Yahweh your healer

“Yahweh your healer” Exodus 15v26
The Australian Cerebral Palsy Research Study – Epidemiological and Genetic Associations with Cerebral Palsy

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Abstract

Introduction

Twenty two mostly small studies have reported associations between cerebral palsy (CP) and specific single nucleotide polymorphisms (SNPs). These data require prospective confirmation in a large cohort. Only one study has examined maternal genetic risk factors for CP. The current large study of mothers and children examines the contributions of genetic and epidemiological factors to CP and their interactions.

Methods

Caucasian children aged between five and 18 years who were born in Australia were recruited with their mothers. Results from 587 case pairs and 1,154 control pairs were analysed. Each mother and child provided DNA using buccal swabs. Multiplex PCR was used to genotype individuals and 35 specific SNPs were included in the analysis. These candidate SNPs have been putatively associated with thrombophilia, inflammation and preterm birth. Mothers completed a health, pregnancy and delivery questionnaire. State perinatal data for each participant provided further epidemiological data, while CP registers provided cerebral palsy diagnosis data. Univariable analysis examined each epidemiological and genetic risk factor individually with Bonferroni correction for multiple testing. Subsequent multivariable analyses were performed combining risks and examining interactions. Odds ratio (OR) and 95% confidence intervals are reported.

Results

Univariable analysis of SNP associations with CP did not confirm the majority of associations reported in the literature after correction for multiple testing. Prothrombin gene mutation in the child remained associated with hemiplegia in term delivered infants where a maternal infection during pregnancy was reported (OR 4.52, 1.70-12.03, p=0.059 after Bonferroni correction). Epidemiological associations with CP included maternal infection during pregnancy
(OR 1.55, 1.26-1.91), small for gestational age (<10th centile, OR 4.35, 2.92-6.48), gestational age <32 weeks (OR 59.20, 28.87-121.38), multiple birth (OR 6.62, 4.00-10.95), a relative with CP (OR 1.61, 1.12-2.32) and male gender (OR 1.68, 1.38-2.06). Iatrogenic heat in labour did not increase the risk of CP. Multivariable analyses of genetic and epidemiological risk factors identified significant associations of CP with male gender (OR 1.5, 1.1-2.1), reported maternal infection (OR 1.9, 1.2-3.0), a relative with CP (OR 1.8, 1.1-2.9) and maternal carriage of TGF-β1-509 (OR 1.3, 1.0-1.6). Subtype analyses showed an increased risk of quadriplegic CP with a family history of CP (OR 3.27, 1.13-9.45). The interaction of maternal TNF-α 308 with infection was inversely related to CP (OR 0.7, 0.5-0.9).

Discussion

Most SNP associations in the literature were not confirmed by this study, probably because those studies did not correct for multiple testing. The study confirmed the reported epidemiological associations with CP in the literature. Combined multivariable analyses suggest an association of some maternal and fetal genotypes with CP outcome, particularly when an infection was reported. These results require further study and the mechanism of association is yet to be determined.

Conclusions

The individual SNPs studied are unlikely to play a large role in CP causation, although maternal SNPs and interactions with infection may be significant. Specific subtypes of CP (particularly term born quadriplegia) are more likely to have a genetic origin than other types. This study has stimulated new genetic and environmental studies of CP.
Statement of declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Michael E O’Callaghan 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.

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M.E. O’Callaghan, March 2011.
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Publications arising from this thesis

1. O’Callaghan ME, MacLennan AH, Haan EA, Dekker G; South Australian Cerebral Palsy Research Group.


This paper was invited by the editors to be part of a special edition of Human Genetics on Neurological and Psychiatric Diseases and Traits.

2. O’Callaghan ME, MacLennan AH, Gibson CS, McMichael GL, Haan EA, Broadbent J, Priest K, Goldwater PN, Dekker GA.

The Australian Cerebral Palsy Research Study - Protocol for a National Collaborative Study Investigating Genomic and Clinical Associations with Cerebral Palsy.

Publications submitted or in preparation


   Fetal and Maternal Candidate SNP Associations with Cerebral Palsy in a Large Case-Control Study.

   *Manuscript submitted March 2011.*


   Epidemiological Risk Factors Associated with Cerebral Palsy in a Large Case-Control Study.

   *Manuscript in preparation.*


   A Multivariable Model of Cerebral Palsy Prediction Utilising Genetic and Epidemiological Risk Factors.

   *Manuscript in preparation.*
Publications relating to this study but not included in this thesis

1. McMichael GL, Gibson CS, O’Callaghan ME, Goldwater PN, Dekker GA, Haan EA, MacLennan AH; South Australian Cerebral Palsy Research Group.
   DNA from Buccal Swabs Suitable for High-throughput SNP Multiplex Analysis.

   Comparison of DNA Extraction Methods from Small Samples of Newborn Screening Cards Suitable for Retrospective Perinatal Viral Research.
   *Journal of Biomolecular Techniques*. Accepted manuscript.

   Maternal Recollection of Perinatal Details over Time – A Comparison of Retrospective Questionnaires and State Held Data in an Australian Setting.
   *Manuscript in preparation*.

   Fetal and Maternal Candidate SNP Associations with Adverse Pregnancy Outcomes in a Large Case-Control Study.
   *Manuscript in preparation*.
Conference abstracts


   Australian Collaborative Study of Genomic and Environmental Factors Associated with Cerebral Palsy. Poster presentation, The 3rd International Cerebral Palsy Conference, February 18-21, 2009 - Sydney, NSW.


   The Australian Cerebral Palsy Research Study – Epidemiological and Genetic Associations with Cerebral Palsy. Accepted plenary presentation, Perinatal Medicine, June 15-17, 2011 - Harrogate, UK. Abstract awarded Student Prize by the British Maternal and Fetal Medicine Society.
# Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADRB2</td>
<td>Beta-2-adrenergic receptor</td>
</tr>
<tr>
<td>AGT</td>
<td>Angiotensinogen</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclo-oxygenase-2</td>
</tr>
<tr>
<td>CP</td>
<td>Cerebral Palsy</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FGB</td>
<td>Fibrinogen beta chain</td>
</tr>
<tr>
<td>FVL</td>
<td>Factor V Leiden</td>
</tr>
<tr>
<td>GA</td>
<td>Gestational age</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
</tr>
<tr>
<td>IL-1B</td>
<td>Interleukin 1β</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>ITGB3</td>
<td>Integrin beta chain beta 3</td>
</tr>
<tr>
<td>IUGR</td>
<td>Intrauterine growth restriction</td>
</tr>
<tr>
<td>LTA</td>
<td>Lymphotoxin-α</td>
</tr>
<tr>
<td>MBL</td>
<td>Mannose binding lectin</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix metalloproteinases</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methylenetetrahydrofolate reductase</td>
</tr>
<tr>
<td>NPPA</td>
<td>Natriuretic peptide A</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PAI</td>
<td>Plasminogen activator</td>
</tr>
<tr>
<td>PGM</td>
<td>Prothrombin gene mutation</td>
</tr>
<tr>
<td>POSU</td>
<td>Perinatal outcome statistics unit</td>
</tr>
<tr>
<td>SCNN1A</td>
<td>Amiloride-sensitive sodium channel subunit alpha</td>
</tr>
<tr>
<td>SELE</td>
<td>Selectin E</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>TLR-4</td>
<td>Toll-like receptor 4</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumour necrosis factor-α</td>
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**Glossary**

- **Quadriplegia**  
  Paralysis of all four limbs

- **Diplegia**  
  Paralysis of corresponding parts on both sides of the body, typically affecting the legs more severely than the arms

- **Hemiplegia**  
  Paralysis of one side of the body

- **Pyrexia**  
  Raised body temperature, fever