relevant TBI Characteristics	Record any summary data (e.g., means and SDs, counts or proportions/percentages) pertaining to any other relevant TBI characteristics reported by the study authors.
Comorbidities	List any comorbidities reported, such as skull fractures, seizures, hospitalisation etc. (as count or proportion/percentage). If authors declare no comorbidities, write 'No comorbidities'. If comorbidities not reported on, write 'Not reported'.
Was data parametric?	If the authors refer to conducting tests for normality, record whether data is parametric or non-parametric. If Parametric, following fields should include values to reflect this (i.e., mean and SD). If non-parametric, values would include median and IQR or Range. If authors do not make reference to this, click "Not Reported". o Parametric o Non-Parametric o Both o Not reported
Data Analysis Methodology	Record how the study has conducted their data analysis. For example, multiple linear regression, logistic regression, correlation analyses, t-tests, etc. Please note - This is only regarding analyses between biomarker concentration and outcomes of interest o Logistic Regression o Linear Regression o ROC Curve Analysis & AUC o T-Tests / Mann Whitney U o Correlation (Pearson or Spearman) o Univariate Analysis o Other (write in comment)
What Behavioural Outcomes were Investigated?	Behavioural Outcomes can be categorised as "Cognition", "Mood", "Motor", "Glasgow Outcome Scale" or "Glasgow Outcome Scale - Extended" . o Cognition

	○ Mood ○ Motor ○ Glasgow Outcome Scale ○ Glasgow Outcome Scale - Extended
Cognitive Outcomes Investigated	Record the type of cognitive outcomes measured throughout the study. For example, working memory, reasoning ability, attention etc. Please record it using the following format: 1. MCI 2. Executive function If none, record "N/A" for this item and the following item
Respective Cognitive Outcome Measures	Record the specific cognitive outcome measures used to assess the respective cognitive outcomes mentioned in previous item. For example, if mild cognitive impairment was measured in the study, then in this section the extractor would record how this was assessed (e.g. something like MoCA scale). using the same convention as the previous item i.e. 1. MCI - MoCA 2. Executive Function - Trail Making Task Record "N/A" if previous item was also "N/A"
Cognitive Outcome Assessment Delivery	Record how the cognitive outcome measure(s) was(were) administered. For example, online, to a caregiver, administered in-person, by a psychologist, structured telephone interview etc. Note if such measure is self-reported by recording "Self-report". If there is no cognitive outcome measured, record "N/A"
Cognitive Outcome Notes	Please use this text box to make any notes regarding the cognitive outcomes or measures you may deem significant i.e., if they only performed certain assessments and particular timepoints etc.
Mood Outcomes Investigated	Record the type of mood outcomes measured throughout the study. For example, anxiety & depression Please record it using the following format: 1. Anxiety 2. Depression If none, record "N/A" for this item and the following item
Respective Mood Outcome Measures	Record the specific mood outcome measures used to assess the respective mood outcomes mentioned in previous item. For example, if anxiety was measured in the study, then in this section the extractor would record how this was assessed (e.g. something like STAI). using the same convention as the previous item i.e. 1. Anxiety - STAI 2. Depression - GDS Record "N/A" if previous item was also "N/A"
Mood Outcome Assessment Delivery	Record how the mood outcome measure(s) was(were) administered i.e., online. If there is no mood outcome measured, record "N/A".

Mood Outcome Notes	Please use this text box to make any notes regarding the mood outcomes or measures you may deem significant i.e., if they only performed certain assessments and particular timepoints etc.
Motor Outcomes Investigated	Record the type of motor outcomes measured throughout the study. For example, gait, tremor, posture etc. Please record it using the following format: 1. Gait 2. Postural Stability If none, record "N/A" for this item and the following item
Respective Motor Outcome Measures	Record the specific motor outcome measures used to assess the respective motor outcomes mentioned in previous item. For example, if gait was measured in the study, then in this section the extractor would record how this was assessed (e.g. something like 10-meter walk test). using the same convention as the previous item i.e. 1. Gait – 10-meter walk test 2. Postural Stability - BESS Record "N/A" if previous item was also "N/A"
Motor Outcome Notes	Please use this text box to make any notes regarding the motor outcomes or measures you may deem significant i.e., if they only performed certain assessments and particular timepoints etc.
Biomarkers Investigated	Check biomarkers to denote what markers of cell injury were assessed in this study. o GFAP o NF-L o UCH-L1 o S100β o NSE o Tau o HMGB1 o SNTF o pNFH o Other
If Other, record biomarker here	
Record Biofluid Source per Biomarker	State the biofluid source of each biomarker i.e. CSF, serum, plasma, urine, saliva etc. Please record using the following convention: Tau - Serum S100B - Serum - CSF
Record Time since Injury Biosample was Collected.	State the time since injury the biomarker was collected (ideally, acute phase up to 7 days post-injury). If several, please record all timepoints using the following convention: Serum - 12 hours - 24 hours - 48 hours Saliva - 1 Week

	If not reported, please record "Not Reported".
Method to extract and quantify biomarkers of interest was extracted and quantified.	Record the method to quantify / measure the amount of biomarker of interest within the biofluid i.e., ELISA, SIMOA, LC-MS etc. If this differs across markers, please record using the following convention. S100B - Serum - SIMOA - Saliva - S100B Tau & UCH-L1 - CSF - ELIZA
Was the data grouped by time since injury (acute vs long-term?)	Report 'Yes' if in the analyses the authors grouped their sample according to a time-post injury, if not then report 'No'. If you report 'No', report "N/A" for the following two items. o Yes o No
If grouped by time since injury, how was grouping/range decided?	If the previous item was responded to with a 'Yes' then indicate what cut-off points authors used to group their sample i.e., did they have a >1year cohort compared to a 1–5-year cohort etc.
Sample Size per Group (N)	If applicable, describe the sample size for each subsample grouped by time since injury.
Was the data grouped by TBI severity?	Report 'Yes' if in the analyses the authors grouped their sample according to a TBI severity, if not then report 'No'. If you report 'No', report "N/A" for the following two items. o Yes o No
If grouped by TBI severity, how was grouping decided?	If the previous item was responded to with a 'Yes' then indicate what cut-off points authors used to group their sample for each outcome variable.
Sample Size per Group (N)	If applicable, describe the sample size for each subsample that is grouped by TBI severity.
Was the data grouped by Glasgow Outcome Scale?	Report 'Yes' if in the analyses the authors grouped their sample according to a Glasgow Outcome Scale, if not then report 'No'. If you report 'No', report "N/A" for the following two items. o Yes o No
If grouped by Glasgow Outcome Scale, how was grouping decided?	If the previous item was responded to with a 'Yes' then indicate what cut-off points authors used to group their sample for each outcome variable.
Sample Size per Group (N)	If applicable, describe the sample size for each subsample that is grouped using GOS.
Was the data grouped by Glasgow Outcome Scale-Extended?	Report 'Yes' if in the analyses the authors grouped their sample according to a Glasgow Outcome Scale-Extended, if not then report 'No'. If you report 'No', report "N/A" for the following two items o Yes o No
If grouped by Glasgow Outcome Scale-Extended, how was grouping decided?	If the previous item was responded to with a 'Yes' then indicate what cut-off points authors used to group their sample for each outcome variable.
Sample Size per Group (N)	If applicable, describe the sample size for each subsample that is grouped using GOSE.

Was the data grouped by another variable of interest?	Report 'Yes' if in the analyses the authors grouped their sample according to another variable of interest and record the variable i.e. cognition, specific motor assessment etc., if not then report 'No'. If you report 'No', report "N/A" for the following two items. o Yes o No
Cognitive Outcome Mean (SD) / Median (IQR or Range)	Record the mean/median scores for participants on the cognitive outcome measure(s) (with standard deviation / IQR or Range in parentheses). If they use a comparator group, then use '/' to separate the scores for different subsamples i.e., favourable vs unfavourable. If there is no cognitive outcome measured, record "N/A"
Was there any missing data for Cognitive Outcome measures.	Report if the study was missing some participant data (e.g., a portion of participants were unable to complete the measure) for the study's cognitive outcome measure(s).
Mood Function Mean (SD) / Median (IQR or Range)	Record the mean/median scores for participants on the mood outcome measure(s) (with standard deviation / IQR or Range in parentheses). If they use a comparator group, then use '/' to separate the scores for different subsamples i.e., favourable vs unfavourable. If there is no mood outcome measured, record "N/A"
Was there any missing data for Mood function measures.	Report if the study was missing some participant data (e.g., a portion of participants were unable to complete the measure) for the study's mood outcome measure(s).
Motor Function Mean (SD) / Median (IQR or Range)	Record the mean/median scores for participants on the motor outcome measure(s) (with standard deviation / IQR or Range in parentheses). If they use a comparator group, then use '/' to separate the scores for different subsamples i.e., favourable vs unfavourable. If there is no motor outcome measured, then respond with N/A.
Was there any missing data for motor function measures?	Report if the study was missing some participant data (e.g., a portion of participants were unable to complete the measure) for the study's motor outcome measure(s).
Biomarker Mean (SD) / Median (IQR or Range)	Record the mean/median scores for participants on the biomarker concentration(s) (with standard deviation/IQR or range in parentheses), as well as the unit of measurement i.e., ng/ml or pg/ml etc. If they use a comparator group, then use '/' to separate the scores for different subsamples i.e., favourable vs unfavourable. If there is no biomarker measured, then respond with N/A. Use the following convention: Overall sample - Mean (SD) Subsample - Favourable - mean (SD) - Unfavourable - mean (SD)
Was there any missing data for biomarker data?	Report if the study was missing some participant data (e.g., a portion of participants were unable to complete the measure) for the study's biomarker measure(s).
Cognitive Outcome Effect Size (type of effect size)	Record the effect size (for the relationship between biomarker measure and cognitive outcome) if reported in the study (in parentheses indicate the type of effect size reported). If no exact effect size is reported, then detail any information that could be used to manually calculate an effect size. For example, if regression values are provided but no effect size then report sample size, number of predictors in the model, variance the

	model accounts for, and statistic for the regression. if there is no measure, then report N/A If multiple were used, record all in list format i.e. Continuous - S100B correlation with MoCA Categorical / Grouped - T-Test for S100B between high and low cognition
Direction of Cognitive Outcome Effect	Report which direction this effect is taking for cognitive outcome. For example, whether higher biomarker concentration corresponds to poorer cognitive outcomes. If several, please report using the following convention: MocA - S100B = Positive - GFAP = Positive - UCH-L1 = Negative
Mood Outcome Effect Size (type of effect size)	Record the effect size (for the relationship between biomarker measure and mood outcome) if reported in the study (in parentheses indicate the type of effect size reported). If no exact effect size is reported, then detail any information that could be used to manually calculate an effect size. For example, if regression values are provided but no effect size then report sample size, number of predictors in the model, variance the model accounts for, and statistic for the regression. if there is no measure, then report N/A If multiple were used, record all in list format i.e. Continuous - S100B correlation with STAI Categorical / Grouped - T-Test for S100B between low and high anxiety groups
Direction of Mood Outcome Effect	Report which direction this effect is taking for mood outcome. For example, whether higher biomarker concentration corresponds to mood outcomes. If several, please report using the following convention: MocA - S100B = Positive - GFAP = Positive - UCH-L1 = Negative
Motor Outcome Effect Size (type of effect size)	Record the effect size (for the relationship between biomarker measure and motor outcome) if reported in the study (in parentheses indicate the type of effect size reported). If no exact effect size is reported, then detail any information that could be used to manually calculate an effect size. For example, if regression values are provided but no effect size then report sample size, number of predictors in the model, variance the model accounts for, and statistic for the regression. If there is no measure, then report N/A If multiple were used, record all in list format i.e. Continuous - S100B correlation with BESS

	Categorical / Grouped - T-Test for S100B between poor vs good gait
Direction of Motor Outcome Effect	Report which direction this effect is taking for motor outcome. For example, whether higher biomarker concentration corresponds to poorer motor outcomes. If several, please report using the following convention: MocA - S100B = Positive - GFAP = Positive - UCH-L1 = Negative
Glasgow Outcome Scale Effect Size (type of effect size)	Record the effect size (for the relationship between biomarker measure and Glasgow outcome scale) if reported in the study (in parentheses indicate the type of effect size reported). If no exact effect size is reported, then detail any information that could be used to manually calculate an effect size. For example, if regression values are provided but no effect size then report sample size, number of predictors in the model, variance the model accounts for, and statistic for the regression. If there is no measure, then report N/A If multiple were used, record all in list format i.e. Continuous - S100B correlation with GOS scores Categorical / Grouped - T-Test for S100B between favourable and unfavourable
Glasgow Outcome Scale-Extended Effect Size (type of effect size)	Record the effect size (for the relationship between biomarker measure and Glasgow outcome scale-extended) if reported in the study (in parentheses indicate the type of effect size reported). If no exact effect size is reported, then detail any information that could be used to manually calculate an effect size. For example, if regression values are provided but no effect size then report sample size, number of predictors in the model, variance the model accounts for, and statistic for the regression. If there is no measure, then report N/A If multiple were used, record all in list format i.e. Continuous - S100B correlation with GOSE scores Categorical / Grouped - T-Test for S100B between favourable and unfavourable
Direction of Glasgow Outcome Scale-Extended Effect	Report which direction this effect is taking for motor outcome. For example, whether higher biomarker concentration corresponds to lower Glasgow outcome scale-extended score.
Covariates?	Record if the study controlled for any potential covariates in their analyses with a 'Yes' or 'No' and if so, which possible confounders were controlled for? For example, did they control for age and injury severity in their analyses.
Does effect size need to be calculated by the reviewer?	Record a 'Yes' if effect size has to be manually calculated or a 'No' if it was already available. o Yes o No
Is reanalysis required?	Indicate whether re-analysis is required for inclusion in the review. Provide a 'Yes', 'No', or 'Unsure' response. Re-analysis here would be

	different than the previous item - here it is referring to if analyses need to be conducted again (using some of their data) to produce values that can be used to estimate an effect size (e.g., significance testing of group differences using means and SDs reported to obtain a p-value for group differences) o Yes o No Unsure
If reanalysis is required - what type of analysis?	If the response to the previous data entry field was 'Yes' or 'Unsure', provide details of the re-analyses that are (or may be) required.
Do authors need to be contacted for data?	o Yes o No o Unsure
What data is missing that we need?	If the response to the previous data entry field was 'Yes' or 'Unsure', specify the exact data that needs (or may need) to be requested from the corresponding author. For example, list the variable name(s) and the data required (e.g., raw data, means, SDs, percentages, etc.) for each variable.
Additional Comments	Record any other comments pertaining to this study that might be of relevance to the current review.

Supplementary Material 3: Quality in Prognostic Studies Tool

Biases	**Issues to consider for judging overall rating of "Risk of bias"**	**Judgement**	
1. Study Participation	**Goal: To judge the risk of selection bias**		
Source of target population	The source population or population of interest is adequately described for key characteristics	0	1
Sample characteristics	Key characteristics of the participant sample are detailed (i.e. age and gender)	0	1
Recruitment strategy	Sample identification, place of recruitment and recruitment process are provided in sufficient detail	0	1
Inclusion and Exclusion Criteria	Inclusion and/or exclusion criteria are clearly and adequately described	0	1
TBI Diagnosis	TBI diagnostic method or criteria are clearly stated and clinically validated	0	1
Injury characteristics	Injury characteristics clearly stated, as was the method for determining severity	0	1
Summary Study Participation	**Total study participation risk of bias score**	**/6**	
Risk of Bias Rating	Classifying domain as low, moderate, or high: - **Low risk of bias** 5-6 - **Moderate risk of bias**: 3-5 - **High risk of bias**: 1-2		

2. Prognostic Factor Measurement	**Goal: To judge the risk of measurement bias related to how biomarkers of cell injury were measured and assessed**			
Biomarkers explored	Biomarkers of cell injury are clearly mentioned throughout the study	0		1
Valid and reliable measurement of biomarkers	Method of biomarker measurement is adequately described	0		1
	Biofluid source clearly defined	0		1
	Unit of measurement for biomarker is reported	0		1
	Time sample was taken after injury was routine and adequately detailed	0	1	2
	Biomarker values were ideally measured as a continuous variable (2), but if it is treated discretely, appropriate cut-offs to define different levels of biomarker i.e. low vs high have been defined (1)	0	1	2
Proportion of data on biomarker concentration available for analysis	More than 80% of the study sample has completed data for biomarker levels	0		1
Summary of Prognostic Factor Measurement	**Total biomarker risk of bias score**	**/9**		
Risk of Bias Rating	Classifying domain as low, moderate, or high: - **Low risk of bias** 7-9 - **Moderate risk of bias**: 4-6 - **High risk of bias**: 1-3			

3. Outcome Measurement	**Goal: To judge the risk of bias related to the measurement of outcome**			
Definition of the Outcome	A clear definition of the clinical outcome is provided	0		1
Valid and reliable measurement of Outcome	The method of outcome measurement used is clinically recognised and validated to limit misclassification bias	0		1
	Outcome scores were ideally assessed as a continuous variable (2), but if it is treated discretely, appropriate cut-offs to define different levels of biomarker i.e. low vs high have been defined (1)	0	1	2
Method and setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants	0		1
	Timepoint post-injury that outcome assessment was administered is clearly stated. A uniform timeframe for each participant is preferred (2) but ranges are also accepted (1)	0	1	2
Proportion of data on outcome measurement	More than 80% of the total sample have available data for the outcome tasks	0		1
Effect sizes	Effect sizes for the association between biomarker levels and outcome variables are clearly presented or can be calculated without author contact	0	1	2
Outcome Measurement Summary	**Outcome of interest is adequately measured in study participants to sufficiently limit potential bias**	**/10**		
Risk of Bias Rating	Classifying domain as low, moderate, or high: - **Low risk of bias** 8-10 - **Moderate risk of bias**: 4-7 - **High risk of bias**: 1-3			

4. Statistical Analysis and Reporting	**Goal: To judge the risk of bias related to the statistical analysis and presentation of results**			
Presentation of analytical strategy	Appropriate statistical analyses were utilised to explore the relationship between biomarker levels and outcome measures	0		1
Assessing normality	Normality was assessed and data was either transformed (e.g. log transformation) or appropriate non-parametric analyses were used if data was non-normally distributed	0		1
Reporting of results	There is description of the association between biomarkers and outcome measures, including information about the statistical significance	0		1
	Continuous variables are analysed, or at least, cut-offs were used with clearly defined cut-off values	0	1	2
	No evidence of publication bias i.e. selectively reporting an outcome measure	0		1
Statistical Analysis and Reporting Summary	**The statistical analysis is appropriate for the design of the study, limiting potential for presentation of invalid or spurious results**	**/6**		
	Classifying domain as low, moderate, or high: - **Low risk of bias** 5-6 - **Moderate risk of bias**: 3-4 - **High risk of bias**: 1-2			

Adapted Quality Assessment Item Scoring Guide

Study participation

1. Key Study Variables and Recruitment	**Item description**	**Scoring procedure**
Source of target population	The source population or population of interest is adequately described	1 point is given if the authors clearly outline that they are investigating a TBI cohort
Sample characteristics	Key characteristics of the participant sample are detailed (i.e. age and gender)	1 point is given if basic sample demographics are described, consisting of at least age and gender
Recruitment methods	Sample identification, place of recruitment and recruitment process are provided in sufficient detail	1 point is awarded if authors clearly describe where they sourced their participants and how they recruited them
Inclusion and exclusion criteria	Inclusion and/or exclusion criteria are clearly and adequately described	1 point is given if inclusion and/or exclusion criteria are described (i.e., whether a specific TBI severity was explored in the study and how they determined said severity)
TBI Diagnosis	TBI diagnostic method or criteria are clearly stated and clinically validated	1 point is given if diagnostic criteria are clearly outlined in the methods of the paper, and the diagnostic criteria are clinically recognised
Injury Characteristics	Injury characteristics were clearly stated, as was the method for determining severity	1 point is given if author refers to injury characteristics i.e. TBI severities, LoC etc. included within the study

2. Prognostic Factor measurement	Item description	Scoring procedure
Biomarkers explored	The biomarkers explored in the study are explicitly mentioned in the methods and utilised in analyses	1 point is given if the authors provide details on biomarkers explored within the study
Valid and reliable measurement of biomarkers	Method of biomarker measurement is adequately described	1 point is awarded if the method of quantifying biomarker levels i.e. SIMOA or ELISA, are provided in the methods
	Biofluid source clearly defined	1 point is awarded if the biofluid source of each biomarker is provided
	Unit of measurement for biomarker is reported	1 point is provided if the unit of measurement i.e. pg/mL is provided for biomarker levels
	Time sample was taken after injury was routine adequately detailed	2 points are awarded if a specific timepoint or timeframe for biofluid extraction after injury is provided i.e. <6 hours or 12-24 hours. 1 point is awarded if a broad timeframe exceeding > 24 hours but < 48 hours is utilised i.e. 24-72 hours. 0 points are awarded if biofluid extraction is inconsistent across participants (> 48 hours) or vague i.e. baseline or admission
	Biomarker values were ideally measured as a continuous variable (2), but if it is treated discretely, appropriate cut-offs to define different levels of biomarker i.e. low vs high have been defined (1)	2 points are given if biomarker levels were assessed on a continuous scale (regardless of whether the authors analysed it as a continuous variable in the results section). 1 point is given if biomarker levels are assessed discretely with explicit cut-off scores. 0 points for a discrete assessment without explicit cut-off scores.
Proportion of data on biomarker concentration available for analysis	More than 80% of the study sample has completed data for biomarker levels	1 point is awarded if more than 80% of their sample has undergone biomarker extraction

3. Outcome measurement	**Item description**	**Scoring procedure**
Definition of the Outcome	A clear definition of the outcome is provided	1 point is awarded if the author explicitly states the behavioural outcome of interest, they are assessing
Valid and reliable measurement of Outcome	The method of outcome measurement used in valid and reliable to limit misclassification bias	1 point is provided if the assessment to measure behavioural outcomes were clearly stated and appropriate
	Outcome scores were ideally assessed as a continuous variable (2), but if it is treated discretely, appropriate cut-offs to define different levels of biomarker i.e. low vs high have been defined (1)	2 points are given if behavioural assessments were assessed on a continuous scale (regardless of whether the authors analysed it as a continuous variable in the results section). 1 point is given if behavioural assessments are assessed discretely with explicit cut-off scores. 0 points for a discrete assessment without explicit cut-off scores.
Method and setting of Outcome Measurement	The method and setting of outcome measurement is the same for all study participants	1 point is awarded if the authors administered their behavioural assessments in the same manner for all participants.
	Timepoint post-injury outcome assessment was administered is clearly stated. A uniform timeframe for each participant is preferred (2) but ranges are also accepted (1)	2 points are awarded if a specific timepoint all behavioural assessments were conducted has been stated. 1 point is awarded if a timeframe is used i.e. 6-12 months. 0 points are awarded if time behavioural assessment was conducted is inconsistent across participants (> 6 months).
Proportion of data on outcome measurement	More than 80% of the total sample have available data for the outcome tasks	1 point is awarded if more than 80% of their sample has undergone behavioural assessments
Effect sizes	Effect sizes for the association between biomarker levels and outcome variables are clearly presented without author contact	If effect sizes are clearly provided (in any format) are clearly presented and easily accessible, then 2 points are awarded. If enough data is provided that allows conversion into an effect size value then 1 point is given, and 0 points are awarded if study required author contact to obtain effect size.

4. Statistical Analysis and Reporting	**Item description**	**Scoring procedure**
Presentation of analytical strategy	Appropriate statistical analyses were utilised to explore the relationship between biomarker levels and outcome measures	1 point is awarded if the authors conduct any sort of associative analysis (continuous or discrete) between biomarkers and behavioural outcome
Assessing normality	Normality was assessed and data was either transformed (e.g. log transformation) or appropriate non-parametric analyses were used if data was non-normally distributed	1 point is awarded if authors assessed normality, with results reflected in consequent analyses
Reporting of results	There is description of the association between biomarkers and outcome measures, including information about the statistical significance	If authors present details about the relationship between biomarker levels and behavioural outcome measures that shows the direction and strength of the relationship/group differences, they are given 1 point.
	Continuous variables are analysed, or at least, cut-offs were used with clearly defined cut offs	2 points are awarded when variables are analysed as continuous variables, 1 point when categorical variables are analysed, and 0 points for poorly presented or absent results
	No evidence of publication bias i.e. selectively reporting an outcome measure	1 point given if there is no evidence of inconsistent reporting of results or the omission of results to achieve a specific outcome

Supplementary Material 4: Cognitive assessments encompassed in review and their respective cognitive domains

Cognitive Domains	Assessments
Global Cognition	Montreal Cognitive Assessment
	Mini Mental State Examination
	Composite scores derived from several cognitive domains
Working Memory	ImPACT – Verbal Memory
	ImPACT – Visual memory
	WSM-R Verbal Memory Index
	WSM-R Visual memory Index
	Digit Span
	Block Span
	Auditory Verbal Learning Test – Immediate
	Complex Figure Test – Immediate
	Buschke Selective Reminding Test
Attention / Executive Function	ImPACT – Reaction Speed
	King-Devick Test
	Stroop Test
	Trail Making Part B/2
	CalCAP Simple Reaction Time 1
	CalCAP Simple Reaction Time 2
	Cat Pred Error
	WSM-R Attention Concentration Index
	K-test
	Paced auditory serial addition test
Learning & Long-Term Memory	Auditory Verbal Learning Test – Learning
	Auditory Verbal Learning Test – Delayed
	Complex Figure Test – Delayed
	WSM-R General Memory Index
	Auditory Verbal Learning Test – 1-5 Total Score
	O-Test
	Free and Cued Selective Reminding Test

Visuospatial	ImPACT – Visual Motor Speed
	Spatial Span
	Complex Figure Test – Copy
Visual Processing Speed	Trail Making 1/A
	Digit Symbol
	Finger tapping
Language	Controlled Oral Word Association
	Excluded Letter Fluency – Total

Chapter 6: Statement of Authorship

Title of Paper	Assessing Nigrosome-1 Voxelwise Differences Between Traumatic Brain Injury and Parkinson's Disease as an Indicator of Disease Development and Predictor of Cognition: A FIND-TBI Preliminary Study
Publication Status	☐ Published ☐ Accepted for Publication ■ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	Angus McNamara		
Contribution to the Paper	Conceptualised, performed and interpreted statistical analyses and wrote the initial manuscript		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01/12/2024

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Mark Jenkinson		
Contribution to the Paper	Contributed to the organisation of the research project, particularly playing a pivotal role in determining analytical techniques and assisting with code development, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Name of Co-Author	Irina Baetu		
Contribution to the Paper	Contributed to the development of the cognitive ability score, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Name of Co-Author	Lyndsey Collins-Praino		
Contribution to the Paper	Contributed to the conceptualisation of the manuscript, organisation of the research project, the design and review of the statistical analysis and the review and critique of the overall manuscript		
Signature		Date	01/12/2024

Chapter 6 - Assessing Nigrosome-1 Voxelwise Differences Between Traumatic Brain Injury and Parkinson's Disease as an Indicator of Disease Development and Predictor of Cognition: A FIND-TBI Preliminary Study

Angus McNamara[1], Irina Baetu[2], Mark Jenkinson[3,4] & Lyndsey Collins-Praino[1]

[1] School of Biomedicine, University of Adelaide, SA 5000, Australia
[2] School of Psychology, University of Adelaide, SA 5000, Australia
[3] Australian Institute for Machine Learning, School of Computer and Mathematical Sciences, University of Adelaide, SA 5000, Australia
[4] South Australian Health and Medical Research Institute, SA 5000, Australia

6.1 ABSTRACT

Introduction: A history of traumatic brain injury (TBI) is a significant risk factor for developing neurodegenerative diseases, particularly Parkinson's Disease (PD). However, it is largely unknown who, following injury, will eventually progress in have PD. Recent advances in magnetic resonance imaging (MRI) allow early regions affected in PD to be visualised, such as using susceptibility weighted imaging (SWI) to assess nigrosome-1 (N1) in the substantia nigra (SN) *pars compacta*. However, evaluation of N1 within a TBI cohort is yet to be conducted. This study assessed voxelwise features of N1 across several cohorts, including TBI, to explore their potential as an early diagnostic marker and its association with cognition.

Methods: SWI was conducted across several cohorts, including healthy controls (n = 62), TBI (n = 88) and PD (n = 39). Additionally, the healthy controls and TBI patients were stratified according to age: young healthy controls (n = 28), young TBI (n = 37), aged healthy controls (n = 34) and aged TBI (n = 51). Principal component analysis was used to derive a composite score of cognitive ability from several cognitive assessments. Generalised linear models were used to determine voxelwise differences across groups, as well as assessing the association between voxel features and cognitive ability while controlling for relevant confounders.

Results: The PD cohort was significantly older than the healthy control and TBI cohorts, as well as having significantly lower cognitive ability. When stratified according to age, the aged TBI and PD cohorts both demonstrated significantly lower cognitive ability relative to the young healthy control and young TBI groups. Significant differences in SN voxel features, particularly N1, were present between PD and all other cohorts; however, no other differences were present. Likewise, no significant associations between voxelwise features and cognitive ability were found.

Conclusion: Findings from SWI-measured voxel features suggest that N1 may act as a marker capable of discriminating PD from other cohorts, but this utility does not seem to extend to TBI. This may indicate that alterations to N1 are closely associated with PD pathophysiology, but not seen with TBI alone. Further, N1 imaging demonstrated little utility in improving prognostic utility for cognitive function above and beyond clinically recognised predictors. Future research should validate such findings in larger sample sizes with an extensively phenotyped TBI cohort and explore alternative neuroimaging markers to identify individuals presenting the highest neurodegenerative risk.

6.2 BACKGROUND

A prior history of traumatic brain injury (TBI) corresponds to a substantially elevated risk of developing several neurodegenerative disorders, including Parkinson's Disease (PD). Gardner and colleagues report that even a single case of mild TBI (mTBI) corresponds to a 56% increased risk of developing PD, which increases up to 83% in the case of single moderate to severe TBI (M/sTBI) (Gardner et al., 2015). Such risk has been extensively explored across several studies, with a meta-analysis of 22 studies reporting a pooled risk ratio of 1.57 for risk of developing PD following a TBI (Jafari et al., 2013b). Further, the injury-dependent nature of increased risk was highlighted in two prospective cohorts (the Religious Orders Study and Memory and Aging Project), demonstrating that for individuals with a history of TBI, a loss of consciousness greater than one hour was associated with accelerated progression of parkinsonian features (Crane et al., 2016). While it is clear TBI is a considerable risk factor for PD, it is still largely unknown who, following injury, will develop the disorder. This is emphasised by considerable discrepancies in the number of cases of TBI compared to confirmed PD cases, with a global incidence of 27.1 million new cases of TBI in 2019 (Huang et al., 2024), compared to approximately 8.5 million confirmed PD cases in the same year

(Organization, 2022b). Given the substantial burden PD places on quality of life on those diagnosed (Zhao et al., 2021) and their carers (Lee et al., 2019a), as well as considerable economic burden (Yang et al., 2020), it is critical to identify who is most susceptible to PD development, allowing for earlier identification and more focused implementation of therapeutic interventions.

To this end, identifying early biomarkers of potential PD development could offer a path forward, with one promising option being the nigrosome-1 (N1) complex. Identified by Damier and colleagues, the N1 complex is a dopaminergic neuron rich region, located in the dorsal portions of the substantia nigra *pars compacta* (SNc) (Damier et al., 1999a). Importantly, the N1 region demonstrates considerable vulnerability relative to other portions of the SNc, and is one of the earliest regions to degenerate in PD (Damier et al., 1999b), corroborated by several other studies reporting earlier and more rapid deterioration of the ventrolateral SNc compared to medial portions in PD (Fearnley and Lees, 1991, German et al., 1989). Given the N1 territory is one of the earliest sites of degeneration in PD, it may act as a promising biomarker for early diagnosis several years prior to clinical onset.

Further, recent advances in magnetic resonance imaging (MRI) have made delineation of the N1 region possible *in vivo*, using susceptibility weighted imaging (SWI). The SWI modality is well suited for visualising N1 due to its high sensitivity to magnetic susceptibility differences in tissues, which are influenced by factors such as calcium, blood products and, most importantly for PD, iron and neuromelanin (Halefoglu and Yousem, 2018). Regional iron accumulation and consequent cell death is well established in PD, being significantly higher in the SNc of PD participants compared to healthy controls (Mochizuki and Yasuda, 2012, Zecca et al., 2004), and SWI is optimised for detection of subtle structural changes within such

regions. Under normal conditions, when imaged via SWI, N1 is a hyperintense region between two hypointensities within the dorsal SN (for visual representation of N1, refer to Figure 1 in study conducted by Lee and colleagues (Lee et al., 2021a)), and therefore, the absence of this hyperintensity acts as a promising diagnostic marker of PD (Cosottini et al., 2015). In fact, a meta-analysis of 19 studies, including a sample of 903 idiopathic PD and 605 healthy control participants, determined that visual assessment of N1 yielded excellent diagnostic discrimination, with a pooled area under the curve of 0.98 (Chau et al., 2020). Further, SWI demonstrates high enough sensitivity to be applied to at-risk populations, with a study comparing 46 REM-sleep behaviour disorder (RBD) patients, 27 PD patients, and 32 control subjects finding that over 25% of RBD participants demonstrated an abnormal N1 reminiscent of PD, determined via assessment of a dorsal nigral hyperintensity marker by two independent raters (Barber et al., 2020). This shows that SWI could potentially be beneficial in identifying who within an at-risk population are at risk of eventual PD conversion, highlighting the need to assess SWI-measured N1 in other at-risk groups, namely TBI. However, to the best of our knowledge, comprehensive assessment of N1 via SWI is yet to been conducted within a TBI population.

Beyond diagnostics, imaging of the N1 territory may also demonstrate prognostic capabilities, particularly considering that both PD and TBI are associated with cognitive dysfunction. No longer considered a purely motor disorder, it is now widely recognised that PD corresponds to decline in various cognitive domains, with executive function, attention and working memory being the most affected (see review, (Fang et al., 2020)). While several transmitter pathways changes are present throughout PD (Barone, 2010), progressive dopaminergic loss leads to altered activity of dopamine-dependant pathway, such as the nigrostriatal and mesocorticolimbic tracts, which are heavily implicated in cognitive decline during disease

course (Halliday et al., 2014). Like PD, TBI is considered a hypodopaminergic condition, leading to alterations in the same dopamine-dependant tracts (see review, (Chen et al., 2017)), which may, in part, help to explain why up to 67% of TBI cases result in cognitive impairments (Jourdan et al., 2016). Further, there is considerable overlap in the cognitive domains affected between PD and TBI. For example, following moderate-severe TBI (M/sTBI), persistent cognitive complaints in attention (Ponsford et al., 2014) and episodic memory (Turkstra et al., 2023) haven been reported several years following injury. Such cognitive complaints are also common in less severe cases of injury, particularly working memory (Hudac et al., 2018, Gosselin et al., 2012). Given this overlap in clinical presentation, it is possible that similar N1 alterations may exist across both conditions and might demonstrate important associations with cognition.

Therefore, this study used SWI to assess voxelwise differences in N1 for a TBI cohort relative to healthy control and PD cohorts, acting as negative and positive controls, respectively. Exploratory analysis stratified the healthy control and TBI cohorts into young and aged cohorts, to account for the potentially confounding effects of age. Further, comprehensive cognitive assessments were conducted, to determine the potential utility of voxelwise changes in N1 as a predictor of cognitive ability across cohorts. This study is the first, to our knowledge, to explicitly explore SWI-measured N1 changes within a TBI cohort, a critical first step in improved identification of individuals at-risk of PD conversion within an at-risk population.

6.3 METHODS

This study was approved by the Human Research Ethics Committee at the University of Adelaide (Ethics approvals: H-2016-219; H-2020-017; H-2020-016; H-2021-120) and conducted in accordance with national guidelines. Participants provided written informed

consent prior to participating in both behavioural testing and magnetic resonance imaging (MRI) acquisition sessions, which were conducted on separate days.

6.3.1 Participants and Cohorts

A total of 189 participants are included in this analysis, recruited via online advertising and community outreach in Adelaide, South Australia. The participants were categorised into three cohorts: 1) healthy controls (n = 62); 2) TBI (n = 88); and 3) idiopathic PD (n = 39).

6.3.1.1 Inclusion and Exclusion Criteria

General exclusion criteria for all participants included:

1) < 18 years of age
2) Substantial hearing and visual impairments not corrected by glasses/contacts
3) Previous diagnosis of a learning disability
4) Lack of English fluency
5) Deemed ineligible to undergo an MRI upon screening, due to:
    - Suffering from claustrophobia
    - Being pregnant or suspecting pregnancy
    - Possessing a metal implant incompatible with MRI

Cohort specific inclusion and exclusion criteria are outlined in Table 6-1:

Table 6-1 Cohort specific inclusion criteria

Cohort	Inclusion Criteria
Healthy Control	No prior history of TBI

TBI	Reported prior history of brain injury resulting from external force (i.e. not stroke or other vasculature complications) Injury sustained above 10 years of age Absence of other neurodegenerative disorder diagnosis
PD	Formal diagnosis of idiopathic PD by a registered neurologist Absence of diagnosis of another neurodegenerative disorder

6.3.1.2 TBI Severity Determination

The TBI cohort was dichotomised according to injury severity: mild (mTBI) or moderate/severe injury (M/sTBI). Participants were categorised as mTBI if they reported receiving a Glasgow Coma Scale (GCS) score of 13-15, or in the event that a GCS was not administered, mTBI was determined using the following injury characteristics: 1) a loss of consciousness of less than one hour, 2) post-traumatic amnesia lasting less than 24 hours, 3) no skull fracture and 4) no imaging abnormalities noted, if imaging was collected at time of injury. If any of these criteria were not met, participants were categorised as M/sTBI. Further, in the event where participants reported several TBIs within their lifetime, information on both their earliest and most recent injury was collected and injury severity for both injuries was separately determined. Where possible, confirmation of injury severity was confirmed with a registered neurosurgeon.

6.3.1.3 Age Stratification

To account for the potentially confounding effects of age, particularly for cognition which demonstrates well-established change throughout the lifespan across multiple domains (Murman, 2015), exploratory analyses were conducted where the healthy control and TBI cohorts were stratified into young (< 55 years of age) and aged (≥ 55 years of age) groups. As

PD is considered an age-related neurodegenerative disorder, this cohort was not further subdivided, as only one PD participant would have been considered young using the above criteria. A total of five groups were included for subsequent analyses: young healthy control (n = 28), young TBI (n = 37), aged healthy control (n = 34), aged TBI (n = 51) and PD (n = 39).

6.3.2 Deriving a Global Cognitive Ability Score

During the behavioural testing sessions, participants undertook a suite of cognitive tasks. The PD cohort underwent cognitive assessment while taking medication, including dopamine replacement therapy, as prescribed. Cognitive assessments collected and included within subsequent analyses are outlined in Table 6-2.

Table 6-2 Cognitive measures used to derive a composite cognitive ability score, including the domains they assess and the way they were scored

Cognitive Assessment	Domain	Scoring Method
Raven's Progressive Matrices (Raven and Raven, 2003)	Fluid intelligence	Total score of 18 trials
Dot Matrix (Alloway et al., 2008)	Working memory	Total number of correct dot locations recalled, with higher scores indicative of better performance
Digit Span (Woods et al., 2011)	Short-term verbal memory	Summative score of total number of correct trials recalled, including both forward and reverse assessments
Stop-Signal Reaction Time Task (SSRT) (Dagenbach and Carr, 1994)	Measure of inhibitory control	Average reaction time to correctly inhibit response
Most Likely Inspection Time (Garaas and Pomplun, 2008)	Visual processing speed	Shortest inspection time to identify the correct direction of the stimulus (before it is covered) with 75% accuracy.
Reinforcement Learning Task (RL) (Frank et al., 2004)	Reinforcement learning	Average proportion of correct responses in both AB and CD stimuli pairings.

To derive an overall cognitive ability measure, principal component analysis (PCA) was conducted on all assessed cognitive variables, and the individual variable loadings were used to construct a weighted average. Of note, healthy control and PD participants included within this study were enrolled in a wider study assessing cognition across the lifespan (n = 1258) and within PD, respectively (n = 106). Consequently, the PCA was conducted on all participants with available cognitive data (n = 1542); however, only the subset that had available MRI data were included for assessment within this study (n = 189). Outputs of the PCA, including eigenvalues, variance explained and loadings per cognitive assessment are summarised in Table 6-3.

Table 6-3 PCA outputs for cognitive ability scores

	Loadings	**Eigenvalue**	**Variance accounted by first factor**
Cognitive Ability		2.1	35%
Ravens	0.72		
Dot Matrix	0.74		
SSRT	-0.57		
Digit Span	0.52		
Most Likely IT	-0.52		
Average RL	0.40		

6.3.3 Image Acquisition and Pre-Processing

All neuroimaging sequences were collected using a Siemens Magnetom Skyra 3.0 T MRI scanner, across a 45-minute protocol. This consisted of 11 different imaging sequences; however, the only sequences utilised within this study are: 1) 3D T1-weighted magnetisation-prepared rapid gradient echo sequence (MPRAGE) (5 minutes and 30 seconds acquisition time, 192 1mm sagittal slices, voxel size = 1 x 1 x 1mm, echo time (TE) = 2.96ms, repetition time (TR) = 2300ms, flip angle = 9°, phase encoding direction anterior to posterior, with an acquired matrix size of 201 x 269 x 271 and 2) a midbrain susceptibility weighted imaging (SWI) sequence (4 minute and 37 second acquisition time, 52 0.8 mm axial slices, voxel size = 0.8 x

0.8 x 0.8 mm, TE = 25ms, TR = 42ms, flip angle = 15°, phase encoding direction right to left and an acquired matrix size of = 176 x 205 x 93). Preprocessing of both imaging sequences included skull-stripping (removal of non-brain tissue) and registration to MNI standard space.

6.3.4 Statistical Analyses

All demographic and cognitive data were analysed using R (version 1.4.1717) (Team, 2010). A Shapiro-Wilk test was conducted and determined that age and cognition deviated significantly from normality, with only education being normally distributed. Therefore, normally distributed variables are displayed as mean ± standard deviation, whereas non-normally distributed variables are reported as median (interquartile range). In addition, frequency data were summarised as counts (percentage). For group-level comparisons of demographic and clinical outcomes, a one-way ANOVA or Kruskal-Wallis test was conducted for normal and non-normally-distributed variables, respectively. If significance was detected, post-hoc Tukey or Dunn tests were conducted for normal and non-normal data, respectively, with a Bonferroni multiple comparison correction applied.

To compare potential differences in nigrosome features across groups, as well as to explore the association of the nigrosome with cognitive ability, a generalised linear model (GLM) was used to compare voxelwise differences in SWI intensity using FSL (Jenkinson et al., 2012). Statistics were performed in FSL using the permutation-based inference tool randomise, with 5000 permutations using the threshold free cluster enhancement (TFCE) statistic. Several potential confounders were controlled for in this analysis. Of note is that throughout the testing period, a scanner change-over occurred, and therefore, scans were normalised by scaling the intensity levels, as well as adding a confounder into the GLM that represented the individual scanner that each scan was collected on. When comparing the healthy control, TBI and PD groups,

additional confounders included age, sex and, for the TBI group, injury severity and time since injury. If participants reported multiple TBIs, severity and time since their earliest and most recent injury were accounted for by including them as separate confounder columns. When stratifying by age, the age confounder column was split using the 55-year cut-off.

When assessing the relationship between voxelwise features of the nigrosome and cognition, group and education were added as confounders. All confounders were demeaned, to allow slope to be estimated independently from the intercept. If voxelwise features significantly differed between groups, t-statistic images highlighting voxels that were deemed significantly different between groups were generated, as well as supporting boxplots reflective of the magnitude of the differences. Likewise, if voxels were identified to have a significant association with cognitive ability, representative images and boxplots were generated.

6.4 RESULTS

6.4.1 Clinical Variable Comparisons

For the TBI cohort, given that some participants reported multiple injuries (n = 28), when performing descriptive statistics for injury severity and time since injury, the earliest reported TBI was used. To this end, an equal split of mTBI and M/sTBI was reported (n = 44 per cohort), with a median time since injury of 20.5 years (range: 0.04 – 59.5 years). For the subset with multiple injuries, injury characteristics for their most recent injury were also calculated, with the majority reporting a mTBI (n = 16; 57%), and a median time since injury of 20.7 years (range: 0.1 – 50.8 years). Additionally, the PD cohort demonstrated a median time since diagnosis of 4 years, (range: 0.1 – 18 years).

When comparing demographic variables of interest and cognition across groups, Kruskal-Wallis tests determined that significant differences were present in age ($H(2)=40.9$, $p<0.0001$), cognitive ability ($H(2) = 16.5$, $p = 0.0003$), as well as sex ($X^2 = 10.8$, $p = 0.005$) as determined via a Chi-square test. Descriptive statistics for each group are reported in Table 6-4

Table 6-4 Differences in variables of interest between healthy controls, TBI and PD*. Normally distributed variables are displayed as mean ± SD, with statistical differences determined via one-way ANOVAs. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Kruskal-Wallis tests. Statistically significant differences are denoted by bolded p-values.*

	Healthy Control		TBI		PD		
	n	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**n**	**Mean ± SD / Median (IQR)**	**p-value**
Age	62	56 (43.5 – 64)	88	59.5 (41 – 68)	39	71 (67 – 74)	**< 0.0001**
Education	61	16.14 ± 2.98	88	16.14 ± 2.98	34	15.94 ± 3.11	0.90
Sex							
Male		25 (40%)		53 (60%)		28 (72%)	**0.005**
Female		37 (60%)		35 (40%)		11 (28%)	
Cognitive Ability	55	0.28 (-0.19 – 0.6)	80	0.02 (-0.49 – 0.57)	31	-0.85 (1.56 – 0.07)	**0.0003**

Post-hoc Dunn tests determined that the PD cohort was significantly older relative to both the healthy control ($Z(2, 86) = 6.09$, $p < 0.0001$) and TBI ($Z(2, 109) = 5.5$, $p < 0.0001$) cohorts. Likewise, the PD cohort presented with the poorest cognitive performance, having significantly lower scores than both healthy controls ($Z(2, 99) = -6.09$, $p < 0.0001$) and TBI ($Z(2, 125) = -5.5$, $p < 0.0001$). Additionally, it was found that the TBI and PD cohort presented with a significantly higher percentage of males (60% and 72%, respectively) compared to healthy controls (40%, $p = 0.005$). Significant differences in age and cognitive ability between groups are detailed in Figure 6-1.

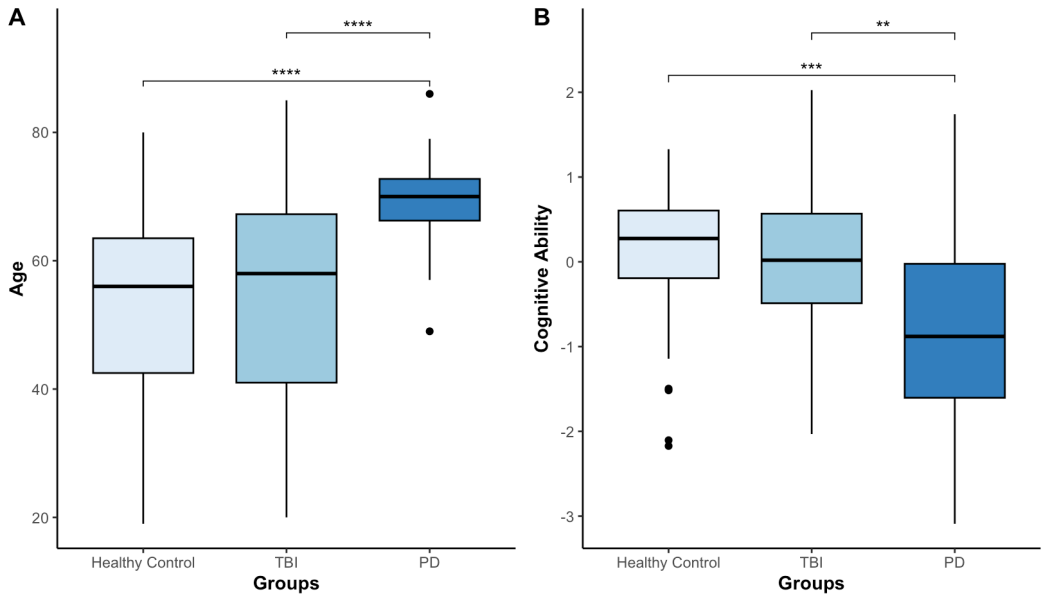


Figure 6-1 Kruskal-Wallis tests determined significant variance in age and cognitive ability between groups*. Post-hoc Dunn tests determined that, relative to healthy control and TBI cohorts, the PD cohort was (A) significantly older, as well as having (B) significantly lower cognitive ability.*

6.4.2 Voxelwise Group Comparisons

When comparing voxelwise features in the midbrain, the GLM found significant differences for voxels where the intensity (in the SWI scan) was lower in the PD cohort relative to both the healthy control (Figure 6-2A) and TBI (Figure 2B) cohorts. Figure 6-2 is supplemented with post-hoc boxplots summarising the intensity differences between the compared groups across the voxels showing significant differences. No voxelwise differences were present between healthy controls and the TBI cohort.

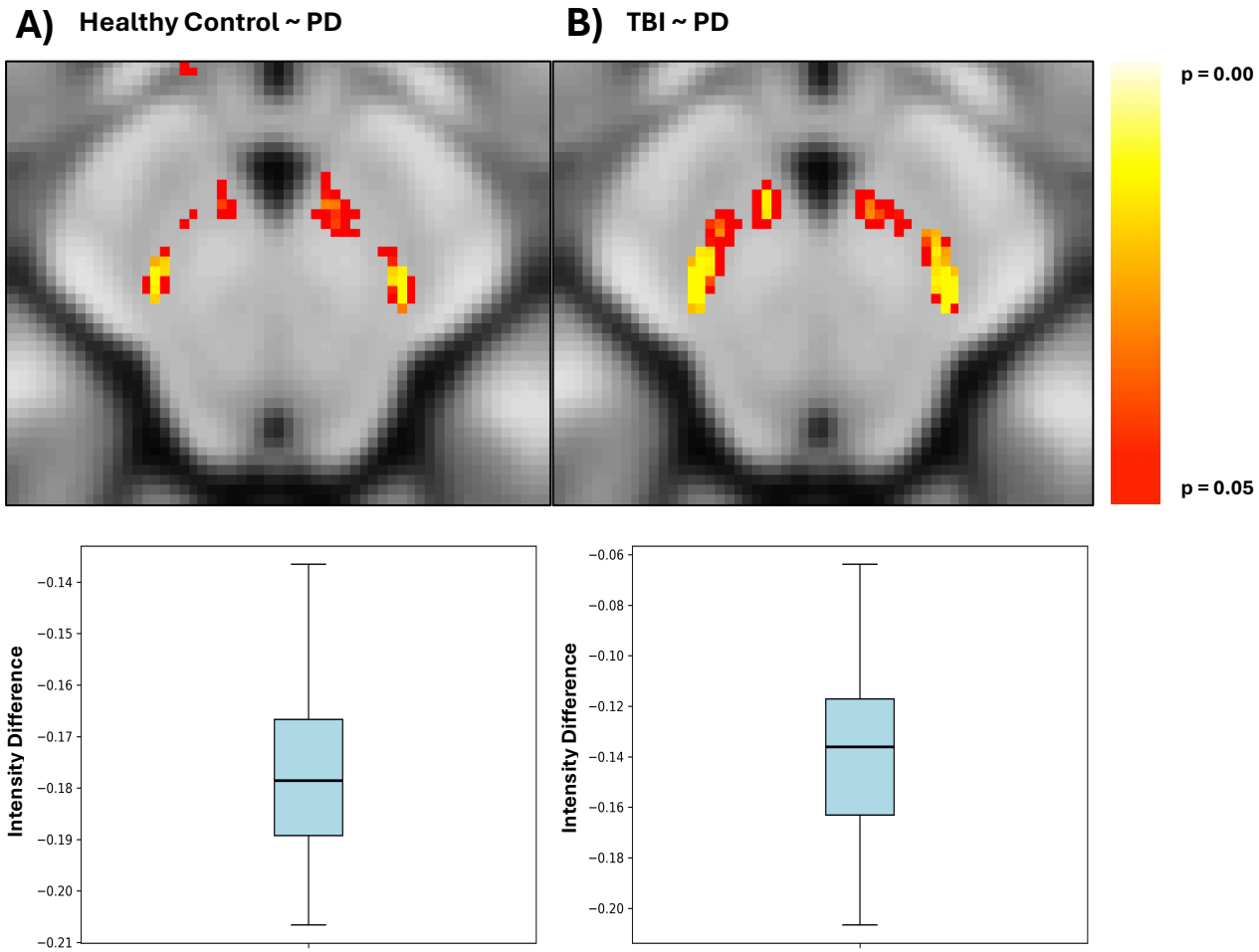


Figure 6-2 ***Voxelwise differences in the midbrain were detected between the PD cohort and both the (A) healthy control and (B) TBI cohorts****, with significant voxels presented in colour. The scale represents the p-value from the GLM after multiple comparison correction, with yellow-white voxels indicative of voxels that were most significantly different (p-value near 0).*

6.4.3 Clinical Variable Comparison After Stratifying by Age

After stratifying by age, when comparing demographic variables and cognition across groups, it was determined that significant differences were present in age ($H(4)= 133$, $p < 0.0001$), cognitive ability ($H(4) = 39.2$, $p = 0.0003$) and sex ($X^2 = 12.5$, $p = 0.02$). Descriptive statistics for each group are reported in Table 6-5.

Table 6-5 Group differences while controlling for potentially confounding effects of age. *Normally distributed variables are displayed as mean ± SD, with statistical differences determined via one-way ANOVAs. Non-parametric variables are displayed as median (IQR), with statistical differences determined via Kruskal-Wallis. Statistically significant differences are denoted by bolded p-values*

	Young Healthy Control		Young TBI		Aged Healthy Control		Aged TBI		PD		
	n	Mean ± SD / Median (IQR)	n	Mean ± SD / Median (IQR)	n	Mean ± SD / Median (IQR)	n	Mean ± SD / Median (IQR)	n	Mean ± SD / Median (IQR)	p-value
Age	28	42 (30.25 – 50.25)	37	39 (30 – 49)	34	63.5 (58 – 69)	51	67 (63 – 70.5)	39	71 (67 – 74)	**<0.0001**
Education	27	17.35 ± 3.08	37	16.86 ± 3.41	34	15.18 ± 2.57	51	15.69 ± 4.14	39	15.94 ± 3.11	0.07
Sex											
Male		9 (32%)		21 (57%)		16 (47%)		32 (63%)		28 (72%)	**0.02**
Female		19 (78%)		16 (43%)		18 (53%)		19 (37%)		11 (28%)	
Cognitive Ability	26	0.44 (0.16 – 0.77)	36	0.30 (0.12 – 0.96)	29	-0.11 (-0.84 – 0.50)	44	-0.37 (-0.71 – 0.09)	31	-0.85 (1.56 – 0.07)	**<0.0001**

Post-hoc Dunn tests determined that aged cohorts were significantly older ($p < 0.0001$) than both the young healthy control (Aged healthy control: $Z(4,58) = 5.39$; Aged TBI: $Z(4,75) = 7.11$; PD: $Z(4,63) = 8.07$) and young TBI groups (Aged healthy control: $Z(4,67) = 5.99$; Aged TBI: $Z(4,84) = 7.96$; PD: $Z(4,72) = 8.91$). Additionally, despite stratification, the aged healthy control cohort were still significantly younger than the PD group ($Z(4, 69) = 2.65$, $p = 0.032$), a difference not seen between aged TBI and PD ($Z(4,86) = 1.53$, $p = 0.38$). Cognitive ability demonstrated a negative relationship with age, with both PD and the aged TBI cohort having significantly lower scores ($p < 0.05$) relative to both the young healthy control (Aged TBI: $Z(4,66) = -3.90$, $p = 0.0006$; PD: $Z(4,53) = -4.72$, $p < 0.0001$) and young TBI groups (Aged TBI: $Z(4,76) = -4.02$, $p = 0.0005$; PD: $Z(4,63) = -4.88$, $p < 0.0001$). Although, cognition did not significantly differ between the Aged TBI and PD groups ($Z(4,71) = -1.24$, $p = 0.64$). Further, sex distribution across groups was deemed significantly different ($X^2 = 12.5$, $p = 0.01$), with young healthy controls presenting with the lowest proportion of males (37%) compared to PD presenting with the highest proportion (73%). Significant differences in age and cognitive ability between groups are detailed in Figure 6-3.

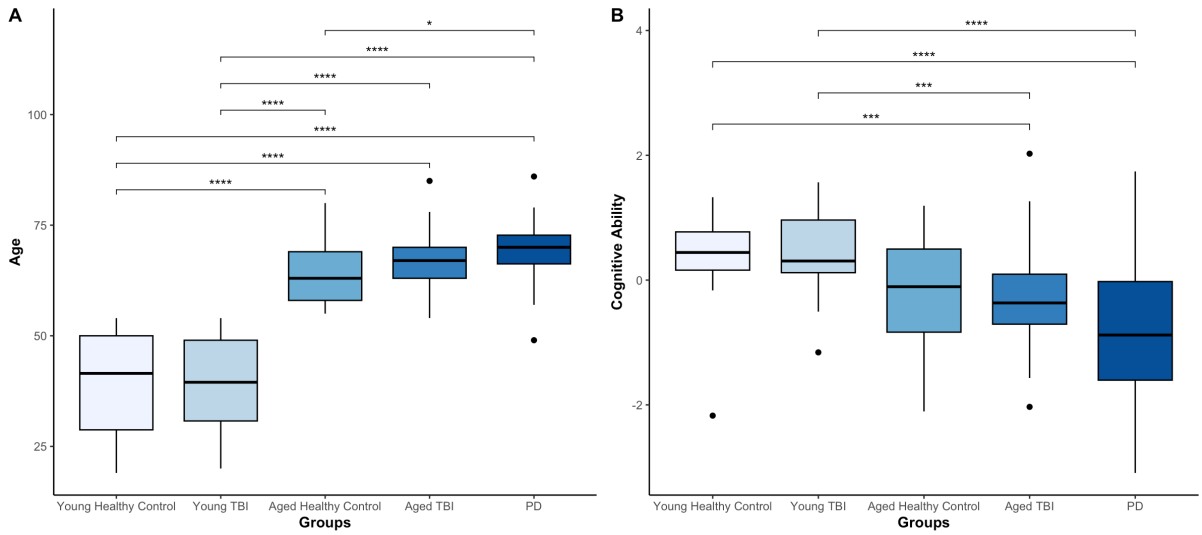


Figure 6-3 ***Kruskal-Wallis tests determined significant differences in age and cognitive ability between groups testing while controlling for age.*** *Post-hoc Dunn tests determined that: (1) relative to the young healthy control and TBI cohorts, the aged cohorts, as well as PD, were significantly older, with the PD cohort also being significantly older than the aged healthy control cohort; (2) a negative relationship was*

present between age and cognitive ability, with the aged TBI and PD cohorts demonstrating significantly lower cognitive ability compared to both the young healthy control and TBI cohorts.

6.4.4 Voxelwise Comparisons Across Age-Stratified Groups

Like the previous voxelwise comparisons, the GLM found significant differences for voxels where the intensity (in the SWI scan) was lower in the PD cohort relative to all other groups, as detailed in Figure 6-4. However, when assessing group differences across other cohorts, no significant differences were detected

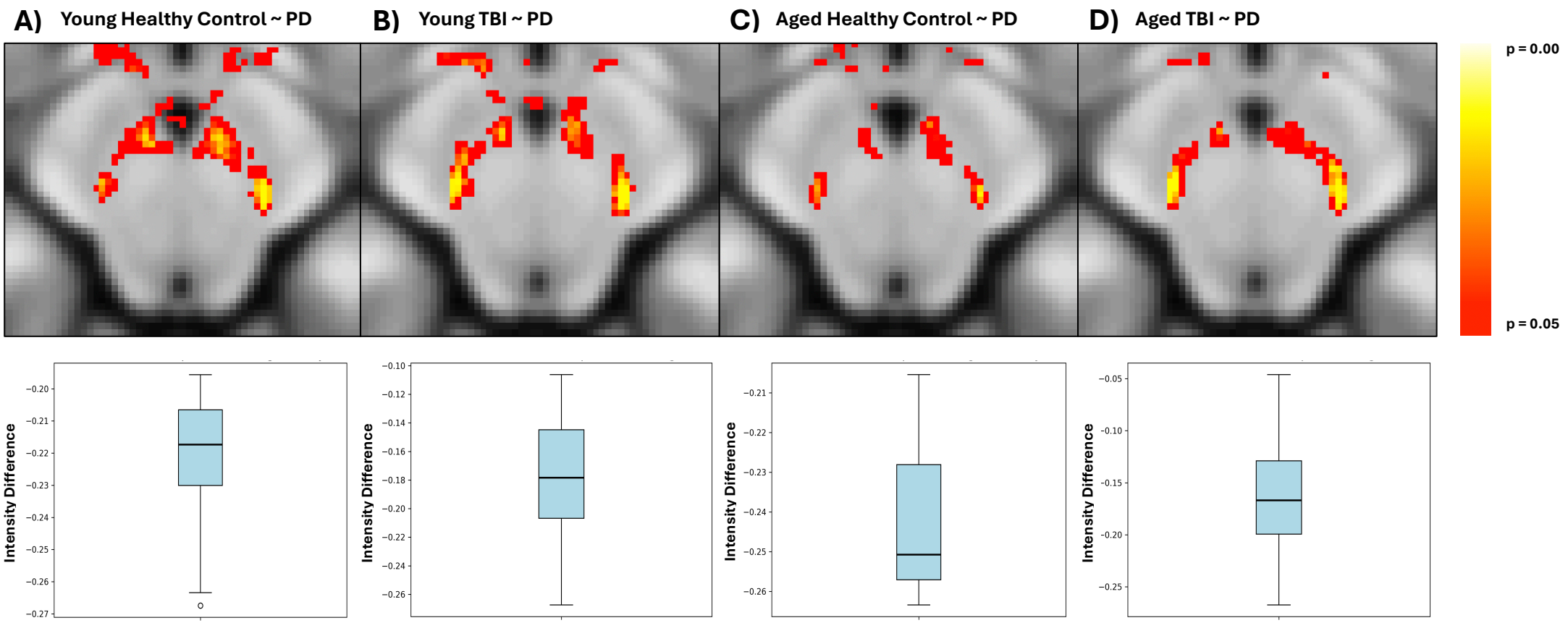


Figure 6-4 Voxelwise differences in the midbrain were detected between the PD cohort and the (A) young healthy control, (B) young TBI, (C) aged healthy control and (D) aged TBI cohorts*, with significant voxels presented in colour. The scale represents the p-value from the GLM after multiple comparison correction, with yellow-white voxels indicative of voxels that were most significantly different (p-value near 0).*

6.4.5 Utility of the Nigrosome to Predict Cognitive Ability

In the GLM that tested if any voxels within the midbrain demonstrated associations with cognitive ability, no significance was found and therefore a representative figure could not be generated. To determine if variables included as confounders in the GLM significantly predicted cognitive ability, further linear regressions were conducted (Table 6-6). It was found that several confounders significantly predicted cognitive ability, such as age (β = -0.03, $p < 0.001$), education (β = 0.05, p = 0.01) and having a diagnosis of PD (β = -0.46, p = 0.017). Further, a moderate amount of variance was predicted by the model (37%) and therefore, it is probable that, within the GLM, when weighted against such confounders, any nigrosome differences probably contributed very little to additional proportion of the variance explained in cognitive ability.

Table 6-6 Multivariate linear regressions were conducted to determine whether confounders present within the voxelwise GLM were significant predictors of cognitive ability*. Regressions determined that both older age and membership in the PD cohort relative to healthy controls yielded significant negative associations with cognitive ability, with a higher number of years of education demonstrating a significant positive association.*

Cognitive Ability

Predictors	*Estimates (β)*	*CI*	*p-value*
Intercept	0.81	-0.05 – 1.67	0.066
Age	-0.03	-0.04 – -0.02	**<0.001**
Female Sex	-0.09	-0.33 – 0.15	0.457
Education	0.05	0.01 – 0.08	**0.010**
TBI	-0.04	-0.31 – 0.23	0.771
PD	-0.46	-0.83 – -0.08	**0.017**
Observations	165		
R^2 / R^2 adjusted	0.368 / 0.348		

6.5 DISCUSSION

This study was, to the best of our knowledge, the first to explore voxelwise differences in N1 within a TBI population, relative to a healthy control and PD cohort, as an indicator of potential disease development and potentially aid in earlier diagnosis. Additionally, to control for the potentially confounding effects of age, exploratory analyses involved stratifying the healthy control and TBI cohorts into a young and aged cohort. Finally, the association between midbrain voxelwise features and cognitive ability was explored. Overall, voxelwise differences were present in PD relative to all other cohorts, with the most significant voxels being present within the N1 region; however, significant voxelwise differences were not present between any other groups. Additionally, when determining associations between voxel features and cognitive ability, no significant results were reported, likely on account of the presence of confounders that were themselves significant predictors of cognition, such as age, education and PD membership, which was confirmed via multivariate regression findings. While such findings indicate N1 as a poor predictor of cognitive ability and indicate that N1 is not significantly altered in those with a history of TBI relative to healthy controls, further research is warranted, and these findings should be interpreted with limitations in mind.

When comparing voxelwise features of the midbrain across groups, it was determined that the SN, specifically N1, were profoundly different in PD relative to all groups. This finding in itself is unsurprising, given that in PD more than 60% of dopaminergic neurons are already lost prior to clinical onset (Dauer and Przedborski, 2003), and that the N1 territory is also amongst the most vulnerable and earliest to degenerate (Damier et al., 1999b). Even in the context of ageing, previous studies have found that dopaminergic neuron loss due to age is different to what is observed throughout PD, with loss being greatest in the lateral ventral tier of the SN which is relatively spared in PD compared to the dorsal tier in particular (Fearnley and Lees,

1991), perhaps explaining the why PD significantly differed from both the young and aged cohorts. In fact, the former study notes that age-related changes to dopaminergic neuron content are distinct to what is reported throughout PD, meaning while accounting for age is important for effective comparisons in clinical presentation, such as cognitive ability, it may be less vital when assessing N1 features.

Surprisingly, however, voxelwise features were not different between other cohorts, namely TBI and healthy controls, even when stratified according to age. This is perhaps indicative of SWI not possessing sensitivity high enough to detect more subtle voxelwise differences, with previous studies reporting that N1 imaging showed little utility in discriminating PD from atypical parkinsonism's (Meijer et al., 2016, Kathuria et al., 2021), which also present with substantial dopaminergic neuron loss. This is also surprising given that previous studies have supported that there are alterations in the dopaminergic system in those with a history of TBI. Several clinical studies assessing dopamine transporter (DaT) via single photon emission tomography (SPECT) imaging have elucidated dopaminergic reductions following TBI, with a study using the I-β-CIT tracer reporting over 50% striatal DaT binding reduction following head trauma resulting in a vegetative state (average time post injury = 141 days) (Donnemiller et al., 2000). Further, less extreme albeit still M/sTBI cohorts have reported likewise patterns, ranging from 15 – 20% striatal DaT reductions (Jenkins et al., 2018, Wagner et al., 2014). This suggests that DaT imaging is a more direct measure of dopaminergic system function, supported by its approval, specifically Ioflupane or [^{123}I]-FP-CIT, for use as a tool to assist in PD diagnosis (de la Fuente-Fernández, 2012) by the Food and Drug Administration. Therefore, it is possible that DaT offers increased sensitivity compared to SWI. However, several of the aforementioned studies primarily assessed severe cases of injury, whereas the present study included a broader range of injury, potentially increasing variability in the overall TBI cohort.

However, another important consideration is that limited prior research exploring N1 features in at-risk populations has been conducted, with only a single study assessing RBD relative to healthy controls and PD. Individuals presenting with RBD have a particularly high risk of developing PD, with Postuma and colleagues highlighting that in a cohort of 89 individuals, 30% developed PD at three-year follow-up, increasing to 66% at 7.5 years (Postuma et al., 2015b). Interestingly, in the study assessing N1 across 46 RBD participants, only 25% demonstrated an SN reminiscent of PD (Barber et al., 2020). This highlights that, even within at-risk populations, only a small percentage may show signs of PD-like pathophysiology and eventual development of PD. Within TBI populations, only a relatively small subset of individuals go on to present with chronic functional impairments or develop PD. For example, investigation in the TRACK-TBI cohort found that, of 1453 mTBI participants assessed, only 53% reported functional impairments, as measured via the Glasgow Outcome Scale Extended (GOSE), at 12-months post-injury (Nelson et al., 2019). Likewise, only 28% of 61 individuals presenting with mTBI at a UK Major Trauma Centre reported incomplete recovery on the GOS two-years post injury (Carroll et al., 2020). As it is hypothesised that individuals presenting with persistent impairments have a higher risk of PD development following injury, it is possible that individuals in the community dwelling TBI cohort within this study did not demonstrate such long-term persistent impairments (noting that the median time since injury in this cohort was 20.5 years (range: 0.04 – 59.5 years)) and thus risk of PD development may not be as high as that reported in previous large epidemiological studies. Additional analysis of behavioural data collected as part of the wider FIND-TBI study is necessary in order to probe this further.

When assessing cognition across cohorts, overall cognitive ability was significantly lower in PD compared to healthy controls and TBI. Even when accounting for the confounding effects

of age, a clear negative relationship was present, with aged TBI and PD being the oldest cohorts and demonstrating significantly lower cognitive ability compared to the young cohorts. This is consistent with previous literature identifying ageing as a well-established predictor of cognition across conditions, including healthy ageing (Murman, 2015, Mikkelsen et al., 2024), TBI (Downing et al., 2024a, Skilbeck et al., 2019) and PD (Guo et al., 2021, Pontone et al., 2009). This may also explain the lack of significant associations observed between N1 voxels and cognitive ability within the present study, as age, in addition to other recognised predictors of cognition, such as education (Albert et al., 1995, Lyketsos et al., 1999) and PD diagnosis, were found to be significant predictors in the multivariate linear regression. Taken together, it appears that there is little additional association between the SWI intensities in N1 and cognitive performance across the included cohorts, over and above what is explained by the non-imaging predictors.

Given N1 imaging in this study demonstrated limited utility in detecting potentially more subtle voxelwise differences, as well as the associations with cognitive ability, future work should aim to determine its specificity for PD and as a marker of dopaminergic function. To this end, the relationship between N1 and other pathophysiological markers of PD, namely positron emission tomography (PET) fluorodopa (F-Dopa) uptake, should be explored. As F-DOPA is a more direct measure of dopaminergic system integrity (Berti et al., 2011), assessing striatal F-DOPA content along with N1 imaging features will allow us to determine how reflective N1 imaging is of dopaminergic dysfunction across our TBI and PD cohorts. Validating N1 as a biomarker could prove highly valuable in biologically defining risk in a TBI population, and other at-risk populations, as F-DOPA PET and Dat SPECT is not only costly, but not as readily accessible as MRI. This would be a crucial next step in determining the clinical utility of N1 as a tool for earlier diagnosis in PD. It should be noted that FIND-TBI is an ongoing research

project, aiming to recruit more participants to address the age and sex disparity present within this study, as well as actively collecting data such as F-DOPA and fluid-based pathological measures which could be instrumental in validating SWI, and providing more robust characterisation of PD-like presentation in our TBI cohort.

However, limitations relating to SWI as a modality should also be considered. While there is large consensus on SWI-measured SN changes demonstrating excellent discrimination when comparing PD and healthy controls, limited research has been conducted in more at-risk populations. Therefore, it is possible that the SWI protocol utilised within this study may lack the resolution and specificity required to detect more subtle anatomical changes in the N1 territory. This ties into limitations resulting from a lack of standard protocols to visualise N1 at present (Cheng et al., 2020), which should be considered for future studies, as variability across studies may complicate the interpretation and transability of research findings. Further, assessing voxelwise features using the present technique may lack power due to only assessing individual voxel values for each test. Therefore, alternatives such as creating a feature across multiple voxels, such as an average intensity based on the anatomical region of interest, would benefit from averaging and thus reducing overall noise. Therefore, the field may benefit from standardisation in SWI acquisition and assessment, improving comparability and consequent reliability in studies assessing N1. Further, limitations pertaining to the dataset itself must be considered. For example, alternative techniques rather than PCA to derive a composite cognitive ability score may be more suitable, such as deriving z-scores, a well-established technique for assessing cognition in neurodegenerative research (Weintraub et al., 2018, Weintraub et al., 2009). Further, the primary research question is detecting which individuals with TBI are likely to develop PD, which would require a well characterised longitudinal cohort rather than the cross-sectional design employed in the present study. Therefore, future work

should aim to track such cohorts longitudinally, as well as changes to voxelwise features and cognitive ability to elucidate who presents highest risk of PD conversion.

6.6 CONCLUSION

To the best of our knowledge, the present study is the first to explore the utility of assessing N1 via SWI within a TBI cohort, to determine its utility as a potential early diagnostic biomarker, as well as a prognostic marker of cognitive ability. While assessing voxelwise features determined that the N1 of individuals with PD significantly differed to all other cohorts, even when accounting for age, group differences between other cohorts, namely TBI and healthy controls, were not present. Further, N1 voxelwise features were not significantly associated with cognitive ability, likely on account of controlling for demographics accounting for a substantially higher amount of variance within the model. However, such findings are largely preliminary, with a scarcity of studies assessing N1 features within at-risk populations, as well as large variability in SWI protocols and assessment demanding standardisation across studies. Further, the present study would greatly benefit from a prospective longitudinal design, whereby all cohorts and their N1 features are tracked over-time to better characterise which individuals within in at-risk population demonstrate higher risk for PD conversion.

6.7 FUNDING

Funding sources and conflict of interest: This work was funded by grants to LCP, MJ and IB by the Medical Research Future Fund (2020/MRF1202188), James and Diana Ramsay Foundation and NeuroSurgical Research Foundation. AM was supported by an Australian government Research Training Program scholarship. The authors declare that there are no conflicts of interest relevant to this work.

6.8 AUTHOR INFORMATION

6.8.1 Author Affiliation

School of Biomedicine, University of Adelaide, SA 5000, Australia

Angus McNamara & Lyndsey Collins-Praino

School of Psychology, University of Adelaide, SA 5000, Australia

Irina Baetu

Australian Institute for Machine Learning, School of Computer and Mathematical Sciences, University of Adelaide, SA 5000, Australia

Mark Jenkinson

South Australian Health and Medical Research Institute, SA 5000, Australia

Mark Jenkinson

6.8.2 Contributions

AM, conceptualized, analysed data, and wrote the original manuscript. LCP contributed to experimental concept and design, interpretation of results, as well as playing a key role in manuscript conceptualisation. MJ was critical in developing and interpreting voxelwise analyses and conceptualising initial study plans. IB contributed to the development of the composite cognitive score and stratification of cohorts according to age. All authors were involved in editing the original manuscript. All authors read and approved the submitted manuscript.

6.9 ACKNOWLEDGEMENTS

The authors would like to acknowledge Angela Walls for playing a pivotal role in developing and optimisation of the MRI protocols utilised in the current study.

6.10 CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. MJ receives royalties from Oxford University Innovations for licensing of the FSL software for commercial, non-academic use.

6.11 ETHICS DECLARATIONS

Ethics approval and consent to participate

The authors declare that all research was conducted in accordance with the Human Research ethical guidelines. Written informed consent was obtained from each participant at enrolment, in accordance with the Declaration of Helsinki. All methods were performed in accordance with the relevant guidelines and regulations

Chapter 7 - Thesis Discussion, Future Considerations and Conclusions

7.1 Research summary

It is now well-established that Parkinson's Disease (PD) is more than a motor disorder and is also associated with several non-motor symptoms, as well as atypical features, including but not limited to profound early postural instability, rapid disease progression and other neurological signs such as cerebellar-related challenges (Postuma et al., 2015a). Such features can aid in distinguishing PD from other forms of parkinsonism, which has led to several diagnostic reforms, with current Movement Disorders Society (MDS) criteria (Postuma et al., 2015a) substantially improving upon previous criteria, such as the United Kingdom Parkinson's Disease Society Brain Bank Diagnostic Criteria **(**UKPDSBB) (Gibb and Lees, 1988) and Gelb's (Gelb et al., 1999) criteria. These improvements result from incorporating comprehensive assessment encompassing both motor and non-motor features, rather than exclusive reliance on cardinal motor symptoms, and by introducing defined levels of diagnostic confidence. However, such criteria are still heavily reliant on clinical presentation and, consequently, present with several limitations, including high rates of misdiagnosis (~20% of cases) (Holroyd et al., 2008) and consequent mistreatment due to considerable clinical overlap with other parkinsonian disorders, as well as relatively late diagnosis. The determinants of delayed diagnosis are multi-faceted, however, several non-motor features are often overlooked as they are also signs of ageing and not necessarily specific to PD (Postuma et al., 2012). Further, in a cohort of 239 individuals newly diagnosed with PD, Breen and colleagues identified male sex and primarily presenting with gait disturbance rather than tremor as factors contributing to late diagnosis (Breen et al., 2013). Taken together, such limitations greatly diminish the efficacy of current treatment strategies (Armstrong and Okun, 2020). The need for improved diagnostic confidence and earlier intervention is reflected by recent efforts to

redefine PD diagnosis based on its biological presentation, rather than clinical symptoms (Chahine et al., 2023). In line with this, recent advancements in biological assessment, such as the alpha synuclein (α-syn) seed amplification assay (SAA) (Siderowf et al., 2023), skin biopsies (Gibbons et al., 2024) and the development of specialised neuroimaging modalities, offer a promising avenue to better assess biomarkers of PD *in vivo*.

While promising, however, such advancements must also be paired with enhanced ability to identify individuals or groups presenting high risk of PD conversion in order to be utilised effectively. To this end, several at-risk populations have been identified, notably individuals with a history of traumatic brain injury (TBI). It is well-recognised that risk of PD development increases substantially following a TBI, with even a single case of mild TBI (mTBI) increasing risk up to 56% and this further increasing up to 83% with more severe injury (Gardner et al., 2018). In line with this, in a large scale prospective epidemiological study conducted by Crane and colleagues, sustaining a loss of consciousness for an hour or greater was associated with significantly elevated risk for both Lewy body pathology and consequent PD development (Crane et al., 2016). While increased risk of PD development in those with a history of head injury is well-established, it remains largely unknown who will develop PD following a TBI. However, it is known that a subset of individuals develop persistent impairments following both severe TBI (Deb et al., 1999a, McMillan et al., 2012) and mTBI (Nelson et al., 2019, Carroll et al., 2020), with these individuals hypothesised to present the high risk of incident PD. Therefore, pathophysiological assessment, paired with comprehensive behavioural phenotyping, within a TBI cohort could help pave the way for improving PD diagnosis and the implementation of disease modifying treatments.

Firstly, this thesis presents a detailed review of the literature pertaining to PD and how improved characterisation of its clinical presentation has influenced diagnostic reforms, as well as how research efforts are currently being made to move towards a biologically defined diagnosis (**Chapter 1**). To this end, several pathophysiological features of PD and their associations with clinical outcomes were explored, including recent advancements in *in vivo* techniques utilised to assess such pathology. This was followed by an analysis using the longitudinal Parkinson's Progression Markers Initiative (PPMI) database, with hierarchical clustering conducted on year-5 MDS Unified Parkinson's Disease Rating Scale (UPDRS) in order to identify clinical subtypes (**Chapter 2**). Additionally, the prognostic utility of a multi-modal panel of baseline measures collected near time of diagnosis, including cerebrospinal fluid (CSF) biomarkers for pathological proteins (i.e. alpha synuclein (α-syn), phosphorylated tau^{181} (p-tau) and amyloid $beta^{1-42}$ (Aβ)), neuroimaging markers targeted at dopaminergic system alterations, prodromal symptom presentation, MDS-UPDRS-derived motor scores and key demographic variables, was explored in order to identify significant predictors of symptom progression. This analysis determined that, of the several predictors assessed, CSF concentrations of α-syn and p-tau were particularly promising, with multi-model assessment significantly improving prediction of cluster membership compared to baseline MDS-UPDRS features alone. This highlighted that biological assessment is a promising path forward, although it should be noted that prodromal features, such as impaired smell and autonomic dysfunction, also acted as significant predictors.

This set the stage for follow-up on other features preceding motor presentation that may explain differential progression of PD symptoms. To this end, several risk factors for PD were considered (**Chapter 3**), with a past of history of TBI noted as one factor of significant research interest. While the increased risk of PD development following TBI is well-established, less is

known about how a past history of TBI may influence symptom progression in PD. A previous longitudinal study identified a past history of TBI as a significant predictor of cognitive change, although not motor or mood outcomes (Schiehser et al., 2016). This however was conducted in a relatively small cohort of TBI and healthy control participants (n = 25 each), as well as a limited follow-up period (two-years) (Schiehser et al., 2016). Given this gap in the literature, an assessment of progression of clinical and pathophysiological features in individuals with PD with and without a history of TBI was conducted in both the PPMI and PostCEPT cohorts (**Chapter 4**). In both cohorts, a past history of TBI did not predict differential rates of progression in any of the assessed features; however, exploratory analyses assessing the potential moderating effects of TBI severity determined that increased severity of injury was a significant predictor of striatal dopamine transporter (DaT) binding and mood dysfunction at follow-up years 4 and 5, respectively. Nevertheless, the proportion of the variance explained in both models was small, suggesting that neither TBI nor injury severity are major drivers of progression of outcomes in clinically established PD.

Therefore, the final chapters within the thesis explore biological factors and their association with clinical outcomes in TBI cohorts. Given that **Chapter 2** highlighted the prognostic utility of biomarkers collected acutely following diagnosis in PD, a systematic review and meta-analysis was performed exploring biomarkers of cell injury, collected acutely following injury, and their association with behavioural outcomes at any timepoint post-injury (**Chapter 5**). Given the nature of the data extracted, a multilevel design was applied to meta-analytic models, allowing for the exploration of moderators that may influence association strength. Overall, it was found that behavioural changes following injury were primarily assessed with global outcome measures, with moderators such as sample source, time of sampling and timepoint at type of behavioural assessment not significantly influencing associations between biomarkers

and outcome. Overall, biomarkers of cell injury demonstrated a moderate negative relationship with global outcome following injury, however no biomarker in particular demonstrated substantially higher associations with outcome above the rest. Further, several gaps in the existing literature were identified, namely, a lack of assessment of the relationship between biomarker concentrations and specific behavioural outcomes following injury, including cognition, mood and motor function. Therefore, the final chapter of this thesis investigated the utility of susceptibility weighted imaging (SWI) to assess voxelwise features of nigrosome-1 (N1) in a cross-sectional TBI cohort of varying severities and time since injury, relative to healthy controls and PD patients (**Chapter 6**). Specifically, this was conducted to determine whether alterations in N1 could act as a potential early marker of heighted PD risk in those with a history of TBI, as well as a potential predictor of cognitive ability across cohorts. Given the critical role that age plays in both PD development and cognition, the healthy control and TBI cohorts were stratified into a young and aged cohort. Unsurprisingly, N1 voxelwise features significantly differed in PD compared to all other cohorts; however, no other group differences were present, nor was N1 significantly associated with cognition when controlling for important confounders. Therefore, it is possible that N1 demonstrates little promise as a predictor of risk of either PD development or persistent cognitive impairment in a TBI cohort; however, our sample size was limited, warranting further research and consideration.

Taken together, the findings from this thesis provide critical insights into factors that drive poorer outcomes in both PD and TBI. However, such outcomes are relatively broad, with additional research assessing the association between biological assessments and specific clinical outcomes critically needed. Further, this thesis highlights several gaps in the current literature, particularly pertaining to heterogeneity in the way TBI is assessed, as well as operationalisation differences in outcome assessment, highlighting the need for harmonisation

across studies in order to improve reproducibility and translatability of findings. This will be essential when further investigating the role that biological assessments may play in identifying individuals at-risk of PD development, and consequently refining current diagnostic and prognostic criteria.

7.2 Fluid Pathophysiological Markers Demonstrate Promise in Forecasting Impairment in PD and TBI

Chapters 2 and **5** assessed the utility of biofluid markers collected acutely following diagnosis or injury in PD and TBI, respectively. **Chapter 2** incorporated several pathophysiological markers known to be associated with PD, namely CSF measures of α-syn, p-tau and amyloid beta$^{1-42}$ (Aβ). α-syn is widely considered to be the primary pathological protein implicated in PD (Gómez-Benito et al., 2020), and has been shown by previous studies to be associated with clinical outcomes. For example, Majbour and colleagues demonstrated that CSF-derived oligomeric α-syn, albeit not total or phosphorylated (Ser219), significantly correlated with severity of PD motor symptoms ($r = -0.37$) (Majbour et al., 2016). However, emerging evidence suggests that both p-tau and Aβ may be drivers of pathology in PD and contribute to heterogeneity in clinical presentation. In the former study, it was reported that a combination of α-syn in all assessed forms (total, oligomeric and phosphorylated) in conjunction with p-tau was the best fitted model for discriminating PD from healthy controls (Majbour et al., 2016). Further, a recent study demonstrated that tau, rather than α-syn aggregation, may facilitate nigrostriatal dopaminergic degeneration (Chu et al., 2024), although few studies to date have specifically assessed the association between p-tau and disease progression in PD. Of the limited evidence, a single study reported that increased levels of CSF p-tau acted as a significant predictor of motor impairment over a two-year period, however, conflicting findings regarding its role in cognitive decline have been reported (Siderowf et al., 2010, Chan et al.,

2023). For example, while a recent study reported that tau derived from plasma extracellular vesicles was significantly associated with cognitive decline in PD (Siderowf et al., 2010, Chan et al., 2023), Siderowf and colleagues found no such associations when assessing CSF-derived tau and p-tau (Siderowf et al., 2010). Conflicting findings may also indicate that plasma extracellular vesicles rather than CSF are an optimal biomarker source to assess the relationship with cognition in PD, warranting further research. Beyond p-tau, reductions in CSF levels Aβ have been shown to be associated with more profound cognitive decline in PD, significantly predicting impairments in delayed memory recall, measured using the cognition subsection of the Alzheimer's Disease Assessment Scale, over a two-year period (Hall et al., 2015). It is important to note, however, that these previous longitudinal studies only assessed the predictive utility of such pathological markers over a minimal follow-up period, with **Chapter 2** addressing this limitation by assessing the predictive power of baseline pathological markers five-years following study entry in an early PD cohort.

Interestingly, at baseline, the clusters that we derived in **Chapter 2** did not demonstrate any differences in CSF levels of pathological markers. This conflicts with findings from other studies, where compared to the tremor-dominant subtype (TD-PD), plasma levels of α-syn and Aβ were significantly higher and lower, respectively, in the postural instability-gait disturbance subtype of PD (PIGD-PD) (Ding et al., 2017), with higher concentrations of CSF p-tau also seen in PIGD-PD (Zuo et al., 2017). However, it should be noted that the PPMI dataset at baseline (i.e., near time of diagnosis) is highly homogenous (Kang et al., 2016), and therefore divergence of pathological presentation between subtypes may not occur until later stages of the disease, as reflected by the finding described in **Chapter 2** and other studies (Johansson et al., 2023, Dadu et al., 2022, Jeong et al., 2023). Further, a lack of differences at baseline does not necessarily correspond to poor prognostic performance. In line with this, baseline CSF

levels of both α-syn and p-tau, but not Aβ, were found to significantly predict membership in a cluster with more marked impairment at year-5 follow-up. While this seems to suggest a clear pathological distinction between clusters, it is important to note that literature supporting this is contentious. For example while CSF α-syn levels have previously been shown to demonstrate significant associations with motor performance (Majbour et al., 2016), other studies have reported conflicting results (Hong et al., 2010, Mollenhauer et al., 2011, van Dijk et al., 2014, Majbour et al., 2016).

Thus, the prognostic utility of such fluid markers must be interpreted with caution. In fact, several limitations and considerations should be kept in mind when validating such findings in future studies. Most strikingly is that the multivariate linear regression used to identify these pathological markers as key predictors of subtype membership could only be conducted on a limited subset of individuals based on data completeness. This sample was rather small (TBI cohort n = 25), and it is difficult to determine how well said findings relate to the wider cohort utilised within the study or to PD more generally. Therefore, future studies aiming to replicate such findings should do so in larger samples and should consider attrition and consistent variable collection as part of their study design to allow for more robust statistical analyses.

Further, while CSF assessment has played a pivotal role in characterising PD pathophysiology, it cannot not be readily implemented clinically due to its invasiveness. However, advances in novel, non-invasive assessment of PD pathophysiology may mitigate this. Of current research interest is the recently developed α-syn SAA, which distinguished PD from healthy controls with a sensitivity of 98.6% in the PPMI cohort (Siderowf et al., 2023). However, while α-syn SAA shows diagnostic potential, its role in prognosis has not yet been determined. Further, neuronally derived extracellular vesicle α-syn (i.e. L1EV-associated α-syn) is a proposed

biomarker of PD, with a study of 40 individuals who later developed PD and related dementia finding that blood tests were positive for L1EV α-syn in more than 80% of individuals (Yan et al., 2024). Beyond α-syn, recent work has assessed plasma extracellular vesicles in 103 individuals with PD, with elevated concentrations being associated with significant decline in both motor and cognitive function (Chan et al., 2023). It should be noted however that despite promise, extracellular vesicles present with technical challenges that may limit clinical use, such as lack of standardised techniques on vesicle isolation and characterisation, warranting further research and harmonisation across studies. Given such promise and an increased likelihood of being implemented routinely in clinic compared to CSF-derived assessments, due to their less invasive nature, future research assessing prognosis of long-term outcomes in PD should consider incorporating such pathological assessments.

As **Chapter 2** highlighted the high prognostic potential of pathological markers collected relatively acutely following time of diagnosis for predicting outcome trajectory in PD and given the link between a history of TBI and later development of PD, the association between biomarkers of cell injury collected within a week post-injury and behavioural outcomes was explored (**Chapter 5**). A systematic review and meta-analytic strategy were utilised to provide a comprehensive assessment of the current state of the literature, as well as synthesise findings across several studies to effectively draw conclusions on the relationship of interest. Biomarkers of cellular injury have shown promise in clinical settings as a screening tool to streamline the detection of intracranial abnormalities following injury. In fact, findings from a multi-centre observational study of 1,959 TBI participants (Bazarian et al., 2018) resulted in the Food and Drug Administration (FDA) approving serum GFAP and UCH-L1 collected within 12 hours of injury for use in emergency room settings to screen for intracranial

abnormalities (Food and Administration, 2018). Despite such diagnostic reforms, no biomarker of cell injury has yet been validated for prediction of behavioural changes following TBI.

In **Chapter 5**, the vast majority of studies utilised global outcome measures to assess behavioural outcome following injury, with the multilevel meta-analysis determining a moderate relationship between biomarkers of cell injury and global outcome. Interestingly, when conducting moderator analysis, in general, no significant differences in moderators were present, including biofluid source, individual biomarker utility, time of sample extraction and timepoint at which outcome assessment was conducted post-injury. While such findings suggest that markers of cell injury, regardless of when they were collected within the week immediately following injury or when outcome was assessed, are associated with global outcome, the GOS and GOSE as outcome measures possess several psychometric limitations that should be considered. A 2019 TRACK-TBI study assessing GOSE utility found that the scale does not present with clear distinctions between scale items, along with a scarcity of items reflective of subtler disability (Ranson et al., 2019). Likewise, a workshop sponsored by the National Institute of Neurological Disorders and Stroke noted that global outcome measures only crudely reflect TBI outcomes and are largely skewed towards severe disability, with limited sensitivity to detect individual differences and milder functional changes (Narayan et al., 2002). This is particularly relevant given that most of the included studies assessed TBI cohorts with moderate-severe TBI, where such measures would be suitable, whereas improved representation of mTBI and its clinical presentation following injury may have altered effect size strength.

Therefore, while biofluid markers assessed in **Chapters 2** and **5** demonstrate high promise for predicting outcomes in both PD and TBI, it is important to note that such outcomes are rather

broad. Thus, it is imperative that future work better characterises associations between biological assessments and more specific outcomes, in order to determine their potential utility for predicting the presentation of specific impairments that may help inform clinical decision making regarding appropriate personalised treatment strategies.

7.3 Critical Need to Assess Specific Outcomes in PD & TBI

Given that the global outcome measures assessed in **Chapter 5** present with several limitations, more specific and sensitive outcome measures should be utilised; however, their representation within TBI literature is critically lacking. Of the included studies, very few assessed cognition as an outcome of interest, with only five being considered eligible for meta-analysis, with a markedly weaker relationship observed between biomarkers of cell injury and cognition. However, the meta-analysis itself is largely underpowered. Further, mood and motor outcomes were even further underrepresented, with only a single study assessing each. Thus, the utility of biomarkers of cell injury as predictors of specific outcomes following injury cannot be determined.

This is important given cognitive; mood and motor outcomes demonstrate critical relationships with aspects such as functional independence and QoL following injury. A recent systematic review of 30 studies highlighted that cognitive changes play a considerable role in return-to-work post-injury (Mani et al., 2017). Likewise, cognition seems to play a pivotal role in functional independence. This is supported by a study, on a sample of 122 individuals admitted to inpatient rehabilitation due to TBI, finding that cognitive impairment, as measured by Orientation Group Monitoring System, was significantly associated with functional independence measures including total ($rho = .49$, $p < .001$), motor ($rho = .40$, $p < .001$) and cognitive scores ($rho = .64$ $p < .001$) (Kaplan and Corrigan, 1994). Likewise, both mood,

(Howlett et al., 2022) and motor (Corrigan et al., 2023) impairments following injury have been identified as predictors of QoL (Rapaport et al., 2005, Kalpinski et al., 2013) and overall life satisfaction (Williamson et al., 2016). Therefore, it is evident that functional ability following injury is a broad term encompassing impairments and specific domains, each of which are vital predictors of individual wellbeing post-injury. Additionally, as highlighted by Kaplan and Corrigan, such impairments do not often occur in isolation and demonstrate significant associations with one another, and therefore, future studies should conduct a comprehensive battery of all domains. Doing so effectively would require utilising specific assessment tools to properly reflect such outcomes, as well as keeping consistency of what assessments are used across studies in mind, to better draw meaningful comparisons and consolidated conclusions across literature.

Likewise, while **Chapter 2** highlights the promise of subtyping in PD, a recent meta-analysis of 38 studies highlights the shortcomings of such subtyping studies, including questionable clinical and biological relevance (Mestre et al., 2021). The latter limitation was at least somewhat addressed by the results in our study, with more marked impairment also associated with a more profound pathological biomarker profile. Further, the authors of the recent meta-analysis recommend that future studies address heterogeneity at the individual level (Mestre et al., 2021). However, a study based on consultations with 52 PD participants determined that personalised medicine and treatment strategies are challenging, with a major contributor being that characterisation of individual outcomes is severely lacking (van den Heuvel et al., 2022). While this is an important step moving forward, given individual variability, this would require a much larger sample size and number of predictors than were available for the analyses presented in **Chapter 2**.

Not only is there a need to better assess PD progression, but also risk factors of PD and their relationship with consequent disease development. While the study conducted by Crane and colleagues was a seminal paper in the field (Crane et al., 2016), proposing TBI and the moderating effects of injury severity as drivers of PD progression and disease development, this was not conducted longitudinally. However, the former studies are amongst the only studies assessing the potential effects of TBI on PD progression longitudinally. Further, findings from **Chapter 4**, whereby TBI and injury severity did not predict differential progression of outcomes in PD, directly oppose findings from Crane, whereby TBI and loss of consciousness (LoC) of an hour or greater corresponded to more rapid progression as determined by a parkinsonian sign score (Crane et al., 2016). However, similar to limitations present in assessing outcome via GOS and GOSE within the TBI literature, the parkinsonian sign score is non-specific and only accounted for motor features of PD, perhaps explaining conflicting findings between studies whereby specific motor and non-motor features were separated. Moving forward, future research assessing TBI as a driver of PD development progression should do so with a prospective longitudinal cohort, allowing for improved characterisation of specific outcomes and individual differences that may be contributing to elevated risk, as well as improved temporal characterisation of clinical outcomes.

7.4 Neuroimaging Demonstrated Little Utility as a Prognostic Factor Across Conditions

While fluid-based pathological assessments demonstrated high prognostic utility in **Chapter 2**, the inclusion of neuroimaging-based markers did not demonstrate as much promise. Neither the SN-related hypointensity or striatal DaT binding significantly predicted more severe functional impairment at year-5 follow-up and, in particular, the former measure did not significantly differ between clusters at baseline. This highlights a limitation present in the wider PPMI study: lack of specialised MRI modalities, optimised for PD. T2-weighted MRI was the

primary structural modality available, which may lack the sensitivity required to distinguish SN-related differences between clusters (for review, see (Santos García et al., 2021)), as also highlighted by a previous study utilising the same proxy marker that we utilised in our study, where that marker did not correlate with clinical outcomes such as UPDRS part three total score ($p = 0.964$; $R^2 = 0.0005$) or disease duration ($p= 0.149$; $R^2 = 0.45$) (Langley et al., 2017). In recent years, however, neuroimaging of PD has undergone significant advancement, as reviewed in **Chapter 1** of this thesis. These specialised MRI modalities may offer the greater sensitivity required to predict progression of clinical symptoms in PD. For example, susceptibility weighted imaging (SWI) and neuromelanin-sensitive MRI (NM-MRI) have been utilised to detect both higher regional iron content and neuromelanin loss, respectively, in the SN of individuals characterised as the akinetic-rigid subtype (AR-PD) relative to TD-PD (Xiong et al., 2020, Lakhani et al., 2024).

Bearing in mind these limitations, in the design of **Chapter 6**, we elected to use SWI, optimised for the visualisation of midbrain structures, to assess voxelwise features of N1 in TBI, PD and healthy controls. Given that N1 is a particularly vulnerable region within the SN, and amongst the earliest to degenerate in PD (Damier et al., 1999b), this was a particularly interesting marker to probe in a cohort with a prior exposure to a known risk factor for PD (i.e. a history of TBI). There is significant consensus that SWI is sensitive enough to discriminate PD from healthy controls (Chau et al., 2020). Further, SWI also appears to demonstrate promise for characterising neurodegeneration within another at-risk population, REM-sleep behaviour disorder (RBD), with a previous study comparing 46 RBD patients, 27 Parkinson's patients, and 32 control subjects finding that over 25% of RBD participants demonstrated an abnormal N1 reminiscent of PD, determined by assessment of a dorsal nigral hyperintensity marker, by two independent raters (Barber et al., 2020).

Notably, while, as expected, significant differences in N1 were present in PD compared to other cohorts, even when stratifying the TBI and healthy control cohorts according to age, no other significant group differences were present. While this may indicate that SWI lacks sensitivity to characterise early neurodegeneration within TBI, it is also important to note that Barber and colleagues determined that, of the RBD participants they assessed, only 25% demonstrated abnormal SN features (Barber et al., 2020). This shows that, even within at-risk populations, not all individuals convert to clinically established PD, highlighted by the finding of Postuma and colleagues that in a cohort of 89 individuals with RBD, 30% developed PD at three-year follow-up, increasing to 66% at 7.5 years (Postuma et al., 2015b). Thus, future work should focus on identifying who is at highest risk of PD development following TBI, such as those with greater symptom persistence or those with additional exposure to other known risk factors (e.g. pesticide exposure or genetic mutations), which may act synergistically with TBI to increase risk. For example, Lee et al reported that while TBI and pesticide exposure in isolation were associated with a moderately increased risk of PD development, dual exposure increased risk three-fold (Lee et al., 2012). Likewise, a randomised controlled trial reported significant interactions between a history of TBI and polymorphisms in the REP1 gene, resulting in a significantly younger age of disease onset relative to individuals with no history of TBI (Goldman et al., 2012). A consideration of which other risk factors might be worth investigating was presented in **Chapter 3**. Considered risk factors encompassed several domains, including demographic variables such as age and sex, external exposures, namely TBI and pesticides, as well as previously researched genetic risk factors.

In addition to not distinguishing the TBI cohort from healthy controls, no significant associations between N1 voxelwise features and cognitive ability were present for any group.

It is important to note that associations were assessed using a generalised linear model, accounting for confounders recognised as important predictors of cognition, such as education (Albert et al., 1995, Lyketsos et al., 1999) and age, with the latter being identified as one of the most critical predictors of cognition across conditions, including PD (Guo et al., 2021, Pontone et al., 2009), TBI (Downing et al., 2024a, Skilbeck et al., 2019) and healthy ageing (Murman, 2015, Mikkelsen et al., 2024). This was validated using multivariate linear regression, with age and education and a clinical diagnosis of PD, but not N1 voxelwise features, significantly predicting cognitive ability. While this seems to suggest that N1 voxelwise features do not have prognostic utility, at least for cognitive function in our cohorts of interest, several caveats should be taken into account when interpreting the results of **Chapter 6**. Namely, limited research has been conducted to assess N1 imaging via SWI in at-risk populations, meaning it is possible that the SWI protocol in **Chapter 6** may lack the resolution and specificity required to detect anatomical changes in the N1 area due to TBI. This ties into a limitation of the wider literature: lack of standardised protocols to visualise N1 (Cheng et al., 2020), therefore, future studies should explore a range of protocols to determine if any are highlighted as particularly promising. However, given that limited studies have assessed this, future research must be conducted to further investigate this. Additionally, voxelwise features may not be the most suitable method to assess prognostic utility, as such features may lack power due to only looking at individual voxel values per test. Therefore, alternative methods such as creating a feature that combines across multiple voxels, such as average intensity based of the SN and N1. Additionally, machine learning methods applied to MRI would be able to derive more complex imaging features compared to voxelwise analysis. Taken together, several considerations surrounding SWI protocols and the way they are assessed should be considered in future research exploring its utility for both diagnostics and prognostics.

7.5 Inconsistencies in TBI and Outcome Assessment Demand Harmonisation Across Studies

A major limitation of TBI research currently is the high variability in how functional outcomes are assessed between studies, or even how studies characterise injury itself. Regarding the relationship between TBI and PD specifically, despite its well-established link to PD development, only limited research has been conducted into the role that a past history of TBI may play in disease progression in PD. In fact, to date, only a single longitudinal study has been conducted to assess differences in symptom progression in individuals that have PD with and without a history of TBI, with that study demonstrating that a history of TBI corresponded to more profound cognitive decline over a two-year follow-up, albeit with no differences in progression noted for either motor or mood function (Schiehser et al., 2016). However, this study was conducted in a rather limited sample (n = 25 per group), and only over a minimal follow-up period. This is particularly problematic for cognition, given difficulties surrounding its assessment in early PD, as highlighted by a meta-analysis exploring conversion to PD with mild cognitive impairment (PD-MCI) or dementia (PD-D), which reported high reversion rates within the first 3 years of diagnosis (Saredakis et al., 2019).

To probe this further, and considering the limitations associated with relying on a single cohort as in **Chapter 2**, the potential influence of TBI on progression of pathophysiological and clinical outcomes was assessed in two longitudinal cohorts: PPMI and PostCEPT up to five-years and four-years, respectively (**Chapter 4**). It was found that, with the exception of a history of TBI corresponding to a younger age of onset in the PPMI cohort and higher depression scores at baseline in the PostCEPT cohort, no other differences in baseline measures or rates of progression were present for either cohort, in contrast with Schiehser and colleagues' earlier findings. This is also contrary to a study conducted in two large prospective cohorts, which reported that a loss of consciousness for an hour or greater corresponded to faster

progression of parkinsonian symptoms, along with elevated risk of both Lewy pathology and incident PD (Crane et al., 2016). However, substantial differences in the way that outcome and progression were assessed between studies were noted, making comparison challenging. As stated previously, Crane and colleagues derived a summary global parkinsonian sign score, solely based on a modified version of the UPDRS which was then re-coded into an ordinal format (Crane et al., 2016). Consequently, unlike **Chapter 3**, such assessment does not consider both motor and non-motor domains, as well as presenting with large differences in the way outcome was operationalised and progression was considered, perhaps explaining differences between study findings. Similarly, large variation was noted between the PPMI and PostCEPT cohorts in the way that TBI was assessed. Unlike PPMI, which included information on multiple injury characteristics, including LoC, PostCEPT classified TBI based on LoC alone. Given that only approximately 10% of concussions result in LoC (Delaney et al., 2002, Guskiewicz et al., 2003), this may have meant that the incidence of TBI was underestimated within the PostCEPT dataset. In line with this, PPMI reported a 40% incidence rate, whereas PostCEPT incidence was substantially lower, at approximately 20%.

Further, such studies place large emphasis on LoC as a core injury feature, however, several other characteristics relevant to clinical outcomes exist. In the World Health Organisation's (WHO) standardised definition of injury severity ratings, post-traumatic amnesia (PTA) less than 24 hours is indicative of mTBI, whereas anything greater can be considered M/sTBI (Control and Prevention, 2003). Like LoC, PTA has been identified as an important predictor of functional outcomes post-injury, with Perrin and colleagues finding that in a retrospective study of 100 TBI participants, PTA duration was a significant predictor of outcome as measured by the Functional Independence Measure ($\beta = -0.46$, $p = .001$) (Perrin et al., 2015). This effect was present even after controlling for other clinical predictors, such as age, gender, educational

level and cause of injury (Perrin et al., 2015). Such findings were corroborated by a recent systematic review of 29 studies, with nine assessed meta-analytically, determining that PTA duration was a reliable indicator of outcome within a week of injury, as well as >42 days, albeit PTA duration was not reliable indicator of outcome between said timepoints, warranting further investigation (Vile et al., 2022). Additionally, the WHO also concluded that intracranial or neurological abnormalities are indicative of more severe cases of injury rather than mTBI (Control and Prevention, 2003). Therefore, not considering such features as part of injury characterisation is a critical oversight. Thus, when determining injury classification in **Chapter 6,** such features were considered and allowed for more robust determination of TBI, as well as improved confidence in injury severity assessment.

Further, a major criticism of the TBI literature in general is considerable sample heterogeneity between studies, highlighted in **Chapter 5,** whereby variability in age, gender and injury severity distribution were present. Consequently, it is difficult to consolidate findings across studies, given such features correspond to variability in clinical and pathophysiological presentation. In fact, a recent comment in the Lancet noted that in general, homogenisation in the what injury features and clinical outcomes are assessed is critically required in the TBI field, in particular for ascertaining the utility of biomarkers and neuroimaging-assessed injury patterns to characterise monitoring of clinical outcomes and therapeutic interventions (Rubiano and Lee, 2023). Likewise, of the limited studies assessing cognition in **Chapter 5**, large variability existed across studies in which cognitive domains were assessed and in the specific cognitive assessments utilised. Taken together, this highlights the need for greater harmonisation in the way several features are assessed in future, including both outcomes post-injury and indeed injury itself, as well as sample characteristics, to increase translatability between studies.

7.6 Thesis strengths and limitations

The overall thesis presents with several strengths, such as expansive follow-up time points and gauging findings across several cohorts and studies. Generally, studies throughout the present thesis utilise a comprehensive approach towards how outcomes were operationalised, the number of outcomes explored as well as assessing range of predictors of clinical outcome. Further, rather than drawing conclusions from a single sample, potentially limiting generalisability to a wider cohort, this thesis assesses several cohorts as well as several forms of study design, such as retrospective longitudinal studies, prospective cohorts as well as cross-sectional. In terms of specific strengths, the study design utilised in **Chapters 2 and 4**, whereby progression of PD features was assessed, addresses several limitations in the current literature. A systematic review assessing cognitive decline in PD noted that very few studies encompassed an expansive follow-up period (> 4 years), as well as often utilising broad assessments such as the Mini Mental State Examination, with only one eligible study assessing the five cognitive domains required for a diagnosis of cognitive impairment (Roheger et al., 2018). Therefore, a particular strength of these Chapters is assessing progression up to five-years and four-years follow-up, for PPMI and PostCEPT respectively, as well as determining specific outcomes across motor, cognition and mood via standardised assessments. Additionally, while several systematic reviews have been conducted looking at biomarkers of cell injury for predicting outcome following injury (Undén and Romner, 2010, Cheng et al., 2014, Behzadi et al., 2024), utilising a multilevel design for **Chapter 5** meta-analyses allowed for several effect sizes per study to be extracted, rather than limiting these to a single one and thus potentially limiting research findings. Finally, **Chapter 6** addresses several study-specific limitations of previous chapters, such as utilising a specialised imaging modality and more robust analytical technique to assess SN and N1 alterations. Further, as in **Chapters 2 and 4**, a battery of specific cognitive assessments was conducted, rather than utilising global cognitive measures. Finally, TBI

ascertainment in **Chapter 6** was the most robust, utilising several injury characteristics as outlined in WHO guidelines, allowing for improved confidence in injury characterisation. Additionally, while several SWI studies often compare PD to healthy controls, to the best of our knowledge, only a single study outside of **Chapter 6** has included an at-risk cohort as an additional comparator group, allowing for potentially more subtle group differences and findings to be determined.

Despite several strengths, findings presented within the current thesis should be interpreted with several limitations in mind. While it is the most developed PD dataset in the world, the PPMI dataset used in **Chapters 2 and 4** has nevertheless several limitations. Due to using a 'wide-net' approach towards variable collection, rather than a hypothesis-driven approach, several variables were inconsistently collected. In line with this, only approximately 30% of the cohort assessed in **Chapter 2** possessed data for all baseline predictors assessed in the multi-modal multivariate regression, limiting consequent regression and machine learning analyses to this subset. Similarly, for **Chapter 4,** PPMI was not designed to assess the effect of TBI on disease progression, with the head injury questionnaire only administered at a single site. This greatly diminished the pool of participants available for analysis for the current work, with inconsistent collection and reporting of variables further contributing to the relatively small sample size. While this was somewhat ameliorated by the inclusion of the PostCEPT cohort for validation, this cohort did not possess pathophysiological data to allow for an investigation of how TBI might affect progression of these markers over time, a key research aim of this thesis. Further, distinctions in the way TBI was ascertained, as well how clinical outcomes were assessed, across the cohorts made direct comparisons and data pooling to increase statistical power infeasible.

Likewise, limitations were present in **Chapter 6**, where the sample size for each cohort was relatively small. This may have consequences for translatability across studies, as well as to clinical populations, as sufficient statistical power is required for both (Fraley and Vazire, 2014). This is particularly critical for **Chapter 6**, given that assessing N1 features and other early markers of PD within a TBI population is still a field in its infancy and depends on consensus across studies for future recommendations. Further, unlike the TBI cohorts analysed in **Chapter 5,** who were often recruited and provided with clinical diagnoses in emergency room settings, the TBI cohorts analysed in **Chapters 4 and 6** are community-dwelling and, consequently, provided self-report regarding TBI. While an extensive questionnaire pertaining to injury characteristics was administered both in the PPMI cohort in **Chapter 4** and the TBI cohort in **Chapter 6**, not only did questionnaire items differ, further reiterating the need for harmonisation between studies, but such self-report is subject to potential biases, given its reliance on participant memory of injury details, which, for the majority, occurred several decades before study participation. In line with this, multiple previous studies have reported that TBI screening tools that rely on self-report information are associated with several limitations, including under- or over-reporting of TBI events and biased recall of the injury itself (Bailie et al., 2017, Corrigan and Bogner, 2007, Dams-O'Connor et al., 2014, Diamond et al., 2007).

6.7 Thesis Conclusions

Overall, this thesis highlights the complex nature of forecasting clinical trajectories in PD and TBI, and the potential role that biological assessment may play in improved outcome characterisation. While fluid-derived biomarkers demonstrated promise, it should be noted that they improved prediction of gross impairment in PD and TBI, rather than being associated with specific outcomes. In fact, insight into associations between biomarkers and specific clinical

outcomes, such as cognition, mood and motor function, are severely lacking, particularly in the TBI literature, and should be a future area of research focus. Conversely, the neuroimaging markers assessed within the present thesis demonstrated little prognostic utility. Despite this, and considering recent advances in the field, it would be premature to state that neuroimaging markers in general are not beneficial for predicting symptom progression in either TBI or PD, with further research warranted. It is critical that future research in this area consider improved harmonisation, including outcome operationalisation and assessment, standardised imaging protocols and how TBI and PD sub-groups are derived, as, currently, substantial heterogeneity is present across studies, limiting translatability of research findings. This would allow for enhanced identification and corroboration of potential pathophysiological features that show the highest promise for forecasting clinical trajectories in both PD and TBI and will be an essential first step towards biologically defining PD and providing earlier diagnoses and interventions.

Chapter 8 - References

AARSLAND, D. & KURZ, M. W. 2010. The epidemiology of dementia associated with Parkinson disease. *Journal of the neurological sciences,* 289**,** 18-22.

ACOSTA, G., RACE, N., HERR, S., FERNANDEZ, J., TANG, J., ROGERS, E. & SHI, R. 2019. Acrolein-mediated alpha-synuclein pathology involvement in the early post-injury pathogenesis of mild blast-induced Parkinsonian neurodegeneration. *Mol Cell Neurosci,* 98**,** 140-154.

ACOSTA, S. A., TAJIRI, N., DE LA PENA, I., BASTAWROUS, M., SANBERG, P. R., KANEKO, Y. & BORLONGAN, C. V. 2015. Alpha-synuclein as a pathological link between chronic traumatic brain injury and Parkinson's disease. *J Cell Physiol,* 230**,** 1024-32.

ADAMS, C., SUESCUN, J., HAQUE, A., BLOCK, K., CHANDRA, S., ELLMORE, T. M. & SCHIESS, M. C. 2023. Updated Parkinson's disease motor subtypes classification and correlation to cerebrospinal homovanillic acid and 5-hydroxyindoleacetic acid levels. *Clin Park Relat Disord,* 8**,** 100187.

ADLER, C. H., BEACH, T. G., HENTZ, J. G., SHILL, H. A., CAVINESS, J. N., DRIVER-DUNCKLEY, E., SABBAGH, M. N., SUE, L. I., JACOBSON, S. A., BELDEN, C. M. & DUGGER, B. N. 2014. Low clinical diagnostic accuracy of early vs advanced Parkinson disease: clinicopathologic study. *Neurology,* 83**,** 406-12.

AFSHEEN, S., REHMAN, A. S., JAMAL, A., KHAN, N. & PARVEZ, S. 2024. Understanding role of pesticides in development of Parkinson's disease: Insights from Drosophila and rodent models. *Ageing Research Reviews,* 98**,** 102340.

AGOSTON, D. V., SHUTES-DAVID, A. & PESKIND, E. R. 2017. Biofluid biomarkers of traumatic brain injury. *Brain Inj,* 31**,** 1195-1203.

AHMED, H., ABUSHOUK, A. I., GABR, M., NEGIDA, A. & ABDEL-DAIM, M. M. 2017. Parkinson’s disease and pesticides: A meta-analysis of disease connection and genetic alterations. *Biomedicine & Pharmacotherapy,* 90**,** 638-649.

AKDEMIR, Ü. Ö., BORA, H. A. T. & ATAY, L. Ö. 2021. Dopamine transporter spect imaging in Parkinson's disease and parkinsoniandisorders. *Turkish journal of medical sciences,* 51**,** 400-410.

AKHTAR, R. S., XIE, S. X., CHEN, Y. J., RICK, J., GROSS, R. G., NASRALLAH, I. M., VAN DEERLIN, V. M., TROJANOWSKI, J. Q., CHEN-PLOTKIN, A. S. & HURTIG, H. I. 2017. Regional brain amyloid-β accumulation associates with domain-specific cognitive performance in Parkinson disease without dementia. *PLoS One,* 12**,** e0177924.

AL NIMER, F., THELIN, E., NYSTROM, H., DRING, A. M., SVENNINGSSON, A., PIEHL, F., NELSON, D. W. & BELLANDER, B. M. 2015. Comparative Assessment of the Prognostic Value of Biomarkers in Traumatic Brain Injury Reveals an Independent Role for Serum Levels of Neurofilament Light. *Plos One,* 10**,** 19.

ALAM, M. M., LEE, J. & LEE, S. Y. 2017. Recent Progress in the Development of TSPO PET Ligands for Neuroinflammation Imaging in Neurological Diseases. *Nucl Med Mol Imaging,* 51**,** 283-296.

ALBERICO, S. L., CASSELL, M. D. & NARAYANAN, N. S. 2015. The Vulnerable Ventral Tegmental Area in Parkinson's Disease. *Basal Ganglia,* 5**,** 51-55.

ALBERT, M. S., JONES, K., SAVAGE, C. R., BERKMAN, L., SEEMAN, T., BLAZER, D. & ROWE, J. W. 1995. Predictors of cognitive change in older persons: MacArthur studies of successful aging. *Psychology and aging,* 10**,** 578.

ALCALAY, R. N., CACCAPPOLO, E., MEJIA-SANTANA, H., TANG, M. X., ROSADO, L., REILLY, M. O., RUIZ, D., LOUIS, E. D., COMELLA, C. L. & NANCE, M. A. 2014. Cognitive and motor function in long-duration PARKIN-associated Parkinson disease. *JAMA neurology,* 71**,** 62-67.

ALCALAY, R. N., MEJIA-SANTANA, H., MIRELMAN, A., SAUNDERS-PULLMAN, R., RAYMOND, D., PALMESE, C., CACCAPPOLO, E., OZELIUS, L., ORR-URTREGER, A. & CLARK, L. 2015. Neuropsychological performance in LRRK2 G2019S carriers with Parkinson's disease. *Parkinsonism & related disorders,* 21**,** 106-110.

ALCARO, A., HUBER, R. & PANKSEPP, J. 2007. Behavioral functions of the mesolimbic dopaminergic system: an affective neuroethological perspective. *Brain research reviews,* 56**,** 283-321.

ALLOWAY, T. P., GATHERCOLE, S. E., KIRKWOOD, H. & ELLIOTT, J. 2008. Evaluating the validity of the automated working memory assessment. *Educational Psychology,* 28**,** 725-734.

ALOE, A. M. & BECKER, B. J. 2012. An effect size for regression predictors in meta-analysis. *Journal of Educational and Behavioral Statistics,* 37**,** 278-297.

ANIRUDHAN, A., MATTETHRA, G. C., ALZAHRANI, K. J., BANJER, H. J., ALZAHRANI, F. M., HALAWANI, I. F., PATIL, S., SHARMA, A., PARAMASIVAM, P. & AHMED, S. 2023. Eleven Crucial Pesticides Appear to Regulate Key Genes That Link MPTP Mechanism to Cause Parkinson's Disease through the Selective Degeneration of Dopamine Neurons. *Brain Sci,* 13.

ARCINIEGAS, D. B., ANDERSON, C. A., TOPKOFF, J. & MCALLISTER, T. W. 2005. Mild traumatic brain injury: a neuropsychiatric approach to diagnosis, evaluation, and treatment. *Neuropsychiatr Dis Treat,* 1**,** 311-27.

ARMSTRONG, M. J. & OKUN, M. S. 2020. Diagnosis and treatment of Parkinson disease: a review. *Jama,* 323**,** 548-560.

ARNAO, V., PIGNOLO, A., DAVÌ, C., RAGONESE, P., ARIDON, P., MASTRILLI, S. & D'AMELIO, M. 2021. Autonomic symptoms in motor subtypes of Parkinson's disease. *Journal of the Neurological Sciences,* 429.

ARTZI, M., EVEN-SAPIR, E., LERMAN SHACHAM, H., THALER, A., URTERGER, A. O., BRESSMAN, S., MARDER, K., HENDLER, T., GILADI, N. & BEN BASHAT, D. 2017. DaT-SPECT assessment depicts dopamine depletion among asymptomatic G2019S LRRK2 mutation carriers. *PloS one,* 12**,** e0175424.

ASCHERIO, A., CHEN, H., WEISSKOPF, M. G., O'REILLY, E., MCCULLOUGH, M. L., CALLE, E. E., SCHWARZSCHILD, M. A. & THUN, M. J. 2006. Pesticide exposure and risk for Parkinson's disease. *Ann Neurol,* 60**,** 197-203.

ASKEN, B. M., YANG, Z. H., XU, H. Y., WEBER, A. G., HAYES, R. L., BAUER, R. M., DEKOSKY, S. T., JAFFEE, M. S., WANG, K. K. W. & CLUGSTON, J. R. 2020. Acute Effects of Sport-Related Concussion on Serum Glial Fibrillary Acidic Protein, Ubiquitin C-Terminal Hydrolase L1, Total Tau, and Neurofilament Light Measured by a Multiplex Assay. *Journal of Neurotrauma,* 37**,** 1537-1545.

ASSINK, M. & WIBBELINK, C. J. 2016. Fitting three-level meta-analytic models in R: A step-by-step tutorial. *The Quantitative Methods for Psychology,* 12**,** 154-174.

AVILA, J., LUCAS, J. J., PEREZ, M. & HERNANDEZ, F. 2004. Role of tau protein in both physiological and pathological conditions. *Physiol Rev,* 84**,** 361-84.
AZIZI, S., HIER, D. B., ALLEN, B., OBAFEMI-AJAYI, T., OLBRICHT, G. R., THIMGAN, M. S. & WUNSCH, D. C. 2021. A Kinetic Model for Blood Biomarker Levels After Mild Traumatic Brain Injury. *Frontiers in Neurology,* 12**,** 14.
BAEK, M. S., LEE, M. J., KIM, H.-K. & LYOO, C. H. 2021. Temporal trajectory of biofluid markers in Parkinson's disease. *Scientific Reports,* 11**,** 14820.
BAILIE, J., BABAKHANYAN, I., JOLLY, M., EKANAYAKE, V., SARGENT, P., DUCKWORTH, J., EKANAYKE, V. & EKANAYAKE, V. 2017. Traumatic Brain Injury-2Accuracy of Self-Reported Questions for Assessment of TBI History. *Archives of Clinical Neuropsychology,* 32**,** 656-666.
BAKER, A. J., RHIND, S. G., MORRISON, L. J., BLACK, S., CRNKO, N. T., SHEK, P. N. & RIZOLI, S. B. 2009. Resuscitation with Hypertonic Saline-Dextran Reduces Serum Biomarker Levels and Correlates with Outcome in Severe Traumatic Brain Injury Patients. *Journal of Neurotrauma,* 26**,** 1227-1240.
BALABANDIAN, M., NOORI, M., LAK, B., KARIMIZADEH, Z. & NABIZADEH, F. 2023. Traumatic brain injury and risk of Parkinson's disease: a meta-analysis. *Acta Neurol Belg,* 123**,** 1225-1239.
BANTOUNOU, M. A., SHOAIB, K., MAZZOLENI, A., MODALAVALASA, H., KUMAR, N. & PHILIP, S. 2024. The association between type 2 diabetes mellitus and Parkinson's disease; a systematic review and meta-analysis. *Brain Disorders,* 15**,** 100158.
BARBER, T. R., GRIFFANTI, L., BRADLEY, K. M., MCGOWAN, D. R., LO, C., MACKAY, C. E., HU, M. T. & KLEIN, J. C. 2020. Nigrosome 1 imaging in REM sleep behavior disorder and its association with dopaminergic decline. *Ann Clin Transl Neurol,* 7**,** 26-35.
BARBOSA, J. H., SANTOS, A. C., TUMAS, V., LIU, M., ZHENG, W., HAACKE, E. M. & SALMON, C. E. 2015. Quantifying brain iron deposition in patients with Parkinson's disease using quantitative susceptibility mapping, R2 and R2. *Magn Reson Imaging,* 33**,** 559-65.
BARBU, M., JÓNSSON, K., ZETTERBERG, H., BLENNOW, K., KOLSRUD, O., RICKSTEN, S. E., DELLGREN, G., BJÖRK, K. & JEPPSSON, A. 2022. Serum biomarkers of brain injury after uncomplicated cardiac surgery: Secondary analysis from a randomized trial. *Acta Anaesthesiologica Scandinavica,* 66**,** 447-453.
BARONE, P. 2010. Neurotransmission in Parkinson's disease: beyond dopamine. *Eur J Neurol,* 17**,** 364-76.
BARTHEL, H. 2020. First Tau PET Tracer Approved: Toward Accurate In Vivo Diagnosis of Alzheimer Disease. *Journal of Nuclear Medicine,* 61**,** 1409-1410.
BASUKALA, D., MUKUNDAN, R., LIM, A., HURRELL, M. A., KEENAN, R. J., DALRYMPLE-ALFORD, J. C., ANDERSON, T. J., MYALL, D. J. & MELZER, T. R. 2021. Automated segmentation of substantia nigra and red nucleus using quantitative susceptibility mapping images: Application to Parkinson's disease. *Computers & Electrical Engineering,* 91**,** 107091.
BAZARIAN, J. J., BIBERTHALER, P., WELCH, R. D., LEWIS, L. M., BARZO, P., BOGNER-FLATZ, V., BROLINSON, P. G., BÜKI, A., CHEN, J. Y. & CHRISTENSON, R. H. 2018. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on

head CT (ALERT-TBI): a multicentre observational study. *The Lancet Neurology,* 17**,** 782-789.
BEHZADI, F., LUY, D. D., SCHAIBLE, P. A., ZYWICIEL, J. F., PUCCIO, A. M. & GERMANWALA, A. V. 2024. A systematic review and meta-analysis of major blood protein biomarkers that predict unfavorable outcomes in severe traumatic brain injury. *Clin Neurol Neurosurg,* 242**,** 108312.
BELLOMO, G., DE LUCA, C. M. G., PAOLETTI, F. P., GAETANI, L., MODA, F. & PARNETTI, L. 2022. α-Synuclein Seed Amplification Assays for Diagnosing Synucleinopathies: The Way Forward. *Neurology,* 99**,** 195-205.
BENAMER, H. T., PATTERSON, J., GROSSET, D. G., BOOIJ, J., DE BRUIN, K., VAN ROYEN, E., SPEELMAN, J., HORSTINK, M., SIPS, H. & DIERCKX, R. 2000a. Accurate differentiation of parkinsonism and essential tremor using visual assessment of [123I]-FP-CIT SPECT imaging: the [123I]-FP-CIT study group. *Movement disorders: official journal of the Movement Disorder Society,* 15**,** 503-510.
BENAMER, H. T., PATTERSON, J., WYPER, D. J., HADLEY, D. M., MACPHEE, G. J. & GROSSET, D. G. 2000b. Correlation of Parkinson's disease severity and duration with 123I-FP-CIT SPECT striatal uptake. *Mov Disord,* 15**,** 692-8.
BENÍTEZ-RIVERO, S., MARÍN-OYAGA, V. A., GARCÍA-SOLÍS, D., HUERTAS-FERNÁNDEZ, I., GARCÍA-GÓMEZ, F. J., JESÚS, S., CÁCERES, M. T., CARRILLO, F., ORTIZ, A. M. & CARBALLO, M. 2013. Clinical features and 123I-FP-CIT SPECT imaging in vascular parkinsonism and Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry,* 84**,** 122-129.
BENNINGER, D. H., MICHEL, J., WALDVOGEL, D., CANDIA, V., PORYAZOVA, R., VAN HEDEL, H. J. & BASSETTI, C. L. 2010. REM sleep behavior disorder is not linked to postural instability and gait dysfunction in Parkinson. *Mov Disord,* 25**,** 1597-604.
BERARDELLI, A., ROTHWELL, J. C., THOMPSON, P. D. & HALLETT, M. 2001. Pathophysiology of bradykinesia in Parkinson's disease. *Brain,* 124**,** 2131-2146.
BERG, D., POSTUMA, R. B., ADLER, C. H., BLOEM, B. R., CHAN, P., DUBOIS, B., GASSER, T., GOETZ, C. G., HALLIDAY, G. & JOSEPH, L. 2015. MDS research criteria for prodromal Parkinson's disease. *Movement Disorders,* 30**,** 1600-1611.
BERTI, V., PUPI, A. & MOSCONI, L. 2011. PET/CT in diagnosis of movement disorders. *Annals of the New York Academy of Sciences,* 1228**,** 93-108.
BETARBET, R., SHERER, T. B., MACKENZIE, G., GARCIA-OSUNA, M., PANOV, A. V. & GREENAMYRE, J. T. 2000. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci,* 3**,** 1301-6.
BIEGON, A. 2021. Considering biological sex in traumatic brain injury. *Frontiers in neurology,* 12**,** 576366.
BILLINGSLEY, K., BANDRES-CIGA, S., SAEZ-ATIENZAR, S. & SINGLETON, A. 2018. Genetic risk factors in Parkinson's disease. *Cell and tissue research,* 373**,** 9-20.
BISPO, J. M. M., MELO, J. E. C., GOIS, A. M., LEAL, P. C., LINS, L., SOUZA, M. F., MEDEIROS, K., RIBEIRO, A. M., SILVA, R. H., MARCHIORO, M. & SANTOS, J. R. 2019. Sex differences in the progressive model of parkinsonism induced by reserpine in rats. *Behav Brain Res,* 363**,** 23-29.
BLOEM, B. R., MARINUS, J., ALMEIDA, Q., DIBBLE, L., NIEUWBOER, A., POST, B., RUZICKA, E., GOETZ, C., STEBBINS, G., MARTINEZ-MARTIN, P. & SCHRAG, A. 2016. Measurement instruments to assess posture, gait, and balance in Parkinson's disease: Critique and recommendations. *Mov Disord,* 31**,** 1342-55.

BOGOSLOVSKY, T., WILSON, D., CHEN, Y., HANLON, D., GILL, J., JEROMIN, A., SONG, L., MOORE, C., GONG, Y., KENNEY, K. & DIAZ-ARRASTIA, R. 2017. Increases of Plasma Levels of Glial Fibrillary Acidic Protein, Tau, and Amyloid β up to 90 Days after Traumatic Brain Injury. *J Neurotrauma,* 34**,** 66-73.

BORENSTEIN, M., HEDGES, L. V., HIGGINS, J. P. & ROTHSTEIN, H. R. 2010. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res Synth Methods,* 1**,** 97-111.

BOSTANTJOPOULOU, S., KATSAROU, Z., DANGLIS, I., KARAKASIS, H., MILIONI, D. & FALUP-PECURARIU, C. 2016. Self-reported autonomic symptoms in Parkinson's disease: properties of the SCOPA-AUT scale. *Hippokratia,* 20**,** 115.

BOUTIN, H., MURRAY, K., PRADILLO, J., MAROY, R., SMIGOVA, A., GERHARD, A., JONES, P. A. & TRIGG, W. 2015. 18F-GE-180: a novel TSPO radiotracer compared to 11C-R-PK11195 in a preclinical model of stroke. *Eur J Nucl Med Mol Imaging,* 42**,** 503-11.

BRAAK, H., DEL TREDICI, K., RÜB, U., DE VOS, R. A., STEUR, E. N. J. & BRAAK, E. 2003. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of aging,* 24**,** 197-211.

BREEN, D. P., EVANS, J. R., FARRELL, K., BRAYNE, C. & BARKER, R. A. 2013. Determinants of delayed diagnosis in Parkinson's disease. *J Neurol,* 260**,** 1978-81.

BROCKMANN, K., SRULIJES, K., HAUSER, A. K., SCHULTE, C., CSOTI, I., GASSER, T. & BERG, D. 2011. GBA-associated PD presents with nonmotor characteristics. *Neurology,* 77**,** 276-80.

BROCKMANN, K., SRULIJES, K., PFLEDERER, S., HAUSER, A. K., SCHULTE, C., MAETZLER, W., GASSER, T. & BERG, D. 2015. GBA-associated Parkinson's disease: reduced survival and more rapid progression in a prospective longitudinal study. *Movement Disorders,* 30**,** 407-411.

BROOKE, M. M., QUESTAD, K. A., PATTERSON, D. R. & BASHAK, K. J. 1992. Agitation and restlessness after closed head injury: a prospective study of 100 consecutive admissions. *Archives of physical medicine and rehabilitation,* 73**,** 320-323.

BROOKS, D. J. 2000. Morphological and functional imaging studies on the diagnosis and progression of Parkinson's disease. *J Neurol,* 247 Suppl 2**,** Ii11-8.

BROOKS, D. J., IBANEZ, V., SAWLE, G. V., QUINN, N., LEES, A. J., MATHIAS, C. J., BANNISTER, R., MARSDEN, C. D. & FRACKOWIAK, R. S. 1990. Differing patterns of striatal 18F-dopa uptake in Parkinson's disease, multiple system atrophy, and progressive supranuclear palsy. *Ann Neurol,* 28**,** 547-55.

BROUSSOLLE, E., DENTRESANGLE, C., LANDAIS, P., GARCIA-LARREA, L., POLLAK, P., CROISILE, B., HIBERT, O., BONNEFOI, F., GALY, G., FROMENT, J. C. & COMAR, D. 1999. The relation of putamen and caudate nucleus 18F-Dopa uptake to motor and cognitive performances in Parkinson's disease. *J Neurol Sci,* 166**,** 141-51.

BRUIJNEN, C., DIJKSTRA, B. A. G., WALVOORT, S. J. W., BUDY, M. J. J., BEURMANJER, H., DE JONG, C. A. J. & KESSELS, R. P. C. 2020. Psychometric properties of the Montreal Cognitive Assessment (MoCA) in healthy participants aged 18-70. *Int J Psychiatry Clin Pract,* 24**,** 293-300.

BRUMBERG, J., TRAN-GIA, J., LAPA, C., ISAIAS, I. U. & SAMNICK, S. 2019. PET imaging of noradrenaline transporters in Parkinson's disease: focus on scan time. *Ann Nucl Med,* 33**,** 69-77.

BUNSRI, S., MUENCHAMNAN, N., NAKSEN, W. & ONG-ARTBORIRAK, P. 2023. The hematological and biochemical effects from pesticide exposure on Thai vegetable farmers. *Toxics,* 11**,** 707.

BUONGIORNO, M., ANTONELLI, F., COMPTA, Y., FERNANDEZ, Y., PAVIA, J., LOMEÑA, F., RÍOS, J., RAMÍREZ, I., GARCÍA, J. R. & SOLER, M. 2017. Cross-sectional and longitudinal cognitive correlates of FDDNP PET and CSF amyloid-β and Tau in Parkinson's disease. *Journal of Alzheimer's Disease,* 55**,** 1261-1272.

BURN, D. J., ROWAN, E. N., ALLAN, L. M., MOLLOY, S., O'BRIEN, J. T. & MCKEITH, I. G. 2006. Motor subtype and cognitive decline in Parkinson's disease, Parkinson's disease with dementia, and dementia with Lewy bodies. *J Neurol Neurosurg Psychiatry,* 77**,** 585-9.

CALABRESI, P., DI LAZZARO, G., MARINO, G., CAMPANELLI, F. & GHIGLIERI, V. 2023. Advances in understanding the function of alpha-synuclein: implications for Parkinson's disease. *Brain,* 146**,** 3587-3597.

CALLIER, S., MORISSETTE, M., GRBOIS, M. & DI PAOLO, T. 2000. Stereospecific prevention by 17β-estradiol of MPTP-induced dopamine depletion in mice. *Synapse,* 37**,** 245-251.

CALNE, D., SNOW, B. & LEE, C. 1992. Criteria for diagnosing Parkinson's disease. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society,* 32**,** S125-S127.

CAPPA, K. A., CONGER, J. C. & CONGER, A. J. 2011. Injury severity and outcome: a meta-analysis of prospective studies on TBI outcome. *Health Psychol,* 30**,** 542-60.

CARROLL, E. L., OUTTRIM, J. G., FORSYTH, F., MANKTELOW, A. E., HUTCHINSON, P. J. A., TENOVUO, O., POSTI, J. P., WILSON, L., SAHAKIAN, B. J., MENON, D. K. & NEWCOMBE, V. F. J. 2020. Mild traumatic brain injury recovery: a growth curve modelling analysis over 2 years. *J Neurol,* 267**,** 3223-3234.

CASSIDY, C. M., ZUCCA, F. A., GIRGIS, R. R., BAKER, S. C., WEINSTEIN, J. J., SHARP, M. E., BELLEI, C., VALMADRE, A., VANEGAS, N., KEGELES, L. S., BRUCATO, G., KANG, U. J., SULZER, D., ZECCA, L., ABI-DARGHAM, A. & HORGA, G. 2019. Neuromelanin-sensitive MRI as a noninvasive proxy measure of dopamine function in the human brain. *Proc Natl Acad Sci U S A,* 116**,** 5108-5117.

CASSIDY, J. D., CARROLL, L., PELOSO, P., BORG, J., VON HOLST, H., HOLM, L., KRAUS, J. & CORONADO, V. 2004. Incidence, risk factors and prevention of mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. *Journal of rehabilitation medicine,* 36**,** 28-60.

CASTAÑO-LEON, A. M., SÁNCHEZ CARABIAS, C., HILARIO, A., RAMOS, A., NAVARRO-MAIN, B., PAREDES, I., MUNARRIZ, P. M., PANERO, I., EIRIZ FERNÁNDEZ, C., GARCÍA-PÉREZ, D., MORENO-GOMEZ, L. M., ESTEBAN-SINOVAS, O., GARCIA POSADAS, G., GOMEZ, P. A. & LAGARES, A. 2022. Serum assessment of traumatic axonal injury: the correlation of GFAP, t-Tau, UCH-L1, and NfL levels with diffusion tensor imaging metrics and its prognosis utility. *J Neurosurg***,** 1-11.

CELEJ, M. S., SARROUKH, R., GOORMAGHTIGH, E., FIDELIO, G. D., RUYSSCHAERT, J.-M. & RAUSSENS, V. 2012. Toxic prefibrillar α-synuclein amyloid oligomers adopt a distinctive antiparallel β-sheet structure. *Biochemical journal,* 443**,** 719-726.

CERRI, S., MUS, L. & BLANDINI, F. 2019. Parkinson's Disease in Women and Men: What's the Difference? *J Parkinsons Dis,* 9**,** 501-515.

CHABOK, S. Y., MOGHADAM, A. D., SANEEI, Z., AMLASHI, F. G., LEILI, E. K. & AMIRI, Z. M. 2012. Neuron-specific enolase and S100BB as outcome predictors in severe diffuse axonal injury. *Journal of Trauma and Acute Care Surgery,* 72**,** 1654-1657.

CHAHINE, L. M., BEACH, T. G., BRUMM, M. C., ADLER, C. H., COFFEY, C. S., MOSOVSKY, S., CASPELL-GARCIA, C., SERRANO, G. E., MUNOZ, D. G., WHITE, C. L., 3RD, CRARY, J. F., JENNINGS, D., TAYLOR, P., FOROUD, T., ARNEDO, V., KOPIL, C. M., RILEY, L., DAVE, K. D. & MOLLENHAUER, B. 2020. In vivo distribution of α-synuclein in multiple tissues and biofluids in Parkinson disease. *Neurology,* 95**,** e1267-e1284.

CHAHINE, L. M., MERCHANT, K., SIDEROWF, A., SHERER, T., TANNER, C., MAREK, K. & SIMUNI, T. 2023. Proposal for a Biologic Staging System of Parkinson's Disease. *J Parkinsons Dis,* 13**,** 297-309.

CHAHINE, L. M., SIDEROWF, A., BARNES, J., SEEDORFF, N., CASPELL-GARCIA, C., SIMUNI, T., COFFEY, C. S., GALASKO, D., MOLLENHAUER, B., ARNEDO, V., DAEGELE, N., FRASIER, M., TANNER, C., KIEBURTZ, K. & MAREK, K. 2019. Predicting Progression in Parkinson's Disease Using Baseline and 1-Year Change Measures. *J Parkinsons Dis,* 9**,** 665-679.

CHAN, D., WOO, J., HO, S., PANG, C., LAW, L., NG, P., HUNG, W., KWOK, T., HUI, E. & ORR, K. 1998. Genetic and environmental risk factors for Parkinson's disease in a Chinese population. *Journal of Neurology, Neurosurgery & Psychiatry,* 65**,** 781-784.

CHAN, L., CHUNG, C. C., HSIEH, Y. C., WU, R. M. & HONG, C. T. 2023. Plasma extracellular vesicle tau, β-amyloid, and α-synuclein and the progression of Parkinson's disease: a follow-up study. *Ther Adv Neurol Disord,* 16**,** 17562864221150329.

CHAPLEAU, M., IACCARINO, L., SOLEIMANI-MEIGOONI, D. & RABINOVICI, G. D. 2022. The Role of Amyloid PET in Imaging Neurodegenerative Disorders: A Review. *J Nucl Med,* 63**,** 13s-19s.

CHARRAD, M., GHAZZALI, N., BOITEAU, V. & NIKNAFS, A. 2014. NbClust: an R package for determining the relevant number of clusters in a data set. *Journal of statistical software,* 61**,** 1-36.

CHATFIELD, D. A., ZEMLAN, F. P., DAY, D. J. & MENON, D. K. 2002. Discordant temporal patterns of S100beta and cleaved tau protein elevation after head injury: a pilot study. *Br J Neurosurg,* 16**,** 471-6.

CHAU, M. T., TODD, G., WILCOX, R., AGZARIAN, M. & BEZAK, E. 2020. Diagnostic accuracy of the appearance of Nigrosome-1 on magnetic resonance imaging in Parkinson's disease: A systematic review and meta-analysis. *Parkinsonism & related disorders,* 78**,** 12-20.

CHAUVEAU, F., BOUTIN, H., VAN CAMP, N., THOMINIAUX, C., HANTRAYE, P., RIVRON, L., MARGUET, F., CASTEL, M. N., ROONEY, T., BENAVIDES, J., DOLLÉ, F. & TAVITIAN, B. 2011. In vivo imaging of neuroinflammation in the rodent brain with [11C]SSR180575, a novel indoleacetamide radioligand of the translocator protein (18 kDa). *Eur J Nucl Med Mol Imaging,* 38**,** 509-14.

CHAVES, M. L. F., GODINHO, C. C., PORTO, C. S., MANSUR, L., CARTHERY-GOULART, M. T., YASSUDA, M. S. & BEATO, R. 2011. Cognitive, functional and behavioral assessment: Alzheimer's disease. *Dement Neuropsychol,* 5**,** 153-166.

CHEN, L., JIANG, H., XING, G., GUAN, B., YANG, Y., AHMED, A. & MA, X. 2021a. Effects of Yunanan Baiyao adjunct therapy on postoperative recovery and clinical prognosis of patients with traumatic brain injury: A randomized controlled trial. *Phytomedicine,* 89**,** 153593.

CHEN, L., JIN, J., DAVIS, J., ZHOU, Y., WANG, Y., LIU, J., LOCKHART, P. J. & ZHANG, J. 2007. Oligomeric α-synuclein inhibits tubulin polymerization. *Biochemical and biophysical research communications,* 356**,** 548-553.

CHEN, P., YU, X., HUANG, T., DENG, Y. B. & HUANG, J. R. 2021b. Role of Diffusion Tensor Imaging Combined with Neuron-Specific Enolase and S100 Calcium-binding Protein B Detection in Predicting the Prognosis of Moderate and Severe Traumatic Brain Injury. *Iranian Red Crescent Medical Journal,* 23**,** 6.

CHEN, Q., SCHERBAUM, R., GOLD, R., PITAROKOILI, K., MOSIG, A., ZELLA, S. & TÖNGES, L. 2023. Data-driven subtyping of Parkinson's disease: comparison of current methodologies and application to the Bochum PNS cohort. *Journal of Neural Transmission,* 130**,** 763-776.

CHEN, W. L., WANG, G. J., YAO, C. Y., ZHU, Z. J., CHEN, R., SU, W. & JIANG, R. C. 2022. The ratio of serum neuron-specific enolase level to admission glasgow coma scale score is associated with diffuse axonal injury in patients with moderate to severe traumatic brain injury. *Frontiers in Neurology,* 13**,** 8.

CHEN, Y. H., HUANG, E. Y., KUO, T. T., MILLER, J., CHIANG, Y. H. & HOFFER, B. J. 2017. Impact of Traumatic Brain Injury on Dopaminergic Transmission. *Cell Transplant,* 26**,** 1156-1168.

CHENG, F., YUAN, Q., YANG, J., WANG, W. & LIU, H. 2014. The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. *PLoS One,* 9**,** e106680.

CHENG, Z., HE, N., HUANG, P., LI, Y., TANG, R., SETHI, S. K., GHASSABAN, K., YERRAMSETTY, K. K., PALUTLA, V. K., CHEN, S., YAN, F. & HAACKE, E. M. 2020. Imaging the Nigrosome 1 in the substantia nigra using susceptibility weighted imaging and quantitative susceptibility mapping: An application to Parkinson's disease. *Neuroimage Clin,* 25**,** 102103.

CHEUNG, M. W. 2019. A Guide to Conducting a Meta-Analysis with Non-Independent Effect Sizes. *Neuropsychol Rev,* 29**,** 387-396.

CHEUNG, M. W., HO, R. C., LIM, Y. & MAK, A. 2012. Conducting a meta-analysis: basics and good practices. *Int J Rheum Dis,* 15**,** 129-35.

CHEUNG, M. W.-L. 2014. Modeling dependent effect sizes with three-level meta-analyses: a structural equation modeling approach. *Psychological methods,* 19**,** 211.

CHO, S. J., BAE, Y. J., KIM, J.-M., KIM, D., BAIK, S. H., SUNWOO, L., CHOI, B. S. & KIM, J. H. 2021a. Diagnostic performance of neuromelanin-sensitive magnetic resonance imaging for patients with Parkinson's disease and factor analysis for its heterogeneity: a systematic review and meta-analysis. *European radiology,* 31**,** 1268-1280.

CHO, S. J., BAE, Y. J., KIM, J. M., KIM, D., BAIK, S. H., SUNWOO, L., CHOI, B. S. & KIM, J. H. 2021b. Diagnostic performance of neuromelanin-sensitive magnetic resonance imaging for patients with Parkinson's disease and factor analysis for its heterogeneity: a systematic review and meta-analysis. *Eur Radiol,* 31**,** 1268-1280.

CHOI, Y., KIM, E. Y., SUN, J., KIM, H. K., LEE, Y. S., OH, B. M., PARK, H. Y. & LEIGH, J. H. 2022. Incidence of Depression after Traumatic Brain Injury: A Nationwide Longitudinal Study of 2.2 Million Adults. *J Neurotrauma,* 39**,** 390-397.
CHU, Y., HIRST, W. D., FEDEROFF, H. J., HARMS, A. S., STOESSL, A. J. & KORDOWER, J. H. 2024. Nigrostriatal tau pathology in parkinsonism and Parkinson's disease. *Brain,* 147**,** 444-457.
COELHO, C. A. Management of discourse deficits following traumatic brain injury: Progress, caveats, and needs. Seminars in Speech and Language, 2007. Copyright© 2007 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ..., 122-135.
COHEN, J. 2013. *Statistical power analysis for the behavioral sciences*, routledge.
COHEN, J. 2016. A power primer.
CONDE, M. A., ALZA, N. P., FUNK, M. I., MANISCALCHI, A., BENZI JUNCOS, O. N., BERGE, I., URANGA, R. M. & SALVADOR, G. A. 2023. α-Synuclein Attenuates Maneb Neurotoxicity through the Modulation of Redox-Sensitive Transcription Factors. *Oxid Med Cell Longev,* 2023**,** 5803323.
CONTROL, C. F. D. & PREVENTION 2003. Report to Congress on mild traumatic brain injury in the United States: steps to prevent a serious public health problem. *Atlanta, GA: Centers for Disease Control and Prevention,* 45.
CORRIGAN, F., WEE, I. C. & COLLINS-PRAINO, L. E. 2023. Chronic motor performance following different traumatic brain injury severity-A systematic review. *Front Neurol,* 14**,** 1180353.
CORRIGAN, J. D. & BOGNER, J. 2007. Initial reliability and validity of the Ohio State University TBI Identification Method. *J Head Trauma Rehabil,* 22**,** 318-29.
COSOTTINI, M., FROSINI, D., PESARESI, I., DONATELLI, G., CECCHI, P., COSTAGLI, M., BIAGI, L., CERAVOLO, R., BONUCCELLI, U. & TOSETTI, M. 2015. Comparison of 3T and 7T susceptibility-weighted angiography of the substantia nigra in diagnosing Parkinson disease. *American Journal of Neuroradiology,* 36**,** 461-466.
COSTA, G., PORCEDDU, P. F., SERRA, M., CASU, M. A., SCHIANO, V., NAPOLITANO, F., PINNA, A., USIELLO, A. & MORELLI, M. 2019. Lack of Rhes Increases MDMA-Induced Neuroinflammation and Dopamine Neuron Degeneration: Role of Gender and Age. *Int J Mol Sci,* 20.
COSTANZO, M., GALOSI, E., DE BARTOLO, M. I., GALLO, G., LEODORI, G., BELVISI, D., CONTE, A., FABBRINI, G., TRUINI, A., BERARDELLI, A. & VIVACQUA, G. 2024. Evaluating the Diagnostic Potential of Combined Salivary and Skin Biomarkers in Parkinson's Disease. *Int J Mol Sci,* 25.
COSTELLO, S., COCKBURN, M., BRONSTEIN, J., ZHANG, X. & RITZ, B. 2009. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *American journal of epidemiology,* 169**,** 919-926.
COX, D., CARVER, J. A. & ECROYD, H. 2014. Preventing α-synuclein aggregation: the role of the small heat-shock molecular chaperone proteins. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease,* 1842**,** 1830-1843.
CRANE, P. K., GIBBONS, L. E., DAMS-O'CONNOR, K., TRITTSCHUH, E., LEVERENZ, J. B., KEENE, C. D., SONNEN, J., MONTINE, T. J., BENNETT, D. A., LEURGANS, S., SCHNEIDER, J. A. & LARSON, E. B. 2016. Association of Traumatic Brain Injury

With Late-Life Neurodegenerative Conditions and Neuropathologic Findings. *JAMA Neurol,* 73**,** 1062-9.
CREMADES, N., COHEN, S. I., DEAS, E., ABRAMOV, A. Y., CHEN, A. Y., ORTE, A., SANDAL, M., CLARKE, R. W., DUNNE, P. & APRILE, F. A. 2012. Direct observation of the interconversion of normal and toxic forms of α-synuclein. *Cell,* 149**,** 1048-1059.
CROWTHER, R. A., DANIEL, S. E. & GOEDERT, M. 2000. Characterisation of isolated α-synuclein filaments from substantia nigra of Parkinson's disease brain. *Neuroscience letters,* 292**,** 128-130.
CZEITER, E., MONDELLO, S., KOVACS, N., SANDOR, J., GABRIELLI, A., SCHMID, K., TORTELLA, F., WANG, K. K., HAYES, R. L., BARZO, P., EZER, E., DOCZI, T. & BUKI, A. 2012a. Brain injury biomarkers may improve the predictive power of the IMPACT outcome calculator. *J Neurotrauma,* 29**,** 1770-8.
CZEITER, E., MONDELLO, S., KOVACS, N., SANDOR, J., GABRIELLI, A., SCHMID, K., TORTELLA, F., WANG, K. K. W., HAYES, R. L., BARZO, P., EZER, E., DOCZI, T. & BUKI, A. 2012b. Brain Injury Biomarkers May Improve the Predictive Power of the IMPACT Outcome Calculator. *Journal of Neurotrauma,* 29**,** 1770-1778.
DADU, A., SATONE, V., KAUR, R., HASHEMI, S. H., LEONARD, H., IWAKI, H., MAKARIOUS, M. B., BILLINGSLEY, K. J., BANDRES-CIGA, S., SARGENT, L. J., NOYCE, A. J., DANESHMAND, A., BLAUWENDRAAT, C., MAREK, K., SCHOLZ, S. W., SINGLETON, A. B., NALLS, M. A., CAMPBELL, R. H. & FAGHRI, F. 2022. Identification and prediction of Parkinson's disease subtypes and progression using machine learning in two cohorts. *NPJ Parkinsons Dis,* 8**,** 172.
DAGENBACH, D. & CARR, T. H. 1994. *Inhibitory processes in attention, memory, and language*, Academic Press San Diego.
DAMIER, P., HIRSCH, E., AGID, Y. & GRAYBIEL, A. 1999a. The substantia nigra of the human brain: I. Nigrosomes and the nigral matrix, a compartmental organization based on calbindin D28K immunohistochemistry. *Brain,* 122**,** 1421-1436.
DAMIER, P., HIRSCH, E., AGID, Y. & GRAYBIEL, A. 1999b. The substantia nigra of the human brain: II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain,* 122**,** 1437-1448.
DAMS-O'CONNOR, K., CANTOR, J. B., BROWN, M., DIJKERS, M. P., SPIELMAN, L. A. & GORDON, W. A. 2014. Screening for traumatic brain injury: findings and public health implications. *J Head Trauma Rehabil,* 29**,** 479-89.
DAUER, W. & PRZEDBORSKI, S. 2003. Parkinson's disease: mechanisms and models. *Neuron,* 39**,** 889-909.
DAVIS, K. A., CULLEN, B., ADAMS, M., BRAILEAN, A., BREEN, G., COLEMAN, J. R., DREGAN, A., GASPAR, H. A., HÜBEL, C. & LEE, W. 2019. Indicators of mental disorders in UK Biobank—A comparison of approaches. *International journal of methods in psychiatric research,* 28**,** e1796.
DE BARTOLO, M. I., VIVACQUA, G., BELVISI, D., MANCINELLI, R., FABBRINI, A., MANZO, N., COSTANZO, M., LEODORI, G., CONTE, A., FABBRINI, G., MORINI, S. & BERARDELLI, A. 2023. A Combined Panel of Salivary Biomarkers in de novo Parkinson's Disease. *Ann Neurol,* 93**,** 446-459.
DE HOND, A. A., STEYERBERG, E. W. & VAN CALSTER, B. 2022. Interpreting area under the receiver operating characteristic curve. *The Lancet Digital Health,* 4**,** e853-e855.

DE KRUIJK, J., LEFFERS, P., MENHEERE, P., MEERHOFF, S. & TWIJNSTRA, A. 2001. S-100B and neuron-specific enolase in serum of mild traumatic brain injury patients A comparison with healthy controls. *Acta neurologica scandinavica,* 103**,** 175-179.

DE KRUIJK, J. R., LEFFERS, P., MENHEERE, P. P., MEERHOFF, S., RUTTEN, J. & TWIJNSTRA, A. 2002. Prediction of post-traumatic complaints after mild traumatic brain injury: early symptoms and biochemical markers. *J Neurol Neurosurg Psychiatry,* 73**,** 727-32.

DE LA FUENTE-FERNÁNDEZ, R. 2012. Role of DaTSCAN and clinical diagnosis in Parkinson disease. *Neurology,* 78**,** 696-701.

DE RIJK, M. C., BRETELER, M. M., GRAVELAND, G. A., OTT, A., GROBBEE, D. E., VAN DER MECHÉ, F. G. & HOFMAN, A. 1995. Prevalence of Parkinson's disease in the elderly: the Rotterdam Study. *Neurology,* 45**,** 2143-6.

DE ROCHEFORT, L., BROWN, R., PRINCE, M. R. & WANG, Y. 2008. Quantitative MR susceptibility mapping using piece-wise constant regularized inversion of the magnetic field. *Magn Reson Med,* 60**,** 1003-9.

DEB, S., LYONS, I. & KOUTZOUKIS, C. 1999a. Neurobehavioural symptoms one year after a head injury. *Br J Psychiatry,* 174**,** 360-5.

DEB, S., LYONS, I., KOUTZOUKIS, C., ALI, I. & MCCARTHY, G. 1999b. Rate of psychiatric illness 1 year after traumatic brain injury. *Am J Psychiatry,* 156**,** 374-8.

DEHBOZORGI, M., MAGHSOUDI, M. R., MOHAMMADI, I., FIROUZABADI, S. R., MOHAMMADITABAR, M., ORAEE, S., AARABI, A., GOODARZI, M., SHAFIEE, A. & BAKHTIYARI, M. 2024a. Incidence of anxiety after traumatic brain injury: a systematic review and meta-analysis. *BMC neurology,* 24**,** 293.

DEHBOZORGI, M., MAGHSOUDI, M. R., RAJAI, S., MOHAMMADI, I., NEJAD, A. R., RAFIEI, M. A., SOLTANI, S., SHAFIEE, A. & BAKHTIYARI, M. 2024b. Depression after traumatic brain injury: A systematic review and Meta-analysis. *The American Journal of Emergency Medicine*.

DELANEY, J. S., LACROIX, V. J., LECLERC, S. & JOHNSTON, K. M. 2002. Concussions among university football and soccer players. *Clinical Journal of Sport Medicine,* 12**,** 331-338.

DELIC, V., BECK, K. D., PANG, K. C. H. & CITRON, B. A. 2020. Biological links between traumatic brain injury and Parkinson's disease. *Acta Neuropathol Commun,* 8**,** 45.

DEWAN, M. C., RATTANI, A., GUPTA, S., BATICULON, R. E., HUNG, Y. C., PUNCHAK, M., AGRAWAL, A., ADELEYE, A. O., SHRIME, M. G., RUBIANO, A. M., ROSENFELD, J. V. & PARK, K. B. 2019. Estimating the global incidence of traumatic brain injury. *J Neurosurg,* 130**,** 1080-1097.

DEY, S., GANGADHARAN, J., DEEPIKA, A., KUMAR, J. K., CHRISTOPHER, R., RAMESH, S. S., DEVI, B. I. & SHUKLA, D. P. 2017. Correlation of ubiquitin C terminal hydrolase and S100 beta with cognitive deficits in young adults with mild traumatic brain injury. *Neurology India,* 65**,** 761-766.

DI BATTISTA, A. P., BUONORA, J. E., RHIND, S. G., HUTCHISON, M. G., BAKER, A. J., RIZOLI, S. B., DIAZ-ARRASTIA, R. & MUELLER, G. P. 2015. Blood biomarkers in moderate-to-severe traumatic brain injury: Potential utility of a multi-marker approach in characterizing outcome. *Frontiers in Neurology,* 6**,** 00110.

DI PUCCHIO, A., VANACORE, N., MARZOLINI, F., LACORTE, E., DI FIANDRA, T. & GASPARINI, M. 2018. Use of neuropsychological tests for the diagnosis of dementia: a survey of Italian memory clinics. *BMJ Open,* 8**,** e017847.

DIAMOND, P. M., HARZKE, A. J., MAGALETTA, P. R., CUMMINS, A. G. & FRANKOWSKI, R. 2007. Screening for traumatic brain injury in an offender sample: a first look at the reliability and validity of the Traumatic Brain Injury Questionnaire. *J Head Trauma Rehabil,* 22**,** 330-8.

DIAZ-ARRASTIA, R., WANG, K. K. W., PAPA, L., SORANI, M. D., YUE, J. K., PUCCIO, A. M., MCMAHON, P. J., INOUE, T., YUH, E. L., LINGSMA, H. F., MAAS, A. I. R., VALADKA, A. B., OKONKWO, D. O., MANLEY, G. T., CASEY, S. S., CHEONG, M., COOPER, S. R., DAMS-O'CONNOR, K., GORDON, W. A., HRICIK, A. J., MENON, D. K., MUKHERJEE, P., SCHNYER, D. M., SINHA, T. K., VASSAR, M. J. & INVESTIGATORS, T.-T. 2014. Acute Biomarkers of Traumatic Brain Injury: Relationship between Plasma Levels of Ubiquitin C-Terminal Hydrolase-L1 and Glial Fibrillary Acidic Protein. *Journal of Neurotrauma,* 31**,** 19-25.

DIAZ-CORRALES, F. J., SANZ-VIEDMA, S., GARCIA-SOLIS, D., ESCOBAR-DELGADO, T. & MIR, P. 2010. Clinical features and 123I-FP-CIT SPECT imaging in drug-induced parkinsonism and Parkinson's disease. *Eur J Nucl Med Mol Imaging,* 37**,** 556-64.

DIMANT, H., KALIA, S. K., KALIA, L. V., ZHU, L. N., KIBUUKA, L., EBRAHIMI-FAKHARI, D., MCFARLAND, N. R., FAN, Z., HYMAN, B. T. & MCLEAN, P. J. 2013. Direct detection of alpha synuclein oligomers in vivo. *Acta neuropathologica communications,* 1**,** 1-10.

DING, J., ZHANG, J., WANG, X., ZHANG, L., JIANG, S., YUAN, Y., LI, J., ZHU, L. & ZHANG, K. 2017. Relationship between the plasma levels of neurodegenerative proteins and motor subtypes of Parkinson's disease. *J Neural Transm (Vienna),* 124**,** 353-360.

DING, Y. S., LIN, K. S., GARZA, V., CARTER, P., ALEXOFF, D., LOGAN, J., SHEA, C., XU, Y. & KING, P. 2003. Evaluation of a new norepinephrine transporter PET ligand in baboons, both in brain and peripheral organs. *Synapse,* 50**,** 345-52.

DING, Y. S., LIN, K. S. & LOGAN, J. 2006. PET imaging of norepinephrine transporters. *Curr Pharm Des,* 12**,** 3831-45.

DING, Y. S., LIN, K. S., LOGAN, J., BENVENISTE, H. & CARTER, P. 2005. Comparative evaluation of positron emission tomography radiotracers for imaging the norepinephrine transporter: (S,S) and (R,R) enantiomers of reboxetine analogs ([11C]methylreboxetine, 3-Cl-[11C]methylreboxetine and [18F]fluororeboxetine), (R)-[11C]nisoxetine, [11C]oxaprotiline and [11C]lortalamine. *J Neurochem,* 94**,** 337-51.

DISABATO, D. J., QUAN, N. & GODBOUT, J. P. 2016. Neuroinflammation: the devil is in the details. *J Neurochem,* 139 Suppl 2**,** 136-153.

DLUZEN, D. E., MCDERMOTT, J. L. & LIU, B. 1996. Estrogen as a neuroprotectant against MPTP-induced neurotoxicity in C57/B1 mice. *Neurotoxicology and teratology,* 18**,** 603-606.

DOHERTY, K. M., SILVEIRA-MORIYAMA, L., PARKKINEN, L., HEALY, D. G., FARRELL, M., MENCACCI, N. E., AHMED, Z., BRETT, F. M., HARDY, J. & QUINN, N. 2013. Parkin disease: a clinicopathologic entity? *JAMA neurology,* 70**,** 571-579.

DONADIO, V., INCENSI, A., EL-AGNAF, O., RIZZO, G., VAIKATH, N., DEL SORBO, F., SCAGLIONE, C., CAPELLARI, S., ELIA, A., STANZANI MASERATI, M., PANTIERI, R.

& LIGUORI, R. 2018. Skin α-synuclein deposits differ in clinical variants of synucleinopathy: an in vivo study. *Sci Rep,* 8**,** 14246.
DONADIO, V., INCENSI, A., LETA, V., GIANNOCCARO, M. P., SCAGLIONE, C., MARTINELLI, P., CAPELLARI, S., AVONI, P., BARUZZI, A. & LIGUORI, R. 2014. Skin nerve α-synuclein deposits: a biomarker for idiopathic Parkinson disease. *Neurology,* 82**,** 1362-9.
DONADIO, V., INCENSI, A., RIZZO, G., SCAGLIONE, C., CAPELLARI, S., FILECCIA, E., AVONI, P. & LIGUORI, R. 2017. Spine Topographical Distribution of Skin α-Synuclein Deposits in Idiopathic Parkinson Disease. *J Neuropathol Exp Neurol,* 76**,** 384-389.
DONNEMILLER, E., BRENNEIS, C., WISSEL, J., SCHERFLER, C., POEWE, W., RICCABONA, G. & WENNING, G. K. 2000. Impaired dopaminergic neurotransmission in patients with traumatic brain injury: a SPECT study using 123I-beta-CIT and 123I-IBZM. *Eur J Nucl Med,* 27**,** 1410-4.
DOORDUIN, J., KLEIN, H. C., DIERCKX, R. A., JAMES, M., KASSIOU, M. & DE VRIES, E. F. 2009. [11C]-DPA-713 and [18F]-DPA-714 as new PET tracers for TSPO: a comparison with [11C]-(R)-PK11195 in a rat model of herpes encephalitis. *Mol Imaging Biol,* 11**,** 386-98.
DORSEY, E. R. & BLOEM, B. R. 2018. The Parkinson pandemic—a call to action. *JAMA neurology,* 75**,** 9-10.
DOTY, R. L., SHAMAN, P., KIMMELMAN, C. P. & DANN, M. S. 1984. University of Pennsylvania Smell Identification Test: a rapid quantitative olfactory function test for the clinic. *Laryngoscope,* 94**,** 176-8.
DOUBLE, K. L., ROWE, D. B., HAYES, M., CHAN, D. K., BLACKIE, J., CORBETT, A., JOFFE, R., FUNG, V. S., MORRIS, J. & HALLIDAY, G. M. 2003. Identifying the pattern of olfactory deficits in Parkinson disease using the brief smell identification test. *Archives of neurology,* 60**,** 545-549.
DOWNING, M. G., CARTY, M., OLVER, J., PONSFORD, M., ACHER, R., MCKENZIE, D. & PONSFORD, J. L. 2024a. The impact of age on outcome 2 years after traumatic brain injury: Case control study. *Annals of Physical and Rehabilitation Medicine,* 67**,** 101834.
DOWNING, M. G., CARTY, M., OLVER, J., PONSFORD, M., ACHER, R., MCKENZIE, D. & PONSFORD, J. L. 2024b. The impact of age on outcome 2 years after traumatic brain injury: Case control study. *Ann Phys Rehabil Med,* 67**,** 101834.
DU, G., LIU, T., LEWIS, M. M., KONG, L., WANG, Y., CONNOR, J., MAILMAN, R. B. & HUANG, X. 2016. Quantitative susceptibility mapping of the midbrain in Parkinson's disease. *Mov Disord,* 31**,** 317-24.
DU, Q., WENG, J. F., LUO, L. F., CEN, M., YU, W. H., ZHENG, Y. K., HU, W., PAN, J. W. & DONG, X. Q. 2018. Serum ST2 as a potential prognostic biomarker for traumatic brain injury. *Clinica Chimica Acta,* 487**,** 145-152.
DUNNING, D. L., WESTGATE, B. & ADLAM, A. R. 2016. A meta-analysis of working memory impairments in survivors of moderate-to-severe traumatic brain injury. *Neuropsychology,* 30**,** 811-819.
EGGERS, C., PEDROSA, D. J., KAHRAMAN, D., MAIER, F., LEWIS, C. J., FINK, G. R., SCHMIDT, M. & TIMMERMANN, L. 2012. Parkinson subtypes progress differently in clinical course and imaging pattern.

EL-AGNAF, O. M., SALEM, S. A., PALEOLOGOU, K. E., CURRAN, M. D., GIBSON, M. J., COURT, J. A., SCHLOSSMACHER, M. G. & ALLSOP, D. 2006. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *Faseb j,* 20**,** 419-25.

EMIN, D., ZHANG, Y. P., LOBANOVA, E., MILLER, A., LI, X., XIA, Z., DAKIN, H., SIDERIS, D. I., LAM, J. Y. & RANASINGHE, R. T. 2022. Small soluble α-synuclein aggregates are the toxic species in Parkinson's disease. *Nature communications,* 13**,** 5512.

ERIXON-LINDROTH, N., FARDE, L., WAHLIN, T. B., SOVAGO, J., HALLDIN, C. & BÄCKMAN, L. 2005. The role of the striatal dopamine transporter in cognitive aging. *Psychiatry Res,* 138**,** 1-12.

ERRO, R., SCHNEIDER, S. A., STAMELOU, M., QUINN, N. P. & BHATIA, K. P. 2016. What do patients with scans without evidence of dopaminergic deficit (SWEDD) have? New evidence and continuing controversies. *Journal of Neurology, Neurosurgery & Psychiatry,* 87**,** 319-323.

ESHUIS, S., JAGER, P., MAGUIRE, R., JONKMAN, S., DIERCKX, R. & LEENDERS, K. 2009. Direct comparison of FP-CIT SPECT and F-DOPA PET in patients with Parkinson's disease and healthy controls. *European journal of nuclear medicine and molecular imaging,* 36**,** 454-462.

ESHUIS, S., MAGUIRE, R., LEENDERS, K., JONKMAN, S. & JAGER, P. 2006a. Comparison of FP-CIT SPECT with F-DOPA PET in patients with de novo and advanced Parkinson's disease. *European journal of nuclear medicine and molecular imaging,* 33**,** 200-209.

ESHUIS, S. A., MAGUIRE, R. P., LEENDERS, K. L., JONKMAN, S. & JAGER, P. L. 2006b. Comparison of FP-CIT SPECT with F-DOPA PET in patients with de novo and advanced Parkinson's disease. *Eur J Nucl Med Mol Imaging,* 33**,** 200-9.

ESPAY, A. J., LEWITT, P. A. & KAUFMANN, H. 2014. Norepinephrine deficiency in Parkinson's disease: the case for noradrenergic enhancement. *Movement Disorders,* 29**,** 1710-1719.

EUSEBI, P., GIANNANDREA, D., BISCETTI, L., ABRAHA, I., CHIASSERINI, D., ORSO, M., CALABRESI, P. & PARNETTI, L. 2017. Diagnostic utility of cerebrospinal fluid α-synuclein in Parkinson's disease: a systematic review and meta-analysis. *Movement disorders,* 32**,** 1389-1400.

EVERS, L. J. W., KRIJTHE, J. H., MEINDERS, M. J., BLOEM, B. R. & HESKES, T. M. 2019. Measuring Parkinson's disease over time: The real-world within-subject reliability of the MDS-UPDRS. *Mov Disord,* 34**,** 1480-1487.

FANG, C., LV, L., MAO, S., DONG, H. & LIU, B. 2020. Cognition deficits in Parkinson's disease: mechanisms and treatment. *Parkinson's disease,* 2020**,** 2076942.

FEARNLEY, J. M. & LEES, A. J. 1991. Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain,* 114 (Pt 5)**,** 2283-301.

FEIGIN, V. L., ABAJOBIR, A. A., ABATE, K. H., ABD-ALLAH, F., ABDULLE, A. M., ABERA, S. F., ABYU, G. Y., AHMED, M. B., AICHOUR, A. N. & AICHOUR, I. 2017. Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *The Lancet Neurology,* 16**,** 877-897.

FENG, Y., LI, K., ROTH, E., CHAO, D., MECCA, C. M., HOGAN, Q. H., PAWELA, C., KWOK, W.-M., CAMARA, A. K. & PAN, B. 2021. Repetitive mild traumatic brain injury in rats impairs cognition, enhances prefrontal cortex neuronal activity, and reduces

pre-synaptic mitochondrial function. *Frontiers in cellular neuroscience,* 15**,** 689334.
FERESHTEHNEJAD, S.-M. & POSTUMA, R. B. 2017. Subtypes of Parkinson's disease: what do they tell us about disease progression? *Current neurology and neuroscience reports,* 17**,** 1-10.
FERESHTEHNEJAD, S.-M., ZEIGHAMI, Y., DAGHER, A. & POSTUMA, R. B. 2017. Clinical criteria for subtyping Parkinson's disease: biomarkers and longitudinal progression. *Brain,* 140**,** 1959-1976.
FERNÁNDEZ-CASTILLA, B., DECLERCQ, L., JAMSHIDI, L., BERETVAS, S. N., ONGHENA, P. & VAN DEN NOORTGATE, W. 2021. Detecting selection bias in meta-analyses with multiple outcomes: A simulation study. *The Journal of Experimental Education,* 89**,** 125-144.
FLORESCO, S. B. & MAGYAR, O. 2006. Mesocortical dopamine modulation of executive functions: beyond working memory. *Psychopharmacology,* 188**,** 567-585.
FOLKERSMA, H., FOSTER DINGLEY, J. C., VAN BERCKEL, B. N., ROZEMULLER, A., BOELLAARD, R., HUISMAN, M. C., LAMMERTSMA, A. A., VANDERTOP, W. P. & MOLTHOFF, C. F. 2011. Increased cerebral (R)-[(11)C]PK11195 uptake and glutamate release in a rat model of traumatic brain injury: a longitudinal pilot study. *J Neuroinflammation,* 8**,** 67.
FOOD, U. & ADMINISTRATION, D. 2018. Evaluation of automatic Class III designation for Banyan Brain Trauma Indicator: decision memorandum. *Silver Spring, MD: US Food and Drug Administration***,** 1-32.
FØRLAND, M. G., ÖHRFELT, A., DALEN, I., TYSNES, O. B., BLENNOW, K., ZETTERBERG, H., PEDERSEN, K. F., ALVES, G. & LANGE, J. 2018. Evolution of cerebrospinal fluid total α-synuclein in Parkinson's disease. *Parkinsonism Relat Disord,* 49**,** 4-8.
FRALEY, R. C. & VAZIRE, S. 2014. The N-pact factor: evaluating the quality of empirical journals with respect to sample size and statistical power. *PLoS One,* 9**,** e109019.
FRANK, M. J., SEEBERGER, L. C. & O'REILLY R, C. 2004. By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science,* 306**,** 1940-3.
FRANKEL, M., FAN, L. Q., YEATTS, S. D., JEROMIN, A., VOS, P. E., WAGNER, A. K., WOLF, B. J., PAULS, Q., LUNNEY, M., MERCK, L. H., HALL, C. L., PALESCH, Y. Y., SILBERGLEIT, R. & WRIGHT, D. W. 2019. Association of Very Early Serum Levels of S100B, Glial Fibrillary Acidic Protein, Ubiquitin C-terminal Hydrolase-L1, and Spectrin Breakdown Product with Outcome in ProTECT III. *Journal of Neurotrauma,* 36**,** 2863-2871.
FROST, R. B., FARRER, T. J., PRIMOSCH, M. & HEDGES, D. W. 2013. Prevalence of traumatic brain injury in the general adult population: a meta-analysis. S. Karger AG Basel, Switzerland.
GAENSLEN, A., SWID, I., LIEPELT-SCARFONE, I., GODAU, J. & BERG, D. 2011. The patients' perception of prodromal symptoms before the initial diagnosis of Parkinson's disease. *Mov Disord,* 26**,** 653-8.
GALLEGOS, S., PACHECO, C., PETERS, C., OPAZO, C. M. & AGUAYO, L. G. 2015. Features of alpha-synuclein that could explain the progression and irreversibility of Parkinson's disease. *Frontiers in neuroscience,* 9**,** 59.

GAN-OR, Z., LEBLOND, C. S., MALLETT, V., ORR-URTREGER, A., DION, P. A. & ROULEAU, G. A. 2015. LRRK2 mutations in Parkinson disease; a sex effect or lack thereof? A meta-analysis. *Parkinsonism & related disorders,* 21**,** 778-782.

GAO, J. & ZHENG, Z. 2015. Development of prognostic models for patients with traumatic brain injury: a systematic review. *Int J Clin Exp Med,* 8**,** 19881-5.

GARAAS, T. W. & POMPLUN, M. 2008. Inspection time and visual–perceptual processing. *Vision research,* 48**,** 523-537.

GARDNER, R. C., BURKE, J. F., NETTIKSIMMONS, J., GOLDMAN, S., TANNER, C. M. & YAFFE, K. 2015. Traumatic brain injury in later life increases risk for Parkinson disease. *Ann Neurol,* 77**,** 987-95.

GARDNER, R. C., BYERS, A. L., BARNES, D. E., LI, Y., BOSCARDIN, J. & YAFFE, K. 2018. Mild TBI and risk of Parkinson disease: A Chronic Effects of Neurotrauma Consortium Study. *Neurology,* 90**,** e1771-e1779.

GARON, M., WEIS, L., FIORENZATO, E., PISTONESI, F., CAGNIN, A., BERTOLDO, A., ANGLANI, M., CECCHIN, D., ANTONINI, A. & BIUNDO, R. 2021. Quantification of Brain β-Amyloid Load in Parkinson's Disease With Mild Cognitive Impairment: A PET/MRI Study. *Front Neurol,* 12**,** 760518.

GATSON, J. W., BARILLAS, J., HYNAN, L. S., DIAZ-ARRASTIA, R., WOLF, S. E. & MINEI, J. P. 2014. Detection of neurofilament-H in serum as a diagnostic tool to predict injury severity in patients who have suffered mild traumatic brain injury. *J Neurosurg,* 121**,** 1232-8.

GAURAV, R., YAHIA-CHERIF, L., PYATIGORSKAYA, N., MANGONE, G., BIONDETTI, E., VALABRÈGUE, R., EWENCZYK, C., HUTCHISON, R. M., CEDARBAUM, J. M., CORVOL, J. C., VIDAILHET, M. & LEHÉRICY, S. 2021. Longitudinal Changes in Neuromelanin MRI Signal in Parkinson's Disease: A Progression Marker. *Mov Disord,* 36**,** 1592-1602.

GAYED, I., JOSEPH, U., FANOUS, M., WAN, D., SCHIESS, M., ONDO, W. & WON, K.-S. 2015. The impact of DaTscan in the diagnosis of Parkinson disease. *Clinical nuclear medicine,* 40**,** 390-393.

GELB, D. J., OLIVER, E. & GILMAN, S. 1999. Diagnostic criteria for Parkinson disease. *Archives of neurology,* 56**,** 33-39.

GELMAN, N., GORELL, J. M., BARKER, P. B., SAVAGE, R. M., SPICKLER, E. M., WINDHAM, J. P. & KNIGHT, R. A. 1999. MR imaging of human brain at 3.0 T: preliminary report on transverse relaxation rates and relation to estimated iron content. *Radiology,* 210**,** 759-67.

GENTIL, B. J., TIBSHIRANI, M. & DURHAM, H. D. 2015. Neurofilament dynamics and involvement in neurological disorders. *Cell and tissue research,* 360**,** 609-620.

GERA, G., CHESNUTT, J., MANCINI, M., HORAK, F. B. & KING, L. A. 2018. Inertial sensor-based assessment of central sensory integration for balance after mild traumatic brain injury. *Military medicine,* 183**,** 327-332.

GERHARD, A., PAVESE, N., HOTTON, G., TURKHEIMER, F., ES, M., HAMMERS, A., EGGERT, K., OERTEL, W., BANATI, R. B. & BROOKS, D. J. 2006. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis,* 21**,** 404-12.

GERMAN, D. C., MANAYE, K., SMITH, W. K., WOODWARD, D. J. & SAPER, C. B. 1989. Midbrain dopaminergic cell loss in Parkinson's disease: computer visualization. *Ann Neurol,* 26**,** 507-14.

GHONEMI, M. O., RABAH, A. A., SABER, H. M. & RADWAN, W. 2013. Role of Phosphorylated Neurofilament H as a diagnostic and prognostic marker in traumatic brain injury. *The Egyptian Journal of Critical Care Medicine,* 1**,** 139-144.
GHOSH, A., ROY, A., LIU, X., KORDOWER, J. H., MUFSON, E. J., HARTLEY, D. M., GHOSH, S., MOSLEY, R. L., GENDELMAN, H. E. & PAHAN, K. 2007. Selective inhibition of NF-kappaB activation prevents dopaminergic neuronal loss in a mouse model of Parkinson's disease. *Proc Natl Acad Sci U S A,* 104**,** 18754-9.
GIBB, W. R. & LEES, A. J. 1988. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. *J Neurol Neurosurg Psychiatry,* 51**,** 745-52.
GIBBONS, C. H., LEVINE, T., ADLER, C., BELLAIRE, B., WANG, N., STOHL, J., AGARWAL, P., ALDRIDGE, G. M., BARBOI, A. & EVIDENTE, V. G. 2024. Skin biopsy detection of phosphorylated α-synuclein in patients with synucleinopathies. *JAMA*.
GILBERT, M., SNYDER, C., CORCORAN, C., NORTON, M. C., LYKETSOS, C. G. & TSCHANZ, J. T. 2014. The association of traumatic brain injury with rate of progression of cognitive and functional impairment in a population-based cohort of Alzheimer's disease: the Cache County Dementia Progression Study. *International psychogeriatrics,* 26**,** 1593-1601.
GOETZ, C. G., TILLEY, B. C., SHAFTMAN, S. R., STEBBINS, G. T., FAHN, S., MARTINEZ-MARTIN, P., POEWE, W., SAMPAIO, C., STERN, M. B. & DODEL, R. 2008. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Movement disorders: official journal of the Movement Disorder Society,* 23**,** 2129-2170.
GOLDMAN, J. E., YEN, S. H., CHIU, F. C. & PERESS, N. S. 1983. Lewy bodies of Parkinson's disease contain neurofilament antigens. *Science,* 221**,** 1082-4.
GOLDMAN, S. M., KAMEL, F., ROSS, G. W., JEWELL, S. A., BHUDHIKANOK, G. S., UMBACH, D., MARRAS, C., HAUSER, R. A., JANKOVIC, J., FACTOR, S. A., BRESSMAN, S., LYONS, K. E., MENG, C., KORELL, M., ROUCOUX, D. F., HOPPIN, J. A., SANDLER, D. P., LANGSTON, J. W. & TANNER, C. M. 2012. Head injury, α-synuclein Rep1, and Parkinson's disease. *Ann Neurol,* 71**,** 40-8.
GÓMEZ-BENITO, M., GRANADO, N., GARCÍA-SANZ, P., MICHEL, A., DUMOULIN, M. & MORATALLA, R. 2020. Modeling Parkinson's Disease With the Alpha-Synuclein Protein. *Front Pharmacol,* 11**,** 356.
GONZALEZ-ORTIZ, F., DULEWICZ, M., ASHTON, N. J., KAC, P. R., ZETTERBERG, H., ANDERSSON, E., YAKOUB, Y., HANRIEDER, J., TURTON, M., HARRISON, P., NELLGARD, B., KARIKARI, T. K. & BLENNOW, K. 2023. Association of Serum Brain-Derived Tau With Clinical Outcome and Longitudinal Change in Patients With Severe Traumatic Brain Injury. *JAMA network open,* 6**,** e2321554.
GORELL, J., ORDIDGE, R., BROWN, G., DENIAU, J., BUDERER, N. & HELPERN, J. 1995. Increased iron-related MRI contrast in the substantia nigra in Parkinson's disease. *Neurology,* 45**,** 1138-1143.
GORELL, J. M., JOHNSON, C., RYBICKI, B., PETERSON, E. & RICHARDSON, R. 1998. The risk of Parkinson's disease with exposure to pesticides, farming, well water, and rural living. *Neurology,* 50**,** 1346-1350.
GOSSELIN, N., BOTTARI, C., CHEN, J. K., HUNTGEBURTH, S. C., DE BEAUMONT, L., PETRIDES, M., CHEUNG, B. & PTITO, A. 2012. Evaluating the cognitive

consequences of mild traumatic brain injury and concussion by using electrophysiology. *Neurosurg Focus,* 33**,** E7: 1-7.
GOYAL, A., FAILLA, M. D., NIYONKURU, C., AMIN, K., FABIO, A., BERGER, R. P. & WAGNER, A. K. 2013. S100b as a Prognostic Biomarker in Outcome Prediction for Patients with Severe Traumatic Brain Injury. *Journal of Neurotrauma,* 30**,** 946-957.
GRANDBOIS, M., MORISSETTE, M., CALLIER, S. & DI PAOLO, T. 2000. Ovarian steroids and raloxifene prevent MPTP-induced dopamine depletion in mice. *Neuroreport,* 11**,** 343-346.
GRAPHPAD. (n.d). *T Test Calculator* [Online]. Available: https://www.graphpad.com/quickcalcs/ttest1/?format=SD [Accessed].
GREALISH, S., JÖNSSON, M. E., LI, M., KIRIK, D., BJÖRKLUND, A. & THOMPSON, L. H. 2010. The A9 dopamine neuron component in grafts of ventral mesencephalon is an important determinant for recovery of motor function in a rat model of Parkinson's disease. *Brain,* 133**,** 482-495.
GREENLAND, J. C., WILLIAMS-GRAY, C. H. & BARKER, R. A. 2019. The clinical heterogeneity of Parkinson's disease and its therapeutic implications. *Eur J Neurosci,* 49**,** 328-338.
GREFFARD, S., VERNY, M., BONNET, A.-M., BEINIS, J.-Y., GALLINARI, C., MEAUME, S., PIETTE, F., HAUW, J.-J. & DUYCKAERTS, C. 2006. Motor score of the unified Parkinson disease rating scale as a good predictor of Lewy body–associated neuronal loss in the substantia nigra. *Archives of neurology,* 63**,** 584-588.
GROOTEN, W. J. A., TSELI, E., ÄNG, B. O., BOERSMA, K., STÅLNACKE, B.-M., GERDLE, B. & ENTHOVEN, P. 2019a. Elaborating on the assessment of the risk of bias in prognostic studies in pain rehabilitation using QUIPS—aspects of interrater agreement. *Diagnostic and prognostic research,* 3**,** 1-11.
GROOTEN, W. J. A., TSELI, E., ÄNG, B. O., BOERSMA, K., STÅLNACKE, B. M., GERDLE, B. & ENTHOVEN, P. 2019b. Elaborating on the assessment of the risk of bias in prognostic studies in pain rehabilitation using QUIPS-aspects of interrater agreement. *Diagn Progn Res,* 3**,** 5.
GUAN, X., XUAN, M., GU, Q., HUANG, P., LIU, C., WANG, N., XU, X., LUO, W. & ZHANG, M. 2017. Regionally progressive accumulation of iron in Parkinson's disease as measured by quantitative susceptibility mapping. *NMR Biomed,* 30.
GUCCIARDI, D. F., LINES, R. L. & NTOUMANIS, N. 2022. Handling effect size dependency in meta-analysis. *International Review of Sport and Exercise Psychology,* 15**,** 152-178.
GUINEY, S. J., ADLARD, P. A., LEI, P., MAWAL, C. H., BUSH, A. I., FINKELSTEIN, D. I. & AYTON, S. 2020. Fibrillar α-synuclein toxicity depends on functional lysosomes. *Journal of Biological Chemistry,* 295**,** 17497-17513.
GUO, Y., LIU, F. T., HOU, X. H., LI, J. Q., CAO, X. P., TAN, L., WANG, J. & YU, J. T. 2021. Predictors of cognitive impairment in Parkinson's disease: a systematic review and meta-analysis of prospective cohort studies. *J Neurol,* 268**,** 2713-2722.
GUSKIEWICZ, K. M., MCCREA, M., MARSHALL, S. W., CANTU, R. C., RANDOLPH, C., BARR, W., ONATE, J. A. & KELLY, J. P. 2003. Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA Concussion Study. *Jama,* 290**,** 2549-2555.

GUSTOT, A., GALLEA, J. I., SARROUKH, R., CELEJ, M. S., RUYSSCHAERT, J.-M. & RAUSSENS, V. 2015. Amyloid fibrils are the molecular trigger of inflammation in Parkinson's disease. *Biochemical Journal,* 471**,** 323-333.

GUYATT, G. H., OXMAN, A. D., SCHÜNEMANN, H. J., TUGWELL, P. & KNOTTNERUS, A. 2011. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. *Journal of clinical epidemiology,* 64**,** 380-382.

HAINING, R. L. & ACHAT-MENDES, C. 2017. Neuromelanin, one of the most overlooked molecules in modern medicine, is not a spectator. *Neural Regen Res,* 12**,** 372-375.

HALEFOGLU, A. M. & YOUSEM, D. M. 2018. Susceptibility weighted imaging: clinical applications and future directions. *World journal of radiology,* 10**,** 30.

HALL, S., SUROVA, Y., ÖHRFELT, A., ZETTERBERG, H., LINDQVIST, D. & HANSSON, O. 2015. CSF biomarkers and clinical progression of Parkinson disease. *Neurology,* 84**,** 57-63.

HALLIDAY, G., MCCANN, H. & SHEPHERD, C. 2012. Evaluation of the Braak hypothesis: how far can it explain the pathogenesis of Parkinson's disease? *Expert Rev Neurother,* 12**,** 673-86.

HALLIDAY, G. M., LEVERENZ, J. B., SCHNEIDER, J. S. & ADLER, C. H. 2014. The neurobiological basis of cognitive impairment in Parkinson's disease. *Movement disorders,* 29**,** 634-650.

HANSEN, C., ANGOT, E., BERGSTRÖM, A.-L., STEINER, J. A., PIERI, L., PAUL, G., OUTEIRO, T. F., MELKI, R., KALLUNKI, P. & FOG, K. 2011. α-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *The Journal of clinical investigation,* 121**,** 715-725.

HARRER, M., CUIJPERS, P., FURUKAWA, T. & EBERT, D. 2021. *Doing meta-analysis with R: A hands-on guide*, Chapman and Hall/CRC.

HARRER, M., CUIJPERS, P., FURUKAWA, T. & EBERT, D. D. 2019. Dmetar: companion R package for the guide'Doing meta-analysis in R'. *R package version 0.0,* 9000.

HARVEY, P. D. 2019. Domains of cognition and their assessment. *Dialogues in clinical neuroscience,* 21**,** 227-237.

HAWTHORNE, G., GRUEN, R. L. & KAYE, A. H. 2009. Traumatic brain injury and long-term quality of life: findings from an Australian study. *J Neurotrauma,* 26**,** 1623-33.

HAYASHI, J. & CARVER, J. A. 2020. The multifaceted nature of αB-crystallin. *Cell Stress and Chaperones,* 25**,** 639-654.

HAYDEN, J. A., VAN DER WINDT, D. A., CARTWRIGHT, J. L., CÔTÉ, P. & BOMBARDIER, C. 2013. Assessing bias in studies of prognostic factors. *Annals of internal medicine,* 158**,** 280-286.

HE, R., ZHAO, Y., HE, Y., ZHOU, Y., YANG, J., ZHOU, X., ZHU, L., ZHOU, X., LIU, Z. & XU, Q. 2020. Olfactory dysfunction predicts disease progression in Parkinson's disease: a longitudinal study. *Frontiers in neuroscience,* 14**,** 569777.

HEALY, D. G., FALCHI, M., O'SULLIVAN, S. S., BONIFATI, V., DURR, A., BRESSMAN, S., BRICE, A., AASLY, J., ZABETIAN, C. P. & GOLDWURM, S. 2008. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *The Lancet Neurology,* 7**,** 583-590.

HEIM, B., KRISMER, F., DE MARZI, R. & SEPPI, K. 2017. Magnetic resonance imaging for the diagnosis of Parkinson's disease. *J Neural Transm (Vienna),* 124**,** 915-964.

HELMRICH, I., CZEITER, E., AMREIN, K., BÜKI, A., LINGSMA, H. F., MENON, D. K., MONDELLO, S., STEYERBERG, E. W., VON STEINBÜCHEL, N., WANG, K. K. W., WILSON, L., XU, H., YANG, Z., VAN KLAVEREN, D. & MAAS, A. I. R. 2022. Incremental prognostic value of acute serum biomarkers for functional outcome after traumatic brain injury (CENTER-TBI): an observational cohort study. *Lancet Neurol,* 21**,** 792-802.

HENDRICKS, R. M. & KHASAWNEH, M. T. 2021. A Systematic Review of Parkinson's Disease Cluster Analysis Research. *Aging Dis,* 12**,** 1567-1586.

HERBST, M., MACLAREN, J., LOVELL-SMITH, C., SOSTHEIM, R., EGGER, K., HARLOFF, A., KORVINK, J., HENNIG, J. & ZAITSEV, M. 2014. Reproduction of motion artifacts for performance analysis of prospective motion correction in MRI. *Magn Reson Med,* 71**,** 182-90.

HERRMANN, M., CURIO, N., JOST, S., GRUBICH, C., EBERT, A. D., FORK, M. L. & SYNOWITZ, H. 2001. Release of biochemical markers of damage to neuronal and glial brain tissue is associated with short and long term neuropsychological outcome after traumatic brain injury. *Journal of Neurology Neurosurgery and Psychiatry,* 70**,** 95-100.

HERRMANN, M., JOST, S., KUTZ, S., EBERT, A. D., KRATZ, T., WUNDERLICH, M. T. & SYNOWITZ, H. 2000. Temporal profile of release of neurobiochemical markers of brain damage after traumatic brain injury is associated with intracranial pathology as demonstrated in cranial computerized tomography. *J Neurotrauma,* 17**,** 113-22.

HIRSCH, L., JETTE, N., FROLKIS, A., STEEVES, T. & PRINGSHEIM, T. 2016. The incidence of Parkinson's disease: a systematic review and meta-analysis. *Neuroepidemiology,* 46**,** 292-300.

HOBAN, D. B., SHRIGLEY, S., MATTSSON, B., BREGER, L. S., JARL, U., CARDOSO, T., NELANDER WAHLESTEDT, J., LUK, K. C., BJÖRKLUND, A. & PARMAR, M. 2020. Impact of α-synuclein pathology on transplanted hESC-derived dopaminergic neurons in a humanized α-synuclein rat model of PD. *Proceedings of the National Academy of Sciences,* 117**,** 15209-15220.

HOFFMAN-ZACHARSKA, D., KOZIOROWSKI, D., ROSS, O. A., MILEWSKI, M., POZNAŃSKI, J., JUREK, M., WSZOLEK, Z. K., SOTO-ORTOLAZA, A., SŁAWEK, J. & JANIK, P. 2013. Novel A18T and pA29S substitutions in α-synuclein may be associated with sporadic Parkinson's disease. *Parkinsonism & related disorders,* 19**,** 1057-1060.

HOLROYD, S., CURRIE, L. J. & WOOTEN, G. F. 2008. Validity, sensitivity and specificity of the mentation, behavior and mood subscale of the UPDRS. *Neurological research,* 30**,** 493-496.

HONG, Z., SHI, M., CHUNG, K. A., QUINN, J. F., PESKIND, E. R., GALASKO, D., JANKOVIC, J., ZABETIAN, C. P., LEVERENZ, J. B., BAIRD, G., MONTINE, T. J., HANCOCK, A. M., HWANG, H., PAN, C., BRADNER, J., KANG, U. J., JENSEN, P. H. & ZHANG, J. 2010. DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. *Brain,* 133**,** 713-26.

HOSSAIN, I., MOHAMMADIAN, M., MAANPAA, H. R., TAKALA, R. S. K., TENOVUO, O., VAN GILS, M., HUTCHINSON, P., MENON, D. K., NEWCOMBE, V. F., TALLUS, J., HIRVONEN, J., ROINE, T., KURKI, T., BLENNOW, K., ZETTERBERG, H. & POSTI, J. P.

2023. Plasma neurofilament light admission levels and development of axonal pathology in mild traumatic brain injury. *BMC Neurology,* 23**,** 304.
HOWLETT, J. R., NELSON, L. D. & STEIN, M. B. 2022. Mental Health Consequences of Traumatic Brain Injury. *Biol Psychiatry,* 91**,** 413-420.
HUANG, W.-S., CHIANG, Y.-H., LIN, J.-C., CHOU, Y.-H., CHENG, C.-Y. & LIU, R.-S. 2003. Crossover study of 99mTc-TRODAT-1 SPECT and 18F-FDOPA PET in Parkinson's disease patients. *Journal of Nuclear Medicine,* 44**,** 999-1005.
HUANG, X.-F., MA, S.-F., JIANG, X.-H., SONG, R.-J., LI, M., ZHANG, J., SUN, T.-J., HU, Q., WANG, W.-R. & YU, A.-Y. 2024. Causes and global, regional, and national burdens of traumatic brain injury from 1990 to 2019. *Chinese journal of traumatology*.
HUDAC, C. M., CORTESA, C. S., LEDWIDGE, P. S. & MOLFESE, D. L. 2018. History of concussion impacts electrophysiological correlates of working memory. *Int J Psychophysiol,* 132**,** 135-144.
HUEBSCHMANN, N. A., LUOTO, T. M., KARR, J. E., BERGHEM, K., BLENNOW, K., ZETTERBERG, H., ASHTON, N. J., SIMREN, J., POSTI, J. P., GILL, J. M. & IVERSON, G. L. 2020. Comparing Glial Fibrillary Acidic Protein (GFAP) in Serum and Plasma Following Mild Traumatic Brain Injury in Older Adults. *Frontiers in Neurology,* 11**,** 12.
HUGHES, A. J., BEN-SHLOMO, Y., DANIEL, S. E. & LEES, A. J. 1992a. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. *Neurology,* 42**,** 1142-6.
HUGHES, A. J., DANIEL, S. E., KILFORD, L. & LEES, A. J. 1992b. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry,* 55**,** 181-4.
HUGHES, A. J., DANIEL, S. E. & LEES, A. J. 2001. Improved accuracy of clinical diagnosis of Lewy body Parkinson's disease. *Neurology,* 57**,** 1497-1499.
HUGUET, A., HAYDEN, J. A., STINSON, J., MCGRATH, P. J., CHAMBERS, C. T., TOUGAS, M. E. & WOZNEY, L. 2013. Judging the quality of evidence in reviews of prognostic factor research: adapting the GRADE framework. *Systematic reviews,* 2**,** 1-12.
HUNTER, J. E. 2004. Methods of meta-analysis: Correcting error and bias in research findings. *Thousand Oaks, CA*.
HUTSON, C. B., LAZO, C. R., MORTAZAVI, F., GIZA, C. C., HOVDA, D. & CHESSELET, M.-F. 2011. Traumatic brain injury in adult rats causes progressive nigrostriatal dopaminergic cell loss and enhanced vulnerability to the pesticide paraquat. *Journal of neurotrauma,* 28**,** 1783-1801.
HWANG, K. S., LANGLEY, J., TRIPATHI, R., HU, X. P. & HUDDLESTON, D. E. 2023. In vivo detection of substantia nigra and locus coeruleus volume loss in Parkinson's disease using neuromelanin-sensitive MRI: Replication in two cohorts. *PLoS One,* 18**,** e0282684.
IMPELLIZZERI, D., CAMPOLO, M., BRUSCHETTA, G., CRUPI, R., CORDARO, M., PATERNITI, I., CUZZOCREA, S. & ESPOSITO, E. 2016. Traumatic Brain Injury Leads to Development of Parkinson's Disease Related Pathology in Mice. *Front Neurosci,* 10**,** 458.
ISAIAS, I. U., CANESI, M., BENTI, R., GERUNDINI, P., CILIA, R., PEZZOLI, G. & ANTONINI, A. 2008. Striatal dopamine transporter abnormalities in patients with essential tremor. *Nuclear Medicine Communications,* 29**,** 349-353.

ISHIKAWA, T., DHAWAN, V., KAZUMATA, K., CHALY, T., MANDEL, F., NEUMEYER, J., MARGOULEFF, C., BABCHYCK, B., ZANZI, I. & EIDELBERG, D. 1996. Comparative nigrostriatal dopaminergic imaging with iodine-123-beta CIT-FP/SPECT and fluorine-18-FDOPA/PET. *J Nucl Med,* 37**,** 1760-5.

ISLAM, M. S., AZIM, F., SAJU, H., ZARGARAN, A., SHIRZAD, M., KAMAL, M., FATEMA, K., REHMAN, S., AZAD, M. A. M. & EBRAHIMI-BAROUGH, S. 2021. Pesticides and Parkinson's disease: Current and future perspective. *Journal of Chemical Neuroanatomy,* 115**,** 101966.

IWAKI, T., WISNIEWSKI, T., IWAKI, A., CORBIN, E., TOMOKANE, N., TATEISHI, J. & GOLDMAN, J. 1992. Accumulation of alpha B-crystallin in central nervous system glia and neurons in pathologic conditions. *The American journal of pathology,* 140**,** 345.

JACK JR, C. R., ALBERT, M. S., KNOPMAN, D. S., MCKHANN, G. M., SPERLING, R. A., CARRILLO, M. C., THIES, B. & PHELPS, C. H. 2011. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association,* 7**,** 257-262.

JAFARI, S., ETMINAN, M., AMINZADEH, F. & SAMII, A. 2013a. Head injury and risk of Parkinson disease: a systematic review and meta-analysis. *Mov Disord,* 28**,** 1222-9.

JAFARI, S., ETMINAN, M., AMINZADEH, F. & SAMII, A. 2013b. Head injury and risk of Parkinson disease: a systematic review and meta-analysis. *Movement disorders,* 28**,** 1222-1229.

JANKOVIC, J. & KAPADIA, A. S. 2001. Functional decline in Parkinson disease. *Archives of neurology,* 58**,** 1611-1615.

JENKINS, P. O., DE SIMONI, S., BOURKE, N. J., FLEMINGER, J., SCOTT, G., TOWEY, D. J., SVENSSON, W., KHAN, S., PATEL, M. & GREENWOOD, R. 2018. Dopaminergic abnormalities following traumatic brain injury. *Brain,* 141**,** 797-810.

JENKINS, P. O., ROUSSAKIS, A. A., DE SIMONI, S., BOURKE, N., FLEMINGER, J., COLE, J., PICCINI, P. & SHARP, D. 2020. Distinct dopaminergic abnormalities in traumatic brain injury and Parkinson's disease. *J Neurol Neurosurg Psychiatry,* 91**,** 631-637.

JENKINSON, M., BECKMANN, C. F., BEHRENS, T. E., WOOLRICH, M. W. & SMITH, S. M. 2012. Fsl. *Neuroimage,* 62**,** 782-790.

JENNETT, B. & BOND, M. 1975. Assessment of outcome after severe brain damage: a practical scale. *The Lancet,* 305**,** 480-484.

JEONG, E. H., LEE, J. Y., HAN, S. K. & SONG, Y. S. 2023. Motor subtypes and clinical characteristics in sporadic and genetic Parkinson's disease groups: analysis of the PPMI cohort. *Front Neurol,* 14**,** 1276251.

JIANG, H., JAIN, M. K. & CAI, H. 2021a. HPLC-free and cassette-based nucleophilic production of [18F] FDOPA for clinical use. *American Journal of Nuclear Medicine and Molecular Imaging,* 11**,** 290.

JIANG, H., JAIN, M. K. & CAI, H. 2021b. HPLC-free and cassette-based nucleophilic production of [(18)F]FDOPA for clinical use. *Am J Nucl Med Mol Imaging,* 11**,** 290-299.

JOHANSSON, M. E., VAN LIER, N. M., KESSELS, R. P., BLOEM, B. R. & HELMICH, R. C. 2023. Two-year clinical progression in focal and diffuse subtypes of Parkinson's disease. *npj Parkinson's Disease,* 9**,** 29.

JOHN, J. A., SAJAN, J. E., OOMMEN, A., JOSEPH, M. & BHATTACHARJI, S. 2022. Predicting functional outcomes in severe traumatic brain injury: Role of S100B along with other clinical and imaging parameters. *Current Medical Issues,* 20**,** 74-81.

JORGE, R. E., ROBINSON, R. G., MOSER, D., TATENO, A., CRESPO-FACORRO, B. & ARNDT, S. 2004. Major depression following traumatic brain injury. *Archives of general psychiatry,* 61**,** 42-50.

JOURDAN, C., BAYEN, E., PRADAT-DIEHL, P., GHOUT, I., DARNOUX, E., AZERAD, S., VALLAT-AZOUVI, C., CHARANTON, J., AEGERTER, P., RUET, A. & AZOUVI, P. 2016. A comprehensive picture of 4-year outcome of severe brain injuries. Results from the PariS-TBI study. *Ann Phys Rehabil Med,* 59**,** 100-6.

JOYCE, J. M., MONCHI, O., ISMAIL, Z., KIBREAB, M., CHEETHAM, J., KATHOL, I., SARNA, J., MARTINO, D. & DEBERT, C. T. 2020. The impact of traumatic brain injury on cognitive and neuropsychiatric symptoms of Parkinson's disease. *Int Rev Psychiatry,* 32**,** 46-60.

KAASINEN, V. & VAHLBERG, T. 2017. Striatal dopamine in P arkinson disease: a meta-analysis of imaging studies. *Annals of neurology,* 82**,** 873-882.

KALIA, L. V. & LANG, A. E. 2015. Parkinson's disease. *The Lancet,* 386**,** 896-912.

KALPINSKI, R. J., WILLIAMSON, M. L., ELLIOTT, T. R., BERRY, J. W., UNDERHILL, A. T. & FINE, P. R. 2013. Modeling the prospective relationships of impairment, injury severity, and participation to quality of life following traumatic brain injury. *Biomed Res Int,* 2013**,** 102570.

KANG, G. A., BRONSTEIN, J. M., MASTERMAN, D. L., REDELINGS, M., CRUM, J. A. & RITZ, B. 2005. Clinical characteristics in early Parkinson's disease in a central California population-based study. *Movement disorders: official journal of the Movement Disorder Society,* 20**,** 1133-1142.

KANG, J. H., MOLLENHAUER, B., COFFEY, C. S., TOLEDO, J. B., WEINTRAUB, D., GALASKO, D. R., IRWIN, D. J., VAN DEERLIN, V., CHEN-PLOTKIN, A. S., CASPELL-GARCIA, C., WALIGÓRSKA, T., TAYLOR, P., SHAH, N., PAN, S., ZERO, P., FRASIER, M., MAREK, K., KIEBURTZ, K., JENNINGS, D., TANNER, C. M., SIMUNI, T., SINGLETON, A., TOGA, A. W., CHOWDHURY, S., TROJANOWSKI, J. Q. & SHAW, L. M. 2016. CSF biomarkers associated with disease heterogeneity in early Parkinson's disease: the Parkinson's Progression Markers Initiative study. *Acta Neuropathol,* 131**,** 935-49.

KAPLAN, C. P. & CORRIGAN, J. D. 1994. The relationship between cognition and functional independence in adults with traumatic brain injury. *Arch Phys Med Rehabil,* 75**,** 643-7.

KASSAMBARA, A. 2016. Factoextra: extract and visualize the results of multivariate data analyses. *R package version,* 1.

KASTEN, M., HARTMANN, C., HAMPF, J., SCHAAKE, S., WESTENBERGER, A., VOLLSTEDT, E. J., BALCK, A., DOMINGO, A., VULINOVIC, F. & DULOVIC, M. 2018. Genotype-phenotype relations for the Parkinson's disease genes Parkin, PINK1, DJ1: MDSGene systematic review. *Movement Disorders,* 33**,** 730-741.

KATAYAMA, T., SAWADA, J., KIKUCHI-TAKEGUCHI, S., KANO, K., TAKAHASHI, K., SAITO, T., OKIZAKI, A. & HASEBE, N. 2020. Cerebrospinal fluid levels of alpha-synuclein, amyloid β, tau, phosphorylated tau, and neuron-specific enolase in patients with Parkinson's disease, dementia with Lewy bodies or other neurological disorders:

Their relationships with cognition and nuclear medicine imaging findings. *Neurosci Lett,* 715**,** 134564.

KATHURIA, H., MEHTA, S., AHUJA, C. K., CHAKRAVARTY, K., RAY, S., MITTAL, B. R., SINGH, P. & LAL, V. 2021. Utility of imaging of nigrosome-1 on 3T MRI and its comparison with 18F-DOPA PET in the diagnosis of idiopathic Parkinson disease and atypical parkinsonism. *Movement disorders clinical practice,* 8**,** 224-230.

KELLY, V. E., JOHNSON, C. O., MCGOUGH, E. L., SHUMWAY-COOK, A., HORAK, F. B., CHUNG, K. A., ESPAY, A. J., REVILLA, F. J., DEVOTO, J., WOOD-SIVERIO, C., FACTOR, S. A., CHOLERTON, B., EDWARDS, K. L., PETERSON, A. L., QUINN, J. F., MONTINE, T. J., ZABETIAN, C. P. & LEVERENZ, J. B. 2015. Association of cognitive domains with postural instability/gait disturbance in Parkinson's disease. *Parkinsonism Relat Disord,* 21**,** 692-7.

KEVIN, K. W. W., ZHANG, Z., O.S, I. S., GLUSHAKOVA, V., PRIMA, F., KOBAISSY, M., LIU, C. C., ROBERTSON, S., PAPA, L., LAWRENCE, M. L., FORMISANO, R., BOSSU, P., CIARAMELLA, A., BARBA, C., CATANI, S., GABRIELLI, A., ROBICSEK, S., WAGNER, A., MONDELLO, S., RONALD & HAYES, L. 2010. Discovery and translational utilities of acute, subacute and chronic brain injury biomarkers. *Brain Injury,* 24**,** 400-401.

KHAN, N. L., GRAHAM, E., CRITCHLEY, P., SCHRAG, A. E., WOOD, N. W., LEES, A. J., BHATIA, K. P. & QUINN, N. 2003. Parkin disease: a phenotypic study of a large case series. *Brain,* 126**,** 1279-1292.

KIA, D. A., ZHANG, D., GUELFI, S., MANZONI, C., HUBBARD, L., REYNOLDS, R. H., BOTÍA, J., RYTEN, M., FERRARI, R., LEWIS, P. A., WILLIAMS, N., TRABZUNI, D., HARDY, J., WOOD, N. W., UNITED KINGDOM BRAIN EXPRESSION, C. & THE INTERNATIONAL PARKINSON'S DISEASE GENOMICS, C. 2021. Identification of Candidate Parkinson Disease Genes by Integrating Genome-Wide Association Study, Expression, and Epigenetic Data Sets. *JAMA Neurology,* 78**,** 464-472.

KIM, J. J., VITALE, D., OTANI, D. V., LIAN, M. M., HEILBRON, K., ASLIBEKYAN, S., AUTON, A., BABALOLA, E., BELL, R. K., BIELENBERG, J., BRYC, K., BULLIS, E., CANNON, P., COKER, D., PARTIDA, G. C., DHAMIJA, D., DAS, S., ELSON, S. L., ERIKSSON, N., FILSHTEIN, T., FITCH, A., FLETEZ-BRANT, K., FONTANILLAS, P., FREYMAN, W., GRANKA, J. M., HERNANDEZ, A., HICKS, B., HINDS, D. A., JEWETT, E. M., JIANG, Y., KUKAR, K., KWONG, A., LIN, K.-H., LLAMAS, B. A., LOWE, M., MCCREIGHT, J. C., MCINTYRE, M. H., MICHELETTI, S. J., MORENO, M. E., NANDAKUMAR, P., NGUYEN, D. T., NOBLIN, E. S., O'CONNELL, J., PETRAKOVITZ, A. A., POZNIK, G. D., REYNOSO, A., SCHLOETTER, M., SCHUMACHER, M., SHASTRI, A. J., SHELTON, J. F., SHI, J., SHRINGARPURE, S., SU, Q. J., TAT, S. A., TCHAKOUTÉ, C. T., TRAN, V., TUNG, J. Y., WANG, X., WANG, W., WELDON, C. H., WILTON, P., WONG, C. D., IWAKI, H., LAKE, J., SOLSBERG, C. W., LEONARD, H., MAKARIOUS, M. B., TAN, E.-K., SINGLETON, A. B., BANDRES-CIGA, S., NOYCE, A. J., GATTO, E. M., KAUFFMAN, M., KHACHATRYAN, S., TAVADYAN, Z., SHEPHERD, C. E., HUNTER, J., KUMAR, K., ELLIS, M., RENTERÍA, M. E., KOKS, S., ZIMPRICH, A., SCHUMACHER-SCHUH, A. F., RIEDER, C., AWAD, P. S., TUMAS, V., CAMARGOS, S., FON, E. A., MONCHI, O., FON, T., GALLEGUILLOS, B. P., MIRANDA, M., BUSTAMANTE, M. L., OLGUIN, P., CHANA, P., TANG, B., SHANG, H., GUO, J., CHAN, P., LUO, W., et al. 2024. Multi-ancestry genome-wide association meta-analysis of Parkinson's disease. *Nature Genetics,* 56**,** 27-36.

KITADA, T., ASAKAWA, S., HATTORI, N., MATSUMINE, H., YAMAMURA, Y., MINOSHIMA, S., YOKOCHI, M., MIZUNO, Y. & SHIMIZU, N. 1998. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *nature,* 392**,** 605-608.

KITAO, S., MATSUSUE, E., FUJII, S., MIYOSHI, F., KAMINOU, T., KATO, S., ITO, H. & OGAWA, T. 2013. Correlation between pathology and neuromelanin MR imaging in Parkinson's disease and dementia with Lewy bodies. *Neuroradiology,* 55**,** 947-953.

KOBORI, N., CLIFTON, G. L. & DASH, P. K. 2006. Enhanced catecholamine synthesis in the prefrontal cortex after traumatic brain injury: implications for prefrontal dysfunction. *Journal of neurotrauma,* 23**,** 1094-1102.

KOHAT, A. K., NG, S. Y., WONG, A. S., CHIA, N. S., CHOI, X., HENG, D. L., LI, W., NG, H.-L., CHUA, S.-T. & NEO, S. X. 2021. Stability of MDS-UPDRS motor subtypes over three years in early Parkinson's disease. *Frontiers in Neurology,* 12**,** 704906.

KOMPOLITI, K., GOETZ, C. G., LITVAN, I., JELLINGER, K. & VERNY, M. 1998. Pharmacological therapy in progressive supranuclear palsy. *Arch Neurol,* 55**,** 1099-102.

KORAT, Š., BIDESI, N. S. R., BONANNO, F., DI NANNI, A., HOÀNG, A. N. N., HERFERT, K., MAURER, A., BATTISTI, U. M., BOWDEN, G. D. & THONON, D. 2021. Alpha-synuclein PET tracer development—an overview about current efforts. *Pharmaceuticals,* 14**,** 847.

KORDOWER, J. H., DODIYA, H. B., KORDOWER, A. M., TERPSTRA, B., PAUMIER, K., MADHAVAN, L., SORTWELL, C., STEECE-COLLIER, K. & COLLIER, T. J. 2011. Transfer of host-derived alpha synuclein to grafted dopaminergic neurons in rat. *Neurobiology of disease,* 43**,** 552-557.

KOSSMEIER, M., TRAN, U. S. & VORACEK, M. 2020. Visualizing meta-analytic data with R package metaviz. *R Package Version,* 3**,** 1.

KOTLYAR, S., LARKIN, G. L., MOORE, C. L. & D'ONOFRIO, G. 2011. S100b immunoassay: an assessment of diagnostic utility in minor head trauma. *J Emerg Med,* 41**,** 285-93.

KOULI, A., TORSNEY, K. M. & KUAN, W.-L. 2018. Parkinson's disease: etiology, neuropathology, and pathogenesis. *Exon Publications***,** 3-26.

KRAEMMER, J., KOVACS, G. G., PERJU-DUMBRAVA, L., PIRKER, S., TRAUB-WEIDINGER, T. & PIRKER, W. 2014. Correlation of striatal dopamine transporter imaging with post mortem substantia nigra cell counts. *Mov Disord,* 29**,** 1767-73.

KREUTZER, J. S., SEEL, R. T. & GOURLEY, E. 2001. The prevalence and symptom rates of depression after traumatic brain injury: a comprehensive examination. *Brain injury,* 15**,** 563-576.

KRISTMAN, V. L., CÔTÉ, P., HOGG-JOHNSON, S., CASSIDY, J. D., EERD, D. V., VIDMAR, M., REZAI, M. & WENNBERG, R. A. 2010. The burden of work disability associated with mild traumatic brain injury in Ontario compensated workers: a prospective cohort study. *The Open Occupational Health & Safety Journal,* 2.

KRÜGER, R., KUHN, W., MÜLLER, T., WOITALLA, D., GRAEBER, M., KÖSEL, S., PRZUNTEK, H., EPPLEN, J. T., SCHOLS, L. & RIESS, O. 1998. AlaSOPro mutation in the gene encoding α-synuclein in Parkinson's disease. *Nature genetics,* 18**,** 106-108.

KUHN, M. 2015. Caret: classification and regression training. *Astrophysics Source Code Library***,** ascl: 1505.003.

KUMAR, A., SIDHU, J., GOYAL, A. & TSAO, J. W. 2023. Alzheimer Disease. *StatPearls*. Treasure Island (FL): StatPearls Publishing
Copyright © 2023, StatPearls Publishing LLC.
KUMARESAN, M. & KHAN, S. 2021. Spectrum of Non-Motor Symptoms in Parkinson's Disease. *Cureus,* 13**,** e13275.
KUO, S. H., TASSET, I., CHENG, M. M., DIAZ, A., PAN, M. K., LIEBERMAN, O. J., HUTTEN, S. J., ALCALAY, R. N., KIM, S., XIMÉNEZ-EMBÚN, P., FAN, L., KIM, D., KO, H. S., YACOUBIAN, T., KANTER, E., LIU, L., TANG, G., MUÑOZ, J., SARDI, S. P., LI, A., GAN, L., CUERVO, A. M. & SULZER, D. 2022. Mutant glucocerebrosidase impairs α-synuclein degradation by blockade of chaperone-mediated autophagy. *Sci Adv,* 8**,** eabm6393.
KWON, H. S. & KOH, S.-H. 2020. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Translational Neurodegeneration,* 9**,** 42.
LAGERSTEDT, L., AZURMENDI, L., TENOVUO, O., KATILA, A. J., TAKALA, R. S. K., BLENNOW, K., NEWCOMBE, V. F. J., MAANPAA, H. R., TALLUS, J., HOSSAIN, I., VAN GILS, M., MENON, D. K., HUTCHINSON, P. J., ZETTERBERG, H., POSTI, J. P. & SANCHEZ, J. C. 2020. Interleukin 10 and Heart Fatty Acid-Binding Protein as Early Outcome Predictors in Patients With Traumatic Brain Injury. *Frontiers in Neurology,* 11**,** 9.
LAHUE, S. C., COMELLA, C. L. & TANNER, C. M. 2016. The best medicine? The influence of physical activity and inactivity on Parkinson's disease. *Movement Disorders,* 31**,** 1444-1454.
LAKHANI, D. A., ZHOU, X., TAO, S., PATEL, V., WEN, S., OKROMELIDZE, L., GRECO, E., LIN, C., WESTERHOLD, E. M., STRAUB, S., WSZOLEK, Z. K., TIPTON, P. W., UITTI, R. J., GREWAL, S. S. & MIDDLEBROOKS, E. H. 2024. Diagnostic utility of 7T neuromelanin imaging of the substantia nigra in Parkinson's disease. *NPJ Parkinsons Dis,* 10**,** 13.
LANCIEGO, J. L., LUQUIN, N. & OBESO, J. A. 2012. Functional neuroanatomy of the basal ganglia. *Cold Spring Harb Perspect Med,* 2**,** a009621.
LANGKAMMER, C., LIU, T., KHALIL, M., ENZINGER, C., JEHNA, M., FUCHS, S., FAZEKAS, F., WANG, Y. & ROPELE, S. 2013. Quantitative susceptibility mapping in multiple sclerosis. *Radiology,* 267**,** 551-9.
LANGKAMMER, C., PIRPAMER, L., SEILER, S., DEISTUNG, A., SCHWESER, F., FRANTHAL, S., HOMAYOON, N., KATSCHNIG-WINTER, P., KOEGL-WALLNER, M., PENDL, T., STOEGERER, E. M., WENZEL, K., FAZEKAS, F., ROPELE, S., REICHENBACH, J. R., SCHMIDT, R. & SCHWINGENSCHUH, P. 2016. Quantitative Susceptibility Mapping in Parkinson's Disease. *PLoS One,* 11**,** e0162460.
LANGLEY, J., HUDDLESTON, D. E., SEDLACIK, J., BOELMANS, K. & HU, X. P. 2017. Parkinson's disease–related increase of-weighted hypointensity in substantia nigra pars compacta. *Movement Disorders,* 32**,** 441-449.
LARA, A. H. & WALLIS, J. D. 2015. The role of prefrontal cortex in working memory: a mini review. *Frontiers in systems neuroscience,* 9**,** 173.
LARSEN, J. P., DUPONT, E. & TANDBERG, E. 1994. Clinical diagnosis of Parkinson's disease. Proposal of diagnostic subgroups classified at different levels of confidence. *Acta neurologica scandinavica,* 89**,** 242-251.

LASHUEL, H. A., OVERK, C. R., OUESLATI, A. & MASLIAH, E. 2013. The many faces of α-synuclein: from structure and toxicity to therapeutic target. *Nature Reviews Neuroscience,* 14**,** 38-48.

LAU, A., SO, R. W. L., LAU, H. H. C., SANG, J. C., RUIZ-RIQUELME, A., FLECK, S. C., STUART, E., MENON, S., VISANJI, N. P., MEISL, G., FAIDI, R., MARANO, M. M., SCHMITT-ULMS, C., WANG, Z., FRASER, P. E., TANDON, A., HYMAN, B. T., WILLE, H., INGELSSON, M., KLENERMAN, D. & WATTS, J. C. 2020. α-Synuclein strains target distinct brain regions and cell types. *Nat Neurosci,* 23**,** 21-31.

LAVISSE, S., GUILLERMIER, M., HÉRARD, A. S., PETIT, F., DELAHAYE, M., VAN CAMP, N., BEN HAIM, L., LEBON, V., REMY, P., DOLLÉ, F., DELZESCAUX, T., BONVENTO, G., HANTRAYE, P. & ESCARTIN, C. 2012. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. *J Neurosci,* 32**,** 10809-18.

LEE, J., KIM, S. H., KIM, Y., KIM, Y. L. & SOHN, Y. 2019a. Quality of life of caregivers of individuals with Parkinson's disease. *Rehabilitation Nursing Journal,* 44**,** 338-348.

LEE, J. Y., LEE, C. Y., KIM, H. R., LEE, C. H., KIM, H. W. & KIM, J. H. 2015. A Role of Serum-Based Neuronal and Glial Markers as Potential Predictors for Distinguishing Severity and Related Outcomes in Traumatic Brain Injury. *J Korean Neurosurg Soc,* 58**,** 93-100.

LEE, P. C., BORDELON, Y., BRONSTEIN, J. & RITZ, B. 2012. Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease. *Neurology,* 79**,** 2061-6.

LEE, T.-W., CHEN, C.-Y., CHEN, K., TSO, C.-W., LIN, H.-H., LAI, Y.-L. L., HSU, F.-T., CHUNG, H.-W. & LIU, H.-S. 2021a. Evaluation of the swallow-tail sign and correlations of neuromelanin signal with susceptibility and relaxations. *Tomography,* 7**,** 107-119.

LEE, Y. G., JEON, S., KANG, S. W., PARK, M., BAIK, K., YOO, H. S., CHUNG, S. J., JEONG, S. H., JUNG, J. H., LEE, P. H., SOHN, Y. H., EVANS, A. C. & YE, B. S. 2021b. Interaction of CSF α-synuclein and amyloid beta in cognition and cortical atrophy. *Alzheimers Dement (Amst),* 13**,** e12177.

LEE, Y. H., CHA, J., CHUNG, S. J., YOO, H. S., SOHN, Y. H., YE, B. S. & LEE, P. H. 2019b. Beneficial effect of estrogen on nigrostriatal dopaminergic neurons in drug-naïve postmenopausal Parkinson's disease. *Sci Rep,* 9**,** 10531.

LEI, J., GAO, G., FENG, J., JIN, Y., WANG, C., MAO, Q. & JIANG, J. 2015. Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: A prospective cohort study. *Critical Care,* 19**,** 362.

LEMA TOMÉ, C. M., TYSON, T., REY, N. L., GRATHWOHL, S., BRITSCHGI, M. & BRUNDIN, P. 2013. Inflammation and α-synuclein's prion-like behavior in Parkinson's disease—is there a link? *Molecular neurobiology,* 47**,** 561-574.

LENNON, M. J., BROOKER, H., CREESE, B., THAYANANDAN, T., RIGNEY, G., AARSLAND, D., HAMPSHIRE, A., BALLARD, C., CORBETT, A. & RAYMONT, V. 2023. Lifetime Traumatic Brain Injury and Cognitive Domain Deficits in Late Life: The PROTECT-TBI Cohort Study. *J Neurotrauma,* 40**,** 1423-1435.

LESKO, M. M., O'BRIEN, S. J., CHILDS, C., BOUAMRA, O., RAINEY, T. & LECKY, F. 2014. Comparison of several prognostic tools in traumatic brain injury including S100B. *Brain Injury,* 28**,** 987-994.

LI, J., CAO, F., YIN, H.-L., HUANG, Z.-J., LIN, Z.-T., MAO, N., SUN, B. & WANG, G. 2020. Ferroptosis: past, present and future. *Cell death & disease,* 11**,** 88.

LI, Y., WANG, C., WANG, J., ZHOU, Y., YE, F., ZHANG, Y., CHENG, X., HUANG, Z., LIU, K., FEI, G., ZHONG, C., ZENG, M. & JIN, L. 2019. Mild cognitive impairment in de novo Parkinson's disease: A neuromelanin MRI study in locus coeruleus. *Mov Disord,* 34**,** 884-892.

LILIANG, P. C., LIANG, C. L., WENG, H. C., LU, K., WANG, K. W., CHEN, H. J. & CHUANG, J. H. 2010. Tau proteins in serum predict outcome after severe traumatic brain injury. *J Surg Res,* 160**,** 302-7.

LIM, K.-L. & TAN, J. M. 2007. Role of the ubiquitin proteasome system in Parkinson's disease. *BMC biochemistry,* 8**,** 1-10.

LIN, L.-F., LIOU, T.-H., HU, C.-J., MA, H.-P., OU, J.-C., CHIANG, Y.-H., CHIU, W.-T., TSAI, S.-H. & CHU, W.-C. 2015. Balance function and sensory integration after mild traumatic brain injury. *Brain injury,* 29**,** 41-46.

LIOU, H., TSAI, M., CHEN, C., JENG, J., CHANG, Y., CHEN, S. & CHEN, R. 1997. Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. *Neurology,* 48**,** 1583-1588.

LITVAN, I., GOLDMAN, J. G., TRÖSTER, A. I., SCHMAND, B. A., WEINTRAUB, D., PETERSEN, R. C., MOLLENHAUER, B., ADLER, C. H., MARDER, K., WILLIAMS-GRAY, C. H., AARSLAND, D., KULISEVSKY, J., RODRIGUEZ-OROZ, M. C., BURN, D. J., BARKER, R. A. & EMRE, M. 2012. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. *Mov Disord,* 27**,** 349-56.

LIU, C., LIU, Z., FANG, Y., DU, Z., YAN, Z., YUAN, X., DAI, L., YU, T., XIONG, M., TIAN, Y., LI, H., LI, F., ZHANG, J., MENG, L., WANG, Z., JIANG, H. & ZHANG, Z. 2022a. Exposure to the environmentally toxic pesticide maneb induces Parkinson's disease-like neurotoxicity in mice: A combined proteomic and metabolomic analysis. *Chemosphere,* 308**,** 136344.

LIU, S.-Y., QIAO, H.-W., SONG, T.-B., LIU, X.-L., YAO, Y.-X., ZHAO, C.-S., BARRET, O., XU, S.-L., CAI, Y.-N., TAMAGNAN, G. D., SOSSI, V., LU, J. & CHAN, P. 2022b. Brain microglia activation and peripheral adaptive immunity in Parkinson’s disease: a multimodal PET study. *Journal of Neuroinflammation,* 19**,** 209.

LJUNGQVIST, J., ZETTERBERG, H., MITSIS, M., BLENNOW, K. & SKOGLUND, T. 2017. Serum Neurofilament Light Protein as a Marker for Diffuse Axonal Injury: Results from a Case Series Study. *Journal of Neurotrauma,* 34**,** 1124-1127.

LU, W., JIANG, C., WANG, Z., CHEN, Y., BAI, R., YAN, G., WANG, G. & REN, H. 2020. Lactic acid, neuron-specific enolase, and blood-brain barrier index after a severe traumatic brain injury: a prospective study. *Br J Neurosurg***,** 1-5.

LUBBERS, V. F., VAN DEN HOVEN, D. J., VAN DER NAALT, J., JELLEMA, K., VAN DEN BRAND, C. & BACKUS, B. 2024. Emergency department risk factors for post-concussion syndrome after mild traumatic brain injury: a systematic review. *Journal of neurotrauma*.

LÜCKING, C. B., DÜRR, A., BONIFATI, V., VAUGHAN, J., DE MICHELE, G., GASSER, T., HARHANGI, B. S., MECO, G., DENÈFLE, P. & WOOD, N. W. 2000. Association between early-onset Parkinson's disease and mutations in the parkin gene. *New England Journal of Medicine,* 342**,** 1560-1567.

LÜDECKE, D. 2021. sjPlot: data visualization for statistics in social science. R package version 2.8. 10. *R Foundation for Statistical Computing*.
LUO, L., ANDREWS, H., ALCALAY, R. N., POYRAZ, F. C., BOEHME, A. K., GOLDMAN, J. G., XIE, T., TUITE, P., HENCHCLIFFE, C. & HOGARTH, P. 2019. Motor phenotype classification in moderate to advanced PD in BioFIND study. *Parkinsonism & related disorders,* 65**,** 178-183.
LYKETSOS, C. G., CHEN, L.-S. & ANTHONY, J. C. 1999. Cognitive decline in adulthood: an 11.5-year follow-up of the Baltimore Epidemiologic Catchment Area study. *American Journal of Psychiatry,* 156**,** 58-65.
MA, S. Y., RÖYTT, M., COLLAN, Y. & RINNE, J. O. 1999. Unbiased morphometrical measurements show loss of pigmented nigral neurones with ageing. *Neuropathol Appl Neurobiol,* 25**,** 394-9.
MACDONALD, S. 2017. Introducing the model of cognitive-communication competence: A model to guide evidence-based communication interventions after brain injury. *Brain injury,* 31**,** 1760-1780.
MAFUIKA, S. N., NAICKER, T., HARRICHANDPARSAD, R. & LAZARUS, L. 2022. The potential of serum S100 calcium-binding protein B and glial fibrillary acidic protein as biomarkers for traumatic brain injury. *Translational Research in Anatomy,* 29**,** 100228.
MAHMOOD, Z., VAN PATTEN, R., NAKHLA, M. Z., TWAMLEY, E. W., FILOTEO, J. V. & SCHIEHSER, D. M. 2020. REM Sleep Behavior Disorder in Parkinson's Disease: Effects on Cognitive, Psychiatric, and Functional outcomes. *J Int Neuropsychol Soc,* 26**,** 894-905.
MAHUL-MELLIER, A.-L., BURTSCHER, J., MAHARJAN, N., WEERENS, L., CROISIER, M., KUTTLER, F., LELEU, M., KNOTT, G. W. & LASHUEL, H. A. 2020. The process of Lewy body formation, rather than simply α-synuclein fibrillization, is one of the major drivers of neurodegeneration. *Proceedings of the National Academy of Sciences,* 117**,** 4971-4982.
MAJBOUR, N. K., VAIKATH, N. N., EUSEBI, P., CHIASSERINI, D., ARDAH, M., VARGHESE, S., HAQUE, M. E., TOKUDA, T., AUINGER, P., CALABRESI, P., PARNETTI, L. & EL-AGNAF, O. M. 2016. Longitudinal changes in CSF alpha-synuclein species reflect Parkinson's disease progression. *Mov Disord,* 31**,** 1535-1542.
MANI, K., CATER, B. & HUDLIKAR, A. 2017. Cognition and return to work after mild/moderate traumatic brain injury: A systematic review. *Work,* 58**,** 51-62.
MANNING-BOG, A. B., MCCORMACK, A. L., LI, J., UVERSKY, V. N., FINK, A. L. & DI MONTE, D. A. 2002. The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha-synuclein. *J Biol Chem,* 277**,** 1641-4.
MAREK, K., JENNINGS, D., LASCH, S., SIDEROWF, A., TANNER, C., SIMUNI, T., COFFEY, C., KIEBURTZ, K., FLAGG, E. & CHOWDHURY, S. 2011. The Parkinson progression marker initiative (PPMI). *Progress in neurobiology,* 95**,** 629-635.
MARQUEZ DE LA PLATA, C. D., HART, T., HAMMOND, F. M., FROL, A. B., HUDAK, A., HARPER, C. R., O'NEIL-PIROZZI, T. M., WHYTE, J., CARLILE, M. & DIAZ-ARRASTIA, R. 2008. Impact of age on long-term recovery from traumatic brain injury. *Arch Phys Med Rehabil,* 89**,** 896-903.

MARSILI, L., RIZZO, G. & COLOSIMO, C. 2018. Diagnostic criteria for Parkinson's disease: from James Parkinson to the concept of prodromal disease. *Frontiers in neurology,* 9**,** 156.

MARTÌ, M. J., TOLOSA, E. & CAMPDELACREU, J. 2003. Clinical overview of the synucleinopathies. *Movement disorders: official journal of the Movement Disorder Society,* 18**,** 21-27.

MARTIN, W. R., WIELER, M. & GEE, M. 2008. Midbrain iron content in early Parkinson disease: a potential biomarker of disease status. *Neurology,* 70**,** 1411-7.

MARTIN, W. W. 2009. Quantitative estimation of regional brain iron with magnetic resonance imaging. *Parkinsonism & Related Disorders,* 15**,** S215-S218.

MARTÍNEZ-MARTÍN, P., GIL-NAGEL, A., GRACIA, L. M., GÓMEZ, J. B., MARTINEZ-SARRIES, J., BERMEJO, F. & GROUP, C. M. 1994. Unified Parkinson's disease rating scale characteristics and structure. *Movement disorders,* 9**,** 76-83.

MATSUURA, K., MAEDA, M., TABEI, K. I., UMINO, M., KAJIKAWA, H., SATOH, M., KIDA, H. & TOMIMOTO, H. 2016. A longitudinal study of neuromelanin-sensitive magnetic resonance imaging in Parkinson's disease. *Neurosci Lett,* 633**,** 112-117.

MCCORMACK, A. L., THIRUCHELVAM, M., MANNING-BOG, A. B., THIFFAULT, C., LANGSTON, J. W., CORY-SLECHTA, D. A. & DI MONTE, D. A. 2002. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. *Neurobiol Dis,* 10**,** 119-27.

MCGUINNESS, L. A. & HIGGINS, J. P. 2021. Risk-of-bias VISualization (robvis): an R package and Shiny web app for visualizing risk-of-bias assessments. *Research synthesis methods,* 12**,** 55-61.

MCKHANN, G. M., KNOPMAN, D. S., CHERTKOW, H., HYMAN, B. T., JACK, C. R., JR., KAWAS, C. H., KLUNK, W. E., KOROSHETZ, W. J., MANLY, J. J., MAYEUX, R., MOHS, R. C., MORRIS, J. C., ROSSOR, M. N., SCHELTENS, P., CARRILLO, M. C., THIES, B., WEINTRAUB, S. & PHELPS, C. H. 2011. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement,* 7**,** 263-9.

MCMILLAN, T. M., TEASDALE, G. M. & STEWART, E. 2012. Disability in young people and adults after head injury: 12-14 year follow-up of a prospective cohort. *J Neurol Neurosurg Psychiatry,* 83**,** 1086-91.

MEIJER, F. J., STEENS, S. C., VAN RUMUND, A., VAN WALSUM, A.-M. V. C., KÜSTERS, B., ESSELINK, R. A., VERBEEK, M. M., BLOEM, B. R. & GORAJ, B. 2016. Nigrosome-1 on susceptibility weighted imaging to differentiate Parkinson's disease from atypical parkinsonism: an in vivo and ex vivo pilot study. *Polish Journal of Radiology,* 81**,** 363.

MERIC, E., GUNDUZ, A., TUREDI, S., CAKIR, E. & YANDI, M. 2010. The Prognostic Value of Neuron-Specific Enolase in Head Trauma Patients. *Journal of Emergency Medicine,* 38**,** 297-301.

MESTRE, T. A., FERESHTEHNEJAD, S. M., BERG, D., BOHNEN, N. I., DUJARDIN, K., ERRO, R., ESPAY, A. J., HALLIDAY, G., VAN HILTEN, J. J., HU, M. T., JEON, B., KLEIN, C., LEENTJENS, A. F. G., MARINUS, J., MOLLENHAUER, B., POSTUMA, R., RAJALINGAM, R., RODRÍGUEZ-VIOLANTE, M., SIMUNI, T., SURMEIER, D. J., WEINTRAUB, D., MCDERMOTT, M. P., LAWTON, M. & MARRAS, C. 2021.

Parkinson's Disease Subtypes: Critical Appraisal and Recommendations. *J Parkinsons Dis,* 11**,** 395-404.
METTING, Z., WILCZAK, N., RODIGER, L. A., SCHAAF, J. M. & VAN DER NAALT, J. 2012. GFAP and S100B in the acute phase of mild traumatic brain injury. *Neurology,* 78**,** 1428-1433.
MIELKE, M. M., RANSOM, J. E., MANDREKAR, J., TURCANO, P., SAVICA, R. & BROWN, A. W. 2022. Traumatic Brain Injury and Risk of Alzheimer's Disease and Related Dementias in the Population. *J Alzheimers Dis,* 88**,** 1049-1059.
MIHAESCU, A. S., VALLI, M., URIBE, C., DIEZ-CIRARDA, M., MASELLIS, M., GRAFF-GUERRERO, A. & STRAFELLA, A. P. 2022. Beta amyloid deposition and cognitive decline in Parkinson's disease: a study of the PPMI cohort. *Molecular Brain,* 15**,** 79.
MIKKELSEN, M. B., O'TOOLE, M. S., ELKJÆR, E. & MEHLSEN, M. 2024. The effect of age on emotion regulation patterns in daily life: Findings from an experience sampling study. *Scandinavian Journal of Psychology,* 65**,** 231-239.
MIKOLIĆ, A., VAN KLAVEREN, D., GROENIGER, J. O., WIEGERS, E. J. A., LINGSMA, H. F., ZELDOVICH, M., VON STEINBÜCHEL, N., MAAS, A. I. R., ROETERS VAN LENNEP, J. E. & POLINDER, S. 2021. Differences between Men and Women in Treatment and Outcome after Traumatic Brain Injury. *J Neurotrauma,* 38**,** 235-251.
MILENKOVIC, V. M., BADER, S., SUDRIA-LOPEZ, D., SIEBERT, R., BRANDL, C., NOTHDURFTER, C., WEBER, B. H. F., RUPPRECHT, R. & WETZEL, C. H. 2018. Effects of genetic variants in the TSPO gene on protein structure and stability. *PLoS One,* 13**,** e0195627.
MOCHIZUKI, H. & YASUDA, T. 2012. Iron accumulation in Parkinson's disease. *Journal of Neural Transmission,* 119**,** 1511-1514.
MOGI, M., HARADA, M., NARABAYASHI, H., INAGAKI, H., MINAMI, M. & NAGATSU, T. 1996. Interleukin (IL)-1 beta, IL-2, IL-4, IL-6 and transforming growth factor-alpha levels are elevated in ventricular cerebrospinal fluid in juvenile parkinsonism and Parkinson's disease. *Neurosci Lett,* 211**,** 13-6.
MOHER, D., SHAMSEER, L., CLARKE, M., GHERSI, D., LIBERATI, A., PETTICREW, M., SHEKELLE, P., STEWART, L. A. & GROUP, P.-P. 2015. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic reviews,* 4**,** 1-9.
MOLLENHAUER, B., CASPELL-GARCIA, C. J., COFFEY, C. S., TAYLOR, P., SINGLETON, A., SHAW, L. M., TROJANOWSKI, J. Q., FRASIER, M., SIMUNI, T., IRANZO, A., OERTEL, W., SIDEROWF, A., WEINTRAUB, D., SEIBYL, J., TOGA, A. W., TANNER, C. M., KIEBURTZ, K., CHAHINE, L. M., MAREK, K. & GALASKO, D. 2019a. Longitudinal analyses of cerebrospinal fluid α-Synuclein in prodromal and early Parkinson's disease. *Mov Disord,* 34**,** 1354-1364.
MOLLENHAUER, B., CASPELL-GARCIA, C. J., COFFEY, C. S., TAYLOR, P., SINGLETON, A., SHAW, L. M., TROJANOWSKI, J. Q., FRASIER, M., SIMUNI, T. & IRANZO, A. 2019b. Longitudinal analyses of cerebrospinal fluid α-Synuclein in prodromal and early Parkinson's disease. *Movement disorders,* 34**,** 1354-1364.
MOLLENHAUER, B., LOCASCIO, J. J., SCHULZ-SCHAEFFER, W., SIXEL-DÖRING, F., TRENKWALDER, C. & SCHLOSSMACHER, M. G. 2011. α-Synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study. *Lancet Neurol,* 10**,** 230-40.

MOLLENHAUER, B., TRAUTMANN, E., TAYLOR, P., MANNINGER, P., SIXEL-DÖRING, F., EBENTHEUER, J., TRENKWALDER, C. & SCHLOSSMACHER, M. G. 2013. Total CSF α-synuclein is lower in de novo Parkinson patients than in healthy subjects. *Neuroscience Letters,* 532**,** 44-48.

MONDELLO, S., BUKI, A., ITALIANO, D. & JEROMIN, A. 2013. α-Synuclein in CSF of patients with severe traumatic brain injury. *Neurology,* 80**,** 1662-8.

MONDELLO, S., ROBICSEK, S. A., GABRIELLI, A., BROPHY, G. M., PAPA, L., TEPAS, J., ROBERTSON, C., BUKI, A., SCHARF, D., JIXIANG, M., AKINYI, L., MULLER, U., WANG, K. K. & HAYES, R. L. 2010. αII-spectrin breakdown products (SBDPs): diagnosis and outcome in severe traumatic brain injury patients. *J Neurotrauma,* 27**,** 1203-13.

MOORTHY, D., RAJESH, K., PRIYA, S. M., ABHINOV, T. & DEVENDRA PRASAD, K. J. 2021. Prediction of Outcome Based on Trauma and Injury Severity Score, IMPACT and CRASH Prognostic Models in Moderate-to-Severe Traumatic Brain Injury in the Elderly. *Asian J Neurosurg,* 16**,** 500-506.

MOUSSAUD, S., JONES, D. R., MOUSSAUD-LAMODIÈRE, E. L., DELENCLOS, M., ROSS, O. A. & MCLEAN, P. J. 2014. Alpha-synuclein and tau: teammates in neurodegeneration? *Mol Neurodegener,* 9**,** 43.

MOUSTAFA, A. A., CHAKRAVARTHY, S., PHILLIPS, J. R., GUPTA, A., KERI, S., POLNER, B., FRANK, M. J. & JAHANSHAHI, M. 2016. Motor symptoms in Parkinson's disease: A unified framework. *Neurosci Biobehav Rev,* 68**,** 727-740.

MOZLEY, L. H., GUR, R. C., MOZLEY, P. D. & GUR, R. E. 2001. Striatal dopamine transporters and cognitive functioning in healthy men and women. *Am J Psychiatry,* 158**,** 1492-9.

MU, J., CHAUDHURI, K. R., BIELZA, C., DE PEDRO-CUESTA, J., LARRAÑAGA, P. & MARTINEZ-MARTIN, P. 2017. Parkinson's disease subtypes identified from cluster analysis of motor and non-motor symptoms. *Frontiers in aging neuroscience,* 9**,** 301.

MÜLLER, A., REICHMANN, H., LIVERMORE, A. & HUMMEL, T. 2002. Olfactory function in idiopathic Parkinson's disease (IPD): results from cross-sectional studies in IPD patients and long-term follow-up of de-novo IPD patients. *J Neural Transm (Vienna),* 109**,** 805-11.

MURAKAMI, Y., KAKEDA, S., WATANABE, K., UEDA, I., OGASAWARA, A., MORIYA, J., IDE, S., FUTATSUYA, K., SATO, T., OKADA, K., UOZUMI, T., TSUJI, S., LIU, T., WANG, Y. & KOROGI, Y. 2015. Usefulness of quantitative susceptibility mapping for the diagnosis of Parkinson disease. *AJNR Am J Neuroradiol,* 36**,** 1102-8.

MURMAN, D. L. 2015. The Impact of Age on Cognition. *Semin Hear,* 36**,** 111-21.

NA, S., JEONG, H., PARK, J. S., CHUNG, Y. A. & SONG, I. U. 2020. The Impact of Amyloid-Beta Positivity with 18F-Florbetaben PET on Neuropsychological Aspects in Parkinson's Disease Dementia. *Metabolites,* 10.

NAEIMI, Z. S., WEINHOFER, A., SARAHRUDI, K., HEINZ, T. & VECSEI, V. 2006. Predictive value of S-100B protein and neuron specific-enolase as markers of traumatic brain damage in clinical use. *Brain Injury,* 20**,** 463-468.

NAKAGAWA, S., LAGISZ, M., JENNIONS, M. D., KORICHEVA, J., NOBLE, D. W., PARKER, T. H., SÁNCHEZ-TÓJAR, A., YANG, Y. & O'DEA, R. E. 2022. Methods for testing publication bias in ecological and evolutionary meta-analyses. *Methods in Ecology and Evolution,* 13**,** 4-21.

NAKAGAWA, S., LAGISZ, M., O'DEA, R. E., POTTIER, P., RUTKOWSKA, J., SENIOR, A. M., YANG, Y. & NOBLE, D. W. 2023. orchaRd 2.0: An R package for visualizing meta-analyses with orchard plots.

NAKAMURA, Y., KITAMURA, T., KAWANO, Y., HOSHINO, K., IRIE, Y., MURANISHI, K., IWAASA, M. & ISHIKURA, H. 2022. Glial fibrillary acidic protein level on admission can predict severe traumatic brain injury in patients with severe multiple trauma: A single-center retrospective observational study. *Curr Res Neurobiol,* 3**,** 100047.

NALLS, M. A., BLAUWENDRAAT, C., VALLERGA, C. L., HEILBRON, K., BANDRES-CIGA, S., CHANG, D., TAN, M., KIA, D. A., NOYCE, A. J., XUE, A., BRAS, J., YOUNG, E., VON COELLN, R., SIMÓN-SÁNCHEZ, J., SCHULTE, C., SHARMA, M., KROHN, L., PIHLSTRØM, L., SIITONEN, A., IWAKI, H., LEONARD, H., FAGHRI, F., GIBBS, J. R., HERNANDEZ, D. G., SCHOLZ, S. W., BOTIA, J. A., MARTINEZ, M., CORVOL, J.-C., LESAGE, S., JANKOVIC, J., SHULMAN, L. M., SUTHERLAND, M., TIENARI, P., MAJAMAA, K., TOFT, M., ANDREASSEN, O. A., BANGALE, T., BRICE, A., YANG, J., GAN-OR, Z., GASSER, T., HEUTINK, P., SHULMAN, J. M., WOOD, N. W., HINDS, D. A., HARDY, J. A., MORRIS, H. R., GRATTEN, J., VISSCHER, P. M., GRAHAM, R. R., SINGLETON, A. B., ADARMES-GÓMEZ, A. D., AGUILAR, M., AITKULOVA, A., AKHMETZHANOV, V., ALCALAY, R. N., ALVAREZ, I., ALVAREZ, V., BANDRES-CIGA, S., BARRERO, F. J., BERGARECHE YARZA, J. A., BERNAL-BERNAL, I., BILLINGSLEY, K., BLAUWENDRAAT, C., BLAZQUEZ, M., BONILLA-TORIBIO, M., BOTÍA, J. A., BOUNGIORNO, M. T., BRAS, J., BRICE, A., BROCKMANN, K., BUBB, V., BUIZA-RUEDA, D., CÁMARA, A., CARRILLO, F., CARRIÓN-CLARO, M., CERDAN, D., CHELBAN, V., CLARIMÓN, J., CLARKE, C., COMPTA, Y., COOKSON, M. R., CORVOL, J.-C., CRAIG, D. W., DANJOU, F., DIEZ-FAIREN, M., DOLS-ICARDO, O., DUARTE, J., DURAN, R., ESCAMILLA-SEVILLA, F., ESCOTT-PRICE, V., EZQUERRA, M., FAGHRI, F., FELIZ, C., FERNÁNDEZ, M., FERNÁNDEZ-SANTIAGO, R., FINKBEINER, S., FOLTYNIE, T., GAN-OR, Z., GARCIA, C., et al. 2019. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. *The Lancet Neurology,* 18**,** 1091-1102.

NARAYAN, R. K., MICHEL, M. E., ANSELL, B., BAETHMANN, A., BIEGON, A., BRACKEN, M. B., BULLOCK, M. R., CHOI, S. C., CLIFTON, G. L. & CONTANT, C. F. 2002. Clinical trials in head injury. *Journal of neurotrauma,* 19**,** 503-557.

NASREDDINE, Z. S., PHILLIPS, N. A., BÉDIRIAN, V., CHARBONNEAU, S., WHITEHEAD, V., COLLIN, I., CUMMINGS, J. L. & CHERTKOW, H. 2005. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society,* 53**,** 695-699.

NAVARRO-ROMERO, A., FERNANDEZ-GONZALEZ, I., RIERA, J., MONTPEYO, M., ALBERT-BAYO, M., LOPEZ-ROYO, T., CASTILLO-SANCHEZ, P., CARNICER-CACERES, C., ARRANZ-AMO, J. A. & CASTILLO-RIBELLES, L. 2022. Lysosomal lipid alterations caused by glucocerebrosidase deficiency promote lysosomal dysfunction, chaperone-mediated-autophagy deficiency, and alpha-synuclein pathology. *npj Parkinson's Disease,* 8**,** 126.

NELSON, L. D., TEMKIN, N. R., BARBER, J., BRETT, B. L., OKONKWO, D. O., MCCREA, M. A., GIACINO, J. T., BODIEN, Y. G., ROBERTSON, C., CORRIGAN, J. D., DIAZ-ARRASTIA, R., MARKOWITZ, A. J. & MANLEY, G. T. 2023. Functional Recovery,

Symptoms, and Quality of Life 1 to 5 Years After Traumatic Brain Injury. *JAMA Netw Open,* 6**,** e233660.

NELSON, L. D., TEMKIN, N. R., DIKMEN, S., BARBER, J., GIACINO, J. T., YUH, E., LEVIN, H. S., MCCREA, M. A., STEIN, M. B., MUKHERJEE, P., OKONKWO, D. O., ROBERTSON, C. S., DIAZ-ARRASTIA, R., MANLEY, G. T., ADEOYE, O., BADJATIA, N., BOASE, K., BODIEN, Y., BULLOCK, M. R., CHESNUT, R., CORRIGAN, J. D., CRAWFORD, K., DUHAIME, A. C., ELLENBOGEN, R., FEESER, V. R., FERGUSON, A., FOREMAN, B., GARDNER, R., GAUDETTE, E., GONZALEZ, L., GOPINATH, S., GULLAPALLI, R., HEMPHILL, J. C., HOTZ, G., JAIN, S., KORLEY, F., KRAMER, J., KREITZER, N., LINDSELL, C., MACHAMER, J., MADDEN, C., MARTIN, A., MCALLISTER, T., MERCHANT, R., NOEL, F., PALACIOS, E., PERL, D., PUCCIO, A., RABINOWITZ, M., ROSAND, J., SANDER, A., SATRIS, G., SCHNYER, D., SEABURY, S., SHERER, M., TAYLOR, S., TOGA, A., VALADKA, A., VASSAR, M. J., VESPA, P., WANG, K., YUE, J. K. & ZAFONTE, R. 2019. Recovery After Mild Traumatic Brain Injury in Patients Presenting to US Level I Trauma Centers: A Transforming Research and Clinical Knowledge in Traumatic Brain Injury (TRACK-TBI) Study. *JAMA Neurol,* 76**,** 1049-1059.

NI, P., QIAO, Y., TONG, W., ZHAO, C. & ZHENG, P. 2020a. Associations between serum tau, neurological outcome, and cognition following traumatic brain injury. *Neurol India,* 68**,** 462-467.

NI, P., QIAO, Y. T., TONG, W. S., ZHAO, C. F. & ZHENG, P. 2020b. Associations between Serum Tau, Neurological Outcome, and Cognition following Traumatic Brain Injury. *Neurology India,* 68**,** 462-467.

NISSEN, T., MALEK, N., GROSSET, K. A., NEWMAN, E. J., PATTERSON, J., HADLEY, D. & GROSSET, D. G. 2014. Baseline [(123) I]FP-CIT SPECT (DaTSCAN) severity correlates with medication use at 3 years in Parkinson's disease. *Acta Neurol Scand,* 129**,** 204-8.

NIYONKURU, C., WAGNER, A. K., OZAWA, H., AMIN, K., GOYAL, A. & FABIO, A. 2013. Group-Based Trajectory Analysis Applications for Prognostic Biomarker Model Development in Severe TBI: A Practical Example. *Journal of Neurotrauma,* 30**,** 938-945.

NOBLE, D. W., LAGISZ, M., O'DEA, R. E. & NAKAGAWA, S. 2017. Nonindependence and sensitivity analyses in ecological and evolutionary meta-analyses. Wiley Online Library.

NOELKER, C., MOREL, L., LESCOT, T., OSTERLOH, A., ALVAREZ-FISCHER, D., BRELOER, M., HENZE, C., DEPBOYLU, C., SKRZYDELSKI, D., MICHEL, P. P., DODEL, R. C., LU, L., HIRSCH, E. C., HUNOT, S. & HARTMANN, A. 2013. Toll like receptor 4 mediates cell death in a mouse MPTP model of Parkinson disease. *Sci Rep,* 3**,** 1393.

NOGUCHI, K., GEL, Y. R., BRUNNER, E. & KONIETSCHKE, F. 2012. nparLD: an R software package for the nonparametric analysis of longitudinal data in factorial experiments.

NYGREN DE BOUSSARD, C., LUNDIN, A., KARLSTEDT, D., EDMAN, G., BARTFAI, A. & BORG, J. 2005. S100 and cognitive impairment after mild traumatic brain injury. *Journal of Rehabilitation Medicine,* 37**,** 53-57.

OGAWA, T., FUJII, S., KUYA, K., KITAO, S. I., SHINOHARA, Y., ISHIBASHI, M. & TANABE, Y. 2018. Role of Neuroimaging on Differentiation of Parkinson's Disease and Its Related Diseases. *Yonago Acta Med,* 61**,** 145-155.

OHTSUKA, C., SASAKI, M., KONNO, K., KOIDE, M., KATO, K., TAKAHASHI, J., TAKAHASHI, S., KUDO, K., YAMASHITA, F. & TERAYAMA, Y. 2013. Changes in substantia nigra and locus coeruleus in patients with early-stage Parkinson's disease using neuromelanin-sensitive MR imaging. *Neurosci Lett,* 541**,** 93-8.

OIKAWA, H., SASAKI, M., TAMAKAWA, Y., EHARA, S. & TOHYAMA, K. 2002. The substantia nigra in Parkinson disease: proton density-weighted spin-echo and fast short inversion time inversion-recovery MR findings. *AJNR Am J Neuroradiol,* 23**,** 1747-56.

OLANOW, C. W., PERL, D. P., DEMARTINO, G. N. & MCNAUGHT, K. S. P. 2004. Lewy-body formation is an aggresome-related process: a hypothesis. *The Lancet Neurology,* 3**,** 496-503.

OLIVECRONA, M. & KOSKINEN, L. O. 2012. The IMPACT prognosis calculator used in patients with severe traumatic brain injury treated with an ICP-targeted therapy. *Acta Neurochir (Wien),* 154**,** 1567-73.

OLIVECRONA, M. & OLIVECRONA, Z. 2013. Use of the CRASH study prognosis calculator in patients with severe traumatic brain injury treated with an intracranial pressure-targeted therapy. *J Clin Neurosci,* 20**,** 996-1001.

OLIVECRONA, M., RODLING-WAHLSTROM, M., NAREDI, S. & KOSKINEN, L. O. D. 2009. S-100B and neuron specific enolase are poor outcome predictors in severe traumatic brain injury treated by an intracranial pressure targeted therapy. *Journal of Neurology Neurosurgery and Psychiatry,* 80**,** 1241-1247.

OLIVECRONA, Z., BOBINSKI, L. & KOSKINEN, L. O. D. 2015. Association of ICP, CPP, CT findings and S-100B and NSE in severe traumatic head injury. Prognostic value of the biomarkers. *Brain Injury,* 29**,** 446-454.

OLIVERA, A., LEJBMAN, N., JEROMIN, A., FRENCH, L. M., KIM, H.-S., CASHION, A., MYSLIWIEC, V., DIAZ-ARRASTIA, R. & GILL, J. 2015. Peripheral total tau in military personnel who sustain traumatic brain injuries during deployment. *JAMA neurology,* 72**,** 1109-1116.

ORGANIZATION, W. H. 2022a. Global health estimates: Leading causes of DALYs. *World Health Organization. Retrieved April,* 25**,** 2022.

ORGANIZATION, W. H. 2022b. Parkinson disease: a public health approach: technical brief.

OSBORN, A., MATHIAS, J. & FAIRWEATHER-SCHMIDT, A. 2014. Depression following adult, non-penetrating traumatic brain injury: a meta-analysis examining methodological variables and sample characteristics. *Neuroscience & Biobehavioral Reviews,* 47**,** 1-15.

OSBORN, A. J., MATHIAS, J. L. & FAIRWEATHER-SCHMIDT, A. K. 2016. Prevalence of anxiety following adult traumatic brain injury: A meta-analysis comparing measures, samples and postinjury intervals. *Neuropsychology,* 30**,** 247-61.

OST, M., NYLEN, K., CSAJBOK, L., OHRFELT, A. O., TULLBERG, M., WIKKELSO, C., NELLGARD, P., ROSENGREN, L., BLENNOW, K. & NELLGARD, B. 2006. Initial CSF total tau correlates with 1-year outcome in patients with traumatic brain injury. *Neurology,* 67**,** 1600-1604.

OTSUKA, M., ICHIYA, Y., KUWABARA, Y., HOSOKAWA, S., SASAKI, M., YOSHIDA, T., FUKUMURA, T., MASUDA, K. & KATO, M. 1996. Differences in the reduced 18F-Dopa uptakes of the caudate and the putamen in Parkinson's disease: correlations with the three main symptoms. *Journal of the neurological sciences,* 136**,** 169-173.

OU, Z., PAN, J., TANG, S., DUAN, D., YU, D., NONG, H. & WANG, Z. 2021. Global trends in the incidence, prevalence, and years lived with disability of parkinson's disease in 204 countries/territories from 1990 to 2019. *Frontiers in public health,* 9**,** 776847.

OWEN, D. R., YEO, A. J., GUNN, R. N., SONG, K., WADSWORTH, G., LEWIS, A., RHODES, C., PULFORD, D. J., BENNACEF, I., PARKER, C. A., STJEAN, P. L., CARDON, L. R., MOOSER, V. E., MATTHEWS, P. M., RABINER, E. A. & RUBIO, J. P. 2012. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J Cereb Blood Flow Metab,* 32**,** 1-5.

PADMAKUMAR, S., KULKARNI, P., FERRIS, C. F., BLEIER, B. S. & AMIJI, M. M. 2022. Traumatic brain injury and the development of parkinsonism: Understanding pathophysiology, animal models, and therapeutic targets. *Biomed Pharmacother,* 149**,** 112812.

PAGANO, G., FERRARA, N., BROOKS, D. J. & PAVESE, N. 2016. Age at onset and Parkinson disease phenotype. *Neurology,* 86**,** 1400-1407.

PALERMO, G., GIANNONI, S., DEPALO, T., FROSINI, D., VOLTERRANI, D., SICILIANO, G., BONUCCELLI, U. & CERAVOLO, R. 2022. Negative DAT-SPECT in Old Onset Parkinson's Disease: An Additional Pitfall? *Movement Disorders Clinical Practice,* 9**,** 530-534.

PAN, L., MENG, L., HE, M. & ZHANG, Z. 2021. Tau in the Pathophysiology of Parkinson's Disease. *J Mol Neurosci,* 71**,** 2179-2191.

PANDEY, S., SINGH, K., PANDEY, D., CHAND, G., SHARMA, V., JHA, R. P., SINGH, R., RAI, S. K. & GUPTA, R. 2017a. "Cleaved Tau Protein": A Novel Biomarker Candidate in Mild Neurotrauma in Emergency Settings. *Indian Journal of Neurotrauma,* 14**,** 26-34.

PANDEY, S., SINGH, K., SHARMA, V., PANDEY, D., JHA, R. P., RAI, S. K., CHAUHAN, R. S. & SINGH, R. 2017b. A prospective pilot study on serum cleaved tau protein as a neurological marker in severe traumatic brain injury. *British Journal of Neurosurgery***,** 1-8.

PANG, S. Y.-Y., HO, P. W.-L., LIU, H.-F., LEUNG, C.-T., LI, L., CHANG, E. E. S., RAMSDEN, D. B. & HO, S.-L. 2019. The interplay of aging, genetics and environmental factors in the pathogenesis of Parkinson's disease. *Translational Neurodegeneration,* 8**,** 1-11.

PAPA, L., AKINYI, L., LIU, M. C., PINEDA, J. A., TEPAS, J. J., 3RD, OLI, M. W., ZHENG, W., ROBINSON, G., ROBICSEK, S. A., GABRIELLI, A., HEATON, S. C., HANNAY, H. J., DEMERY, J. A., BROPHY, G. M., LAYON, J., ROBERTSON, C. S., HAYES, R. L. & WANG, K. K. 2010. Ubiquitin C-terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. *Crit Care Med,* 38**,** 138-44.

PAPA, L., LEWIS, L. M., SILVESTRI, S., FALK, J. L., GIORDANO, P., BROPHY, G. M., DEMERY, J. A., LIU, M. C., MO, J. & AKINYI, L. 2012. Serum levels of ubiquitin C-terminal hydrolase distinguish mild traumatic brain injury from trauma controls and are elevated in mild and moderate traumatic brain injury patients with

intracranial lesions and neurosurgical intervention. *Journal of Trauma and Acute Care Surgery,* 72**,** 1335-1344.
PAREDES-RODRIGUEZ, E., VEGAS-SUAREZ, S., MORERA-HERRERAS, T., DE DEURWAERDERE, P. & MIGUELEZ, C. 2020. The Noradrenergic System in Parkinson's Disease. *Front Pharmacol,* 11**,** 435.
PARENT, A., MACKEY, A. & DE BELLEFEUILLE, L. 1983. The subcortical afferents to caudate nucleus and putamen in primate: a fluorescence retrograde double labeling study. *Neuroscience,* 10**,** 1137-50.
PASCOAL, T. A., LEUZY, A., THERRIAULT, J., CHAMOUN, M., LUSSIER, F., TISSOT, C., STRANDBERG, O., PALMQVIST, S., STOMRUD, E., FERREIRA, P. C. L., FERRARI-SOUZA, J. P., SMITH, R., BENEDET, A. L., GAUTHIER, S., HANSSON, O. & ROSA-NETO, P. 2023. Discriminative accuracy of the A/T/N scheme to identify cognitive impairment due to Alzheimer's disease. *Alzheimers Dement (Amst),* 15**,** e12390.
PASQUINI, J., DEUSCHL, G., PECORI, A., SALVADORI, S., CERAVOLO, R. & PAVESE, N. 2023. The Clinical Profile of Tremor in Parkinson's Disease. *Mov Disord Clin Pract,* 10**,** 1496-1506.
PASQUINI, J., DURCAN, R., WIBLIN, L., STOKHOLM, M. G., ROCHESTER, L., BROOKS, D. J., BURN, D. & PAVESE, N. 2019. Clinical implications of early caudate dysfunction in Parkinson's disease. *Journal of Neurology, Neurosurgery & Psychiatry,* 90**,** 1098-1104.
PAUL, K. C., KROLEWSKI, R. C., LUCUMI MORENO, E., BLANK, J., HOLTON, K. M., AHFELDT, T., FURLONG, M., YU, Y., COCKBURN, M., THOMPSON, L. K., KREYMERMAN, A., RICCI-BLAIR, E. M., LI, Y. J., PATEL, H. B., LEE, R. T., BRONSTEIN, J., RUBIN, L. L., KHURANA, V. & RITZ, B. 2023. A pesticide and iPSC dopaminergic neuron screen identifies and classifies Parkinson-relevant pesticides. *Nat Commun,* 14**,** 2803.
PAVESE, N. & TAI, Y. F. 2018. Nigrosome Imaging and Neuromelanin Sensitive MRI in Diagnostic Evaluation of Parkinsonism. *Mov Disord Clin Pract,* 5**,** 131-140.
PAVLOVIC, D., PEKIC, S., STOJANOVIC, M. & POPOVIC, V. 2019. Traumatic brain injury: neuropathological, neurocognitive and neurobehavioral sequelae. *Pituitary,* 22**,** 270-282.
PEDREGOSA, F., VAROQUAUX, G., GRAMFORT, A., MICHEL, V., THIRION, B., GRISEL, O., BLONDEL, M., PRETTENHOFER, P., WEISS, R. & DUBOURG, V. 2011. Scikit-learn: Machine learning in Python. *the Journal of machine Learning research,* 12**,** 2825-2830.
PEI, Y., TANG, X., ZHANG, E., LU, K., XIA, B., ZHANG, J., HUANG, Y., ZHANG, H. & DONG, L. 2022. The diagnostic and prognostic value of glial fibrillary acidic protein in traumatic brain injury: a systematic review and meta-analysis. *Eur J Trauma Emerg Surg*.
PELINKA, L. E., KROEPFL, A., SCHMIDHAMMER, R., KRENN, M., BUCHINGER, W., REDL, H. & RAABE, A. 2004. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. *J Trauma,* 57**,** 1006-12.
PELTZ, C. B., KENNEY, K., GILL, J., DIAZ-ARRASTIA, R., GARDNER, R. C. & YAFFE, K. 2020. Blood biomarkers of traumatic brain injury and cognitive impairment in older veterans. *Neurology,* 95**,** E1126-E1133.
PERALTA, C., STRAFELLA, A. P., VAN EIMEREN, T., CERAVOLO, R., SEPPI, K., KAASINEN, V., ARENA, J. E., LEHERICY, S. & GROUP, I. P. M. D. S. N. S. 2022. Pragmatic

approach on neuroimaging techniques for the differential diagnosis of parkinsonisms. *Movement Disorders Clinical Practice,* 9**,** 6-19.
PEREL, P., ARANGO, M., CLAYTON, T., EDWARDS, P., KOMOLAFE, E., POCCOCK, S., ROBERTS, I., SHAKUR, H., STEYERBERG, E. & YUTTHAKASEMSUNT, S. 2008. Predicting outcome after traumatic brain injury: practical prognostic models based on large cohort of international patients. *Bmj,* 336**,** 425-9.
PERRIN, P. B., NIEMEIER, J. P., MOUGEOT, J. L., VANNOY, C. H., HIRSCH, M. A., WATTS, J. A., ROSSMAN, W., GRAFTON, L. M., GUERRIER, T. D., PERSHAD, R., KINGSBURY, C. A., BARTEL, S. W. & WHITNEY, M. P. 2015. Measures of injury severity and prediction of acute traumatic brain injury outcomes. *J Head Trauma Rehabil,* 30**,** 136-42.
PETIT-TABOUÉ, M. C., BARON, J. C., BARRÉ, L., TRAVÈRE, J. M., SPECKEL, D., CAMSONNE, R. & MACKENZIE, E. T. 1991. Brain kinetics and specific binding of [11C]PK 11195 to omega 3 sites in baboons: positron emission tomography study. *Eur J Pharmacol,* 200**,** 347-51.
PIERI, L., MADIONA, K. & MELKI, R. 2016. Structural and functional properties of prefibrillar α-synuclein oligomers. *Scientific reports,* 6**,** 24526.
PITZ, V., MAKARIOUS, M. B., BANDRES-CIGA, S., IWAKI, H., ASLIBEKYAN, S., AUTON, A., BABALOLA, E., BELL, R. K., BIELENBERG, J., BRYC, K., BULLIS, E., COKER, D., PARTIDA, G. C., DHAMIJA, D., DAS, S., ELSON, S. L., ERIKSSON, N., FILSHTEIN, T., FITCH, A., FLETEZ-BRANT, K., FONTANILLAS, P., FREYMAN, W., GRANKA, J. M., HERNANDEZ, A., HICKS, B., HINDS, D. A., JEWETT, E. M., JIANG, Y., KUKAR, K., KWONG, A., LIN, K.-H., LLAMAS, B. A., LOWE, M., MCCREIGHT, J. C., MCINTYRE, M. H., MICHELETTI, S. J., MORENO, M. E., NANDAKUMAR, P., NGUYEN, D. T., NOBLIN, E. S., O'CONNELL, J., PETRAKOVITZ, A. A., POZNIK, G. D., REYNOSO, A., SCHUMACHER, M., SHASTRI, A. J., SHELTON, J. F., SHI, J., SHRINGARPURE, S., SU, Q. J., TAT, S. A., TCHAKOUTÉ, C. T., TRAN, V., TUNG, J. Y., WANG, X., WANG, W., WELDON, C. H., WILTON, P., WONG, C. D., SINGLETON, A. B., NALLS, M., HEILBRON, K., BLAUWENDRAAT, C. & ANDME RESEARCH, T. 2024. Analysis of rare Parkinson's disease variants in millions of people. *npj Parkinson's Disease,* 10**,** 11.
POLYMEROPOULOS, M. H., LAVEDAN, C., LEROY, E., IDE, S. E., DEHEJIA, A., DUTRA, A., PIKE, B., ROOT, H., RUBENSTEIN, J. & BOYER, R. 1997. Mutation in the α-synuclein gene identified in families with Parkinson's disease. *science,* 276**,** 2045-2047.
PONSFORD, J. L., DOWNING, M. G., OLVER, J., PONSFORD, M., ACHER, R., CARTY, M. & SPITZ, G. 2014. Longitudinal follow-up of patients with traumatic brain injury: outcome at two, five, and ten years post-injury. *J Neurotrauma,* 31**,** 64-77.
PONTONE, G. M., WILLIAMS, J. R., ANDERSON, K. E., CHASE, G., GOLDSTEIN, S. A., GRILL, S., HIRSCH, E. S., LEHMANN, S., LITTLE, J. T., MARGOLIS, R. L., RABINS, P. V., WEISS, H. D. & MARSH, L. 2009. Prevalence of anxiety disorders and anxiety subtypes in patients with Parkinson's disease. *Mov Disord,* 24**,** 1333-8.
POST, B., MERKUS, M. P., DE BIE, R. M., DE HAAN, R. J. & SPEELMAN, J. D. 2005. Unified Parkinson's disease rating scale motor examination: are ratings of nurses, residents in neurology, and movement disorders specialists interchangeable? *Mov Disord,* 20**,** 1577-84.

POSTUMA, R. B., AARSLAND, D., BARONE, P., BURN, D. J., HAWKES, C. H., OERTEL, W. & ZIEMSSEN, T. 2012. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Movement Disorders,* 27**,** 617-626.

POSTUMA, R. B. & BERG, D. 2019. Prodromal Parkinson's disease: the decade past, the decade to come. *Movement disorders,* 34**,** 665-675.

POSTUMA, R. B., BERG, D., STERN, M., POEWE, W., OLANOW, C. W., OERTEL, W., OBESO, J., MAREK, K., LITVAN, I., LANG, A. E., HALLIDAY, G., GOETZ, C. G., GASSER, T., DUBOIS, B., CHAN, P., BLOEM, B. R., ADLER, C. H. & DEUSCHL, G. 2015a. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord,* 30**,** 1591-601.

POSTUMA, R. B., GAGNON, J. F., BERTRAND, J. A., GÉNIER MARCHAND, D. & MONTPLAISIR, J. Y. 2015b. Parkinson risk in idiopathic REM sleep behavior disorder: preparing for neuroprotective trials. *Neurology,* 84**,** 1104-13.

POSTUMA, R. B., POEWE, W., LITVAN, I., LEWIS, S., LANG, A. E., HALLIDAY, G., GOETZ, C. G., CHAN, P., SLOW, E., SEPPI, K., SCHAFFER, E., RIOS-ROMENETS, S., MI, T., MAETZLER, C., LI, Y., HEIM, B., BLEDSOE, I. O. & BERG, D. 2018. Validation of the MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord,* 33**,** 1601-1608.

PRINGSHEIM, T., JETTE, N., FROLKIS, A. & STEEVES, T. D. 2014. The prevalence of Parkinson's disease: a systematic review and meta-analysis. *Mov Disord,* 29**,** 1583-90.

PUÑAL-RIOBÓO, J., SERENA-PUIG, A., VARELA-LEMA, L., ALVAREZ-PÁEZ, A. M. & RUANO-RAVINA, A. 2009. [Clinical utility of (18)F-DOPA-PET in movement disorders. A systematic review]. *Rev Esp Med Nucl,* 28**,** 106-13.

PUSTEJOVSKY, J. 2023. clubSandwich: Cluster-Robust (Sandwich) Variance Estimators with Small-Sample
Corrections.

PYATHA, S., KIM, H., LEE, D. & KIM, K. 2022. Association between heavy metal exposure and Parkinson’s disease: a review of the mechanisms related to oxidative stress. *Antioxidants,* 11**,** 2467.

QIN, X. Y., ZHANG, S. P., CAO, C., LOH, Y. P. & CHENG, Y. 2016. Aberrations in Peripheral Inflammatory Cytokine Levels in Parkinson Disease: A Systematic Review and Meta-analysis. *JAMA Neurol,* 73**,** 1316-1324.

QIN, Z., HU, D., HAN, S., REANEY, S. H., DI MONTE, D. A. & FINK, A. L. 2007. Effect of 4-hydroxy-2-nonenal modification on alpha-synuclein aggregation. *J Biol Chem,* 282**,** 5862-70.

RAABE, A. & SEIFERT, V. 2000. Protein S-100B as a serum marker of brain damage in severe head injury: preliminary results. *Neurosurgical Review,* 23**,** 136-138.

RAHEJA, A., SINHA, S., SAMSON, N., BHOI, S., SELVI, A., SHARMA, P. & SHARMA, B. 2016. Serum biomarkers as predictor of long term outcome in severe traumatic brain injury. *Journal of Neurosurgery,* 124**,** A1186-A1187.

RAINEY, T., LESKO, M., SACHO, R., LECKY, F. & CHILDS, C. 2009. Predicting outcome after severe traumatic brain injury using the serum S100B biomarker: Results using a single (24 h) time-point. *Resuscitation,* 80**,** 341-345.

RAJPUT, A. H., VOLL, A., RAJPUT, M. L., ROBINSON, C. A. & RAJPUT, A. 2009. Course in Parkinson disease subtypes: A 39-year clinicopathologic study. *Neurology,* 73**,** 206-12.

RAMANI, L., MALEK, N., PATTERSON, J., NISSEN, T. & NEWMAN, E. J. 2017. Relationship between [(123) I]-FP-CIT SPECT and clinical progression in Parkinson's disease. *Acta Neurol Scand,* 135**,** 400-406.

RAMLACKHANSINGH, A. F., BROOKS, D. J., GREENWOOD, R. J., BOSE, S. K., TURKHEIMER, F. E., KINNUNEN, K. M., GENTLEMAN, S., HECKEMANN, R. A., GUNANAYAGAM, K., GELOSA, G. & SHARP, D. J. 2011. Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol,* 70**,** 374-83.

RANSON, J., MAGNUS, B. E., TEMKIN, N., DIKMEN, S., GIACINO, J. T., OKONKWO, D. O., VALADKA, A. B., MANLEY, G. T. & NELSON, L. D. 2019. Diagnosing the GOSE: Structural and Psychometric Properties Using Item Response Theory, a TRACK-TBI Pilot Study. *J Neurotrauma,* 36**,** 2493-2505.

RAPAPORT, M. H., CLARY, C., FAYYAD, R. & ENDICOTT, J. 2005. Quality-of-life impairment in depressive and anxiety disorders. *American Journal of Psychiatry,* 162**,** 1171-1178.

RAVANFAR, P., LOI, S. M., SYEDA, W. T., VAN RHEENEN, T. E., BUSH, A. I., DESMOND, P., CROPLEY, V. L., LANE, D. J., OPAZO, C. M. & MOFFAT, B. A. 2021. Systematic review: quantitative susceptibility mapping (QSM) of brain iron profile in neurodegenerative diseases. *Frontiers in neuroscience,* 15**,** 618435.

RAVEN, J. & RAVEN, J. 2003. Raven Progressive Matrices. *Handbook of nonverbal assessment*. New York, NY, US: Kluwer Academic/Plenum Publishers.

RAVINA, B., TANNER, C., DIEULIIS, D., EBERLY, S., FLAGG, E., GALPERN, W. R., FAHN, S., GOETZ, C. G., GRATE, S., KURLAN, R., LANG, A. E., MAREK, K., KIEBURTZ, K., OAKES, D., ELLIOTT, R. & SHOULSON, I. 2009. A longitudinal program for biomarker development in Parkinson's disease: a feasibility study. *Mov Disord,* 24**,** 2081-90.

REES, J., RYAN, J., LAWS, M. & DEVINE, A. 2024. A comprehensive examination of the evidence for whole of diet patterns in Parkinson's disease: a scoping review. *Nutritional neuroscience,* 27**,** 547-565.

REIJNDERS, J., EHRT, U., LOUSBERG, R., AARSLAND, D. & LEENTJENS, A. 2009. The association between motor subtypes and psychopathology in Parkinson's disease. *Parkinsonism & related disorders,* 15**,** 379-382.

REIMÃO, S., PITA LOBO, P., NEUTEL, D., CORREIA GUEDES, L., COELHO, M., ROSA, M. M., FERREIRA, J., ABREU, D., GONÇALVES, N., MORGADO, C., NUNES, R. G., CAMPOS, J. & FERREIRA, J. J. 2015a. Substantia nigra neuromelanin magnetic resonance imaging in de novo Parkinson's disease patients. *Eur J Neurol,* 22**,** 540-6.

REIMÃO, S., PITA LOBO, P., NEUTEL, D., GUEDES, L. C., COELHO, M., ROSA, M. M., AZEVEDO, P., FERREIRA, J., ABREU, D., GONÇALVES, N., NUNES, R. G., CAMPOS, J. & FERREIRA, J. J. 2015b. Substantia nigra neuromelanin-MR imaging differentiates essential tremor from Parkinson's disease. *Mov Disord,* 30**,** 953-9.

REN, J., HUA, P., LI, Y., PAN, C., YAN, L., YU, C., ZHANG, L., XU, P., ZHANG, M. & LIU, W. 2020. Comparison of Three Motor Subtype Classifications in de novo Parkinson's Disease Patients. *Front Neurol,* 11**,** 601225.

RIEMANN, B. L. & GUSKIEWICZ, K. M. 2000. Effects of mild head injury on postural stability as measured through clinical balance testing. *Journal of athletic training,* 35**,** 19.

RIETDIJK, C. D., PEREZ-PARDO, P., GARSSEN, J., VAN WEZEL, R. J. & KRANEVELD, A. D. 2017. Exploring Braak's Hypothesis of Parkinson's Disease. *Front Neurol,* 8**,** 37.
RILEY, R. D., MOONS, K. G., SNELL, K. I., ENSOR, J., HOOFT, L., ALTMAN, D. G., HAYDEN, J., COLLINS, G. S. & DEBRAY, T. P. 2019. A guide to systematic review and meta-analysis of prognostic factor studies. *bmj,* 364.
RINNE, J. O. 2009. Positron emission tomography in the differential diagnosis of parkinsonism. *J Mov Disord,* 2**,** 53-7.
RINNE, O. J., NURMI, E., RUOTTINEN, H. M., BERGMAN, J., ESKOLA, O. & SOLIN, O. 2001. [(18)F]FDOPA and [(18)F]CFT are both sensitive PET markers to detect presynaptic dopaminergic hypofunction in early Parkinson's disease. *Synapse,* 40**,** 193-200.
RISBROUGH, V. B., VAUGHN, M. N. & FRIEND, S. F. 2022. Role of inflammation in traumatic brain injury–associated risk for neuropsychiatric disorders: State of the evidence and where do we go from here. *Biological psychiatry,* 91**,** 438-448.
RIZZO, G., COPETTI, M., ARCUTI, S., MARTINO, D., FONTANA, A. & LOGROSCINO, G. 2016. Accuracy of clinical diagnosis of Parkinson disease: a systematic review and meta-analysis. *Neurology,* 86**,** 566-576.
ROGAN, A., SIK, A., DICKINSON, E., PATEL, V., PECKLER, B., MCQUADE, D. & LARSEN, P. D. 2023. Diagnostic performance of S100B as a rule-out test for intracranial pathology in head-injured patients presenting to the emergency department who meet NICE Head Injury Guideline criteria for CT-head scan. *Emerg Med J,* 40**,** 159-166.
ROHEGER, M., KALBE, E. & LIEPELT-SCARFONE, I. 2018. Progression of cognitive decline in Parkinson's disease. *Journal of Parkinson's disease,* 8**,** 183-193.
ROSE, K. N., SCHWARZSCHILD, M. A. & GOMPERTS, S. N. 2024. Clearing the smoke: what protects smokers from Parkinson's disease? *Movement Disorders,* 39**,** 267-272.
ROSENTHAL, R., COOPER, H. & HEDGES, L. 1994. Parametric measures of effect size. *The handbook of research synthesis,* 621**,** 231-244.
ROSS, G. W., ABBOTT, R. D., PETROVITCH, H., TANNER, C. M. & WHITE, L. R. 2012. Pre-motor features of Parkinson's disease: the Honolulu-Asia Aging Study experience. *Parkinsonism Relat Disord,* 18 Suppl 1**,** S199-202.
RUBENSTEIN, R., CHANG, B., YUE, J. K., CHIU, A., WINKLER, E. A., PUCCIO, A. M., DIAZ-ARRASTIA, R., YUH, E. L., MUKHERJEE, P. & VALADKA, A. B. 2017. Comparing plasma phospho tau, total tau, and phospho tau–total tau ratio as acute and chronic traumatic brain injury biomarkers. *JAMA neurology,* 74**,** 1063-1072.
RUBIANO, A. M. & LEE, K. 2023. Traumatic brain injury research: homogenising heterogeneity. *The Lancet Neurology,* 22**,** 973-975.
RUIZ-MARTÍNEZ, J., GOROSTIDI, A., IBANEZ, B., ALZUALDE, A., OTAEGUI, D., MORENO, F., DE MUNAIN, A. L., BERGARECHE, A., GÓMEZ-ESTEBAN, J. C. & MASSÓ, J. F. M. 2010. Penetrance in Parkinson's disease related to the LRRK2 R1441G mutation in the Basque country (Spain). *Movement disorders,* 25**,** 2340-2345.
RUTHERFORD, N. J., MOORE, B. D., GOLDE, T. E. & GIASSON, B. I. 2014. Divergent effects of the H50Q and G51D SNCA mutations on the aggregation of α-synuclein. *Journal of neurochemistry,* 131**,** 859-867.

SAARI, L., KIVINEN, K., GARDBERG, M., JOUTSA, J., NOPONEN, T. & KAASINEN, V. 2017. Dopamine transporter imaging does not predict the number of nigral neurons in Parkinson disease. *Neurology,* 88**,** 1461-1467.

SAKAKIBARA, S., HASHIMOTO, R., KATAYAMA, T., KENJYO, M., YOKOKAWA, Y., SAITO, Y., HIRAKAWA, A., ITO, M., NAKAMURA, T. & HARA, K. 2020. Longitudinal change of DAT SPECT in Parkinson's disease and multiple system atrophy. *Journal of Parkinson's Disease,* 10**,** 123-130.

SALGADO, J. F. 2018. Transforming the area under the normal curve (AUC) into Cohen'sd, Pearson's rpb, odds-ratio, and natural log odds-ratio: two conversion tables. *European Journal of Psychology Applied to Legal Context,* 10**,** 35-47.

SANTOS GARCÍA, D., DE DEUS FONTICOBA, T., CORES, C., MUÑOZ, G., PAZ GONZÁLEZ, J. M., MARTÍNEZ MIRÓ, C., SUÁREZ, E., JESÚS, S., AGUILAR, M. & PASTOR, P. 2021. Predictors of clinically significant quality of life impairment in Parkinson's disease. *npj Parkinson's Disease,* 7**,** 118.

SAREDAKIS, D., COLLINS-PRAINO, L. E., GUTTERIDGE, D. S., STEPHAN, B. C. M. & KEAGE, H. A. D. 2019. Conversion to MCI and dementia in Parkinson's disease: a systematic review and meta-analysis. *Parkinsonism Relat Disord,* 65**,** 20-31.

SASAKI, M., SHIBATA, E., TOHYAMA, K., TAKAHASHI, J., OTSUKA, K., TSUCHIYA, K., TAKAHASHI, S., EHARA, S., TERAYAMA, Y. & SAKAI, A. 2006. Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. *Neuroreport,* 17**,** 1215-8.

SCHIEHSER, D. M., FILOTEO, J. V., LITVAN, I., PIROGOVSKY-TURK, E., LESSIG, S. L. & SONG, D. S. 2016. Cognitive functioning in individuals with Parkinson's disease and traumatic brain injury: A longitudinal study. *Parkinsonism Relat Disord,* 30**,** 58-61.

SCHONHAUT, D. R., MCMILLAN, C. T., SPINA, S., DICKERSON, B. C., SIDEROWF, A., DEVOUS, M. D., SR., TSAI, R., WINER, J., RUSSELL, D. S., LITVAN, I., ROBERSON, E. D., SEELEY, W. W., GRINBERG, L. T., KRAMER, J. H., MILLER, B. L., PRESSMAN, P., NASRALLAH, I., BAKER, S. L., GOMPERTS, S. N., JOHNSON, K. A., GROSSMAN, M., JAGUST, W. J., BOXER, A. L. & RABINOVICI, G. D. 2017. (18) F-flortaucipir tau positron emission tomography distinguishes established progressive supranuclear palsy from controls and Parkinson disease: A multicenter study. *Ann Neurol,* 82**,** 622-634.

SCHRETLEN, D. J. & SHAPIRO, A. M. 2003. A quantitative review of the effects of traumatic brain injury on cognitive functioning. *Int Rev Psychiatry,* 15**,** 341-9.

SCHWARZ, S. T., XING, Y., TOMAR, P., BAJAJ, N. & AUER, D. P. 2017. In Vivo Assessment of Brainstem Depigmentation in Parkinson Disease: Potential as a Severity Marker for Multicenter Studies. *Radiology,* 283**,** 789-798.

SEEL, R. T., KREUTZER, J. S., ROSENTHAL, M., HAMMOND, F. M., CORRIGAN, J. D. & BLACK, K. 2003. Depression after traumatic brain injury: a National Institute on Disability and Rehabilitation Research Model Systems multicenter investigation. *Archives of physical medicine and rehabilitation,* 84**,** 177-184.

SENGUPTA, U. & KAYED, R. 2022. Amyloid β, Tau, and α-Synuclein aggregates in the pathogenesis, prognosis, and therapeutics for neurodegenerative diseases. *Progress in neurobiology,* 214**,** 102270.

SHAHIM, P., GREN, M., LIMAN, V., ANDREASSON, U., NORGREN, N., TEGNER, Y., MATTSSON, N., ANDREASEN, N., OST, M., ZETTERBERG, H., NELLGARD, B. &

BLENNOW, K. 2016. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. *Scientific Reports,* 6**,** 9.
SHAHIM, P., TEGNER, Y., WILSON, D. H., RANDALL, J., SKILLBACK, T., PAZOOKI, D., KALLBERG, B., BLENNOW, K. & ZETTERBERG, H. 2014. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurology,* 71**,** 684-692.
SHAHIM, P. & ZETTERBERG, H. 2022. Neurochemical Markers of Traumatic Brain Injury: Relevance to Acute Diagnostics, Disease Monitoring, and Neuropsychiatric Outcome Prediction. *Biol Psychiatry,* 91**,** 405-412.
SHARON, R., BAR-JOSEPH, I., FROSCH, M. P., WALSH, D. M., HAMILTON, J. A. & SELKOE, D. J. 2003. The formation of highly soluble oligomers of α-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. *Neuron,* 37**,** 583-595.
SHMUELI, K., DE ZWART, J. A., VAN GELDEREN, P., LI, T. Q., DODD, S. J. & DUYN, J. H. 2009. Magnetic susceptibility mapping of brain tissue in vivo using MRI phase data. *Magn Reson Med,* 62**,** 1510-22.
SHRESTHA, S., PARKS, C. G., UMBACH, D. M., RICHARDS-BARBER, M., HOFMANN, J. N., CHEN, H., BLAIR, A., BEANE FREEMAN, L. E. & SANDLER, D. P. 2020. Pesticide use and incident Parkinson's disease in a cohort of farmers and their spouses. *Environ Res,* 191**,** 110186.
SIDEROWF, A., CONCHA-MARAMBIO, L., LAFONTANT, D. E., FARRIS, C. M., MA, Y., URENIA, P. A., NGUYEN, H., ALCALAY, R. N., CHAHINE, L. M., FOROUD, T., GALASKO, D., KIEBURTZ, K., MERCHANT, K., MOLLENHAUER, B., POSTON, K. L., SEIBYL, J., SIMUNI, T., TANNER, C. M., WEINTRAUB, D., VIDENOVIC, A., CHOI, S. H., KURTH, R., CASPELL-GARCIA, C., COFFEY, C. S., FRASIER, M., OLIVEIRA, L. M. A., HUTTEN, S. J., SHERER, T., MAREK, K. & SOTO, C. 2023. Assessment of heterogeneity among participants in the Parkinson's Progression Markers Initiative cohort using α-synuclein seed amplification: a cross-sectional study. *Lancet Neurol,* 22**,** 407-417.
SIDEROWF, A., XIE, S. X., HURTIG, H., WEINTRAUB, D., DUDA, J., CHEN-PLOTKIN, A., SHAW, L. M., VAN DEERLIN, V., TROJANOWSKI, J. Q. & CLARK, C. 2010. CSF amyloid {beta} 1-42 predicts cognitive decline in Parkinson disease. *Neurology,* 75**,** 1055-61.
SILVER, J. M., KRAMER, R., GREENWALD, S. & WEISSMAN, M. 2001. The association between head injuries and psychiatric disorders: findings from the New Haven NIMH Epidemiologic Catchment Area Study. *Brain injury,* 15**,** 935-945.
SIMÕES, R. M., CASTRO CALDAS, A., GRILO, J., CORREIA, D., GUERREIRO, C., PITA LOBO, P., VALADAS, A., FABBRI, M., CORREIA GUEDES, L., COELHO, M., ROSA, M. M., FERREIRA, J. J. & REIMÃO, S. 2020. A distinct neuromelanin magnetic resonance imaging pattern in parkinsonian multiple system atrophy. *BMC Neurol,* 20**,** 432.
SIMUNI, T., CHAHINE, L. M., POSTON, K., BRUMM, M., BURACCHIO, T., CAMPBELL, M., CHOWDHURY, S., COFFEY, C., CONCHA-MARAMBIO, L. & DAM, T. 2024. A biological definition of neuronal α-synuclein disease: towards an integrated staging system for research. *The Lancet Neurology,* 23**,** 178-190.
SIMUNI, T., SIDEROWF, A., LASCH, S., COFFEY, C. S., CASPELL-GARCIA, C., JENNINGS, D., TANNER, C. M., TROJANOWSKI, J. Q., SHAW, L. M., SEIBYL, J., SCHUFF, N.,

SINGLETON, A., KIEBURTZ, K., TOGA, A. W., MOLLENHAUER, B., GALASKO, D., CHAHINE, L. M., WEINTRAUB, D., FOROUD, T., TOSUN, D., POSTON, K., ARNEDO, V., FRASIER, M., SHERER, T., CHOWDHURY, S. & MAREK, K. 2018. Longitudinal Change of Clinical and Biological Measures in Early Parkinson's Disease: Parkinson's Progression Markers Initiative Cohort. *Mov Disord,* 33**,** 771-782.

SIXEL-DÖRING, F., TRAUTMANN, E., MOLLENHAUER, B. & TRENKWALDER, C. 2011. Associated factors for REM sleep behavior disorder in Parkinson disease. *Neurology,* 77**,** 1048-54.

SKAANNING, L. K., SANTORO, A., SKAMRIS, T., MARTINSEN, J. H., D'URSI, A. M., BUCCIARELLI, S., VESTERGAARD, B., BUGGE, K., LANGKILDE, A. E. & KRAGELUND, B. B. 2020. The Non-Fibrillating N-Terminal of α-Synuclein Binds and Co-Fibrillates with Heparin. *Biomolecules,* 10.

SKILBECK, C., THOMAS, M. & HOLM, K. 2019. Predicting mood outcome following traumatic brain injury (TBI): PTA & demographic variables. *Brain Impairment*.

SKRAHINA, V., GABER, H., VOLLSTEDT, E. J., FÖRSTER, T. M., USNICH, T., CURADO, F., BRÜGGEMANN, N., PAUL, J., BOGDANOVIC, X. & ZÜLBAHAR, S. 2021. The Rostock International Parkinson's Disease (ROPAD) study: protocol and initial findings. *Movement Disorders,* 36**,** 1005-1010.

SMITH, C. J., XIONG, G., ELKIND, J. A., PUTNAM, B. & COHEN, A. S. 2015. Brain Injury Impairs Working Memory and Prefrontal Circuit Function. *Front Neurol,* 6**,** 240.

SMITH, R., CAPOTOSTI, F., SCHAIN, M., OHLSSON, T., VOKALI, E., MOLETTE, J., TOUILLOUX, T., HLIVA, V., DIMITRAKOPOULOS, I. K., PUSCHMANN, A., JÖGI, J., SVENNINGSSON, P., ANDRÉASSON, M., SANDIEGO, C., RUSSELL, D. S., MIRANDA-AZPIAZU, P., HALLDIN, C., STOMRUD, E., HALL, S., BRATTEBY, K., TAMPIO L'ESTRADE, E., LUTHI-CARTER, R., PFEIFER, A., KOSCO-VILBOIS, M., STREFFER, J. & HANSSON, O. 2023. The α-synuclein PET tracer [18F] ACI-12589 distinguishes multiple system atrophy from other neurodegenerative diseases. *Nature Communications,* 14**,** 6750.

SOFIC, E., RIEDERER, P., HEINSEN, H., BECKMANN, H., REYNOLDS, G., HEBENSTREIT, G. & YOUDIM, M. 1988. Increased iron (III) and total iron content in post mortem substantia nigra of parkinsonian brain. *Journal of neural transmission,* 74**,** 199-205.

SOMMERAUER, M., FEDOROVA, T. D., HANSEN, A. K., KNUDSEN, K., OTTO, M., JEPPESEN, J., FREDERIKSEN, Y., BLICHER, J. U., GEDAY, J., NAHIMI, A., DAMHOLDT, M. F., BROOKS, D. J. & BORGHAMMER, P. 2018. Evaluation of the noradrenergic system in Parkinson's disease: an 11C-MeNER PET and neuromelanin MRI study. *Brain,* 141**,** 496-504.

SPIEGEL, J., SONE, J. Y., BUTLER, T., PIRRAGLIA, E. & DE LEON, M. J. 2021. Traumatic brain injury accelerates Parkinson's disease onset without altering Parkinson's neuropathology. *Alzheimer's & Dementia,* 17**,** e051517.

STEFANIS, L. 2012. α-Synuclein in Parkinson's disease. *Cold Spring Harbor perspectives in medicine,* 2**,** a009399.

STEFANOVIC, B., DURIC, O., STANKOVIC, S., MIJATOVIC, S., DOKLESTIC, K., JOVANOVIC, B., MARJANOVIC, N. & KALEZIC, N. 2017. Elevated Serum Protein S100B and Neuron Specific Enolase Values as Predictors of Early Neurological

Outcome after Traumatic Brain Injury. *Journal of Medical Biochemistry,* 36**,** 314-321.

STERN, M. B., DOTY, R., DOTTI, M., CORCORAN, P., CRAWFORD, D., MCKEOWN, D., ADLER, C., GOLLOMP, S. & HURTIG, H. 1994. Olfactory function in Parkinson's disease subtypes. *Neurology,* 44**,** 266-266.

STEWART, T., LIU, C., GINGHINA, C., CAIN, K. C., AUINGER, P., CHOLERTON, B., SHI, M. & ZHANG, J. 2014. Cerebrospinal fluid α-synuclein predicts cognitive decline in Parkinson disease progression in the DATATOP cohort. *Am J Pathol,* 184**,** 966-975.

STEYERBERG, E. W., MUSHKUDIANI, N., PEREL, P., BUTCHER, I., LU, J., MCHUGH, G. S., MURRAY, G. D., MARMAROU, A., ROBERTS, I., HABBEMA, J. D. & MAAS, A. I. 2008. Predicting outcome after traumatic brain injury: development and international validation of prognostic scores based on admission characteristics. *PLoS Med,* 5**,** e165; discussion e165.

STIASNY-KOLSTER, K., MAYER, G., SCHÄFER, S., MÖLLER, J. C., HEINZEL-GUTENBRUNNER, M. & OERTEL, W. H. 2007. The REM sleep behavior disorder screening questionnaire--a new diagnostic instrument. *Mov Disord,* 22**,** 2386-93.

SU, E., BELL, M. J., WISNIEWSKI, S. R., ADELSON, P. D., JANESKO-FELDMAN, K. L., SALONIA, R., CLARK, R. S., KOCHANEK, P. M., KAGAN, V. E. & BAYIR, H. 2010. α-Synuclein levels are elevated in cerebrospinal fluid following traumatic brain injury in infants and children: the effect of therapeutic hypothermia. *Dev Neurosci,* 32**,** 385-95.

SULZER, D., BOGULAVSKY, J., LARSEN, K. E., BEHR, G., KARATEKIN, E., KLEINMAN, M. H., TURRO, N., KRANTZ, D., EDWARDS, R. H., GREENE, L. A. & ZECCA, L. 2000. Neuromelanin biosynthesis is driven by excess cytosolic catecholamines not accumulated by synaptic vesicles. *Proc Natl Acad Sci U S A,* 97**,** 11869-74.

SUWIJN, S. R., VAN BOHEEMEN, C. J., DE HAAN, R. J., TISSINGH, G., BOOIJ, J. & DE BIE, R. M. 2015. The diagnostic accuracy of dopamine transporter SPECT imaging to detect nigrostriatal cell loss in patients with Parkinson's disease or clinically uncertain parkinsonism: a systematic review. *EJNMMI research,* 5**,** 1-8.

SUZUKI, K., OKUMA, Y., UCHIYAMA, T., MIYAMOTO, M., SAKAKIBARA, R., SHIMO, Y., HATTORI, N., KUWABARA, S., YAMAMOTO, T. & KAJI, Y. 2017. Impact of sleep-related symptoms on clinical motor subtypes and disability in Parkinson's disease: a multicentre cross-sectional study. *Journal of Neurology, Neurosurgery & Psychiatry*.

SVINGOS, A. M., ROBICSEK, S. A., HAYES, R. L., WANG, K. K., ROBERTSON, C. S., BROPHY, G. M., PAPA, L., GABRIELLI, A., HANNAY, H. J., BAUER, R. M. & HEATON, S. C. 2022. Predicting Clinical Outcomes 7-10 Years after Severe Traumatic Brain Injury: Exploring the Prognostic Utility of the IMPACT Lab Model and Cerebrospinal Fluid UCH-L1 and MAP-2. *Neurocritical Care,* 37**,** 172-183.

TAI, Y. F., AHSAN, R. L., DE YÉBENES, J. G., PAVESE, N., BROOKS, D. J. & PICCINI, P. 2007. Characterization of dopaminergic dysfunction in familial progressive supranuclear palsy: an 18F-dopa PET study. *J Neural Transm (Vienna),* 114**,** 337-40.

TAN, S., HARTONO, S., WELTON, T., ANN, C. N., LIM, S. L., KOH, T. S., LI, H., SETIAWAN, F., NG, S., CHIA, N., LIU, S., MARK HAACKE, E., KING TAN, E., CHEW SENG TAN, L. & LING CHAN, L. 2021. Utility of quantitative susceptibility mapping and

diffusion kurtosis imaging in the diagnosis of early Parkinson's disease. *Neuroimage Clin,* 32**,** 102831.
TANG, Y., LI, L., HU, T., JIAO, F., HAN, L., LI, S., XU, Z., FAN, Y., SUN, Y., LIU, F., YEN, T. C., ZUO, C. & WANG, J. 2023. In Vivo (18) F-Florzolotau Tau Positron Emission Tomography Imaging in Parkinson's Disease Dementia. *Mov Disord,* 38**,** 147-152.
TANNER, C. Follow-up of persons with neurologic diseases (FOUND): Up to 46 months follow-up. MOVEMENT DISORDERS, 2006. WILEY-LISS DIV JOHN WILEY & SONS INC, 111 RIVER ST, HOBOKEN, NJ 07030 USA, S127-S127.
TANNER, C. M. & GOLDMAN, S. M. 1996. Epidemiology of Parkinson's disease. *Neurol Clin,* 14**,** 317-35.
TATENO, A., JORGE, R. E. & ROBINSON, R. G. 2003. Clinical correlates of aggressive behavior after traumatic brain injury. *The Journal of neuropsychiatry and clinical neurosciences,* 15**,** 155-160.
TEAM, R. D. C. 2010. R: A language and environment for statistical computing. *(No Title)*.
TEASDALE, G. & JENNETT, B. 1974. Assessment of coma and impaired consciousness: a practical scale. *The Lancet,* 304**,** 81-84.
THELIN, E. P., JEPPSSON, E., FROSTELL, A., SVENSSON, M., MONDELLO, S., BELLANDER, B. M. & NELSON, D. W. 2016. Utility of neuron-specific enolase in traumatic brain injury; relations to S100B levels, outcome, and extracranial injury severity. *Crit Care,* 20**,** 285.
THELIN, E. P., JOHANNESSON, L., NELSON, D. & BELLANDER, B. M. 2013. S100B Is an Important Outcome Predictor in Traumatic Brain Injury. *Journal of Neurotrauma,* 30**,** 519-528.
THIRUCHELVAM, M., BROCKEL, B., RICHFIELD, E. K., BAGGS, R. B. & CORY-SLECHTA, D. A. 2000a. Potentiated and preferential effects of combined paraquat and maneb on nigrostriatal dopamine systems: environmental risk factors for Parkinson's disease? *Brain research,* 873**,** 225-234.
THIRUCHELVAM, M., MCCORMACK, A., RICHFIELD, E. K., BAGGS, R. B., TANK, A. W., DI MONTE, D. A. & CORY-SLECHTA, D. A. 2003. Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. *European Journal of Neuroscience,* 18**,** 589-600.
THIRUCHELVAM, M., RICHFIELD, E. K., BAGGS, R. B., TANK, A. W. & CORY-SLECHTA, D. A. 2000b. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. *Journal of Neuroscience,* 20**,** 9207-9214.
THOMAS, G. E. C., HANNAWAY, N., ZARKALI, A., SHMUELI, K. & WEIL, R. S. 2024. Longitudinal Associations of Magnetic Susceptibility with Clinical Severity in Parkinson's Disease. *Mov Disord,* 39**,** 546-559.
THOMAS, G. E. C., LEYLAND, L. A., SCHRAG, A. E., LEES, A. J., ACOSTA-CABRONERO, J. & WEIL, R. S. 2020. Brain iron deposition is linked with cognitive severity in Parkinson's disease. *J Neurol Neurosurg Psychiatry,* 91**,** 418-425.
TOMITA, K., NAKADA, T., OSHIMA, T., MOTOSHIMA, T., KAWAGUCHI, R. & ODA, S. 2019. Tau protein as a diagnostic marker for diffuse axonal injury. *Plos One,* 14**,** 9.
TRINH, J., GUELLA, I. & FARRER, M. J. 2014. Disease penetrance of late-onset parkinsonism: a meta-analysis. *JAMA neurology,* 71**,** 1535-1539.
TRNKA, S., STEJSKAL, P., JABLONSKY, J., KRAHULIK, D., POHLODEK, D. & HRABALEK, L. 2023. S100B protein as a biomarker and predictor in traumatic brain injury.

Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia.

TSALENCHUK, M., GENTLEMAN, S. M. & MARZI, S. J. 2023. Linking environmental risk factors with epigenetic mechanisms in Parkinson's disease. *NPJ Parkinsons Dis,* 9**,** 123.

TURKSTRA, L. S., HOSSEINI-MOGHADDAM, S., WOHLTJEN, S., NURRE, S. V., MUTLU, B. & DUFF, M. C. 2023. Facial affect recognition in context in adults with and without TBI. *Frontiers in Psychology,* 14.

UMEH, C. C., SZABO, Z., PONTONE, G. M. & MARI, Z. 2013. Dopamine transporter imaging in psychogenic parkinsonism and neurodegenerative parkinsonism with psychogenic overlay: a report of three cases. *Tremor and Other Hyperkinetic Movements,* 3.

UNDÉN, J. & ROMNER, B. 2010. Can low serum levels of S100B predict normal CT findings after minor head injury in adults?: an evidence-based review and meta-analysis. *J Head Trauma Rehabil,* 25**,** 228-40.

URYU, K., CHEN, X. H., MARTINEZ, D., BROWNE, K. D., JOHNSON, V. E., GRAHAM, D. I., LEE, V. M., TROJANOWSKI, J. Q. & SMITH, D. H. 2007. Multiple proteins implicated in neurodegenerative diseases accumulate in axons after brain trauma in humans. *Exp Neurol,* 208**,** 185-92.

VAN BEILEN, M., PORTMAN, A. T., KIERS, H. A., MAGUIRE, R. P., KAASINEN, V., KONING, M., PRUIM, J. & LEENDERS, K. L. 2008. Striatal FDOPA uptake and cognition in advanced non-demented Parkinson's disease: a clinical and FDOPA-PET study. *Parkinsonism Relat Disord,* 14**,** 224-8.

VAN DEN HEUVEL, L., MEINDERS, M. J., POST, B., BLOEM, B. R. & STIGGELBOUT, A. M. 2022. Personalizing decision-making for persons with Parkinson's disease: where do we stand and what to improve? *Journal of Neurology,* 269**,** 3569-3578.

VAN DEN NOORTGATE, W., LÓPEZ-LÓPEZ, J. A., MARÍN-MARTÍNEZ, F. & SÁNCHEZ-MECA, J. 2013. Three-level meta-analysis of dependent effect sizes. *Behavior research methods,* 45**,** 576-594.

VAN DER HEEDEN, J. F., MARINUS, J., MARTINEZ-MARTIN, P., RODRIGUEZ-BLAZQUEZ, C., GERAEDTS, V. J. & VAN HILTEN, J. J. 2016. Postural instability and gait are associated with severity and prognosis of Parkinson disease. *Neurology,* 86**,** 2243-2250.

VAN DER HEIDE, A., MEINDERS, M. J., SPECKENS, A. E., PEERBOLTE, T. F., BLOEM, B. R. & HELMICH, R. C. 2021. Stress and mindfulness in Parkinson's disease: clinical effects and potential underlying mechanisms. *Movement Disorders,* 36**,** 64-70.

VAN DIJK, K. D., BIDINOSTI, M., WEISS, A., RAIJMAKERS, P., BERENDSE, H. W. & VAN DE BERG, W. D. 2014. Reduced α-synuclein levels in cerebrospinal fluid in Parkinson's disease are unrelated to clinical and imaging measures of disease severity. *Eur J Neurol,* 21**,** 388-94.

VAN LAAR, A. D., WEBB, K. R., KEENEY, M. T., VAN LAAR, V. S., ZHARIKOV, A., BURTON, E. A., HASTINGS, T. G., GLAJCH, K. E., HIRST, W. D. & GREENAMYRE, J. T. 2023. Transient exposure to rotenone causes degeneration and progressive parkinsonian motor deficits, neuroinflammation, and synucleinopathy. *npj Parkinson's Disease,* 9**,** 121.

VAN REEKUM, R., COHEN, T. & WONG, J. 2000. Can traumatic brain injury cause psychiatric disorders? *The Journal of neuropsychiatry and clinical neurosciences,* 12**,** 316-327.
VARÇIN, M., BENTEA, E., MICHOTTE, Y. & SARRE, S. 2012. Oxidative stress in genetic mouse models of Parkinson's disease. *Oxid Med Cell Longev,* 2012**,** 624925.
VAUPEL, J. W. 2010. Biodemography of human ageing. *Nature,* 464**,** 536-542.
VIECHTBAUER, W. & VIECHTBAUER, M. W. 2015. Package 'metafor'. *The Comprehensive R Archive Network. Package 'metafor'. http://cran. r-project. org/web/packages/metafor/metafor. pdf*.
VILE, A. R., JANG, K., GOURLAY, D. & MARSHMAN, L. A. G. 2022. Posttraumatic Amnesia: A Systematic Review and Meta-Analysis. Proposal for a New Severity Classification. *World Neurosurgery,* 162**,** e369-e393.
VISSER, E., KEUKEN, M. C., FORSTMANN, B. U. & JENKINSON, M. 2016. Automated segmentation of the substantia nigra, subthalamic nucleus and red nucleus in 7T data at young and old age. *Neuroimage,* 139**,** 324-336.
VIVACQUA, G., LATORRE, A., SUPPA, A., NARDI, M., PIETRACUPA, S., MANCINELLI, R., FABBRINI, G., COLOSIMO, C., GAUDIO, E. & BERARDELLI, A. 2016. Abnormal Salivary Total and Oligomeric Alpha-Synuclein in Parkinson's Disease. *PLoS One,* 11**,** e0151156.
VIVACQUA, G., SUPPA, A., MANCINELLI, R., BELVISI, D., FABBRINI, A., COSTANZO, M., FORMICA, A., ONORI, P., FABBRINI, G. & BERARDELLI, A. 2019. Salivary alpha-synuclein in the diagnosis of Parkinson's disease and Progressive Supranuclear Palsy. *Parkinsonism Relat Disord,* 63**,** 143-148.
VOLKOW, N. D., DING, Y. S., FOWLER, J. S., WANG, G. J., LOGAN, J., GATLEY, S. J., HITZEMANN, R., SMITH, G., FIELDS, S. D. & GUR, R. 1996. Dopamine transporters decrease with age. *J Nucl Med,* 37**,** 554-9.
VOLKOW, N. D., GUR, R. C., WANG, G. J., FOWLER, J. S., MOBERG, P. J., DING, Y. S., HITZEMANN, R., SMITH, G. & LOGAN, J. 1998. Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. *Am J Psychiatry,* 155**,** 344-9.
VOLPICELLI-DALEY, L. A., LUK, K. C., PATEL, T. P., TANIK, S. A., RIDDLE, D. M., STIEBER, A., MEANEY, D. F., TROJANOWSKI, J. Q. & LEE, V. M.-Y. 2011. Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron,* 72**,** 57-71.
VOORMOLEN, D. C., POLINDER, S., VON STEINBUECHEL, N., VOS, P. E., CNOSSEN, M. C. & HAAGSMA, J. A. 2019. The association between post-concussion symptoms and health-related quality of life in patients with mild traumatic brain injury. *Injury,* 50**,** 1068-1074.
VOS, P. E., JACOBS, B., ANDRIESSEN, T. M., LAMERS, K. J., BORM, G. F., BEEMS, T., EDWARDS, M., ROSMALEN, C. F. & VISSERS, J. L. 2010. GFAP and S100B are biomarkers of traumatic brain injury: an observational cohort study. *Neurology,* 75**,** 1786-93.
VOS, P. E., LAMERS, K. J. B., HENDRIKS, J. C. M., VAN HAAREN, M., BEEMS, T., ZIMMERMAN, C., VAN GEEL, W., DE REUS, H., BIERT, J. & VERBEEK, M. M. 2004. Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. *Neurology,* 62**,** 1303-1310.

WAGNER, A. K., SCANLON, J. M., BECKER, C. R., RITTER, A. C., NIYONKURU, C., DIXON, C. E., CONLEY, Y. P. & PRICE, J. C. 2014. The influence of genetic variants on striatal dopamine transporter and D2 receptor binding after TBI. *J Cereb Blood Flow Metab,* 34**,** 1328-39.

WAKABAYASHI, K., TANJI, K., ODAGIRI, S., MIKI, Y., MORI, F. & TAKAHASHI, H. 2013. The Lewy body in Parkinson's disease and related neurodegenerative disorders. *Molecular neurobiology,* 47**,** 495-508.

WALDER, B., ROBIN, X., REBETEZ, M. M. L., COPIN, J. C., GASCHE, Y., SANCHEZ, J. C. & TURCK, N. 2013. The Prognostic Significance of the Serum Biomarker Heart-Fatty Acidic Binding Protein in Comparison with S100b in Severe Traumatic Brain Injury. *Journal of Neurotrauma,* 30**,** 1631-1637.

WALKER, Q. D., ROONEY, M. B., WIGHTMAN, R. M. & KUHN, C. M. 2000. Dopamine release and uptake are greater in female than male rat striatum as measured by fast cyclic voltammetry. *Neuroscience,* 95**,** 1061-70.

WALKER, W. C. & PICKETT, T. C. 2007. Motor impairment after severe traumatic brain injury: A longitudinal multicenter study. *Journal of Rehabilitation Research & Development,* 44.

WALLERT, E., LETORT, E., VAN DER ZANT, F., WINOGRODZKA, A., BERENDSE, H., BEUDEL, M., DE BIE, R., BOOIJ, J., RAIJMAKERS, P. & VAN DE GIESSEN, E. 2022. Comparison of [18F]-FDOPA PET and [123I]-FP-CIT SPECT acquired in clinical practice for assessing nigrostriatal degeneration in patients with a clinically uncertain parkinsonian syndrome. *EJNMMI research,* 12**,** 68.

WANG, J., LI, J., HAN, L., GUO, S., WANG, L., XIONG, Z., CHEN, Z., CHEN, W. & LIANG, J. 2016. Serum τ protein as a potential biomarker in the assessment of traumatic brain injury. *Exp Ther Med,* 11**,** 1147-1151.

WANG, J., LI, Y., HUANG, Z., WAN, W., ZHANG, Y., WANG, C., CHENG, X., YE, F., LIU, K., FEI, G., ZENG, M. & JIN, L. 2018a. Neuromelanin-sensitive magnetic resonance imaging features of the substantia nigra and locus coeruleus in de novo Parkinson's disease and its phenotypes. *Eur J Neurol,* 25**,** 949-e73.

WANG, K. K., MUNOZ-PAREJA, J. C., LAUTENSLAGER, L. A., TYNDALL, J. A., YANG, Z., KERRIGAN, M. R., DIAZ-ARRASTIA, R., KORLEY, F. K., OKONKWO, D. & PUCCIO, A. M. 2023a. Diagnostic performance of point-of-care ubiquitin carboxy-terminal Hydrolase-L1 assay in distinguishing imaging abnormalities in traumatic brain injury: A TRACK-TBI cohort study. *Advances in Biomarker Sciences and Technology,* 5**,** 38-49.

WANG, K. K., YANG, Z., ZHU, T., SHI, Y., RUBENSTEIN, R., TYNDALL, J. A. & MANLEY, G. T. 2018b. An update on diagnostic and prognostic biomarkers for traumatic brain injury. *Expert review of molecular diagnostics,* 18**,** 165-180.

WANG, K. Y., YU, G. F., ZHANG, Z. Y., HUANG, Q. & DONG, X. Q. 2012a. Plasma high-mobility group box 1 levels and prediction of outcome in patients with traumatic brain injury. *Clinica Chimica Acta,* 413**,** 1737-1741.

WANG, L., YAN, Y., ZHANG, L., LIU, Y., LUO, R. & CHANG, Y. 2021. Substantia nigra neuromelanin magnetic resonance imaging in patients with different subtypes of Parkinson disease. *J Neural Transm (Vienna),* 128**,** 171-179.

WANG, S., WU, T., CAI, Y., YU, Y., CHEN, X. & WANG, L. 2023b. Neuromelanin magnetic resonance imaging of substantia nigra and locus coeruleus in Parkinson's disease with freezing of gait. *Front Aging Neurosci,* 15**,** 1060935.

WANG, W., JIN, J., HE, R. & GONG, H. 2017. Gender differences in pesticide use knowledge, risk awareness and practices in Chinese farmers. *Science of the Total Environment,* 590**,** 22-28.
WANG, X., HUANG, P., HAACKE, E. M., LIU, Y., ZHANG, Y., JIN, Z., LI, Y., XU, Q., LIU, P., CHEN, S., HE, N. & YAN, F. 2023c. Locus coeruleus and substantia nigra neuromelanin magnetic resonance imaging differentiates Parkinson's disease and essential tremor. *Neuroimage Clin,* 38**,** 103420.
WANG, Y., BUTROS, S. R., SHUAI, X., DAI, Y., CHEN, C., LIU, M., HAACKE, E. M., HU, J. & XU, H. 2012b. Different iron-deposition patterns of multiple system atrophy with predominant parkinsonism and idiopathetic Parkinson diseases demonstrated by phase-corrected susceptibility-weighted imaging. *AJNR Am J Neuroradiol,* 33**,** 266-73.
WATT, S. E., SHORES, E. A., BAGULEY, I. J., DORSCH, N. & FEARNSIDE, M. R. 2006. Protein S-100 and neuropsychological functioning following severe traumatic brain injury. *Brain Inj,* 20**,** 1007-17.
WEINREB, P. H., ZHEN, W., POON, A. W., CONWAY, K. A. & LANSBURY, P. T., JR. 1996. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry,* 35**,** 13709-15.
WEINSHENKER, D. 2018. Long Road to Ruin: Noradrenergic Dysfunction in Neurodegenerative Disease. *Trends Neurosci,* 41**,** 211-223.
WEINTRAUB, S., BESSER, L., DODGE, H. H., TEYLAN, M., FERRIS, S., GOLDSTEIN, F. C., GIORDANI, B., KRAMER, J., LOEWENSTEIN, D. & MARSON, D. 2018. Version 3 of the Alzheimer Disease Centers' neuropsychological test battery in the Uniform Data Set (UDS). *Alzheimer Disease & Associated Disorders,* 32**,** 10-17.
WEINTRAUB, S., SALMON, D., MERCALDO, N., FERRIS, S., GRAFF-RADFORD, N. R., CHUI, H., CUMMINGS, J., DECARLI, C., FOSTER, N. L. & GALASKO, D. 2009. The Alzheimer's disease centers' Uniform Data Set (UDS): The neuropsychologic test battery. *Alzheimer Disease & Associated Disorders,* 23**,** 91-101.
WERRY, E. L., BRIGHT, F. M., PIGUET, O., ITTNER, L. M., HALLIDAY, G. M., HODGES, J. R., KIERNAN, M. C., LOY, C. T., KRIL, J. J. & KASSIOU, M. 2019. Recent Developments in TSPO PET Imaging as A Biomarker of Neuroinflammation in Neurodegenerative Disorders. *Int J Mol Sci,* 20.
WHO, A. 2020. WHO methods and data sources for life tables 1990–2019. World Health Organization Geneva.
WIESMANN, M., STEINMEIER, E., MAGERKURTH, O., LINN, J., GOTTMANN, D. & MISSLER, U. 2010. Outcome prediction in traumatic brain injury: comparison of neurological status, CT findings, and blood levels of S100B and GFAP. *Acta Neurologica Scandinavica,* 121**,** 178-185.
WILLIAMS-GRAY, C. H., MASON, S. L., EVANS, J. R., FOLTYNIE, T., BRAYNE, C., ROBBINS, T. W. & BARKER, R. A. 2013. The CamPaIGN study of Parkinson's disease: 10-year outlook in an incident population-based cohort. *Journal of Neurology, Neurosurgery & Psychiatry,* 84**,** 1258-1264.
WILLIAMSON, M. L., ELLIOTT, T. R., BOGNER, J., DREER, L. E., ARANGO-LASPRILLA, J. C., KOLAKOWSKY-HAYNER, S. A., PRETZ, C. R., LEQUERICA, A. & PERRIN, P. B. 2016. Trajectories of Life Satisfaction Over the First 10 Years After Traumatic Brain Injury: Race, Gender, and Functional Ability. *J Head Trauma Rehabil,* 31**,** 167-79.

WILLS, J., CREDLE, J., OAKS, A. W., DUKA, V., LEE, J.-H., JONES, J. & SIDHU, A. 2012. Paraquat, but not maneb, induces synucleinopathy and tauopathy in striata of mice through inhibition of proteasomal and autophagic pathways. *PloS one,* 7**,** e30745.

WILSON, L., BOASE, K., NELSON, L. D., TEMKIN, N. R., GIACINO, J. T., MARKOWITZ, A. J., MAAS, A., MENON, D. K., TEASDALE, G. & MANLEY, G. T. 2021. A manual for the glasgow outcome scale-extended interview. *Journal of neurotrauma,* 38**,** 2435-2446.

WINNER, B., JAPPELLI, R., MAJI, S. K., DESPLATS, P. A., BOYER, L., AIGNER, S., HETZER, C., LOHER, T., VILAR, M. & CAMPIONI, S. 2011. In vivo demonstration that α-synuclein oligomers are toxic. *Proceedings of the National Academy of Sciences,* 108**,** 4194-4199.

WISE, R. A. 2009. Roles for nigrostriatal—not just mesocorticolimbic—dopamine in reward and addiction. *Trends in neurosciences,* 32**,** 517-524.

WITT, S. T., LOVEJOY, D. W., PEARLSON, G. D. & STEVENS, M. C. 2010. Decreased prefrontal cortex activity in mild traumatic brain injury during performance of an auditory oddball task. *Brain Imaging Behav,* 4**,** 232-47.

WOERTGEN, C., ROTHOERL, R. D. & BRAWANSKI, A. 2002. Early S-100B serum level correlates to quality of life in patients after severe head injury. *Brain Injury,* 16**,** 807-816.

WOERTGEN, C., ROTHOERL, R. D., HOLZSCHUH, M., METZ, C. & BRAWANSKI, A. 1997. Comparison of serial S-100 and NSE serum measurements after severe head injury. *Acta Neurochirurgica,* 139**,** 1161-1165.

WOERTGEN, C., ROTHOERL, R. D., METZ, C. & BRAWANSKI, A. 1999. Comparison of clinical, radiologic, and serum marker as prognostic factors after severe head injury. *J Trauma,* 47**,** 1126-30.

WOODS, D. L., KISHIYAMAA, M. M., LUND, E. W., HERRON, T. J., EDWARDS, B., POLIVA, O., HINK, R. F. & REED, B. 2011. Improving digit span assessment of short-term verbal memory. *J Clin Exp Neuropsychol,* 33**,** 101-11.

WU, Y., LE, W. & JANKOVIC, J. 2011. Preclinical biomarkers of Parkinson disease. *Archives of neurology,* 68**,** 22-30.

WUNDERLICH, M. T., TOWNEND, W., MARTIN, B., YATES, D. & GUY, M. 2003. Head injury outcome prediction in the emergency department: A role for protein S-100b? [9]. *Journal of Neurology Neurosurgery and Psychiatry,* 74**,** 827-828.

XIANG, J., ZHANG, Z. & YE, K. 2023. A promising PET tracer candidate targeting α-synuclein inclusions. *Clin Transl Med,* 13**,** e1408.

XIONG, W., LI, L.-F., HUANG, L., LIU, Y., XIA, Z.-C., ZHOU, X.-X., TANG, B.-S., GUO, J.-F. & LEI, L.-F. 2020. Different iron deposition patterns in akinetic/rigid-dominant and tremor-dominant Parkinson's disease. *Clinical neurology and neurosurgery,* 198**,** 106181.

XIONG, X., HE, H., YE, Q., QIAN, S., ZHOU, S., FENG, F., FANG, E. F. & XIE, C. 2023. Alzheimer's disease diagnostic accuracy by fluid and neuroimaging ATN framework. *CNS Neurosci Ther*.

YAMAZAKI, Y., YADA, K., MORII, S., KITAHARA, T. & OHWADA, T. 1995. Diagnostic significance of serum neuron-specific enolase and myelin basic protein assay in patients with acute head injury. *Surg Neurol,* 43**,** 267-70; discussion 270-1.

YAMIN, G. & TEPLOW, D. B. 2017. Pittsburgh Compound-B (PiB) binds amyloid β-protein protofibrils. *J Neurochem,* 140**,** 210-215.
YAN, S., JIANG, C., JANZEN, A., BARBER, T. R., SEGER, A., SOMMERAUER, M., DAVIS, J. J., MAREK, K., HU, M. T., OERTEL, W. H. & TOFARIS, G. K. 2024. Neuronally Derived Extracellular Vesicle α-Synuclein as a Serum Biomarker for Individuals at Risk of Developing Parkinson Disease. *JAMA Neurol,* 81**,** 59-68.
YANG, W., HAMILTON, J. L., KOPIL, C., BECK, J. C., TANNER, C. M., ALBIN, R. L., RAY DORSEY, E., DAHODWALA, N., CINTINA, I., HOGAN, P. & THOMPSON, T. 2020. Current and projected future economic burden of Parkinson's disease in the U.S. *NPJ Parkinsons Dis,* 6**,** 15.
YASUNO, F., OTA, M., KOSAKA, J., ITO, H., HIGUCHI, M., DORONBEKOV, T. K., NOZAKI, S., FUJIMURA, Y., KOEDA, M., ASADA, T. & SUHARA, T. 2008. Increased binding of peripheral benzodiazepine receptor in Alzheimer's disease measured by positron emission tomography with [11C]DAA1106. *Biol Psychiatry,* 64**,** 835-41.
YINGYING, Z. 2015. Potential influencing factors of time from onset to clinical diagnosis and misdiagnosis rate of Parkinson's patients in Shanghai. *Chin J Neurol,* 48**,** 995-999.
ZAMAN, V., SHIELDS, D. C., SHAMS, R., DRASITES, K. P., MATZELLE, D., HAQUE, A. & BANIK, N. L. 2021. Cellular and molecular pathophysiology in the progression of Parkinson's disease. *Metabolic brain disease,* 36**,** 815-827.
ZAREI, H., DIZAJI, S. R., TOLOUI, A., YOUSEFIFARD, M. & ESMAEILI, A. 2024. Diagnostic and Prognostic Values of S100B versus Neuron Specific Enolase for Traumatic Brain Injury; a Systematic Review and Meta-analysis. *Archives of Academic Emergency Medicine,* 12.
ZAREI, H., VAZIRIZADEH-MAHABADI, M., ADEL RAMAWAD, H., SARVEAZAD, A. & YOUSEFIFARD, M. 2023. Prognostic Value of CRASH and IMPACT Models for Predicting Mortality and Unfavorable Outcome in Traumatic Brain Injury; a Systematic Review and Meta-Analysis. *Arch Acad Emerg Med,* 11**,** e27.
ZAROW, C., LYNESS, S. A., MORTIMER, J. A. & CHUI, H. C. 2003. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. *Arch Neurol,* 60**,** 337-41.
ZARZAUR, B. L., BELL, T. M. & ZANSKAS, S. A. 2017. Resiliency and quality of life trajectories after injury. *J Trauma Acute Care Surg,* 82**,** 939-945.
ZECCA, L., STROPPOLO, A., GATTI, A., TAMPELLINI, D., TOSCANI, M., GALLORINI, M., GIAVERI, G., AROSIO, P., SANTAMBROGIO, P., FARIELLO, R. G., KARATEKIN, E., KLEINMAN, M. H., TURRO, N., HORNYKIEWICZ, O. & ZUCCA, F. A. 2004. The role of iron and copper molecules in the neuronal vulnerability of locus coeruleus and substantia nigra during aging. *Proc Natl Acad Sci U S A,* 101**,** 9843-8.
ZEMLAN, F. P., JAUCH, E. C., MULCHAHEY, J. J., GABBITA, S. P., ROSENBERG, W. S., SPECIALE, S. G. & ZUCCARELLO, M. 2002. C-tau biomarker of neuronal damage in severe brain injured patients: association with elevated intracranial pressure and clinical outcome. *Brain Research,* 947**,** 131-139.
ZENG, W., CAI, J., ZHANG, L. & PENG, Q. 2024. Iron Deposition in Parkinson's Disease: A Mini-Review. *Cellular and Molecular Neurobiology,* 44**,** 26.
ZHANG, J., FITSANAKIS, V. A., GU, G., JING, D., AO, M., AMARNATH, V. & MONTINE, T. J. 2003. Manganese ethylene-bis-dithiocarbamate and selective dopaminergic

neurodegeneration in rat: a link through mitochondrial dysfunction. *J Neurochem,* 84**,** 336-46.
ZHANG, J., JIN, J., SU, D., FENG, T. & ZHAO, H. 2023. Tau-PET imaging in Parkinson's disease: a systematic review and meta-analysis. *Front Neurol,* 14**,** 1145939.
ZHANG, J., LI, X. & LI, J.-D. 2019a. The roles of post-translational modifications on α-synuclein in the pathogenesis of Parkinson's diseases. *Frontiers in neuroscience,* 13**,** 381.
ZHANG, Y., SHU, L., SUN, Q., PAN, H., GUO, J. & TANG, B. 2018. A comprehensive analysis of the association between SNCA polymorphisms and the risk of Parkinson's disease. *Frontiers in Molecular Neuroscience,* 11**,** 391.
ZHANG, Z. Y., LI, J., YE, Q., DONG, Y., BAO, G. M., SHEN, Y. K., WENG, J. F., LUO, L. F. & CEN, M. 2019b. Usefulness of serum interleukin-33 as a prognostic marker of severe traumatic brain injury. *Clinica Chimica Acta,* 497**,** 6-12.
ZHANG, Z. Y., ZHANG, L. X., DONG, X. Q., YU, W. H., DU, Q., YANG, D. B., SHEN, Y. F., WANG, H., ZHU, Q., CHE, Z. H., LIU, Q. J., JIANG, L. & DU, Y. F. 2014. Comparison of the performances of copeptin and multiple biomarkers in long-term prognosis of severe traumatic brain injury. *Peptides,* 60**,** 13-17.
ZHAO, N., YANG, Y., ZHANG, L., ZHANG, Q., BALBUENA, L., UNGVARI, G. S., ZANG, Y. F. & XIANG, Y. T. 2021. Quality of life in Parkinson's disease: A systematic review and meta-analysis of comparative studies. *CNS Neurosci Ther,* 27**,** 270-279.
ZUO, L.-J., PIAO, Y.-S., LI, L.-X., YU, S.-Y., GUO, P., HU, Y., LIAN, T.-H., WANG, R.-D., YU, Q.-J. & JIN, Z. 2017. Phenotype of postural instability/gait difficulty in Parkinson disease: relevance to cognitive impairment and mechanism relating pathological proteins and neurotransmitters. *Scientific reports,* 7**,** 44872.