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The Role of Genetic Factors in the Aetiology of Focal Epilepsy and in the Outcomes of Epilepsy Treatment

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To Silvia, Tita and Emilio

Abstract

Epilepsy is one of the most common neurological disorders, affecting people of all ages, races, social classes, and nationalities. It is characterised by an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological and social consequences of this condition. The aetiology of epilepsy has long been regarded as 'unknown' in about two-thirds of cases. However, evidence mainly from the past two decades has allowed to establish that many epilepsies initially considered to be of unknown cause have in fact a genetic basis. The importance of genetics in epilepsy is not limited to aetiology, and growing interest exists on the genetic contributions to epilepsy treatment outcomes.

The overarching aim of this thesis was to investigate and characterise the role of genetic factors in determining the aetiology of focal epilepsies and influencing outcomes of epilepsy treatment. Firstly, I ascertained the extent to which newly diagnosed non-lesional mesial temporal lobe epilepsy (MTLE) actually represents *familial* MTLE (FMTLE). By directly assessing relatives of patients presenting to a First Seizure Clinic with non-lesional MTLE, the familial syndrome of FMTLE was found to account for about one-fifth of new diagnoses of non-lesional MTLE. Secondly, I examined the diagnostic yield and management implications of genetic testing in the routine clinical care of focal epilepsies. By applying whole exome sequencing (WES) with targeted gene analysis on patients with non-lesional focal epilepsy in whom a genetic aetiology was suspected, pathogenic or likely pathogenic variants were detected in one-eighth of cases. In an individual with drug-resistant epilepsy, the genetic finding altered clinical management, resulting in complete seizure control. Thirdly, I evaluated whether genetic

factors influence seizure outcome after surgery for drug-resistant MTLE. Using a multicentre case-control WES study design, no definitive evidence for genetic influences on postsurgical outcome was found. However, a signal emerged for surgical failures, as a group, being enriched for individuals with deleterious variants in established epilepsy genes. Lastly, I investigated the genetic underpinnings of neural tube defects (NTDs) resulting from prenatal exposure to valproic acid (VPA). Adopting a family-based WES study design, two rare variants possibly conferring a substantial risk of VPA-associated NTDs were identified in the planar cell polarity genes *CELSR1* and *SCRIB*. However, this interpretation remains speculative due to sample size limitations.

Clearly, genetics has an important role in the aetiology of focal epilepsy. The results of this thesis also substantiate the excitement around the potential for genetics to improve outcomes of epilepsy treatment.

Declaration

This is to certify that:

(i) This thesis comprises only my original work towards the Doctor of Philosophy except where indicated in the preface;

(ii) Due acknowledgement has been made in the text to all other material used;

(iii) This thesis is fewer than 100,000 words in length, exclusive of tables, maps, biographies and appendices.

Signature**:**  Date: 1-November-2017

Dr Piero Cesare Perucca

Preface

In this thesis, each chapter presenting original research is a stand-alone manuscript written for publication, and as such there is some necessary repetition. In all chapters, I am the primary author and principle contributor. Chapters 4 and 5 are published journal articles (see section entitled 'Publications, Presentations and Awards' for details), and are included in this thesis with permission of coauthors.

For chapter 4, I designed the project; collected, analysed and interpreted the data; performed the statistical analysis; and drafted and revised the manuscript. Prof Doug Crompton, Ms Susannah Bellows, Dr Anne McIntosh, Dr Mark Newton, Prof Frank Vajda, Prof Patrick Kwan, Prof Terence O'Brien, Dr Meng Tan, Prof Samuel Berkovic contributed to the data collection, analysis and interpretation. Prof Tomas Kalincik contributed to the statistical analysis. Prof Samuel Berkovic, Prof Doug Crompton, and Prof Ingrid Scheffer were involved in the conception and design of the project. Prof Samuel Berkovic supervised the project. All coauthors contributed to the revision of the manuscript. Two anonymous reviewers provided comments that improved the manuscript.

For chapter 5, I collected, analysed and interpreted the data; performed the statistical analyses; and drafted and revised the manuscript. Prof Ingrid Scheffer, Dr Simon Harvey, Prof Paul James, Dr Sebastian Lunke, Ms Brigid Regan, Mr John Damiano, Dr Michael Hildebrand, Prof Samuel Berkovic, Prof Terence O'Brien, and Prof Patrick Kwan contributed to the data collection, analysis and interpretation. Dr Natalie Thorne implemented the bioinformatics pipeline. Prof Ingrid Scheffer, Dr Paul James, Prof Clara Gaff, Prof Terence O'Brien, and Prof Patrick Kwan conceived and designed the project.

Prof Ingrid Scheffer, Dr Natalie Thorne, Prof Clara Gaff, Dr Natalie Thorne, Dr Michael Hildebrand, Prof Terence O'Brien, and Prof Patrick Kwan were involved in the project supervision or coordination. All coauthors contributed to the revision of the manuscript. Two anonymous reviewers provided comments that improved the manuscript.

For chapter 6, I collected, analysed and interpreted the data; supervised data collection at the participating EPIGEN centres; performed the statistical analyses; and drafted the manuscript. Prof Terence O'Brien, Prof Patrick Kwan, Prof Samuel Berkovic, Dr Anne McIntosh, Dr Chantal Depondt, Prof Mohamad Mikati, Prof Orrin Devinsky, Dr Patricia Dugan and Prof Danielle Andrade contributed to the data collection, analysis and interpretation. Dr Erin Heinzen and Dr Nicole Griffin performed the genetic analysis. Prof Terence O'Brien, Prof Norman Delanty and Prof David Goldstein conceived and designed the project, and were involved in the project supervision and coordination. Prof Terence O'Brien, Prof Patrick Kwan and Prof Samuel Berkovic revised the manuscript.

For chapter 7, I conceived and designed the project; collected, analysed and interpreted the data; supervised data collection at the Karolinska Institute, Stockholm, Sweden; performed the genetic analysis; and drafted and revised the manuscript. Prof Terence O'Brien, Prof Frank Vajda, Prof Patrick Kwan, Prof Samuel Berkovic, Prof Torbjorn Tomson, Dr Alison Anderson, Ms Dana Jazayeri, Ms Janet Graham, Ms Alison Hitchcock, and Dr Marian Todaro contributed to the data collection, analysis and interpretation. Dr Slave Petrovski, Prof David Goldstein, and Dr Alison Anderson contributed to the genetic analysis. Prof Terence O'Brien and Prof Frank Vajda were involved in the project conception and design. Prof Terence O'Brien supervised the

project. Prof Terence O'Brien, Prof Patrick Kwan and Prof Samuel Berkovic revised the manuscript.

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I would like to express my deepest gratitude to my supervisors Prof Patrick Kwan, Prof Terry O'Brien and Prof Sam Berkovic, for making this PhD an intellectually stimulating and rewarding journey through their outstanding mentorship, guidance and support. They have always made time for me in their busy schedule. This PhD is also a testament to the extraordinary spirit of collaboration that they continuously foster, allowing for research to be undertaken across different sites.

I would like to thank my colleagues at the Royal Melbourne Hospital for their incredible support during my PhD. A special thanks to Prof Frank Vajda for his outstanding support and friendship. He has provided tremendous guidance early in my career – without him, I would have not chosen to study Medicine – and continues to be an inspiration in my daily life. I am particularly grateful to Dr Anne McIntosh, Dr Meng Tan, Prof Tomas Kalincik, Dr Alison Anderson, Dr Marian Todaro, Ms Janet Graham, Ms Alison Hitchcock, and Ms Sue Belbin for their help with data collection and analysis.

I would also like to thank my colleagues at the Epilepsy Research Centre at Austin Health, particularly Prof Ingrid Scheffer, Prof Doug Crompton, Dr Michael Hildebrand, Mr John Damiano, Dr Slave Petrovski, Dr Mark Newton, Ms Brigid Regan, Ms Caitlin Bennett, and Ms Susannah Bellows for their tremendous contribution to various aspects of the work presented in this thesis.

I would like to express my appreciation to Prof Clara Gaff, Dr Natalie Thorne, Dr Simon Harvey, Prof Paul James, Dr Sebastian Lunke and the entire staff of the Melbourne Genomics Health Alliance for allowing me to undertake the project presented in chapter

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Finally, I would like to acknowledge the tireless love, encouragement and support of my family, especially my wife Silvia and my parents Tita and Emilio.

Publications, Presentations and Awards

A major part of the work presented in this thesis has resulted in publications, presentations and awards, as detailed below:

Manuscripts Resulting from the Work Presented in this Thesis

Perucca P, Crompton DE, Bellows ST, McIntosh AM, Kalincik T, Newton MR, Vajda FJE, Scheffer IE, Kwan P, O'Brien TJ, Tan KM, Berkovic SF. Familial mesial temporal lobe epilepsy and the borderland of déjà vu. *Ann Neurol* 2017;82:166-176.

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von Ellenrieder N, Beltrachini L, **Perucca P**, Gotman J. Size of cortical generators of epileptic interictal events and visibility on scalp EEG. *Neuroimage* 2014;94:47-54.

Abstracts and Proceedings

Sivathamboo S, O'Brien TJ, Jones NC, Velakoulis D, Constantino TN, Chen Z, Sparks PB, Goldin J, Kwan P, **Perucca P**. Prolonged cardiorespiratory disturbance following convulsive seizures. *Presented in poster form at the 29th Annual Scientific Meeting of the Australasian Sleep Association and Australasian Sleep Technologists Association: "Sleep DownUnder Annual Scientific Meeting 2017", Auckland, New Zealand, 25-28 October 2017.*

Sivathamboo S, O'Brien TJ, Kwan P, **Perucca P**, White EJ, Hollis C, Carino J, Chen Z, Velakoulis D, Jones NC, Goldin J. Prevalence of sleep-disordered breathing among patients admitted for prolonged video-EEG monitoring. *Presented in platform form at the 29th Annual Scientific Meeting of the Australasian Sleep Association and Australasian Sleep Technologists Association: "Sleep DownUnder Annual Scientific Meeting 2017", Auckland, New Zealand, 25-28 October 2017.*

Perucca P, Crompton DE, Bellows ST, McIntosh AM, Kalincik T, Newton MR, Vajda FJE, Scheffer IE, Kwan P, O'Brien TJ, Tan KM, Berkovic SF. Familial mesial temporal lobe epilepsy and the borderland of déjà vu. *Presented in platform form at the 32nd International Epilepsy Congress, Barcelona, Spain, 2-6 September 2017.*

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Perucca P, Crompton DE, Bellows S, McIntosh AM, Newton MR, Kwan P, O'Brien TJ, Scheffer IE, Berkovic SF. How Frequent Is Familial Mesial Temporal Lobe Epilepsy? *Presented in poster form at the Annual Melbourne Brain Centre (MBC) Symposium, Melbourne, VIC, Australia, 24-25 November 2016.*

EPIGEN Consortium. Seizure outcome after mesial temporal lobe epilepsy surgery: effect of genetic factors. *Delivered as oral presentation at 2016 ERC Epilepsy Research Retreat, Marysville, VIC, Australia, 11-13 August 2016.*

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Sivathamboo S, Jones NC, White EJ, Hollis C, Strawhorn A, Carino J, Tan M, French CR, Yerra R, **Perucca P**, Goldin J, Velakoulis D, Kwan P, O'Brien TJ. Cardiopulmonary function during the peri-ictal state of epileptic and psychogenic non-epileptic seizures. *Presented in poster form at the 2016 Melbourne Health Research Week Symposium, The Royal Melbourne Hospital, Melbourne, VIC, Australia, 16-23 June 2016.*

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Perucca P, Crompton DE, Bellows S, McIntosh AM, Newton MR, Kwan P, O'Brien TJ, Scheffer IE, Berkovic SF. How Frequent Is Familial Mesial Temporal Lobe Epilepsy? *Presented in poster form at the American Epilepsy Society meeting, Philadelphia, PA, USA, 4-8 December 2015.*

Perucca P, Scheffer IE, Harvey AS, James PA, Lunke S, Thorne N, Gaff C, Regan BM, Damiano JA, Hildebrand MS, Berkovic SF, O'Brien TJ, Kwan P. Clinical whole-exome sequencing in focal epilepsies of suspected genetic etiology. *Presented in poster and platform form at the 5th Annual Melbourne Brain Centre (MBC) Symposium, Melbourne, VIC, Australia, 27-28 November 2015.*

Perucca P, Scheffer IE, Harvey AS, James PA, Lunke S, Thorne N, Gaff C, Berkovic SF, O'Brien TJ, Kwan P, Melbourne Genomics Health Alliance Clinical whole-exome sequencing in focal epilepsies of suspected genetic aetiology. *Presented in platform form at the Epilepsy Society of Australia meeting, Adelaide, SA, Australia, 21-23 October 2015.*

Perucca P, Scheffer IE, O'Brien TJ, Harvey AS, James PA, Lunke S, Thorne N, Gaff C, Kwan P, Melbourne Genomics Health Alliance**.** Clinical whole-exome sequencing in

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Jacobs J, Wu JY, **Perucca P**, Zelmann R, Mader M, Dubeau F, Schulze-Bonhage A, Mathern G, Gotman J. Surgical removal of high-frequency oscillations correlates with postsurgical outcome – a multicenter study. *Epilepsy Curr* 2015;15(Suppl 1):355. *Presented in poster form at the American Epilepsy Society meeting, Seattle, WA, USA, 5-9 December 2014.*

Ferrari-Marinho T, **Perucca P**, Mok K, Olivier A, Hall J, Dubeau F, Gotman J. Intracranial EEG seizure-onset patterns correlate with interictal HFOs in patients with refractory epilepsy. *Epilepsia* 2014;55(Suppl 2):202. *Presented in poster form at the 11th European Congress on Epileptology, Stockholm, Sweden, 29 June-3 July 2014.*

Selected Lectures in which Work from this Thesis was Presented

Genetic testing for focal epilepsy: ready for prime time? *Delivered at the 8th Annual Queensland Epilepsy Symposium "Thinking outside the box", Brisbane, QLD, Australia, 27 October 2017.*

The genetics of temporal lobe epilepsy: time to re-think in the clinic? *Delivered at Neurology Grand Rounds, The Royal Melbourne Hospital, Melbourne, VIC, Australia, 8 February 2017.*

Genomic contributions to epilepsy surgery outcomes. *Delivered at the CURE Frontiers in Research Seminar Series, Acquired Epileptogenesis Symposium 2017, Melbourne, VIC, Australia, 6 February 2017.*

The genetics of focal epilepsies. *Delivered at Epilepsy Foundation, Melbourne, VIC, Australia, 16 November 2016.*

Can genetics predict the outcome of surgery for temporal lobe epilepsy? *Delivered at the MBC@RMH Neuroscience Seminar, Melbourne, VIC, Australia, 17 October 2016.*

Demonstration project flagship: Focal epilepsy. *Delivered at Melbourne Genomics Symposium, Demonstrating success: Outcomes 2014-2015, Melbourne, VIC, Australia, 1 September 2016.*

Can genomics predict outcome following epilepsy surgery for TLE. *Delivered at Melbourne Epilepsy@MBC, Melbourne, VIC, Australia, 31 August-1 September 2016.*

Focal epilepsy: is it time to incorporate genetic testing into routine clinical care? *Delivered at the MBC@RMH Neuroscience Seminar, Melbourne, VIC, Australia, 30 November 2015.*

Focal epilepsy: integrating genetic testing into routine clinical care. *Delivered at the 5th Epilepsy Congress of South China, Guangzhou, China, 14 November 2015.*

An investigation of genetic factors predisposing to valproic acid-associated neural tube defects. *Delivered at Fetal Medicine @ Mercy: Brains at Twilight, Mercy Hospital for Women, Melbourne, VIC, Australia, 13 May 2015.*

Exome sequencing in epilepsy syndromes. *Delivered at the 4th Annual Symposium of the Melbourne Brain Centre at the Royal Melbourne Hospital: Precision Neuroscience, Melbourne, VIC, Australia, November 28, 2014.*

Prizes and Awards

2017 Mendelsohn Student Lecturer Runner-up Award from the Melbourne Neuroscience Institute, University of Melbourne, and The Florey Institute of Neuroscience and Mental Health.

Best Poster Award for best poster presentation at the Annual Melbourne Brain Centre (MBC) Symposium, November 24-25, 2016, Melbourne, VIC, Australia.

Best Poster Award for best poster presentation at the 5th Annual Melbourne Brain Centre (MBC) Symposium, November 27-28, 2015, Melbourne, VIC, Australia.

Best Platform Award for best platform presentation at the Epilepsy Society of Australia (ESA) 29th Annual Scientific meeting, October 21-23, 2015, Adelaide, SA, Australia.

Epilepsy Society of Australia (ESA) Travel Scholarship to attend and present at the 69th Annual Meeting of the American Epilepsy Society, December 4-8, 2015, Philadelphia, PA, USA.

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Figure 1.1 from Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshé SL, Peltola J, Roulet Perez E, Scheffer IE, Zuberi SM, Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology, *Epilepsia*, 2017, vol. 58, no. 4, pp. 522-530. Figure from p. 525.	p. 42	Y
Figure 1.2 from Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, Hirsch E, Jain S, Mathern GW, Moshé SL, Nordli DR, Perucca E, Tomson T, Wiebe S, Zhang YH, Zuberi SM, ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology, *Epilepsia*, 2017, vol. 58, no. 4, pp. 512-521. Figure from p. 515.	p. 47	Y
Figure 1.3 from Hauser WA, Annegers JF, Kurland LT, Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935-1984, *Epilepsia*, 1993, vol. 34, no. 3, pp. 453-468. Figure from p. 456.	p. 48	Y
Table 1.3 from Peljto AL, Barker-Cummings C, Vasoli VM, Leibson CL, Hauser WA, Buchhalter JR, Ottman R, Familial risk of epilepsy: a population-based study, *Brain*, 2014, vol. 137, Pt 3, pp. 795-805. Table from p. 798.	p. 57	Y
Figure 1.5 from Helbig I, Heinzen EL, Mefford HC; ILAE Genetics Commission, Primer part 1-The building blocks of epilepsy genetics, *Epilepsia*, 2016, vol. 57, no. 6, pp. 861-868. Figure from p. 863.	p. 66	Y
Figure 1.6 from Scheffer IE, Heron SE, Regan BM, Mandelstam S, Crompton DE, Hodgson BL, Licchetta L, Provini F, Bisulli F, Vadlamudi L, Gecz J, Connelly A, Tinuper P, Ricos MG, Berkovic SF, Dibbens LM, Mutations in mammalian target of rapamycin regulator DEPDC5 cause focal epilepsy with brain malformations, *Ann Neurol*, 2014, vol. 75, no. 5, pp. 782-787. Figure from p. 786.	p. 68	Y
Table 1.5 from Moshé SL, Perucca E, Ryvlin P, Tomson T, Epilepsy: new advances, *Lancet*, 2015, vol. 385, no. 9971, pp. 884-898. Panel from p. 889.	p. 73	Y
Table 1.6 from Perucca P, Gilliam FG. Adverse effects of antiepileptic drugs, *Lancet Neurol*, 2012, vol. 11, no. 9. pp. 792-802. Table from p. 795.	p. 76	Y
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List of Abbreviations

A	Disease causing automatic
AAN	American Academy of Neurology
ADEAF	Autosomal dominant epilepsy with auditory features
ADNFLE	Autosomal dominant nocturnal frontal lobe epilepsy
AED	Antiepileptic drug
ATL	Anterior temporal lobectomy
BECTS	Benign epilepsy with centro-temporal spikes
BWA	Burrows-Wheeler Alignment Tool
C	Conserved
CADD	Combined Annotation Dependent Depletion
CBZ	Carbamazepine
CCDS	Consensus coding sequence
CI	Confidence interval
CLB	Clobazam
CNS	Central nervous system
CNVs	Copy number variants
CNZ	Clonazepam
CP	Centro-parietal
CT	Computed tomography
D	Damaging (SIFT), probably damaging (PolyPhen2) or disease causing (MutationTaster)
DRESS	Drug reaction with eosinophilia and systemic symptoms
DZ	Dizygotic

EEG	Encephalogram
EIMFS	Epilepsy in infancy with migrating focal seizures
EOAE	Early-onset absence epilepsy
ESP	Exome Sequencing Project
ETX	Ethosuximide
EURAP	European and International Registry of Antiepileptic Drugs and Pregnancy
EUROCAT	European Surveillance of Congenital Anomalies
EVS	Exome Variant Server
ExAC	Exome Aggregation Consortium
F	Female
F	Frontal
FAS	Focal aware seizures
FBTCS	Bilateral tonic-clonic seizures
Fe	Female
FFEVF	Familial focal epilepsy with variable foci
FIAS	Focal impaired awareness seizures
FLE	Frontal lobe epilepsy
FMTLE	Familial mesial temporal lobe epilepsy
FT	Fronto-temporal
GAS	Generalized absence seizures;
GATK	Genome Analysis Toolkit
GBP	Gabapentin
GERP	Genomic Evolutionary Rate Profiling

GGE	Genetic generalized epilepsy
GLUT-1	Glucose transporter type 1
GQ	Genotype quality
GTCS	Generalized tonic-clonic seizures
HS	Hippocampal sclerosis
ID	Intellectual disability
IGM	Institute for Genomic Medicine
ILAE	International League Against Epilepsy
L	Left
LCM	Lacosamide
LEV	Levetiracetam
LTG	Lamotrigine
M	Male
MAF	Minor allele frequency
mo	Months
MRI	Magnetic resonance imaging
MTLE	Mesial temporal lobe epilepsy
mTOR	Mammalian target of rapamycin
MTS	Mesial temporal sclerosis
MZ	Monozygotic
n/a	Not applicable
NMDA	N-methyl-D-aspartate
ns	Not significant
NTD	Neural tube defect

O	Occipital;
OIRDA	Occipital intermittent rhythmic delta activity
OLE	Occipital lobe epilepsy
OR	Odds ratio
OXC	Oxcarbazepine;
PET	Positron emission tomography
PLE	Parietal lobe epilepsy
QD	Quality by depth
QUAL	Quality score
R	Right
RR	Relative risk
RVIS	Residual Variation Intolerance Score
ShW	Sharp waves
SIR	Standardised incidence ratio
SMR	Standardised morbidity ratio
SNVs	Single nucleotide variants
SPECT	Single-photon emission computed tomography
SpW	Spike-and-waves
subRVIS	sub-region Residual Variation Intolerance Scores
SUDEP	Sudden unexpected death in epilepsy
Susp Man	Suspicious manifestations
T	Temporal
TIRDA	Temporal intermittent rhythmic delta activity
TLE	Temporal lobe epilepsy

TPM	Topiramate
TPO	Temporo-parieto-occipital
UOTCS	Unknown onset tonic-clonic seizures
VPA	Valproic acid
WES	Whole exome sequencing
WGS	Whole genome sequencing
WHO	World Health Organisation
wks	Weeks
y	Years
ZNS	Zonisamide

Chapter 1:

Literature Review

Epileptic Seizures and Epilepsy: Definition, Pathophysiology and Classification

It is important to distinguish between epilepsy and epileptic seizures. Epilepsy is disease of the brain characterised by an *enduring* predisposition to generate epileptic seizures. Accordingly, seizures constitute the primary 'symptom' of epilepsy. In addition, epileptic seizures can also occur as isolated events not associated with the aforementioned enduring predisposition and, as such, occurrence of a seizure may not necessarily imply a diagnosis of epilepsy.

Epileptic Seizures

In 1870, the father of modern epileptology, John Hughlings Jackson, defined an epileptic seizure as a "symptom" resulting from "an occasional, an excessive, and a disorderly discharge of nerve tissue" (Jackson, 1970). This definition has withstood the test of time and greatly influenced subsequent thought. Following in Jackson's footsteps, the International League Against Epilepsy (ILAE) devised in 2005 a consensus definition, which conceptualises an epileptic seizure as "a transient occurrence of signs and/or

symptoms due to abnormal excessive or synchronous neuronal activity in the brain" (Fisher *et al.*, 2005).

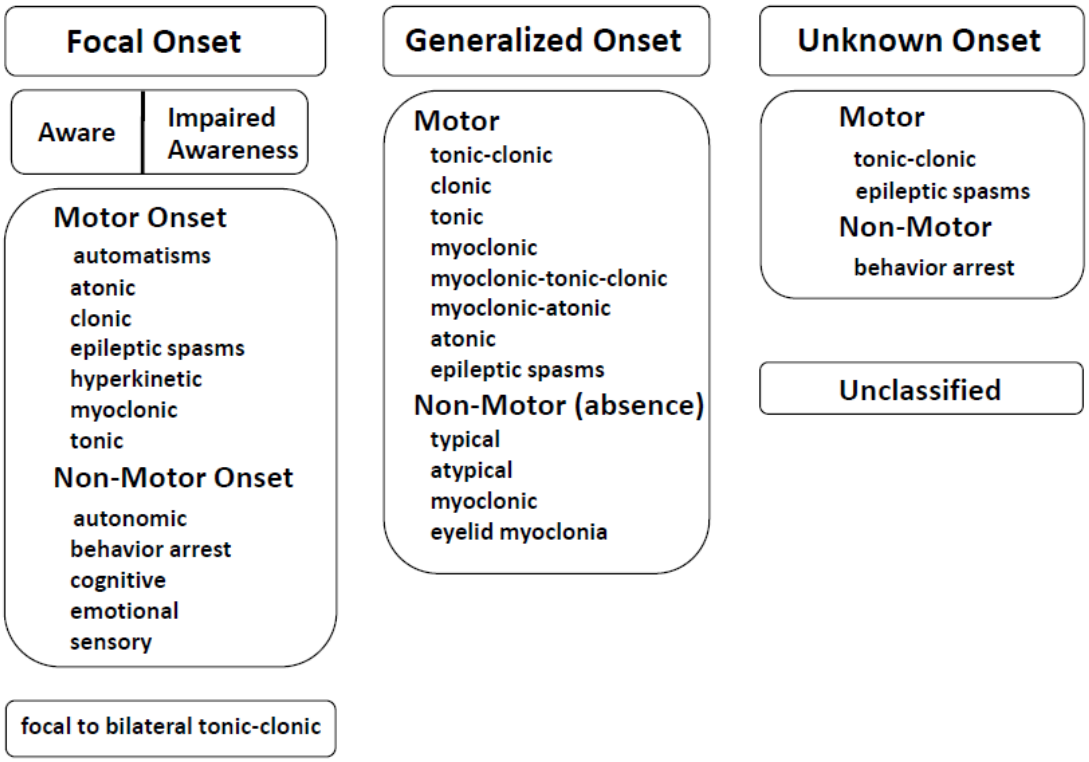
Descriptions of seizures date back to ancient Mesopotamia (Temkin, 1945). Henri Gastaut proposed a modern classification of seizures in 1964 (Gastaut *et al.*, 1964), and since then a number of classification systems have been formulated (Gastaut, 1969b, 1970; 1981; Engel and International League Against, 2001; Engel, 2006; Berg *et al.*, 2010). A highly influential classification was developed by the ILAE in 1981, which divided seizures in three main categories: partial (or focal) seizures, reflecting initial activation of a system of neurons limited to part of one hemisphere; generalised seizures, reflecting initial involvement of both hemispheres; and unclassified seizures, in which inadequate or incomplete data did not allow to classify a certain seizure into one of the two previous categories (**Table 1.1**) (1981). Focal and generalised seizures were further divided into specific sub-types. Although still widely used today, the 1981 classification has been criticised for not reflecting the subsequent advances in the understanding of the pathogenesis of epileptic seizures.

Table 1.1: 1981 ILAE classification of epileptic seizures.

<table>
<tr>
<td rowspan="2">1) Partial (focal, local) seizures
▪ Simple partial seizures (consciousness not impaired)
o With motor signs
o With somatosensory or special-sensory symptoms
o With autonomic symptoms or signs
o With psychic symptoms
▪ Complex partial seizures (with impairment of consciousness; may sometimes begin with simple symptomatology)
o Simple partial onset followed by impairment of consciousness
o With impairment of consciousness at onset
▪ Partial seizures evolving to secondarily generalized seizures (This may be generalized tonic-clonic, tonic, or clonic)
o Simple partial seizures evolving to generalized seizures
o Complex partial seizures evolving to generalized seizures
o Simple partial seizures evolving to complex partial seizures evolving to generalized seizures</td>
<td>2) Generalized seizures (convulsive or nonconvulsive)
▪ Absence seizures
o Typical absence
o Atypical absence
▪ Myoclonic seizures
▪ Clonic seizures
▪ Tonic seizures
▪ Toni-clonic seizures
▪ Atonic seizures</td>
</tr>
<tr>
<td>3) Unclassified epileptic seizures</td>
</tr>
</table>

Most recently, the ILAE developed a revised operational classification of seizure types (**Figure 1.1**), which builds on previous frameworks, including the 1981 classification (Fisher *et al.*, 2017). In this revised scheme, seizures are divided into: focal (onset) seizures, originating within networks limited to one hemisphere; generalised (onset) seizures, originating at some point within and rapidly engaging bilaterally distributed networks; and seizures of unknown onset, referring to cases in which there is insufficient information to classify a seizure as focal or generalised. Focal seizures can be distinguished into focal aware seizures (corresponding to simple partial seizures in the 1981 classification), indicating that awareness was retained throughout the seizures, and focal impaired awareness seizures (corresponding to complex partial seizures in the 1981 classification), indicating that awareness was impaired during any part of the seizure. Focal aware seizures and focal impaired awareness seizures can be further sub-grouped according to the presence of motor or non-motor manifestations. Focal to bilateral tonic-clonic seizures (corresponding to partial seizures evolving to secondarily generalised seizures in the 1981 classification) are a separate sub-type of focal seizures indicating a convulsive seizure originating from a network limited to one hemisphere. Generalized seizures are sub-divided into motor (tonic-clonic; clonic; tonic; myoclonic; myoclonic-tonic-clonic; myoclonic-atonic; atonic; epileptic spasms) and non-motor (absence: typical, atypical, myoclonic, eyelid myoclonia) seizures. Seizures of unknown onset can be characterised according to the presence of motor or non-motor phenomena, or just be considered as 'unclassified' if available information does not allow further sub-grouping.

Figure 1.1: 2017 ILAE classification of epileptic seizures. Reproduced by permission from John Wiley and Sons (Fisher *et al.*, 2017).



Epilepsy

Affecting approximately 65 million people worldwide, epilepsy is a common chronic neurological illness with deleterious effects on social, vocational, physical and psychological well-being (Baker *et al.*, 1997; Kanner, 2003; Aldenkamp and Bodde, 2005; Sillanpaa and Shinnar, 2005; Perucca *et al.*, 2009b; Fiest *et al.*, 2014). In the World Health Organisation (WHO)'s Global Burden of Disease 2010 study, epilepsy was ranked as the second most burdensome neurological condition as measured by disability-adjusted life years (Murray *et al.*, 2012).

Epilepsy has been conceptualised by the ILAE as a "disorder of the brain characterised by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition" (Fisher *et al.*, 2005). Practically, epilepsy is defined by any of the following conditions: at least two unprovoked (or reflex) seizures occurring more than 24 hours apart; a single unprovoked (or reflex) seizure and a probability of further seizures being similar to the general recurrence risk (≥60%) after two unprovoked seizures, occurring over the next 10 years; or a diagnosis of an epilepsy syndrome (Fisher *et al.*, 2014). Central to this definition is the concept of 'unprovoked' seizures, which are seizures occurring in the absence of precipitating factors, as opposed to 'acute symptomatic' seizures, which occur at the time of a systemic insult or in close temporal association with a documented brain insult (Beghi *et al.*, 2010). Another important aspect of the practical definition of epilepsy is the required '≥60% recurrence risk' after a single unprovoked seizure: although this quantifies the core principle of the conceptual definition of epilepsy (i.e. an 'enduring predisposition' to generate seizures), it may be difficult to estimate such risk in the

individual patient. Generally, this risk applies to structural lesions, such as a stroke, a central nervous system (CNS) infection or a severe head injury; a diagnosis of epilepsy syndrome; or certain circumstances in the presence of other risk factors (Fisher *et al.*, 2014).

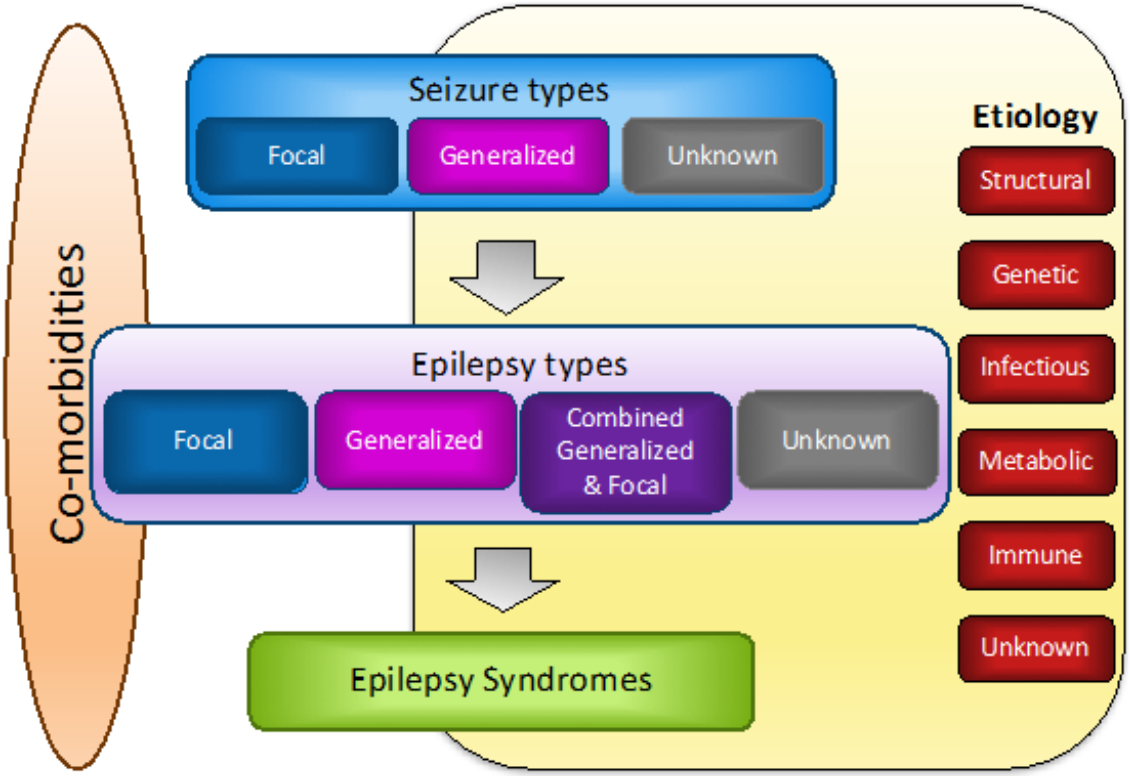
Similarly to the classification of epileptic seizures, the classification of the epilepsies has been a subject of major debate since the 1960s, when modern concepts of classification were introduced by Henri Gastaut (Gastaut *et al.*, 1964; Gastaut, 1969a, b). Several frameworks have been proposed in the past 50 years (Gastaut, 1969a; 1985, 1989; Engel and International League Against, 2001; Engel, 2006; Berg *et al.*, 2010). An important milestone in this process was the 1989 ILAE classification, which categorised epilepsies and epileptic syndromes into four major classes: those with focal seizures (focal epilepsies and syndromes); those with generalised seizures (generalised epilepsies and syndromes); epilepsies and syndromes undetermined whether focal or generalised; and special syndromes (**Table 1.2**) (1989). Focal and generalised epilepsies and syndromes were further sub-grouped into: symptomatic, indicating the presence of a known underlying cause; cryptogenic, indicating the presence of a presumed ("hidden" or "occult") underlying cause; and idiopathic, indicating the absence of an underlying cause other than a possible hereditary predisposition. The 1989 classification continues to be widely used nowadays, but has the inherent limitation of not taking into account the subsequent scientific discoveries which have profoundly re-shaped our understanding of epilepsy.

Table 1.2: 1989 ILAE Classification of the epilepsies and epileptic syndromes.

<table>
<tr><th colspan="2">1) Localization-related (focal, local, partial) epilepsies and syndromes</th></tr>
<tr><td><u>Idiopathic (with age-related onset)</u>
▪ Benign childhood epilepsy with centro-temporal spikes
▪ Childhood epilepsy with occipital paroxysms
▪ Primary reading epilepsy</td><td><u>Symptomatic or Cryptogenic</u>
▪ Chronic progressive epilepsia partialis continua of childhood (Kojewnikow’s syndrome)
▪ Syndromes characterized by seizures with specific modes of precipitation
▪ Temporal lobe epilepsies
▪ Frontal lobe epilepsies
▪ Parietal lobe epilepsies
▪ Occipital lobe epilepsies</td></tr>
<tr><th colspan="2">2) Generalized epilepsies and syndromes</th></tr>
<tr><td><u>Idiopathic (with age-related onset)</u>
▪ Benign neonatal familial convulsions
▪ Benign neonatal convulsions
▪ Benign myoclonic epilepsy in infancy
▪ Childhood absence epilepsy (pyknolepsy)
▪ Juvenile absence epilepsy
▪ Juvenile myoclonic epilepsy (impulsive petit mal)
▪ Epilepsy with grand mal (GTCS) seizures on awakening
▪ Other generalized idiopathic epilepsies not defined above
▪ Epilepsies with seizures precipitated by specific modes of activation</td><td><u>Symptomatic or Cryptogenic</u>
▪ West syndrome (infantile spasms, Blitz-Nick-Salaam Krämpfe)
▪ Lennox-Gastaut syndrome
▪ Epilepsy with myoclonic-astatic seizures
▪ Epilepsy with myoclonic absences
▪ Early myoclonic encephalopathy
▪ Early infantile epileptic encephalopathy with suppression burst
▪ Other symptomatic generalized epilepsies</td></tr>
<tr><th colspan="2">3) Epilepsies and syndromes undetermined whether focal or generalized</th></tr>
<tr><td><u>With both generalized and focal seizures</u>
▪ Neonatal seizures
▪ Severe myoclonic epilepsy in infancy
▪ Epilepsy with continuous spike-waves during slow wave sleep
▪ Acquired epileptic aphasia (Landau-Kleffner-syndrome)
▪ Other undetermined epilepsies</td><td><u>Without unequivocal generalized or focal features</u></td></tr>
<tr><th colspan="2">4) Special syndromes</th></tr>
<tr><td colspan="2"><u>Situation-related seizures</u>
▪ Febrile convulsions
▪ Isolated seizures or isolated status epilepticus
▪ Seizures occurring only when there is an acute metabolic or toxic event due to factors such as alcohol, drugs, eclampsia, non-ketotic hyperglycemia</td></tr>
</table>

To address this important limitation, the ILAE has recently produced a revised classification of the epilepsies (Scheffer *et al.*, 2017), which is organised on three levels (**Figure 1.2**). The first level is the seizure type, defined according to the revised seizure classification by the ILAE (Fisher *et al.*, 2017). Definition of the seizure type(s) is followed by the diagnosis of the epilepsy type, which includes four main classes: focal epilepsy, generalised epilepsy, combined focal and generalised epilepsy, and unknown epilepsy. The third level consists of defining the epilepsy syndrome, when a specific syndromic diagnosis can be made. The classification encourages establishing the aetiology at each of three levels, because often this has important implications for clinical management. Aetiology is classified into six separate categories: structural, genetic, infectious, metabolic, immune and unknown. Notably, the revised classification allows the use of the term 'idiopathic generalised epilepsies', which encompasses four syndromes: childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy and generalised tonic-clonic seizures alone (formerly known as generalised tonic-clonic seizures on awakening). These syndromes can also be grouped as genetic generalized epilepsies; there is very strong evidence that these are genetic disorders, but sociocultural factors in some countries make the designation 'genetic' problematic.

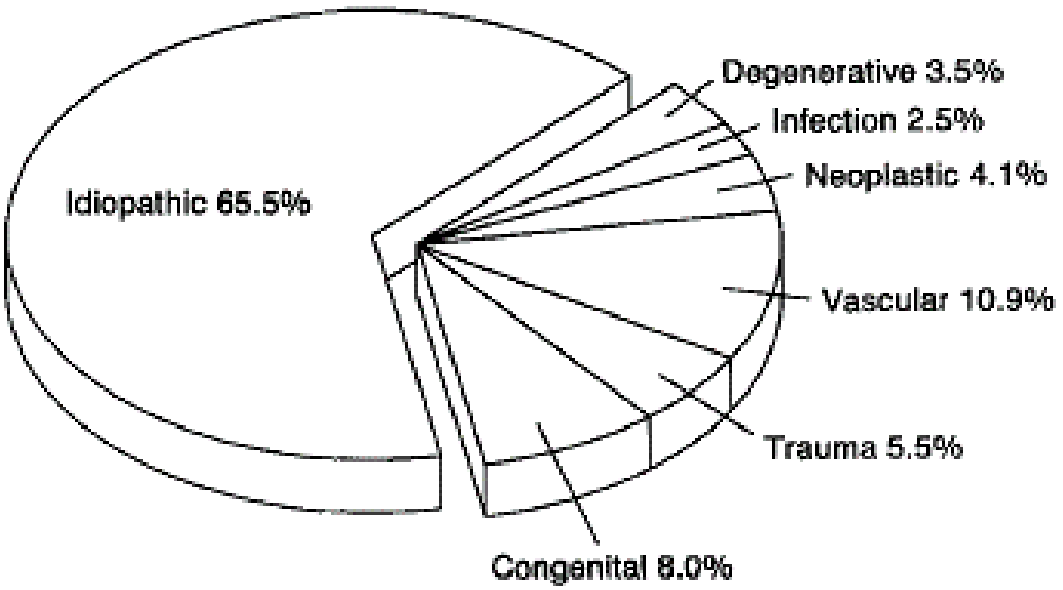
Figure 1.2: Framework of the 2017 ILAE classification of the epilepsies. Reproduced by permission from John Wiley and Sons (Scheffer *et al.*, 2017).



The Aetiology of Epilepsy

As emphasised by the new classification of the epilepsies by the ILAE, identifying the aetiology of epilepsy is important due to its treatment implications (Scheffer *et al.*, 2017). In classic epidemiological studies, however, no underlying cause could be identified in approximately two-thirds of cases (**Figure 1.3**) (Hauser *et al.*, 1993; Olafsson *et al.*, 1996; Olafsson *et al.*, 2005). In the remaining cases, common causes included stroke, brain tumours, traumatic brain injuries, CNS infections, and dementia (Hauser *et al.*, 1993; Olafsson *et al.*, 1996; Olafsson *et al.*, 2005).

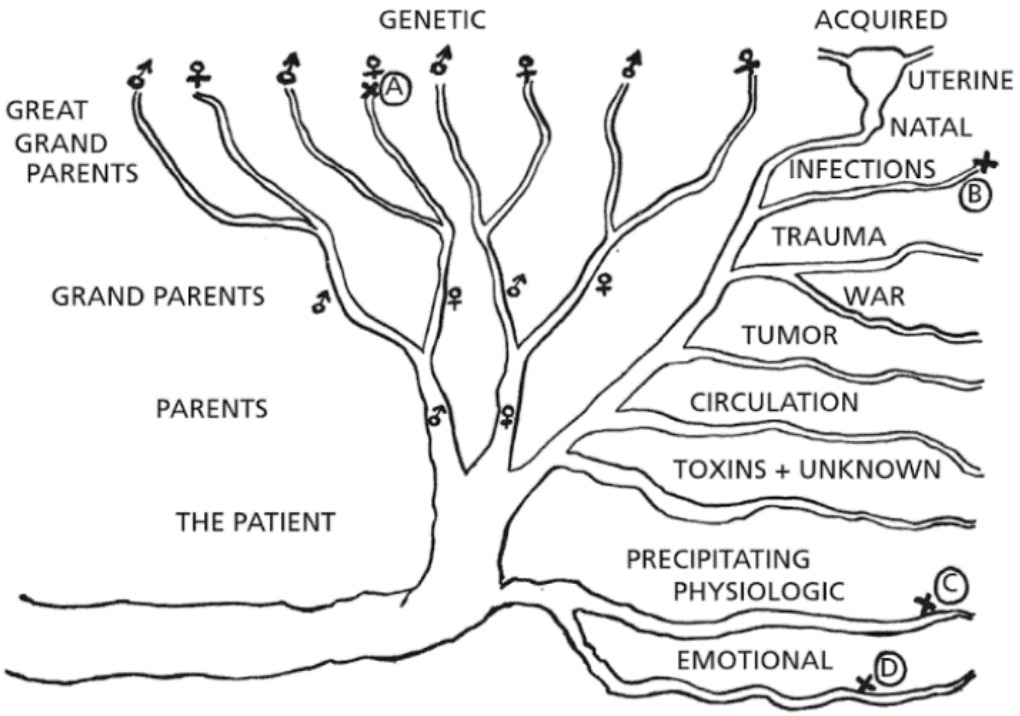
Figure 1.3: Aetiology of epilepsy among incident cases in Rochester, Minnesota (1935-1984). Reproduced with permission from John Wiley and Sons (Hauser *et al.*, 1993).



Even when a major aetiology is identified, other factors (including genetic and environmental) can contribute to the phenotypic expression of a certain form of epilepsy. This had already been recognised by William Lennox in 1960, who used the metaphor of the *river* to illustrate the multifactorial nature of epilepsy (**Figure 1.4**) (Lennox and Lennox, 1960). This was also well conceptualised by his metaphor of the *reservoir*:

> "Causes may be represented as the sources of the reservoir. At the bottom is the already present volume of water, which represents the person's predisposition, a fundamental cause. But the reservoir is supplied also by streams which represent the contributory conditions, such as lesions of the brain acquired since conception, certain disorders of bodily function and emotional disturbances" (Lennox and Lennox, 1960).

Figure 1.4: Lennox's river of epilepsy, illustrating the various factors which can contribute to an individual's epilepsy. From (Lennox and Lennox, 1960).



Research in ensuing years has progressively highlighted the important role of genetics in the aetiology of epilepsy, validating Lennox's intuition. It is now known that epilepsies generally considered as 'acquired', such as those related to a stroke (Yang *et al.*, 2014), a tumour (Frattini *et al.*, 2013), a head trauma (Christensen *et al.*, 2009; Diamond *et al.*, 2014), an infection (Kariuki *et al.*, 2013) or congenital malformations (Barkovich *et al.*, 2012), have a genetic contribution. For example, in a population-based Danish study of more than 1.6 million people, a positive family history of epilepsy was associated with approximately a 10-fold increased risk of developing epilepsy following a severe traumatic brain injury (relative risk, RR: 10.09 [95% confidence interval, CI: 4.20-24.26]) (Christensen *et al.*, 2009). Furthermore, accrued evidence has also allowed to establish that many epilepsies initially considered to be of unknown cause have in fact a genetic aetiology (Thomas and Berkovic, 2014).

A common misconception is that genetics are mainly implicated in the aetiology of the generalised epilepsies. In reality, evidence mainly from the past two decades, clearly indicate that genetic factors also play a major role in the pathogenesis of focal epilepsies, and this will be discussed in detail in the next section. **Box 1.1** provides an explanation of terms and concepts commonly used in genetics, which will be adopted in the next section as well as in other parts of this thesis.

Box 1.1: Common terms and concepts used in genetics.

Exome: The complete protein-coding (exonic) region of the human genome, in which most of the currently known disease-causing variation is located. The exome constitutes ~1.5% of the genome.

Allele: One of two or more alternative forms of a gene. At each position on a chromosome, an individual inherits two alleles, one from the mother and one from the father. If the alleles are identical, the individual is 'homozygote' at that position; If the alleles are different, the individual is 'heterozygote' at that positon.

Variant: A difference in a DNA sequence compared to the reference sequence. Variants can be inherited from parents (familial variant) or arise *de novo*, occurring at any stage during gametogenesis or post-zygotically. As such, testing of DNA samples from both parents and the proband (trio) is required to determine whether a variant is familial or *de novo* (i.e. parental samples testing negative). A variant found in multiple individuals in the general population (often defined as having a frequency of ≥1%) and which does not cause any severe disease is termed a 'polymorphism'. Polymorphisms include variants impacting on susceptibility to a disease or a response to a certain treatment (risk alleles). In contrast to risk alleles, a 'rare variant of large effect size' is seen rarely in the population (frequency of <1%) and contributes substantially to the pathogenesis of a certain disease. Rare variants of large effect size include 'mutations', very rare variants resulting in a detrimental phenotype. Establishing variant pathogenicity is challenging due to 'genomic noise' in the human genome. With current sequencing technologies, up to 50,000 variants that differ from the reference human genome can be identified in any given exome. Of these 50,000 variants, up to 1,000 result in altered amino acid sequence of their encoded proteins and have not been previously reported. If parent-offspring trios are analysed, 150-200 of these variants are transmitted from parents to the child. Furthermore, 1-2 *de novo* mutations altering protein function are seen in every child, irrespective of whether he or she is affected or not (Conrad *et al.*, 2011; Iossifov *et al.*, 2012). Therefore, to assess variant pathogenicity, ample information is considered, including variant type, location, impact on encoded protein, frequency in population datasets, and segregation within families.

Penetrance: The likelihood that an individual carrying a mutation also expresses the associated disease.

Monogenic (Mendelian) inheritance: Inheritance determined by transmission of a single gene, as per the basic laws of inheritance described by Gregor Mendel in 1865. Patterns of monogenic inheritance include autosomal dominant, autosomal recessive and sex-linked. 'Autosomal' refers to genes on chromosomes 1-22 (autosomes). 'Sex-linked' refers to genes on chromosomes X or Y. Autosomal dominant inheritance is characterised by a phenotype which is expressed when the corresponding allele is either homozygous or heterozygous. Autosomal recessive inheritance is characterised by a phenotype which is only expressed when the corresponding allele is in a homozygous state. X-linked inheritance is characterised by a phenotype which is expressed when the corresponding allele on the X chromosome is either in a homozygous or

heterozygous state. Diseases displaying monogenic inheritance (i.e. Mendelian diseases) are relatively rare and are typically first recognised clinically by their predictable mode of inheritance within families. Classic examples of Mendelian diseases include cystic fibrosis, Huntington's disease and Duchenne muscular dystrophy.

Complex inheritance: Multifactorial inheritance, involving multiple genes, possibly in combination with environmental influences. Complex inheritance is displayed by many common diseases, such as hypertension, coronary heart disease, type II diabetes mellitus and asthma. The precise genetic architecture of these complex diseases remains to be fully understood (Gibson, 2012). Although rare variants of large effect size explain a fraction of the total risk of any complex disease, genome-wide association studies suggest that the majority of the risk may be attributable to a constellation of common variants each conferring a small or modest effect. However, most of these common polymorphisms are yet to be uncovered.

Next-generation sequencing (massively parallel sequencing): DNA sequencing that targets multiple genes simultaneously. Next-generation sequencing technologies include multi-gene panels, as well as whole exome sequencing (WES) and whole genome sequencing (WGS). WES targets the protein-coding regions of the genome (exons), in which most of the currently known disease-causing variants are located. WGS targets the exons as well as non-coding regions (introns) (Biesecker and Green, 2014). The advent of next-generation sequencing in 2005 has provided unprecedented opportunities to understand the genetic basis of human diseases and has fuelled an explosion of gene discoveries. In this respect, an important example is provided by Mendelian diseases. The proportion of Mendelian diseases for which genetic underpinnings have been identified has raised from <2% in 1990 to ~50% in the next-generation sequencing era (Boycott *et al.*, 2013).

Genome-wide association studies: Genome screens of unrelated individuals and appropriately matched controls or parent-proband trios typically aimed at assessing whether common polymorphisms are associated with a certain disease.

Genetics of Focal Epilepsies

Focal epilepsies have traditionally been regarded as predominantly acquired disorders. This is likely related to the common observation that the epilepsy resulting from an environmental insult, such as a stroke, head injury or tumour, is focal. However, data accumulated mainly in the past two decades have confuted historical views and have highlighted the importance of genetics in the aetiology of the focal epilepsies. These data are largely derived from four lines of evidence: familial aggregation studies; twin studies; clinical descriptions of familial focal epilepsy syndromes; and gene discoveries.

Familial Aggregation Studies

Familial aggregation studies estimate the magnitude of risk of a certain disease among relatives of affected probands (Zimmerman *et al.*, 2009). As such, they have inherent important implications for genetic counselling (Winawer and Shinnar, 2005). In addition, they can provide clues to the mode of inheritance of underlying genes and shed lights on phenotypic and genetic heterogeneity (Ottman *et al.*, 1997; Winawer *et al.*, 2003).

Early studies suggested an increased risk of seizure disorders among relatives of probands with focal epilepsy (1.7-4.3%) compared to the general population (0.5-1%), but to a lesser degree than among relatives of probands with generalised epilepsy (3.4-16.8%) (Eisner *et al.*, 1959; Lennox and Lennox, 1960; Beaussart and Loiseau, 1969; Burkhardt, 1972; Tsuboi and Endo, 1977). However, these studies were hampered by serious methodological limitations which might have potentially affected risk estimates, including selection bias, small sample size, lack of controls, reliance on patient interviews

to collect information about family history, failure to adjust for age in relatives, and ambiguous definitions of epilepsy (Peljto *et al.*, 2014).

Only a few familial aggregation studies have been population-based (Annegers *et al.*, 1976; Annegers *et al.*, 1982; Ottman *et al.*, 1989; Hemminki *et al.*, 2006; Peljto *et al.*, 2014). One of these studies used resources from the Rochester-Olmsted County Record Linkage Project in the US, and assessed the risk of unprovoked seizures in the offspring of parents with epilepsy (Ottman *et al.*, 1989). This risk was found to be approximately three-fold greater compared to the general population, irrespective of whether the parent's epilepsy was focal (standardised morbidity ratio, SMR: 3.2 [95% CI: 1.8-5.4]) or generalised (SMR: 3.3 [95% CI: 1.5-6.2]). Notably, another study using the same resources examined the risk of seizure disorders in the offspring of affected mothers, and found that the offspring of mothers with focal epilepsy had a higher ratio of observed-to-expected epilepsy than the offspring of mothers with generalised epilepsy (3.47 vs 2.22) (Annegers *et al.*, 1976). Hemminki et al. (Hemminki *et al.*, 2006) linked several national Swedish databases, including the Swedish Hospital Discharge Register, and assessed the familial risks for siblings to be hospitalised for epilepsy. The risk for a sibling to have focal epilepsy when the co-sibling had any type of epilepsy was not greater than expected. However, there were increased risks for siblings to be hospitalised for the same type of epilepsy, including focal epilepsy (standardised incidence ratio, SIR: 2.56 [95% C.I.: 1.10-5.60]) and idiopathic generalised epilepsy (SIR: 8.43 [95% CI: 4.22-16.39]).

A limited number of familial aggregation studies have controlled for the confounding effect of the aetiology of epilepsy (Annegers *et al.*, 1982; Ottman *et al.*, 1989; Ottman *et al.*, 1998; Hemminki *et al.*, 2006; Peljto *et al.*, 2014). Focal epilepsies are more likely to

be associated with an identifiable environmental insult of the CNS than generalised epilepsies, and genetic contributions are smaller in acquired epilepsies compared to those without an identifiable cause (Ottman, 2001). A population-based study using the resources of the Rochester-Olmsted County Record Linkage Project eliminated the confounding effect of epilepsy aetiology and assessed the risk of seizure disorders in relatives of probands with childhood-onset idiopathic epilepsy (Annegers *et al.*, 1982). In an analysis by probands' seizure type, the risk of epilepsy or isolated seizures in siblings and children of probands with focal seizures was found to be three-fold greater than in the general population (RR: 3.0 [95% CI: 1.2-6.3]).

A recent study by Peljto et al. addressed methodological limitations of earlier studies, and used the resources of the Rochester-Olmsted County Record Linkage Project to perform a comprehensive analysis of the risk of epilepsy among all first-degree relatives of probands with different epilepsy syndromes (**Table 1.3**) (Peljto *et al.*, 2014). The risk was significantly greater among relatives of probands with generalised epilepsy than among those of probands with focal epilepsy (SIR, 5.0 [95% CI: 3.18-7.45] vs 2.1 [1.27-3.10]), but it was significantly increased in both groups compared to the general population. Among relatives of probands with focal epilepsy, the risk was increased by more than two-fold for relatives of probands with focal epilepsy of unknown cause. Similar risk estimates were found for relatives of probands with idiopathic focal epilepsy, but they were based on small number and did not reach statistical significance. Interestingly, the risk was high (SIR=4.8) for relatives of probands with focal epilepsy of prenatal/developmental cause. In line with previous observations (Bianchi *et al.*, 2003; Winawer *et al.*, 2003; Jain *et al.*, 2004; Hemminki *et al.*, 2006), the authors also found that among relatives of probands with generalised epilepsy, the risk was increased to a

greater degree for generalised epilepsy (SIR, 8.3 [95% CI: 2.93-15.31]) than for focal epilepsy (2.5 [0.92-4.00]); similarly, among relatives of probands with focal epilepsy, the risk was increased to a greater degree for focal epilepsy (2.6 [1.19–4.26]) than for generalised epilepsy (1.0 [0.00-2.19]).

Overall, familial aggregation studies indicate that relatives of individuals with focal epilepsy carry a two to three-fold increased risk of epilepsy compared to the general population. In addition, they suggest that the genetic influences on focal vs generalised epilepsies may differ.

Table 1.3: Standardised incidence ratios (SIRs) for epilepsy in first-degree relatives of probands with different epilepsy syndromes in Olmsted County, Minnesota, USA[a]. Reproduced from: Peljto AL, Barker-Cummings C, Vasoli VM, Leibson CL, Hauser WA, Buchhalter JR, Ottman R, Familial risk of epilepsy: a population-based study, Brain, 2014, Vol. 137, Pt 3, 795-805, by permission of Oxford University Press.

Proband epilepsy syndrome	N of probands	First-degree relatives		Cumulative incidence to age 40 (%) (SE)	SIR (95% CI)
		N	With epilepsy		
All epilepsy[b]	660	2439	75	4.7 (0.60)	3.3 (2.45-4.32)
Idiopathic	136	571	28	7.3 (1.52)	5.5 (3.52-7.93)
Unknown cause	279	984	25	3.8 (0.84)	2.7 (1.71-3.97)
Structural/metabolic	245	884	22	3.8 (0.94)	2.6 (1.54-3.93)
Prenatal/developmental	91	317	13	6.1 (1.88)	4.3 (2.27-7.22)
Identified genetic[c]	14	54	0	-	-
Post-natal cause[d]	140	513	9	2.7 (1.03)	1.8 (0.66-3.14)
Generalised	175	708	32	7.3 (1.40)	5.0 (3.18-7.45)
Idiopathic	116	495	26	8.1 (1.72)	6.0 (3.75-8.93)
Unknown cause[e]	10	43	0	-	-
Structural/metabolic	49	170	6	6.3 (2.85)	3.9 (1.01-8.20)
Prenatal/developmental	30	112	5	7.3 (3.66)	4.7 (0.93-10.29)
Identified genetic	7	21	0	-	-
Post-natal cause	12	37	1	4.9 (4.38)	2.7 (0.00-13.45)
Focal	337	1239	25	2.9 (0.65)	2.1 (1.27-3.10)
Idiopathic	20	76	2	2.0 (2.01)	2.7 (0.00-6.81)
Unknown cause	167	603	13	2.9 (0.94)	2.2 (1.07–3.48)
Structural/metabolic	150	560	10	2.9 (0.95)	1.9 (0.90-3.27)
Prenatal/developmental	39	121	5	8.5 (3.50)	4.8 (1.56-9.88)
Identified genetic	7	33	0	-	-
Post-natal cause	104	406	5	1.6 (0.76)	1.3 (0.26-2.53)
Unclassifiable	140	461	17	5.2 (1.46)	4.2 (2.37-6.31)
Unknown cause	97	322	12	6.2 (1.82)	4.7 (2.53-7.51)
Structural/metabolic	43	139	5	3.2 (2.39)	3.3 (0.67-6.66)
Prenatal/developmental	20	75	3	1.9 (1.89)	3.6 (0.00-8.01)
Post-natal cause	23	64	2	5.1 (4.85)	2.8 (0.00-8.95)

[a] restricted to relatives born in 1920 or later and to residency periods from 1935-2008 in Olmsted County, Minnesota, USA; [b] includes relatives of eight probands with both generalized and focal epilepsy (31 relatives, one affected) who are excluded from generalized, focal, and unclassifiable subgroups; [c] identified genetic causes such as tuberous sclerosis, phenylketonuria, and Down syndrome. [d] postnatal causes in probands include: traumatic brain injury (n=39), stroke (n=26), neoplasm (n=24), CNS infection (n=17), autoimmunity (n=3), neurodegeneration (n=5), alcoholism (n=9), encephalopathy (n=3), and other or multiple causes (n=14). [e] includes West syndrome (n=2), epilepsy with myoclonic-astatic seizures (n=1), epilepsy with myoclonic absences (n=1), and other generalized epilepsies of unknown cause other than idiopathic generalized epilepsies (n=6).

Twin Studies

Twin studies are a powerful method to elucidate the genetic underpinnings of complex disorders, such as epilepsy (Berkovic *et al.*, 1998; Boomsma *et al.*, 2002). Under certain assumptions, higher concordance for a disorder in monozygotic (MZ) twins than in dizygotic (DZ) twins suggests a genetic component to the disorder (Boomsma *et al.*, 2002). Furthermore, the magnitude of concordance in MZ twins, coupled with the extent to which this exceeds that in DZ twins, are indicators of the size the genetic component (Boomsma *et al.*, 2002). Several studies have reported higher concordance rates for epilepsy in MZ twins than in DZ twins, supporting the concept that genetics contribute to the pathogenesis of the common epilepsies (Rosanoff *et al.*, 1934; Inouye, 1960; Lennox and Lennox, 1960; Marshall *et al.*, 1962; Corey *et al.*, 1991; Sillanpaa *et al.*, 1991; Berkovic *et al.*, 1998; Kjeldsen *et al.*, 2003). However, very few studies have used twin samples to investigate the role of genetics in the focal epilepsies.

Berkovic et al. applied the 1989 ILAE classification of the epilepsies and epileptic syndromes (1989) on 253 twin pairs in whom one or both had epilepsy or febrile seizures, ascertained from two community-based Australian twin registries or by referral (Berkovic *et al.*, 1998). Concordance rates were significantly greater in MZ twins than in DZ twins for both generalised epilepsies (0.82 vs 0.26, $p<0.001$) and focal epilepsies (0.36 vs 0.05, $p=0.01$), although overall estimates were greater in twins with generalised epilepsies than in those with focal epilepsies. The findings in the focal epilepsy group were largely attributable to 30 pairs with cryptogenic focal epilepsies, in which the concordance was 0.55 in MZ twins vs 0 in DZ twins ($p<0.001$). Interestingly, concordance rates did not

differ significantly between MZ twins and DZ twins for idiopathic focal epilepsies (0.33 vs 0.033) or symptomatic focal epilepsies (0 vs 0).

Vadlamudi et al. (Vadlamudi *et al.*, 2004a) compared concordance rates in 149 twin pairs with seizures from William Lennox's twin series vs the aforementioned 253 Australian twin pairs using the same ILAE classifications of seizures (1981) and epilepsies (1989). The MZ and DZ concordance estimates were strikingly similar between Lennox's series and the Australian series for generalised epilepsies (Lennox: 0.80 vs 0.27, $p<0.001$; Australian: 0.82 vs 0.26, $p<0.001$). As opposed to the Australian series, in Lennox's series concordance rates did not differ significantly between MZ twins and DZ twins for focal epilepsies (0.09 vs 0.06). This discrepancy might be related to the authors' inability to classify all of Lennox's concordant MZ pairs, some of which may actually represent concordant focal epilepsies; notably, MZ concordance rates for unclassified epilepsy tended to higher in Lennox's series than in the Australian series (0.83 vs 0.53. $p=0.08$).

In an update of the Australian series including 418 twin pairs with seizures (Vadlamudi *et al.*, 2014), concordances rates remained significantly higher in MZ pairs than in DZ pairs for both generalised epilepsies (0.79 vs 0.29, $p<0.0001$) and focal epilepsies (0.40 vs 0.03, $p<0.0001$). Concordances in MZ and DZ twin pairs were significantly higher for generalised epilepsies than for focal epilepsies. Among the focal epilepsies, concordance rates were significantly higher in MZ twins than in DZ twins for non-lesional temporal lobe epilepsy (0.82 vs 0, $p=0.003$). Notably, concordance rates did not differ between MZ and DZ pairs for idiopathic focal epilepsies (0 vs 0.17, $p=0.3$), comprising benign epilepsy with centro-temporal spikes and benign occipital epilepsies, which have long

been considered to be largely genetic in origin. No differences were seen between the two types of twins for symptomatic focal epilepsies (MZ: 0.08 vs DZ: 0, p=0.2).

In summary, twin studies provide strong evidence for genetic contributions to focal epilepsies. These contributions are particularly evident in non-lesional temporal lobe epilepsy. Although benign epilepsy with centro-temporal spikes and benign epilepsies of childhood have long been assumed to be predominantly genetic disorders, twin data (Vadlamudi *et al.*, 2004b; Taylor *et al.*, 2008; Vadlamudi *et al.*, 2014) do not support this view and suggest that non-conventional genetic factors or environmental factors may have an important pathogenic role.

Clinical Descriptions of Familial Focal Epilepsy Syndromes

In the past 25 years, several familial focal epilepsy syndromes have been identified, most of which display Mendelian inheritance (Ottman, 2001; Thomas and Berkovic, 2014). Not only have these syndromes provided further evidence for the contributions of genetics to the pathogenesis of focal epilepsies, but they have also led to important gene discoveries. In this section, we will review the clinical characteristics of four main syndromes: autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), familial mesial temporal lobe epilepsy (FMTLE), autosomal dominant epilepsy with auditory features (ADEAF), and familial focal epilepsy with variable foci (FFEVF). Genes implicated in these syndromes will be discussed in the next section.

Autosomal Dominant Nocturnal Frontal Lobe Epilepsy (ADNFLE)

First described in 1994 in six families from Australia, UK and Canada (Scheffer *et al.*, 1994; Scheffer *et al.*, 1995), ADNFLE has subsequently been recognized in other parts of the world (Oldani *et al.*, 1998; Nakken *et al.*, 1999). Seizures typically begin in the first two decades of life (peak at age 8-10 years) (Scheffer *et al.*, 1994; Scheffer *et al.*, 1995), and persist into adulthood. The predominant pattern is clusters of brief (<1 minute) nocturnal focal motor seizures, with hyperkinetic or tonic manifestations, occurring shortly after falling asleep or before awakening. Many affected individuals experience an aura, and retain awareness throughout the events. These seizures can be misdiagnosed as normal sleep behaviour, parasomnias, or psychiatric or medical conditions. About two-thirds of cases have also focal to bilateral tonic-clonic seizures (previously known as secondarily generalised tonic-clonic seizures) (Fisher *et al.*, 2017). A characteristic feature of the syndrome is also the marked intra-familial variation of the epilepsy severity.

Affected individuals are generally of normal intellect with no abnormalities no neurological examination. Interictal encephalogram (EEG) recordings are normal in most cases, whereas ictal video-EEG monitoring can demonstrate that the captured events are seizures of frontal lobe origin. Neuroimaging is normal. Many patients respond well to antiepileptic drug (AED) therapy with carbamazepine. However, a severe form of ADNFLE, associated with drug-resistant epilepsy, psychiatric comorbidities and intellectual disability, has also been described (Khatami *et al.*, 1998; Derry *et al.*, 2008).

ADNFLE is inherited in an autosomal dominant manner with a penetrance of approximately 70%.

Familial Mesial Temporal Lobe Epilepsy (FMTLE)

FMTLE was first described in 1994 in an Australian community-based study in twins (Berkovic *et al.*, 1994), and subsequently also recognized in other cohorts, including non-twin families (Berkovic *et al.*, 1996; Striano *et al.*, 2008; Gambardella *et al.*, 2009; Crompton *et al.*, 2010). FMTLE is typically a benign syndrome with onset in adolescence or early adulthood and no antecedent febrile seizures (Berkovic *et al.*, 1994; Berkovic *et al.*, 1996; Striano *et al.*, 2008; Gambardella *et al.*, 2009; Crompton *et al.*, 2010). The predominant seizure type are focal aware seizures (previously known as simple partial seizures) (Fisher *et al.*, 2017) with intense déjà vu and, less commonly, epigastric discomfort, perceptual distortions and fear. Within such families, these symptoms are often perceived as 'normal' experiences by affected individuals and may not even be detected by clinicians without specific inquiries, thus explaining why FMTLE can go easily undiagnosed. About two-thirds of cases have also infrequent focal impaired awareness seizures (previously known as complex partial seizures) (Fisher *et al.*, 2017) and rare focal to bilateral tonic-clonic seizures.

Neurological examination of affected individuals is normal. EEG recordings often do not reveal any epileptiform abnormalities and neuroimaging is normal. The epilepsy is generally very mild and responsive to AED therapy. However, a clinically heterogeneous form of FMTLE, often associated with antecedent febrile seizures, magnetic resonance imaging (MRI) signs of hippocampal sclerosis (HS) and drug resistance, has also been described (Cendes *et al.*, 1998).

FMTLE typically exhibits a complex pattern of inheritance (Crompton *et al.*, 2010), but rare dominant families have also been reported (Gambardella *et al.*, 2000; Hedera *et al.*, 2007; Dibbens *et al.*, 2013; Ishida *et al.*, 2013).

Autosomal Dominant Epilepsy with Auditory Features (ADEAF)

ADEAF was first described in 1995 in a large American family (Ottman *et al.*, 1995), and subsequently appreciated in other families (Poza *et al.*, 1999; Brodtkorb *et al.*, 2002; Winawer *et al.*, 2002; Michelucci *et al.*, 2003). Seizure onset is typically in adolescence or early adulthood, in the setting of no antecedent risk factors. A characteristic feature are focal aware seizures with auditory symptoms and/or receptive aphasia. Auditory symptoms mainly consist of elementary hallucinations, such as humming, buzzing or ringing; less commonly, they are in the form of illusions (e.g. volume changes) or complex hallucinations (e.g. voices or songs). Ictal receptive aphasia is the sudden inability to understand language in the absence of general confusion. Overall, these symptoms are suggestive of lateral temporal localisation; hence, this syndrome has been also labelled 'autosomal dominant lateral temporal epilepsy' (Poza *et al.*, 1999). In addition to focal aware seizures, affected individuals have also focal impaired awareness seizures and focal to bilateral tonic-clonic seizures.

Neurological examination in these individuals is normal. In up to two-thirds of cases, the EEG may show interictal epileptiform abnormalities. Neuroimaging is normal. The clinical course of ADEAF is typically benign, with seizures being easily controlled with AED therapy.

ADEAF is inherited in an autosomal dominant manner, with an estimated penetrance of 67% in an analysis of 24 published families (Rosanoff and Ottman, 2008).

Familial Focal Epilepsy with Variable Foci (FFEVF)

FFEVF was first described in 1998 in a four-generation Australian family with 10 affected individuals (Scheffer *et al.*, 1998; Picard *et al.*, 2000; Berkovic *et al.*, 2004b; Morales-Corraliza *et al.*, 2010). A number of families with FFEVF have since been reported (Callenbach *et al.*, 2003). The striking feature of this syndrome is the marked intra-familial variability in seizure semiology and EEG abnormalities, with evidence of focal seizures arising from different brain regions (i.e. frontal, temporal, parietal or occipital) in different family members. However, focal seizure semiology and congruent EEG abnormalities remain constant in each individual. Age of seizure onset is also variable, ranging from infancy to adulthood.

Individuals have typically normal intellect with no abnormalities found on neurological examination. Neuroimaging does not reveal any epileptogenic lesion. Response to AED therapy is variable, but in most individuals seizures can be easily controlled.

FFEVF displays an autosomal dominant pattern of inheritance, with penetrance of approximately 60%.

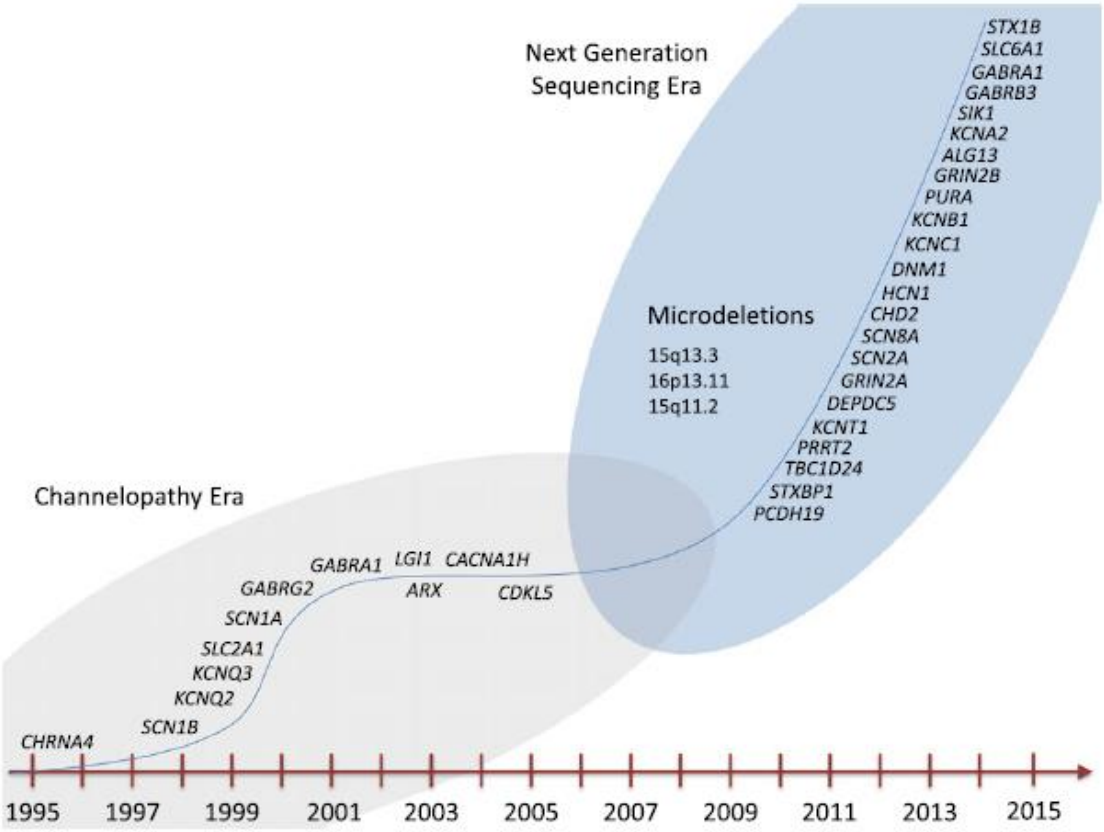
Gene Discoveries

The ultimate evidence for the genetic contribution to a certain disease is the identification of one or more specific genes. Remarkably, the very first epilepsy gene to be discovered was for focal epilepsy: *CHRNA4*, encoding the nicotinic acetylcholine receptor α4 subunit, identified in 1995 in a large Australian pedigree with ADNFLE. (Steinlein *et al.*, 1995). This finding paved the way for a pioneer era of gene discoveries – mainly ion channel genes – in Mendelian familial epilepsy syndromes ("channelopathy era"; **Figure 1.5**). These comprised a number of focal epilepsy genes, including: the voltage-gated potassium channel genes *KCNQ2* and *KCNQ3*, associated with benign familial neonatal seizures (Biervert *et al.*, 1998; Charlier *et al.*, 1998; Singh *et al.*, 1998); the voltage-gated sodium channel α2 subunit gene *SCN2A*, linked with benign familial neonatal-infantile seizures (Heron *et al.*, 2002); and *LGI1*, encoding a neuronal secreted protein, associated with ADEAF (Kalachikov *et al.*, 2002). However, these early discoveries did not lead to uncover the genetic underpinnings of all focal epilepsies. Rather, a relatively dormant period followed, characterised by negative candidate gene studies or claims of having identified putative focal epilepsy genes which did not eventually withstand replication (Cavalleri *et al.*, 2005; Thomas and Berkovic, 2014; Helbig *et al.*, 2016a).

The advent of next-generation sequencing (or massively parallel sequencing) approximately 10 years ago has provided unprecedented opportunities to decipher the genetic basis of human diseases (Goodwin *et al.*, 2016), and this has re-fuelled interest in genes associated with focal epilepsies (**Figure 1.5**) (Thomas and Berkovic, 2014). Next-generation sequencing technologies, including multi-gene panels as well as whole exome sequencing (WES) and whole genome sequencing (WGS), enable interrogation of

variation in several genes simultaneously (Biesecker and Green, 2014). WES targets all protein-coding regions (exons) in the human genome, in which most highly penetrant disease-causing variants are found (Biesecker and Green, 2014). WGS targets the entire genome, including exons as well as non-coding regions (introns), which may incorporate disease-causing variants affecting of the messenger RNA encoded by the gene (Biesecker and Green, 2014).

Figure 1.5: History of epilepsy gene discoveries. Reproduced by permission from John Wiley and Sons (Helbig *et al.*, 2016a).

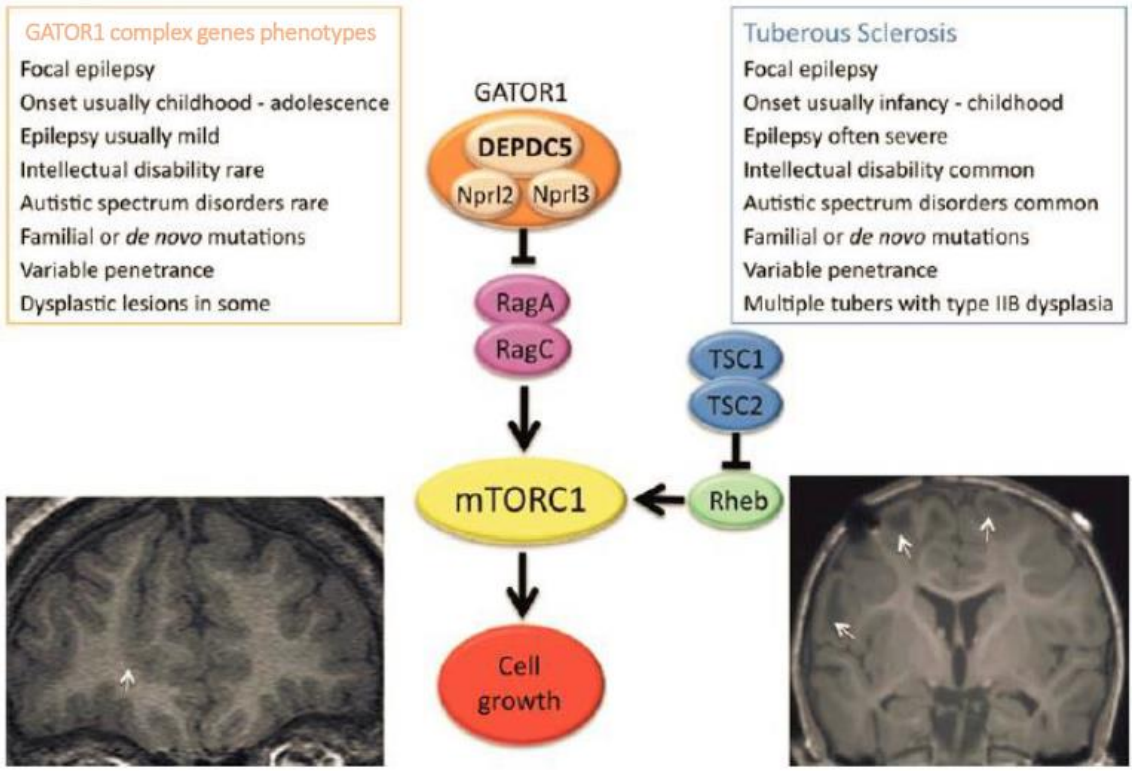


A major discovery in the "next-generation sequencing era" has been that genes encoding components of the GATOR1 complex (*DEPDC5*, *NPRL2*, *NPRL3*), a negative modulator of the mammalian target of rapamycin (mTOR) pathway, have a prominent role in the pathogenesis of focal epilepsies (**Figure 1.6**). Germline mutations in *DEPDC5* were initially identified in seven of eight large pedigrees with FFEVF, and in 10/82 (12%) smaller families with non-lesional focal epilepsy (Dibbens *et al.*, 2013). *DEPDC5* mutations were subsequently detected in other, mostly familial, forms of focal epilepsy, including ADNFLE, FMTLE, ADEAF and benign epilepsy with centro-temporal spikes (Ishida *et al.*, 2013; Lal *et al.*, 2014; Picard *et al.*, 2014; Baulac *et al.*, 2015; Pippucci *et al.*, 2015; Striano *et al.*, 2015). They were also found in focal epilepsies with malformations of cortical development, such as focal cortical dysplasia, subcortical band heterotopia and hemimegaloencephaly (Scheffer *et al.*, 2014; Baulac *et al.*, 2015; D'Gama *et al.*, 2015; Scerri *et al.*, 2015). To date, germline mutations in *NPRL2* and *NPRL3* have been reported in a few families and individuals with focal epilepsy, with and without brain malformations (Korenke *et al.*, 2016; Ricos *et al.*, 2016; Sim *et al.*, 2016; Weckhuysen *et al.*, 2016).

The mTOR pathway regulates a wide range of biological process, including cell growth, proliferation and apoptosis, in response to intra- and extracellular cues (Laplante and Sabatini, 2012). The identification of the GATOR1-related focal epilepsies expands the spectrum of the neurological disorders resulting from dysregulation of the mTOR pathway - the "mTORopathies" - the archetype of which is tuberous sclerosis (**Figure 1.6**) (Wong and Crino, 2012; Baulac, 2016). Notably, recent research has also shown that brain somatic mutations in mTOR pathway genes play a major role in the pathogenesis of malformations of cortical development, such as focal cortical dysplasia and

hemimegalencephaly, which are often associated with focal epilepsy (Lee *et al.*, 2012; Jamuar *et al.*, 2014; Lim *et al.*, 2015; Nakashima *et al.*, 2015; Lim *et al.*, 2017).

Figure 1.6: Mutations in genes encoding components of the GATOR1 complex (DEPDC5, NPRL2 and NPRL3), a negative modulator of the mammalian target of rapamycin (mTOR) pathway, can result in focal epilepsy. GATOR1-related focal epilepsies share phenotypic features with tuberous sclerosis, the archetype of neurological disorders associated with dysregulation of the mTOR pathway ("mTORopathies"). Reproduced by permission from John Wiley and Sons (Scheffer *et al.*, 2014).



The next-generation sequencing era has been characterised by other important gene discoveries in focal epilepsies (**Figure 1.5**). *PRRT2*, encoding a protein expressed in the CNS which is thought to be involved in synaptic transmission release, has been associated with an expanding spectrum of paroxysmal disorders, including benign infantile seizures,

infantile convulsions and paroxysmal choreoatetosis, and paroxysmal kinesigenic dyskinesia (Chen *et al.*, 2011b; Heron *et al.*, 2012a; Ebrahimi-Fakhari *et al.*, 2015). *KCNT1*, encoding a sodium-gated potassium channel subunit, has been linked with severe early-onset epilepsies, including ADNFLE with drug-resistant seizures, psychiatric comorbidities and intellectual disability, and epilepsy in infancy with migrating focal seizures (EIMFS), a severe epileptic encephalopathy characterised by focal seizures migrating from one brain region to another (Barcia *et al.*, 2012; Heron *et al.*, 2012b). *GRIN2A*, encoding the N-methyl-D-aspartate (NMDA) receptor NR2A subunit, has been found to have a major role in the epilepsy-aphasia spectrum comprising typical and atypical benign epilepsy with centro-temporal spikes, Landau-Kleffner syndrome and epileptic encephalopathy with continuous spike-and-wave in slow-wave sleep (Carvill *et al.*, 2013; Lemke *et al.*, 2013; Lesca *et al.*, 2013); interestingly, *GRIN2A* mutations appear to be more common in the more severe phenotypes, occurring in 0-5% of individuals with typical benign centro-temporal spikes compared to approximately 20% of those with epileptic encephalopathy with continuous spike-and-wave in slow-wave sleep (Carvill *et al.*, 2013; Lemke *et al.*, 2013; Lesca *et al.*, 2013).

In addition to new gene discoveries, the role of established epilepsy genes in the pathogenesis of focal epilepsies has recently been re-appraised. In a large genome-wide association study, variation in the sodium channel α1 subunit gene *SCN1A*, a well-recognised cause of genetic epilepsy with febrile seizures plus and Dravet syndrome, was found to possibly raise the risk for mesial temporal lobe epilepsy (MTLE) with HS and febrile seizures (Kasperaviciute *et al.*, 2013).

Overall, these results highlight the progress made in the past 20 years in understanding the genetics of different, mostly rare, forms of focal epilepsy (**Table 1.4**). Recently, a joint effort by two large international collaborations – the Epi4K consortium and the Epilepsy Phenome/Genome Project – assessed the extent to which genes responsible for rare epilepsies contribute to common epilepsies and whether, as in the rare epilepsies, the genetic risk arises mainly from ultra-variants of large effect (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). The authors separately compared WES data from 640 individuals with familial genetic generalised epilepsy and 525 individuals with familial non-acquired focal epilepsy to 3877 unaffected controls, and found a higher frequency of ultra-rare deleterious variants in established dominant epilepsy genes in both epilepsy groups (familial genetic generalised epilepsy: odds ratio [OR] 2.3, 95% CI 1.7-3.2, $p=9.1 \times 10^{-8}$; familial non-acquired focal epilepsy: 3.6, 2.7–4.9, $p=1.1 \times 10^{-17}$). Comparison of an additional group of 662 individuals with sporadic non-acquired focal epilepsy to the same controls did not identify any significant signals. In the group with familial non-acquired focal epilepsy, five established epilepsy genes (*DEPDC5*, *LGI1*, *PCDH19*, *SCN1A* and *GRIN2A*) ranked as the top genes enriched for ultra-rare deleterious variants, although only *DEPDC5* achieved study-wide significance (OR 8.1, 95% CI 3.6-18.3, $p=1.8 \times 10^{-7}$); the population attributable risk of familial non-acquired epilepsy with these five genes was approximately 8%. No individual gene was significantly associated with familial genetic generalised epilepsy. These data are important as they provide evidence for a definite link between the genetics of rare and common epilepsies.

Table 1.4: Familial focal epilepsy syndromes and genes implicated in their pathogenesis.

Familial focal epilepsy syndrome	**Gene(s)**
Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)	*CHRNA4*, *CHRNA2*, *CHRNB2*, *DEPDC5*, *KCNT1*, *NPRL2*, *NPRL3*
Autosomal dominant epilepsy with auditory features	*LGI1*, *RELN*
Autosomal dominant rolandic epilepsy with speech dyspraxia	*GRIN2A*
Benign familial neonatal seizures	*KCNQ2*, *KCNQ3*
Benign familial neonatal-infantile seizures	*KCNQ2*, *SCN2A*
Benign familial infantile seizures	*ATP1A2*, *KCNQ2*, *KCNQ3*, *PRRT2**, *SCN2A*, *SCN8A*
Familial focal epilepsy with variable foci	*DEPDC5*, *NPRL2*, *NPRL3*
Familial mesial temporal lobe epilepsy (FMTLE)	*DEPDC5*
Partial epilepsy with pericentral spikes	?

* main gene for benign familial infantile seizures; ? = genes implicated in partial epilepsy with pericentral spikes are yet unknown

The Treatment of Epilepsy

Medical Treatment

Antiepileptic drugs (AEDs) are the mainstay of the treatment of epilepsy. Approximately two-thirds of people become seizure-free on appropriate AED therapy, with varying response rates according to a number of factors, including epilepsy syndrome, underlying aetiology and number or frequency of seizures in the early phase of the disorder (Guerrini, 2006; Perucca, 2009; Perucca and Tomson, 2011). Independently of prognostic factors, most patients attaining seizure freedom respond to the first-ever prescribed AED (Kwan and Brodie, 2000; Perucca *et al.*, 2011a) and typically do so on low-to-moderate doses (Kwan and Brodie, 2001), which are associated with better AED tolerability (Perucca *et al.*, 2011b).

There is not a single AED which is ideal for first-line therapy in all patients with epilepsy. Rather, AED selection should take into account patient preferences and characteristics, including seizure type (**Table 1.5**), epilepsy syndrome, and other factors such as age, sex, ethnicity and comorbid conditions (Perucca, 2009; Perucca and Tomson, 2011). Although older AEDs such as carbamazepine and valproic acid (VPA) remain valid first-line therapies, newer AEDs are increasingly being used as initial treatment options, mainly due to their lower propensity for drug interactions (Mattson *et al.*, 1985; Mattson *et al.*, 1992; Brodie *et al.*, 2007; Marson *et al.*, 2007a, b; Glauser *et al.*, 2010; Baulac *et al.*, 2012; Baulac *et al.*, 2017). In the specific case of women of childbearing potential, the use of VPA as first-line therapy should be avoided owing to its risks of fetal toxicity (Tomson *et al.*, 2015b), a topic which will discussed in detail in a section below.

Table 1.5: Efficacy spectrum of the main AEDs in different types of seizures. Reproduced from The Lancet, 385, Moshé SL, Perucca E, Ryvlin P, Tomson T, Epilepsy: new advances, 884-898, Copyright (2015), with permission from Elsevier.

Effective against focal seizures and most generalised seizure types

Valproic acid

Benzodiazepines

- Benzodiazepines occasionally exacerbate tonic seizures, particularly after intravenous use in patients with Lennox-Gastaut syndrome.

Phenobarbital, primidone

- Phenobarbital and primidone are not effective against absence seizures.

Lamotrigine

- Lamotrigine can aggravate myoclonic seizures in some patients. The efficacy of lamotrigine is best documented against focal and secondarily generalised tonic-clonic seizures, primarily generalised tonic-clonic seizures, absence seizures, and drop attacks associated with the Lennox-Gastaut syndrome.

Levetiracetam

- Efficacy against tonic and atonic seizures has not been documented. The efficacy of levetiracetam is best documented against focal and secondarily generalised tonic-clonic seizures, primarily generalised tonic-clonic seizures, and myoclonic seizures.

Topiramate

- Efficacy against absence seizures has not been documented. The efficacy of topiramate is best documented against focal and secondarily generalised tonic-clonic seizures, primarily generalised tonic-clonic seizures, and drop attacks associated with the Lennox–Gastaut syndrome.

Zonisamide

- Efficacy against most generalised seizures types is poorly documented. The efficacy of zonisamide is best documented against focal and secondarily generalised tonic-clonic seizures.

Rufinamide

- Efficacy of rufinamide against absence and primarily generalised tonic-clonic seizures has not been documented. The efficacy of rufinamide is best documented against focal and secondarily generalised tonic-clonic seizures, and drop attacks associated with the Lennox-Gastaut syndrome.

Felbamate

- Efficacy against absence, myoclonic, and primarily generalised tonic-clonic seizures has not been documented. The efficacy of felbamate is best documented against focal and secondarily generalised tonic-clonic seizures, and drop attacks associated with the Lennox–Gastaut syndrome.

Primarily effective against focal seizures, with or without secondary generalisation

Carbamazepine, phenytoin, oxcarbazepine, eslicarbazepine acetate, tiagabine

- Carbamazepine, phenytoin, and oxcarbazepine can be efficacious also against primarily generalised tonic-clonic seizures. Carbamazepine, phenytoin, oxcarbazepine, tiagabine and, presumably, eslicarbazepine acetate can precipitate or aggravate absence and myoclonic seizures.

Lacosamide, perampanel, retigabine

- Tentative classification. Lacosamide, retigabine, and perampanel have not been assessed in patients with primarily generalised seizures.

Gabapentin, pregabalin

- Gabapentin and pregabalin can precipitate or aggravate myoclonic seizures.

Vigabatrin

- Vigabatrin can precipitate or aggravate myoclonic seizures. It is efficacious against infantile spasms.

Effective against absence seizures

Ethosuximide

- Ethosuximide can also be efficacious against myoclonic seizures.

In patients who do not respond to the first appropriate AED, the probability of attaining seizure-freedom decreases proportionally with the number of AEDs tried (Kwan and Brodie, 2000; Arts *et al.*, 2004; Schiller and Najjar, 2008; Brodie *et al.*, 2012). Based on these observations, the ILAE has defined drug-resistant epilepsy as "failure of adequate trials of two tolerated, appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" (Kwan *et al.*, 2010). Drug-resistant epilepsy is associated with excess disability, morbidity and even mortality (Perucca *et al.*, 2011a). Disappointingly, the introduction of several new AEDs in the past 20 years has had minimal impact on the overall outcomes of the pharmacological treatment of epilepsy, with seizures remaining uncontrolled in approximately a third of patients (Brodie *et al.*, 2012).

Adverse Effects of Antiepileptic Drugs (AEDs)

Adverse effects are an important cause of failure of AED treatment (Perucca and Gilliam, 2012; Zaccara and Perucca, 2016). They are the most common impediment to achieving fully effective AED dosages, and result in treatment discontinuation in up to 25% of patients (Kwan and Brodie, 2000; Perucca *et al.*, 2009a). They also negatively impact on health-related quality of life (Gilliam *et al.*, 2004a; Perucca *et al.*, 2009a; Perucca *et al.*, 2009b), and their reduction through modifications of AED therapy has been associated with improved subjective well-being without concomitant deterioration of seizure control (Gilliam *et al.*, 2004b).

Adverse drug effects can be classified in different ways (Perucca *et al.*, 2009a; Perucca and Gilliam, 2012). Using a modified version of the WHO classification, adverse effects

of AEDs can be distinguished into five types (**Table 1.6**): those that may arise acutely, as a consequence of the known pharmacological properties of the drug (Type A); those that are idiosyncratic (Type B); those that are chronic, resulting from the cumulative dose of the drug (Type C); those that are delayed, including teratogenesis and carcinogenesis (Type D); and those that are secondary to drug interactions (Type E) (Perucca and Gilliam, 2012). A separate discussion of the teratogenic effects of AEDs will be provided in the section below.

Table 1.6: Classification of adverse effects of AEDs. Reprinted from The Lancet Neurology, Vol. 11, Perucca P and Gilliam FG, Adverse effects of antiepileptic drugs, 792-802, Copyright (2012), with permission from Elsevier.

Type of adverse effects	**Description**	**Examples**	**Management**
Type A	▪ Related to the known mechanism of action of the drug ▪ Common (1-10%) or very common (>10%) ▪ Acute ▪ Dose- or serum concentration-dependent ▪ Predictable ▪ Reversible	▪ Drowsiness, lethargy, tiredness, fatigue, insomnia ▪ Dizziness, unsteadiness, vertigo, imbalance, ataxia, diplopia, tremor ▪ Cognitive impairment ▪ Irritability, aggressive behavior, depression ▪ Gastrointestinal symptoms ▪ Hyponatremia ▪ Paresthesias	*Prevention* ▪ Select an AED with a profile of tolerability suitable to the characteristics and preferences of the patient ▪ Start at low doses ▪ Up-titrate gradually ▪ Target the lowest effective maintenance dose *Management* ▪ Reduce dose ▪ Modify the dosing scheme ▪ Discontinue AED if measures to prevent/ameliorate toxicity are ineffective
Type B	▪ Related to the individual vulnerability (immunologic, genetic or other mechanism) ▪ Uncommon (0.1-1%) or rare (<0.1%) ▪ Develop during the first few weeks of treatment ▪ Unpredictable ▪ High morbidity and mortality ▪ Reversible	▪ Skin rashes, severe mucocutaneous reactions (DRESS, TEN, SJS) ▪ Aplastic anemia, agranulocytosis ▪ Hepatotoxicity, pancreatitis ▪ Angle closure glaucoma ▪ Aseptic meningitis	*Prevention* ▪ Avoid (or use very cautiously) specific AEDs in high-risk groups ▪ Start at low doses ▪ Up-titrate gradually *Management* ▪ Discontinue AED promptly ▪ Symptomatic or supportive management

			▪ Substitute AED with least risk for cross-reactivity reactions or worsening of underlying condition
Type C	▪ Related to the cumulative dose of the drug ▪ Common (1-10%) ▪ Chronic ▪ Mostly reversible	▪ Decreased bone mineral density ▪ Weight gain, weight loss ▪ Folate deficiency ▪ Connective tissue disorders ▪ Hirsutism, gingival hypertrophy ▪ Alopecia ▪ Visual field loss	*Prevention* ▪ Select an AED with a tolerability profile suitable to the characteristics and preferences of the patient *Management* ▪ Symptomatic or replacement therapy (e.g. calcium, vitamin D, folic acid) as required ▪ Discontinuation of AED if required
Type D	▪ Related to prenatal exposure to the drug (e.g. teratogenesis) or carcinogenesis ▪ Uncommon (0.1-1%) ▪ Delayed ▪ Dose-dependent ▪ Irreversible	▪ Birth defects ▪ Neurodevelopmental delay in the offspring ▪ Pseudolymphoma	*Prevention* ▪ If possible, avoid valproate, phenobarbital and polytherapy in women of childbearing potential ▪ Aim at low-risk monotherapies at the lowest effective dose before pregnancy ▪ Avoid discontinuation or major treatment changes during pregnancy
Type E	▪ Adverse drug interactions ▪ Common (1-10%) ▪ Predictable ▪ Reversible	▪ Increased risk of skin rash after adding lamotrigine to valproate ▪ Reduced seizure control after adding the combined contraceptive pill to lamotrigine ▪ Reduced effectiveness of warfarin after adding carbamazepine ▪ Increased risk for CNS neurotoxicity after combination of sodium-channel blocking AEDs	*Prevention* ▪ Avoid unnecessary polytherapy ▪ Choose co-medications with low potential for adverse drug interactions *Management* ▪ Adjust doses according to clinical response and, if necessary, serum drug concentrations

Teratogenic Effects of AEDs

Teratogenic effects of AEDs are a major concern in the treatment of women of childbearing potential (Thomas *et al.,* 2010). Prenatal exposure to AEDs, particularly in the first trimester of gestation, is associated with a two- to three-fold increased risk of major congenital malformations (Tomson and Battino, 2012). The risk varies across different monotherapies (**Table 1.7**), with VPA and phenobarbital carrying a higher risk compared to carbamazepine, phenytoin and lamotrigine (Tomson and Battino, 2012). For VPA, the risk is clearly dose-dependent (Tomson *et al.*, 2011; Hernandez-Diaz *et al.*, 2012; Campbell *et al.*, 2014), with doses ≤500 mg/day carrying the lowest risk (Hernandez-Diaz *et al.*, 2012); for carbamazepine, phenobarbital, lamotrigine, the risk may also be dose-dependent (Tomson *et al.*, 2011; Campbell *et al.*, 2014), although this was not confirmed in one large registry (Hernandez-Diaz *et al.*, 2012). Information on the teratogenic potential of newer AEDs, other than lamotrigine, is limited (Tomson and Battino, 2012; Vajda *et al.*, 2014); a few studies have suggested a higher risk for topiramate compared to lamotrigine (Hernandez-Diaz *et al.*, 2012), whereas accruing data for levetiracetam are reassuring to date (Mawhinney *et al.*, 2013).

Table 1.7: Rates of major congenital malformations for five AED monotherapies from four main pregnancy registries. Data are number of major congenital malformations/number of pregnancies exposed to AED monotherapy (%).

Registry	**Valproate (VPA)**	**Phenobarbital**	**Phenytoin**	**Carbamazepine**	**Lamotrigine**
UK Epilepsy and pregnancy register (Morrow *et al.*, 2006; Campbell *et al.*, 2014)	82/1220 (6.7%)	-	3/82 (4%)	43/1657 (2.6%)	49/2098 (2.3%)
North American AED Pregnancy Registry (Hernandez-Diaz *et al.*, 2012)	30/323 (9%)	11/199 (6%)	12/416 (3%)	31/1033 (3%)	31/1562 (2%)
International Registry of Antiepileptic Drugs and Pregnancy (EURAP) (Tomson *et al.*, 2011)	98/1010 (10%)	16/217 (7%)	6/103 (6%)	79/1402 (6%)	37/1280 (7%)
Australian Register of Antiepileptic Drugs in Pregnancy (Vajda *et al.*, 2014)	35/253 (13.8%)	0/4* (0%)	1/41 (2.4%)	19/346 (5.5%)	14/307 (4.6%)

*include pregnancies exposed to phenobarbital or primidone

The types of major congenital malformations appear to vary across different AEDs (Tomson and Battino, 2012). In the European Surveillance of Congenital Anomalies (EUROCAT) database, including data on congenital abnormalities from 14 countries, use of VPA monotherapy during the first trimester was associated with increased risks of spina bifida (OR 12.7 [95% CI: 7.7-20.7]), atrial septal defect, (2.5 [1.4-4.4]), cleft palate (5.2 [2.8-9.9]), hypospadias (4.8 [2.9-8.1]), polydactyly (2.2 [1.0-4.5]), and craniosynostosis (6.8 [1.8-18.8]) compared to no AED use (Jentink *et al.*, 2010b). In another study using the EUROCAT database, the only malformation associated with carbamazepine monotherapy was spina bifida (OR 2.6 [95% CI: 1.2-5.3] compared to no AED use) (Jentink *et al.*, 2010a).

AED polytherapy has been consistently found to be associated with a greater risk of major congenital malformations compared to monotherapy (Tomson and Battino, 2012). Growing evidence suggests that the malformation risk associated with polytherapy depends on the specific AEDs included in the combination rather than just the number of drugs (Vajda *et al.*, 2010; Holmes *et al.*, 2011; Vajda *et al.*, 2016). It appears that the increased risk with polytherapy is largely dependent on whether VPA is part of the combination (Vajda *et al.*, 2010; Tomson *et al.*, 2015a).

In addition to major congenital malformations, *in utero* AED exposure may impact on post-natal cognitive and behavioural development. Pre-natal VPA exposure has been associated with reduced cognitive abilities (Meador *et al.*, 2013; Shallcross *et al.*, 2014; Gerard and Meador, 2015; Bromley and Baker, 2017) and growing evidence suggest that it also carries an increased risk of autism spectrum disorders (Christensen *et al.*, 2013). The effect of VPA on neurodevelopmental outcomes appears to be dose-related (Meador

et al., 2013; Wood *et al.*, 2015). Exposure to lamotrigine, carbamazepine, levetiracetam or phenytoin monotherapy is associated with more favourable neurodevelopmental outcomes compared to VPA, but more data are required to evaluate whether these AEDs have subtle effects on post-natal cognition and behaviour (Gerard and Meador, 2015; Bromley and Baker, 2017). Information on neurodevelopmental outcomes with other AEDs is either inadequate or missing (Gerard and Meador, 2015; Bromley and Baker, 2017).

Considering the risks of adverse fetal outcomes associated with maternal VPA use, current guidelines recommend avoidance of VPA in women of childbearing potential where possible (Harden *et al.*, 2009; Tomson *et al.*, 2015b). However, challenges often arise, as VPA may be the only agent capable of fully controlling seizures in many such patients, particularly those with juvenile myoclonic epilepsy and other generalised epilepsy syndromes (Marson *et al.*, 2007b; Perucca *et al.*, 2015).

Epilepsy Surgery

Resective, disconnective or destructive epilepsy surgery is the most effective way of controlling seizures in selected patients with drug-resistant epilepsy (Ryvlin *et al.*, 2014). Assessing whether a patient is a candidate for epilepsy surgery relies on a number of investigations, including scalp video-EEG monitoring, neuropsychological testing and neuroimaging, aimed at delineating the epileptogenic zone (i.e. the minimum amount of cortex which if resected, disconnected or destructed will result in complete seizure freedom) as well as defining the risk of post-operative morbidity (Luders *et al.*, 2006;

Ryvlin *et al.*, 2014). Some patients may also require intracranial EEG investigations to better localise the epileptogenic zone (Spencer and Huh, 2008; Ryvlin *et al.*, 2014).

Seizure-freedom after epilepsy surgery is attained in a variable proportion of patients depending on a number of factors, including epilepsy type, results of presurgical investigations, underlying pathology, and duration of follow-up (McIntosh *et al.*, 2004; Jeha *et al.*, 2006; Jeha *et al.*, 2007; Spencer and Huh, 2008; de Tisi *et al.*, 2011; Ryvlin *et al.*, 2014). In a well-designed trial, Wiebe et al. (Wiebe *et al.*, 2001) randomised 80 patients with drug-resistant temporal lobe epilepsy (TLE) to either surgery (anterior temporal lobectomy with amygdalohippocampectomy) or continued AED therapy. At one year follow-up, 23 of the 40 (58%) surgically treated patients were free of disabling seizures vs only 3 of 40 (8%) medically treated patients ($p<0.001$). Patients in the surgical group reported better health-related quality of life than those in the medical group. Notably, one patient in the medical group died of sudden unexpected death in epilepsy (SUDEP); no deaths occurred in the surgical group. In a subsequent decision analysis, surgery for a 35-year-old man with drug-resistant TLE was found to increase life expectancy by 5 years (95% CI, 2.1-9.2) compared to continued medical therapy (Choi *et al.*, 2008) This benefit compared favourably with that of established medical interventions: eliminating lifelong coronary heart disease mortality in a 35-year-old individual had been previously shown to increase survival by 3.1-3.3 years (Tsevat *et al.*, 1991) and coronary grafting or beta-blocker use after myocardial infarction had been shown to increase survival by 0.25-1.1 years (Goldman *et al.*, 1988; Wong *et al.*, 1990).

In 2003, the American Academy of Neurology (AAN) recommended that "patients with disabling complex partial (focal) seizures, with or without secondarily generalized

seizures, who have failed appropriate trials of first-line antiepileptic drugs should be considered for referral to an epilepsy surgery center" (Engel *et al.*, 2003). Of concern, however, the utilisation of epilepsy surgery in the US has not increased since the publication of the AAN recommendations (Englot *et al.*, 2012). Furthermore, referral for epilepsy surgery evaluation occurs late in the course of the disease, typically after 20 years of seizures (Elliott *et al.*, 2013), despite evidence of surgery being effective after failure of two AEDs (Engel *et al.*, 2012) as well as data suggesting that longer duration of epilepsy increases the risk of surgical failure (Simasathien *et al.*, 2013).

In circumstances in which surgical cure is not feasible, palliative epilepsy surgery, such as corpus callosotomy, may be undertaken with the goal of decreasing seizure frequency and severity, as well as improving quality of life (Spencer and Huh, 2008; Ryvlin *et al.*, 2014).

Other Therapies for Epilepsy

A number of therapeutic options are available for patients with drug-resistant epilepsy who are not candidates for epilepsy surgery. A widely used treatment is vagus nerve stimulation, which has been shown to result in ≥50% seizure reduction in approximately half of treated patients (Englot *et al.*, 2011). However, less than 5% of treated patients become seizure-free (Englot *et al.*, 2011). Transcutaneous stimulation of the vagus (Stefan *et al.*, 2012) or trigeminal nerve (DeGiorgio *et al.*, 2013) are promising neurostimulation techniques, which need to be validated in large well-designed studies.

Two treatments which have been proven to be effective in patients with drug-resistant epilepsy are deep brain stimulation of the anterior nucleus of the thalamus (Fisher *et al.*, 2010; Salanova *et al.*, 2015) and responsive cortical stimulation, which delivers electrical stimulation only upon detection of abnormal electrocortigraphic activity via a closed-loop implanted device (Morrell and Group, 2011; Bergey *et al.*, 2015). These treatments can result in seizure reduction, but rarely render patients seizure-free (Fisher *et al.*, 2010; Morrell and Group, 2011).

The ketogenic diet is a high-fat, adequate protein, low-carbohydrate diet which is a first-line therapy for glucose transporter type 1 (GLUT-1) deficiency syndrome and pyruvate dehydrogenase complex deficiency (Rogovik and Goldman, 2010), and an effective treatment for children with various drug-resistant epilepsy syndromes (Winesett *et al.*, 2015). In a randomised trial in drug-resistant childhood epilepsy, 28 of 73 (38%) children assigned to the diet had >50% seizure reduction at 3-month follow-up vs only 4 of 72 (6%) controls (<0.0001) (Neal *et al.*, 2008); 5 children (7%) in the diet group had >90% seizure reduction vs no controls (p=0.06). A limitation of the diet has traditionally been its poor long-term compliance, which less strict modifications have attempted to address (Winesett *et al.*, 2015). The diet holds promise in the treatment of epilepsy in adults and of refractory status epilepticus (Klein *et al.*, 2014; Cervenka *et al.*, 2017), but early observations need to be substantiated by well-designed studies.

Pharmacogenomics

Pharmacogenomics is the study of variation in genes encoding drug-metabolising enzymes, transporters and drug targets, and how these variations influence drug disposition and response, including efficacy and toxicity (Crews *et al.*, 2012; Franco and Perucca, 2015; Zaccara and Perucca, 2016). To date, efforts to identify genomic predictors of AED efficacy have been fairly disappointing. In an elegant genome-wide association study of 889 patients with newly-treated epilepsy, including patients from the Royal Melbourne Hospital and Austin Health in Melbourne, Speed et al. concluded that, at the population level, it is unlikely any single common variant explains >4.4% of the variation in treatment outcomes (Speed *et al.*, 2014). On the other hand, major progress has been made in the identification of genomic predictors of adverse AED effects, particularly idiosyncratic reactions (Franco and Perucca, 2015). A very important discovery is that, in Han Chinese and other South Asian populations, *HLA-B*1502* is strongly associated with the risk of carbamazepine-induced serious cutaneous reactions, namely Stevens-Johnson syndrome and toxic epidermal necrolysis (Chung *et al.*, 2004; Hung *et al.*, 2006; Man *et al.*, 2007; Tassaneeyakul *et al.*, 2010; Wu *et al.*, 2010; Franco and Perucca, 2015; Zaccara and Perucca, 2016). This finding led the U.S. Food and Drug Administration to recommend that Han Chinese and South Asian ethnic groups be genotyped for *HLA-B*1502* before initiation of carbamazepine treatment and that the drug be avoided in individuals who test positive "unless the expected benefit clearly outweighs the increased risk of serious skin reactions" (US Food and Drug Administration). The clinical utility of *HLA-B*1502* genotyping was demonstrated in a

large Taiwanese study of 4877 patients with epilepsy who were candidates to receive treatment with carbamazepine (Chen *et al.*, 2011a). Those testing positive for *HLA-B*1502* (7.7%) were given an alternative AED, whereas *HLA-B*1502*-negative patients (92.3%) were commenced on carbamazepine. No serious cutaneous reactions occurred in the latter group. Based on the incidence of serious cutaneous reactions in this population, the authors estimated that screening for *HLA-B*1502* protected approximately 10 cases from developing Stevens-Johnsons syndrome or toxic epidermal necrolysis.

Some studies in Han Chinese and Thai populations have also described an association between *HLA-B*1502* and serious cutaneous reactions induced by phenytoin, lamotrigine and oxcarbazepine, which are structurally related to carbamazepine, suggesting that it is safer to avoid these AEDs as alternative agents to carbamazepine in *HLA-B*1502*-positive individuals (Man *et al.*, 2007; Locharernkul *et al.*, 2008; Hung *et al.*, 2010; Cheung *et al.*, 2013).

Among the HLA genotypes predisposing to AED-induced idiosyncratic reactions, *HLA-A*3101* has been associated with an increased risk of a variety of reactions, including maculopapular exanthema, Stevens-Johnsons syndrome, toxic epidermal necrolysis and drug reaction with eosinophilia and systemic symptoms (DRESS) (McCormack *et al.*, 2011; Ozeki *et al.*, 2011). As opposed to *HLA-B*1502*, *HLA-A*3101* is common in most ethnic groups, including Europeans and Asian populations (Amstutz *et al.*, 2014). However, the degree of elevated risk with *HLA-A*3101* is less than that with *HLA-B*1502* (Amstutz *et al.*, 2014). Overall, the clinical utility of *HLA-A*3101* genotyping is still unknown, and a study to address this knowledge gap is currently underway in Japan (Kaniwa and Saito, 2013).

Recently, light has been shed on the genomic underpinnings of phenytoin-induced idiosyncratic reactions. Phenytoin is metabolised primarily by the cytochrome P450 (CYP) 2C9 enzyme (Franco and Perucca, 2015). Genetic variants causing reduced CYP2C9 enzyme activity result in decreased phenytoin clearance and, consequently, increased serum phenytoin concentrations and heightened risk of toxicity (Franco and Perucca, 2015). In a large case-control study conducted in Taiwan, Japan and Malaysia, *CYP2C* variants, including *CYP2C9*3*, known to cause 93-95% reduction in phenytoin clearance, were found to increase the risk of phenytoin-induced severe cutaneous reactions (Chung *et al.*, 2014). Delayed phenytoin clearance was detected in individuals who developed severe cutaneous reactions, particularly *CYP2C9*3* carriers. Whether these reactions can be prevented by a slow up-titration schedule of phenytoin or regular monitoring of phenytoin serum concentrations has not been assessed.

Precision Medicine

The idea of delivering treatments specifically targeted towards the molecular causes of a disease has been a long-sought, yet largely elusive, goal in medicine. The advent of next-generation sequencing has fuelled renewed hope and, besides oncology, epilepsy likely offers the greatest opportunity to achieve precision medicine (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). This is related to the recent wave of novel gene discoveries, the availability of various experimental models in which pathway-directed therapies can be developed, and the possibility of evaluating the effectiveness of promising agents in cost-effective, small-sized, brief clinical trials (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017).

For some genetic epilepsies, precision medicine is already becoming reality (Franco and Perucca, 2015). A prime example is GLUT-1 deficiency syndrome, in which mutations in the *SLC2A1* gene result in impaired glucose transport across the blood-brain barrier and wide phenotypic variability (Klepper *et al.*, 2005; Pong *et al.*, 2012; Thomas and Berkovic, 2014). These patients respond well to the ketogenic diet, which provides the brain with an alternative energy substrate to glucose (Klepper *et al.*, 2005). Another example is pyridoxine-dependent epilepsy, usually caused by mutations in *ALDH7A1*, the detection of which allow prompt treatment with pyridoxine (vitamin B6) (Mills *et al.*, 2006). Identifying the molecular cause of a certain form of epilepsy can also prevent or minimise adverse AED effects. In *SCN1A*-related epilepsies, use of sodium channel-blocking AEDs can aggravate seizures (Franco and Perucca, 2015), although exceptions have been reported (Dalic *et al.*, 2015). In patients with epilepsy due to *POLG* mutations, avoidance of valproate therapy has been recommended due to the increased risk of hepatic failure (Tzoulis *et al.*, 2006; Engelsen *et al.*, 2008).

Other genetic epilepsies may soon be amenable to precision therapy approaches. These may include *KCNT1*-related epilepsies. In an elegant *in vitro* study, *KCNT1* mutations implicated in EIMFS and ADNFLE were found to be associated with an increase in function (Milligan *et al.*, 2014). Furthermore, the gain of function was reversed by the antiarrhythmic drug quinidine, a known antagonist of KCNT1 channels. Quinidine was later reported to dramatically improve seizure control in two patients with EIMFS due to gain-of-function *KCNT1* mutations (Bearden *et al.*, 2014; Mikati *et al.*, 2015), whereas conflicting results were obtained with other *KCNT1*-related phenotypes (Mikati *et al.*, 2015; Chong *et al.*, 2016; Fukuoka *et al.*, 2017). Well-designed clinical trials are needed. Additional targeted therapies under investigation include: ezogabine (retigabine) for

KCNQ2-related epilepsies (Orhan *et al.*, 2014); memantine for *GRIN2A*-related epilepsies (Pierson *et al.*, 2014); and mTOR inhibitors for mTORopathies, such as everolimus which has already been demonstrated to have some effect in tuberous sclerosis (Krueger *et al.*, 2010; French *et al.*, 2016).

Precision medicine may also apply to epilepsy surgery (Thomas and Berkovic, 2014). A common concern in genetic focal epilepsies is that the mutation causing the epilepsy may be widely expressed in the brain, ultimately undermining the effectiveness of a focal resection, disconnection or destruction (Surges and Elger, 2013). There may also be genetic factors which are not directly implicated in the process of focal epileptogenesis, which may nevertheless impact on the outcome of epilepsy surgery (Catarino *et al.*, 2011). Available data on the effect of genetics on surgical outcomes are derived from anecdotal reports or small series, and are conflicting. Some authors reported poor outcomes in operated patients with *SCN1A* mutations, and recommended that these variants be considered a contraindication to epilepsy surgery (Barba *et al.*, 2014; Skjei *et al.*, 2015). Mutations in other genes, including *SCN1B* or GATOR1 complex genes, may instead not pose an increased risk of surgical failure (Scerri *et al.*, 2015; Sim *et al.*, 2016; Scheffer *et al.*, 2017). Clearly, well-designed large studies are required to address the question of whether genetics influence the outcome of epilepsy surgery.

Chapter 2:

Thesis Aims

By building on the advances in epilepsy genetics mainly from the past two decades (Thomas and Berkovic, 2014), the overall aim of my thesis was to investigate and characterise the role of genetic factors in determining the aetiology of focal epilepsies and influencing outcomes of epilepsy treatment.

The specific aims of this thesis were the following:

1) To ascertain the frequency of familial mesial temporal lobe epilepsy (FMTLE) among patients presenting with non-lesional mesial temporal lobe epilepsy (MTLE);
2) To evaluate the diagnostic yield and management implications of genetic testing in the routine clinical care of focal epilepsies;
3) To assess whether genetic factors influence the seizure outcome of surgery for drug-resistant MTLE;
4) To investigate the genetic underpinnings of neural tube defects (NTDs) resulting from prenatal exposure to valproic acid (VPA).

Aim 1 is addressed in chapter 4, aim 2 in chapter 5, aim 3 in chapter 6, and aim 4 in chapter 7.

Chapter 3:

General Methods

Introduction

This chapter provides a description of the general methods used across the series of studies presented in this thesis. Specific methodology, protocols and endpoints applicable to individual studies are described in detail in the relevant chapter.

For each study, I recruited participants, collected relevant data, conducted statistical analyses, interpreted findings and prepared tables and figures. For the study described in chapter 6, I also coordinated subject recruitment and data collection which took place at different participating sites within the framework of the EPIGEN collaboration (Cavalleri *et al.*, 2007; Petrovski *et al.*, 2009). For the study described in chapter 7, I also oversaw subject recruitment and data acquisition performed at the Karolinska Institute, Stockholm, Sweden.

Two important shared components of the research presented in this thesis were phenotyping and sequencing, which will be discussed in detail in the sections below.

Phenotyping

Careful phenotyping is a critical step in the diagnostic pathway and remains instrumental for understanding the genetic underpinnings of human disease (Kohane, 2014). Participants of the studies described in chapters 4 and 5 underwent a comprehensive historical assessment, including a validated epilepsy questionnaire (Reutens *et al.*, 1992), which was conducted by myself in the form of a face-to-face or telephone interview. This assessment (see **Appendices**) aimed at capturing a wide range of information, including: demographic data; antenatal, birth and postnatal history; developmental, educational and occupational history; seizure history, comprising a verbatim record of the description of any seizure by the participant and/or any available witness as well as direct questions screening for common or subtle ictal and peri-ictal manifestations; past medical history; previous neuroimaging and EEG investigations; treatment history, including information on previous and current exposure to antiepileptic drugs; family history; and a detailed pedigree. Interviews lasted 30 minutes to 3 hours, depending on the complexity of the participant's history. Strenuous efforts were made to obtain previous medical records, including clinicians' letters, hospital records, neuroimaging studies and EEGs.

For participants of the studies described in chapters 6 and 7, study-specific data were collected using *ad hoc* case report forms.

Sequencing

Whole exome sequencing (WES) was utilised in three of the four studies presented in this thesis, which are described in detail in chapters 5, 6 and 7.

In the study described in chapter 5, WES was performed at two laboratories in Melbourne, Australia: the Australian Genome Research Facility and the Centre for Translational Pathology, The University of Melbourne. Participants' DNA samples were sequenced using Illumina HiSeq 2500 sequencers after capture by Nextera V1.2 exome capture kit (Illumina, San Diego, CA, USA). Sequenced data were processed using Cpipe (Sadedin *et al.*, 2015) version 0.9.5 (bwa, GATK Unified Genotyper, Anno-var). Bioinformatics analyses were performed by Dr Sebastian Lunke at the Centre for Translational Pathology.

In the studies described in chapters 6 and 7, all sequencing was performed at the Institute for Genomic Medicine (IGM), Columbia University, NY, USA - formerly, the Center for Human Genome Variation, Duke University, Durham, USA. Participants' DNA samples were exome sequenced with the Agilent All Exon (50MB or 65MB) or the Nimblegen SeqCap EZ V2.0 or 3.0 Exome Enrichment kit (Roche NimbleGen, Madison, USA) or whole genome sequenced with Illumina HiSeq 2000 or 2500 (Illumina, San Diego, CA, USA) sequencers according to standard protocols. Sequenced data were processed using an in-house bioinformatics pipeline as described previously (Cirulli *et al.*, 2015; Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). Bioinformatics analyses were performed by Dr Nicole Griffin or Dr Slave Petrovski at the IGM.

Ethics Principles and Regulations

All studies described in this thesis were approved by the relevant ethics committees. Written informed consents were obtained from all participants or from their parents/legal guardians in the case of minors.

All participants' data were de-identified, and securely stored. Only researchers involved in the studies had access to the participants' data.

Chapter 4:

The Frequency of Familial Mesial Temporal Lobe Epilepsy among Patients Presenting with Non-Lesional Mesial Temporal Lobe Epilepsy*

Abstract

Objective: The cause of mesial temporal lobe epilepsy (MTLE) is often unknown. This study ascertained the extent to which newly diagnosed non-lesional MTLE actually represents familial MTLE (FMTLE).

Methods: All consecutive patients presenting to the Austin Heath First Seizure Clinic with MTLE and a normal MRI or MRI-evidence of hippocampal sclerosis over a 10-year period were identified. Patients' first-degree relatives and pairwise age- and sex-matched controls underwent a comprehensive epilepsy interview. Each interview transcript was reviewed independently by two epileptologists, blinded to relative or control status. Reviewers classified each subject as follows: epilepsy, specifying if MTLE; manifestations suspicious of epilepsy; or unaffected. Physiological déjà vu was noted.

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* This study was published in *Ann Neurol* 2017;82:166-176.

Results: Forty-four patients were included. At the Clinic, MTLE had been recognized to be familial in 2 patients only. Among 242 subjects interviewed, MTLE was diagnosed in 9/121 relatives vs 0/121 controls (p=0.008). All affected relatives had seizures with intense déjà vu and accompanying features; 6 relatives had not been previously diagnosed. Déjà vu experiences which were suspicious, but not diagnostic, of MTLE occurred in 6 additional relatives vs none of the controls (p=0.04). Physiological déjà vu was common, and did not differ significantly between relatives and controls. After completing the relatives' interviews, FMTLE was diagnosed in 8 of 44 patients (18.2%).

Conclusion: FMTLE accounts for almost one-fifth of newly diagnosed non-lesional MTLE, and it is largely unrecognized without direct questioning of relatives. Relatives of patients with MTLE may experience déjà vu phenomena which clinically lie in the 'borderland' between epileptic seizures and physiological déjà vu.

Introduction

Temporal lobe epilepsy (TLE) is the most common focal epilepsy syndrome in adulthood (Zarrelli *et al.*, 1999), and traditionally has been regarded as a predominantly acquired disorder. The identification of familial types of TLE, including mesial and lateral forms (Berkovic *et al.*, 1994; Ottman *et al.*, 1995), led to revision of this historical view and to recognition of the important contribution of genetic factors to the aetiology of TLE. Familial mesial TLE (FMTLE) was the first familial type of TLE to be identified, in the setting of a community-based study in twins (Berkovic *et al.*, 1998). FMTLE was originally described as a benign syndrome, with onset in adolescence or early adulthood and no antecedent febrile seizures. Seizures were mild and often subtle, consisting predominantly of focal aware seizures (formerly known as simple partial seizures) (Fisher *et al.*, 2017) with prominent déjà vu and, less commonly, nausea, perceptual distortions and fear. In many affected individuals, these symptoms were the only ictal manifestations and went completely unrecognised. When present, focal impaired awareness seizures and focal to bilateral tonic-clonic seizures (formerly known as complex partial seizures and secondarily generalised tonic-clonic seizures) (Fisher *et al.*, 2017) were infrequent and rare respectively, and were easily controlled with antiepileptic drug therapy. EEG recordings often did not reveal epileptiform abnormalities and MRI investigations were normal. Similar characteristics were later appreciated in other cohorts, including non-twin families (Berkovic *et al.*, 1996; Gambardella *et al.*, 2000; Striano *et al.*, 2008; Crompton *et al.*, 2010). In hospital-based series, families with a heterogeneous clinical presentation, often associated with antecedent febrile seizures, hippocampal sclerosis (HS) and drug resistance, were also identified (Cendes *et al.*, 1998; Kobayashi *et al.*, 2001; Scheffer *et al.*, 2007; Gambardella *et al.*, 2009). FMTLE typically displays complex inheritance

(Crompton *et al.*, 2010), but rare dominant families, with linkage to candidate loci (Hedera *et al.*, 2007; Chahine *et al.*, 2013; Fanciulli *et al.*, 2014) or association with mutations in GATOR1 complex genes in small families (Dibbens *et al.*, 2013; Ishida *et al.*, 2013; Striano *et al.*, 2015; Ricos *et al.*, 2016), have been reported. In contrast to FMTLE, the rare syndrome of familial lateral TLE is due to mutations in *LGI1* in 50% of families, with Reelin mutations described in a few pedigrees (Ottman *et al.*, 1995; Kalachikov *et al.*, 2002; Berkovic *et al.*, 2004a; Ottman *et al.*, 2004; Dazzo *et al.*, 2015).

The epidemiology of FMTLE is unknown. Its assessment is hampered by the mild and often subtle nature of the symptoms, which may be perceived as 'normal' phenomena by affected individuals. These individuals typically remain undiagnosed, unless a focal to bilateral tonic-clonic seizure occurs which may prompt them to seek medical attention. In addition, FMTLE may not be recognised by clinicians if careful inquiries into specific symptoms are not made. Here, these methodological challenges were addressed within the framework of a case-control study by directly assessing relatives of patients presenting to a First Seizure Clinic with non-lesional mesial TLE (MTLE). Cases with MRI-evidence of HS were included in this group, but not those with structural lesions. This study shows that FMTLE accounts for a substantial proportion of new diagnoses of non-lesional MTLE, and yet it is largely unrecognised. In addition, this study highlights that, unlike matched controls, relatives of patients with non-lesional MTLE may experience déjà vu phenomena which clinically are in the 'borderland' between physiological déjà vu and epileptic seizures of mesial temporal lobe origin.

Methods

Study Design and Subjects

I first identified patients presenting to a First Seizure Clinic with non-lesional MTLE. A case-control design was then applied to compare the frequency of MTLE among patients' first-degree relatives vs pairwise age- and sex-matched controls. The case-control study findings were used to ascertain the frequency of FMTLE, defined as the proportion of patients with ≥1 first-degree relatives with MTLE.

Patients and Their First-Degree Relatives

Patients with non-lesional MTLE were identified through the First Seizure Clinic at Austin Health, a major teaching hospital serving a community of ~500,000 people in the north-east metropolitan area of Melbourne, Australia. The First Seizure Clinic is a dedicated service for adolescents and adults with new-onset seizures and suspected newly-diagnosed epilepsy referred by local general practitioners or hospital accident and emergency departments (King *et al.*, 1998). The diagnostic procedures at the Clinic have been described previously (King *et al.*, 1998). Briefly, patients are assessed by an experienced epileptologist (Dr Mark R. Newton), who routinely screens for minor manifestations of potentially epileptic nature, including subtle features of temporal lobe seizures, and any family history of epilepsy or seizures. Attempts are made to obtain an EEG within 24 hours of a seizure, and when the first EEG is normal, a sleep-deprived EEG is organised. A brain MRI is also part of the diagnostic protocol, except for patients with idiopathic generalised epilepsies or benign epilepsy with centro-temporal spikes, in

whom a decision to perform an MRI is made on a case-by-case basis. Diagnoses are based on clinical history and the investigations' results.

The identification of patients with non-lesional MTLE was a two-step process. Firstly, I searched the First Seizure Clinic database for all patients who received a diagnosis of TLE from 1-January-2005 to 31-December-2014. Secondly, I reviewed their medical records to identify patients who had: 1) a clear MTLE seizure semiology, defined as the presence at seizure onset of ≥1 features strongly supportive of mesial temporal localization, i.e. déjà vu, stereotyped flashbacks of a past event, a rising epigastric/visceral sensation, or stereotyped olfactory or gustatory hallucinations (French *et al.*, 1993; Crompton *et al.*, 2010); and 2) no epileptogenic lesion on brain MRI. Patients with MRI-evidence of HS were included in the study, provided that they had no additional epileptogenic abnormalities. Patients with a diagnosis of TLE made prior to their first appointment at the Clinic were excluded.

Identified patients were invited to participate in the study. This involved providing a detailed family pedigree and asking all eligible first-degree relatives if they were available to undergo a face-to-face or telephone interview about their health. Eligible criteria for relatives were: 1) age ≥13 years; and 2) ability to communicate effectively in a semi-structured interview.

Controls

Each relative was paired with an age- (within ±3 years) and sex-matched control subject who underwent the same interview. Controls were recruited from the Royal Melbourne

Hospital, Melbourne, Australia, and comprised outpatients undergoing nerve conduction studies mainly for suspected carpal tunnel syndrome, visitors of inpatients with acute stroke, and students. Eligibility criteria for controls were: 1) age ≥13 years; 2) ability to communicate effectively in a semi-structured interview; and 3) no first-degree relative with MTLE.

The study was approved by the Austin Health and Melbourne Health Human Research Ethics Committees. Written informed consent was obtained from all participants or their parents/guardians.

Subjects' Assessment

Relatives and controls underwent a semi-structured face-to-face or telephone interview, including a validated epilepsy questionnaire (Reutens *et al.*, 1992), to collect demographic information; pre-natal, birth, post-natal and developmental history; past medical history; information on any previous EEG or neuroimaging (computed tomography [CT] or MRI) investigations; current medications; information on any prior exposure to antiepileptic drugs; and family history (**Appendix 1**). As previously described (Reutens *et al.*, 1992), the epilepsy questionnaire is similar in form to a clinical assessment. It comprises a verbatim record of the description of any seizure by the subject and, if available, an observer. This is complemented by direct questions screening for a wide range of ictal and peri-ictal manifestations, including features suggestive of mesial temporal lobe origin (Reutens *et al.*, 1992; Berkovic *et al.*, 1996; Crompton *et al.*, 2010). For subjects who reported undergoing EEG or neuroimaging studies, efforts were made to obtain formal results which were incorporated into the interview transcript.

To ensure that the assessment of subjects' interviews was carried out blindly to group allocation, the family pedigree and any information that could lead to identifying the subject as a relative or control (e.g. names, references to family members, etc.) were removed from the interview transcripts, each of which was assigned a random numerical code before review. Each coded interview transcript was then reviewed independently by two experienced epileptologists from different institutions (Professor Samuel F. Berkovic from Austin Health, Melbourne, and Dr K. Meng Tan from The Royal Melbourne Hospital, Melbourne), who classified the corresponding subject as having: 1) *epilepsy*, specifying when possible the type of epilepsy. This category referred to individuals in whom a diagnosis of epilepsy could be confidently made on clinical grounds. Reviewers were specifically asked to comment on whether the subject had MTLE, based primarily on their interpretation of the transcript, using agreed *ad hoc* criteria as a general guide (**Table 4.1**); 2) *suspicious manifestations*, detailing the specific manifestations. This category referred to symptoms which alone would not be sufficient to make a diagnosis of epilepsy. However, they would typically lead in clinical practice to request EEG and imaging studies; or 3) *no epilepsy or suspicious manifestations* (unaffected subjects). Reviewers also made note of any previous history of febrile seizures. In case of disagreement between the two reviewers, this was resolved through joint re-assessment of the coded interview transcript, blinded to each of the reviewers' initial classification.

Particular attention was given to reported déjà vu experiences, as they can represent a subtle feature of MTLE. Déjà vu was categorized as epileptic (ictal), suspicious or physiological. Déjà vu was defined as epileptic when its characteristics were aligned with the *ad hoc* criteria listed in **Table 4.1**. Déjà vu was designated as suspicious when it had some elements consistent with mesial temporal lobe seizures, but also other

characteristics which the reviewers considered atypical for an epileptic etiology (e.g. duration of several minutes, occurrence mainly in specific circumstances, etc). Physiological déjà vu referred to brief déjà vu experiences without other features.

Table 4.1: *Ad hoc* criteria used by reviewers as a general guide for the diagnosis of MTLE. These criteria were based on previous studies (French *et al.*, 1993; Crompton *et al.*, 2010; Warren-Gash and Zeman, 2014), and were agreed by both reviewers prior to assessing the subjects' interview transcripts.

Presence of a feature strongly supportive of mesial temporal localization: déjà vu, stereotyped flashbacks of a past event, a rising epigastric/visceral sensation, or stereotyped olfactory or gustatory hallucinations	
AND	At least one of the following: ✓ Association with impaired awareness ✓ Progression to loss of consciousness and convulsion OR At least two of the following: ✓ Prolonged duration of feature (i.e. several seconds or more) ✓ Moderate/high frequency of feature (i.e. several times per year or more) ✓ Severe intensity of feature, disrupting ongoing activities (e.g. thinking, conversation, etc.) ✓ Occurrence of feature in clusters ✓ Association with another feature strongly supportive of mesial temporal lobe localization (see above) or any of the following features: dreamlike sensation, fear, nausea, warmth, sweating, flushing and pallor ✓ Subsequent period of recovery, which may include symptoms such as fatigue, drowsiness, confusion or headache ('post-ictal phase')

Analysis Strategy

The primary outcome of the study was to assess the frequency of MTLE among relatives vs controls. This was followed by the comparison between the two groups of the proportion of subjects with: suspicious manifestations; physiological déjà vu; forms of epilepsy other than MTLE (other epilepsies); and previous history of febrile seizures.

The study estimated the frequency of FMTLE among patients presenting to the First Seizure Clinic with non-lesional MTLE who consented to participate in the study. A patient was considered to have FMTLE if he or she had ≥1 first-degree relatives with MTLE. The proportion of patients who had first-degree relatives with suspicious manifestations, other epilepsies or previous history of febrile seizures was also determined.

Statistical Analysis

Descriptive summary statistics were reported as frequencies and percentages or median and range (minimum-maximum), as appropriate. Fisher's exact test was used for comparisons of categorical data, and the Mann-Whitney U test for comparisons of continuous data.

Cohen's kappa statistic (κ) (Cohen, 1960) was used to quantify the initial degree of agreement between reviewers in classifying the coded interview transcripts, adjusting for agreement due to chance. κ equals 0 when the observed agreement is entirely due to chance, +1 when the agreement is maximal, and -1 when observers totally disagree (Cohen, 1960). For intermediate κ values, Landis and Koch (Landis and Koch, 1977)

proposed the following agreement categories: poor ($\kappa<0$); slight ($\kappa=0$-0.20); fair ($\kappa=0.21$-0.40); moderate ($\kappa=0.41$-0.60); substantial ($\kappa=0.61$-0.80); almost perfect ($\kappa>0.80$).

McNemar's chi-square test with continuity correction was applied to relative-control pairs to assess differences in the frequency of MTLE and other outcomes between relatives and controls.

Significance was set at $p\leq0.05$. All analyses were performed with R V.3.1.0 (https://www.r-project.org/).

Results

Subjects' Characteristics

Of 179 patients who received a diagnosis of TLE at the First Seizure Clinic from 1-January-2005 to 31-December-2014, 72 met eligibility criteria for non-lesional MTLE and, of these, 44 (61.1%) agreed to participate in the study (**Figure 4.1**). There were no differences in demographic and clinical characteristics between patients who were enrolled and those who were not, except for a higher representation of females in the enrolled group (63.6% vs 35.7%; **Table 4.2**).

Participants presented to the Clinic at a median age (range) of 35 (12-73) years, typically (n=33, 75%) following their first focal to bilateral tonic-clonic seizure. However, most (n=31, 70.4%) had experienced unrecognised focal seizures consistent with MTLE semiology (e.g. déjà vu, rising epigastric sensation, etc.) long before their presentation. Thirty patients (68.2%) had an abnormal EEG. Forty patients (90.9%) had a normal brain MRI and 4 (9.1%) had MRI-evidence of HS. At enrolment, information on family history collected initially at the Clinic (**Table 4.2**) was revisited through completion of a detailed pedigree. Fifteen patients (34.1%) reported a family history of epilepsy or single afebrile seizures, and 6 (13.6%) a family history of febrile seizures. In only 2 patients (4.5%), MTLE had been recognized to be familial at the Clinic. One was the only proband who reported having a relative diagnosed with TLE; this was a sister who had "convulsions and déjà vu". The other proband had a brother diagnosed with epilepsy who had "convulsions and episodes of hot flushes and déjà vu".

Figure 4.1: Flow diagram of patients included in the study. HS=hippocampal sclerosis; MTLE=mesial temporal lobe epilepsy; TLE=temporal lobe epilepsy. * indicates that 19 patients had ≥2 reasons for exclusion.

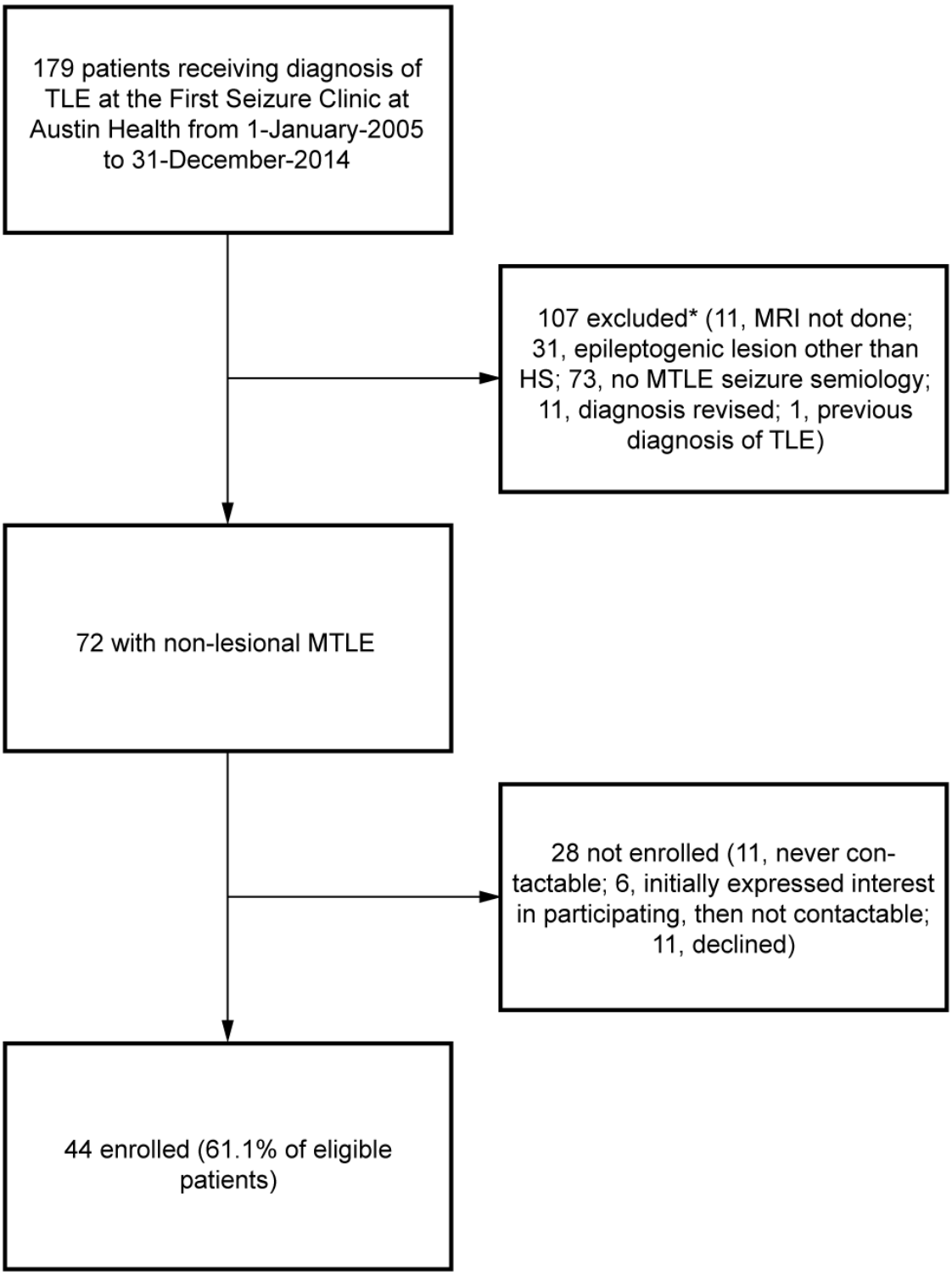


Table 4.2: Characteristics of eligible patients who were enrolled in the study and those who were not enrolled.

	Enrolled (n=44)	Not enrolled (n=28)	p value
Sex, n. (%)			
- Males	16 (36.4%)	18 (64.3%)	0.03
- Females	28 (63.6%)	10 (35.7%)	
Age at presentation to the First Seizure Clinic, years, median (range)	35 (12-73)	33 (14-63)	0.77
History of febrile seizures, n. (%)	3 (6.8%)	2 (7.1%)	1.00
Age at seizure onset[a], n. (%)			
- Childhood (age <18 years)	7 (15.9%)	6 (21.4%)	0.55
- Adulthood	37 (84.1%)	22 (78.6%)	
- Elderly (age ≥65 years)	0 (0%)	0 (0%)	
Seizure types, n. (%)			
- FAS only	3 (6.8%)	0 (0%)	0.32
- FAS + FIAS	5 (11.4%)	1 (3.6%)	
- FAS + FBTCS	18 (40.9%)	16 (57.1%)	
- FAS + FIAS + FBTCS	18 (40.9%)	11 (39.3%)	
EEG, n. (%)			
- Normal	14 (31.8%)	16 (57.1%)	0.08
- Non-specific temporal slowing only	13 (29.5%)	3 (10.7%)	
- TIRDA only	0 (0%)	0 (0%)	
- Temporal interictal epileptiform discharges only	3 (6.8%)	0 (0%)	
- Temporal lobe seizure only	1 (2.3%)	0 (0%)	
- Other findings only	2 (4.6%)	0 (0%)	
- Two or more abnormal findings	11 (25%)	9 (32.2%)	
MRI, n. (%)			
- No epileptogenic lesion	40 (90.9%)	26 (92.9%)	1.00
- Hippocampal sclerosis	4 (9.1%)	2 (7.1%)	
Family history[b], n. (%)			
- Epilepsy or single afebrile seizures	11 (25%)	13 (46.4%)	0.08
- Febrile seizures	4 (9.1%)	1 (3.6%)	0.64

FAS=focal aware seizures; FBTCS=focal to bilateral tonic-clonic seizures; FIAS=focal impaired awareness seizures; TIRDA=temporal intermittent rhythmic delta activity. [a] Patients typically experienced unrecognized focal seizures with MTLE semiology (e.g. déjà vu, rising epigastric sensation, etc.) long before their presenting seizure, not allowing accurate ascertainment of the age of seizure onset; [b] as collected at presentation to the First Seizure Clinic

The 44 patients had a total of 180 first-degree relatives who were eligible for the interview. Of these, 59 were not available to be interviewed, including 1 with undefined focal epilepsy, 3 with a previous history of febrile seizures and all eligible first-degree relatives of 3 patients. Therefore, 121 (67.2%) first-degree relatives of 41 probands underwent the interview [median number of relatives interviewed per proband (range): 3 (1-7)]. They had a median age (range) of 50 (13-86) years, with 66 (54.5%) being males. All 121 individuals [median age (range): 49 (14-87) years] who were asked to serve as pairwise age- (within ±3 years) and sex-matched controls consented to participate in the study and underwent the same interview.

Frequency of MTLE among Relatives vs Controls

There was initial inter-reviewer agreement on 218/242 coded interview transcripts (moderate agreement, κ=0.54; asymptotic SE: 0.07; 95% confidence interval: 0.39-0.68; **Table 4.3**).

Table 4.3: Inter-reviewer agreement in classifying the 242 coded interview transcripts. Agreement between the two reviewers is indicated by bold numbers.

			Reviewer #2										
			Epilepsy						Susp Man		Unaffected		
			MTLE	MTLE. Previous history of febrile seizures	TLE	Childhood absence epilepsy	Epilepsy, suspected generalized	Unclassified epilepsy	Suspicious visual episodes	Suspicious déjà vu	Unaffected. Previous history of febrile seizures	Unaffected	Total
Reviewer #1	Epilepsy	MTLE	**7**	0	0	0	0	0	0	3	0	7	17
		MTLE. Previous history of febrile seizures	0	**1**	0	0	0	0	0	0	0	0	1
		TLE	1	0	**0**	0	0	0	0	0	0	0	1
		Childhood absence epilepsy	0	0	0	**2**	0	0	0	0	0	0	2
		Epilepsy, suspected generalized	0	0	0	0	**0**	1	0	0	0	0	1
		Unclassified epilepsy	1	0	0	0	0	**1**	0	0	0	0	2
	Susp Man	Suspicious visual episodes	0	0	0	0	0	0	**0**	0	0	0	0
		Suspicious déjà vu	0	0	0	0	0	0	0	**0**	0	4	4
	Unaffected	Unaffected. Previous history febrile seizures	0	0	0	0	0	0	0	0	**2**	0	2
		Unaffected	2	0	0	0	0	1	1	3	0	**205**	212
Total			11	1	0	2	0	3	1	6	2	216	242

MTLE=mesial temporal lobe epilepsy; Susp Man=suspicious manifestations; TLE=temporal lobe epilepsy

After reviewers resolved disagreements (24 transcripts), MTLE was diagnosed in 9 of the 121 (7.4%) relatives and in none of the 121 (0%) controls (p=0.008; **Table 4.4**). Of note, 8 of the 9 relatives had been classified initially as having MTLE independently by both reviewers.

Table 4.4: Frequency of MTLE and other outcomes among first-degree relatives of patients with MTLE vs controls.

	Relatives	Controls	p value
MTLE	9/121 (7.4%)	0/121 (0%)	0.008
Suspicious manifestations (i.e. suspicious déjà vu)[a]	6/112 (5.4%)	0/112 (0%)	0.04
Physiological déjà vu[b]	77/106 (72.6%)	65/106 (61.3%)	0.08
Other epilepsies	5/121 (4.1%)	1/121 (0.8%)	0.22
Febrile seizures	2/121 (1.6%)	1/121 (0.8%)	1.00

MTLE=mesial temporal lobe epilepsy. [a] the 9 relative-control pairs with MTLE were excluded from this analysis; [b] the 15 relative-control pairs with MTLE or suspicious déjà vu were excluded from this analysis.

The characteristics of the 9 relatives with MTLE are detailed in **Table 4.5**. None had a history of pre- or perinatal insults, central nervous system infections or severe traumatic brain injury. One had a single febrile seizure at age 2 years. All 9 relatives had longstanding (up to 4 decades) focal aware seizures with déjà vu. Ictal déjà vu was not triggered by specific circumstances, was typically intense and intrusive, and was always associated with other features, including visceral/epigastric sensation, nausea, lightheadedness, slow motion and perceptual distortion. The déjà vu and accompanying symptoms lasted several seconds to a few minutes, and were often followed by tiredness or fatigue. In 3 relatives, these symptoms tended to occur in clusters, with symptom-free

periods lasting for up to several months. Two relatives had focal aware seizures only. Six had also focal impaired awareness seizures and 3 focal to bilateral tonic-clonic seizures. Remarkably, only the 3 relatives with focal to bilateral tonic-clonic seizures previously underwent EEG and MRI investigations; all 3 received a diagnosis of epilepsy and were started on antiepileptic drugs which resulted in excellent seizure control with persistence of only rare focal aware seizures. In the remaining 6 relatives, the mild and subtle epileptic phenomena went completely unrecognised, as illustrated by the following example:

> Subject 21 is a 62-year-old right-handed man who had normal birth and development. His past medical history is unremarkable. He has an older sister with non-lesional MTLE. He acknowledges experiencing recurrent episodes of déjà vu since age 27 years, but did not have any for the past 15 years. The déjà vu was a very intense feeling of familiarity, similar to "a vertical hold on TV". The feeling was often accompanied by altered perception of the surroundings: for example, an object in front of him would gradually become "more significant and consuming". These symptoms lasted about a minute, after which he could "blackout" for 10 seconds or more. Afterwards, he was usually "slow" and depressed for up to 30 minutes. There have been no witnessed convulsions. The episodes tended to occur in clusters every 6 months or so. He never sought medical attention.

Table 4.5: Characteristics of the 9 relatives with MTLE.

Subject n., group	Relative of patient n.	Sex	Age	History of febrile seizures	Age of onset	Seizure type	EEG	Neuro-imaging	AED therapy (outcome)	Classification consensus	Previous diagnosis of epilepsy (specific diagnosis)
12, Relative	3	F	50 y	No	Childhood	FAS (intense déjà vu and stereotyped flashbacks of past event, lasting a few min and occurring a few times a year)	Not done	Not done	None	MTLE	No
21, Relative	5	M	62 y	No	27 y	FAS (intense déjà vu and perceptual distortions, lasting 1 min and followed by feeling slow and depressed; used to occur in clusters every 6 months), FIAS	Not done	Not done	None	MTLE	No
44, Relative	10	M	43 y	No	10 y	FAS (sick feeling in stomach and déjà vu, lasting up to 2 min, followed by tiredness; used to occur every few months), FBTCS	Done, but results not available	Done, but results not available	CBZ 600 mg/day (since starting CBZ 18 y ago, no FBTCS and only very rare FAS)	MTLE	Yes (unknown)[a]
114, Relative	16	M	49 y	No	Childhood	FAS (intense déjà vu and depersonalization, lasting several sec and occurring every 3-5 months)	Not done	Normal MRI[b]	None	MTLE	No
120, Relative	26	F	55 y	No	31 y	FAS (intense déjà vu, nausea and flushing, lasting up to 30 sec, followed by fatigue and occurring a few times a year), FIAS	Not done	Not done	None	MTLE	No
176, Relative	28	M	53 y	No	20 y	FAS (déjà vu, light-headedness, slow motion, depersonalization and derealization, lasting 20 sec; used to occur every few months), FIAS, FBTCS	L T slowing	Normal CT and MRI	LTG 100 mg/day (since starting LTG 5 y ago, no FBTCS and	MTLE	Yes (TLE)[c]

									only very rare FAS)		
199, Relative	26	F	34 y	No	12 y	FAS (déjà vu, gustatory hallucinations, nausea, feeling flustered, perceptual distortions, lightheadedness and aphasia, lasting 15-30 sec, followed by tiredness and feeling sick; used to occur in clusters every 3 months), FIAS, FBTCS	L T slowing and ShW	Normal CT and MRI	LTG 200 mg/day (since starting LTG 3 y ago, no FBTCS and only very rare FAS)	MTLE	Yes (TLE)[d]
207, Relative	21	M	30 y	Yes (single febrile seizure)	19 y	FAS (intense déjà vu, slow motion, light-headedness and dizziness, lasting up to 2 min, followed by tiredness, and occurring in clusters monthly), FIAS	Not done	Not done	None	MTLE. Previous history of febrile seizure	No
221, Relative	39	M	15 y	No	6 y	FAS (intense déjà vu, "butterflies" in stomach and feeling confused, lasting 30 sec, and occurring weekly), FIAS	Not done	Not done	None	MTLE	No

CBZ; carbamazepine; F=female; FAS=focal aware seizures; FBTCS=bilateral tonic-clonic seizures; FIAS=focal impaired awareness seizures; L=left; LTG=lamotrigine; M=male; MTLE=mesial temporal lobe epilepsy; ShW=sharp waves; T=temporal; TLE=temporal lobe epilepsy. [a] the proband reported that the relative had "frontal lobe epilepsy"; [b] performed for headaches and tinnitus; [c] the proband reported that the relative had "epilepsy with convulsions and episodes of hot flushes and déjà vu"; [d] the proband reported that the relative had "TLE with déjà vu and convulsions"

Suspicious Manifestations, Physiological Déjà Vu, Other Epilepsies and Febrile Seizures in Relatives vs Controls

Suspicious Manifestations

All 'suspicious' manifestations consisted of déjà vu experiences which were possibly compatible with MTLE, but not regarded as diagnostic by the blinded reviewers (i.e. 'suspicious déjà vu'). Suspicious déjà vu occurred in 6 of 112 (5.4%) relatives vs none of 112 (0%) controls (p=0.04; **Table 4.4**). Because déjà vu was part of their seizures, the 9 relatives with MTLE and their pairwise matched controls were excluded from the comparison of the proportion of subjects with suspicious manifestations between relatives and controls. Of note, 4 of the 6 relatives had been classified initially as having MTLE by one of the two reviewers, but on consensus blinded review this was changed to suspicious déjà vu.

The characteristics of the 6 relatives with suspicious déjà vu are shown in **Table 4.6**. None had antecedent risk factors for epilepsy other than a positive family history. All 6 relatives had recurrent unusual déjà vu experiences, which were not necessarily related to any specific circumstance and were typically intense and distracting. They did not, however, prevent the individual from carrying on with activities. In 4 relatives, there were no associated features. In one of the remaining 2 relatives, the déjà vu was "frightening"; in the other, it was at times accompanied by epigastric discomfort. The déjà vu usually lasted a few minutes, but on occasions it was only momentary. There were no subsequent symptoms, but one relative reported being at times "more introspective" afterwards. In 3 relatives, the déjà vu experiences occurred in clusters. None of the relatives received a diagnosis of epilepsy or was started on antiepileptic drugs. One relative underwent EEG

and MRI investigations for events of orthostatic intolerance: the EEG showed right temporal slowing but no epileptiform discharges and the MRI did not reveal any epileptogenic lesion. An example of déjà vu that is suspicious of MTLE is provided below:

> Subject 209 is a 50-year-old left-handed woman with normal birth and development. Her past medical history is unremarkable. She has a son with non-lesional MTLE and an older sister with unclassified epilepsy. At age 10 years, she started experiencing episodes of intense déjà vu, in which a "scenario would seem very familiar" to the point of actually believing that she had seen it before. The last episode was about 10 years ago. The déjà vu experience was not associated with any symptom or loss of awareness; in fact, she would be able to continue on with her activities. Episodes were reported as lasting up to 5-10 minutes, but there were instances in which they were only fleeting. She felt well afterwards. The episodes tended to cluster over a one-week period, after which no déjà vu experience would occur for several months. She never sought medical attention.

Table 4.6: Characteristics of the 6 relatives with suspicious déjà vu.

Subject n., group	Relative of patient n.	Sex	Age	History of febrile seizure	Age of onset	Event type	EEG	Neuro-imaging	AED (outcome)	Classification consensus	Previous diagnosis of epilepsy (specific diagnosis)
24, Relative	19	F	75 y	No	12 y	Episodes of intense frightening déjà vu, momentary or lasting several min, at times followed by being "more introspective", and occurring in clusters every few months	Not done	Not done	None	Suspicious déjà vu	No
78, Relative	25	M	28 y	No	Childhood	Episodes intense déjà vu, lasting 3-10 min and occurring twice a month	Not done	Not done	None	Suspicious déjà vu	No
121, Relative	29	F	60 y	No	Childhood	Episodes of intense déjà vu, lasting 2-3 min and occurring in clusters every few months	Not done	Not done	None	Suspicious déjà vu	No
185, Relative	31	F	19 y	No	Childhood	Episodes of mild déjà vu, at times associated with sick feeling in stomach, lasting up to 1 min and occurring every 2 months	R T slowing[a]	MRI showing no epileptogenic lesion. Non-specific white matter changes[a]	None	Suspicious déjà vu	No
209, Relative	32	F	50 y	No	10 y	Episodes of intense déjà vu, lasting 5-10 min but sometimes only fleeting; used to occur in clusters every few months	Not done	Not done	None	Suspicious déjà vu	No
239, Relative	38	F	16 y	No	9 y	Episodes of intense déjà vu, lasting 2 min and occurring four times a week	Not done	Not done	None	Suspicious déjà vu	No

F=female; M=male; R=right; T=temporal. [a] performed for events of orthostatic intolerance.

Physiological Déjà Vu

Physiological déjà vu was common, and did not differ in frequency between relatives and controls (76.2% vs 61.3%, p=0.08; **Table 4.4**). The 15 relatives with MTLE (n=9) or suspicious déjà vu (n=6) and their pairwise matched controls were excluded from this comparison as it was not possible to diagnose physiological déjà vu in the presence of the more severe manifestations. The common déjà vu experiences were typically mild, momentary and rare, and were often triggered by specific circumstances (e.g. visiting a new place, engaging in a certain conversation, performing certain actions, etc.). They did not affect the person's ability to carry on with activities. There were no accompanying or subsequent features, and there was no tendency to occur in clusters. The characteristics of physiological déjà vu are well illustrated by the following example:

> Subject 76 is a 52-year-old right-handed man who had normal birth and delivery. His past medical history is notable only for a traumatic left brachial plexus injury. He has no family history of epilepsy or seizures. He acknowledges experiencing déjà vu on average once a year, in the form of a mild sensation of "having done or seen this before". The feeling was only fleeting, and did not interrupt his ongoing activities. There were no concomitant or subsequent symptoms. He has had these experiences as long as he can remember.

Other Epilepsies and Febrile Seizures

There were no significant differences between relatives and controls in the proportion of subjects with other epilepsies (4.1% vs 0.8%, p=0.22) or a previous history of febrile

seizures (1.7% vs 0.8%, p=1.00; **Table 4.4**). The characteristics of the 5 relatives and the single control with other epilepsies are detailed in **Table 4.7**. Relatives included: undefined focal epilepsy (n=2); childhood absence epilepsy (n=1); epilepsy, suspected generalised (n=1); and unclassified epilepsy (n=1). The single control had childhood absence epilepsy.

Table 4.7: Characteristics of the 6 subjects with other epilepsies.

Subject n., group	Relative of patient n.	Sex	Age	History of febrile seizure	Age of onset	Seizure type	EEG	Neuro-imaging	AED (outcome)	Classification consensus	Previous diagnosis of epilepsy (specific diagnosis)
15, Relative	6	M	56 y	No	30 y	UOTCS	Normal	Done, but results not available	CBZ 400 mg/day (seizure-free since starting CBZ 23 y ago)	Unclassified epilepsy	Yes (unknown)[a]
49, Control	n/a	F	24 y	No	5 y	GAS, GTCS	3Hz generalized SpW	Normal MRI	VPA 400 mg/day, LEV 2500 mg/day, ETX 100 mg/day, trial drug (uncontrolled seizures)	Childhood absence epilepsy	Yes (Idiopathic generalized epilepsy)
103, Relative	17	M	14 y	No	5 y	GAS, GTCS	OIRDA	Normal CT	VPA 100 mg/day (seizure-free since starting VPA 7 y ago)	Childhood absence epilepsy	Yes (unknown)[b]
131, Relative	18	M	71 y	No	69 y	FIAS, FBTCS	Normal	Normal CT	CBZ 200 mg/day (seizure-free shortly after starting CBZ 2 y ago)	Undefined focal epilepsy	Yes (focal epilepsy)[c]
215, Relative	24	F	39 y	No	2 y	Unclassified seizures	Done, but results not available	Normal CT	VPA 400 mg/day, CBZ 400 mg/day (seizure-free for past 20 months)	Epilepsy, suspected generalized	Yes (unknown)[d]
233, Relative	38	F	79 y	No	71 y	FIAS	Done, but results not available	Normal MRI	CBZ 400 mg/day (seizure-free since starting CBZ 8 y ago)	Undefined focal epilepsy	Yes (focal epilepsy)[e]

CBZ; carbamazepine; ETX=ethosuximide; F=female; FAS=focal aware seizures; FBTCS=bilateral tonic-clonic seizures; FIAS=focal impaired awareness seizures; GAS=generalized absence seizures; GTCS=generalized tonic-clonic seizures; L=left; LEV=levetiracetam; M=male; n/a=not applicable; OIRDA=occipital intermittent rhythmic delta activity; SpW=spike-and-waves; UOTCS=unknown onset tonic-clonic seizures; VPA=valproic acid. [a] the proband reported that the relative had "epilepsy with three convulsions in sleep"; [b] the proband reported that the relative had "childhood absence epilepsy"; [c] the proband did not report that the relative had epilepsy; [d] the proband reported that the relative had "petit mal epilepsy"; [e] the proband reported that the relative had "epilepsy".

Frequency of FMTLE and Other Outcomes in Patients Presenting to the First Seizure Clinic with non-lesional MTLE

Figure 4.2 illustrates the number of first-degree relatives with MTLE, suspicious déjà vu, other epilepsies or a previous history of febrile seizures for each of the 44 probands who presented to the First Seizure Clinic with non-lesional MTLE.

Eight of the 44 (18.2%) patients had one or more first-degree relatives with MTLE, meeting criteria for FMTLE. These included the 2 patients who had received a diagnosis of FMTLE at the Clinic. Seven probands had a single first-degree relative with MTLE, and 1 proband had two. Of note, in all relatives with MTLE, the onset of seizures predated the probands' diagnosis at the Clinic.

Six patients (13.6%) had a first-degree relative with suspicious déjà vu. None of these probands overlapped with those with FMTLE. Six patients (13.6%) had first-degree relatives with other epilepsies. These included one proband who had also a relative with suspicious déjà vu. Five patients (11.4%) had a first-degree relative with a history of febrile seizures; one of these relatives had MTLE. Of these 5 probands, one had also a relative with MTLE without antecedent febrile seizures and another had a relative with undefined focal epilepsy. Overall, 21 of the 44 (47.7%) patients had one or more first-degree relatives in any of the aforementioned categories.

Figure 4.2: Number of first-degree relatives with MTLE, suspicious déjà vu, other epilepsies or febrile seizures for each of the 44 probands presenting to the First Seizure Clinic with non-lesional MTLE. To ease interpretation, yellow cells are used for first-degree relatives with MTLE, green cells for those with suspicious déjà vu, blue cells for those with other epilepsies, and red cells for those with febrile seizures. Grey cells are used to highlight the lack of relatives in a specific category. ([a]) indicates that the relative was not interviewed (in this case, information on his/her status was obtained from the detailed pedigree completed with the proband at enrolment); ([b]) indicates that the relative of patient n.21 had both MTLE and a history of febrile seizures; ([c]) indicates that proband received a diagnosis of FMTLE at the First Seizure Clinic.

		Number of first-degree relatives interviewed	Number of first-degree relatives per category				
			MTLE	Suspicious déjà vu	Other epilepsies	Febrile seizures	Total
Patient n.	1	2					
	2	1					
	3	5	1			1	2
	4	4					
	5	2	1				1
	6	3			1		1
	7	0					
	8	6					
	9	1					
	10	4	1				1
	11	3				1[a]	1
	12	4					
	13	5			1[a]	1[a]	2
	14	2					
	15	0					
	16	5	1				1
	17	3			1		1
	18	3			1		1
	19	2		1			1
	20	4					
	21	2	1[b]			1[b]	1[b]
	22	1					
	23	3					
	24	7			1		1
	25	2		1			1
	26[c]	3	2				2
	27	1					
	28[c]	4	1				1
	29	5		1			1
	30	2					
	31	4		1			1
	32	1		1			1
	33	2					
	34	2					
	35	3					
	36	0					
	37	2				1[a]	1
	38	3		1	1		2
	39	2	1				1
	40	2					
	41	3					
	42	4					
	43	2					
	44	2					
Total		121	9[b]	6	6	5[b]	25[b]

Discussion

The frequency of FMTLE is unknown. The inherent challenges of assessing a syndrome which is often unrecognised were addressed by interviewing all available first-degree relatives of all consenting patients who presented to a First Seizure Clinic with non-lesional MTLE over a 10-year period. Each relative was paired with an age- and sex-matched control who underwent the same interview. Each interview transcript was reviewed independently by two epileptologists, blinded as to whether the transcript belonged to a relative or a control. Inter-reviewer disagreements were resolved by joint re-evaluation, still blinded to relative or control status as well as to the reviewers' initial assessments.

FMTLE was found to account for almost one-fifth (18.2%) of new diagnoses of non-lesional MTLE. Reinforcing earlier observations (Berkovic *et al.*, 1996; Crompton *et al.*, 2010), FMTLE was also found to be mostly undiagnosed. Only a minority of patients presenting to the First Seizure Clinic with this syndrome actually received a diagnosis of FMTLE. This discrepancy was largely attributable to patients reporting no family history of epilepsy, when in fact they had a first-degree relative with unrecognised MTLE. Among the first-degree relatives who were found in the study to have MTLE, only the subset who developed tonic-clonic seizures received a diagnosis of epilepsy and were started on antiepileptic drugs. The undiagnosed relatives had unrecognised focal aware seizures with intense déjà vu and other symptoms, and some also had unrecognised focal impaired awareness seizures. Overall, the findings of this study highlight the usefulness of collecting a detailed history from available relatives of patients presenting with non-lesional MTLE, an approach which is likely to uncover many unrecognised cases of

MTLE and ultimately allow to make a diagnosis of FMTLE. This diagnosis is important for patient counselling, including reassurance that, in most cases, the syndrome is associated with a benign course with a tendency to undergo prolonged periods of spontaneous remission and/or good responsiveness to antiepileptic drug therapy (Berkovic *et al.*, 1996; Striano *et al.*, 2008; Crompton *et al.*, 2010).

An additional 13.6% of patients with non-lesional MTLE had a first-degree relative with 'suspicious déjà vu'. Interestingly, none of the controls reported a similar experience. On the other hand, relatives and controls did not differ in the frequency of individuals with physiological déjà vu (72.6% vs 61.3%), which was consistent with that reported in the general population (Brown, 2003). 'Suspicious manifestations' were defined as any symptoms which alone would not be sufficient to make a diagnosis of epilepsy, but which in clinical practice would typically lead to request investigations. Such suspicious manifestations comprised an unusual experience with déjà vu, which was clinically in the borderland between epileptic déjà vu and physiological déjà vu (**Table 4.8**). Like epileptic déjà vu, these suspicious manifestations were typically intense, prolonged and frequent, and could occur in clusters. Like physiological déjà vu, they were not followed by a post-ictal phase and did not progress to loss of awareness/consciousness or a convulsion. The experiences were also occasionally fleeting or triggered by specific circumstances, and were rarely associated with additional manifestations. The ambiguous nature of this form of déjà vu was reflected by its poor inter-reviewer agreement (**Table 4.3**), with 4 of 6 relatives with suspicious déjà vu being initially classified by one of the reviewers as having MTLE. In this context, investigations may not necessarily lead to a definite diagnosis. One relative underwent EEG and MRI studies, which were non-contributory.

Table 4.8: Typical characteristics of epileptic déjà vu, suspicious déjà vu and physiological déjà vu.

	Epileptic déjà vu	Suspicious déjà vu	Physiological déjà vu
Triggered by specific circumstances[a]	Rarely	Occasionally	Frequently
Intensity	Severe	Severe	Mild
Duration	Several seconds to a few minutes	Usually a few minutes, but can be fleeting	Usually fleeting
Frequency	Several times per year or more	Several times per year or more	Yearly or less
Possible occurrence in clusters	Yes	Yes	No
Association with other symptoms	Yes	Rarely	No
Subsequent phase of recovery	Yes	No	No
Possible progression to impaired awareness	Yes	No	No
Possible progression to loss of consciousness and convulsion	Yes	No	No

[a] These refer to specific contexts, such as visiting a new place, engaging in a certain conversation, performing specific actions, or encountering specific smells. They do not include known provoking factors for seizures, such as sleep deprivation, photic stimulation, etc.

The significance of these suspicious déjà vu experiences is uncertain. Considering that they were found only among relatives of patients with MTLE, it can be speculated that they represent focal aware seizures and that these individuals therefore also had FMTLE. In 3 of the 6 relatives with suspicious déjà vu, however, such experiences were recalled as lasting several minutes (up to 10), which is atypical for focal seizures with preserved awareness. The identification of suspicious déjà vu may also reflect the presence of a continuum between physiological déjà vu and epileptic déjà vu (Illman *et al.*, 2012; Moulin, 2014). Despite evidence of being associated with different neuroanatomical substrates (Labate *et al.*, 2015), both physiological déjà vu and epileptic déjà vu appear to result from altered neural signalling within memory-related mesial temporal structures (Shaw *et al.*, 2016). The minor disturbance of function responsible for déjà vu in the healthy brain may possibly propagate further and with greater intensity in the brain of some relatives of patients with MTLE, leading to suspicious déjà vu, and even more so in the epileptic brain, resulting in epileptic déjà vu (Moulin, 2014). A subgroup (13.6%) of patients had first-degree relatives with other epilepsies, including undefined focal epilepsy, childhood absence epilepsy and unclassified epilepsy. Although the frequency of occurrence of these other forms of epilepsy tended to be greater among relatives than among controls, the difference was not significant. Remarkably, as opposed to relatives who were found to have MTLE, all of the individuals with other epilepsies underwent EEG and MRI investigations, received a diagnosis of epilepsy and were treated with antiepileptic drugs. These findings further highlight the deceptive nature of FMTLE, which, unlike other common forms of epilepsy, often escapes diagnosis.

These findings also contribute to an improved understanding of ‘hidden’ causes of TLE. Clearly, genetics plays an important role. Other hidden causes include autoimmune

encephalitis and occult focal cortical dysplasia (Bell *et al.*, 2009), although information on their frequency among cases with new-onset, non-lesional TLE is lacking. In cohorts comprising new-onset and established epilepsies, 15-22% of individuals with focal epilepsy of unknown cause had detectable serum neurological autoantibodies (Brenner *et al.*, 2013; Dubey *et al.*, 2017); whether these cases have a true autoimmune etiology remains uncertain.

This study has limitations. Firstly, it was not population-based. Rather, patients were recruited through a First Seizure Clinic at a major teaching hospital serving a large metropolitan community. Due to its design (including interviewing all available first-degree relatives of enrolled patients), this study required strenuous efforts to be completed and it would be challenging to replicate a similar endeavour at a population level. Secondly, not all eligible patients could be enrolled in the study, but this is unlikely to have influenced the results. The demographic and clinical characteristics of participants and non-participants were largely similar; the only exception was a higher proportion of females among participants, which is to be expected (Galea and Tracy, 2007). Thirdly, EEG and MRI investigations were not obtained from relatives found to have unrecognised MTLE. However, epilepsy is primarily a clinical diagnosis and these relatives had strong clinical evidence of MTLE, with both reviewers being concordant in reaching this diagnosis in 8 of the 9 relatives at initial assessment. Furthermore, even when performed, EEG and MRI studies in individuals with FMTLE are often normal (Berkovic *et al.*, 1996; Crompton *et al.*, 2010). Lastly, since second- and third-degree relatives of patients presenting with MTLE were not interviewed, the study results might underestimate the actual frequency of FMTLE in our cohort.

More than 20 years since FMTLE was first described in twins and small families (Berkovic *et al.*, 1994; Berkovic *et al.*, 1996), epidemiological data are now available indicating that this syndrome affects about 1 in 5 individuals with newly-diagnosed non-lesional MTLE, and possibly an even greater proportion if those relatives classified in this study as having 'suspicious déjà vu' represent in fact additional MTLE cases. Clearly, FMTLE is a common genetic syndrome, which will likely go undetected in clinical practice unless a detailed history is collected directly from relatives. To date, the genetic determinants for most patients with FMTLE remain unknown. Discovery of the molecular underpinnings of this syndrome will likely aid diagnosis and solve the dilemma regarding whether the borderlands of déjà vu are a mild manifestation of FMTLE.

Chapter 5:

The Clinical Utility of Genetic Testing for Focal Epilepsies*

Abstract

Objective: Driven by advances in genomic technology and reduction in costs, next-generation sequencing is venturing into routine clinical care. The 'real-world' clinical utility of next-generation sequencing remains to be determined in focal epilepsies, which account for 60% of all epilepsies and for which the importance of genetic factors is just beginning to emerge. This study investigated the diagnostic yield and management implications of whole exome sequencing (WES)-based screening of selected genes in the routine care of common focal epilepsies suspected to have a genetic basis.

Methods: WES, followed by targeted analysis of 64 epilepsy genes, was performed on 40 consecutive children and adults enrolled prospectively from routine clinical practice who had MRI-negative focal epilepsy and a family history of febrile seizures or any type of epilepsy in at least one first- or second-degree relative. Exclusion criteria were previous genetic testing, severe intellectual disability and benign focal epilepsies of childhood.

* This study was published in *Epilepsy Res* 2017;131:1-8.

Results: 5/40 (12.5%) patients had a pathogenic or likely pathogenic variant, detected in *SCN1A*, *DEPDC5*, *PCDH19, GABRG2* or *NPRL2*. Identifying a pathogenic *SCN1A* variant in a patient with drug-resistant epilepsy prompted to halt presurgical investigations due to concern of unfavorable post-surgical outcome. It also led in the same patient to discontinue long-standing carbamazepine therapy (a potentially aggravating drug in epilepsies due to *SCN1A* mutations), resulting in complete seizure control. Patients with pathogenic or likely pathogenic variants had a younger median age of seizure onset (range) compared to those without [18 months (8 months-18 years) *vs* 18 years (18 months-70 years), p=0.02].

Conclusion: These data demonstrate that WES with targeted gene analysis is an effective diagnostic tool for patients with common focal epilepsies in whom a genetic etiology is suspected. It can also influence clinical decision-making, including antiepileptic drug selection and consideration of epilepsy surgery, hence supporting its incorporation in the routine clinical care of this patient group.

Introduction

By accounting for 60% of all epilepsy cases, focal epilepsies are a clinically important group of disorders which have traditionally been regarded as being largely acquired (Thomas and Berkovic, 2014). It was thus somewhat surprising when the first epilepsy gene to be discovered was for focal epilepsy, identified in a large multiplex family with a rare autosomal dominant disorder (Steinlein *et al.*, 1995). Despite this early hint that genetic factors may underlie focal epilepsies, gene discovery has been slow and genetic factors considered unlikely to be relevant to common focal epilepsies (Cavalleri *et al.*, 2005; Thomas and Berkovic, 2014; Helbig *et al.*, 2016a).

Recent advances in gene sequencing technologies have rekindled the interest in the genetics of focal epilepsies, leading to several important breakthroughs. A key discovery was that mutations in *DEPDC5*, which encodes a negative regulator of the mammalian target of rapamycin (mTOR) pathway, are a major cause of inherited focal epilepsies, including familial focal epilepsy with variable foci, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), familial temporal lobe epilepsy (FMTLE), and other small families with non-lesional focal epilepsy (Dibbens *et al.*, 2013; Ishida *et al.*, 2013; Picard *et al.*, 2014). *DEPDC5* mutations can also result in focal epilepsy with brain malformations (Scheffer *et al.*, 2014; Baulac *et al.*, 2015). In addition, the role of established epilepsy genes in focal epilepsies has been reappraised. In a genome-wide association study, variation in *SCN1A* was found to increase susceptibility to mesial temporal lobe epilepsy (MTLE) associated with hippocampal sclerosis and febrile seizures (Kasperaviciute *et al.*, 2013).

Whole exome sequencing (WES) is now commonplace in research endeavours and moving into clinical use, yet its utility for focal epilepsy in the 'real-world' setting has not been specifically determined. This study prospectively evaluated the diagnostic yield and management implications of WES, followed by targeted gene analysis, in patients with common focal epilepsies seen in everyday clinical practice.

Methods

Study Design and Participants

This was a prospective two-centre study designed to assess the utility of incorporating WES in the routine care of patients with focal epilepsies in whom there was a justification for considering the possibility of a genetic cause. From 10 February 2014 to 10 December 2014, consecutive eligible patients seen at the epilepsy outpatient clinics or inpatient video-EEG monitoring units of the Royal Children's Hospital and Royal Melbourne Hospital were invited to participate in the study. Inclusion criteria were age >4 weeks, a diagnosis of focal epilepsy, no epileptogenic lesion detected on brain MRI, and a family history of febrile seizures or any type of epilepsy in at least one first- or second-degree relative. Exclusion criteria were previous genetic testing (except for chromosomal microarray), severe intellectual disability, benign epilepsy with centro-temporal spikes, and benign occipital epilepsies.

Participants underwent an extensive historical assessment using an *ad hoc* instrument, including a validated questionnaire for epilepsy phenotyping (Reutens *et al.*, 1992), to collect: demographic information; pre-natal, birth, post-natal and developmental history; seizure characteristics; information on previous EEG and neuroimaging investigations; comorbidities; treatment; and family history (**Appendix 2**). Seizure descriptions were also sought by witnesses. Efforts were made to obtain previous medical records, including physicians' letters, hospital records, EEGs and MRIs. A blood sample was obtained from each participant for DNA extraction.

The study was approved by the Melbourne Health and Royal Children's Hospital Human Research Ethics Committees. Written informed consent was provided by all participants or participants' parents or legal guardians in the case of minors.

Whole Exome Sequencing (WES)

Following genomic DNA extraction, coding exon sequences were enriched with Nextera Rapid Capture Exome and sequenced using HiSeq 2500 Rapid chemistry (Illumina, San Diego, USA). Alignment and variant calling was performed using Cpipe (Sadedin *et al.*, 2015) version 0.9.5 (bwa, GATK Unified Genotyper, Annovar). Non-synonymous variants, small insertions/deletions (up to 10-20bp) and canonical splice site variants with a minor allele frequency <1% in the Exome Aggregation Consortium (ExAC), Exome Sequencing Project (ESP), and 1000 Genomes repositories were further analysed, after excluding systematic artefacts.

Sixty-four genes were selected for interpretation (**Table 5.1**). Twenty-nine were known focal epilepsy genes. Of note, only 27 of these genes were examined initially. Following the recent discovery that *NPRL2* and *NPRL3* contribute to common focal epilepsies (Ricos *et al.*, 2016), WES data from each participant were re-analysed to screen for mutations in these two genes. Given the phenotypic variability of genetic epilepsies, 35 epilepsy genes which have not been associated with focal epilepsies were also considered in the initial analysis. These mostly comprised genes associated with epilepsies in which focal seizures can occur, such as the epileptic encephalopathies.

Table 5.1: List of 64 epilepsy genes analysed in the study.

Genes associated with focal epilepsies (n=29)		
CHRNA2	*KCNT1*	*SCN1A*
CHRNA4	*LGI1*	*SCN1B*
CHRNB2	*MECP2*	*SCN2A*
CNTNAP2	*NPRL2*[a]	*SLC2A1*
DEPDC5	*NPRL3*[a]	*SRPX2*
FLNA	*PCDH19*	*SYN1*
GABRG2	*POLG*	*TBC1D24*
GRIN2A	*PRIMA1*	*TSC1*
KCNQ2	*PRRT2*	*TSC2*
KCNQ3	*RELN*	
Genes associated with other epilepsies*[b] *(n=35)		
ADSL	*EFHC1*	*PPT1*
ALDH7A1	*EPM2A*	*PRICKLE1*
ARX	*FOXG1*	*SCN9A*
ATP6AP2	*GABRA1*	*SLC25A22*
CACNB4	*GAMT*	*SLC9A6*
CDKL5	*GATM*	*SPTAN1*
CLN3	*MFSD8*	*STXBP1*
CLN5	*NHLRC1*	*TCF4*
CLN6	*NRXN1*	*TPP1*
CLN8	*PLCB1*	*UBE3A*
CSTB	*PNKP*	*ZEB2*
CTSD	*PNPO*	

[a] *NPRL2* and *NPRL3* were analysed at a second stage, following the recent discovery that mutations in these genes can cause common focal epilepsies (Ricos *et al.*, 2016); [b] these genes were considered in light of the phenotypic variability of genetic epilepsies. They mostly included genes associated with epilepsies in which focal seizures can occur as a seizure type, such as the epileptic encephalopaties.

WES Interpretation

Variants identified for each patient were rigorously reviewed in multidisciplinary meetings involving adult and paediatric epileptologists, bioinformaticians, clinical geneticists, and genetic counsellors, taking into account the patient's phenotype, the

family history, information from clinical and population databases, previous reports in the literature, and in silico tools' scores (SIFT, PolyPhen, MutationTaster, PhyloP, CADD) (Liu *et al.*, 2011). Variants in genes associated with a phenotype concordant with the patient's phenotype were classified in a five-class scheme, based on the latest guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards *et al.*, 2015) (**Table 5.2**). Under this scheme, 'pathogenic' or 'likely pathogenic' variants are deemed suitable for use in clinical decision-making when combined with other evidence of the disease in question. Variants in an epilepsy gene not previously associated with the patient's phenotype were also considered. For these 'possibly concordant' genes, the same principles for classification were applied and were deemed 'potentially pathogenic' where a causal link had not previously been shown. The final classification represented the consensus reached at the multidisciplinary meetings.

Variants classified as pathogenic, likely pathogenic and potentially pathogenic were validated by Sanger sequencing, followed by segregation testing in families where possible. Results were forwarded to the treating clinician for discussion with the patient or family. Formal genetic counselling was also provided.

Table 5.2: Variant classification scheme.

Pathogenic	• Protein-truncating variant in a concordant gene* affecting known functional domain (except were protein-truncating variants are frequent in population datasets) • Missense mutation in a concordant gene, <1:1000 in population datasets AND previously described in the literature: - in multiple (≥3) families segregating the same phenotype OR - with evidence of convincing segregation (to at least 3rd degree relative) OR - with functional data from an established assay
Likely pathogenic	Novel missense variant in a concordant gene with strong support from *in silico* analysis or formal segregation analysis
Potentially pathogenic	• Protein-truncating variant in a 'possibly concordant' gene* affecting known functional domain • Novel missense variant in a 'possibly concordant' gene with strong support from *in silico* analysis or formal segregation analysis
Variant of unknown significance	• Protein-truncating variant in a concordant gene where protein-truncating variants are frequent in population datasets or not known to affect a functional domain • Missense variant: - reported but <1:100 in population datasets OR - inconsistent support from *in silico* analysis, segregation studies or functional assays OR - discordant gene*
Likely benign	Missense variant: - >1:100 in population dataset OR - Consistent evidence of neutrality from *in silico* analysis, segregation or functional studies
Polymorphism	• Common variants >3% • Variant with established evidence of neutrality from population studies

*A concordant gene is one where mutations have previously been associated with the patient's clinical features, while a 'possibly concordant' gene has been described as a cause for epilepsy but not the specific type observed in the patient. A discordant gene would not be expected to cause the patient's phenotype.

Statistical Analysis

The primary outcome of the study was the proportion of patients with pathogenic or likely pathogenic variants, and the potential impact of detecting these variants on patient management. The proportion of patients with potentially pathogenic variants, other variants or no identified variants was also assessed, and factors associated with the identification of pathogenic or likely pathogenic variants were explored.

Categorical data were compared using the Fisher's exact test, and continuous data using the Mann-Whitney U-test. The Bonferroni correction was applied to adjust for multiple comparisons, maintaining the level of significance at 0.05. All statistical analyses were performed with SPSS 19.0 (IBM, Chicago, USA).

Results

Participants' Characteristics

Of 42 consecutive patients meeting eligibility criteria, 40 provided informed consent and were enrolled in the study. Twenty-four (60%) were males and 16 (40%) were females. Their median age (range) was 32.5 (2-74) years [12 children (i.e. age <18 years) and 28 adults], and median age of seizure onset (range) was 17.5 years (8 months-70 years). Most patients (n=32, 80%) underwent brain MRI studies at 3T; the remaining 8 patients (20%) were imaged at 1.5T. Temporal lobe epilepsy (TLE) was the most common type of focal epilepsy (n=24, 60%), followed by frontal lobe epilepsy (n=6, 15%), parietal lobe epilepsy (n=1, 2.5%) and occipital lobe epilepsy (n=1, 2.5%); localization was undefined in 8 patients (20%). Thirty-two patients (80%) had only a single affected first- or second-degree relative, 5 (12.5%) had two, and 3 (7.5%) had three or more. One patient (2.5%) had mild intellectual disability.

Genetic Diagnosis

High-coverage WES data were generated (mean 97.9-fold, **Table 5.3**) and targeted analysis of 64 epilepsy genes was performed. Of the 40 subjects, 5 (12.5%) had a pathogenic (n=2) or likely pathogenic (n=3) variant (**Table 5.4**, **Figure 5.1**). These included 4 variants (2 pathogenic and 2 likely pathogenic) identified in the initial analysis of WES data, and 1 likely pathogenic variant detected after the data were re-analysed to screen for mutations in the two novel focal epilepsy genes (Ricos *et al.*, 2016).

Table 5.3: WES coverage metrics.

Patient	Total reads	Mean read depth	% of bases covered by >1 read	% of bases covered by >10 reads	% of bases covered by >20 reads
1	3418766387	75.4	99.4	93.2	85.1
2	4194614344	92.5	99.7	96.3	91.6
3	4042372081	89.2	99.6	96.2	90.8
4	3552193478	78.4	99.4	95.3	89.1
5	4615664607	101.8	99.6	97.4	93.1
6	3292408744	72.6	99.4	93.8	86.2
7	5313822344	117.2	99.5	95.0	89.8
8	5932949470	130.9	99.6	96.3	91.9
9	3772668662	83.2	99.5	95.1	88.8
10	4129339125	91.1	99.6	96.1	90.7
11	3917030189	86.4	99.5	96.2	91.0
12	4134652769	91.2	99.5	96.3	91.1
13	3865988078	85.3	99.6	95.4	89.3
14	3400657766	75.0	99.4	94.6	87.7
15	5243067595	115.7	99.5	95.9	91.1
16	4315260521	95.2	99.5	96.3	91.3
17	3843241793	84.8	99.5	96.0	90.3
18	5241115197	115.6	99.6	96.3	91.7
19	4592464985	101.3	99.7	96.2	91.3
20	4036797040	89.1	99.3	94.2	88.0
21	5352763974	118.1	99.6	95.6	90.6
22	5185267627	114.4	99.5	95.2	89.9
23	4447074844	98.1	99.6	96.3	91.4
24	3950746846	87.2	99.4	93.2	86.0
25	3678455843	81.2	99.0	91.8	84.1
26	6056895268	133.6	99.5	96.2	92.0
27	3930081543	86.7	99.4	95.7	90.0
28	5498077047	121.3	99.7	96.0	91.3
29	4238551668	93.5	99.7	97.0	92.4
30	5630366732	124.2	99.8	98.2	95.6
31	5907555674	130.3	99.7	97.2	93.5

32	4343794657	95.8	99.4	95.4	90.0
33	4350677021	96.0	99.6	95.5	90.0
34	4069674819	89.8	99.5	95.9	90.6
35	4719610671	104.1	99.7	96.5	91.9
36	5689888947	125.5	99.5	96.3	92.2
37	4607360437	101.7	99.5	94.4	88.6
38	4300878468	94.9	99.7	96.7	91.6
39	2649111447	58.4	99.6	94.7	86.2
40	3998011559	88.2	99.7	96.5	91.9
Average	***4436498007***	***97.9***	***99.5***	***95.7***	***90.2***
SD	***810343615***	***17.9***	***0.1***	***1.2***	***2.3***

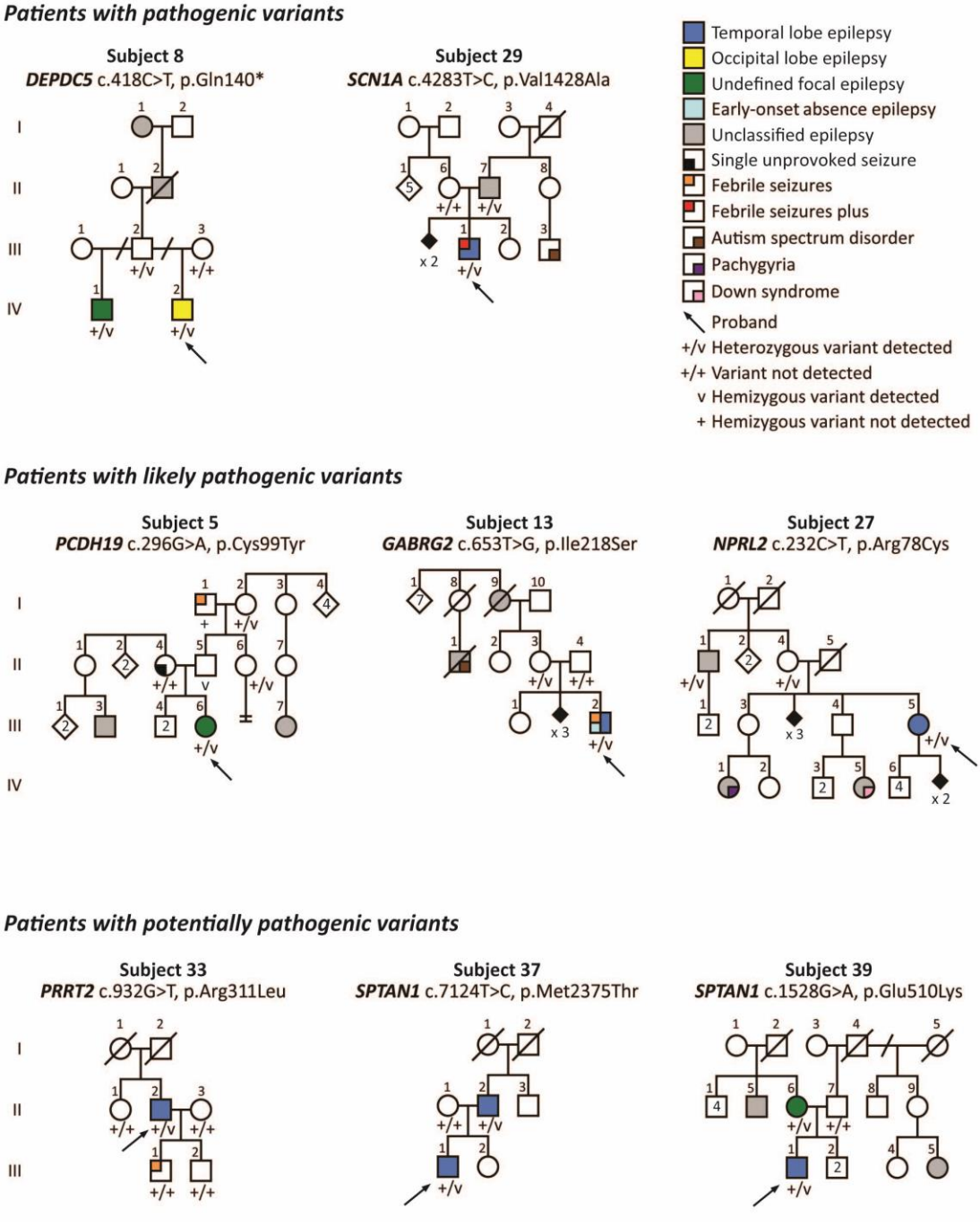
Table 5.4: Characteristics of patients with pathogenic and likely pathogenic variants. (Family history in **Figure 5.1**).

Subject n., Sex	**Age**	**Age of seizure onset**	**Seizure type/semiology**	**EEG**	**MRI (scanner field strength)**	**Epilepsy syndrome**	**ID**	**AED therapy (daily dose) at study enrolment**	**Seizure outcome at study enrolment**	**Gene (RefSeq access number): variant**	***In silico* tools*** **SIFT, PolyPhen2, MutationTaster, PhyloP, CADD**	**Allele frequency in ExAC**	**Previously reported pathogenic variant (reference)**
Patients with pathogenic variants													
8, M	10 y	2 y	Complex visual hallucination, followed by loss of consciousness and fall	*Interictal:* R O SpW	Normal (3T)	R OLE	No	OXC 1200 mg	Seizure-freedom (1 y)	*DEPDC5* (NM_001136029): c.418C>T (p.Gln140*)	n/a, n/a, A, C, 4.4	Not found	Yes (Scheffer *et al.*, 2014)
29, M	22 y	8 mo	Febrile seizures (8 mo-16 y) Episodes of "weird" feeling in the head, followed by behavioral arrest, unresponsiveness, progressing to R head version and convulsion (from 16 y)	*Interictal:* R T SpW *Ictal:* R T onset	Normal (3T)	Febrile seizures plus R TLE	No	CBZ 1200 mg, LEV 3000 mg, ZNS 400 mg	Uncontrolled seizures	*SCN1A* (NM_001165963): c.4283T>C (p.Val1428Ala)	D, D, D, C, 4.4	Not found	Yes (Sugawara *et al.*, 2001)
Patients with likely pathogenic variants													
5, Fe	7 y	9 mo	Two clusters of seizures consisting of staring, some diffuse stiffening and subsequent bilateral clonic jerking, precipitated by fever (9-12 mo) Clusters of seizures comprising fearful screaming, R limb tonic posturing, L head version and unresponsiveness, typically precipitated by fever (from 2 y)	*Interictal:* Midline spikes *Ictal:* L TPO onset	Normal (3T)	*PCDH19* female epilepsy	No	LEV 500 mg	Seizure-freedom (9 mo)	*PCDH19* (NM_001105243): c.296G>A (p.Cys99Tyr)	D, D, D, C, 3.6	Not found	No

13, M	36 y	18 mo	Single febrile seizure (11 mo) Absence seizures (18 mo-16 y) Generalized tonic-clonic seizures (18 mo-20 y) Episodes of déjà vu, fear, head rush, followed by loss of awareness (from 27 y)	*Interictal (16 y):* Fragments of 3Hz generalized SpW *Interictal (34 y):* L TIRDA	Normal (3T)	Febrile seizure EOAE (childhood) L TLE (adulthood)	No	CBZ 400 mg	Seizure-freedom (2y)	*GABRG2* (NM_000816): c.653T>G (p.Ile218Ser)	D, D, D, C, 4.8	Not found	No
27, Fe	40 y	18 y	Episodes of behavioral arrest, blank staring and unresponsiveness Clusters of convulsions in sleep	*Interictal:* L T slowing and SpW *Ictal:* L T onset	Normal (3T)	L TLE	No	LTG 100 mg, VPA 1000 mg	Uncontrolled seizures	*NPRL2* (NM_006545): c.232C>T (p.Arg78Cys)	D, D, D, C, 2.0	Not found	No

AED=antiepileptic drug; CBZ=carbamazepine; EOAE=early-onset absence epilepsy; Fe=female; ID=intellectual disability; L=left; LEV=levetiracetam; LTG=lamotrigine; M=male; mo=months; O=occipital; OLE=occipital lobe epilepsy; OXC=oxcarbazepine; R=right; SpW=spike-and-wave; T=temporal; TIRDA=temporal intermittent rhythmic delta activity; TLE=temporal lobe epilepsy; TPO=temporo-parieto-occipital; VPA=valproic acid; y=years; ZNS=zonisamide; *In silico tools prediction: n/a=not applicable; A=disease causing automatic; D=damaging (SIFT), probably damaging (PolyPhen2), disease causing (MutationTaster); C=conserved

Figure 5.1: Pedigrees for patients with pathogenic, likely pathogenic and potentially pathogenic variants. +/v denotes individuals heterozygous for the variant, v individuals hemizygous for the variant, +/+ individuals homozygous for the wild-type allele, and + individuals hemizygous for the wild-type allele. Filled symbols are individuals with epilepsy, with the epilepsy type being specified by color. Filled quarters indicate single unprovoked seizures (black), febrile seizures (orange), febrile seizures plus (red), autism spectrum disorder (brown), pachygyria (purple), or Down syndrome (pink). Squares are males, circles females. Slashes indicate deceased individuals. Filled black diamonds indicate miscarriages. Arrows indicate probands.



One of the two pathogenic variants was a truncation mutation in *DEPDC5* found in a 10-year-old boy with occipital lobe epilepsy; the mutation was inherited from his unaffected father and also identified in his paternal half-brother with non-lesional focal epilepsy. The other pathogenic variant was found in *SCN1A* in a 22-year-old man with a history of febrile seizures plus from ages 8 months to 16 years and subsequent development of drug-resistant TLE; the variant was inherited from his father who had unclassified epilepsy.

The first of the three likely pathogenic variants was found in *PCDH19* in a 7-year-old female with clusters of focal motor seizures precipitated by fever; the variant was inherited from her unaffected father and also detected in her unaffected paternal aunt and grandmother. The second likely pathogenic variant was identified in *GABRG2* in a 36-year-old male with a history of a single febrile seizure and early-onset absence epilepsy prior to the development of TLE; the variant was inherited from his unaffected mother. The third likely pathogenic variant was detected through WES re-analysis in *NPRL2* in a 40-year-old female with TLE; the variant was inherited from her unaffected mother and also identified in her maternal uncle with unclassified epilepsy.

The characteristics of the 35 patients in whom no pathogenic or likely pathogenic variants were detected are shown in **Table 5.5**. Three patients (7.5% of the entire cohort) had 'potentially pathogenic' variants: one in *PRRT2* and two in *SPTAN1* (**Figure 5.1**). Twenty-six patients (67.5%) had variants of unknown significance, benign variants or polymorphisms. In six patients (15%), no variants were found.

Table 5.5: Characteristics of patients in whom no pathogenic or likely pathogenic variants were found.

Subject n., Sex	Age	Age of seizure onset	Seizure type/semiology	EEG	MRI	Family history	Epilepsy syndrome	ID	AED therapy (daily dose) at study enrolment	Seizure outcome at study enrolment
1, M	34 y	17 y	"Pulsating" sensation in both frontotemporal regions, dizziness, "eye flickering" sensation, nausea, feeling that "something is coming", progressing to loss of consciousness and convulsion	Normal	Normal	Brother (unclassified epilepsy)	Undefined focal epilepsy	No	CBZ 600 mg, LEV 500 mg	Seizure-freedom (4 y)
2, Fe	17 y	5 y	Clusters of seizures arising mainly from sleep consisting of lip-smacking, unresponsiveness, progressing to convulsion	Normal	Normal	Maternal grandmother and maternal first cousin (unclassified epilepsy)	TLE	No	VPA 1400 mg	Seizure-freedom (5 y)
3, M	34 y	23 y	Episodes of feeling that "something is coming", déjà vu, happiness, complex auditory ("circus music") and visual hallucinations, progressing to inability to speak, metallic taste, fear and subsequent loss of awareness Convulsions in sleep	Normal	Normal	Twin brother (Familial TLE) and paternal second cousin (unclassified epilepsy)	Familial TLE	No	CBZ 400 mg	Seizure-freedom (2 y)
4, Fe	48 y	42 y	Episodes of feeling "hot", followed by loss of awareness and wandering Convulsions in sleep	*Interictal:* L T slowing	Normal	Mother, cross second cousin and maternal distant cousin (unclassified epilepsy)	L TLE	No	CBZ 400 mg	Seizure-freedom (2 y)
6, M	23 y	16 y	Episodes of R hemiface and hemitongue numbness, extending to R hemibody Convulsions in sleep	Normal	Normal	Mother (undefined focal epilepsy), maternal cousin (unclassified epilepsy)	L PLE	No	CBZ 1200 mg	Uncontrolled seizures

7, M	2 y	18 mo	Clusters of seizures consisting of stiffening with facial grimacing, jaw clenching, L head and eye deviation, upper limb extension, finger in "jazz" position, unresponsiveness, progressing to convulsion	*Interictal:* L F SpW, and L CP spikes	Normal	Sister (undefined focal epilepsy), maternal great grandfather (unclassified epilepsy)	Undefined focal epilepsy	No	CBZ 340 mg	Seizure-freedom (2 y)
9, M	71 y	70 y	Febrile seizures (4-5 y) Intense unpleasant smell ("nail polish"), followed by staring, unresponsiveness, chewing, L hand "tremor" (from 70 y)	Normal	Normal	Daughter (febrile seizures and unclassified epilepsy)	Febrile seizures TLE	No	Unknown (AED trial)	Seizure-free (9 mo)
10, M	17 y	16 y	Feeling that "something is coming", déjà vu, followed by staring, unresponsiveness, chewing, "garbled" speech or inability to speak	*Interictal:* L T slowing and SpW *Ictal:* L T onset	Normal	Sister (BECTS)	L TLE	No	LTG 200 mg, LEV 1000 mg, VPA 400 mg	Uncontrolled seizures
11, Fe	22 y	22 y	"Stabbing-like" headache in both frontotemporal regions, dizziness, followed by loss of consciousness, L head version, and subsequent convulsion	Normal	Normal	Brother [L TLE with L mesial temporal structures lesion (differential diagnosis: low-grade glioma vs dysplasia)]	Undefined focal epilepsy	No	LTG 50 mg	Undefined (on AED therapy for 3 wks)
12, Fe	7 y	3 y	Febrile seizures (6 mo-2y) Episodes of staring, unresponsiveness, L eye deviation, oral automatisms, progressing to convulsion (from 3 y)	*Interictal:* L T slowing and spikes, bursts of generalized SpW *Ictal:* L T onset	Normal	Mother (febrile seizures), paternal second cousin (unclassified epilepsy)	Febrile seizures L TLE	No	TPM 75 mg	Uncontrolled seizures
14, Fe	65 y	56 y	Episodes of feeling "something is about to happen", déjà vu, inability to speak or	*Interictal:* R T spikes	No epileptogenic lesion. White matter	Son (undefined focal epilepsy)	R TLE	No	LTG 500 mg	Seizure-freedom (3 y; had R ATL at age 59 y)

			communicate, followed by loss of awareness Episodes of feeling "something is about to happen", followed by convulsion	*Ictal:* R T onset	changes consistent with chronic small vessel ischemia					
15, Fe	64 y	62 y	Episodes arising from sleep consisting of chewing, followed by eye opening, R arm elevation, R head version and R upper limb and trunk clonic contractions Convulsions in sleep	*Interictal:* L TIRDA and L T spikes *Ictal:* L T onset	No epileptogenic lesion. White matter changes consistent with chronic small vessel ischemia	Sister (post-traumatic focal epilepsy)	L TLE	No	GBP 1200 mg, TPM 50 mg	Uncontrolled seizures
16, Fe	32 y	2 y	Feeling "weak", feeling that "something is coming", unpleasant taste ("gritty dirty sand"), followed by inability to speak, loss of awareness, bilateral hand fidgeting, aggressive behaviour and subsequent convulsion	*Interictal:* L T slowing	Normal	Mother and maternal uncle (single seizure), maternal aunt (unclassified epilepsy), maternal first cousin (focal epilepsy undefined), maternal first cousin (FLE)	L TLE	No	LTG 800 mg	Seizure freedom (1 y)
17, Fe	9 y	2 y	Clusters of seizures consisting shoulder elevation and abduction (more prominent on R side), L head deviation, followed by bilateral upper limb "shivering"	*Interictal:* L F slowing and SpW *Ictal:* L F onset	Normal	Maternal aunt (unclassified epilepsy), paternal distant cousin (febrile seizures and unclassified epilepsy)	L FLE	Yes (mild)	CLB 15 mg, OXC 750 mg	Uncontrolled seizures
18, M	15 y	13 y	Single febrile seizure (18 mo) Clusters of seizures arising out of sleep consisting of screaming, yelling, trashing limb movements, rotating trunk	*Interictal:* Bi-F slowing and bi-F (R>L) SpW	Normal	Maternal uncle (GGE)	Febrile seizure R FLE	No	CBZ 1000 mg	Seizure freedom (7 mo)

			movements, fearful expression, progressing to loss of consciousness and convulsion (from 13 y)							
19, M	74 y	61 y	Episodes of staring, unresponsiveness, lip-smacking, R upper limb extension, R head deviation, followed by convulsion Episodes of staring, unresponsiveness, lip-smacking R hand fidgeting, followed by convulsion	*Ictal:* L T, R T or bi-T onset	No epileptogenic lesion. White matter changes consistent with chronic small vessel ischemia	Brother (unclassified epilepsy)	Bi-TLE	No	LEV 4000 mg	Seizure freedom (1 y)
20, Fe	22 y	20 y	Clusters of convulsions, occasionally preceded by sensation that "something is about to happen", impaired vision and possibly "out of body" experience Episodes consisting of difficulty understanding what people are saying, and inability to speak or "jumbled" speech	Normal	Normal	Father (TLE)	Undefined focal epilepsy	No	LTG 200 mg, LEV 1500 mg	Uncontrolled seizures
21, M	30 y	22 y	Febrile seizures (6 mo-2 y) "Sick" feeling in the stomach rising to throat, headache, followed by staring, unresponsiveness, lip-smacking (from 22 y) Convulsions without warning (from 22 y)	*Interictal:* L T or R T spikes *Ictal:* R T or LT onset	Normal	Brother (Familial TLE)	Febrile seizures Familial TLE	No	LCM 400 mg, LEV 2000 mg, OXC 600 mg	Uncontrolled seizures
22, M	41 y	25 y	R hand contraction, progressing to R hand jerking, extending to R arm, hemiface and leg and subsequent convulsion	Normal	Normal	Three nephews (all with OLE)	L FLE	No	CBZ 400 mg	Seizure freedom (6 y)

23, M	17 y	15 y	Feeling “groggy”, followed by loss of consciousness and convulsion	*Interictal:* L F slowing and SpW *Ictal:* L F onset	Normal	Maternal uncle (TLE), maternal great grandmother (unclassified epilepsy), maternal second cousin (febrile seizures and unclassified epilepsy), paternal first cousin (febrile seizure)	L FLE	No	OXC 1200 mg	Uncontrolled seizures
24, M	56 y	54 y	Episodes of feeling tired, feeling “something is about to happen”, followed by feeling of being in another place, sensation that “a disaster is about to happen”, inability to speak Convulsion in sleep	*Interictal:* L T spikes	Normal	Mother (TLE)	L TLE	No	No AED	Undefined (to be commenced on AED therapy)
25, Fe	61 y	56 y	Very “familiar” feeling that is difficult to describe, followed by inability to speak and lip-smacking, progressing to loss of consciousness and convulsion	*Interictal:* bi-FT SpW, LT slowing	No epileptogenic lesion. White matter changes consistent with chronic small vessel ischemia	Mother (TLE)	TLE	No	LEV 1500 mg	Undefined (on AED treatment for 1 mo)
26, Fe	37 y	10 y	Episodes of rising “heat” sensation starting in the abdomen, followed by feeling overwhelmed with thoughts, déjà vu, inability to speak and staring Convulsions in sleep	Normal	Normal	Brother (unclassified epilepsy)	TLE	No	OXC 1200 mg	Uncontrolled seizures
28, M	59 y	49 y	Episodes of blank staring and unresponsiveness, progressing to L head version and convulsion	*Ictal:* R T ictal onset	Normal	Sister (unclassified epilepsy), daughter (undefined focal epilepsy)	R TLE	No	LTG 200 mg, LEV 2000 mg	Uncontrolled seizures

			Episodes of intense déjà vu and euphoria							
30, M	67 y	63 y	Clusters of seizures consisting of feeling "strange", followed by staring, unresponsiveness, chewing, swallowing	*Interictal:* L TIRDA	Normal	Sister (unclassified epilepsy)	L TLE	No	LTG 100 mg	Uncontrolled seizures
31, M	53 y	18 y	Reading-induced jaw jerking and inability to speak, progressing to loss of consciousness and convulsion if reading continues	Normal	Normal	Paternal uncle (reading epilepsy), paternal great aunt and paternal distant cousin (unclassified epilepsy)	Reading epilepsy	No	CBZ 400 mg, VPA 2000 mg	Uncontrolled seizures
32, Fe	33 y	12 y	Episodes of L hemibody "heavy" sensation, followed by "walking in circles", fall and convulsion Episodes of chest pain, occipital headache, metallic taste, flower-like smell, R hemicranium numbness, progressing to loss of behavioral arrest, staring, unresponsiveness, lip-smacking, bilateral hand automatisms, R hand and finger twitching	Normal	Normal	Mother (febrile seizures), maternal grandmother and paternal distant cousin (unclassified epilepsy)	Undefined focal epilepsy	No	OXC 900 mg, TPM 350 mg, VPA 2500 mg	Uncontrolled seizures
33, M	60 y	18 y	Metallic taste and feeling vague, followed by loss of consciousness, R head deviation and convulsion	Normal	No epileptogenic lesion. A few non-specific white matter changes	Son (febrile seizures)	TLE	No	CBZ 1200 mg, LEV 1000 mg	Seizure freedom (5 y)
34, Fe	11 y	18 mo	Clusters of seizures arising from sleep consisting of R leg jerking, extending to R arm and hemiface, progressing to loss of consciousness and convulsion	Normal	Normal	Maternal grandfather (post-traumatic epilepsy)	L FLE	No	OXC 1050 mg, CLB 10 mg	Uncontrolled seizures
35, M	17 y	12 y	"Awkward" feeling in the head, followed by behavioral arrest,	*Interictal:* R T slowing	Normal	Brother (FLE), maternal uncle	R TLE	No	CBZ 600 mg	Seizure freedom (2 y)

			staring, unresponsiveness, subsequent L head deviation and convulsion			(unclassified epilepsy)				
36, Fe	28 y	14 y	Déjà vu, rising "adrenaline rush" starting in the stomach , fear, followed by decreased awareness, mumbling and staring	Normal	Normal	Maternal grandfather (undefined focal epilepsy)	TLE	No	LEV 500 mg	Seizure-freedom (2 y)
37, M	42 y	38 y	Unpleasant smell ("nail polish remover"), followed by decreased awareness ("like being struck by lightning")	Normal	No epileptogenic lesion. A few non-specific white matter changes	Father (Familial TLE)	Familial TLE	No	CBZ 400 mg	Seizure-freedom (2 y)
38, M	13 y	11 y	Clusters of seizures arising from sleep consisting of bilateral hand posturing, body rocking	*Ictal:* bi-F onset	Normal	Mother and two brothers (ADNFLE), two maternal aunts and two maternal first cousins (unclassified epilepsy), maternal cousin (febrile seizures)	ADNFLE	No	LEV 750 mg	Uncontrolled seizures
39, M	34 y	34 y	Episodes of déjà vu, nausea, feeling "sick", dizziness, feeling that "everything is in slow motion", followed by loss of consciousness and convulsion Convulsions in sleep	Normal	Normal	Mother (undefined focal epilepsy), maternal uncle and paternal second cousin (unclassified epilepsy)	TLE	No	CBS 400 mg	Undefined (on AED therapy for 5 wks)
40, Fe	22 y	21 y	Single febrile seizure (1 y) Two bilateral convulsive seizures. One preceded by feeling unwell, dizziness, elementary visual hallucinations (multiple uncolored spots evolving in shape and size), sensation of	Normal	Normal	Mother (post-traumatic epilepsy)	Febrile seizure Undefined focal epilepsy	No	LEV 500 mg, VPA 500 mg (in process of switching from VPA to LEV monotherapy)	Seizure-free (5 mo; since being on AED therapy)

			"everything being in slow motion". The other preceded by violent L arm jerking (from 21 y)							

ADNFLE=autosomal dominant nocturnal frontal lobe epilepsy; AED=antiepileptic drug; ATL=anterior temporal lobectomy; BECTS=benign epilepsy with centro-temporal spikes; CBZ=carbamazepine; CLB=clobazam; CP=centro-parietal; F=frontal; Fe=female; FLE=frontal lobe epilepsy; FT=fronto-temporal; GBP=gabapentin; GGE=genetic generalized epilepsy; ID=intellectual disability; L=left; LCM=lacosamide; LEV=levetiracetam; LTG=lamotrigine; M=male; mo=months; O=occipital; OLE=occipital lobe epilepsy; OXC=oxcarbazepine; PLE=parietal lobe epilepsy; R=right; SpW=spike-and-wave; T=temporal; TIRDA=temporal intermittent rhythmic delta activity; TLE=temporal lobe epilepsy; TPM=topiramate; VPA=valproic acid; wks=weeks; y=years; ZNS=zonisamide

Impact of Genetic Diagnosis on Patient Management

Clinical management was altered as a result of the genetic finding in 1 of the 5 patients with pathogenic or likely pathogenic variants (Subject 29 in **Table 5.4** and **Figure 5.1**). The patient is a 22-year-old male with drug-resistant MRI-negative TLE. Prior to obtaining the WES results, he had been considered for presurgical inpatient video-EEG monitoring due to uncontrolled monthly seizures. The finding of a *SCN1A* mutation led to the decision to halt presurgical evaluation due to the possibility of poor outcome of epilepsy surgery in patients with *SCN1A* mutations (Barba *et al.*, 2014; Skjei *et al.*, 2015). The patient was instead started on perampanel (up to 12 mg/day) added onto his baseline antiepileptic drug regimen consisting of carbamazepine 1200 mg/day, levetiracetam 3000 mg/day and zonisamide 400 mg/day (then reduced to 200 mg/day), but seizures persisted at a virtually unchanged frequency. Because sodium-channel blocking antiepileptic drugs, such as carbamazepine, have been reported to aggravate seizures in individuals with *SCN1A* mutations (Franco and Perucca, 2015), the decision was then made to discontinue carbamazepine and the patient has become seizure-free since that time (19 months; drug regimen at last follow-up: levetiracetam 3000 mg/day, zonisamide 200 mg/day and perampanel 10 mg/day).

Factors Associated with Pathogenic or Likely Pathogenic Variants

Patients with pathogenic or likely pathogenic variants had a younger median age of seizure onset compared to those without an identified genetic aetiology [18 months (range: 8 months-18 years) vs 18 years (18 months-70 years), p=0.02]. There was no

significant association between finding a mutation and other factors, including sex, age at recruitment, and number of affected relatives (**Table 5.6**).

Table 5.6: Exploration of factors associated with the detection of pathogenic or likely pathogenic variant.

Variables	**Pathogenic or likely pathogenic variants**		**P value***
	Yes (n=5)	**No (n=35)**	
Sex, n. (%)			
- Women	2 (40%)	14 (40%)	ns
- Men	3 (60%)	21 (60%)	
Age group at recruitment, n. (%)			
- Children (i.e. age <18 years)	2 (40%)	10 (29%)	ns
- Adults	3 (60%)	25 (71%)	
Age at seizure onset, years, median (range)	1.5 (0.66-18)	18 (1.5-70)	0.02
Number of affected first- or second-degree relatives, n. (%)			
- One	4 (80%)	28 (80%)	ns
- Two or more	1 (20%)	7 (20%)	

ns = not significant; * Comparisons performed using the Fisher's exact test or the Mann-Whitney U-test as appropriate, with Bonferroni correction for multiplicity.

Discussion

Driven by its high throughput and increased affordability, next-generation sequencing is being increasingly applied in the clinical care of patients with various conditions, including epilepsy. Its clinical utility has been demonstrated in the epileptic encephalopaties, in neurodevelopmental disorders in which epilepsy is a comorbid feature, and in mixed epilepsy cohorts (Lemke *et al.*, 2012; Srivastava *et al.*, 2014; Della Mina *et al.*, 2015; Mercimek-Mahmutoglu *et al.*, 2015; Allen *et al.*, 2016; Helbig *et al.*, 2016b). Despite the growing number of focal epilepsy genes (Thomas and Berkovic, 2014), however, the value of incorporating next-generation sequencing in the clinical care of patients with focal epilepsies has not been specifically investigated to date. The present study addresses this important knowledge gap.

WES-based screening of 64 epilepsy genes was performed in 40 consecutive patients with common focal epilepsies of suspected genetic aetiology (family history of febrile seizures or any type of epilepsy in at least one first- or second-degree relative and no MRI epileptogenic lesion) prospectively enrolled from routine clinical practice. Most patients (80%) had only a single first- or second-degree affected relative, in whom expectations of finding a mutation are generally lower than in large multiplex families. Remarkably, WES identified pathogenic or likely pathogenic variants in 12.5% of cases. This rate is likely to increase in the future, as the number of genes identified as having a causative role in focal epilepsies is steadily growing.

Pathogenic or likely pathogenic variants were found in multiple genes, underscoring the molecular heterogeneity of focal epilepsies. Notably, two of these genes were *DEPDC5* and *NPRL2*, which encode components of the GATOR1 complex, a repressor of mTOR

activity (Bar-Peled *et al.*, 2013). This finding further supports the primary role of the mTOR pathway in the pathogenesis of focal epilepsies (Ricos *et al.*, 2016).

In an individual with drug-resistant TLE, identification of a pathogenic *SCN1A* mutation led to the decision to halt presurgical investigations. Although some gene defects may not necessarily preclude epilepsy surgery, *SCN1A* mutations have been associated with poor surgical outcomes and have been suggested to be a contraindication to epilepsy surgery (Barba *et al.*, 2014; Skjei *et al.*, 2015). In the same individual, the genetic finding also led the treating physician to discontinue therapy with carbamazepine, a drug known to potentially aggravate seizures in patients with epilepsies due to *SCN1A* mutations (Franco and Perucca, 2015). Carbamazepine withdrawal resulted in complete seizure control, most likely due to removal of its adverse interaction on the mutated voltage-gated sodium channel. Alternatively, achievement of seizure freedom could have been due to an increase in serum perampanel levels secondary to carbamazepine withdrawal (Patsalos *et al.*, 2016), but this explanation is unlikely because prior perampanel therapy at a dose as high as 12 mg/day had had little or no impact on seizure frequency in this patient. Overall, these observations indicate that the introduction of WES in the investigation of patients with focal epilepsies can influence clinical decision-making.

Interestingly, four of the five patients with pathogenic or likely pathogenic variants had their seizure onset in childhood, as often observed in genetic epilepsies. The diagnostic rate was 20% (4/20) for patients with onset less than 18 years. Although these findings should be viewed with caution given the small number of patients with identified mutations, they suggest that the diagnostic yield of WES with targeted gene analysis

could possibly be higher in a cohort of patients with focal epilepsy with paediatric seizure onset.

In most families, the identified pathogenic or likely pathogenic variants segregated with epilepsy, underscoring the utility of a positive family history in guiding effective genetic testing in focal epilepsy. Notably, these variants were also detected in apparently healthy individuals, which is in line with the known incomplete penetrance of several epilepsy genes. The likely pathogenic variant in *PCDH19* was found in a female proband who had a typical *PCDH19* epilepsy phenotype (clusters of focal motor seizures precipitated by fever and starting in infancy) and in three unaffected relatives (father, paternal aunt and paternal grandmother); it was not detected in the mother who had a history of a single unprovoked seizure or in the paternal grandfather who had a single febrile seizure. Whether these two latter individuals carried modifier genes ultimately influencing the expression of the *PCDH19* epilepsy in the proband is unknown.

In routine practice, a genetic aetiology is sought mainly in patients in whom epilepsy co-occurs with intellectual disability or global developmental delay (Srivastava *et al.*, 2014). In the present study, severe intellectual disability was an exclusion criterion and only one of the 40 participants had intellectual disability, which was of mild degree. Notably, none of the 5 patients with pathogenic or likely pathogenic variants had intellectual disability. Altogether, these findings highlight that the absence of intellectual disability does not exclude a genetic aetiology in focal epilepsy.

The results of this study were obtained by performing WES, followed by exome data filtering of selected epilepsy genes. This approach has limitations. Firstly, WES does not detect all types of genomic variation, such as large insertions/deletions, copy number

variants (CNVs), trinucleotide repeats, intronic alterations, structural chromosomal rearrangements and epigenetic modifications (Biesecker and Green, 2014). Secondly, the findings of this study might have also been attained with targeted sequencing using gene panels, which are associated with lower costs and permit higher coverage than WES. However, the cost of WES is rapidly decreasing. Furthermore, the fast pace of novel gene discovery in epilepsy imposes frequent updates to panel-based approaches, ultimately reducing their cost-effectiveness long-term. In contrast, WES data can be re-interrogated as new genes are identified and can even serve as a research platform for gene discovery. The advantages of WES are exemplified by the present study, in which WES data were re-analysed to screen for mutations in *NPRL2* and *NPRL3* following the recent discovery that these genes contribute to common focal epilepsies (Ricos *et al.*, 2016); WES re-analysis resulted in the identification of a likely pathogenic variant in *NPRL2* (**Table 5.4**, **Figure 5.1**).

In conclusion, this study is a real-world application of one of the latest gene-sequencing technologies available in clinical practice to a cohort in which genetic testing is not usually part of the diagnostic work-up. This study demonstrated that WES with targeted gene analysis is an effective diagnostic tool for patients with common focal epilepsies and can influence clinical decision-making, hence supporting its incorporation into routine care.

Chapter 6:

The Effect of Genetic Factors on Seizure Outcome after Surgery for Drug-Resistant Mesial Temporal Lobe Epilepsy

Abstract

Objective: Although epilepsy surgery is the most effective treatment for drug-resistant mesial temporal lobe epilepsy (MTLE), 20-50% of patients considered to be ideal candidates for MTLE surgery continue to have seizures post-operatively. Whether some of these surgical failures are attributable to genetic factors remains unknown. This study assessed the influence of genetic factors on seizure outcome after MTLE surgery.

Methods: This was a case-control study including whole exome sequencing (WES) data from patients who underwent surgery for drug-resistant unilateral MTLE with a normal MRI or MRI-evidence of mesial temporal sclerosis, and who had ≥2 years of post-operative follow-up. Only patients at the opposite extremes of seizure outcome were included, i.e. those with uncontrolled seizures since surgery (unfavourable outcome) and those who attained sustained seizure-freedom after surgery (favourable outcome). Patients were recruited through the multicentre EPIGEN collaboration, and their exomes

were sequenced at the Institute for Genomic Medicine (IGM), New York, USA. Exomes from controls without epilepsy were also included, sequenced as part of unrelated studies.

Results: WES data from 31 patients with an unfavourable outcome, 115 patients with a favourable outcome and 2,797 controls were compared. The proportion of subjects with deleterious variants in genes established as causative of human epilepsy differed significantly across the three groups (p=0.03), being highest among patients with an unfavourable outcome (19.3%), followed by those with a favourable outcome (9.6%) and controls (7.6%). In pairwise comparisons, this proportion was significantly higher among patients with an unfavourable outcome than among controls (p=0.03), but no significant differences were found between the two outcome groups or between patients with a favourable outcome and controls. No individual gene was associated with post-operative seizure outcome.

Conclusion: Due to sample size limitations, this study does not provide definitive evidence on whether genetic factors influence seizure outcome after MTLE surgery. However, this study provides a signal for surgical failures, as a group, possibly being enriched for individuals with deleterious variants in epilepsy genes. This signal requires verification in larger cohorts.

Introduction

Despite the introduction of more than 20 new antiepileptic drugs (AEDs) in the past 25 years, one-third of people with epilepsy remain resistant to pharmacological therapy (Brodie *et al.*, 2012). Drug-resistant epilepsy is associated with increased disability, morbidity and mortality (Kanner, 2003; Hermann *et al.*, 2006; Perucca *et al.*, 2009b; Friedman and Gilliam, 2010; Hesdorffer *et al.*, 2011; Kwan *et al.*, 2011; Perucca *et al.*, 2011a; Perucca and Gilliam, 2012). For many of these individuals, epilepsy surgery is the most effective way to control seizures (Ryvlin *et al.*, 2014). Mesial temporal lobe epilepsy (MTLE) is among the epilepsy syndromes most likely to be drug-resistant, and is the most common indication for epilepsy surgery (Engel *et al.*, 2003).

Assessing whether a patient is a suitable surgical candidate relies on a battery of investigations, and their concordance is generally associated with more favourable post-operative seizure outcomes (Ryvlin *et al.*, 2014). However, 20-50% of patients undergoing MTLE surgery continue to have seizures despite concordant pre-surgical investigations (Gilliam *et al.*, 1997). The reasons for these surgical failures remain poorly understood (Harroud *et al.*, 2012; Ryvlin *et al.*, 2014). An unanswered question is whether genetic factors play a role (Surges and Elger, 2013). An increasing number of genes have been implicated in the pathogenesis of focal epilepsies (Thomas and Berkovic, 2014), including temporal lobe epilepsy (TLE) (Kalachikov *et al.*, 2002; Scheffer *et al.*, 2007; Dibbens *et al.*, 2013; Ishida *et al.*, 2013; Kasperaviciute *et al.*, 2013; Dazzo *et al.*, 2015; Ricos *et al.*, 2016), and a common concern is that the genetic defect causing the epilepsy may be widely expressed in the brain, ultimately undermining the effectiveness of a focal resection (Surges and Elger, 2013). In addition, there may be genetic factors not directly

implicated in the process of focal epileptogenesis, which may nevertheless affect the outcome of MTLE surgery (Barba *et al.*, 2014).

Current knowledge on the effect of genetic factors on post-surgical seizure outcome is conflicting (Kobayashi *et al.*, 2003; Scheffer *et al.*, 2007; Catarino *et al.*, 2011; Barba *et al.*, 2014; Scerri *et al.*, 2015; Skjei *et al.*, 2015; Sim *et al.*, 2016), with most data being derived from case reports and small series. Large well-designed studies are lacking. Here, these methodological issues were addressed by performing a large, international, multi-centre, case-control study, which used whole exome sequencing (WES) to evaluate the influence of genetic defects on seizure outcome after MTLE surgery.

Methods

Subjects

WES was performed on samples collected from 146 patients who underwent a comprehensive pre-surgical evaluation and subsequent MTLE surgery at centres participating in the EPIGEN collaboration (Cavalleri *et al.*, 2007; Petrovski *et al.*, 2009). Seven patients were recruited at the Erasmus Hospital, Brussels, Belgium; 31 at the Duke University Medical Center, Durham, USA; 3 at the New York University Langone Medical Center, New York, USA; 14 at the Beaumont Hospital, Dublin, Ireland; 18 at the Toronto Western Hospital, Toronto, Canada; 18 at the Royal Melbourne Hospital, Melbourne, Australia; and 56 at Austin Health, Melbourne, Australia. Inclusion criteria were: 1) European ancestry; 2) a diagnosis of drug-resistant (Kwan *et al.*, 2010) unilateral MTLE, with focal impaired awareness seizures (formerly known as complex partial seizures) (Fisher *et al.*, 2017) as the predominant seizure type; 3) a pre-operative MRI at ≥1.5T showing unilateral mesial temporal sclerosis (MTS) only or no epileptogenic lesion. Patients with MRIs at <1.5T demonstrating MTS only were also eligible, provided that MTS was subsequently confirmed by histological examination; 4) pre-surgical investigations (interictal and ictal EEG, MRI and, if available, neuropsychological testing, positron emission tomography [PET], single-photon emission computed tomography [SPECT]) consistent with a unilateral mesial temporal lobe seizure onset; 5) a temporal lobe resection (standard anterior temporal lobectomy or selective amygdalohippocampectomy) performed at age ≥15 years; 7) histological examination of the surgical specimen, if available, consistent with the pre-operative MRI findings, revealing MTS only or no specific pathology; 8) post-operative follow-up of ≥2 years,

allowing to classify seizure outcome into ‘favourable’ or ‘unfavourable’. ‘Favourable outcome’ was defined as: complete seizure freedom after surgery; persistence of focal aware seizures (formerly known as simple partial seizures) (Fisher *et al.*, 2017) only after surgery, with no focal impaired awareness seizures for >2 years at last follow-up; initial post-operative seizure recurrence, followed by complete seizure-freedom for ≥5 years at last follow-up; or post-operative seizures occurring only following antiepileptic drug withdrawal or due to a provoking circumstance, such as missed antiepileptic drug doses or an intercurrent systemic illness. ‘Unfavourable outcome’ was defined as ongoing drug-resistant seizures starting in the first years after surgery and no evidence on post-operative MRI, if available, of inadequate temporal lobe resection.

Exclusion criteria were: 1) interictal EEG epileptiform discharges predominantly contralateral to the resected temporal lobe; 2) extra-temporal or generalised interictal EEG epileptiform discharges; 3) pre-surgical investigations suggesting seizures to be originating outside the resected temporal lobe or from a primary temporal lobe epileptogenic zone extending to neighbouring regions, such as the insula (Barba *et al.*, 2016); 4) evidence of dual pathology on histological examination.

Patients’ data were compared to 2,797 European ancestry control exomes, sequenced as part of various studies using a consistent platform (Cirulli *et al.*, 2015).

The study was approved by the ethics committee at each participating site. All subjects provided written informed consent.

Sequencing

All sequencing was carried out at the Institute for Genomic Medicine (IGM), Columbia University, USA. Samples were either exome sequenced with the Agilent All Exon (50MB or 65MB) or the Nimblegen SeqCap EZ V2.0 or 3.0 Exome Enrichment kit (Roche NimbleGen, Madison, USA) or whole genome sequenced with Illumina HiSeq 2000 or 2500 sequencers (Illumina, San Diego, USA) according to standard protocols.

Sequenced data from the 146 patients with surgically treated MTLE and the 2,797 ethnicity-matched controls were processed by Dr Nicole Griffin using the same bioinformatics pipeline at the IGM (Cirulli *et al.*, 2015; Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017).

Analysis Strategy

The primary endpoint of the study was to evaluate whether the patient group with an unfavourable outcome was enriched for individuals with deleterious variants in epilepsy genes. For this purpose, a set of 48 established human epilepsy genes was considered (**Table 6.1**), modified from a list of genes proposed to form the basis for epilepsy precision medicine (EpiPM Consortium, 2015). The proportion of subjects with ≥1 qualifying variant in this gene set was then compared across three separate groups: patients with an unfavourable outcome; patients with a favourable outcome; and controls. Qualifying variants were non-synonymous variants predicted to be probably damaging or possibly damaging on the *in silico* PolyPhen-2 pathogenicity prediction tool, which had

a test cohort minor allele frequency (MAF) of 0.05% and were absent in the Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC) repositories.

Table 6.1: List of established epilepsy genes included in the primary gene-set analysis. (*) indicates subset included in the secondary gene-set analysis.

ALG13	*GRIN2B*	*PRRT2*
CDKL5	*HCN1*	*RELN*
CHD2	*HNRNPU*	*SCN1A**
CHRNA2	*KCNA2*	*SCN1B*
CHRNA4	*KCNB1*	*SCN2A*
CHRNB2	*KCNC1*	*SCN8A*
CNTNAP2	*KCNMA1*	*SCN9A*
*DEPDC5**	*KCNQ2*	*SIK1*
DNM1	*KCNQ3*	*SLC2A1*
EEF1A2	*KCNT1*	*SLC35A2*
GABRA1	*LGI1**	*SLC6A1*
GABRB3	*MEF2C*	*SPTAN1*
GABRG2	*NPRL2*	*STX1B*
GNAO1	*NPRL3*	*STXBP1*
GRIN1	*PCDH19**	*SYNGAP1*
*GRIN2A**	*PRICKLE2*	*VPS13A*

Secondary analyses included an analogous gene-set analysis limited to a subset of 5 genes implicated in the pathogenesis of focal epilepsies (*SCN1A*, *DEPDC5*, *LGI1*, *GRIN2A* and *PCDH19*) (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017); a comparison of the proportion of patients with a favourable outcome between subjects with ≥1 qualifying variant in the set of 48 epilepsy genes and those without such variants; and a genome-wide gene collapsing analysis, as previously described (Cirulli *et al.*, 2015; Bagnall *et al.*, 2016), separately comparing each outcome group to the controls.

The Fisher's exact test was used for comparisons of categorical variables and the Mann Whitney U test for comparisons of continuous variables. For the gene collapsing analysis, the Bonferroni correction was used to adjust for multiple comparisons, maintaining the level of significance at alpha of 0.05.

Results

Subjects' Characteristics

The median age (range) of the 146 patients included in the study was 49.3 (25-79.3) years, with a higher proportion of women than men (54% vs 46%) (**Table 6.2**). Pre-operatively, most patients (86%) had unilateral MTS detected on brain MRI. Median age at surgery (range) was 39 years (15-64), with the vast majority of patients (94%) undergoing a standard anterior temporal lobectomy. Median post-operative follow-up (range) was 9 (2-23.3) years.

Of the 146 patients, 115 (79%) had a favourable outcome and 31 (21%) had an unfavourable outcome. As shown in **Table 6.2**, patients with a favourable outcome and those with an unfavourable outcome had similar characteristics, except for a higher representation of cases with MTLE and no MRI epileptogenic lesion in the former group compared to the latter group (29% vs 10%, p=0.02).

Table 6.2: Clinical and demographic characteristics of the entire cohort of patients with surgically treated MTLE, and of the two post-operative seizure outcome patient groups.

Variables	All patients (n=146)	Groups by post-operative seizure outcome		*p* value
		Favourable (n=115)	Unfavourable (n=31)	
Gender, n. (%)				
- Men	67 (46%)	54 (47%)	13 (42%)	0.7
- Women	79 (54%)	61 (53%)	18 (58%)	
Age, median (range), years	49.3 (25-79.3)*	49 (25.5-71.7)*	52 (25-79.3)	0.4
Pre-operative MRI, n. (%)				
- Mesial temporal sclerosis	125 (86%)	103 (90%)	22 (71%)	0.02
- No epileptogenic lesion	21 (14%)	12 (10%)	9 (29%)	
Age at surgery, median (range), years	39 (15-64)*	37.7 (15-64)*	41 (16-63)	0.4
Surgical procedure, n. (%)				
- Standard anterior temporal lobectomy	137 (94%)	106 (92%)	31 (100%)	0.2
- Selective amygdalohippocampectomy	9 (6%)	9 (8%)	0 (0%)	
Post-operative follow-up, median (range), years	9 (2-23.3)	9 (2-23.3)	9.4 (2-20.9)	0.4
Post-operative seizure outcome, n. (%)				
- Favourable	115 (79%)	-	-	n/a
- Unfavourable	31 (21%)			

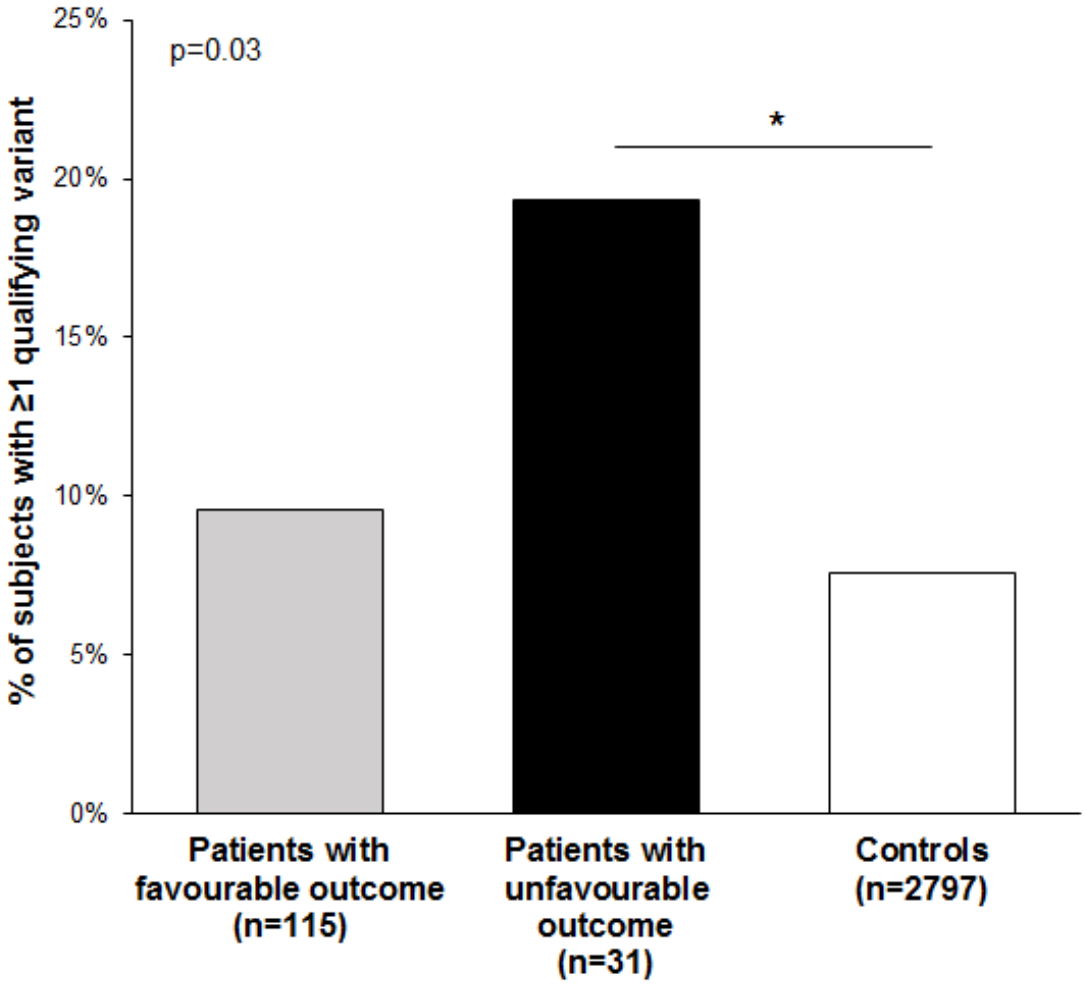
*data missing for one patient; n/a=not applicable

Genetic Factors and Post-Operative Seizure Outcome

The proportion of subjects with ≥1 qualifying variant in the set of 48 established epilepsy genes differed significantly across groups (p=0.03; **Figure 6.1**), being highest among patients with an unfavourable outcome (19.3%), followed by patients with a favourable outcome (9.6%) and controls (7.6%). Between-group comparisons revealed that this proportion was significantly higher among patients with an unfavourable outcome

compared to controls (p=0.03), whereas no significant differences were found between patients with an unfavourable outcome and those with a favourable outcome or between patients with a favourable outcome and controls (**Figure 6.1**).

Figure 6.1: Proportion of subjects with ≥1 qualifying variant in the set of 48 established epilepsy genes across three groups: patients with a favourable outcome, patients with an unfavourable outcome and controls. Proportions differed significantly across the three groups (p=0.03). Between-group comparisons showed that the proportion of subjects with ≥1 qualifying variant was significantly higher among patients with an unfavourable outcome than controls (p=0.03, as indicated by bar with asterisk). No other significant between-group differences were found.



The proportion of subjects with ≥1 qualifying variant in the subset of 5 focal epilepsy genes did not differ significantly across the groups (patients with a favourable outcome, 2.6%; patients with an unfavourable outcome, 3.2%; controls, 1.3%; p=0.2)

The proportion of patients with a favourable outcome did not differ significantly between subjects with ≥1 qualifying variant in the set of 48 epilepsy genes vs those without such variants (n=11/17, 64.7% vs n=104/129, 80.6%; p=0.2).

No gene showed genome-wide significant association with post-operative seizure outcome.

Discussion

A role of genetics as a potential cause for failure of epilepsy surgery has long been postulated (Abou-Khalil, 2007; Tellez-Zenteno *et al.*, 2010; Surges and Elger, 2013). Small studies, mainly based on reports of patients with specific gene defects undergoing surgery (Barba *et al.*, 2014; Scerri *et al.*, 2015; Skjei *et al.*, 2015; Sim *et al.*, 2016; Scheffer *et al.*, 2017), have yielded conflicting results. The advent of next-generation sequencing has revolutionised genomic and genetic research, enabling the simultaneous interrogation of multiple genes in large patient cohorts (Goodwin *et al.*, 2016). Here, next-generation sequencing was applied to investigate the effect of genetic factors on seizure outcome after MTLE surgery from two different angles: 1) are patients who do not become seizure-free after MTLE surgery, as a group, enriched for individuals with deleterious variants in known epilepsy genes? 2) are there specific genes impacting on post-operative seizure outcome at an individual level?

In the largest study of its kind to date, WES was performed on 146 patients who underwent surgery for drug-resistant MTLE. These patients were carefully selected using strict eligibility criteria to minimise the risk of common causes of surgical failures, such as bi-temporal, extra-temporal or temporal plus epilepsy; dual pathology; and insufficient resection of mesial temporal structures (Harroud *et al.*, 2012; Barba *et al.*, 2016). The study also targeted patients at the opposite extremes of long-term post-operative seizure outcome (≥2 years of post-operative follow-up; median 9 years), thus excluding individuals with a 'relapsing-remitting' course (de Tisi *et al.*, 2011). Of the 146 patients, 31 were clear 'surgical failures', with ongoing uncontrolled seizures starting shortly after surgery, and 115 were 'surgical successes', attaining sustained seizure-freedom

immediately or shortly after surgery. These two groups were compared to 2,797 controls from the general population. Interestingly, the proportion of individuals with deleterious variants in 48 established epilepsy genes was found to differ significantly across the three groups, a result largely attributable to the proportion being significantly higher among surgical failures than controls (19.3% vs 7.6%, p=0.03). Although the proportion of individuals with deleterious variants in the 48 genes was nominally higher among failures than successes (19.3% vs 9.6%), this difference did not reach statistical significance. The gene-set analysis limited to the subset of 5 focal epilepsy genes instead did not yield significant results. There were also no significant differences in the proportion of surgical successes between subjects with qualifying variants in the set of 48 epilepsy genes and those without such variants. Therefore, our findings do not provide conclusive evidence for genetic factors affecting the outcome of MTLE surgery. However, they provide a signal - which needs to be verified in larger cohorts - for surgical failures, as a group, possibly being enriched for individuals with deleterious variants in epilepsy genes.

No specific genes were found to be associated with post-operative seizure outcome, which may result from our study being underpowered. A previous, single-centre, case-control study found that the *PRNP* variant allele Asn171Ser was highly prevalent in patients with drug-resistant MTLE with MTS and was associated with a five-fold increased risk of uncontrolled seizures after anterior temporal lobectomy (Walz *et al.*, 2003). The association between the *PRNP* variant and drug-resistant MTLE with MTS, however, was not confirmed in a subsequent larger investigation (Cavalleri *et al.*, 2005). Recently, two case series reported poor seizure outcomes in patients with *SCN1A* mutations undergoing epilepsy surgery (Barba *et al.*, 2014; Skjei *et al.*, 2015), and mutations in *SCN1A* have been proposed to be a contraindication to surgery (Barba *et al.*, 2014). On the other hand,

certain gene defects may not preclude epilepsy surgery. Scheffer et al. (Scheffer *et al.*, 2007) reported two patients with drug-resistant temporal lobe epilepsy and *SCN1B* mutations who became seizure-free after anterior temporal lobectomy. In a series of 10 patients with surgically treated MTLE and large microdeletions, favourable outcomes were attained in 8 (80%) (Catarino *et al.*, 2011). Gene collapsing analysis conducted in larger populations are therefore required to determine whether specific genes impact on an individual's response to surgical therapy for drug-resistant MTLE.

Chapter 7:

An Investigation of Genetic Factors Predisposing to Valproic Acid-Associated Neural Tube Defects

Abstract

Objective: Prenatal exposure to valproic acid (VPA) is associated with a 10- to 20-fold increase in the risk of neural tube defects (NTDs). Experimental and human evidence suggests a genetic predisposition to these teratogenic effects of VPA. This study explored whether VPA-associated NTDs are attributable, at least in part, to rare variants of large effect size.

Methods: This whole exome sequencing (WES) study used a family-based design, including: 12 trios with a NTD case exposed to VPA *in utero* during the first trimester of gestation (VPA-NTD families); 5 trios with unaffected offspring exposed to VPA during the first trimester (VPA-unaffected families). Families were recruited through the Australian Register of Antiepileptic Drugs in Pregnancy and at the Karolinska Institute, Stockholm, Sweden. Samples were sequenced at the Institute for Genomic Medicine (IGM), New York, USA. 'Qualifying variants' designated rare deleterious variation within the sequenced data, and were sought in 31 genes which have been linked with NTDs in general.

Results: Two qualifying variants were identified in two VPA-NTD families. The first was a c.8668G>A missense variant in *CELSR1*, identified in an 11-year-old female with spina bifida and other malformations, who was exposed prenatally to VPA 1500 mg/day. The variant was also detected in her brother and mother, who did not have NTDs and were not exposed to VPA *in utero*. The second variant was a c.416G>A missense substitution in *SCRIB*, identified in a 31-year-old male with spina bifida, who was exposed prenatally to VPA and carbamazepine (unknown doses). The variant was also found in his mother without NTDs, who was not exposed to VPA *in utero*. The two variants were not detected in VPA-unaffected families. Notably, *CELSRI1* and *SCRIB* are involved in the planar cell polarity signalling pathway, which is implicated in various developmental processes, including neural tube formation (neurulation), and which has increasingly been linked to NTDs.

Conclusion: This study identified two rare variants in the planar cell polarity genes *CELSR1* and *SCRIB*, which may possibly confer a substantial risk of developing VPA-associated NTDs. However, this interpretation remains speculative due to the limited sample size. Confirmation in larger cohorts is needed.

Introduction

Neural tube defects (NTDs) are severe congenital malformations characterized by failure of the neural tube to close completely, resulting in incomplete formation of the brain and/or the spinal cord (Botto *et al.*, 1999). Their frequency is approximately one per 1000 births (Botto *et al.*, 1999; Mitchell, 2005; Zaganjor *et al.*, 2016), although higher rates have been reported using miscarriage material (Creasy and Alberman, 1976). The two most common forms of NTDs are anencephaly and spina bifida, which account for approximately 90% of cases with NTDs (Copp *et al.*, 2013). Anencephaly, which is characterized by complete or partial absence of the brain and skull, is invariably lethal before or at birth (Botto *et al.*, 1999; Copp *et al.*, 2013). Spina bifida, which is a herniation of neural tissue through an incompletely formed spine, is compatible with post-natal survival, but its neurological and medical sequelae often result in severe life-long disabilities (Date *et al.*, 1993) including spastic paraplegia, neurogenic incontinence and associated cognitive abnormalities with a high risk of psychosocial maladjustment (Zurmohle *et al.*, 1998).

Prenatal exposure to the antiepileptic drug (AED) valproic acid (VPA) has been associated with a 10- to 20-fold increase in the risk of NTDs, particularly spina bifida, compared to the general population (Jentink *et al.*, 2010b). Although it has been recommended that VPA be avoided in women of childbearing potential when possible (Harden *et al.*, 2009; Tomson *et al.*, 2015b), VPA may be the only agent capable of fully controlling seizures for many women, particularly those with juvenile myoclonic epilepsy and some other generalised epilepsy syndromes (Marson *et al.*, 2007b; Mantoan and

Walker, 2011). VPA has also several non-epilepsy indications (Johannessen Landmark, 2008), reinforcing the need for research into predictors of risk of VPA-associated NTDs.

The teratogenic risk of VPA appears to be dose-dependent, increasing with higher doses of the drug (Omtzigt *et al.*, 1992; Vajda *et al.*, 2013). In addition, there is some evidence suggesting that there may be a genetically determined predisposition to VPA-associated NTDs. Specifically, in animals, there are clear strain differences in susceptibility to VPA-associated NTDs (Finnell *et al.*, 1988; Finnell, 1991; Hall *et al.*, 1997; Faiella *et al.*, 2000), and in one study in mice a susceptibility locus has been suggested to reside on chromosome 7 (Lundberg *et al.*, 2004). In humans, only a small proportion (1-2%) of VPA-exposed pregnancies results in a NTD (Omtzigt *et al.*, 1992; Finnell *et al.*, 2003; Tomson *et al.*, 2011), lending further support to the hypothesis of a differential genetic susceptibility to VPA-associated NTDs. An increased frequency of NTDs in relatives of offspring with NTDs who were exposed to VPA *in utero* has been reported (Robert and Guibaud, 1982). There are also reports of women taking VPA who had multiple pregnancies resulting in a NTD (Duncan *et al.*, 2001; Campbell *et al.*, 2013). One of these women was subsequently switched to another medication and had a healthy child (Duncan *et al.*, 2001).

Despite these observations, genetic factors predisposing to VPA-associated NTDs have yet to be identified. In contrast, progress has been made in understanding the genetic underpinnings of NTDs in general. NTDs have a complex pattern of inheritance, involving multiple genes as well as environmental influences. To date, a number of variants in different genes, such as those regulating the folate one-carbon metabolism and those involved in the planar cell polarity signalling pathway, have been linked with an

increased risk of NTD occurrence (Greene *et al.*, 2009; Copp *et al.*, 2013; De Marco *et al.*, 2014). The question arises as to whether genes implicated in NTDs also play a role in the occurrence of VPA-associated NTDs (Finnell *et al.*, 2003; Copp *et al.*, 2013).

Available evidence suggests that the mode of inheritance of severe adverse drug reactions may not be complex (Zhou and Pearson, 2013). Rather, rare variants of large effect sizes have been implicated (Zhou and Pearson, 2013), suggesting that these phenotypes may display a genetic architecture similar to that of classic Mendelian diseases. This may also be the case for VPA-associated NTDs. As such, the present study applied whole exome sequencing (WES) to investigate whether the genetic risk of VPA-associated NTDs is attributable, at least in part, to rare variants of large effect in NTD genes.

Methods

Study Design and Subjects

This study applied a family-based approach, including complete and incomplete parent-offspring trios to investigate genetic risk factors of VPA-associated NTDs. In this setting, trio designs offer different advantages. First, they permit a complete assessment of Mendelian inheritance patterns, including *de novo* mutagenesis. Second, for rare conditions, such as VPA-associated NTDs, they have considerable more power to detect associations than other designs (Laird and Lange, 2006). Trios were referred to as 'incomplete' if WES data were missing from one or more family members, and 'complete' when there were no missing data.

The study included two separate sets of trios: i) those with VPA-exposed NTD cases and ii) those with VPA-exposed unaffected offspring. 'VPA exposure' referred to first-trimester exposure to VPA *in utero*, either as monotherapy or combination therapy (i.e. VPA plus other AEDs). 'NTD cases' comprised livebirths, induced abortions, stillbirths or spontaneous abortions with a NTD. Trios with VPA-exposed NTD cases were considered as 'VPA-NTD families'. 'Unaffected offspring' referred to offspring without any birth defect. Trios with VPA-exposed unaffected offspring were labelled as 'VPA-unaffected families'. These trios were required not to have had any induced abortions, stillbirths or spontaneous abortions.

A total of 23 subjects from 12 unrelated VPA-NTD families were included, with each family having one VPA-exposed NTD case (**Figure 7.1**). Eight of these families were Australian, and participating family members were identified and recruited either through

the Australian Register of Antiepileptic Drugs in Pregnancy (Vajda *et al.*, 2003; Vajda *et al.*, 2013; Vajda *et al.*, 2016) (14 subjects from 6 families) or by neurologists' referral (4 subjects from 2 families). The remaining four families were Swedish, and participating members (5 subjects) were recruited at the Karolinska Institute, Stockholm. The 23 participants comprised 20 first-degree relatives of VPA-exposed NTD cases (12 mothers, 3 fathers and 5 siblings) as well as all 3 affected livebirths (**Figure 7.1**).

The characteristics of the 12 VPA-exposed NTD cases are shown in **Table 7.1**. With the exception of the 3 affected livebirths recruited in the study, all cases were induced abortions. Most (9/12) had spina bifida, and two had anencephaly; in one case, the type of NTD was not specified. Four cases had other concomitant birth defects. Eight had been exposed *in utero* to VPA monotherapy and four to VPA combined with other AEDs. Of the 10 cases for which there was information on whether folic acid had been used peri-conceptually, 8 had been exposed to folate supplementation. None of the cases had a family history of NTDs.

A total of 15 subjects from 5 unrelated VPA-unaffected families were included (**Figure 7.2**). All of these families were Australian, and participating members were identified and recruited through the Australian Register of Antiepileptic Drugs in Pregnancy. The 15 participants comprised 5 mothers, 4 fathers, and 6 VPA-exposed unaffected livebirths.

The study was approved by the Human Research and Ethics Committees at Melbourne Health, Melbourne, Australia, and at the Karolinska Institute, Stockholm, Sweden. All subjects signed a written informed consent form prior to study participation.

Figure 7.1: Pedigrees of the 12 VPA-NTD families. Families A-H were Australian, with participating members recruited either through the Australian Register of Antiepileptic Drugs in Pregnancy or by neurologists' referral. Families I-L were Swedish, with participating members recruited at the Karolinska Institute, Stockholm. *indicates family members who participated in the study.

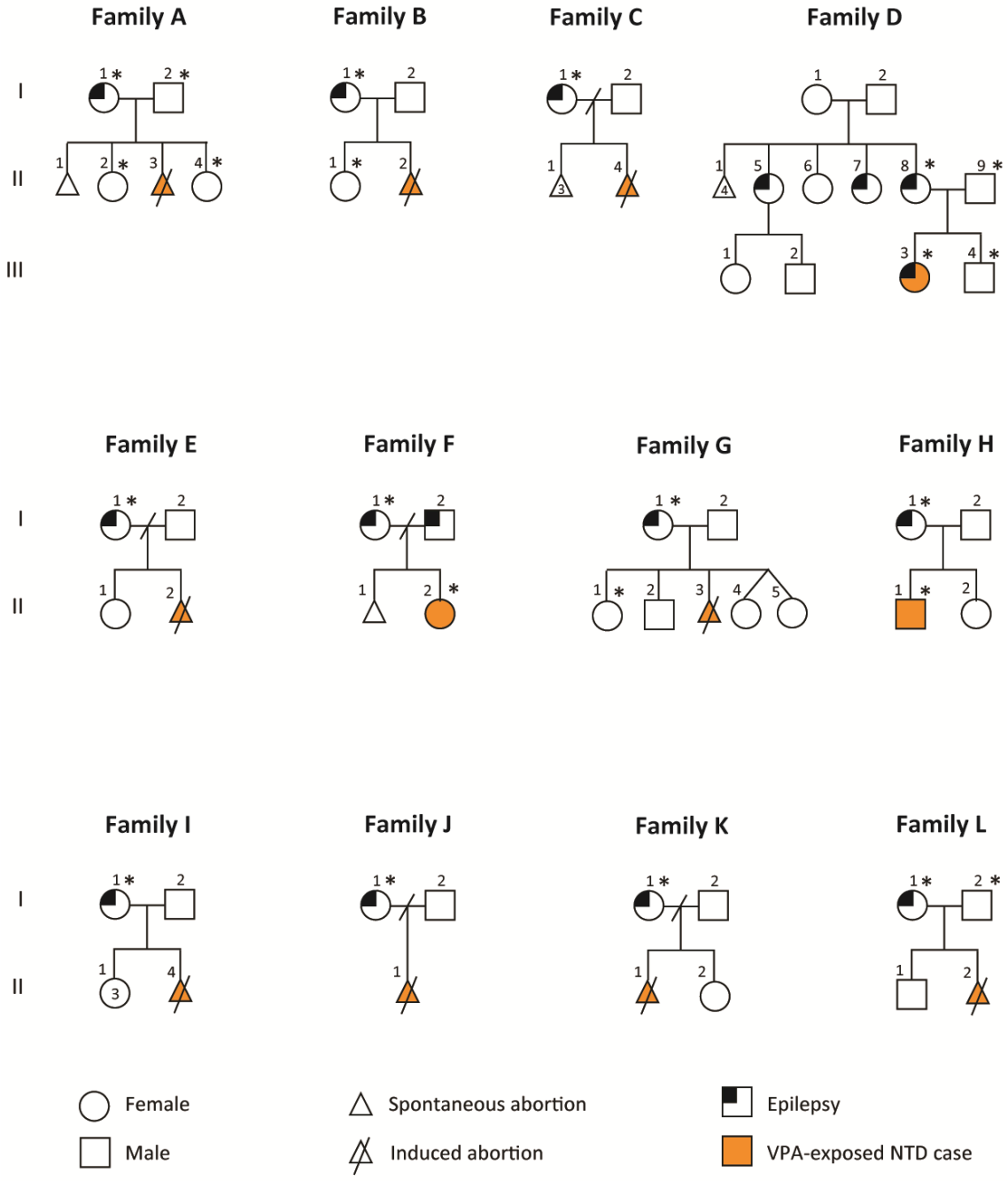
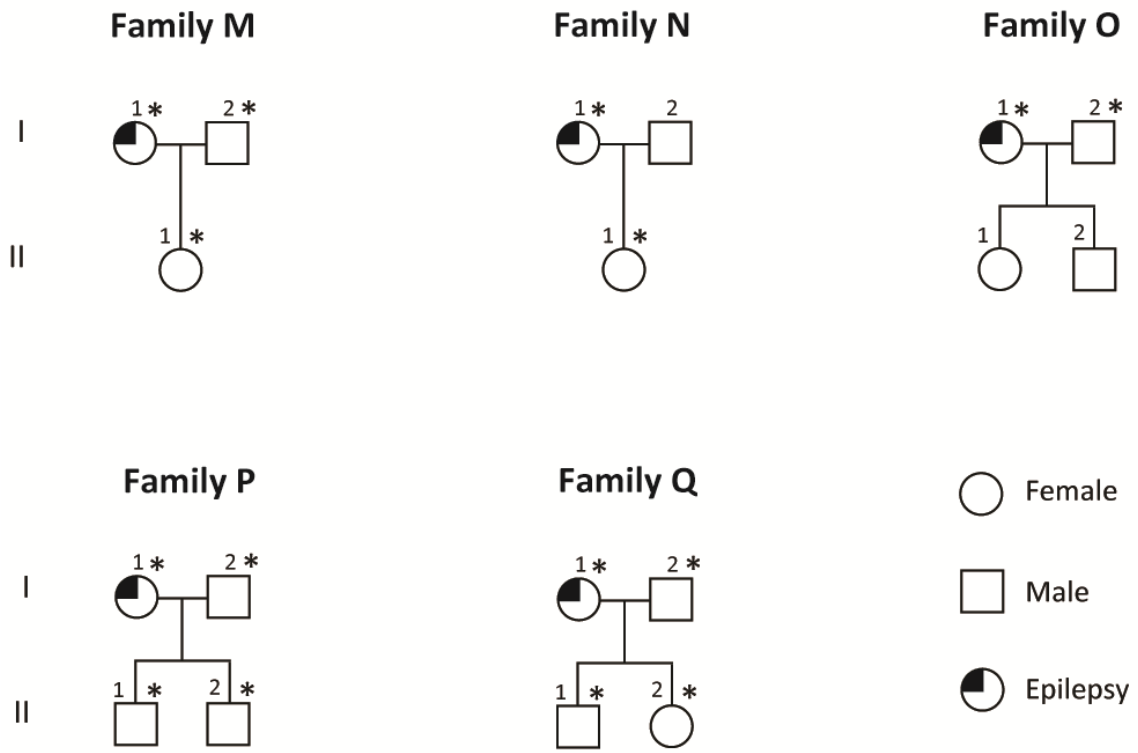


Table 7.1: Characteristics of the 12 VPA-exposed NTD cases.

VPA-exposed NTD case*	Pregnancy outcome	Type of NTD	Additional birth defects	AED exposure *in utero* (mg/day)	Folic acid exposure *in utero*
A/II-3	Induced abortion	Anencephaly	No	VPA (unknown), CBZ, (unknown), CNZ (unknown)	Unknown
B/II-2	Induced abortion	Spina bifida	No	VPA (3000), TPM (75)	Yes
C/II-4	Induced abortion	Spina bifida	No	VPA (3000)	Yes
D/III-3	Livebirth	Spina bifida	Bilateral periventricular nodular heterotopia, hydrocephalus, Chiari II malformation, pulmonary artery stenosis, ventricular septal defect, atrial septal defect, patent ductus arteriosus, clinodactyly	VPA (1500)	Yes
E/II-2	Induced abortion	Spina bifida	Hydrocephalus	VPA (2000), LTG (150)	Yes
F/II-2	Livebirth	Spina bifida	Unspecified urinary tract defect	VPA (1000)	Yes
G/II-3	Induced abortion	Anencephaly	No	VPA (1400)	No
H/II-1	Livebirth	Spina bifida	No	VPA (unknown), CBZ (unknown)	No
I/II-4	Induced abortion	Spina bifida	No	VPA (900)	Yes
J/II-1	Induced abortion	Unspecified NTD	No	VPA (900)	Unknown
K/II-1	Induced abortion	Spina bifida	No	VPA (1200)	Yes
L/II-2	Induced abortion	Spina bifida	Chiari II malformation	VPA (1000)	Yes

*Family/generation-individual; AED=antiepileptic drug; CBZ=carbamazepine; CNZ=clonazepam; LTG=lamotrigine; NTD=neural tube defect; TPM=topiramate; VPA=valproic acid

Figure 7.2: Pedigrees of the 5 VPA-unaffected families. The offspring of each family was exposed to VPA *in utero*. *indicates family members who participated in the study.



Sequencing and Bioinformatics Pipeline

All sequencing was performed at the Institute for Genomic Medicine (IGM), Columbia University, NY, USA. Participants' DNA samples were exome sequenced using the Nimblegen SeqCap EZ V2.0 or 3.0 Exome Enrichment kit (Roche NimbleGen, Madison, USA) using Illumina HiSeq 2500 (Illumina, San Diego, USA) sequencers according to standard protocols. The bioinformatics analysis was carried out by Dr Slave Petrovski at the IGM. After quality filtering the raw sequenced data with CASAVA (Illumina, San Diego, USA), the Illumina lane-level FASTQ files were aligned to the Human Reference Genome (NCBI Build37/hg19) with the Burrows-Wheeler Alignment Tool (BWA) (Li

and Durbin, 2009). Picard (http://picard.sourceforge.net) was applied to remove duplicate reads and process lane-level SAM files, resulting in a sample-level BAM file which was used for variant calling. Variant calling was conducted using the Genome Analysis Toolkit (GATK) best practice protocol with re-alignment around indels and base quality scores recalibration (McKenna *et al.*, 2010).

Only non-synonymous single nucleotide variants (SNVs) residing within the consensus coding sequence (CCDS) release 14 (Pruitt *et al.*, 2009) were considered at an upper minor allele frequency (MAF) of 1%. Variants were excluded if they were among a predefined in-house list of sequencing artefacts or if they were labelled by EVS (http://evs.gs.washington.edu/EVS/) or ExAC (http://exac.broadinstitute.org/about) as being problematic variants. SNVs were required to have: ≥3-fold coverage; quality score (QUAL) of ≥50; genotype quality (GQ) score of ≥20; and quality by depth (QD) score of ≥2. Variants were further screened according to VQSR tranche computed using the known SNV sites from HapMap v3.3, dbSNP, and the Omni chip array from the 1000 Genomes Project. To "PASS", variants were required to attain a tranche of 99.9%. For heterozygous genotypes, the alternative allele ratio was required to be ≥25%.

Analysis Strategy

The 12 VPA-NTD families were initially screened by myself for qualifying variants in 31 genes which have been associated with NTDs (**Table 7.2**) (Greene *et al.*, 2009; Copp *et al.*, 2013). As described previously (Cirulli *et al.*, 2015; Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017), the term 'qualifying variants' refers to the subset of the variation of the sequenced data meeting specific criteria designed to enrich

for deleterious variants. In this study, qualifying variants were defined by the following criteria: prediction as “probably damaging” by PolyPhen-2 (HumDiv) (Adzhubei *et al.*, 2010); Combined Annotation Dependent Depletion (CADD) score >15 (Kircher *et al.*, 2014); and presence in <20 alleles in the ExAC repository. In the three families with an affected livebirth, only qualifying variants present in the livebirth were considered. The 31 NTD genes comprised 19 genes regulating folate one-carbon metabolism, 8 genes involved in planar cell polarity signalling, and 4 genes implicated in other pathways (Greene *et al.*, 2009; Copp *et al.*, 2013).

Subsequently, the presence of any qualifying variant identified among VPA-NTD families was assessed among VPA-unaffected families. The VPA-unaffected families were also screened for additional qualifying variants in candidate VPA-associated NTD genes.

Table 7.2: List of 31 NTD genes analysed in the study.

Folate-related genes (n=19)	**Planar cell polarity genes (n=8)**
ALDH1A2 *AMT* *BHMT* *CBS* *COMT* *CUBN* *DHFR* *GLDC* *MTHFD1* *MTHFD1L* *MTHFR* *MTR* *MTRR* *NAT1* *PCMT1* *PCYT1A* *SARDH* *SLC19A1* *TYMS*	*CELSR1* *DVL2* *FZD6* *PRICKLE1* *SCRIB* *VANGL1* *VANGL2* *FUZ*
	Other genes (n=4)
	CFL1 *HK1* *PAX3* *SLC2A1*

Results

Qualifying Variants in VPA-NTD Families

In the molecular analysis of the 23 subjects from the 12 VPA-NTD families, two heterozygous qualifying variants were identified, both in planar cell polarity genes: one in *CELSR1* and the other in *SCRIB* (**Table 7.3**).

The first variant was at position 46760520 on chromosome 22 and corresponded to a c.8668G>A missense variant in *CELSR1* (NM_014246.1), encoding a p.Val2890Met amino acid change in the C-terminal domain of the Celsr1 protein (**Figure 7.3A**). The variant was predicted to be probably damaging by PolyPhen-2 (HumDiv), had a CADD score >30, and affected a highly evolutionarily conserved amino acid (http://genome.ucsc.edu). The variant was reported in the ExAC repository with an allele frequency of 16/117812 (0.00014%) across all populations, and 6/64274 (0.000093%) in the European population. *CELSR1* is among the most intolerant genes to functional variation (RVIS=0.631) (Petrovski *et al.*, 2013), but the missense variant is positioned in a tolerant exon (exon subRVIS=76.996) (Gussow *et al.*, 2016). The missense variant *CELSR1* c.8668G>A (p.Val2890Met) was identified in family D, an Australian family of European ancestry (**Figure 7.4**). Specifically, it was detected in the affected individual III-3, an 11-year-old female with spina bifida (repaired at birth), periventricular nodular heterotopia, hydrocephalus, Chiari II malformation, pulmonary artery stenosis, ventricular septal defect, atrial septal defect, patent ductus arteriosus, clinodactyly, and unclassified epilepsy, who had been exposed prenatally to VPA 1500 mg/day and folate supplementation. The variant was also identified in the unaffected brother (III-4), who had not been exposed to any AEDs *in utero*, and in the mother (II-8), who had

periventricular nodular heterotopia and focal epilepsy, but no NTDs, and who also had not been exposed to any AEDs *in utero*. Interestingly, family D has previously been reported as several members (I-1, II-5, II-7, II-8 and III-3) were found to have periventricular nodular heterotopia caused by a mutation in the X-linked filamin-1 gene, an adenine to guanine substitution at the splice acceptor of exon 7 (*FLNA* c.9882-2A>G), resulting in missplicing of intron 6 and protein truncation (Fox *et al.*, 1998; Poussaint *et al.*, 2000; Sheen *et al.*, 2001; Sheen *et al.*, 2004). Periventricular nodular heterotopia was also detected on the brain MRI of individual III-1, but genetic testing was not performed.

The second qualifying variant identified among the VPA-NTD families was at position 144895115 on chromosome 8 and corresponded to a c.416G>A missense variant in *SCRIB*, encoding a p.Arg139Gln residue change in the leucine rich repeat 5 domain of the Scrib protein (**Table 7.3**, **Figure 7.3B**). The variant was predicted as probably damaging by Polyphen-2 (HumDiv), had a CADD score >30, and affected a highly evolutionarily conserved amino acid residue. The variant was present in the ExAC database with an allele frequency of 2/109742 (0.000018%) across all populations, and 1/60548 (0.000016%) in the European population. *SCRIB* is among the genes most intolerant to functional variation (RVIS=1.026) (Petrovski *et al.*, 2013), and the variant is also positioned in an intolerant exon (exon subRVIS=3.339) (Gussow *et al.*, 2016). The *SCRIB* c.416G>A (p.Arg139Gln) missense variant was detected in family H, an Australian family of European ancestry (**Figure 7.4**). The variant was identified in a 31-year-old male (II-1) with spina bifida repaired at birth, who had been exposed prenatally to VPA and carbamazepine (unknown daily doses), and in his mother (I-1) with non-lesional temporal lobe epilepsy, but no NTDs, who had not been exposed to any AEDs *in*

utero. Individual II-2, who had autism and was exposed prenatally to VPA and carbamazepine (unknown daily doses), did not participate in the study.

Qualifying Variants in VPA-unaffected Families

Neither of the two qualifying variants identified among VPA-NTD families was detected among the VPA-unaffected families.

No additional qualifying variants in *CELSR1* or *SCRIB* were identified among the VPA-unaffected families.

Table 7.3: Details of the two qualifying variants identified in VPA-NTD families.

Gene	Variant	Variant type	Amino-acid change	PolyPhen-2 (HumDiv)	CADD scores	GERP score	Allele frequency ExAC	RVIS	Exon subRVIS
CELSR1	c.8668G>A	Missense	p.Val2890Met	Probably damaging (1.00)	34	4.62	16	0.631	76.996
SCRIB	c.416G>A	Missense	p.Arg139Gln	Probably damaging (1.00)	34	4.52	2	1.026	3.339

CADD= Combined Annotation Dependent Depletion; GERP=Genomic Evolutionary Rate Profiling; RVIS=Residual Variation Intolerance Score; subRVIS=sub-region Residual Variation Intolerance Scores

Figure 7.3: Location of the amino acid alterations encoded by the two qualifying variants in the Celsr1 (A) and Scrib (B) proteins.

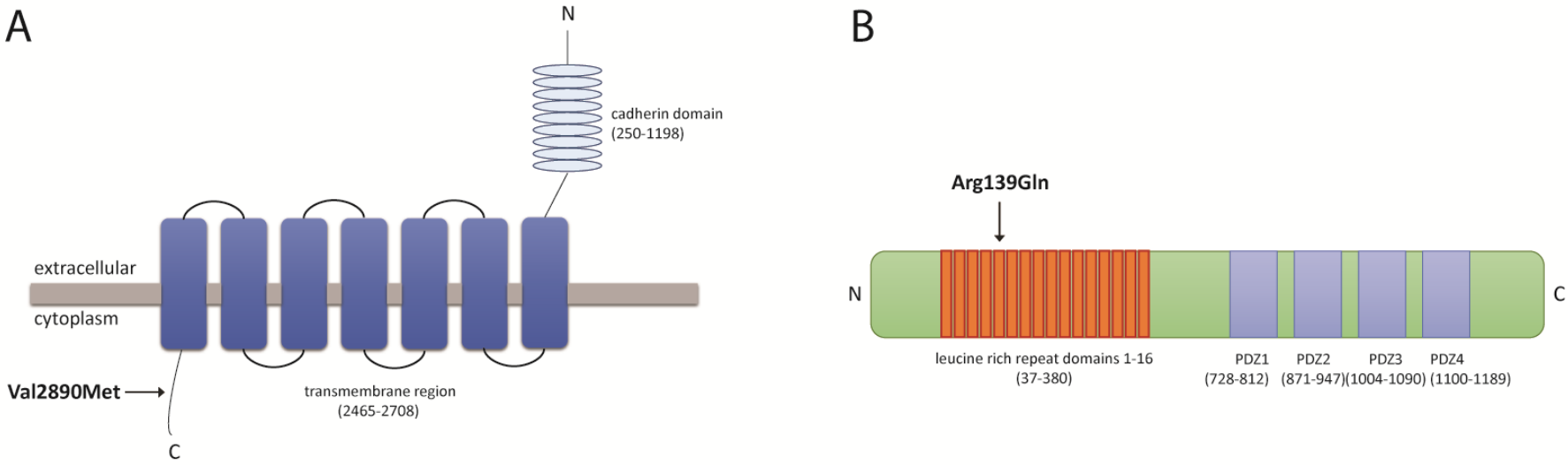
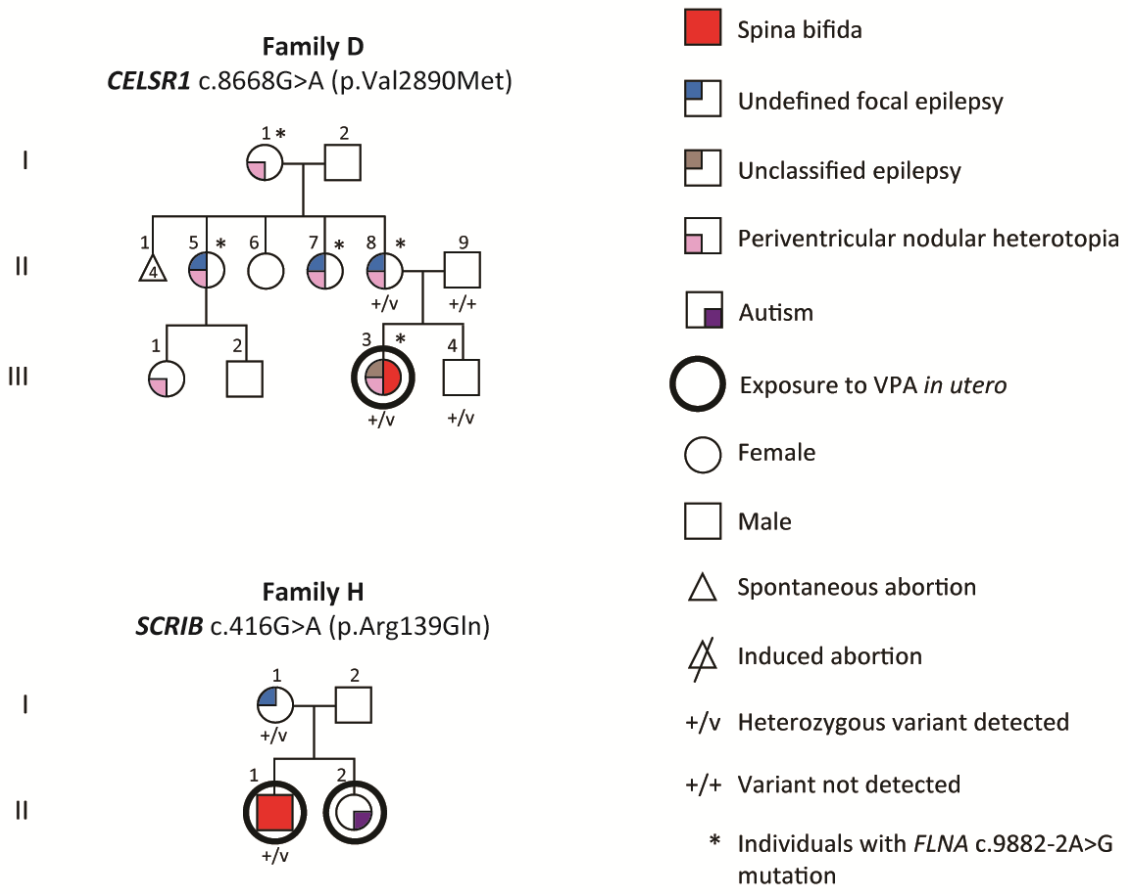


Figure 7.4: Pedigrees of the two VPA-NTD families in which qualifying variants were identified. Family D has been reported previously as several individuals (indicated by an asterisk) were found to have periventricular nodular heterotopia caused by a mutation in the X-linked filamin-1 gene (Fox *et al.*, 1998; Poussaint *et al.*, 2000; Sheen *et al.*, 2001; Sheen *et al.*, 2004). Individual III-1 also had periventricular nodular heterotopia, but no genetic testing was performed.



Discussion

The present study explored genetic risk factors for the occurrence of VPA-associated NTDs. Evidence from both experimental models and humans suggests in fact that there is genetic predisposition to these birth defects (Robert and Guibaud, 1982; Finnell *et al.*, 1988; Finnell, 1991; Duncan *et al.*, 2001; Finnell *et al.*, 2003; Lundberg *et al.*, 2004; Campbell *et al.*, 2013). Using a family-based design, this study tested the hypothesis that one mechanism for VPA-associated NTDs are rare variants of large effect sizes in genes that have been linked to NTDs. VPA-associated NTDs are rare birth defects, occurring in only 1-2% of offspring exposed to VPA *in utero*, and multiple mechanisms are likely involved; VPA dose is also implicated, as the risk of spina bifida increases in a dose-dependent manner with increasing VPA doses (Vajda *et al.*, 2013).

In this study, twelve VPA-NTD families were recruited from two different countries (8 from Australia and 4 from Sweden). Although this sample is far from being large enough for a robust genetic investigation, it represents a sizable cohort from a clinical prospective. Recruitment of VPA-NTD families is very challenging for a number of reasons. First, VPA-associated NTDs are rare occurrences, and their frequency has substantially decreased in the past 15 years as a result of a reduction of VPA use and efforts to prescribe lower VPA doses in women of childbearing potential (Vajda *et al.*, 2013; Perucca *et al.*, 2015). Second, NTDs are a sensitive issue for mothers, who are faced with the distressing decision of whether to terminate pregnancy or not when a NTD is identified prenatally; in the present study, 9 of the 12 pregnancies with a NTD resulted in an induced abortion. For women who underwent the process of induced abortion due to occurrence of a NTD, participation in a study designed to assess whether a genetic factor played a role in causing

the defect can evoke stressful memories and rekindle feelings of guilt often suppressed by unconscious psychological mechanisms of self-protection. Interestingly, 8 additional mothers of VPA-NTD cases were identified through the Australian Register of Antiepileptic Drugs in Pregnancy and all were invited to participate in this study. However, they all declined, either immediately or after verbally agreeing to participate when first approached.

Despite the limited sample size, our study produced some interesting findings which could form the basis for future studies. Specifically, two qualifying variants in planar cell polarity genes were identified in two VPA-NTD families. The non-canonical Wnt-planar cell polarity pathway coordinates the uniform orientation, structure and movement of cells within the plane of a tissue or organ, which are necessary for numerous developmental processes, including neural tube formation (neurulation) (Juriloff and Harris, 2012). The planar cell polarity pathway has a critical role early in neurulation: it mediates a process termed "convergent extension", characterised by the movement of neural plate and mesoderm cells from the sides of the embryo to the midline, intercalating between previous midline cells (Ybot-Gonzalez *et al.*, 2007; Keller *et al.*, 2008). This results in narrowing and elongation of the neural plate, ensuring that the neural folds are sufficiently close together for closure to begin (Ybot-Gonzalez *et al.*, 2007; Keller *et al.*, 2008; Copp *et al.*, 2013). The role of planar cell polarity genes in the pathogenesis of NTDs has recently received considerable attention (Juriloff and Harris, 2012; Copp *et al.*, 2013; De Marco *et al.*, 2014). One of the two qualifying variants was identified in family D (**Figure 7.4**) and consisted of missense substitution in *CELSR1*, a core planar cell polarity gene encoding a seven-pass transmembrane protocadherin (Tissir and Goffinet, 2013). *CELSR1* has been linked with NTDs in a number of recent association studies

(Allache *et al.*, 2012; Robinson *et al.*, 2012; Lei *et al.*, 2014; Qiao *et al.*, 2016), but the c.8668G>A (p.Val2890Met) missense variant has not been previously reported. This variant met study criteria for deleteriousness, but was located in an exon which is tolerant to functional change (**Table 7.3**). The expression of *CELSR1* was shown to be altered by VPA in an *in vitro* gastrulation model (Li and Marikawa, 2016). One may speculate that the *CELSR1* c.8668G>A (p.Val2890Met) variant is generally tolerated, but becomes deleterious with first-trimester exposure to VPA. In family D, the variant was found in three members, but only the member exposed to VPA *in utero* had a NTD (spina bifida). Notably, family D has previously been reported, as a mutation in the X-linked filamin-1 gene (*FLNA* c.9882-2A>G) was found to cause periventricular nodular heterotopia in several members (Fox *et al.*, 1998; Poussaint *et al.*, 2000; Sheen *et al.*, 2001; Sheen *et al.*, 2004). In addition to periventricular nodular heterotopia, *FLNA* mutations have been associated with epilepsy, cardiovascular defects, extremity deformities, other neuroradiological abnormalities and even spina bifida (Fox *et al.*, 1998; Srour *et al.*, 2011), all of which were present in individual III-3. However, it is interesting that this severe phenotype was seen exclusively in the individual exposed to VPA *in utero*, whereas the other members with the mutation had only heterotopia with or without well-controlled epilepsy.

The other qualifying variant was detected in family H (**Figure 7.4**) and was characterised by a missense substitution in *SCRIB*, a gene encoding a large cytoplasmic scaffold protein in the planar cell polarity pathway (Albertson *et al.*, 2004). *SCRIB* has been linked with craniorachischisis and spina bifida in two recent association studies (Robinson *et al.*, 2012; Lei *et al.*, 2013), but the c.416G>A (p p.Arg139Gln) variant has not been previously reported. The variant met study criteria for deleteriousness and was located in

an intolerant exon (**Table 7.3**), suggesting it may be deleterious even in the absence of first-trimester exposure to VPA. Interestingly, it was found in a mother-son pair, but spina bifida occurred only in the son who was exposed to VPA *in utero*.

It is tempting to speculate that the variants identified represent genuine genetic risk factors for the occurrence of VPA-associated NTDs. However, alternative interpretations should be kept in mind. A possibility is that these are variants raising risk for NTDs, irrespective of first-trimester exposure to VPA, and associated with incomplete penetrance. Another possible explanation is that these variants are non-relevant to the pathogenesis of NTDs (associated or not with prenatal VPA exposure); the literature is filled with false claims of genotype-phenotype associations and therefore caution is required when interpreting these findings, especially given that they were obtained from small samples.

Clearly, larger studies, including as controls VPA-unaffected families as well as families with NTD cases which were not exposed prenatally to any teratogen (sporadic NTDs), are required to assess whether the identified variants and, more broadly, planar cell polarity genes have a role in the pathogenesis of VPA-associated NTDs. These studies can only be completed within the framework of a large international collaborative effort, similar to existing endeavours which have resulted in major advances in the field of epilepsy genetics and pharmacogenomics (McCormack *et al.*, 2011; EpiPM Consortium, 2015; Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). Such an initiative is currently under way, and recruitment of VPA-NTD and control families is currently taking place at centres in other countries, including Spain, Italy and China.

Anatomical teratogenesis is not the only concern related to VPA use in pregnancy. In the past decade, VPA-induced *behavioural* teratogenesis, including increased risks of

impaired post-natal neurodevelopment and autism, has also been increasingly recognised (Adab *et al.*, 2004; Meador *et al.*, 2009; Christensen *et al.*, 2013; Meador *et al.*, 2013; Gerard and Meador, 2015; Wood *et al.*, 2015). Given that VPA remains the most effective treatment for common generalised epilepsy syndromes and that it has also non-epilepsy indications, further research into predictors of the multifaceted aspects of VPA-induced fetal toxicity is clearly warranted.

Chapter 8:

Conclusions and Future Directions

The work described in this thesis contributes to an improved understanding of the role of genetic factors in the aetiology of focal epilepsy and treatment outcomes in epilepsy.

Role of Genetic Factors in the Aetiology of Focal Epilepsies

Temporal lobe epilepsy (TLE) is the most common focal epilepsy in adulthood (Zarrelli *et al.*, 1999; Devinsky, 2004), and mesial TLE (MTLE) is its most frequent sub-type (Engel *et al.*, 2003). The cause of MTLE is often unknown. Remarkably, the results described in this thesis indicate that almost one-fifth of new diagnoses of non-lesional MTLE actually represent *familial* MTLE (FMTLE). Due to the mild and subtle nature of its symptoms and underestimation of the role of genetic factors as a cause of focal epilepsy (Berkovic *et al.*, 1996; Striano *et al.*, 2008; Gambardella *et al.*, 2009; Crompton *et al.*, 2010), this familial syndrome is largely unrecognised unless a detailed history is collected directly from patients' relatives.

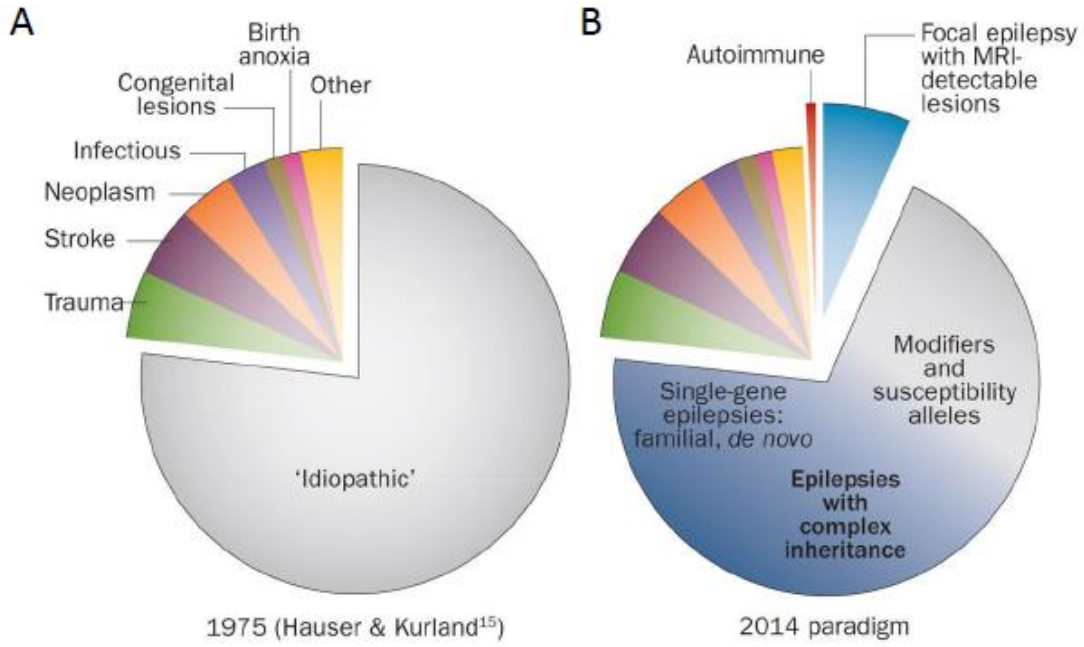
The frequency of FMTLE may be even higher than suggested by the findings reported in this thesis. Here, assessments were limited to first-degree relatives of patients presenting to a First Seizure Clinic with non-lesional MTLE. It is plausible that patients without affected first-degree relatives had in fact affected, yet unrecognised, second- or third-

degree relatives. Furthermore, one-sixth of patients not deemed to have FMTLE had first-degree relatives with déjà vu experiences lying in the 'borderland' between epileptic déjà vu and physiological déjà vu (**Table 4.8**). These experiences were termed 'suspicious' because they had elements compatible with mesial temporal lobe seizures, but also characteristics which were atypical for an epileptic aetiology. Nevertheless, given that these experiences were not reported by any of the age- and sex-matched controls, it can be speculated that at least some of them actually represented epileptic seizures and that these individuals were additional cases of FMTLE.

A number of genes have been associated with the focal epilepsies (Thomas and Berkovic, 2014), including the very first epilepsy gene to be discovered, *CHRNA4*, detected in a large Australian pedigree with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein *et al.*, 1995). Notably, many of these genes were identified after the introduction of next-generation sequencing (**Figure 1.5**) (Helbig *et al.*, 2016a). Considering these discoveries, the question arises as to what is the frequency of mutations in these genes in common focal epilepsies routinely followed in clinical practice. By applying whole exome sequencing (WES) with targeted gene analysis on 40 consecutive out- or inpatients with MRI-negative focal epilepsy and a family history of febrile seizures or any type of epilepsy in a first- or second-degree relatives, pathogenic or likely pathogenic variants were identified in 12.5% (5/40) of cases. These variants were found across different genes, namely *SCN1A*, *DEPDC5*, *PCDH19*, *GABRG2* and *NPRL3*, which underscores the molecular heterogeneity of focal epilepsies. Considering the fast pace of disease-gene discovery, it is likely that this diagnostic rate will increase in the immediate future as novel focal epilepsy genes are identified (Scheffer, 2014).

Overall, the results from this thesis provide additional important evidence for a strong genetic contribution to the aetiology of focal epilepsies. On a larger scale, they also support the paradigm by Thomas and Berkovic (Thomas and Berkovic, 2014), who proposed re-classifying many epilepsies previously termed ‘idiopathic’ as having a genetic cause, based on recent advances in understanding the genetic basis of the epilepsies (**Figure 8.1**). In the classic epidemiological study by Hauser and Kurland published in 1975 (Hauser and Kurland, 1975), no cause was identified in 72% of cases of focal epilepsy (i.e. ‘idiopathic cases’). Today, this proportion would be considerably smaller owing to the identification of epileptogenic lesions only detectable on brain MRI, the discovery of the autoimmune epilepsies (Toledano and Pittock, 2015), and - most importantly - the recognition that many focal epilepsies have a genetic aetiology.

Figure 8.1: Aetiology of epilepsy: traditional and modern concepts. (A) Aetiology epilepsy among incident cases in Rochester, Minnesota (1935-1967) (Hauser and Kurland, 1975). (B) '2014 paradigm' by which many cases previously considered to be 'idiopathic' have been re-classified as having a genetic cause based on advances in the understanding of genetic architecture of epilepsies over the past 40 years. The precise proportions of Mendelian vs complex epilepsies remain uncertain. Please note that a proportion of the idiopathic cases have been replaced by epilepsies associated with a lesion only detectable on brain MRI and the autoimmune epilepsies. Reproduced by permission from Macmillan Publishers Ltd: Nature Reviews Neurology (Thomas and Berkovic, 2014), copyright 2014.



Role of Genetic Factors in the Outcomes of Epilepsy Treatment

Driven by advances in genomic technologies and reduction of costs, next-generation sequencing is going to be increasingly applied in routine clinical practice, providing opportunities not only to make molecular diagnoses, but also to guide treatments (Dixon-Salazar *et al.*, 2012; Biesecker and Green, 2014; Scheffer, 2014; Srivastava *et al.*, 2014). An example is provided in this thesis by the case of a 22-year-old man with drug-resistant MRI-negative TLE, in whom clinical WES with targeted gene analysis identified a pathogenic variant in *SCN1A* (**Table 5.4**, **Figure 5.1**). Prior to obtaining the WES results, he had been considered for presurgical inpatient video-EEG monitoring due to monthly focal to bilateral tonic-clonic seizures. The identification of a *SCN1A* mutation led to the decision to halt presurgical investigations due to the risk of poor surgical outcomes in individuals with *SCN1A* mutations (Barba *et al.*, 2014; Skjei *et al.*, 2015). In the same patient, the genetic finding also led the treating clinician to recommend discontinuation of long-standing carbamazepine therapy, a potentially seizure aggravating drug in *SCN1A*-related epilepsies (Franco and Perucca, 2015), resulting in complete seizure control.

Although *SCN1A* mutations have been associated with unfavourable surgical outcomes (Barba *et al.*, 2014; Skjei *et al.*, 2015), mutations in other genes, such as *SCN1B* or GATOR1 complex genes, do not appear to carry an increased risk of surgical failure (Scheffer *et al.*, 2007; Scerri *et al.*, 2015; Sim *et al.*, 2016). However, these data are derived from small series or anecdotal reports, and as such do not answer the question of whether genetics affect the outcome of epilepsy surgery. The concern is that gene defects causing epilepsy may be widely expressed in the brain, ultimately undermining the

effectiveness of a focal resection, disconnection or destruction (Surges and Elger, 2013). Furthermore, there may be genetic factors not implicated in the process of focal epileptogenesis, which may nevertheless affect surgical outcome (Barba *et al.*, 2014). Aiming to shed light on these issues, work described in this thesis evaluated the effect of genetic factors on the outcome of MTLE surgery, by comparing WES data from 31 patients who continued to have uncontrolled seizures post-operatively (surgical failures), 115 patients who became seizure-free (surgical successes) and 2,797 controls. The proportion of individuals with deleterious variants in established epilepsy genes differed significantly across the three groups, a finding attributable to the proportion being significantly higher among surgical failures than controls (**Figure 6.1**). Although such proportion was nominally higher among failures than successes (19.3% vs 9.6%), the difference did not reach statistical significance. No individual gene was associated with unfavourable post-surgical outcome, which is to be expected given the limited patient sample size (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). Therefore, these results do not allow to draw firm conclusions on the influence (or lack thereof) of genetic factors on MTLE surgery outcome. However, they do provide a signal that surgical failures, as a group, may be enriched for individuals with deleterious variants in epilepsy genes, a possibility which requires verification in larger patient samples.

The last aim of this thesis was to investigate genomic predictors of valproic acid (VPA)-associated neural tube defects (NTDs), for which a genetic contribution has long been suggested (Robert and Guibaud, 1982; Finnell *et al.*, 1988; Finnell, 1991; Omtzigt *et al.*, 1992; Hall *et al.*, 1997; Faiella *et al.*, 2000; Duncan *et al.*, 2001; Lundberg *et al.*, 2004). VPA-associated NTDs remain a major concern as VPA is still the most effective therapy for many common epilepsies, such as juvenile myoclonic epilepsy and other generalised

epilepsy syndromes (Marson *et al.*, 2007b). VPA indications also extend beyond epilepsy, including migraine prophylaxis and bipolar disorder treatment (Johannessen Landmark, 2008). Using a family-based WES approach, this thesis identified, in 2 of 12 VPA-NTD families, two rare variants in the planar cell polarity genes *CELSR1* and *SCRIB* which may possibly confer a substantial risk to develop VPA-associated NTDs. This finding requires validation in larger cohorts. It should be emphasised that, although 12 VPA-NTD families are a small sample for a robust genetic study, they represent a sizable cohort from a clinical point of view. VPA-associated NTDs are in fact rare events, and their frequency has substantially decreased in the past 15 years owing to decreased VPA use and prescription of lower VPA doses in women of childbearing potential (Perucca *et al.*, 2015). Mothers who underwent termination of pregnancy due to the prenatal identification of a NTD are often unwilling to participate in an investigation of genetic determinants of the malformation, as this may carry the risk of re-emergence of stressful memories and sentiments of guilt related to the process of induced abortion.

Future directions

The results of this thesis pave the way to new research questions and avenues:

1) Understanding the borderland of déjà vu. The finding that first-degree relatives of patients with non-lesional MTLE may have déjà vu experiences which lie in the 'borderland' between epileptic déjà vu and physiological déjà vu raises the following question: are these experiences actually seizures, and as such a mild manifestation of FMTLE, or not? An answer might be provided by the discovery of the molecular underpinnings of FMTLE, which continue to remain largely elusive more than 20 years since this syndrome was first described (Berkovic *et al.*, 1996).

2) The clinical value of whole exome sequencing (WES). While the identification of pathogenic or likely pathogenic variants in 5 of 40 (12.5%) patients who underwent clinical WES with targeted gene analysis is an important finding, it also denotes the inability to 'solve' the cause of the epilepsy in the vast majority of the cohort. Efforts are currently under way to determine whether analysis of the entire exome (i.e. not limited to known epilepsy genes) can increase the proportion of 'solved cases'. To facilitate the adoption in clinical practice, future research should also focus on a more systematic evaluation of the clinical impact and cost-effectiveness of WES in the diagnostic pathway of patients with focal epilepsy.

3) The role of genetics factors in determining epilepsy surgery outcome. The EPIGEN investigation described in this thesis is an important first step towards answering the question of whether genetic factors influence the outcome of epilepsy surgery. Larger investigations are now needed to follow up on the signal that patients failing MTLE

surgery, as a group, are possibly enriched for individuals with deleterious variants in established epilepsy genes. The ultimate goal is to allow prediction of outcome on an individual basis, and therefore additional well-designed research is required to evaluate whether there are specific genes associated with post-surgical outcome.

4) Genetic determinants of valproic acid (VPA)-induced teratogenicity. Identifying genetic predictors of VPA-associated NTDs would be of great importance for the counselling of women of childbearing age in whom VPA therapy is being considered, and could allow further reduction of the frequency of NTDs by preventing exposure of individuals at greatest risk. An understanding of genetic risk factors would also have implications for understanding the mechanisms of VPA-induced NTDs, identifying potential protective treatments, and designing novel VPA analogues with improved safety profile. An international, multicentre, collaboration is currently under way to build on the findings of this thesis, and similar endeavours exploring other aspects of VPA-induced teratogenesis, such as behavioural teratogenesis, are warranted.

Epilepsy genetics has entered a new exciting era. The rise of eugenics in the early 20th century, which resulted in downplaying the 'transmissibility' of epilepsy, is a distant memory (Thomas and Berkovic, 2014). Driven by continued improvements in gene sequencing technologies and the efforts of large international collaborations (Perucca and O'Brien, 2015), genetics is anticipated to lead to further major breakthroughs in understanding the pathophysiology of epilepsy and in improving epilepsy diagnosis and treatment.

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Appendices

Appendix 1. Instrument used to collect demographic and clinical data in the study described in Chapter 4 ("The frequency of familial mesial temporal lobe epilepsy among patients presenting with non-lesional mesial temporal lobe epilepsy").

FAMILY STUDIED

I. PERSONAL DATA
NAME
MAIDEN NAME

ADDRESS

TELEPHONE Home
Business

General Practitioner
Address

Telephone

Neurologist/Paediatrician

Telephone

Observer
Relationship
Address

Telephone

AFFECTED STATUS

DATE OF INTERVIEW:

DOB

UR number

UR at

SEX M F

MARITAL STATUS Single Defacto Divorced
Married Widow Separated

PARTNER

Are you related to your partner? Y N
If YES, describe:

CHILDREN

Stillbirths/miscarriages? Y N
If yes, number and weeks gestation:

Full consent
Medical Record consent
Interviewed by:

Dr.

RA

Other things to do:-

Photograph
Blood
EEG
EEG Result
MRI
MRI Result
Request info
Info rec'd

II. PREGNANCY & PERINATAL PERIOD Information obtained from: ----------

A. The Pregnancy

1. Were there any problems during the pregnancy? | Y | N |

---

2. During:		Gestation					Gestation		
	Viral illness/infections	weeks	Y	N	Oedema	weeks	Y	N
	Bleeding	weeks	Y	N	Hypertension	weeks	Y	N
	Drugs	weeks	Y	N	Proteinuria	weeks	Y	N
	Smoking	weeks	Y	N	Polyhydramnios	weeks	Y	N
	Alcohol	weeks	Y	N	Oligohydramnios	weeks	Y	N
	Diabetes	weeks	Y	N				

B. The Birth

3. Gestation weeks

4. Place of birth ----------

---

5. Doctor involved ----------

Objective birth data:

Duration of :

first stage ---------- time: ----------

second stage ---------- ----------

third stage ---------- ----------

6. Onset of labour: Spontaneous
Medical induction
Surgical induction

Analgesia in labour:
N2O
Pethidine
Epidural
Caudal
Pudendal
TENS

7. Presentation Cephalic Shoulder Transverse Other:
Breech Footling Oblique ----------

8. Foetal problems during labour: HR > 160
HR < 120
CTG: Type I decelerations
CTG: Type II decelerations
Loss of baseline variation
Scalp pH at

9. Type of delivery: Normal vaginal
Forceps: Type _____
Vacuum extraction
Caesarian section
Elective for
Emergency for _____

10. Condition at birth: Good
Poor

Placenta
_____

Resuscitation needed: Suction
Bag and mask
Intubation
Ventilation
Drugs given:

Resuscitation needed:
_____
_____
_____
_____
_____
_____

Time until cuddle:

Apgar score _____ at 1 minute _____ at 5 minutes _____ at 10 minutes

11. Maternal complications postpartum _____
_____

C. Neonatal period

12. Birthweight _____ kg _____ lb/oz

13. Taken to special care nursery? Taken to NICU?
Duration _____ days Duration _____ days

14. Neonatal problems _____
_____
_____
_____

Seizures: onset: _____ hours/days _____
offset: _____ treatment: _____

IVH
Sepsis
Antibiotics
Transfusion Hyperbilirubinaemia Peak
Ventilation _____ days _____ %O2 max.

15. Age at discharge from hospital: _____ days of age
16. Problems in first month of life:

II. DEVELOPMENT AND EDUCATION

A. Developmental milestones

1. Milestones: Sat
Walked
Single words
Two words together
Toilet training

Handedness: Right Left

Growth:
Height
Weight

B. Educational and Occupational History

2. Educational attainment:

	Year of completion
Never attended school	
Special School	
Primary School	
Secondary School	
Technical School	
University	
Higher graduate Degree or Diploma	

3. Occupation:

Child		Sales and professional service	
Sheltered workshop		Plant machine operators	
Student		Labourer: skilled	
Home duties		unskilled	
Manager/administration		Pensioner: invalid	
Professional		elderly	
Trades		Unemployed	
Clerks			

4. Comments

III. SEIZURE HISTORY

A. Seizure types:

Number of types: ☐

Type 1 Age at onset _____ yrs

Description _____

_____

_____

_____

Post-Ictal

Frequency _____

Duration _____

Investigations (hospitalisation / medical intervention) _____

_____

Response to medication

_____

Type 2 Age at onset _____ yrs

Description

_____

_____

_____

Post-Ictal _____

Frequency _____

Duration _____

Investigations (hospitalisation / medical intervention) _____

_____

Response to medication

_____

Do the seizures follow each other in a particular pattern? _____

_____

Do you have:

	S	O
Blank spells, switch off the air, or go off the airwaves?	☐	☐
Jerks or twitches, especially just after waking up?	☐	☐

Length of time? _____

B. Auras

Duration

Do you experience: emotional changes _____

flashbacks or forced recollection of past events? ----------

feeling of being in a dream, in an unusually strange or unfamiliar place _____

deja vu (the sense that unfamiliar surroundings or objects are familiar) ----------

the sense that everyday surroundings or objects are unfamiliar (jamais vu)?

intense fear? _____

rage or anger? _____

euphoria, happiness or pleasure? ----------

Do you see or hear things that aren't real? Do you experience any unusual smells or tastes?

Indicate type of hallucination

visual

auditory

olfactory

gustatory

How long do these hallucinations last? ----------

Do you notice any pins and needles, electric shocks, tingling or other changes in sensation?

Where does it start and how does it spread? How long does it last?

Do objects or sounds in the room appear distorted or altered? _____

For example, do sounds seem nearer or farther away or ----------

objects seem shrunken or magnified? _____

Do you feel that everything is in slow motion or sped up? _____

Do you experience: ----------

a funny feeling in your tummy? _____

mouth watering or drooling? ----------

changes in pulse rate (a pounding heart)? _____

Do any of the following make you feel unusual in any way?

Patterns

Reading

Puzzles/arithmetic/calculations

Eye closure

IV. MEDICATION

Have you ever been on any anticonvulsant medication? Y | N

When?

Which drug/s?

How long for?

Have you ever been on:

			Effect		
Valproate	Epilim		N	I	
Carbamazepine	Tegretol		N	I	
Clonazepam	Rivotril		N	I	
Clobazam	Frisium		N	I	
Vigabatrin	Sabril		N	I	
Lamotrigine	Lamictal		N	I	
Gabapentin	Neurontin		N	I	
Topiramate	Topamax		N	I	
Tiagabine	Gabitril		N	I	
Phenytoin	Dilantin		N	I	
Ethosuximide	Zarontin		N	I	
Nitazepam	Mogadon		N	I	
Diazepam	Valium		N	I	
Phenobarb	Phenobarb		N	I	
Primidone	Mysoline		N	I	
Remacimide			N	I	
Acetazolamide	Diamox		N	I	
Felbamate	Felbatol		N	I	
Sulthiame	Ospolot		N	I	
Levetiracetam	Keppra		N	I	

What combinations of drugs have you been on in the past?	Dates	Effect		If effect, describe seizure type and change in frequency:
		N	I	
		N	I	

V. INVESTIGATIONS

1. Have you ever had:

	DATES:	Y	N	PLACE:
an EEG		Y	N	
video-EEG		Y	N	
a CT scan		Y	N	
an MRI scan		Y	N	

2. Twins

Blood typing:
Red cell Ag:
HLA Ag:

3. Results

EEG

Previous records:		Current record:	
Resting		Resting	
Hyperventilation		Hyperventilation	
Photic		Photic	
Monitoring		Monitoring	

CT scan

MRI scan

VI. PAST MEDICAL HISTORY

1. Past medical problems:

Diabetes:	Y	N
Fainting:	Y	N
Migraine:	Y	N
Anxiety/Depression:	Y	N
Cancer	Y	N

2. Are you on any medications?

3. Have you ever had:

			Age
meningitis?	Y	N	
encephalitis?	Y	N	
any other infection of the brain?	Y	N	

4. Have you ever had a serious head injury (sufficient to lose consciousness)? Y N

5. As an infant or child, did you ever fit with a fever? | Y | N |

Age at first F.C.

Age at last F.C.

Number of F.C.

How long did they last?

Were you admitted to hospital? | Y | N | Address

6.1 Do you ever have muscle cramps? | Y | N |

6.2 Do your arms or legs ever move by themselves? | Y | N |

6.3 Do you every get cramps with exercise or prolonged walking? | Y | N |

6.4 If answer to 6.1, 6.2 or 6.3 is YES:

How often does this happen?

Under what circumstances?

How old were you when this started?

Is it just uncomfortable or does the arm or leg twist into a funny position?

How long does it last?

7.1 Do you suffer from headaches? | Y | N |

7.2 If answer to 7.1 is YES:

Where do you feel the headache pain?

Is the pain symmetrical or more at one side than the other?

Is it a throbbing pain? | Y | N |

How severe is it? (1=trivial, 10=excruciating agony)

Is there:

Nausea/vomiting? | Y | N |

Worsening with lights? | Y | N |

Worsening with noise? | Y | N |

Worsening with activity (eg. climbing stairs)? | Y | N |

What can you do to improve it? Does sleep help?

Are there any changes in vision before or during the headache? Describe:

Are there any changes in sensation in vision before or during the headache? Describe:

Are there any changes in your speech before or during the headache? Describe:

7.3 If answer to 7.1 is NO:

Do you get episodes when your vision changes for a few minutes (eg. sparkling lights/shapes/"holes in vision")? Describe:

Do you get episodes where you have numbness or tingling in one side of your face or hand for a few minutes? Describe:

VII. CLINICAL EXAMINATION

1. General:

2. Neurological:

3. **Family Pedigree:**

Checklist: Names
Maiden names
DOB or YOB
Birthplace of ancestors
Consanguinity
Stillbirths/Miscarriages
Febrile convulsions
Family history of seizures in married in relatives

Appendix 2. Instrument used to collect demographic and clinical data in the study described in Chapter 5 ("The clinical utility of genetic testing for focal epilepsies").

STUDY INSTRUMENT

Recruiting Hospital ❑ The Royal Melbourne Hospital (RMH) ❑ The Royal Children's Hospital (RCH)

Subject identification no.: _____	**Date of interview:** ___ ___/ ___ ___/ ___ ___ ___ ___ D D M M Y Y Y Y

The information in this study form was obtained from:

_____

_____

DEMOGRAPHICS

1. Gender

Male.....1
Female.....2

2. Ethnicity (self-declared)

Aboriginal Australia and Torres Strait Islanders.....1
Jewish.....2
Middle Eastern.....3
Caucasian.....4
Asian-Chinese.....5
Asian-Indian.....6
Asian-Indonesian.....7
Asian-Japanese.....8
Asian-Sri Lankan.....9
Asian-Pakistani.....10
Asian-Malaysian.....11
Asian-Korean.....12
Asian-Filipino.....13
Asian-Vietnamese.....14
Pacific Islander.....15
African.....16
Other [**SPECIFY:**_____].....77
Unknown.....99

3. Relatives' ethnicity

A. Mother's ethnicity

Aboriginal Australia and Torres Strait Islanders.....1
Jewish.....2
Middle Eastern.....3
Caucasian.....4
Asian-Chinese.....5
Asian-Indian.....6
Asian-Indonesian.....7
Asian-Japanese.....8
Asian-Sri Lankan.....9
Asian-Pakistani.....10
Asian-Malaysian.....11
Asian-Korean.....12
Asian-Filipino.....13
Asian-Vietnamese.....14
Pacific Islander.....15
African.....16
Other [**SPECIFY:**_____].....77
Unknown.....99

B. Maternal grandmother's ethnicity

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Unknown 99

C. Maternal grandfather's ethnicity

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Unknown 99

D. Father's ethnicity

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Unknown 99

E. Paternal grandmother's ethnicity

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Unknown 99

F. Paternal grandfather's ethnicity

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Unknown 99

4. Affected relatives' ethnicity

A. Family member 1

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Unknown 99

B. Family member 2

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Not applicable 88
Unknown 99

C. Family member 3

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Not applicable 88
Unknown 99

D. Family member 4

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Not applicable 88
Unknown 99

E. Family member 5

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Not applicable 88
Unknown 99

F. Family member 6

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Not applicable 88
Unknown 99

G. Family member 7

[RELATIONSHIP TO SUBJECT]:
_____

Aboriginal Australia and Torres Strait Islanders 1
Jewish 2
Middle Eastern 3
Caucasian 4
Asian-Chinese 5
Asian-Indian 6
Asian-Indonesian 7
Asian-Japanese 8
Asian-Sri Lankan 9
Asian-Pakistani 10
Asian-Malaysian 11
Asian-Korean 12
Asian-Filipino 13
Asian-Vietnamese 14
Pacific Islander 15
African 16
Other [**SPECIFY:**_____] 77
Not applicable 88
Unknown 99

PREGNANCY AND PERINATAL PERIOD

1. The pregnancy

1.1. Were there any problems during the pregnancy?

Yes.....1
No…….**[GO TO 2.]**.....0
Don't know……..**[GO TO 2.]**.....99

A. What were the problems during the pregnancy?

_____

_____

_____

[SPECIFY WHETHER ANY THE FOLLOWING PROBLEMS OCCURRED DURING PREGNANCY]

Problem during pregnancy			**YES**	**NO**	**DON'T KNOW**	**Gestational age (weeks)**
B.	Viral illness/infection		1	0	99	
C.	Bleeding		1	0	99	
D.	Drugs		1	0	99	
	D.1.	**[NAME THEM]**				
E.	Smoking		1	0	99	
F.	Alcohol		1	0	99	
G.	Diabetes		1	0	99	
H.	Abnormal oedema		1	0	99	
I.	Hypertension		1	0	99	
L.	Proteinuria		1	0	99	
M.	Polyhydramnios		1	0	99	
N.	Oligohydramnios		1	0	99	

2. The birth

2.1. Were there any problems during the birth?

Yes.....1
No…….**[GO TO 2.2.]**.....0
Don't know……**[GO TO 2.2.]**.....99

A. What were the problems during the birth?

_____

_____

_____

2.2. Is gestational age at birth known?

Yes [**SPECIFY**:_____].....1
No.....0

2.3. Were there any foetal problems during labour?

Yes.....1
No…….**[GO TO 2.4.]**.....0
Don't know…**[GO TO 2.4.]**.....99

A. What were the foetal problems during labour?

_____

_____

_____

2.4. Type of delivery

Normal vaginal.....**[GO TO 2.5.]**.....1
Forceps **[TYPE:_____]**.. **[GO TO 2.5.]** 2
Vacuum extraction…….**[GO TO 2.5.]**.....3
Caesarian section 4
Don't know……**[GO TO 2.5.]**.....99

A. Type of C-section

Elective.....1
Emergency.....2
Don't know.....99

B. **[SPECIFY REASONS FOR (ELECTIVE/EMERGENCY) C-SECTION]:**

_____

_____

2.5. Foetal condition at birth

Good.....1
Poor 0
Don't know.....99

2.6. Was resuscitation needed?

Yes.....1
No…….**[GO TO 2.7.]**.....0
Don't know……**[GO TO 2.7.]**.....99

[SPECIFY MEASURES USED FOR RESUSCITATION]

Measures		**YES**	**NO**	**DON'T KNOW**	**Reasons for need of resuscitation measure**
A.	Suction	1	0	99	
B.	Bag and mask	1	0	99	
C.	Intubation	1	0	99	
D.	Ventilation	1	0	99	
E.	Other	1	0	99	

[SPECIFY DRUGS GIVEN DURING RESUSCITATION]:

_____

_____

2.7. Is the Apgar score available?

Yes.....1
No…….**[GO TO 3.]**.....0
Don't know……**[GO TO 3.]**.....99

[SPECIFY APGAR SCORES AT EACH TIME POINT]

Apgar score at different time points		YES	NO	DON'T KNOW	Score									
A.	1 minute	1	0	99	1	2	3	4	5	6	7	8	9	10
B.	5 minutes	1	0	99	1	2	3	4	5	6	7	8	9	10
C.	10 minutes	1	0	99	1	2	3	4	5	6	7	8	9	10

3. The neonatal period

3.1. Is the weight at birth known?

Yes [**SPECIFY if KG OR lb/oz**:_____] 1
No.....0

3.2. Did the newborn have any problems?

Yes.....1
No……..**[GO TO NEXT SECTION]** 0
Don't know……..**[GO TO NEXT SECTION]** 99

A. What were the neonatal problems?

_____

_____

[SPECIFY WHETHER THE NEWBORN HAD ANY OF THE FOLLOWING PROBLEMS]

Neonatal problems		YES	NO	DON'T KNOW	Duration [SPECIFY IF HOURS OR DAYS]	TREATMENT
B.	Seizures	1	0	99		
C.	Sepsis	1	0	99		
D.	IVH	1	0	99		
E.	Hyperbilirubinemia	1	0	99		
F.	Other	1	0	99		

G. Was the newborn taken to special care nursery?

Yes **[DURATION (days)**_____] 1
No.....0
Don't know.....99

H. Was the newborn taken to the NICU?

Yes **[DURATION (days)**_____] 1
No.....0
Don't know.....99

DEVELOPMENT AND EDUCATION

1. Developmental milestones

1.1. Specify ages for each developmental milestone

Answers: **Y=Yes; N=NO; N/A=Not applicable; DK=Don't know**

Milestone		Age known?				Age [SPECIFY IF MONTHS OR YEARS]	Comments
		Y	N	N/A	DK		
A.	Sat	1	0	88	99		
B.	Walked	1	0	88	99		
C.	Single words	1	0	88	99		
D.	Two words together	1	0	88	99		

1.2. Handedness

Right 1
Left 2
Ambidextrous 3
Don't know 99

2. Educational and occupational history

2.1. Educational attainment (highest level)

Never attended school 1
Special school 2
Primary school 3
Secondary school 4
Extra assistance in secondary school 5
TAFE 6
University 7
Higher graduate degree or diploma 8
Don't know 99

2.2. Did the subject have any developmental issue?

Yes 1
No……..**[GO TO 2.3.]** 0
Don't know……**[GO TO 2.3.]** 99

[SPECIFY THE TYPE OF DEVELOPMENTAL ISSUE]

Developmental issue			YES	NO	DON'T KNOW	Age identified	Specify severity (mild/moderate/severe) and any other comments
A.	Learning difficulties		1	0	99		
B.	Intellectual disability		1	0	99		
C.	Behavioral problems		1	0	99		
D.	Autism Spectrum Disorder		1	0	99		
E.	Speech/language problems		1	0	99		
F.	Other psychiatric problems		1	0	99		
	F.1.	**[SPECIFY]**					

2.3. Did the subject have any regression of skills?

Yes **[SPECIFY AGE**_____]....1
No……..**[GO TO 2.4.]**....0
Don't know…..**[GO TO 2.4.]**....99

A. [DESCRIBE HOW THE SUBJECT REGRESSED] _____

_____

_____

B. Was the regression related to a seizure(s)?

Yes....1
No....0
Don't know....99

2.4. Subject's current occupation: _____

_____

SEIZURE HISTORY

1.1. How many different types of seizures does the subject (or his/her family) think he/she has *ever* had?

One 1
Two.....2
Three.....3
Four or more **[SPECIFY_____]** 4
Don't know.....99

1.2. [ASK THE SUBJECT TO DESCRIBE WHAT HAPPENS IN EACH TYPE OF SEIZURE. IF MORE THAN ONE SEIZURE TYPE, START WITH THE OLDEST TYPE MOVING TO THE MOST RECENT ONE]

A. Seizure type 1

Currently occurring (i.e. over the past 12 months).....1
No longer occurring.....0
Don't know.....99

A.1. Age at onset **[SPECIFY IF MONTHS OR YEARS]:**

A.2. Age at offset (if applicable) **[SPECIFY IF MONTHS OR YEARS]:**

A.3. Aura:

A.4. Seizure description:

A.5. Post-ictal:

A.6. Frequency (per month, if applicable):

A.7. Duration:

B. Seizure type 2

Currently occurring (i.e. over the past 12 months).....1
No longer occurring.....0
Don't know.....99

B.1. Age at onset **[SPECIFY IF MONTHS OR YEARS]:**

B.2. Age at offset (if applicable) **[SPECIFY IF MONTHS OR YEARS]:**

B.3. Aura:

B.4. Seizure description:

B.5. Post-ictal:

B.6. Frequency (per month, if applicable):

B.7. Duration:

C. Seizure type 3

Currently occurring (i.e. over the past 12 months).....1
No longer occurring.....0
Don't know.....99

C.1. Age at onset **[SPECIFY IF MONTHS OR YEARS]:**

C.2. Age at offset (if applicable) **[SPECIFY IF MONTHS OR YEARS]:**

C.3. Aura:

C.4. Seizure description

C.5. Post-ictal:

C.6. Frequency (per month, if applicable):

C.7. Duration:

D. Seizure type 4

Currently occurring (i.e. over the past 12 months).....1
No longer occurring.....0
Don't know.....99

D.1. Age at onset **[SPECIFY IF MONTHS OR YEARS]:**

D.2. Age at offset (if applicable) **[SPECIFY IF MONTHS OR YEARS]:**

D.3. Aura:

D.4. Seizure description

D.5. Post-ictal:

D.6. Frequency (per month, if applicable):

D.7. Duration:

E. Other seizure types/comments:

1.3. Does the subject have blank spells in which he/she will "switch off or go off the airwaves"?

Yes.....1
No……**[GO TO 2.2.]**.....0
Don't know……**[GO TO 2.2.]**.....99

A. [PROVIDE DESCRIPTION, AND SPECIFY AGE AT ONSET, DURATION, AND TRIGGER FACTORS]

_____

_____

_____

1.4. Drop attacks: has the subject ever dropped to the ground, dropped objects, or had his/her head suddenly drop to his/her chest for no reason?

Yes.....1
No……**[GO TO 2.3.]**.....0
Don't know……**[GO TO 2.3.]**.....99

A. Without losing consciousness, have his/her legs given way?

Yes.....1
No.....0
Don't know.....99

B. Without losing consciousness, has he/she dropped to ground?

Yes.....1
No.....0
Don't know.....99

C. Without losing consciousness, have his/her arms dropped or sagged?

Yes.....1
No.....0
Don't know.....99

D. Without losing consciousness, has he/she dropped objects?

Yes.....1
No.....0
Don't know.....99

E. Without losing consciousness, does his/her head drop or sag?

Yes.....1
No.....0
Don't know.....99

1.5. As an infant or child, did the subject ever fit with a fever?

Yes.....1
No……**[GO TO NEXT SECTION]**.....0
Don't know……**[GO TO NEXT SECTION]**.....99

A. Was the subject admitted to hospital?

Yes.....1
No.....0
Don't know.....99

B. Is the age of the *first* febrile convulsion known?

Yes **[SPECIFY AGE_____]**.....1
No.....0

C. Is the age of the *last* febrile convulsion known?

Yes **[SPECIFY AGE_____]**.....1
No.....0

D. How many febrile convulsions did he/she have?

One.....1
Two.....2
Three.....3
Four or more **[SPECIFY_____]**.....4
Don't know.....99

E. How long did they last?

_____

_____

SEIZURE ONSET AND FREQUENCY

[Complete the questions below separately for each seizure type, specifying if the information was obtained from the subject (S) or a witness (W).]

Answers: **Y=Yes; N=No; NA=Not applicable; DK=Don't know**

Question		Seizure type 1						Seizure type 2						Seizure type 3						Seizure type 4					
						S	W					S	W					S	W					S	W
1.	When did the seizures begin?					1	2					1	2					1	2					1	2
2.	When was the last seizure?					1	2					1	2					1	2					1	2
3.	How often seizures do occur now? **[IF APPLICABLE, PER MONTH]**					1	2					1	2					1	2					1	2
4.	Is the number of seizures prior to starting treatment known? **[IF "N", "NA", "DK" GO TO 5.]**	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2
		1	0	88	99			1	0	88	99			1	0	88	99			1	0	88	99		
	A. How many seizures in total?	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
	B. How many seizures in the 6 months prior to starting treatment?	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**	**1**	**2**	**3-5**	**6-10**	**11-20**	**>20**
		1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
5.	Has seizure frequency changed over the years? **[IF "N", "NA", "DK" GO TO 6.]**	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2
		1	0	88	99			1	0	88	99			1	0	88	99			1	0	88	99		
	A. When did the change occur?																								
	B. Why did the change occur?																								
6.	Has the subject ever had a long period free of seizures? **[IF "N", "NA", "DK" GO TO 7.]**	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2	**Y**	**N**	**NA**	**DK**	1	2
		1	0	88	99			1	0	88	99			1	0	88	99			1	0	88	99		
	A. When was that?																								
	B. How long did it last?																								

7.	Have the seizures ever been so frequent that the subject did not recover between seizures? **[IF "N", "NA", "DK" GO TO NEXT SECTION.]**	**Y**	**N**	**NA**	**DK**			**Y**	**N**	**NA**	**DK**			**Y**	**N**	**NA**	**DK**			**Y**	**N**	**NA**	**DK**		
		1	0	88	99	1	2	1	0	88	99	1	2	1	0	88	99	1	2	1	0	88	99	1	2
	A.	When was that? **[ALSO SPECIFY NUMBER OF EPISODES OF STATUS EPILEPTICUS]**																							

SEIZURE PHENOMENA

[Complete the questions below separately for each seizure type, specifying, when applicable, if the information was obtained from the subject (S) or a witness (W)]

Answers: **Y=Yes; N=No; NA=Not applicable; DK=Don't know**

Question			**Seizure type 1**					**Seizure type 2**					**Seizure type 3**					**Seizure type 4**				
			Y	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
1.	**Aura**																					
1.1.	Does any warning occur before seizures? **[IF "N" or "DK" GO TO 2.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide brief description of the warning																				
	B.	How long does it last?																				
	C.	Does it ever occur on its own?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	D.	If occurring on its own, how often does it occur?				1	2				1	2				1	2				1	2
2.	**Ictal phase**																					
2.1.	During a seizure, does the subject experience a funny feeling in the tummy?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.2.	During a seizure, does he/she experience mouthwatering or drooling?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.3.	During a seizure, does he/she experience palpitations or a pounding heart?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.4.	During a seizure, does he/she "go blank" or lose awareness of the surroundings? **[IF "N" or "DK" GO TO 2.5.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2

	A.	Does he/she go completely blank or retain some awareness of the surroundings?																			
	B.	Does he/she stop what he/she is doing or continue on automatically "like a robot?"																			
	C.	For how long is he/she blank or "out of it?"																			
2.5.	During a seizure, does the subject experience sudden emotional changes for no reason? **[IF "N" or "DK" GO TO 2.6.]**		**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
			1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Does he/she experience intense fear?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	B.	Does he/she experience rage or anger?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	C.	Does he/she experience euphoria, happiness, or pleasure?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	D.	How long do these changes last?																			
	E.	Provide a brief description of these changes																			
2.6.	During a seizure, does the subject experience flashbacks, or a feeling of being in a dream or in an unusually strange or familiar place? **[IF "N" or "DK" GO TO 2.7.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Does he/she experience flashbacks or forced recollection of past events?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	B.	Does he/she experience the sense that unfamiliar surroundings or objects are familiar (déjà vu)?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2

	C.	Does he/she experience the sense that everyday surroundings or objects are unfamiliar (jamais vu)?	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
			1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	D.	Does he/she experience the feeling that he/she is a dream when he/she is awake?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	E.	How long do these feelings last?																				
	F.	Provide a brief description of these feelings																				
2.7.	During a seizure, does the subject <u>see</u> or <u>hear</u> things that aren't actually there? **[IF "N" or "DK" GO TO 2.8.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a brief description of these hallucinations																				
	B.	How long do they last?																				
2.8.	During a seizure, does the subject experience any unusual <u>smells</u> or <u>tastes</u>? **[IF "N" or "DK" GO TO 2.9.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a brief description of the smells or tastes																				
	B.	How long do they last?																				

2.9.	During a seizure, does the subject notice any pins and needles, electric shocks, tingling, or other changes in sensation? **[IF "N" or "DK" GO TO 2.10.]**		**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
			1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Is he/she able to get rid of the feeling by moving?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	B.	Provide a description of this sensation																				
	C.	Where does it start/how does it spread?																				
	D.	How long does it last?																				
2.10.	During an aura or seizure, do objects appear distorted or altered? (e.g. bigger or smaller) **[IF "N" or "DK" GO TO 2.11.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a description of this perception																				
	B.	How long does it last?																				
2.11.	During an aura or seizure, do sounds appear distorted or altered? (e.g. louder or softer) **[IF "N" or "DK" GO TO 2.12.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a description of this perception																				

	B.	How long does it last?																			
2.12.	During a seizure, does he/she feel that everything is in slow motion or sped up? **[IF "N" or "DK" GO TO 2.13.]**		**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
			1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a description of this feeling																			
	B.	How long does it last?																			
2.13.	During a seizure, does the subject feel dizzy or experience vertigo? **[IF "N" or "DK" GO TO 2.14.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a description of this feeling																			
	B.	How long does it last?																			
2.14.	Do <u>patterns</u> make the subject feel funny or unusual in any way?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.15.	Does <u>reading</u> make the subject feel funny or unusual in any way?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.16.	Do <u>puzzles/arithmetic/calculations</u> make the subject feel funny or unusual in any way?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2

2.17.	Does eye closure make the subject feel funny or unusual in any way?		**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
			1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.18.	During the seizure, do his/her head and eyes turn in a particular direction? **[IF "N" or "DK" GO TO 2.19.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Which direction do they turn?																				
	B.	How long does it last?																				
2.19.	During a seizure, are the arms and/or legs held stiff in a particular position? **[IF "N" or "DK" GO TO 2.20.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Provide a description of the arm/leg posturing																				
	B.	Are both arms/legs or is just one arm/leg affected? **[IF JUST ONE ARM /LEG, SPECIFY WHICH ONE]**																				
	C.	Are the arms/legs bent or straight?																				
	D.	How long does it last?																				
2.20.	Do any jerking or twitching movements of the face, arm, or leg occur during the seizure? **[IF "N" or "DK" GO TO 2.21.]**		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Do they involve face or lips?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	B.	Do they involve arms?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	C.	Do they involve legs?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2

	D.	Does the jerking affect both sides of the body at the same time or only one side at a time? **[SPECIFY SIDE]**																			
	E.	Are the jerks infrequent and irregular, or are they repeated and regular?																			
	F.	How long does the jerking last?																			
2.21.	During a seizure, do the eyelids twitch or is there repeated blinking? **[IF "N" or "DK" GO TO 2.22.]**		**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
			1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A.	Does it affect one or both sides? **[SPECIFY SIDE IF APPROPRIATE]**																			
	B.	How long does it last?																			
2.22.	During a seizure, does lip smacking occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.23.	During a seizure, does lip licking occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.24.	During a seizure, does chewing occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.25.	During a seizure, does swallowing occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.26.	During a seizure, does laughing occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.27.	During a seizure, does speaking occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.28.	During a seizure, does picking at or fiddling with things occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.29.	During a seizure, does walking or making stepping or bicycling movements occur?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.30.	Does the subject ever bite his/her tongue during a seizure?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.31.	Does the subject ever wet his/herself during a seizure?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
2.32.	Does the subject ever pass stool during a seizure?		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2

2.33.	During the seizure, does breathing stop or does the subject go blue?	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
3.	**Post-ictal phase**																				
3.1.	After a seizure, is the subject confused or drowsy? **[IF "N" or "DK" GO TO 3.2.]**	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A. How long does the confusion or drowsiness last?																				
3.2.	After a seizure, does the subject have a headache? **[IF "N" or "DK" GO TO 3.3.]**	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A. Is the headache on one side of the head or does it ache all over? **[IF ON ONE SIDE, SPECIFY SIDE]**																				
3.3.	Do seizures have any longer lasting effects on vision, speech, sensation, or muscle power? **[IF "N" or "DK" GO TO 3.4.]**	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A. Provide a description of these effects																				
	B. How long do these effects last?																				
4.	**Evolution**																				
4.1.	Do the seizures follow each other in a particular pattern? **[IF "N" or "DK" GO TO 4.2.]**	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A. Provide a description of the pattern																				

4.2.	Do the features of a seizure appear at the same time or follow a certain sequence? **[IF SEQUENTIAL, PROVIDE DESCRIPTION OF SEQUENCE OF EVENTS]**					

SEIZURE PRECIPITANTS

[Complete the questions below separately for each seizure type, specifying, when applicable, if the information was obtained from the subject (S) or a witness (W)]
Answers: **Y=Yes; N=No; NA=Not applicable; DK=Don't know**

Question		Seizure type 1					Seizure type 2					Seizure type 3					Seizure type 4				
		Y	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**
1.	Are there any triggers or precipitants to your seizures? **[IF "N" or "DK" GO TO 2.]**	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A. Specify any trigger or precipitant																				
2.	Are there any situations in which seizures are more likely to occur? **[IF "NO" or "DK" GO TO 3.]**	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2
	A. Specify these situations																				
3.	What percentage of the seizures occur in the first 2 hours after waking?				1	2				1	2				1	2				1	2
4.	What percentage of the seizures occur late morning?				1	2				1	2				1	2				1	2
5.	What percentage of the seizures occur in the afternoon?				1	2				1	2				1	2				1	2
6.	What percentage of the seizures occur in the evening?				1	2				1	2				1	2				1	2

7.	What percentage of the seizures occur in the first half of sleep?					1	2					1	2					1	2					1	2					1	2
8.	What percentage of the seizures occur in the last half of sleep?					1	2					1	2					1	2					1	2					1	2
9.	What percentage of the seizures have no relation to the time of day?					1	2					1	2					1	2					1	2					1	2
10.	Has he/she had a seizure during a daytime nap?	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**	**Y**	**N**	**DK**	**S**	**W**					
		1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2					
11.	Has he/she had a seizure on awakening from a daytime nap?	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2	1	0	99	1	2					
12.	Quantify how frequent are seizures in relation to particular factors **(N=Never; S=Sometimes; A=Always; DK=Don't know)**																														
	A. Sleep deprivation	**N**	**S**	**A**	**DK**	**S**	**W**	**N**	**S**	**A**	**DK**	**S**	**W**	**N**	**S**	**A**	**DK**	**S**	**W**	**N**	**S**	**A**	**DK**	**S**	**W**						
		1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	B. Increased amount of alcohol in the previous 24 hours?	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	C. Flashing lights/television/computer/disco light/riding in a car along tree lined road	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	D. Physical stress	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	E. Emotional stress	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	F. Overbreathing	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	G. Fever	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
	H. Noises or startle **[GO TO NEXT SECTION IF MALE SUBJECT]**	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2	1	2	3	99	1	2						
13.	In relation to the menstrual cycle, what percentage of the seizures occur during the period?	**%**		**N/A**		1	2	**%**		**N/A**		1	2	**%**		**N/A**		1	2	**%**		**N/A**		1	2						
				88						88						88						88									

14.	What percentage of the seizures occur in the first 14 days after the period?		88	1	2		88	1	2		88	1	2		88	1	2
15.	What percentage of the seizures occur in the few days before the period?		88	1	2		88	1	2		88	1	2		88	1	2

INVESTIGATIONS (enter most representative findings)

1. EEG

1.1. Routine EEG

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done…….. **[GO TO 1.2.]** 0
Don't know……**[GO TO 1.2.]** 99

A. If routine EEG done, what were the findings?

Abnormal 1
Normal 0
Don't know 99

[LIST ALL ABNORMAL FINDINGS, STARTING WITH THE MOST COMMON]

Abnormal findings (e.g. spike, slowing, seizure; if seizure, select location of the seizure-onset zone)	**Location (lobe)**												**Electrode/s at which finding has maximum amplitude** (if known)
	Frontal			**Temporal**			**Parietal**			**Occipital**			
	R	**L**	**Bil**	**R**	**L**	**Bil**	**R**	**L**	**Bil**	**R**	**L**	**Bil**	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	

[ADDITIONAL COMMENTS]: _____

_____

_____

B. If hyperventilation performed, was it abnormal?

Yes 1
No 0
Don't know 99

[DESCRIBE]: _____

_____

_____

C. If photic stimulation performed, was it abnormal?

Yes 1
No 0
Don't know 99

[DESCRIBE]: _____

_____

_____

1.2. Sleep-deprived EEG

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done…….. **[GO TO 1.3.]** 0
Don't know……**[GO TO 1.3.]** 99

A. If sleep-deprived EEG done, what were the findings?

Abnormal 1
Normal 0
Don't know 99

[LIST ALL ABNORMAL FINDINGS, STARTING WITH THE MOST COMMON]

Abnormal findings (e.g. spike, slowing, seizure; if seizure, select location of the seizure-onset zone)	**Location (lobe)**												**Electrode/s at which finding has maximum amplitude** (if known)
	Frontal			**Temporal**			**Parietal**			**Occipital**			
	R	**L**	**Bil**	**R**	**L**	**Bil**	**R**	**L**	**Bil**	**R**	**L**	**Bil**	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	

[ADDITIONAL COMMENTS]: _____

_____

_____

B. If hyperventilation performed, was it abnormal?

Yes 1
No 0
Don't know 99

[DESCRIBE]: _____

_____

_____

C. If photic stimulation performed, was it abnormal?

Yes 1
No 0
Don't know 99

[DESCRIBE]: _____

_____

_____

1.3. Video-EEG monitoring

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done…….. **[GO TO 2.]** 0
Don't know……**[GO TO 2.]** 99

A. If video-EEG monitoring done, what were the findings?

Abnormal 1
Normal 0
Don't know 99

[LIST ALL ABNORMAL FINDINGS, STARTING WITH THE MOST COMMON]

Abnormal findings (e.g. spike, slowing, seizure; if seizure, select location of the seizure-onset zone)	**Location (lobe)**												**Electrode/s at which finding has maximum amplitude** (if known)
	Frontal			**Temporal**			**Parietal**			**Occipital**			
	R	**L**	**Bil**	**R**	**L**	**Bil**	**R**	**L**	**Bil**	**R**	**L**	**Bil**	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	
	1	2	3	4	5	6	7	8	9	10	11	12	

[ADDITIONAL COMMENTS]: _____

_____

_____

B. If hyperventilation performed, was it abnormal?
Yes.....1
No.....0
Don't know.....99

[DESCRIBE]: _____

_____

_____

C. If photic stimulation performed, was it abnormal?
Yes.....1
No.....0
Don't know.....99

[DESCRIBE]: _____

_____

_____

2. CT
Done [**DATE**, DD/MM/YYYY:_____].....1
Not done.....0
Don't know.....99

[REPORT]: _____

_____

3. MRI
Done.....1
Not done……..**[GO TO 4.]**.....0
Don't know……..**[GO TO 4.]**.....99

3.1. Number of MRIs done

One 1
Two.....2
Three.....3
Four or more 4
Don't know.....99

A. MRI 1 [**DATE**, DD/MM/YYYY:_____] **[REPORT]:**

B. MRI 2 [**DATE**, DD/MM/YYYY:_____] **[REPORT]:**

C. MRI 3 [**DATE**, DD/MM/YYYY:_____] **[REPORT]:**

D. Other MRI [**DATE**, DD/MM/YYYY:_____] **[REPORT]:**

4. PET

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done 0
Don't know.....99

[REPORT]:

5. SPECT

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done 0
Don't know.....99

[REPORT]:

6. PSYCHIATRIC ASSESSMENT

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done 0
Don't know.....99

[REPORT]:

7. NEUROPSYCHOLOGICAL EVALUATION

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done 0
Don't know.....99

[REPORT]:

8. **OTHER [SPECIFY_____]**

Done [**DATE**, DD/MM/YYYY:_____] 1
Not done 0
Don't know 99

[REPORT]: _____

_____

9. **NEUROLOGIC EXAM**

Abnormal 1
Normal 0
Don't know 99

[SPECIFY ABNORMAL FINDINGS]: _____

_____

_____

_____

10. Specify how long the subject has been followed at the recruiting hospital _____

11. If the subject has been referred to the recruiting hospital in the past 12 months, please specify reason of referral (e.g. diagnostic query): _____

_____

FAMILY HISTORY

1. Marital status

Single….**[GO TO 2.]** 1
Married 2
Defacto 3
Widow 4
Divorced 5
Separated 6
Don't know….**[GO TO 2.]** 99

A. Is the subject related to his/her partner in other way other than marriage/defacto relationship?

Yes [**SPECIFY**:_____] 1
No 0
Don't know 99

2. Does the subject have any children?

Yes 1
No….**[GO TO 3.]** 0
Don't know….**[GO TO 3.]** 99

A. How many? _____

B. Are all children from the same relationship?

Yes 1
No….**[GO TO 2.C.]** 0
Don't know….**[GO TO 2.C.]** 99

B.1. [SPECIFY]: _____

_____

_____

C. Any miscarriages/stillbirths?

Yes 1
No….**[GO TO 3.]** 0
Don't know….**[GO TO 3.]** 99

C.1. [SPECIFY <u>NUMBER</u> OF MISCARRIAGES/STILLBIRTHS AND <u>WEEKS OF GESTATION</u> FOR EACH MISCARRIAGE/STILLBIRTH] _____

_____

_____

_____

3. Is there consanguinity in the family?

Yes 1
No….**[GO TO 4.]** 0
Don't know….**[GO TO 4.]** 99

A. [SPECIFY] _____

_____

_____

_____

_____

FAMILY HISTORY SCREENING INTERVIEW

Ask the subject the following questions which explore whether febrile convulsions and/or seizures occurred in other family members. In this form, note **only** the family members affected.

Answers: **Y=Yes; POSS=Possible; N=No; DK=Don't know**

Questions			**Family member 1**				**Family member 2**				**Family member 3**				**Family member 4**			
			[Relationship to subject]: _____				**[Relationship to subject]:** _____				**[Relationship to subject]:** _____				**[Relationship to subject]:** _____			
			Male (1)		Female (2)		Male (1)		Female (2)		Male (1)		Female (2)		Male (1)		Female (2)	
			Age_____				Age_____				Age_____				Age_____			
			Y	**POSS**	**N**	**DK**	**Y**	**POSS**	**N**	**DK**	**Y**	**POSS**	**N**	**DK**	**Y**	**POSS**	**N**	**DK**
1.	Did anyone ever tell you that he/she had a seizure or convulsion caused by a high fever when he/she was a child? **[IF "NO" or "DON'T KNOW" GO TO 2.]**		1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	A.	How many seizures did he/she have because of high fever?																
	B.	How old was he/she when that happened [the FIRST time]? **[IF ONLY HAD ONE GO TO 2.]**																
	C.	How old was he/she when that happened [the LAST time]?																
2.	[Other than the seizure/s [he/she] had because of a high fever] Has [he/she] ever had, or has anyone ever told you that [he/she] had a seizure disorder or epilepsy?		1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	A.	How old was he/she the first time he/she had a seizure?																
	B.	What type of seizure/s did he/she have?																
	C.	How many seizures did he/she have?																

	D.	Is he/she taking antiepileptic medications? **[IF "NO" or "DON'T KNOW" GO TO NEXT SECTION]**	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
		D.1. Do you know which one/s?																
[Ask the following questions only if subject said "No" to epilepsy or a seizure disorder in Q2]																		
3.		[Other than the seizure/s he/she had because of a high fever] Has he/she ever had, or has anyone ever told you that he/she had, any of the following																
	A.	A seizure, convulsion, fit, or spell under any circumstances?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	B.	Uncontrolled movements of part or all of the body such as twitching, jerking, shaking, or going limp?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	C.	An unexplained change in mental state or level of awareness, or an episode of "spacing out"?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	D.	Did anyone ever tell you that when he/she was a small child, he/she would daydream or stare into space more than other children?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	E.	Has he/she ever noticed any unusual body movements or feelings when exposed to strobe lights, video games, flickering lights, or sun?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	F.	Shortly after waking up, either in the morning or after a nap, has he/she ever noticed uncontrollable jerking or clumsiness, such as dropping things or things suddenly "flying" from his/her hands?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	G.	Has he/she ever had any other type of repeated unusual spells?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99

FAMILY HISTORY SCREENING INTERVIEW

Ask the subject the following questions which explore whether febrile convulsions and/or seizures occurred in other family members. In this form, note **only** the family members affected.

Answers: **Y=Yes; POSS=Possible; N=No; DK=Don't know**

Questions			**Family member 5**				**Family member 6**				**Family member 7**				**Family member 8**			
			[Relationship to subject]: _____				**[Relationship to subject]:** _____				**[Relationship to subject]:** _____				**[Relationship to subject]:** _____			
			Male (1)		Female (2)		Male (1)		Female (2)		Male (1)		Female (2)		Male (1)		Female (2)	
			Age_____				Age_____				Age_____				Age_____			
			Y	**POSS**	**N**	**DK**	**Y**	**POSS**	**N**	**DK**	**Y**	**POSS**	**N**	**DK**	**Y**	**POSS**	**N**	**DK**
1.	Did anyone ever tell you that he/she had a seizure or convulsion caused by a high fever when he/she was a child? **[IF "NO" or "DON'T KNOW" GO TO 2.]**		1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	A.	How many seizures did he/she have because of high fever?																
	B.	How old was he/she when that happened [the FIRST time]? **[IF ONLY HAD ONE GO TO 2.]**																
	C.	How old was he/she when that happened [the LAST time]?																
2.	[Other than the seizure/s [he/she] had because of a high fever] Has [he/she] ever had, or has anyone ever told you that [he/she] had a seizure disorder or epilepsy?		1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	A.	How old was he/she the first time he/she had a seizure?																
	B.	What type of seizure/s did he/she have?																
	C.	How many seizures did he/she have?																
	D.	Is he/she taking antiepileptic medications?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99

		[IF "NO" or "DON'T KNOW" GO TO NEXT SECTION]																		
		D.1.	Do you know which one/s?																	
[Ask the following questions only if subject said "No" to epilepsy or a seizure disorder in Q2]																				
3.	[Other than the seizure/s he/she had because of a high fever] Has he/she ever had, or has anyone ever told you that he/she had, any of the following																			
	A.	A seizure, convulsion, fit, or spell under any circumstances?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	B.	Uncontrolled movements of part or all of the body such as twitching, jerking, shaking, or going limp?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	C.	An unexplained change in mental state or level of awareness, or an episode of "spacing out"?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	D.	Did anyone ever tell you that when he/she was a small child, he/she would daydream or stare into space more than other children?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	E.	Has he/she ever noticed any unusual body movements or feelings when exposed to strobe lights, video games, flickering lights, or sun?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	F.	Shortly after waking up, either in the morning or after a nap, has he/she ever noticed uncontrollable jerking or clumsiness, such as dropping things or things suddenly "flying" from his/her hands?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99
	G.	Has he/she ever had any other type of repeated unusual spells?	1	2	0	99	1	2	0	99	1	2	0	99	1	2	0	99

FAMILY TREE

Checklist:

☐	Consanguinity	☐	Autism or behavioral problems	☐	Migraine	☐	Déjà vu
☐	Stillbirths/miscarriages	☐	ID or learning disabilities	☐	Anxiety or Depression	☐	Blank spells
☐	Febrile convulsions	☐	Fainting	☐	Diabetes	☐	Jerks
☐	Family Hx of seizures	☐	Breathholding	☐	Unusual events	☐	Other

OTHER MEDICAL HISTORY

1. Does the subject have any other conditions?

Yes 1
No……..**[GO TO 2.]** 0
Don't know……**[GO TO 2.]** 99

A. If yes, which of the following?

Other neurological disorder 1
Psychiatric disorder 2
Neurosurgery 3
Medical disease 4
Other 77
Unknown 99

[SPECIFY]: _____

_____

_____

_____

2. Is the subject taking **non-AED** medications?

Yes 1
No 0
Don't know 99

[LIST ALL CURRENT NON-AED MEDICATION]

DRUG	DAILY DOSE (mg)	START DATE (DD/MM/YYYY; if known)

[IF DRUGS SUGGEST OTHER CONDITIONS WHICH HAVE NOT BEEN EXPLORED, THEN ASK NOW]:

_____

_____

_____

_____

_____

_____

TREATMENT

1. Antiepileptic drugs

1.1. Is the subject taking any AEDs at the moment?

Yes.....1
No……..**[GO TO 1.2.]**.....0
Don't know……..**[GO TO 1.2.]**.....99

[SPECIFY CURRENT AED TREATMENT]

AED (Brand name)		**YES**	**NO**	**DON'T KNOW**	**Daily dose (mg)**	**Start date [DD/MM/YYYY]** (Specify **YEAR** at least)	**Latest serum levels** [Specify **DATE** and if **mg/L or mmol/L**]
A.	**Acetazolamide** (Diamox)	1	0	99			
B.	**Carbamazepine** (Tegretol)	1	0	99			
C.	**Clobazam** (Frisium)	1	0	99			
D.	**Clonazepam** (Rivotril)	1	0	99			
E.	**Diazepam** (Valium)	1	0	99			
F.	**Ethosuximide** (Zarontin)	1	0	99			
G	**Felbamate** (Felbatol)	1	0	99			
H.	**Gabapentin** (Neurontin)	1	0	99			
I.	**Lacosamide** (Vimpat)	1	0	99			
J.	**Lamotrigine** (Lamictal)	1	0	99			
K.	**Levetiracetam** (Keppra)	1	0	99			
L.	**Nitazepam** (Mogadon)	1	0	99			
M.	**Oxcarbazepine** (Trileptal)	1	0	99			
N.	**Phenobarbital** (Phenobarb)	1	0	99			
O.	**Phenytoin** (Dilantin)	1	0	99			
P.	**Pregabalin** (Lyrica)	1	0	99			
Q.	**Primidone** (Mysoline)	1	0	99			
R.	**Sulthiame** (Ospolot)	1	0	99			
S.	**Tiagabine** (Gabitril)	1	0	99			
T.	**Topiramate** (Topamax)	1	0	99			
U.	**Valproate** (Epilim)	1	0	99			
V.	**Vigabatrin** (Sabril)	1	0	99			
W.	**Zonisamide** (Zonegran)	1	0	99			
X.	**Other:** _____	1	0	99			
Y.	**Other:** _____	1	0	99			

1.2. Has the subject ever received any other AEDs? If currently off-treatment, has the subject ever received AEDs in the past?

Yes.....1
No……..**[GO TO 2.]**.....0
Don't know……..**[GO TO 2.]**.....99

[SPECIFY PREVIOUS AED TREATMENT]

AED (Brand name)		YES	NO	DON'T KNOW	Reasons for stopping AED					Start date [DD/MM/YYYY] (specify **YEAR** at least)	Stop date [DD/MM/YYYY] (specify **YEAR** at least))	Maximum daily dose (mg) (if known)
					Seizure-freedom	Lack of efficacy	Side effects	Unknown	Other			
A.	**Acetazolamide** (Diamox)	1	0	99	1	2	3	4	5			
B.	**Carbamazepine** (Tegretol)	1	0	99	1	2	3	4	5			
C.	**Clobazam** (Frisium)	1	0	99	1	2	3	4	5			
D.	**Clonazepam** (Rivotril)	1	0	99	1	2	3	4	5			
E.	**Diazepam** (Valium)	1	0	99	1	2	3	4	5			
F.	**Ethosuximide** (Zarontin)	1	0	99	1	2	3	4	5			
G	**Felbamate** (Felbatol)	1	0	99	1	2	3	4	5			
H.	**Gabapentin** (Neurontin)	1	0	99	1	2	3	4	5			
I.	**Lacosamide** (Vimpat)	1	0	99	1	2	3	4	5			
J.	**Lamotrigine** (Lamictal)	1	0	99	1	2	3	4	5			
K.	**Levetiracetam** (Keppra)	1	0	99	1	2	3	4	5			
L.	**Nitazepam** (Mogadon)	1	0	99	1	2	3	4	5			
M.	**Oxcarbazepine** (Trileptal)	1	0	99	1	2	3	4	5			
N.	**Phenobarbital** (Phenobarb)	1	0	99	1	2	3	4	5			
O.	**Phenytoin** (Dilantin)	1	0	99	1	2	3	4	5			
P.	**Pregabalin** (Lyrica)	1	0	99	1	2	3	4	5			
Q.	**Primidone** (Mysoline)	1	0	99	1	2	3	4	5			
R.	**Sulthiame** (Ospolot)	1	0	99	1	2	3	4	5			
S.	**Tiagabine** (Gabitril)	1	0	99	1	2	3	4	5			
T.	**Topiramate** (Topamax)	1	0	99	1	2	3	4	5			
U.	**Valproate** (Epilim)	1	0	99	1	2	3	4	5			

V.	**Vigabatrin** (Sabril)	1	0	99	1	2	3	4	5			
W.	**Zonisamide** (Zonegran)	1	0	99	1	2	3	4	5			
Z.	**Other:** _____	1	0	99	1	2	3	4	5			

[FOR EACH AED DISCONTINUED DUE TO "SIDE EFFECTS", SPECIFY THE SIDE EFFECT/S" (e.g. carbamazepine: dizziness, ataxia)]

_____

_____

_____

_____

_____

1.3. Is it known when the first AED was started?
Yes [**DATE**, DD/MM/YYYY:_____].....1
No.....0

1.4. How can the subject be classified according to ILAE criteria for drug responsiveness of epilepsy?
Drug responsive.....1
Drug resistant.....2
Undefined.....3
Don't know.....99

ILAE classification of drug responsiveness of epilepsy (Kwan P et al., Epilepsia 2010;51:1069-77)

Drug responsive: seizure freedom for a minimum of three times the longest pretreatment interseizure interval, or 12 months, whichever is longer.
Drug resistant: failure of *adequate* trials of two tolerated and *appropriately* chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom.
Undefined: epilepsy does not fulfill the definition criteria for either drug resistant or drug responsive epilepsy

2. Non-AED therapies

2.1. Has the subject received treatments for his/her epilepsy other than AEDs?
Yes.....1
No……..**[STOP HERE]**.....0
Don't know……..**[STOP HERE]**.....99

2.2. Has the subject undergone epilepsy surgery?
Yes.....1
No……..**[GO TO 2.4]**.....0
Don't know……..**[GO TO 2.3]**.....99

[PROVIDE DETAILS OF THE SURGICAL PROCEDURE/S]

Surgical procedure(s)	**Date [DD/MM/YYYY]** (Specify **YEAR** at least)	**ILAE classification of postsurgical outcome**							**Surgical complications**
		1	**2**	**3**	**4**	**5**	**6**	**Unknown**	
		1	2	3	4	5	6	99	
		1	2	3	4	5	6	99	
		1	2	3	4	5	6	99	

ILAE classification of postsurgical outcome (Wieser HG et al. Epilepsia 2001;42:282–286)

Class 1: Completely seizure free; no auras
Class 2: Only auras; no other seizures
Class 3: One to three seizure days per year; ± auras
Class 4: Four seizure days per year to 50% reduction of baseline seizure days; ± auras
Class 5: Less than 50% reduction of baseline seizure days to 100% increase of baseline seizure days; ± auras
Class 6: More than 100% increase of baseline seizure days; ± auras

2.3. Has the subject ever been on the ketogenic diet?

Yes.....1
No…….**[GO TO 2.4]**.....0
Don't know……..**[GO TO 2.4]**.....99

A. Specify the start and end dates for the diet: **[SPECIFY IF "ONGOING"]:**

_____

_____

B. What has been the effect of the diet?

Seizure-free.....1
At least 50% seizure reduction, but not seizure-free 2
No substantial effect 3
Increased number of seizures.....4
Unknown 99

C. Side effects associated with the diet:

_____

_____

2.4. Has the subject been implanted with the vagal nerve stimulator (VNS)?

Yes.....1
No…….**[GO TO 2.5]**.....0
Don't know……..**[GO TO 2.5]**.....99

A. Specify the start and end dates of VNS therapy: **[SPECIFY IF "ONGOING"]:**

_____

_____

B. What has been the effect of the VNS?

Seizure-free.....1
At least 50% seizure reduction, but not seizure-free 2
No substantial effect 3
Increased number of seizures.....4
Unknown 99

C. Side effects associated with the VNS:

_____

_____

2.5. Other treatments **[SPECIFY DATES, EFFICACY AND TOLERABILITY]:**

_____

_____

_____

_____

_____

I reviewed the pages of this study form and confirm that all the data collected are complete and accurate.

Name (Print): _____

Signature: _____ **Date:** ___ ___/ ___ ___/ ___ ___ ___ ___
D D M M Y Y Y Y