

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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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

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Signed.....

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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 generalised epilepsy
CGH	Comparative genome hybridisation
CNV	Copy number variant
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
dTTP	Deoxythymidine triphosphate
dH₂O	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 <i>in-situ</i> 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 disability
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
MgCl₂	Magnesium chloride
MLPA	Multiplex ligation-dependent probe amplification
MRI	Magnetic resonance imaging
MZ	Monozygous
nAChR	Nicotinic acetylcholine receptor
(NH₄)₂SO₄	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

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

Long-QT syndrome genes

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

Other genes

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