Molecular Mechanisms In The Epilepsies Of Infancy

A thesis submitted for the degree of Doctor of Philosophy to the University of Adelaide

by

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November 2010

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ABSTRACT

Over the past decade and a half, considerable advances have been made in the understanding of the molecular mechanisms underlying the idiopathic epilepsies. Technological advances and completion of the Human Genome Project have enabled continued progress. Much of this has impacted on families with epilepsies developing in infancy.

Benign familial neonatal seizures (BFNS) is often caused by mutations in either of two potassium channel subunit genes, *KCNQ2* and *KCNQ3*. Twenty-three of 36 families investigated (65%) were found to have mutations in one of these genes detectable by sequencing. Multiplex ligation-dependent probe amplification, which detects deletions and duplications affecting a specific gene, was applied to solve a further 17% of families. This revealed that deletions and duplications in *KCNQ2* are a common mechanism for the pathogenesis of BFNS.

The remaining unsolved BFNS families were analysed further to seek other mechanisms. A novel microduplication was identified in one family with BFNS and intellectual disability. This was characterised by comparative genome hybridisation (CGH) and fluorescence *in-situ* hybridisation and demonstrated the value of applying these technologies to familial as well as sporadic cases. A patient with neonatal seizures and long-QT syndrome (LQTS) was found to have a unique combination of changes in two genes associated with LQTS, supporting speculation that he had a “cardio-cerebral” channelopathy.

Two “BFNS” families had mutations in *SCN2A*, the gene usually associated with benign familial neonatal-infantile seizures (BFNIS). BFNIS is distinguished from BFNS by a higher age of seizure onset distribution. The initial clinical misclassification highlights the phenotypic overlap between these two disorders. These families are now reclassified on molecular criteria as BFNIS families with an earlier than usual age of onset. This distinction is of clinical significance since unlike BFNIS with *SCN2A* mutations 15% of BFNS patients with *KCNQ2* mutations have seizures later in life.

The remaining three BFNS families are unsolved. For two families, genotyping of microsatellite markers linked to known BFNS loci showed that they could not have mutations at those loci. Linkage to these loci was excluded by recombination, demonstrating that at least one other gene associated with BFNS exists.

The parental origin of *de novo* mutations in *SCN1A* was investigated. These mutations cause Dravet Syndrome (DS), a severe childhood epileptic encephalopathy. The mutations were found to originate on the paternal chromosome in approximately 75% of cases. The effect of parental age on mutagenesis in *SCN1A* was investigated and found not to be a contributing factor. This is the only epilepsy syndrome where sufficient *de novo* mutations have been identified for meaningful analysis of their parental origin.

Finally, the causative gene for benign familial infantile seizures (BFIS) mapped to chromosome 16p11.2-q12.1 remains elusive. Array CGH revealed no pathogenic copy number changes. Sequence capture and next-generation sequencing of the genes in the linkage region did not detect a mutation in a coding region. Several unique, but non-pathogenic, variants were identified in BFIS families. This paves the way for the next steps aimed at detecting rarer molecular defects such as recurrent inversions or unstable repeats.

STATEMENT

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 Sarah Elizabeth Heron 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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Signed…… Date……...

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ACKNOWLEDGEMENTS

I would like to thank Associate Professor John Mulley, my principal supervisor, for his guidance and support during this project, and Professor Jozef Gécz, my co-supervisor, for his support and advice. I appreciate the support of the Division of Genetics and Molecular Pathology, SA Pathology (and formerly the Department of Genetic Medicine, Women's and Children's Hospital) for the use of their equipment, space and other resources throughout this project, as well as the financial support of the University of Adelaide and the National Health and Medical Research Council of Australia.

I would also like to thank all the past and present team members of the Epilepsy Research Program and the Neurogenetics Program for their support and advice throughout this project. In particular I must thank Bev Johns, Robert Schultz and Tristiana Williams for DNA extractions, Mark Corbett for advice on designing sequence capture arrays and Alison Gardner for analysis of next-generation sequencing data. Thanks must also go to Helen Eyre, Jillian Nicholl and Sharon Bain of the Cytogenetics Laboratory for assistance with designing FISH experiments and for performing FISH, to Kathie Friend for assistance with the use of software for linkage analysis, to Kathy Cox for assistance and advice about MLPA and to the staff of the Molecular Genetics Laboratory for advice on performing parentage testing.

This project would not have been possible without the work of Professors Samuel Berkovic and Ingrid Scheffer and their team at the Epilepsy Research Centre, Austin Health, who provided most of the clinical material on which the project was based. Particular thanks are due to Bronwyn Grinton, Jacinta McMahon, Karen Oliver and Simone Yendle for providing detailed clinical information. I also thank the following clinicians and researchers for additional clinical material and information: Dr Sameer Zuberi, Royal Hospital for Sick Children, Glasgow, United Kingdom; Dr Clair Pridmore and Professor Eric Haan, Women's and Children's Hospital, Adelaide, SA, Australia; Dr Michael Shevell, Montreal Children's Hospital, Montreal, Quebec, Canada; Dr Thorsten Stanley and Natalie Redshaw, University of Otago, Wellington, New Zealand; Dr Marta Hernandez, Pontificia Universidad Católica de Chile, Santiago, Chile.

Finally, I must thank my partner, Ian Nicholson, and my parents, Jenny and the late David Heron, for their support, especially over the last three and a half years.

ABBREVIATIONS AND CHEMICAL SYMBOLS USED IN THIS THESIS

AD	Autosomal dominant
ADNFLE	Autosomal dominant nocturnal frontal lobe epilepsy
ADPEAF	Autosomal dominant partial epilepsy with auditory features
ADPEVF	Autosomal dominant partial epilepsy with variable foci
APS	Ammonium persulphate
AS-PCR	Allele-specific polymerase chain reaction
BAC	Bacterial artificial chromosome
BFNS	Benign familial neonatal seizures
BFNIS	Benign familial neonatal-infantile seizures
BFIS	Benign familial infantile seizures
bp	Base pair
CAE	Childhood absence epilepsy
GFE	Cryptogenic focal epilepsy
CGE	Cryptogenic genralized epilepsy
CGH	Comparative genome hybridisation
CNV	Copy number variant
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
dTTP	Deoxythymidine triphosphate
dH_2O	Deionised water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
ddNTP	Dideoxynucleotide triphosphate
DMSO	Dimethylsulphoxide
DS	Dravet Syndrome
DZ	Dizygous
EDTA	Ethylenediamine-tetraacetic acid
EEG	Electroencephalogram
EFMR	Epilepsy with mental retardation limited to females
FAME	Familial adult myoclonic epilepsy
FISH	Fluorescence *in-situ* hybridisation
FS	Febrile seizures
FS+	Febrile seizures plus
FHM	Familial hemiplegic migraine
GABA	γ-aminobutyric acid
GEFS+	Genetic epilepsy with febrile seizures plus
HCl	Hydrochloric acid
HRM	High-resolution melting
ICCA	Infantile convulsions and choreoathetosis
ID	Intellectual disablity
IGE	Idiopathic generalised epilepsy
IGETCS	Idiopathic generalised epilepsy with tonic-clonic seizures
in/del	Insertion or deletion
IVS	Intervening sequence
JAE	Juvenile absence epilepsy

JME	Juvenile myoclonic epilepsy
kb	Kilo base pair
KCl	Potassium chloride
LQTS	Long QT Syndrome
LGS	Lennox-Gastaut Syndrome
LR-PCR	Long-range polymerase chain reaction
MAE	Myoclonic-astatic epilepsy
Mb	Mega base pair
$MgCl_2$	Magnesium chloride
MLPA	Multiplex ligation-dependent probe amplification
MRI	Magnetic resonance imaging
MZ	Monozygous
nAchR	Nicotinic acetylcholine receptor
$(NH_4)_2SO_4$	Ammonium sulphate
PCR	Polymerase chain reaction
PKC	Paroxysmal kinesigenic choreoathetosis
PKD	Paroxysmal kinesigenic dyskinesia
RE	Restriction enzyme
RNA	Ribonucleic acid
SIMFE	Severe infantile multifocal epilepsy
SMEB	Severe myoclonic epilepsy of infancy borderland variant
SMEI	Severe myoclonic epilepsy of infancy
SNP	Single nucleotide polymorphism
SSCA	Single-stranded conformation polymorphism analysis
SUDEP	Sudden death in epilepsy
TBE	Tris-borate-EDTA
TLE	Temporal lobe epilepsy
TM	Transmembrane
UCSC	University of California, Santa Cruz
UTR	Untranslated region
WCH	Women’s and Children’s Hospital

GENE SYMBOLS USED IN THIS THESIS

Epilepsy-associated genes

ATP1A2	Na^+/K^+ transporting ATPase alpha 2 polypeptide
CACNA1H	T-type voltage-dependent calcium channel alpha 1H subunit
CHRNA2	Nicotinic acetylcholine receptor alpha 2 subunit
CHRNA4	Nicotinic acetylcholine receptor alpha 4 subunit
CHRNA7	Nicotinic acetylcholine receptor alpha 7 subunit
CHRNB2	Nicotinic acetylcholine receptor beta 2 subunit
CLCN2	Chloride channel 2
CRH	Corticotropin releasing hormone
EFHC1	EF-hand domain containing 1
GABRA1	Gamma aminobutyric acid A receptor alpha 1 subunit
GABRB3	Gamma aminobutyric acid A receptor beta 3 subunit
GABRD	Gamma aminobutyric acid A receptor delta subunit
GABRG2	Gamma aminobutyric acid A receptor gamma 2 subunit
HCN2	Hyperpolarization activated cyclic nucleotide-gated potassium channel 2
KCNQ2	Voltage-gated potassium channel, KQT-like subfamily, member 2
KCNQ3	Voltage-gated potassium channel, KQT-like subfamily, member 3
LGI1	Leucine-rich, glioma inactivated 1
NEDD4-2	Neural precursor cell expressed, developmentally down-regulated 4, isoform 2
PCDH19	Protocadherin 19
SCN1A	Voltage-gated sodium channel type I alpha subunit
SCN1B	Voltage-gated sodium channel type I beta subunit
SCN2A	Voltage-gated sodium channel type II alpha subunit
SCN3A	Voltage-gated sodium channel type III alpha subunit
SCN9A	Voltage-gated sodium channel type IX alpha subunit
SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1

Long-QT syndrome genes

KCND2	Voltage-gated potassium channel, Shal-related subfamily, member 2
KCNE1	Voltage-gated potassium channel, Isk-related family, member 1
KCNE2	Voltage-gated potassium channel, Isk-related family, member 2
KCNH2	Voltage-gated potassium channel, subfamily H (eag-related), member 2
KCNQ1	Voltage-gated potassium channel, KQT-like subfamily, member 1
SCN5A	Voltage-gated sodium channel type V alpha subunit

Other genes

APC	Adenomatous polyposis coli
ARMC5	Armadillo repeat containing 5
AVPR2	Arginine vasopressin receptor 2
BCKDK	Branched chain ketoacid dehydrogenase kinase
C16orf92	Chromosome 16 open reading frame 92
COBLL1	Cordon-bleu-like 1
CSRNP3	Cysteine-serine-rich nuclear protein 3
EFNB1	Ephrin-B1
F8	Coagulation factor VIII
F9	Coagulation factor IX
FGFR2	Fibroblast growth factor receptor 2
FGFR3	Fibroblast growth factor receptor 3
GALNT3	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3
GFAP	Glial fibrillary acidic protein
GRB14	Growth factor receptor-bound protein 14
GTF3C1	General transcription factor IIIC, polypeptide 1, alpha 220kDa
HRAS	Harvey rat sarcoma viral oncogene homolog
ITGAM	Integrin, alpha M (complement component 3 receptor 3 subunit)
KCNJ11	Inwardly-rectifying potassium channel, subfamily J, member 11
LMNA	Lamin A/C
MECP2	Methyl CpG binding protein 2 (Rett syndrome)
MVP	Major vault protein
NF1	Neurofibromin 1
NF2	Neurofibromin 2
PLP1	Proteolipid protein 1
PTPN11	Protein tyrosine phosphatase, non-receptor type 11
RET	Ret proto-oncogene
SLC38A11	Solute carrier family 38, member 11 (putative sodium-coupled neutral amino acid transporter 11)
TCOF1	Treacher Collins-Franceschetti syndrome 1
TSC2	Tuberous sclerosis 2
TTC21B	Tetratricopeptide repeat domain 21B
VHL	von Hippel-Lindau tumour suppressor
ZNF668	Zinc finger protein 668