The potential impact of genotype-driven precision medicine for children with epilepsy

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Joseph Daniel Symonds

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Contents

A. Abstract

Introduction:

The development and application of next generation sequencing (NGS) technology has led to an exponential rise in the number of genes and genetic variants associated with epilepsy. The detection of highly penetrant and damaging variants in some patients can be sufficient to provide an adequate explanation for the entire disease process. Particularly high yields from such diagnostic genetic testing are observed in cohorts of children who present with early onset seizures. Obtaining a genetic diagnosis can be helpful to families in terms of informing further reproductive decisions, providing answers, and preventing further costly investigations. Evidence is emerging that certain anti-epileptic therapies may be more effective than others in specific genetic epilepsies.

Aim:

The aim of this thesis is to explore the potential for genetically-guided therapy for children with epilepsy. This will be primarily achieved through describing the epidemiology of the genetic epilepsies of childhood, and through researching the evidence-base for gene-specific therapy.

Methods:

This is a mixed methods study. In chapter 5 The epidemiology of early childhood genetic epilepsy is described using a prospective whole Scotland population based national cohort. This includes all children presenting under three years of age presenting with new onset epilepsy over a defined time period (May 2014 to May 2017, n =315). These children were tested on a panel of 104 epilepsy-associated genes. In chapter 6 the potential for Whole Genome Sequencing (WGS) to identify further genetic diagnoses in deeply-phenotyped families is then explored in a separate cohort of children presenting in the West of Scotland with severe or drug-resistant epilepsy (n = 79). In chapter 4, a systematic review approach is used to

identify any epilepsy-associated genes for which evidence exists to support a specific therapeutic approach. The results from this review considered in both cohorts. Chapter 7 describes a new genetic epilepsy due to *SMC1A* truncation and explores the potential for specific therapy in this condition. Chapter 8 evaluates whether sub-analysis of genetic data within a randomised controlled trial can be harnessed to identify patients most likely to respond to therapy.

Key results:

Epilepsy affects 1 per 383 children before their third birthday. In 22% of these children a single-gene cause can be identified. For 80% per cent of single-gene diagnoses in this group of patients there is some evidence to support a specific therapeutic approach. Evidence is variable in quality and nature. Between 1 in 2,000 and 1 in 2,300 of all children born are likely to have a genetically determined epilepsy for which there is currently available some evidence for a specific treatment choice. The majority of currently achievable genetic diagnoses are concentrated in a small number of genes, with genetic diagnoses beyond the 20 most common being extremely individually rare. Evidence to support specific therapeutic approaches is generally lacking in these rarer genetic epilepsies, particularly in those not associated with ion channel dysfunction. A stronger evidence base is required, and to generate this this will demand wide collaboration, and rigorous study design, and open access to pharmacogenomic data.

B. Acknowledgements

Supervisors

I am grateful to my supervisors Professor Sameer Zuberi and Professor Faisal Ahmed for supporting me throughout this project. Professor Zuberi was the inspiration behind many of the studies included in this thesis. Through his tireless grant application writing and academic networking he obtained the necessary funding to make both the Genetic and Autoimmune Childhood Epilepsy (GACE study) and Whole Genome Sequencing (WGS) study happen. He has kept me motivated and inspired throughout my studies and clinical training, and I have no doubt will continue to do so long into the future.

Collaborators in the Genetic and Autoimmune Childhood Epilepsy (GACE study):

The following individuals were all key collaborators in the GACE study. They recruited participants, obtained informed consent, obtained DNA samples, and submitted clinical details for the project.

Name	Title	Affiliation
Dr. Sarah Abernethy	Consultant Paediatric Neurologist	Royal Hospital for Children, Glasgow
Dr. Ishaq Abu-Arafeh	Consultant Paediatrician	Forth Valley Royal Hospital, Larbert
Dr. Jamie Andrew	Consultant Paediatrician	University Hospital Wishaw
Dr. Philip Brink	Consultant Paediatric Neurologist	Tayside Children's Hospital, Dundee
Dr. Andreas Brunklaus	Consultant Paediatric Neurologist	Royal Hospital for Children, Glasgow
Dr. Mary Callaghan	Consultant Paediatrician	University Hospital Wishaw
Dr. Jamie Cruden	Consultant Paediatrician	Victoria Hospital, Kirkcaldy
Dr. Christine Findlay	Consultant Paediatrician	University Hospital Crosshouse, Kilmarnock
Dr. Rosemary Grattan	Consultant Paediatrician	Forth Valley Royal Hospital, Larbert
Dr. Iain Horrocks	Consultant Paediatric Neurologist	Royal Hospital for Children, Glasgow
Dr. Alice Jollands	Consultant Paediatric Neurologist	Tayside Children's Hospital, Dundee
Dr. Martin Kirkpatrick	Consultant Paediatric Neurologist	Tayside Children's Hospital, Dundee
Dr. Jane MacDonnell	Consultant Paediatrician	Borders General Hospital, Melrose
Dr. Stewart MacLeod	Consultant Paediatric Neurologist	Royal Hospital for Children, Glasgow
Dr. Jean McKnight	Consultant Paediatrician	Dumfries and Galloway Royal Infirmary
Dr. Ailsa McLellan	Consultant Paediatric Neurologist	Royal Hospital for Sick Children, Edinburgh
Dr. Calum Morrison	Consultant Paediatrician	University Hospital Crosshouse, Kilmarnock
Dr. Lelsey Nairn	Consultant Paediatrician	Royal Alexandra Hospital, Paisley
Dr. Mary O'Regan	Consultant Paediatric Neurologist	Royal Hospital for Children, Glasgow
Dr. Jay Shetty	Consultant Paediatric Neurologist	Royal Hospital for Sick Children, Edinburgh
Dr. Elma Stephen	Consultant Paediatrician	Royal Aberdeen Children's Hospital
Dr. Kamath Tallur	Consultant Paediatric Neurologist	Royal Hospital for Sick Children, Edinburgh
Dr. Alan Webb	Consultant Paediatrician	Raigmore Hospital, Inverness
Sister Margaret Wilson	Epilepsy Specialist Nurse	Royal Hospital for Children, Glasgow

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Name	Title	Affiliation
Lynne Adam	Epilepsy Specialist Nurse	University Hospital Wishaw
Gail Alexander	Epilepsy Specialist Nurse	Royal Alexandra Hospital, Paisley
Celia Brand	Epilepsy Specialist Nurse	Royal Hospital for Sick Children, Edinburgh
Jo Campbell	Epilepsy Specialist Nurse	Royal Aberdeen Children's Hospital
Diane Carroll	Epilepsy Specialist Nurse	Royal Hospital for Children, Glasgow
Rhona Dick	Epilepsy Specialist Nurse	University Hospital Wishaw
Caroline Gibson	Epilepsy Specialist Nurse	Forth Valley Royal Hospital, Larbert
Misty MacDonald	Epilepsy Specialist Nurse	Royal Hospital for Children, Glasgow
Kirsteen MacIntosh	Epilepsy Specialist Nurse	Raigmore Hospital, Inverness
Kelly McBeath	Epilepsy Specialist Nurse	Raigmore Hospital, Inverness
Dr. Linda McLennan	Consultant Paediatrician	Raigmore Hospital, Inverness
Laura Mortimer	Epilepsy Specialist Nurse	Victoria Hospital, Kirkcaldy
Joanne Pascual	Epilepsy Specialist Nurse	University Hospital Crosshouse, Kilmarnock
Dr. Elizabeth Pilley	Paediatric Neurology Registrar	Royal Hospital for Sick Children, Edinburgh

The following people, in the genetics department in Glasgow, were involved in the design and implementation of the Glasgow 104 gene epilepsy panel, and in the analysis and interpretation of genetic variants identified in the GACE study:

Name	Title	Affiliation
Rachael Birch	Clinical Scientist	West of Scotland Regional Genetics Department
Dr. Louise Diver	Clinical Scientist	West of Scotland Regional Genetics Department
Sarah Gardiner	Clinical Scientist	West of Scotland Regional Genetics Department
Dr. Shelagh Joss	Consultant Clinical Geneticist	West of Scotland Regional Genetics Department
Prof. Daniela Pilz	Consultant Clinical Geneticist	West of Scotland Regional Genetics Department
Eleanor Reavey	Clinical Scientist	West of Scotland Regional Genetics Department
Meghan Slean	Biomedical sciences student	University of Glasgow
Kirsty Stewart	Clinical Scientist	West of Scotland Regional Genetics Department

The following people helped to facilitate review of EEG records in order to establish Cohort 2:

Name	Title	Affiliation
Gillian Horsburgh	Neurophysiologist	Royal Hospital for Children, Glasgow
Holly MacKenzie	Neurophysiologist	University Hospital Crosshouse, Kilmarnock
Suzanne Moir	Neurophysiologist	Forth Valley Royal Hospital, Larbert
Angela Robertson	Neurophysiologist	Royal Hospital for Children, Glasgow
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Analysis and interpretation of variants identified in the WGS project was done in collaboration with Dr. Katherine Elliott, and under the supervision of Dr. Julian Knight at the Wellcome Trust Centre for Human Genetics (WTCHG) at the University of Oxford. I am grateful to both for their wisdom, enthusiasm for the project, and exceptional hospitality on the many occasions in which I visited Oxford during this PhD. Administrative aspects of sample management, and Sanger sequencing confirmation of selected variants was done by Lawrence Petherbridge in Oxford.

Collaborators in the *SMC1A* gene study

I am grateful to Professor David Fitzpatrick at the University of Edinburgh for first alerting me to the interesting phenotype of patients with *SMC1A* truncations, and to the Deciphering Developmental Disorders (DDD) study in Cambridge for facilitating further investigation of the phenotype.

The following people submitted clinical and genetic details to the *SMC1A* project:

Name	Title	Affiliation
Dr. Jamie Cruden	Consultant Paediatrician	Victoria Hospital, Kirkcaldy
Dr. Anita Devlin	Consultant Paediatric Neurologist	Great North Children's Hospital, Newcastle
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Participants

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Friends and Family

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C. List of tables and figures

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D. Author's declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University of Glasgow's Regulations and Code of Practice for Research Degree Programmes and that is has not been submitted for any other academic reward. I am the sole author of this thesis and was responsible for leading all aspects of this research, under guidance from my supervisors. A number of colleagues collaborated at various stages and these have been formally acknowledged above.

E. Publications and presentations

Publications arising from the primary work of this project

Symonds JD, Zuberi SM, Stewart K, McLellan A, O'Regan M, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. *Brain* 2019; 142:2303-2318

Symonds JD and Zuberi SM. Monogenetics, polygene disorders and the quest for modifying genes. *Neuropharmacology*. 2018; 132:3-19.

Symonds JD, Zuberi M and Johnson MR. Advances in epilepsy gene discovery and implications for epilepsy diagnosis and treatment. *Current Opinion in Neurology*. 2017; 30:193-199

Symonds JD, Joss S, Metcalfe KA, Somarathi S, Cruden J, et al. Heterozygous truncation mutations of the *SMC1A* gene cause a severe early-onset epilepsy with cluster seizures in females: Detailed phenotyping of 10 new cases. *Epilepsia* 2017; 25:565-575

Publications in which participants in this project have been included

Ng BG, Sosicka P, Agadi S, Almannai M, Bacino CA, et al. SLC35A2-CDG: Functional characterisation, expanded molecular, clinical, and biochemical phenotypes of 30 unreported individuals. *Human Mutation* 2019; 40:908-925

Snijders Blok L, Hiatt SM, Bowling KM, Prokop KW, Engel KL, et al. De novo mutations in MED13, a component of the Mediator complex are associated with a novel neurodevelopmental disorder. *Human Genetics* 2018; 137:375-388

Selected presentations arising from this project

Symonds JD, Somarathi S, Devlin A, Donaldson A, Lampe A, et al. Heterozygous truncation mutations of the SMC1A gene cause a severe early-onset epilepsy with cluster seizures in females. *International Child Neurology Congress*. Amsterdam; May 2016 [oral presentation].

Symonds JD, Vincent A, Williams N, Dorris L, Lang B, et al. The Genetic and Autoimmune Childhood Epilepsy (GACE) study. *European Congress on Epileptology*. Prague; September 2016 [oral presentation].

Symonds JD, Lang B, Vincent A, Dorris L, Ellis R, et al. A prospective whole Scottish population study of genetic and immune causes of epilepsy and complex febrile seizures in children under 3 years of age: the Genetic and Autoimmune Childhood Epilepsy (GACE) Study. *British Paediatric Neurology Association Annual Conference*. Cambridge, UK; January 2017 [oral presentation]

Symonds JD, Lang B, Vincent A, Brunklaus, Dorris L, et al. Genetic and immune findings in complex febrile seizures and the epidemiology of Dravet syndrome: a nationwide cohort study. *European Paediatric Neurology Society Congress*; Lyon, France; June 2017 [oral presentation].

Symonds JD, Vincent A, Lang B, Dorris L, Brunklaus, et al. Scottish whole population based prospective genetic and autoimmune testing in new onset epilepsy and complex febrile seizures in children <3 years: diagnostic and clinical utility; *American Epilepsy Society Annual Meeting*; Washington DC, USA; December 2017 [oral presentation]. *Awarded the Grass Young Investigator award.*

Symonds JD, Elliott K, Brunklaus A, Joss S, MacLeod S, et al. Genetic diagnosis of epilepsy. Do we need genomes? *British Paediatric Neurology Association Annual Conference*. London, UK [oral presentation].

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Symonds JD, Elliott K, Knight J, Brunklaus A, Joss S, et al. Aetiological investigations and treatment outcomes in a prospective population-based cohort of children with epilepsy < 3 years. *British Paediatric Neurology Association Annual Conference*. Liverpool, UK; January 2019 [oral presentation].

F. List of Abbreviations

ADHD	Attention Deficit Hyperactivity Disorder
ADNFLE	Autosomal dominant nocturnal frontal lobe epilepsy
AED	Anti-epileptic drug
ASD	Autism Spectrum Disorder
CBD	Cannabidiol
CNS	Central nervous system
EIMFS	Epilepsy of infancy with migrating focal seizures
ExAC	Exome aggregation consortium
GABA	Gamma-amino-butyric acid
GACE	Genetic and autoimmune childhood epilepsy (study)
GEFS+	Genetic epilepsy with febrile seizures plus
GnomAD	Genome aggregation database
GTCS	Generalised tonic-clonic seizure(s)
GTEx	Genotype tissue expression
GWAS	Genome wide association study
ILAE	International League Against Epilepsy
MLPA	Multiplex Ligation Probe Amplification
NGS	Next generation sequencing
pLI	Probability of loss of function intolerance
QoL	Quality of life
SUDEP	Sudden Unexpected Death in Epilepsy
WES	Whole Exome Sequencing
WGS	Whole genome sequencing

1. Introduction

The objective of this thesis is to explore the potential for treating epilepsies based on information about precise genetic cause, focussing primarily on childhood-onset epilepsies. The term *precision medicine* has been chosen, though the terms *stratified medicine,* and *personalised medicine* have been used to describe similar concepts (Smith R, 2012). The term stratified medicine is typically used to describe the identification of subgroups of patients who are likely to benefit, or suffer an adverse outcome, from a particular intervention. An example of this is the now routine testing of the TPMT enzyme prior to starting treatment with the immunosuppressant medication Azathioprine. TPMT testing allows a small subgroup of patients (up to 1 in 170) who are deficient in the enzyme, which is critical to the metabolism of the drug to be identified since these patients are at increased risk of bone marrow toxicity (Drug and Therapeutics Bulletin 2009). Stratification does not always have to be on the basis of a blood test. Phenotypic features, for example as seizure type in epilepsy, may be effective methods of stratification (Marson et al., 2007a, Marson et al., 2007b). The concept of personalised medicine can be conceived as the ultimate extension of stratified medicine. Here we have moved beyond subgroups to and individual level, taking into account multiple variables – perhaps some genetic, some phenotypic, some environmental – when deciding on the optimum treatment approach. The difficulty with the concept of personalised medicine is that clinicians practice this type of medicine every day, albeit without an evidence base. True personalised medicine may not in fact be consistent with evidence-based medicine, since no individual patient has presented in the same set of circumstances ever before.

The term precision medicine is likely to mean different things to different people, but certainly overlaps with the concepts of stratified and personalised medicine. To many in the epilepsy community precision medicine implies a treatment targeted at the underlying disease mechanism (Kearney et al., 2019). The mainstay of epilepsy treatment is anti-epileptic medications, which are used in an attempt

to stop and/or prevent seizures. However, as we begin to better understand the biology of these conditions, seizures are likely to be considered an end point of a disease process rather than the disease in itself. A precision therapy approach may be one that targets underlying biological mechanisms, the clearest example of which would be gene-corrective therapy. Nonetheless at times certain established anti-epileptic treatments do get close to addressing the primary biological mechanisms of the disease: examples include the use of the Ketogenic Diet in Glut1-deficiecy syndrome, and sodium channel modifying anti-epileptic medications in genetic sodium channel epilepsies. König et al. argue that precision medicine "should not be viewed as an end point of a novel stratification process with clinical utility, but rather as a highly sophisticated and more complex process" (König et al., 2017). This concept of precision medicine is well suited to this thesis, in which multiple channels of investigation are applied to explore whether having a better genetic understanding of the epilepsies of early childhood, at varying levels of precision, has the potential to deliver better treatment.

This exploration takes place on backdrop of major recent advances in the understanding of the genetic causes of epilepsies. These advances have taken place largely thanks to the application of Next Generation Sequencing (NGS) technology. The dissertation involves two literature review studies and four primary investigations. Evidence from these studies has been used to understand in what proportion of children presenting with epilepsy a precise genetic diagnosis can be made, and then, of those with a precise genetic diagnosis, what proportion may be amenable to a precision-therapy approach. In the process of this exploration a number of key conceptual issues must be considered. These include what the key therapeutic goals in treating epilepsy are, including addressing co-morbidity; how we think about classifying epilepsies with aetiology in mind; and what is meant by drug-resistance. Since these themes keep recurring, and since they frame much of the ensuing discussion, the introduction to this thesis will provide a general background to them, starting with a review of the epidemiology of childhood-onset epilepsy.

1.1 Epilepsy Epidemiology

1.1.1 Incidence and prevalence of epilepsy

Epilepsy has been defined as a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005). Conceptually this definition positions epilepsy somewhere in a hinterland between a symptom and a disease. As will be discussed in this thesis, our increasing ability to resolve the causes of epilepsy challenges this definition. Nonetheless it is a definition that has proved to be operationally practical. In the majority of epidemiological studies any individual who has had more than one unprovoked epileptic seizure has been classified as having epilepsy.

A meta-analysis of international epidemiological studies has estimated the point prevalence of epilepsy to be 6.38 per 1,000 persons (Fiest et al., 2017). The world population is currently 7.6 billion, so it can be expected that there are approximately 48 million people globally with epilepsy.

The incidence of epilepsy is age-dependent, with the highest incidences found in the young and the elderly. Age specific-incidence is most accurately represented in the data from the long-term population-based cohort of Rochester Minnesota (Hauser, Annegers & Kurland, 1993), though this reflects the epidemiology only in an advanced post-industrial economy.

Figure 1.1a is based on the data from the Rochester cohort, demonstrating that only in age groups over 70 years does the incidence of epilepsy surpass the levels observed in the under five years age groups.

Epidemiological studies of epilepsy in childhood have been relatively few, and these are summarised in table 1.1a. A consistent finding is that the incidence of

epilepsy in the first year of life is higher than in later childhood. Comparison between studies is difficult since case definitions may vary slightly. All the studies in table 1.1a have excluded children with acute symptomatic seizures, febrile seizures, and single seizures. Only the North London study (Eltze et al., 2013) took a prospective approach to case identification so all the other studies are dependent on accurate case recording in registries and notes. In the studies from Helsinki and Nova Scotia cases were identified from single centres, with the assumption that patients with epilepsy would all be reviewed in these centres.

1.1.2 Global and economic burden of epilepsy

According to the World Health Organisation (WHO) Global Burden of Disease Study, Neurological disorders are ranked as the world's leading cause of disability-adjusted life years (DALYs) lost, comprising 10.2% of the total. Epilepsy is ranked as the fifth leading cause of neurological-related DALYs lost, behind stroke, migraine, dementias and meningitis (Feigin et al., 2015). Epilepsy-related morbidity has been calculated to cost 12,418,000 DALYs per year and cause 125,000 deaths (Feigin et al., 2015). Epilepsy was estimated to cost the European economy 15.5 Billion Euros in 2004, with direct costs relating to health and social care provision, and indirect costs relating to loss of productivity (Maura et al., 2007).

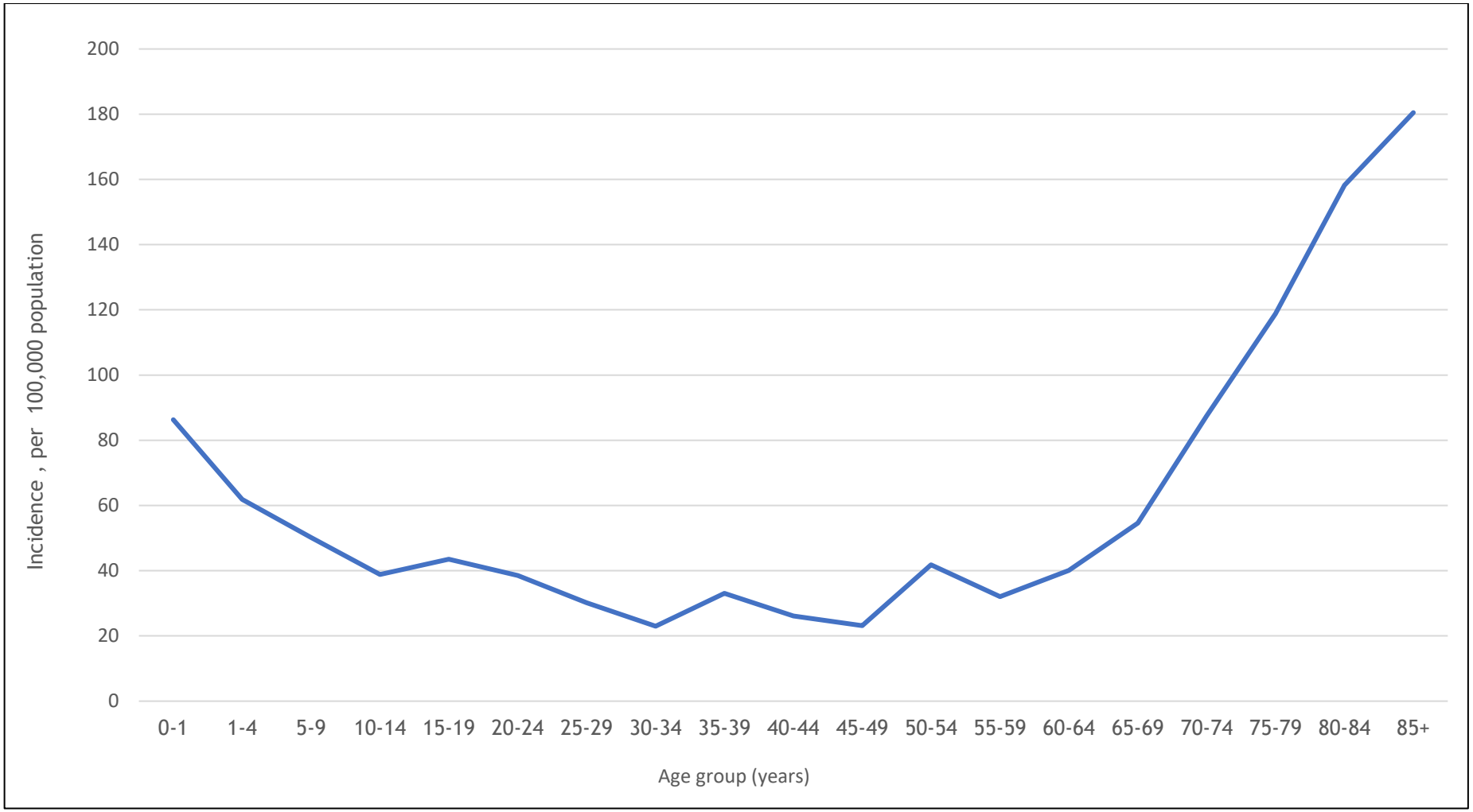


Figure 1.1a: Epilepsy age-specific incidence, adapted from Hauser et al. 1993

Table 1.1a: Epidemiological studies of epilepsy in childhood.

Region, Country	Age group	Methodology	Denominator population (children at risk)	Incident cases of epilepsy	Incidence	Reference
Rochester, MN, USA	< 1 year	1935-1984 whole population cohort (n = 1,102,882). Patients with epilepsy identified from review of medical records.	41,717	36	86 per 100,000 person years	(Hauser, Annegers & Kurland, 1993)
Rochester, MN, USA	< 5 years		193,645	130	67 per 100,000 person years	(Hauser, Annegers & Kurland, 1993)
Rochester, USA	< 10 years		173,464	217	59 per 100,000 person years	(Hauser, Annegers & Kurland, 1993)
Norway	< 1 year	1999-2009 birth cohort. Patients with epilepsy identified from data linkage to the Norwegian Patient Registry	Data not given	Data not given	144 per 100,000 person years	(Aaberg et al., 2017)
Norway	1-10 years		Data not given	Data not given	58 per 100,000 person years	(Aaberg et al., 2017)
Nova Scotia, Canada	< 13 months	Children < 16 years presenting with a second unprovoked seizure between 1977 and 1985. Identified through EEG referrals	94,800	112	118 per 100,000 person years	(Camfield et al., 1996)
Nova Scotia, Canada	< 16 years		1,676,000	693	41 per 100,000 person years	(Camfield et al., 1996)
Helsinki, Sweden	< 1 year	1997-2006 birth cohort. Children with epilepsy identified from a single hospital centre.	127,730	158	124 per 100,000 person years	(Gaily et al., 2016)
North London, UK	< 2 years	Prospective recruitment of new incident cases of epilepsy < 24 months in North London.	98,090	57	54 per 100,000 person years	(Eltze et al., 2013)

1.2 Comorbidity in Epilepsy

1.2.1 Overall comorbidity

Patients with epilepsy experience a significantly higher burden of additional physical and mental health problems than age-matched controls (Weatherburn et al., 2017). Absolute prevalence rates of comorbidity vary markedly between studies, depending on the population included and the diagnostic criteria applied (Athanasios, Sisodiya & Sander, 2012; Gaitatzis, Trimble & Sander, 2004). In a survey of 713 adults with active epilepsy in Northern Sweden, 28.1% had a comorbid cognitive disorder, 14.4% had a motor disorder, and 5.9% had a psychiatric disorder (Lars, 2005). Children with epilepsy are more than three times as likely to be diagnosed with a psychiatric disorder than children with other chronic health conditions such as diabetes (Davies, Hayman & Goodman, 2007).

1.2.2 Comorbidity in childhood-onset epilepsy

The most commonly encountered comorbidities that are seen in association with childhood-onset epilepsy are neurodevelopmental and psychiatric. Overall 30% of children with epilepsy onset before the age of 15 years have developmental delay (Baca et al., 2011). A systematic review and meta-analysis of 19 studies found that 6.3% of people with childhood-onset epilepsy have comorbid Autism Spectrum Disorder (ASD), compared with the general population prevalence of 0.75 to 1.1% (Strasser et al., 2017). Significantly higher rates are observed in those with focal seizures (19.9%), infantile spasms (41.9%), and Dravet Syndrome (47.4%) (Strasser et al., 2017). The prevalence of comorbid Attention Deficit Hyperactivity Disorder (ADHD) was found to be 19.9% in one study (Baca et al., 2011) and 23.7% in another (Kwong et al., 2016a), though estimates of the prevalence of ADHD in the childhood population range from 1% to 20% (Polanczyk et al., 2007). Anxiety and Depression were diagnosed in 32.8% and 22.1% respectively of adolescents with epilepsy in a cross-sectional study of 140 patients in Hong Kong, compared with equivalent rates of 20.4% and 14.3% in controls with asthma (Kwong et al., 2016b).

1.2.3 Comorbidity and age of onset

Additional morbidity in childhood-onset epilepsy appears to be more prevalent in those who receive an epilepsy diagnosis at a young age. Baca et al. followed up a community-based cohort of 613 children with a mean age of epilepsy onset of 4.4 years. Nine years after diagnosis 277 of this cohort underwent re-assessment, including medical chart review, parent interview, and completion of a self-administered generic Health-Related Quality of Life Questionnaire (HRQoL). In this group 39% had a Neurodevelopmental Spectrum Disorder (NDSD) such as developmental delay, dyslexia or autism; 25.6% had a psychiatric disorder, 14.8% had migraine, and 23.8% had another chronic medical condition. The presence of a psychiatric comorbidity was significantly associated with lower quality of life, using validated measurements (Baca et al., 2011).

Mortality is significantly higher in children who are diagnosed with epilepsy at a young age. Jennum et al. interrogated the Danish National Patient Registry (NPR) for patients diagnosed with epilepsy before the age of 20 years between 1998 and 2002. They identified 3123 children who were diagnosed before their sixth birthday, and 5018 who were diagnosed between the ages of six and 20 years. Follow-up was reported after 10 years, and outcomes were compared to 10,036 randomly-chosen controls without epilepsy who were matched for age and gender. In the younger-at diagnosis group the hazard ratio for mortality compared with controls was 14.46 (95% confidence intervals [CI] 11.8-17.7) and in the older-at-diagnosis group it was 5.58 (95% CI 4.9-6.4). These data indicate that being diagnosed with epilepsy before your sixth birthday is associated with a more than 10 fold increased risk of mortality over 10 years compared with the general population, and a more than two fold risk of mortality compared with those diagnosed with epilepsy between the ages of six and 20 years (Jennum et al., 2017).

1.2.4 Drug-resistant epilepsy and comorbidity

The data presented above suggest that children with early-onset epilepsy are a particularly vulnerable group. Within this group there are some who are more vulnerable than others.

A population-based cohort of children with early drug-resistant epilepsy were studied by Wirrell et al. (Wirrell et al., 2012). They reviewed the medical records of 127 children who had been diagnosed with epilepsy before their third birthday and had developed drug-resistance. 85% were followed-up for more than 24 months. Here drug-resistance was defined as either failure, in relation to seizure frequency (more than one seizure in the six months prior to most recent follow-up) of two or more antiepileptic drugs; or having undergone resective epilepsy surgery or callosotomy after failure of two or more antiepileptic drugs. By these criteria 44 (35%) children had drug-resistant epilepsy by the time of latest follow-up. Mortality in the drug-resistant group was 9/44 (20.5%) compared with 0/83 in the non-drug-resistant group ($P<0.001$). In the drug-resistant group 95% of children had developmental delay compared with 36% of the non-drug-resistant group ($p<0.001$). The strongest predictor of drug-resistance in this study was the presence of developmental delay at the time of epilepsy diagnosis (odds ratio = 37.1, 95% confidence intervals 8.38-164.21), suggesting that uncontrolled seizures, rather than being a cause of developmental delay, may be a proxy measure of an underlying disorder which predisposes to both drug-resistant seizures and developmental delay.

1.2.5 Mortality and Sudden Unexpected Death in Epilepsy

As detailed above, people with epilepsy have a higher mortality rate than the general population. There are positive associations between age of onset (Jennum et al., 2017) the development of drug-resistance, and mortality (Wirrell et al., 2012). Salinpåå and Shinnar conducted 40-year follow-up of a Finish cohort of 245 children diagnosed with epilepsy in 1964 (median age of onset was three years). 60

subjects died (24%) over the follow-up period and this mortality rate was estimated to be three times that of a control population. The most common cause of death was Sudden Unexplained Death in Epilepsy (SUDEP), which caused 18 deaths (Sillanpåå & Shinnar, 2010).

Sudden Unexpected Death in Epilepsy (SUDEP) is defined as a "sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death that occurs in benign circumstances in an individual with epilepsy, with or without evidence for a seizure, and excludes documented status epilepticus (Nashef et al., 2011)." The exact mechanisms involved in SUDEP are unclear, but are likely to be complex and multifactorial (Devinsky et al., 2016 Massey et al., 2014). Significant risk factors associated with SUDEP are male sex, high frequency of generalised tonic-clonic seizures (GTCS), young age of onset, and use of Anti Epileptic Drug (AED) polytherapy (Hesdorffer et al., 2011).

SUDEP may be influenced by genetics. In the severe childhood-onset epilepsy Dravet Syndrome, which is caused by mutations in the neuronal sodium channel gene *SCN1A*, mortality has been estimated at 15.84 per 1,000 person-years (Cooper et al., 2016) and SUDEP is thought to account for half of deaths (Cooper et al., 2016; Shmuely et al., 2016). *SCN1A* has been proposed as a candidate SUDEP gene (Bagnall et al., 2016). Other candidate SUDEP genes identified involve regulation of cardiac rhythm (Bagnall et al., 2016). Overall, any genetic contribution to SUDEP risk is likely to involve a complex polygenic picture. Leu et al. analysed Whole Exome Sequencing data from 18 individuals who had succumbed to SUDEP and compared this to 87 living people with epilepsy. They found that the SUDEP group had a significantly greater polygenic burden of rare genetic variations compared to the non-SUDEP group (Leu et al., 2015).

1.3 Epilepsy and Quality of Life

1.3.1 Goals of treatment

The general aims of treating any medical condition can be summarised as to either prolong life, to improve quality of life (QoL), or both. In epilepsy, direct mortality is rare. Epilepsy-related mortality remains poorly understood, and no significant modifiable risk factors have been identified (Massey et al., 2014) so the primary goal of treatment is essentially to improve QoL.

QoL is multi-dimensional, complex, and difficult to measure. In patients with frequent epileptic seizures the aim of therapeutic management is often to stop seizures, or at least reduce their frequency, with the expectation that a reduced seizure-burden will contribute to an enhanced QoL for the individual. For a patient who becomes seizure-free on treatment this difference can lead to substantial differences in lifestyle opportunities, such as the ability to drive. Absolute seizure-freedom in epilepsy consistently associates with improved QoL (Sillanpää & Shinnar, 2005; Jacoby et al., 2007). For patients with severe drug-resistant epilepsy, seizure-freedom is, by definition, not achieved. Despite this, therapeutic approaches largely focus on reducing seizure frequency, though a balance between therapeutic efficacy, and side effects of treatment must be struck.

Though QoL is what matters most for people with epilepsy, it is rarely used as a primary outcome measure in epilepsy therapeutic trials (Noble & Marson, 2016). QoL, as well as being difficult to measure objectively, it is dependent on multiple variables, and is highly vulnerable to placebo effect (Flik et al., 2017). All major therapeutic trials in epilepsy have used seizure frequency as a primary outcome measure. It is therefore important to understand how seizure frequency relates to QoL.

1.3.2 Seizure frequency and quality of life

Baker et al. obtained questionnaire-based QoL data from >5000 adults with epilepsy from 15 European countries. In this sample, at the time of questionnaire completion, 38% had had no seizures for 12 months, 24% were experiencing a maximum of one seizure per month, and 38% were experiencing more than one seizure per month. They identified a significant association between seizure frequency and every single aspect of QoL that they measured. These associations persisted even when those who were seizure-free were removed from the analysis (Baker, Gagnon & McNulty, 1998). This finding has been reproduced in subsequent studies (Baker, Gagnon & McNulty, 1998; Leidy et al., 1999; Bautista, Tannahill Glen, 2009). It is difficult to determine with certainty whether the observed association between seizure frequency and QoL is causal. On the other hand in a study of 87 adult patients with temporal lobe epilepsy, psychiatric comorbidity was shown to have a significantly stronger association with QoL than seizure frequency, though seizure frequency did continue to show an independent association (Johnson et al., 2004).

For younger patients the relative importance of seizure-related factors as an influence on QoL may be less. The QUALITÉ study group in Canada obtained questionnaire data from 480 children aged eight to 14 years with epilepsy. They found a strong relationship between mental health and QoL but not between seizure frequency and QoL (Fayed et al., 2015), a finding that was reproduced in Taylor et al.'s questionnaire study of 248 UK children (Taylor et al., 2011). Other studies have used qualitative methodology to explore younger patients' ideas about how their epilepsy relates to their QoL. These studies also find that seizure-related factors do not emerge as strong themes, and that participants place more emphasis on peer acceptance, and the extent of support from family and friends, as determinants of their QoL (Bishop & Allen, 2003, McEwan et al., 2004).

1.3.3 Parent/carer-reported QoL

Where the very young are concerned we depend on proxy assessments of QoL from parents or carers. Several validated measures of parent/carer reported QoL have been developed including generic questionnaires such as the PedsQL (Varni, Seid & Kurtin, 2001), and epilepsy-specific ones such as the QOLCE (Connolly et al., 2005). Interestingly when both children with epilepsy and their parents complete QoL questionnaires there is only a modest correlation between the two, with parents broadly underestimating QoL in comparison with their children (Taylor et al., 2011, Ronen. et al., 2003, Baca et al., 2010, Verhey et al., 2009). For parents and carers seizure-related factors appear to be more important in their assessment of QoL than they are for people with epilepsy themselves (Cianchetti et al., 2015, Conway et al., 2016, Lagae et al., 2017).

1.3.4 Caregiver burden

In any medical condition for which there is expected to be a significant caregiver burden, including most chronic neurological conditions and most paediatric conditions, it is important to consider the potential impact of any intervention on carer QoL. The caregiver burden of drug-resistant childhood epilepsy is substantial and has a significant impact on carer QoL. Again, non-seizure-related factors such as support systems, and cognitive and behavioural comorbidity appear to be stronger determinants of carer QoL than seizure-related factors (Williams et al., 2003, Lv et al., 2009).

1.3.5 Therapeutics beyond seizures

This thesis interrogates the role that genetics may contribute towards the development of personalised treatment approaches for people with epilepsy. A case that could be made for the promotion of personalised medicine is that because these approaches target the underlying cause of a disease they may have

broader therapeutic benefits than traditional approaches, which focus purely on seizure control.

A complete assessment of any potential benefits that disease-focussed therapeutics in epilepsy may have over symptom-focussed therapeutics must clearly look beyond narrow outcome measures such as seizure frequency. Broader outcome assessments must take into account the impact of any treatment on cognitive, behavioural, social, and QoL measures.

1.4 Drug resistance

1.4.1 Definition and epidemiology of drug-resistance in epilepsy

Kwan and Brodie followed up a single centre cohort of 525 patients aged nine to 93 years who were started on antiepileptic drug (AED) treatment following a new diagnosis of epilepsy. They defined seizure freedom as no seizures for at least 12 months and reported that at last follow-up 333 patients (63%) were seizure-free. They showed that 47% of patients became seizure-free on the first AED they were prescribed, and a further 13% became seizure-free on the second AED prescribed, either as replacement for or in addition to the first. Of all the patients who were prescribed a third AED only 4% became seizure-free. They therefore concluded that failure of two AEDs was predictive of lack of long-term remission, with a sensitivity of 96% (Kwan & Brodie, 2000).

Reflecting the findings from the Kwan and Brodie study, in 2010 the International League Against Epilepsy (ILAE) published a proposed definition of drug-resistant epilepsy. The proposed definition was as follows: *failure of adequate trials of two tolerated, appropriately chosen and used AED schedules (whether as monotherapy or in combination) to achieve sustained seizure-freedom* (Kwan et al., 2009). Precise definitions as to what constitutes an "adequate trial" and "sustained seizure-freedom" have not been refined, making direct comparisons between cohorts difficult. Nonetheless this is a definition that has been widely applied to both clinical and research settings.

1.4.2 Drug resistance in childhood-onset epilepsies

Ramos-Lizana et al. prospectively followed up a single centre cohort of 508 paediatric patients with new onset epilepsy, 459 whom received AED-treatment (Ramos-Lizana et al., 2012). The mean age at epilepsy diagnosis was 4.9 years and follow-up was for a minimum of 24 months. Seizure freedom was defined as having no seizures for either 12 months, or for at least three times the longest previous

seizure-free interval, whichever was longer. 87 patients satisfied the ILAE definition of drug-resistant epilepsy, which represented 17% of the total cohort and 19% of the AED-treated group. Ramos-Lizana et al. reported that 73% of AED-treated cases achieved seizure-freedom on either their first or second AED regimen. When compared to the equivalent figure of 65% in the Kwan and Brodie study this suggests that paediatric-onset epilepsies are more pharmacosensitive than adult-onset epilepsies. The authors also noted that an additional 7% of patients became seizure-free on a third or subsequent AED regimen. This proportion is also significantly greater than what was observed in the Kwan and Brodie study. In a retrospective study of 468 children aged 1-17 years, with median age at diagnosis of 5.4 years, Wirrell et al. found that of 25 patients who had failure of two AEDs eight (32%) achieved a favourable outcome, defined as seizure-free for a minimum of 12 months (Wirrell, Wong-Kisiel & Nickels, 2014).

A consistent finding between the Ramos-Lizana and Wirrell studies is that, in these paediatric populations, a failure of two AEDs appears to be less indicative of a long-term remission than in the largely adult population study by Kwan and Brodie. This may indicate that certain childhood-onset epilepsies are more likely to have a therapy-specific response. Alternatively it may reflect the ultimately self-limited nature of a proportion of childhood-onset epilepsies, including some that are initially resistant to treatment (Ramos-Lizana et al., 2012). However, inconsistency of follow-up may also explain these apparent differences between paediatric and adult groups. Short-term seizure-remission outcomes may not reflect long-term seizure freedom. Berg et al. found that of 128 patients with childhood-onset epilepsy who had failed two AEDs, 73 (57%) had a 12-month remission in seizures, but only 28 (22%) achieved three-year seizure freedom (Berg et al., 2009).

1.4.3 Factors associated with drug-resistant epilepsy in childhood

Looking specifically at a population who were under three years of age at diagnosis of epilepsy, Wirrell et al. found a significantly higher rate of drug-resistance than is reported in other studies. They retrospectively reviewed the cases of 127

children from the Rochester cohort who were consecutively diagnosed with epilepsy before the age of 36 months. They found that 44 (36%) became drug-resistant. Here drug resistance was defined as a seizure frequency of greater than one every six months at final follow-up and failure of two AEDs, or having undergone epilepsy surgery after failure to respond to two or more AEDs (Wirrell et al., 2012). In an Italian single-centre cohort of 266 children diagnosed with epilepsy before the age of 36 months, 173 (65%) satisfied the ILAE definition of drug-resistance (Vignoli et al., 2016).

Infantile onset of epilepsy is consistently associated with drug-resistance (Ramos-Lizana et al., 2012; Wirrell, Wong-Kisiel & Nickels, 2014; Vignoli et al., 2016). In the Wirrell et al. study of children <36 months, the odds ratio for drug-resistance for the group who were diagnosed at ≤12 months of age was 6.76 (95% confidence interval [CI] 2.00-22.84) (Wirrell et al., 2012). In the Ramos-Lizana study 94 children were diagnosed before 12 months of age, of whom 29 (30%) developed drug-resistance, compared to 59/414 (14%) of those diagnosed after 12 months of age (odds ratio 2.68, 95% CI 1.60-4.50) (Ramos-Lizana et al., 2012).

Other factors that were found to be significantly associated with drug-resistance in the Wirrell et al. study were the presence of developmental delay at onset, abnormal neurologic examination, abnormal neuroimaging, abnormal initial EEG, and the identification of an aetiology (all $P<0.005$) (Wirrell et al., 2012). Other studies also show that drug-resistance is more common in those children who have comorbid developmental delay, and in those for whom a genetic, metabolic, or structural cause for the seizures is identified (Ramos-Lizana et al., 2012, Vignoli et al., 2016).

1.4.4 Aetiology and drug-resistance

Children with early onset epilepsy and comorbid developmental delay are more likely to develop drug-resistance and they are also more likely to have an identifiable genetic, structural or metabolic cause for their seizures. However, an

aetiology can only be diagnosed insofar as it is looked for. The studies cited in this section have been limited in the extent to which they have investigated cases for genetic aetiology, so our understanding of the relationship between aetiology, seizure-outcome, and comorbidity is incomplete. By enabling aetiology to be identified in a greater proportion of patients, the emergence of high throughput genetic testing offers the opportunity to more fully understand these relationships.

1.5 The role of genetics in epilepsy

1.5.1 Introduction

Until recently the aetiology of most epilepsies was not well understood. However, over the past 20 years the application of genetic technologies has enabled researchers to gain enormous insights into the genetic basis of many of the epilepsies. Some individually rare epilepsies have revealed themselves as monogenic conditions. These monogenic epilepsies often present in early childhood, are frequently drug-resistant, and are associated with significant co-morbidities. Collectively these epilepsies have a significant impact on clinical resources. More common however are epilepsy syndromes which appear to have complex patterns of inheritance. Hundreds of genes have been implicated in the pathogenesis of the epilepsies, and every year more epilepsy-associated genes continue to be identified.

1.5.2 Heritability of epilepsy

Heritability refers to *the proportion of phenotypic variance that is attributable to genetic variance* (Manolio et al., 2009). Heritability had been understood as a concept long before the discovery of DNA, through the observation of *heredity*, which is the passing on of traits from one generation to the next. Heredity in epilepsy was noted by Hippocrates (460-370 BC) (Turnbull et al., 2005). In 1861 John Russell Reynolds recorded that one third of epilepsy patients had a positive family history (Reynolds, 1861). Epilepsy heritability was first demonstrated experimentally by William G Lennox, who between 1947 and 1960 published many studies of twins with epilepsy. These studies demonstrated a significantly higher concordance for epilepsy in monozygotic compared with dizygotic twins (Vadlamudi et al., 2004). The largest epilepsy twin study to date reported 47,626 twin pairs from USA, Norway, and Denmark in 2005. Concordance rates for epilepsy diagnosis were 28% in monozygotic twins compared with 7% in dizygotic twins (Kjeldsen et al., 2005).

1.5.3 Epilepsy as a feature of genetic syndromes

Historically, another pointer to the important role of genetics in epilepsy has been the prominence of epilepsy as a feature in a number of inherited or genetic syndromes. Tuberous Sclerosis, a condition in which 80-90% of patients develop epilepsy (Webb, Fryer & Osborne, 1991), was recognised to demonstrate an autosomal dominant pattern of inheritance in 1913 (Berg, 1913). The *TSC2* and *TSC1* genes were identified respectively in 1993 (European Chromosome 16 Tuberous Sclerosis Consortium, 1993) and 1997 (van Slegtenhorst et al., 1997).

Even before the discovery of the Tuberous Sclerosis genes, the molecular genetic mechanism for another developmental disorder became apparent when it was found by *fluorescence in situ hybridization* (FISH) that male patients with developmental delay and distinctive dysmorphism, had an unstable regions of DNA on the X chromosome. The term Fragile X syndrome was coined (Yu et al., 1991). The unstable region was subsequently found to be based on trinucleotide repeats in the promoter of the *FMR1* gene (Verkek, et al., 1991). 10-40% of patients with Fragile X syndrome are reported to have epilepsy (Berry-Kravis et al., 2010).

Epilepsy is a prominent feature of a number of other well-characterised genetic syndromes, and in many cases there is a clinically recognisable epilepsy phenotype. These include single gene disorders such as Rett syndrome (Dolce et al., 2013); a number of chromosomal microdeletion syndromes (Mullen et al., 2013, Olson et al., 2014); chromosomal aberrations such as ring chromosome 20 (Daber et al., 2012); and imprinting disorders, for example Angelman syndrome (Thibert et al., 2013). The high prevalence of epilepsy in a wide variety of genetic conditions highlights both the importance of genetic determinants in epilepsy and the complex nature of epilepsy heritability.

1.5.4 Epilepsies recognised as single gene disorders

The first epilepsy to be recognised as a single gene disorder was autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). This condition, with a relatively consistent phenotype and an autosomal dominant pattern of inheritance, was described in five unrelated families in 1995 (Scheffer et al., 1995). A variant in the *CHRNA4* gene which segregated with affected individuals in a large ADNFLE family was described later the same year (Steinlein et al., 1995). *CHRNA4* encodes the alpha 4 subunit of the neuronal nicotinic acetylcholine receptor, which allows sodium (Na^+) and calcium (Ca^{2+}) ions to enter nerve cells when acetylcholine binds to it. The discovery of *CHRNA4* mutations in ADNFLE families paved the way for further single gene epilepsy discoveries. Families in which there was an apparent dominant inheritance pattern of epilepsy became prime subjects for genetic investigation, with the molecular focus being on genes encoding neuronal ion channels.

Genetic epilepsy with febrile seizures Plus (GEFS+), a familial condition in which some members have febrile seizures only and others have febrile and afebrile seizures, was the second monogenic epilepsy breakthrough (Scheffer & Berkovic, 1997). In families affected by GEFS+, the epilepsy appeared to demonstrate an autosomal dominant pattern of inheritance, yet within families there was a wide variation in epilepsy phenotype. Mild cases demonstrated febrile seizures only, and the most severe cases had epileptic encephalopathy. Despite this phenotypic variation, GEFS+ was verified as a genuine monogenic disorder when mutations in the *SCN1A* gene, encoding a subunit of the neuronal sodium channel, were identified in two GEFS+ families (Escayg et al., 2000).

At the most severe end of the spectrum, some GEFS+ family members were diagnosed as severe myoclonic epilepsy of infancy (SMEI) (Singh et al., 2001). SMEI, first described in 1978, is typified by a developmental and epileptic encephalopathy – a condition in which developmental slowing or regression is seen concomitantly with seizure presentation, and in which it is hypothesised that

seizure activity directly contributes to cognitive morbidity (Scheffer et al., 2017). In most cases SMEI occurs sporadically without any familial inheritance pattern (Dravet, 2012). It was subsequently found that *de novo SCN1A* variants were common in SMEI (Claes et al., 2001). Thus SMEI became the first known non-familial genetic epilepsy. Atypical cases of SMEI (initially termed SME "Borderland," SMEB) were also found to harbour *de novo SCN1A* mutations (Fukuma et al., 2004; Kanai et al., 2004; Harkin et al., 2007). This broadening of the phenotype, coupled with the strong association with *SCN1A* variants, has led to the adoption of the term Dravet Syndrome instead of SMEI/SMEB. 60-80% of patients with Dravet Syndrome have a *de novo SCN1A* variant (Dravet, 2012; Depienne et al., 2009; Brunklaus et al., 2013).

Dravet Syndrome is often considered a "model" single gene epilepsy for three main reasons: i) to date it is by far the best studied and may be the commonest; ii) the phenotype is clearly defined; and iii) a causal variant in a single gene (*SCN1A*) is identified in the majority of cases. The picture is less clear for other epilepsy syndromes, in which variants in many different genes appear to associate with same phenotype. For example, epilepsy of infancy with migrating focal seizures (EIMFS) has been associated with variants in at least 10 different genes (McTague et al., 2016).

1.5.5 Genetic and phenotypic heterogeneity

As demonstrated by EIMFS, a single epilepsy syndrome can be associated with variants in many different genes. Conversely, there are examples of single genes that have been associated with more than one distinct epilepsy syndrome – phenotypic heterogeneity. Variants in the *KCNT1* gene have been associated with both ADNFLE (Heron et al., 2012) and EIMFS (Barcia et al., 2012; Ishii et al., 2013). *KCNQ2* variants are associated with both benign familial neonatal seizures (Singh et al., 1998), and early infantile epileptic encephalopathy (Kato et al., 2013).

Factors believed to have a significant impact on the genotype-phenotype relationship in single gene epilepsies include the specific location and functional consequence of the genetic variant, which have been extensively investigated in *SCN1A*-related epilepsy (Harkin et al., 2007; Zuberi et al., 2011; Mulley et al., 2005), the influence of other modifier genes (Zara & Bianchi, 2009; Dibbens, Heron & Mulley, 2007), and environmental influences of gene expression, known as epigenetic factors (Roopra, Dingledine & Hsieh, 2012).

Besides implying the existence of other modifiers, phenotypic heterogeneity raises two key points. Firstly, it highlights the importance of carefully defining clinical phenotypes independently of aetiology, as advocated by the ILAE multi-axial approach (Zara & Bianchi, 2009; Berg et al., 2010; Wilmshurst et al., 2014). Secondly, it supports the approach, when looking for a genetic cause for an epilepsy, of screening panels of genes rather than single genes. There exists a risk of ascertainment bias if clinicians restrict their requesting of genetic investigations to single gene sequencing in cases that conform to the established classical phenotypes (Cattani et al., 2016; O'Donnell-Luria & Miller, 2016).

1.5.6 Copy number variation and epilepsy

Deletions and duplications of large sections of DNA are present in all humans. The average human has 2,100-2,500 copy number variants (CNVs) displacing 20 million bases of DNA as a consequence (Sebat et al., 2004). This dwarfs the extent of human variation due to single nucleotide polymorphisms (SNPs) (Sachidanandam, 2001). A *de novo* CNV is expected to occur in one in 20 individuals (Itsara et al., 2010).

The pathogenicity of a CNV depends on the genes contained within the deleted or duplicated element, and on whether copy loss or copy gain of those genes has any functional consequence.

A number of well characterised pathogenic CNV-related neurodevelopmental syndromes have epilepsy as a prominent feature. These include 22q11 deletion (Di George syndrome), 2q22.3 deletion (Mowat-Wilson syndrome), 4p16.3 deletion (Wolf-Hirschhorn syndrome), and 22q13.3 deletion (Phelan-McDermid syndrome) (Olson et al., 2014). In other situations a rare CNV may disrupt an established epilepsy gene such as *SCN1A* (Lim et al., 2015) or *KCNQ2* (Pascual, Wierenga & Ng, 2013), resulting in an epilepsy phenotype similar to that associated with pathogenic single nucleotide variations (SNV) in that gene.

The most complex interplay between CNVs and epilepsy relates to susceptibility loci. These are regions of the genomic that if subject to CNV result in an increased predisposition to neurodevelopmental disorders, but with incomplete penetrance. Susceptibility variants by definition must, at least in theory, act in combination or interaction with other genetic or environmental factors to result in a clinical effect. Susceptibility loci for epilepsy have been identified at 1q21.1 (Mefford et al., 2008; Mefford et al., 2010; Basel-Vanagaite et al., 2011), 15q11.2 (Mullen et al., 2013; Mefford et al., 2010, Helbig et al., 2009), 15q13.3 (Mullen et al., 2013, Mefford et al., 2010) and 16p13.11 (Mullen et al., 2013; Mefford et al., 2010; Heinzen et al., 2010). All these are also susceptibility loci for other neurodevelopmetnal disorders such as learning disability, autism spectrum disorder, and schizophrenia. People with epilepsy related to these susceptibility loci appear to be more likely to have generalised epilepsies (as opposed to focal epilepsies) and associated learning disability (Mullen et al., 2013).

1.5.7 Complex genetic inheritance

Single genes appear to be the exception rather than the rule in epilepsy (Hildebrand et al., 2016). The most common electro-clinical epilepsy syndromes of childhood, including childhood absence epilepsy (CAE), juvenile myoclonic epilepsy (JME), and benign childhood epilepsy with centro-temporal spikes (BCECTS) have been subject to significant genetic investigation, yet rarely are monogenic causes of these epilepsies identified (Heinzen et al., 2012, Klassen et al., 2011). What has

emerged is that a large number of susceptibility genes exist for these epilepsies. Genetic variants considered to confer susceptibility to epilepsy have been associated with susceptibility to other neurodevelopmental disorders such as autism, learning disability and attention deficit hyperactivity disorder (Sherr et al., 2013; de Ligt J et al., 2012).

Genome wide association studies (GWAS) aim to identify SNPs that are common in the population and contribute to disease susceptibility. Because the genome is six billion base pairs in size, stringent p values are required to prevent overinterpretation of SNP signals. Consequently, very large sample sizes are required to demonstrate genome wide significance. A metanalysis of all epilepsy GWAS studies, totalling 8,696 cases and 26,157 controls, identified just two loci with significance (Hibar et al., 2014). GWAS has been more successful for other neurodevelopmental disorders, particularly autism (Anney et al., 2017) and schizophrenia (Ripke & O'Donovan, 2017). Hundreds of susceptibility loci have been identified in both these conditions, though they collectively explain only a tiny proportion of the variance (Manolio et al., 2009). The relative success of GWAS in autism and schizophrenia compared with epilepsy is most likely due to the significantly larger sample sizes. It may or may not mean that that these conditions are less genetically heterogeneous than epilepsy.

The role of polygenic inheritance in the causation of apparently sporadic severe neurodevelopmental disease has been investigated in autism, using a method of analysis called polygenic transmission disequilibrium. In a landmark study by Weiner et al. participants were recruited from the Simons Simplex Collection (SSC), a resource of more than 2,500 families in which a single child was diagnosed with autism spectrum disorder (ASD) and no other family member to the level of first cousins had ASD. Genotype data from cases, parents, and any unaffected siblings, were interrogated for the presence of common SNPs that, through published genome GWAS, had previously been associated with i) ASD, ii) schizophrenia, and iii) high educational attainment. Remarkably, the genomes of the ASD cases were significantly enriched for SNPs in all three groups compared

with parents and unaffected siblings. This phenomenon was still observed when those cases with relevant *de novo* variants were analysed as a separate group, suggesting that polygenic factors act additively to *de novo* variants (Weiner et al., 2017).

1.5.8 Types of genetic transmission in severe neurodevelopmental disorders – from *de novo* single gene variants to polygenic transmission

Genome wide screening of large numbers of patients with severe neurodevelopmental disorders (NDD), including severe early onset epilepsies of childhood, reveals a significant enrichment of *de novo* mutations (DNM), particularly involving genes for which loss of function variants are not observed in the healthy population. Importantly, not all of the excess DNM observed is explained by mutations in known neurodevelopmental disease-associated genes, implying that many novel NDD-associated genes are yet to be discovered (McRae et al., 2017; Hamdan et al., 2017).

The Deciphering Developmental Disorders study, which recruited 14,000 children and their parents from the UK with wide-ranging developmental disorders, identified an excess of 1,796 DNMs among the first 4,293 cases published, compared with healthy population controls. If every single one of these DNMs were responsible for disease causation in an individual case, that would still leave 2,497 (58%) cases genetically unexplained. Some at least of these remaining cases will be explained by Mendelian mechanisms such as homozygous or compound heterozygous inheritance (Martin et al., 2018), dominantly inherited variants from affected parents (perhaps with incomplete penetrance or imprinting effects), or X-linked inheritance.

Non-Mendelian factors will also play a role, such as polygenic inheritance; non-germline genetic events such as somatic mutations; epigenetic changes; and environmental causes (Figure 1.5a).

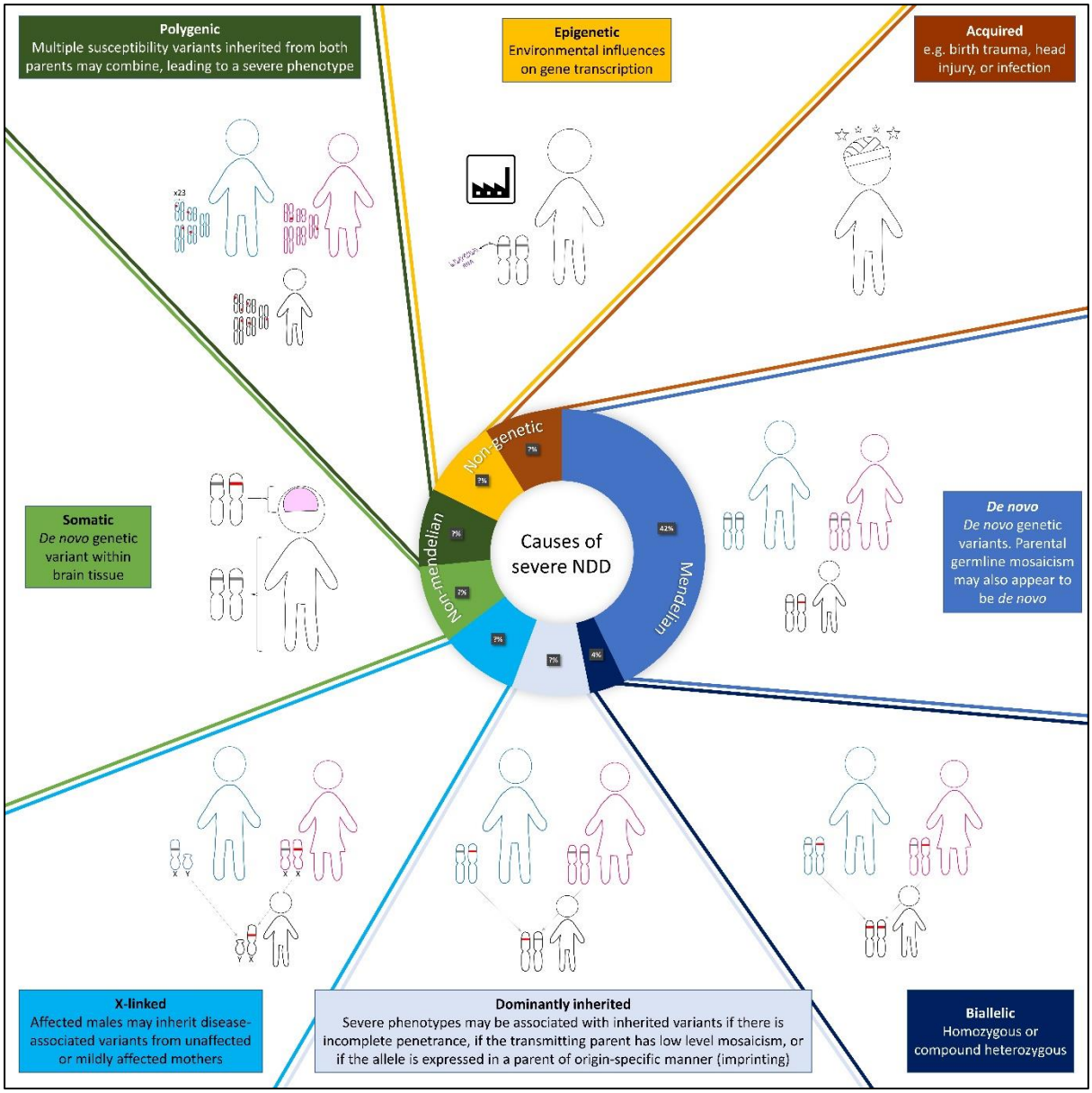


Figure 3.5a: Causes of severe neurodevelopmental disorders: Blue – Mendelian; Green Non-Mendelian genetic; Orange – Non-genetic

1.5.9 Conclusion

Recent advances in our genetic understanding of epilepsy have so far revealed that a proportion of cases are associated with variations in single genes or in genomic copy number. It is likely that many more genetic associations will come to light. More complex genetic patterns are likely to explain much of the remaining heritability of epilepsy, though so far conclusions from GWAS studies have been limited by sample size. Even in cases of epilepsy where a single gene cause is identified, additional genetic modifiers are likely to influence the phenotype.

1.6 Epilepsy Aetiology – old and new concepts

1.6.1 Introduction

The challenge of describing and classifying the epilepsies has occupied the League Against Epilepsy (ILAE) Commission on Terminology, in its various guises, since 1970 (Merlis, 1970). From the very inception of this task, it became clear that any classification of the epilepsies must necessarily take a multi-axial approach, since a number of non-mutually exclusive characteristics can be described in relation to any individual's epilepsy. These include ictal phenomena, seizure types, epilepsy syndrome (see table 1.6a); comorbidity; and aetiology (Scheffer et al., 2017). It can be argued that there are in fact only two axes - aetiology and phenotype – since ictal phenomena, seizure types, epilepsy type, and comorbidity can all be considered as components of phenotype.

Table 2.6a: Definitions of epilepsy phenotype characteristics

Term	Definition	Reference
Ictal phenomena	Non-categorical description of clinical symptoms and signs associated with an epileptic seizure.	(Blume et al., 2001)
Seizure type	Categorisation of epileptic seizures based on ictal phenomena +/- EEG features (e.g. focal, myoclonic, tonic etc.)	(Fisher et al., 2017)
Epilepsy syndrome	Categorisation of epilepsy syndrome based on seizure type(s) and evolution, EEG findings, and comorbidity	(Scheffer et al., 2017)

1.6.2 Diagnostic resolution

Phenotype and aetiology of epilepsy can both be diagnosed at varying degrees of resolution. The degree of resolution achievable depends on the characteristics of the individual case, the availability and limitations of diagnostic resources, as well as the limitations of scientific understanding itself. Taking phenotype first: at low resolution, an individual could be described as having a "focal epilepsy" if their observed seizures have a clinically focal onset. If EEG is not available (or if seizures are never captured on EEG), and if the clinical picture and family history

do not support the diagnosis of any specific epilepsy syndrome, then the epilepsy diagnosis for this individual is likely to remain "unclassified focal epilepsy". Alternatively, if the clinical picture or EEG indicate temporal lobe seizures and there is a family history compatible with dominant inheritance, then a much higher resolution diagnosis could be made: autosomal dominant familial temporal lobe epilepsy. Figure 1.6a gives an example of the spectrum of phenotypic resolution.

The same spectrum of diagnostic resolution exists for aetiology. At low resolution, an aetiological diagnosis of "genetic" may be applied in a case where there is a supportive family history, or where what is known about the epilepsy syndrome itself (e.g. juvenile myoclonic epilepsy) is suggestive of genetic aetiology. Conversely, a high resolution genetic diagnosis may be made if a precise genetic change (single nucleotide variation, copy number variation, or chromosomal rearrangement) is attributed as the cause of the epilepsy. Figure 1.6b gives an example of the spectrum of aetiological resolution.

The recent NGS revolution has occurred on a backdrop of the epilepsy community having made sustained efforts to build a framework for high resolution phenotypic diagnosis. An intricate catalogue of epilepsy syndromes has evolved, many of which were in fact described long before the ILAE's efforts at classification began. The most recent ILAE position paper on classification of seizures and the epilepsies does not provide a list of ILAE designated epilepsy syndromes, though the current nosology commission are defining the syndromes. However, in previous ILAE proposals (not formally adopted), lists have been provided, with up to 38 different epilepsy syndromes catalogued (Engel, 2016; Berg et al., 2010). In the pre-NGS era, where aetiological diagnosis was relatively rare, an epilepsy syndrome diagnosis inevitably became both description *and* explanation for clinicians and families alike. In the NGS era, the role of the epilepsy syndrome as an explanatory diagnosis has lost some traction, but this does not mean it has lost any value as a phenotypic descriptor.

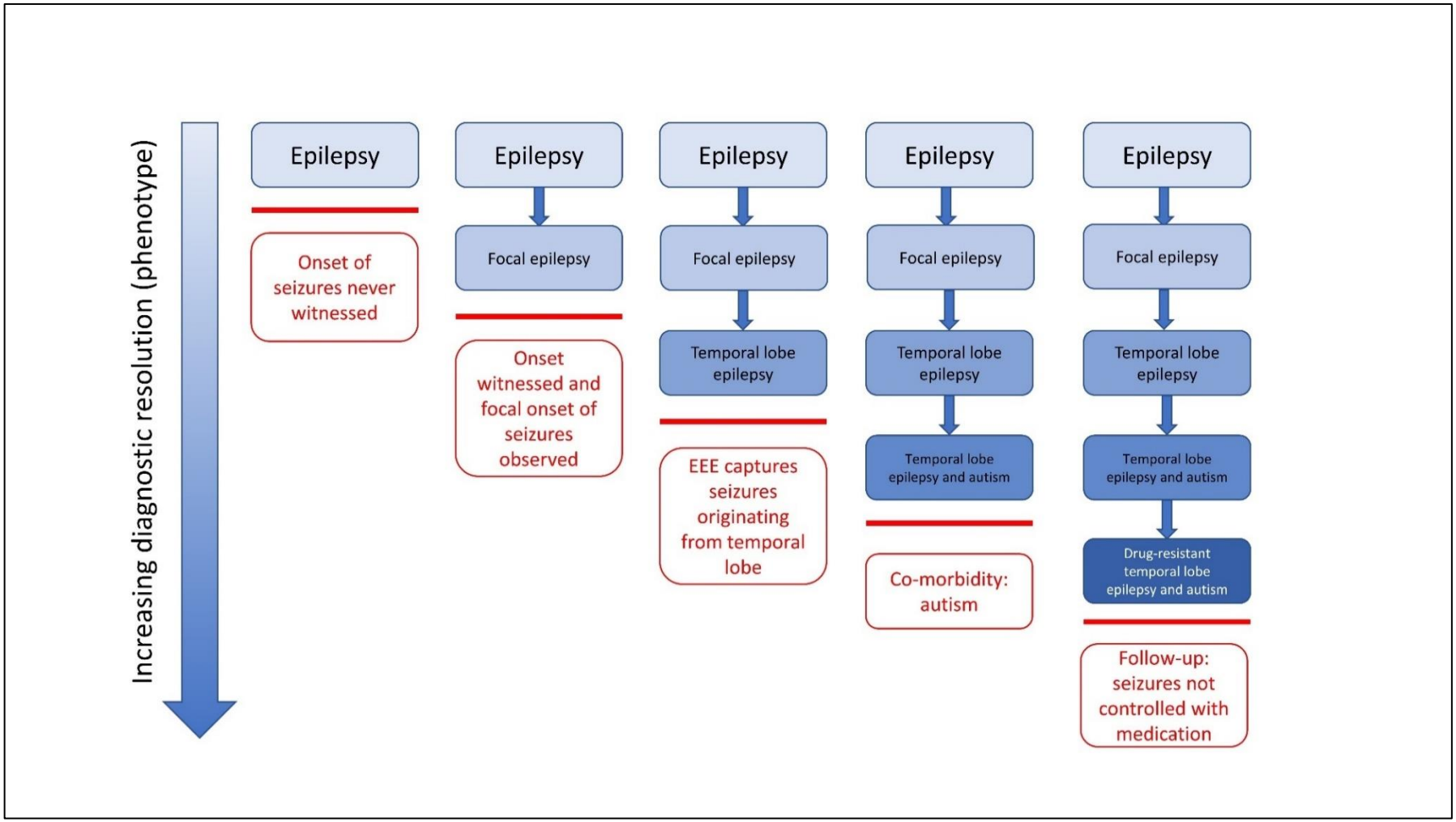


Figure 4.6a: An example of increasing phenotypic resolution

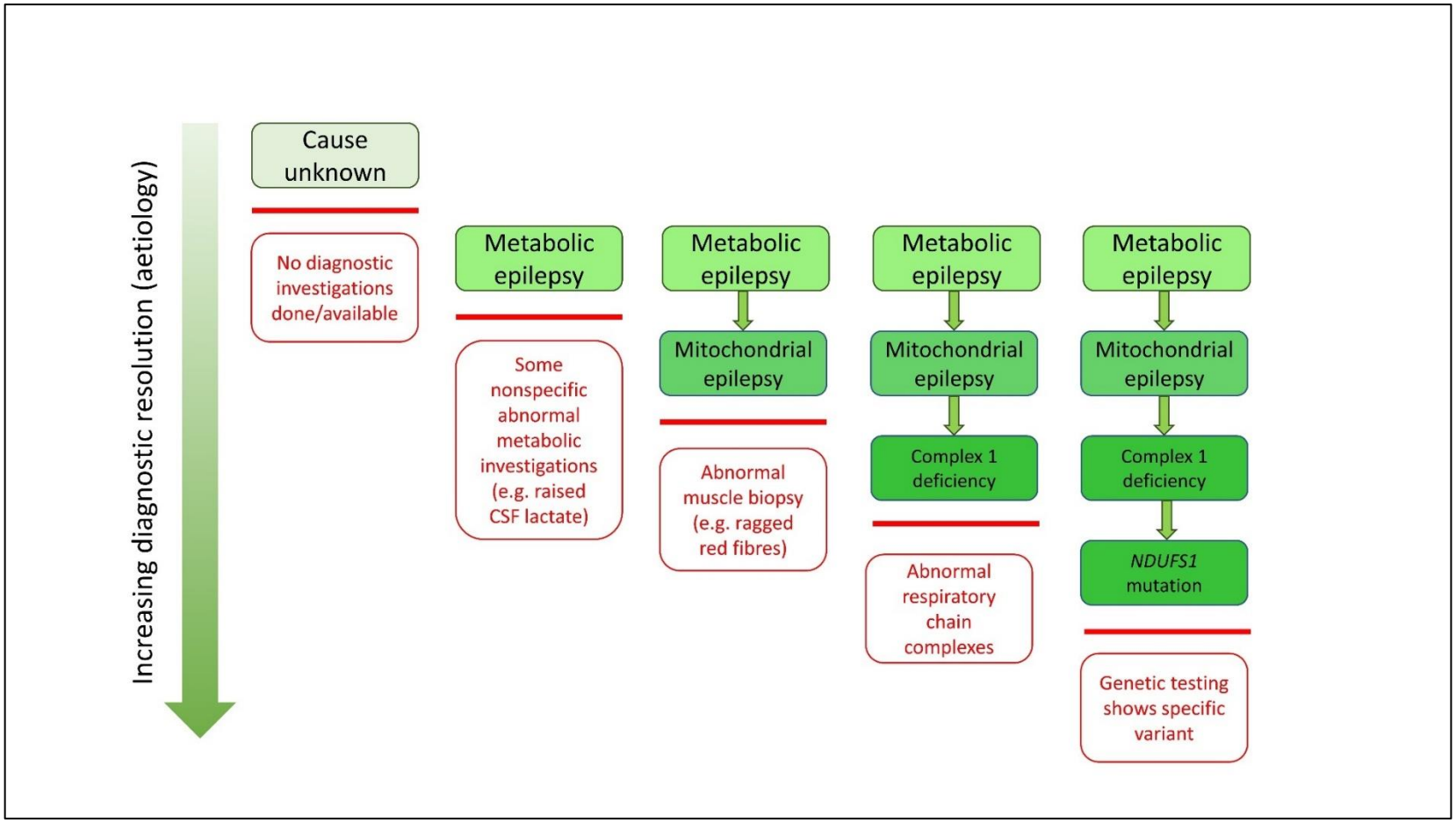


Figure 1.6b: An example of increasing aetiological resolution

1.6.3 Aetiological classification – old concepts

In parallel with efforts to refine phenotypic characterisation of the epilepsies, numerous international consensus attempts to classify epilepsy by aetiology have been made. The ILAE approved classification of aetiology which remained in place from 1989 until the latest revision in 2017, described epilepsy aetiology as being idiopathic, cryptogenic, or symptomatic (Commision on Classification and Terminology of the International League Against Epilepsy, 1989). In this classification epilepsy was considered symptomatic if it was secondary to an identified brain lesion – such as dysplasia, neoplasia, vascular, or hypoxic-ischaemic damage - or to a metabolic disease process; cryptogenic if it behaved phenotypically like a symptomatic epilepsy but no underlying cause could be identified; and idiopathic if it could be characterised as a recognised seizure disorder for which typically no underlying cause could be found. Such an aetiological classification, summarised in Figure 1.6c, does not entirely separate aetiology from phenotype since the distinction between idiopathic and cryptogenic is essentially a phenotypic one.

Between 1989 and 2017 various unratified ILAE proposals introduced new concepts. Among those fundamental to the aetiological concepts being discussed were the exchange of the term cryptogenic for *probable symptomatic* in the 2001 proposal (Engel, 2001), and the suggestion that *idiopathic* epilepsies be better categorised as *genetic* epilepsies in 2010 (Berg et al., 2010) (see figure 1.6d). This latter proposal reflected the findings of family studies which clearly demonstrated a hereditary predisposition to idiopathic epilepsy. Despite this, precise genetic causes of these epilepsies can rarely be identified.

1.6.4 Aetiological classification – new concepts

The recent advance of genomic medicine has prompted further revision of epilepsy classification. The wide genotypic and phenotypic heterogeneity exposed by the

monogenic forms of epilepsy have emphasised the conceptual necessity to keep phenotype and aetiology separated in classification (Scheffer et al., 2017).

We now understand the genetic basis of most inherited metabolic diseases, and an increasing proportion of diseases of cortical malformation. In addition, genetic diagnosis can be made in a significant minority of severe early onset epilepsies. Moreover, in some cases with phenotypes that would have previously been considered "idiopathic", such as childhood absence epilepsy (CAE) (Striano et al., 2012), juvenile myoclonic epilepsy (JME) (Bailey et al., 2018), and benign childhood epilepsy with centrotemporal spikes (BCECTS) (Lemke et al., 2013) a precise genetic aetiology can be identified. The latest ILAE classification (see Figure 1.6e) therefore recognises that the majority of epilepsies are likely to have a genetic basis, though the capability to precisely define this depends on resources available, and on current limits of knowledge. Effectively the concept of *symptomatic* epilepsy has become obsolete, since it is now recognised that "all epilepsies are symptomatic of something" (Berg et al., 2010). The new classification also recognises that the aetiological groups (*genetic, structural, metabolic, immune*) are not mutually exclusive.

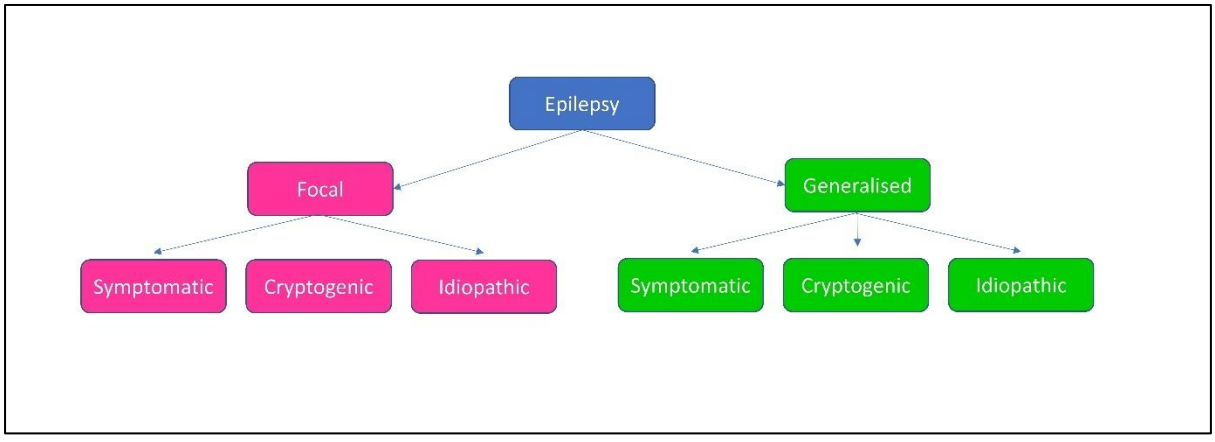


Figure 1.6c: ILAE 1989 Classification

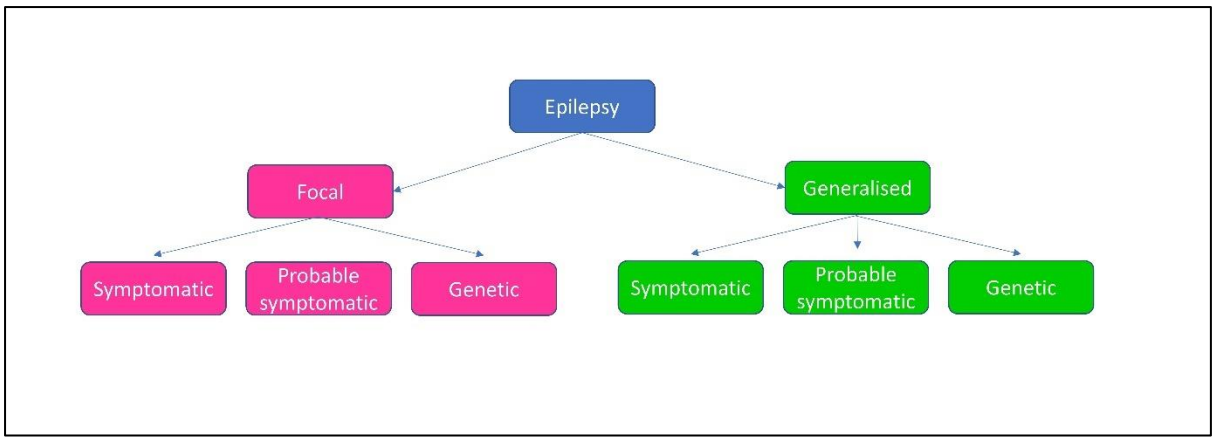


Figure 1.6d: ILAE 2010 concepts

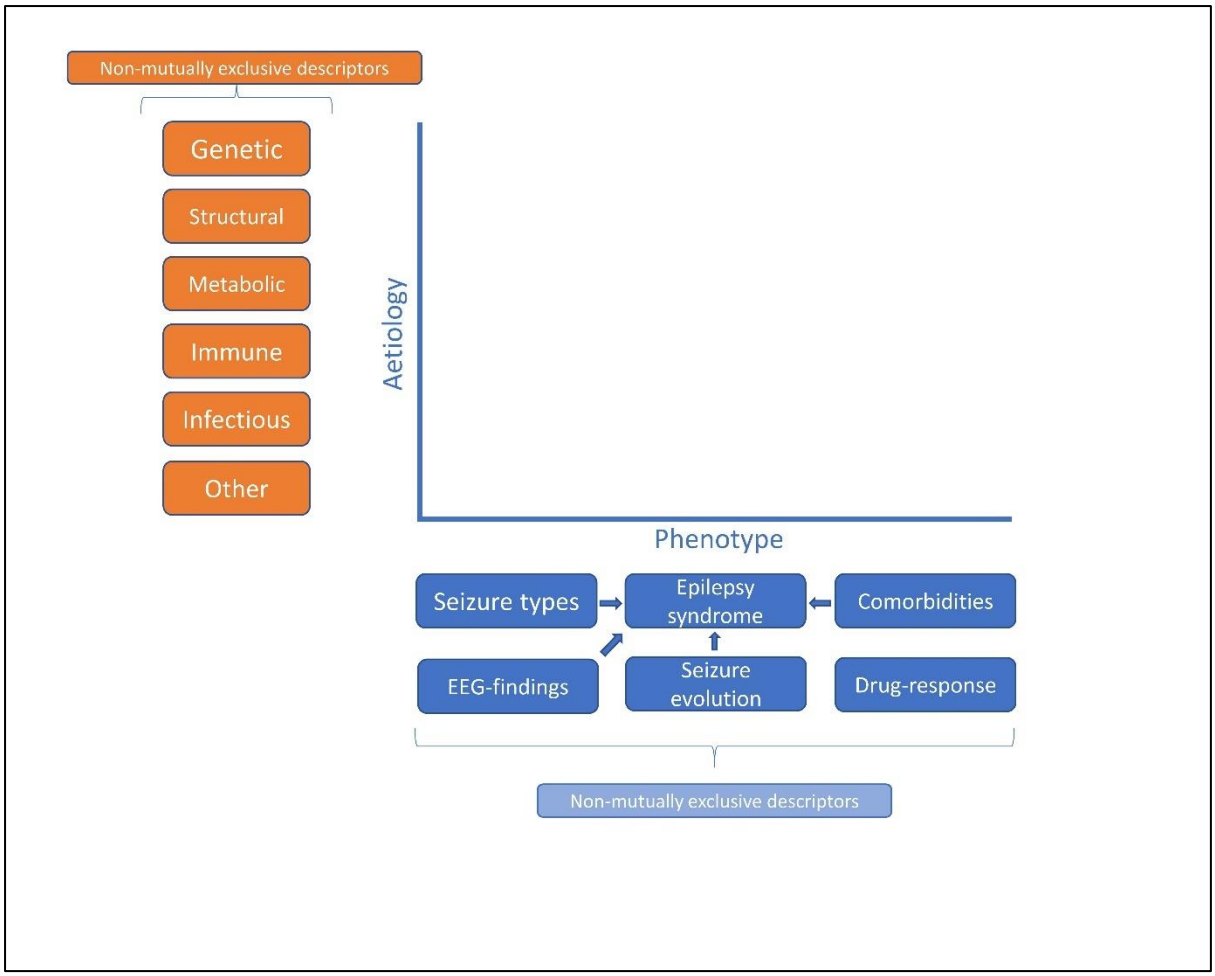


Figure 1.6e: ILAE 2017 Classification

1.6.5 Retro-phenotyping

In the pre-NGS era, genetic investigation of epilepsies would begin with the phenotype and use chromosome linkage approaches and single gene analysis (Sanger sequencing, Multiplex Ligation Probe Amplification) to identify and investigate genes harbouring mutations associated with these phenotypes. This approach inevitably led to a degree of conformational bias since patients with conforming phenotypes would be preferentially recruited to such studies, and consequently the full phenotypic spectrum associated with each gene was rarely represented. In the current NGS era the reverse approach is occurring. Typical NGS studies involve large cohorts of patients with a broad range of phenotypes. Following identification of a genetic association, phenotypes are investigated and described retrospectively. The major consequence of this retro-phenotyping approach is that a more complete phenotypic spectrum associated with many genes has been unveiled. For example, variants in the gene *GABRA1* have been associated with a range of phenotypes, including drug-responsive JME, CAE, West syndrome, Dravet syndrome, and Ohtahara syndrome (Johannesen et al., 2016, Kodera et al., 2016).

Chapter 7 of this thesis describes a new genetic condition in which epilepsy is perhaps the most prominent symptom, but the comorbidities help define it and make it recognisable.

1.7 Conclusion

In this introduction I have shown that epilepsy is a common condition that has a significant impact on the lives of those affected, the lives of their families, and on the health service and economy. Early childhood is associated with a specifically high incidence of new-onset epilepsy. Those patients who present in early childhood are more likely to develop drug-resistance and have a particularly high rate of comorbidity. Those with both early onset and drug-resistance are not only more likely to have comorbidity, but are also more likely to have an identifiable underlying cause. For children with drug-resistant epilepsy and significant cognitive and behavioural comorbidity quality of life is likely to depend on many factors beyond seizure control alone.

Modern genetic testing allows a precise diagnosis to be made in increasing proportions of patients with childhood-onset epilepsy. Achieving a genetic diagnosis may be useful for many reasons, including prognostication, counselling (Brunklaus et al., 2013, Hammond et al., 2010, Shostak, Zarhin & Ottman, 2011, Lingen et al., 2016, Might & Wilsey, 2014), advising parents on recurrence risk, and preventing further costly investigations (Brunklaus et al., 2013, Tan et al., 2017), but any benefits of genetic in terms of guiding treatment have yet to be established. If precision approaches to epilepsy treatment are to be evaluated meaningfully, outcomes beyond the seizure control, including impact on comorbidity and quality of life, must be assessed in a structured and systematic way.

In order to understand what potential impact genetics will have on treatment in childhood-onset epilepsy we first need to know how common monogenic forms of epilepsy are. This will be addressed in chapters 3 (literature review), 5 (using gene panel testing), and 6 (using Whole Genome Sequencing).

Chapter 4 reviews the current status of the evidence for precision therapy in epilepsy. Most of the limited evidence for precision therapy relates to epilepsies in

which the genetic cause relates to an ion channel. Chapter 7 looks at whether precision approaches can be extended to non-ion channel epilepsies, using *SMC1A* as an example.

In order to make progress with precision therapy, we are likely to need to move beyond the rather reductive idea that treatment decisions can be guided by knowledge of the gene alone. Chapter 8 investigates whether the precise nature and location of an *SCN1A* variant is associated with treatment response to Cannabidiol, using RCT methodology.

2. General Methods

2.1 Introduction

In order to assess the potential impact of genotype-driven precision therapy for children with epilepsy, two questions need to be answered:

i) How common are single gene causes of epilepsy?
ii) What is the current status of, and future scope for, precision therapy in these epilepsies?

A mixed methods approach will be used to answer these questions. In each chapter detailed methods will be described separately. Chapters 3 and 4 are literature-based studies, aiming to answer questions i) and ii) above respectively. Chapters 5 to 8 are primary research, involving a variety of patient cohorts detailed in table 2.2a.

2.2 Summary of cohorts

Table 2.2a: Summary of patient cohorts in this thesis

Chapter and cohort	Selection	Phenotyping	Genotyping
Chapter 5, cohort 1	Prospectively recruited patients presenting with epilepsy and/or complex febrile seizures before the age of 36 months from the whole of Scotland, over a three-year period, without determined aetiology (2014-2017).	Clinician-completed structured proforma at recruitment and again 12 months after genetic results communicated. Parent-completed developmental, behavioural and quality of life questionnaires.	104 gene epilepsy panel (Glasgow).
Chapter 5, cohort 2	Retrospectively ascertained patients presenting with epilepsy before the age of 36 months from the West of Scotland, over a three-year period (2014-2017).	Electronic medical record review.	Various methods, as arranged by clinical team, including single gene sequencing, gene panel testing, chromosomal microarray, and whole genome sequencing.
Chapter 6	Childhood-onset complex epilepsies managed within the West of Scotland. No restriction on time of presentation.	Face-to-face interview with parents and patient. Examination of patient. Medical record review, including EEGs and neuroimaging.	Trio Whole Genome Sequencing (Oxford).
Chapter 7	Patients recruited to the Deciphering Developmental Disorders (DDD) study who had *de novo* truncating variants in the *SMC1A* gene identified.	Clinician-completed structured proforma.	Trio Whole Exome Sequencing already completed as part of the Deciphering Developmental Disorders (DDD) study prior to patient selection. *SMC1A* variant information provided.
Chapter 8	Patients recruited to the GWPCARE3 and GWPCARE4 trials of Cannabidiol versus Placebo (multicentre multinational randomised controlled trial)s.	Seizure frequency over 14-week treatment period compared with 4-week baseline.	*SCN1A* variant information provided by recruiting clinicians

2.3 Publicly available tools used for variant interpretation

Testing methodologies have varied between cohorts and are described in detail separately. However, the tools used to interpret the significance genetic variants have been consistent between cohorts. Limitations of these tools will be discussed in section 6.4.

2.3.1 Population frequency and variant tolerance data

The largest publicly available datasets providing population frequency data of genetic variants are the Exome Aggregation Consortium (ExAC) and the Genome Aggregation Database (gnomAD), both managed by the Broad Institute at Harvard University, Massachusetts (Lek et al., 2016). ExAC provides whole exome sequencing (WES) data (all protein-encoding DNA) on 60,706 unrelated individuals. gnomAD provides whole genome sequencing (WGS) data (all DNA, coding and non-coding) on 123,136 unrelated individuals. To allow users to account for population-specific variation, participants were recruited from all major ethnic backgrounds, and variants are reported according to broad ethnic groups. Both databases exclude individuals with severe paediatric disease, so it is expected that these datasets are depleted of variants associated with severe early-onset epilepsies. The gnomAD dataset is more complete and accurate because of larger numbers and because WGS is intrinsically more accurate than WES. In this thesis variants have therefore been checked for population frequency against gnomAD data. For example, in chapter 6 candidate variants have only been considered when observed in fewer than 0.01% of alleles in gnomAD (equivalent to 1 heterozygote per 5,000 individuals for autosomal genes).

The ExAC dataset provides additional data on variant tolerance that has been used when considering candidate genes. For each gene covered by ExAC the number of missense variants per gene has been calculated and compared with the number of expected variants based on the length of the gene (coding regions only), the observed number of synonymous variants observed in the gene, and on missense

variation across the exome as a whole. This is expressed as a z-score (standard deviations in the number of observed variants from the expected number, using chi-squared). The z-score is positive when the observed number is fewer than expected and negative with the observed number is greater than expected. Hence, genes with high z-scores do not tolerate missense variation. A separate scoring system, using a complex "expectation-maximisation algorithm" described in the supplementary section 4.4 of Lek et al. (Lek et al., 2016) is used to create a measure of the probability that a gene is intolerant of loss-of-function, the "Probability of Loss-of-Function Intolerance" (pLI) score. pLI scores of >0.9 are considered to indicate significant intolerance of a gene for loss of function. The authors state that extreme constraint does not necessarily reflect a lethal disease or status as a disease gene, but probably points to genes in which heterozygous loss of function confers some non-trivial survival or reproductive disadvantage. The pLI score is especially useful in the interpretation of *de novo* protein truncating variants (PTVs) in patients with severe phenotypes.

2.3.2 Computational tools for predicating the pathogenicity of missense variants

A wide range of tools are available, all with similar functions. These use complex machine learning algorithms driven by multiple inputs which include nucleotide and amino acid conservation between species, polarity and pH shifts associated with amino acid changes, and sequence and structure descriptors relating to the region affected. These have been comprehensively reviewed and appraised elsewhere (Janita, Ayodeji & Mauno, 2011, Ohanian, Otway & Fatkin, 2012). For largely practical reasons – because their use was already established within the Glasgow clinical genetics laboratory – the two tools which I selected were Polyphen-2® (Adzhubei et al., 2010) and SIFT® (Sorting Intolerant From Tolerant) (Kumar, Henikoff & Ng, 2009).

Polyphen-2 expresses outputs as an overall probability. This is a composite of a sensitivity p-score and a specificity p-score. In chapter 6 to identify candidate variants I filtered out those with a Polyphen-2 scores ≤0.8.

SIFT expresses outputs in terms of scaled probability that a variant is benign. A score of 1 represents a predicted benign variant and a score of 0 represents a predicted damaging variant. When tested for sensitivity and specificity against established pathogenic and benign variants both Polyphen-2 and SIFT demonstrate reasonable sensitivity (68% and 69% respectively in one analysis) but poor specificity (13% and 16%) for predicting pathogenicity (Flanagan, Patch & Ellard, 2010). In other words, these tools are much more useful for filtering out benign variants than they are for selecting pathogenic ones.

2.3.3 Computational tools for predicting splicing effects

In the majority of genes, introns are flanked by the two base sequence *GT*.. …*AG*. These sequences act as specific signals for the spliceosome to remove transcribed intronic RNA from precursor messenger RNA to form mature RNA. Variants affecting these flanking regions of intronic sequence typically result in truncation (Scotti & Swanson, 2015). The splicing process is complex, and more deeply intronic variants can also have an impact on the splicing process. Furthermore, variants within coding sequence can result in the creation of aberrant splice sites, which can also lead to truncation. Various machine learning tools have been developed to predict the impact of variants on splicing. The ones used in this thesis are Spice-Site-Finder-Like®, MaxEntScan®, NNSPLICE®, GeneSplicer®, and Human Splicing Finder® (Jian, Boerwinkle & Liu, 2013).

2.3.4 Brain expression data

Genes that have relatively high expression in brain tissue are more likely to be implicated in neurological disease (Negi & Guda, 2017). Various publicly available datasets of relative RNA expression in tissues are available. The largest of these is

the GTEx consortium dataset, which catalogues quantitative RNA sequencing data from 7,051 samples obtained from 449 donors (Aguet et al., 2017). These are mostly immediate post-mortem samples taken from otherwise healthy young adults who have died from trauma. Data on relative expression of genes in brain are available from GTEx. In chapter 6 I have shown that genes associated with severe forms of childhood-onset epilepsy have significantly higher brain GTEx ratios. I have used these data the prioritise candidate epilepsy genes.

2.3.5 Missense variant localisation

The location of a variant within a gene is a major factor which determines its pathogenicity. Indirectly this is included in prediction algorithms such as Polyphen-2 and SIFT. However, these operate on general principles rather than specific knowledge about genetically determined functional domains of proteins. For some genes, particularly ion channel genes, precise knowledge of the corresponding region of protein affected by a missense variant provides important information. For example, in severe epilepsies associated *SCN1A* variants, epilepsy associated variants cluster in regions which correspond with transmembrane regions of the protein (Zuberi et al., 2011). To obtain information of variant localisation I used the Uniprot portal, a database of 60 million sequences manually curated by experts who critically review experimental and predicted data for each protein (UniProt Consortium, 2017).

2.3.6 Gene matching

One of factors that limits variant interpretation is the current state of knowledge. A gene for which there is little known one day may turn out to be of pathological significance the next. When a very rare variant in a novel gene is identified in a patient its significance will be uncertain, even if the variant appears to be damaging and the gene appears to be a good candidate based on factors such as known mechanism, ExAC constraint scores and GTEx ratio. If more patients with similar phenotypes and similar/identical variants in the same gene are identified, then significance dramatically increases. To facilitate, this various "gene matching" systems have been developed which clinical researchers can use to deposit anonymous phenotype and genotype details of patients, with appropriate consent. Programs that will be used in this project are: Decipher (Firth et al., 2009), Gene Matcher (Sobreira et al., 2015), and Phenome Central (Buske et al., 2015).

3. Meta-analysis of next generation sequencing studies in epilepsy

3.1 Introduction

The Sanger method of DNA sequencing, developed in 1977 (Sanger, Nicklen & Coulson, 1977) remained the mainstay for identifying DNA sequence variants in both clinical and research settings until 2005. Though highly accurate, the Sanger method is low throughput. As a result, research-based testing using this method was largely hypothesis-driven, favouring the sequencing of genes whose function was believed to relate to the disease being investigated. From 2005 onwards the development of Next Generation Sequencing (NGS) has dramatically increased genetic sequencing throughput (Mardis, 2011).

Currently applied NGS approaches include *gene panels* in which selected known disease-associated genes only are sequenced, *clinical exome* sequencing (sometimes referred to as the Mendeliome) in which all known disease-associated genes are sequenced (currently approximately 4,800 genes), a *whole exome* in which all coding DNA is sequenced, and a *whole genome* in which all DNA, coding and non-coding, is sequenced.

The application of NGS has permitted much larger cohorts of patients to be investigated for multiple genetic variants in a more hypothesis-limited manner than was the case previously. This has facilitated a revolution in the genetic understanding of many diseases, including epilepsy. Prior to the advent of NGS, the epilepsy literature emphasised a narrative that heritable disorders of neuronal ion channels were the major genetic factor in epilepsy (Helbig et al., 2008). As a direct result of NGS, the number of genes associated with epilepsy has risen substantially since 2005. Disorders of the ion channel may no longer be the dominant theme.

NGS has become a mainstream diagnostic test for many clinicians looking after patients with epilepsy. A number of research groups have reported their diagnostic yield from NGS testing. However, due to biases and inconsistencies in case selection, testing procedures and publication, is not clear from any individual study which are the most commonly implicated genes in epilepsy.

The aims of this chapter are: i) to systematically analyse the genes that have been associated with epilepsy, and to categorise them into groups based on broad function; ii) to perform a meta-analysis of studies reporting the application of NGS in cohorts of patients with epilepsy in order to understand which are the most commonly-implicated genes.

3.2 Methods

3.2.1 Identification of epilepsy-associated genes:

Genes associated with epilepsy were identified through four routes, sequentially:

1. Personal experience of encountering patients with genetic epilepsy and from reading papers describing cases
2. Review of genes covered by epilepsy diagnostic testing in UK NHS laboratories and US commercial laboratories
3. Search of the Online Mendelian Inheritance in Man (OMIM) resource (Johns Hopkins University, Baltimore, 2018) for using the term [epilepsy]. *Search date February 5th 2018*; *1064 results*.
4. Search of Medline [1860-2017] using the search terms ([epilepsy] *or* [seizure]) *and* [gene]. *Search date April 18th 2018; 8534 results*

For each epilepsy-associated gene identified I reviewed the original research for quality and relevance. Any gene considered relevant to epilepsy was added to a database into which I systematically recorded phenotype features (age of onset, seizure types, comorbidities), mode of inheritance, and date of first published case or case series. I defined six functional groups for genes as follows, and categorised each gene into the group of best fit:

- **Metabolic:** Genes encoding proteins involved in the transport and metabolism of intracellular small molecules, or mitochondrial function
- **Ion channel/G-protein:** Genes whose products form ion channels, ion channel accessory proteins, or G-proteins
- **Cell growth and proliferation:** Genes encoding regulators of cell growth and proliferation
- **Synaptic:** Genes encoding proteins with specifically high expression at the synapse, or with key synaptic functions (excluding ion channels and G-proteins)

- **Regulatory:** Genes thought to play a role in transcriptional regulation, translational regulation, or post-translational protein modification
- **Others:** Miscellaneous, or unknown function

3.2.2 Meta-analysis of diagnostic yield from epilepsy NGS studies

Papers reporting the application of NGS to cohorts of patients with epilepsy were identified using a Medline literature search (*date April 18th 2018*) using the following terms:

- [epilepsy] *or* [epileptic] *or* [seizure] *and*
- [next generation sequencing] or [gene panel] or [exome] or [genome]

Total results = 870

Abstracts of these papers were reviewed to identify studies in which NGS technology (either a targeted gene panel, a whole exome, or a whole genome) had been applied in the diagnostic evaluation of a cohort of patients with epilepsy. Studies which did not include patients with epilepsy were excluded, as were those that did not report diagnostic results, or in which 10 or fewer patients were reported. Diagnostic results from each study were reviewed to check whether they satisfied the American College of Medical Genetics and Genomics criteria for pathogenicity (Richards et al., 2015). Variants that did not meet these criteria were removed from the results. Total number of diagnostic results involving each gene were summated for each paper.

3.3 Results

3.3.1 Epilepsy-associated genes: discovery over time, and functional categories

359 epilepsy-associated genes were added to the database. These genes fell into the following functional categories, in descending order of frequency (Figure 3.3a):

- Metabolic: 116 (32%)
- Cell growth and proliferation: 83 (23%)
- Ion channel-G-protein: 66 (19%)
- Regulatory: 48 (13%)
- Synaptic: 32 (9%)
- Others: 14 (4%)

The proportionate increase in epilepsy gene discovery in each group between 2011 and 2017 was as follows:

- Metabolic: 59 to 116 (200%)
- Cell growth and proliferation: 37 to 83 (220%)
- Ion channel-G-protein: 35 to 66 (190%)
- Regulatory: 17 to 48 (280%)
- Synaptic: 9 to 32 (360%)
- Others: 5 to 14 (360%)

Since the arrival of NGS, there has been a greater proportionate increase in gene discovery within the regulatory and synaptic groups than within the metabolic, cell growth and proliferation and ion channel/G-protein groups.

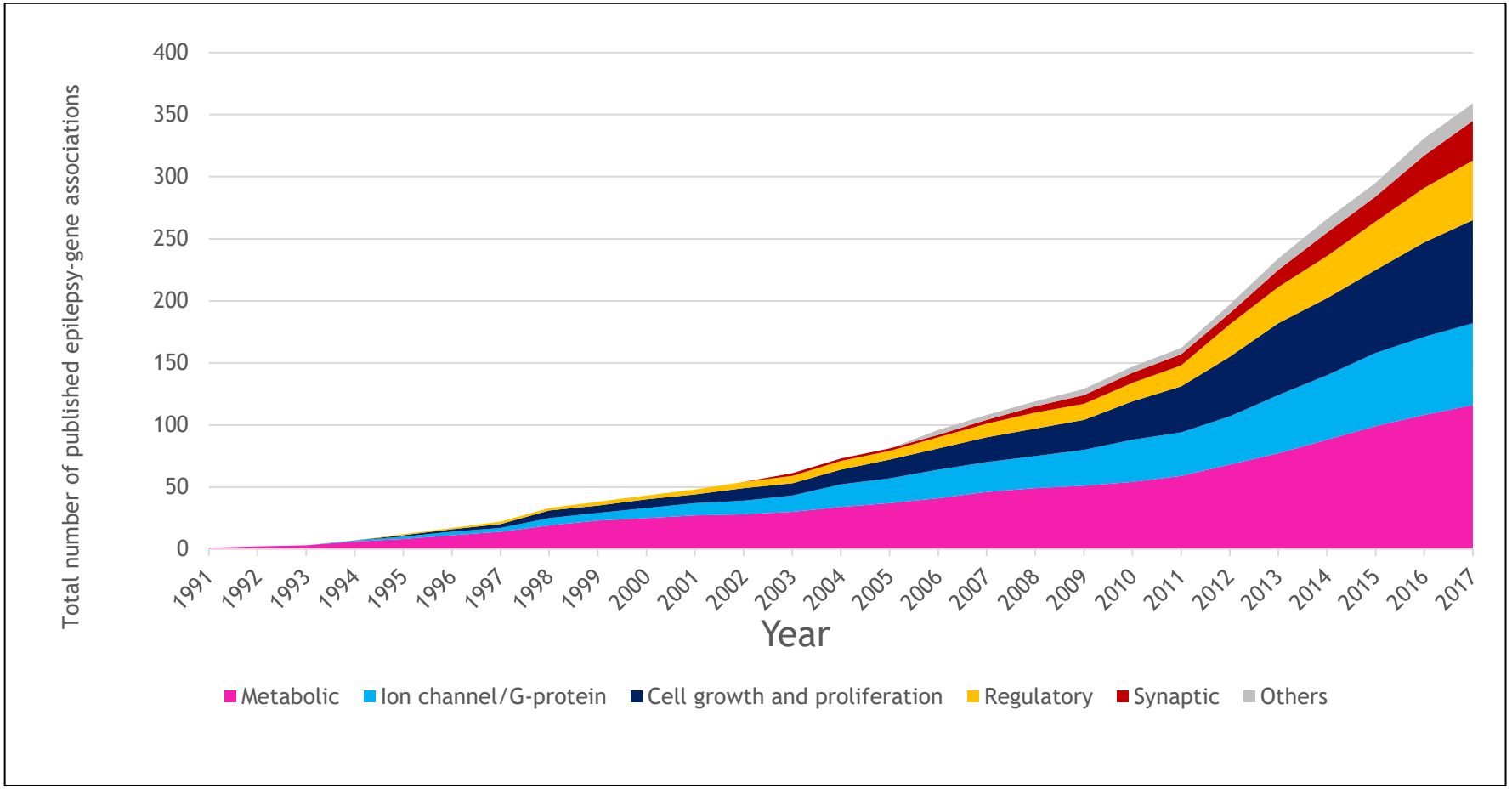


Figure 3.3a: Epilepsy gene discovery 1991-2017

3.3.2 Meta-analysis of diagnostic yield from NGS epilepsy studies

3.3.2.1 Most commonly implicated genes

24 studies were included in the analysis. These are presented in table 3.3a. Diagnostic yield ranged from 3% to 50%.

For 104/210 genes there was only a single patient with a diagnostic result reported.

Figure 3.3a shows the genes recurrently implicated (four or more positive results). The most commonly implicated genes were *SCN1A, KCNQ2, CDKL5, SCN2A, STXBP1*, and *PCDH19*. These six genes were implicated in more than 50% of the diagnostic results. The 27 most commonly-implicated genes explained 80% of the diagnostic results.

In order to adjust for the variability in size of panels I have also represented the most commonly implicated genes as a proportion of the total number of patients tested for variants in each respective gene (Figure 3.3c). Two papers did not report which genes were included in their panels (Zhang 2017 and Butler 2017) and one paper reported changing their panel mid-way through the study (Trump 2016), so these were excluded from this analysis. The most commonly implicated seven genes are exactly the same in both analyses but a few genes appear to gain significance (see *GABRB3, CHD2, WWOX* and *NEXMIF*).

Figure 3.3d presents the number of diagnostic results in the functional category groups and compares these with the total number of known epilepsy-associated genes in each group (see Figure 3.3a). Although ion channel/G-protein genes represent under 20% of the total numerically, variants in these genes account for more than half of the diagnostic results. Metabolic genes – 32% of the total – account for just 11% of the diagnostic results. Genes involved in growth and proliferation make up 23% of the numbers but only 6% of the diagnostic results.

3.3.2.2 Factors associated with diagnostic yield

3.3.2.2.1 Number of genes selected (i.e. size of panel)

Figure 3.3e takes the data from Table 3.3a and shows the relationship between the size of the panel, plotted on a logarithmic scale, and the diagnostic yield. There is a clear association between the number of genes selected for testing and the diagnostic yield, though this explains only about one fifth of the variance in yield observed (R^2 = 0.225). Variation in patient selection between these studies may explain much of the remaining variance. The low diagnostic yield of 4% in the study by Hildebrand et al. may be due to the fact that most of the patients in this study had sporadic temporal lobe epilepsy. The current state of knowledge suggests that sporadic temporal lobe epilepsy is rarely monogenic. The low yield of 3% in the Myers et al. study may be because most of the patients selected had already undergone extensive genetic investigation.

3.3.2.2.2 Phenotypes

Studies that recruited patients with childhood-onset severe epilepsies (e.g. Hamdan 2017, Tumiene 2017, Ko 2018) had higher diagnostic yield than those with broader inclusion (Trump 2015, Butler 2017). Moreover, within individual studies, age of onset was also associated with increased probability of receiving a diagnostic result. In the Trump et al. study (Trump et al., 2016) the odds ratio for a diagnostic result in the children aged less than two months was 5.0 (see table 3.3b) and in Møller et al.'s study presentation in the first month of life was associated with an odds ratio of a diagnostic result of 5.7. Conversely in the Helbig et al. study, a significant difference for early-onset patients was not seen. Nor did Helbig et al. find significantly increased diagnostic yields in subgroups with infantile spasms or early onset epileptic encephalopathy (Helbig et al., 2016). An important difference is that the Helbig study used a panel of 4800 genes, whereas the Trump and Møller studies both used a more selective 46-gene panel. Trump et al. and Møller et al. are likely to have favoured genes associated with early onset

epilepsies. The Ko et al. study, using a 172 gene panel, found a significant association between drug-resistance and a diagnostic result (odds ratio: 3.5) (Ko et al., 2018). In summary, early onset, severe and drug-resistant epilepsies appear to be associated with increased diagnostic yield when relatively small gene panels are used, but this effect is not seen when larger panels are used.

Table 3.3a: Summary of 24 NGS studies of epilepsy, involving 13,063 patients: 2012-2018

Study	Country/region	Patient selection	Platform	Yield	Reference
Lemke 2012	Germany/ Switzerland	Not specified – variable phenotypes	265 gene epilepsy panel	16/33 (48%)	(Lemke et al., 2012)
Kodera 2013	Japan	Early onset epileptic encephalopathy	30 gene epilepsy panel	11/53 (21%)	(Kodera et al., 2013)
Della Mina 2014	Italy	Not specified – variable phenotypes	67 gene epilepsy panel	9/19 (47%)	(Mina et al., 2015)
Carvill 2014	Global	Infantile spasms or Lennox Gastaut Syndrome	Trio Whole Exome Sequencing (WES)	51/356 (14%)	(Carvill et al., 2014)
Allen 2015	Ireland	Unexplained early onset epileptic encephalopathy	137 gene epilepsy panel	13/50 (26%)	(Allen et al., 2016)
Trump 2015	UK	Tertiary referrals to Great Ormond Street Hospital	46 gene epilepsy panel	58/323 (18%)	(Trump et al., 2016)
Zhang 2015	China	Unexplained epilepsy and intellectual disability	300 gene epilepsy panel	46/253 (18%)	(Zhang et al., 2015)
Møller 2016	Denmark, Estonia, the UK, Argentina, Pakistan	Epileptic encephalopathies and familial epilepsies	46 gene epilepsy panel	49/216 (23%)	(Møller et al., 2016)
Myers 2016	Global	Unsolved epileptic encephalopathy cases	27 candidate gene epilepsy panel	18/531 (3%)	(Myers et al., 2016)
Helbig 2016	USA	Clinical referrals to diagnostic lab, all patients with seizures	Diagnostic exome	119/314 (38%)	(Helbig et al., 2016)
Zhang 2016	China	Early onset epileptic encephalopathy	17 gene epilepsy panel	56/175 (32%)	(Zhang et al., 2017)

Parrini 2016	Italy	Drug-resistant epilepsy (0-5 years)	95 gene epilepsy panel	71/349 (20%)	(Parrini et al., 2017)
Hildebrand 2016	Australia	Focal epilepsy	11 gene epilepsy panel	11/251 (4%)	(Hildebrand et al., 2016)
de Kovel 2016	Europe	Seizures and intellectual disability, onset <5 years	26 gene epilepsy panel	31/360 (9%)	(Kovel et al., 2016)
Gokben 2017	Turkey	Early-onset epileptic encephalopathy	16 gene panel	9/30 (30%)	(Gokben et al., 2017)
Butler 2017	USA	Clinical referrals	110 gene epilepsy panel	58/339 (17%)	(Butler et al., 2017)
Hamdan 2017	Canada	Developmental and epileptic encephalopathy	Trio Whole Genome Sequencing (WGS)	63/197 (32%)	(Hamdan et al., 2017)
Ortega-Moreno 2017	Spain	Epilepsy and developmental delay	106 gene epilepsy panel	17/87 (20%)	(Ortega-Moreno et al., 2017)
Newman 2017	USA	Referrals to diagnostic lab.	100 gene epilepsy panel	36/166 (22%)	(Newman et al., 2017)
Tumiene 2017	Slovenia	Epilepsy and developmental delay or dysmorphism	Diagnostic exome (4813 genes)	40/86 (47%)	(Tumienė et al., 2017)
Ko 2018	South Korea	Developmental and epileptic encephalopathy	172 gene epilepsy panel	97/278 (35%)	(Ko et al., 2018)
Palmer 2018	Australia	Epileptic encephalopathies	Diagnostic exome	16/32 (50%)	(Palmer et al., 2018)
Lindy 2018	USA	Clinical referrals	70 gene panel	1324/8565 (15.5%)	(Lindy et al., 2018)

Table 3.3b: Odds ratios for clinical predictors of diagnostic results within epilepsy NGS studies

Study	Clinical feature associated with diagnostic result	Number with diagnostic result/Number with feature (%)	Number with diagnostic result/Number without feature (%)	Odds ratio (95% confidence intervals) and p value (Fisher's exact test)
Trump 2015	Presentation < 2 months	30/77 (39%)	28/246 (11%)	5.0 (2.7-9.1), p<0.0001
Møller 2016	Presentation < 1 month	12/21 (57%)	37/195 (19%)	5.7 (2.2-14.5), p<0.001
Helbig 2016	Presentation < 1 month	12/28 (43%)	107/276 (37%)	1.3 (0.6-2.8), n.s.
Helbig 2016	Infantile spasms	16/41 (39%)	103/273 (38%)	1.4 (0.7-2.7), n.s.
Helbig 2016	Early Onset Epileptic Encephalopathy	28/67 (42%)	91/247 (37%)	1.2 (0.7-2.1), n.s.
Ko 2018	Drug-resistant seizures	74/161 (46%)	23/118 (19%)	3.5 (2.0-6.1), p<0.0001

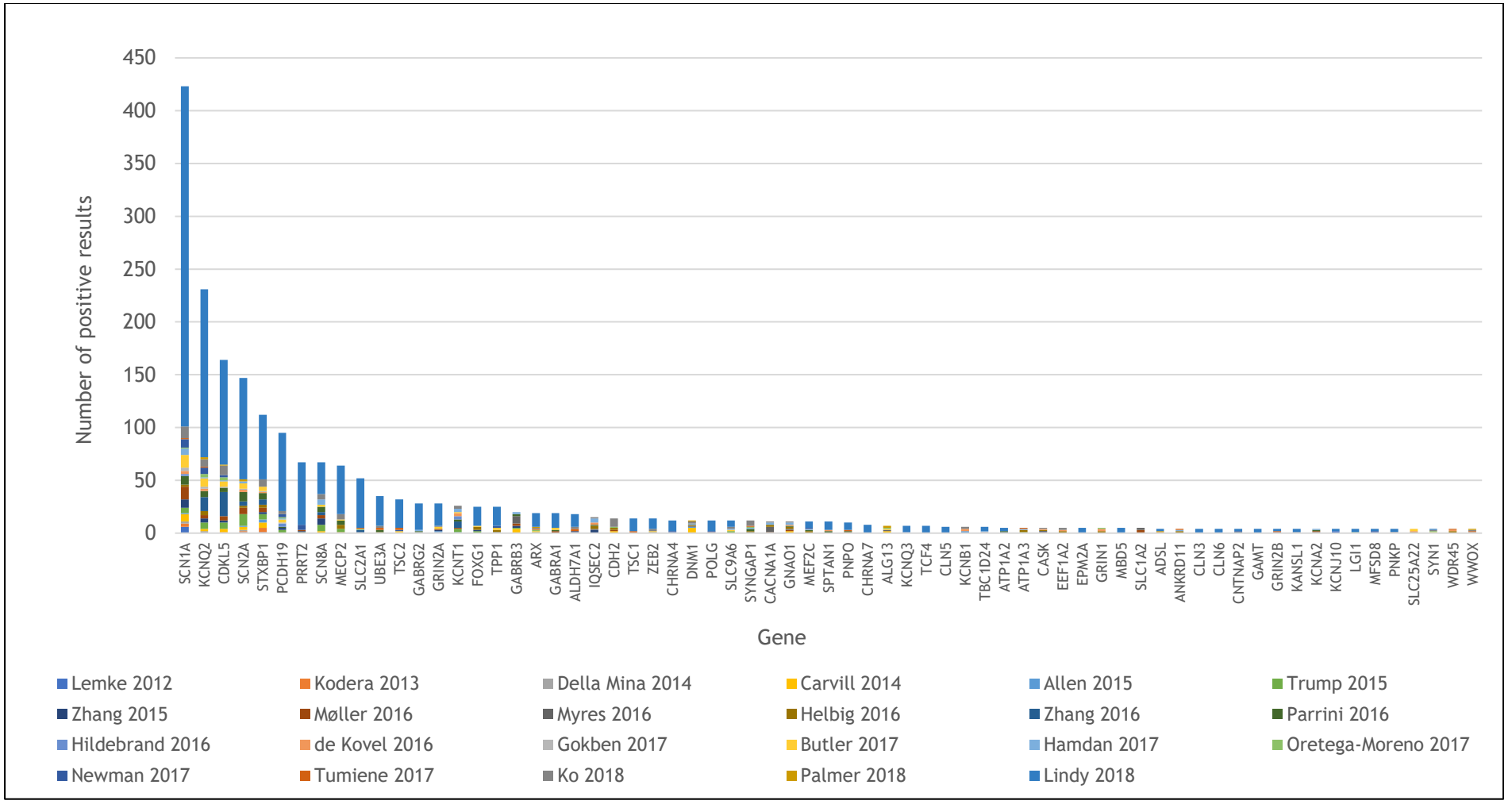


Figure 3.3b: Combined results from 24 NGS studies in epilepsy. Genes implicated on four or more occasions.

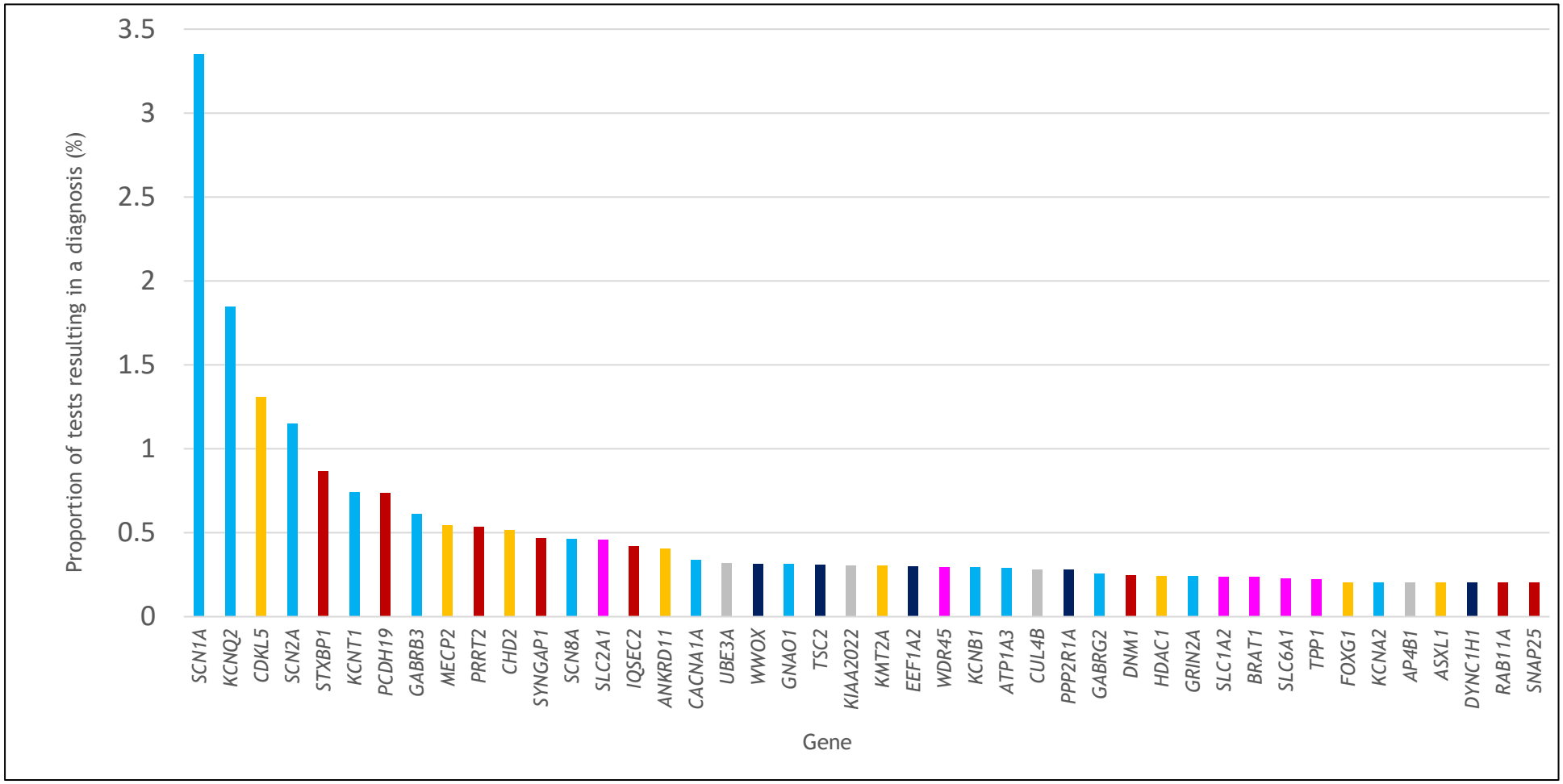


Figure 3.3c: Genes for which >0.2% of tests resulted in a diagnosis

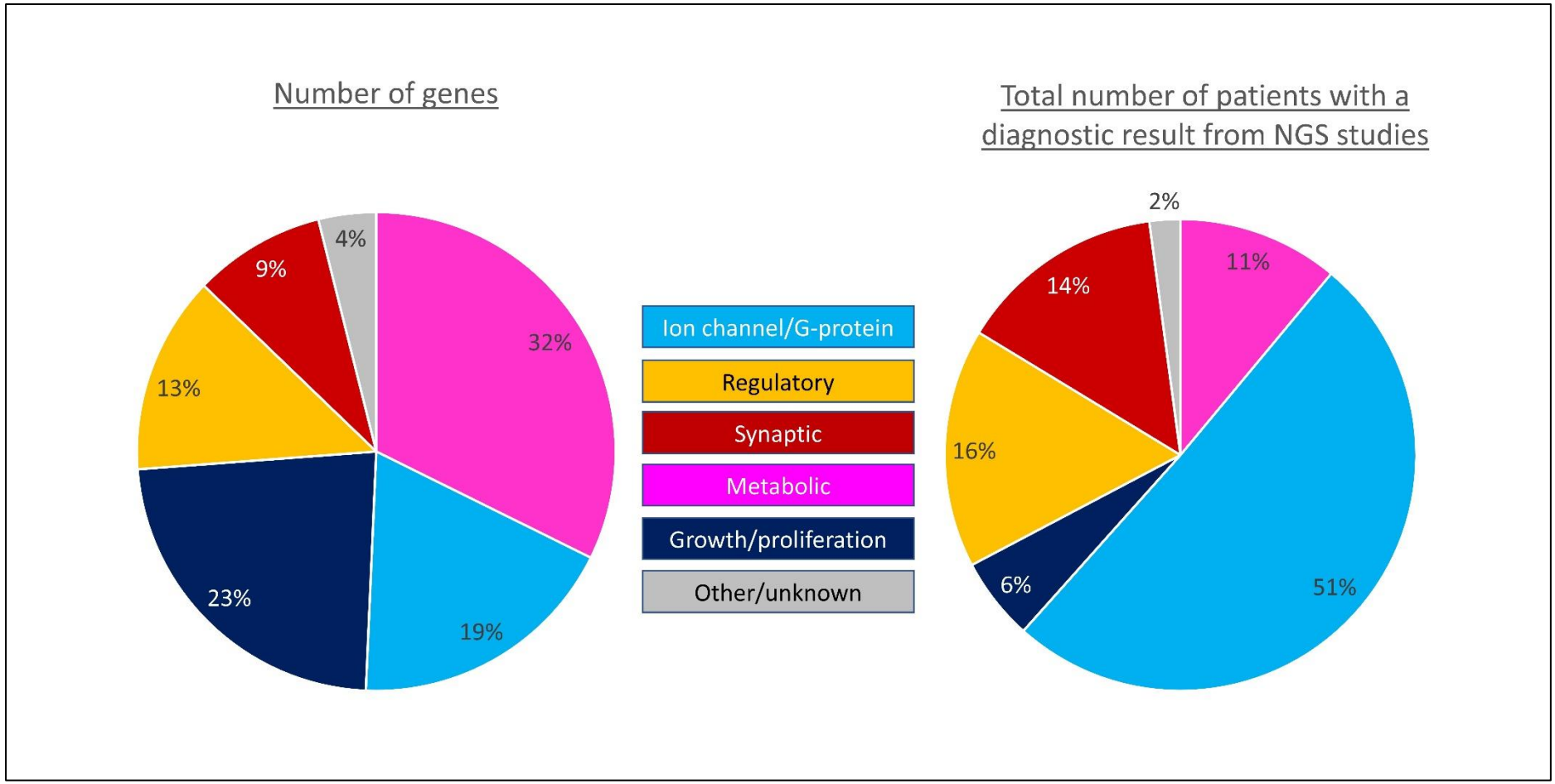


Figure 3.3d: Gene functional groupings: comparison of the diagnostic results from NGS meta-analysis with the total number of epilepsy-associated genes in each group

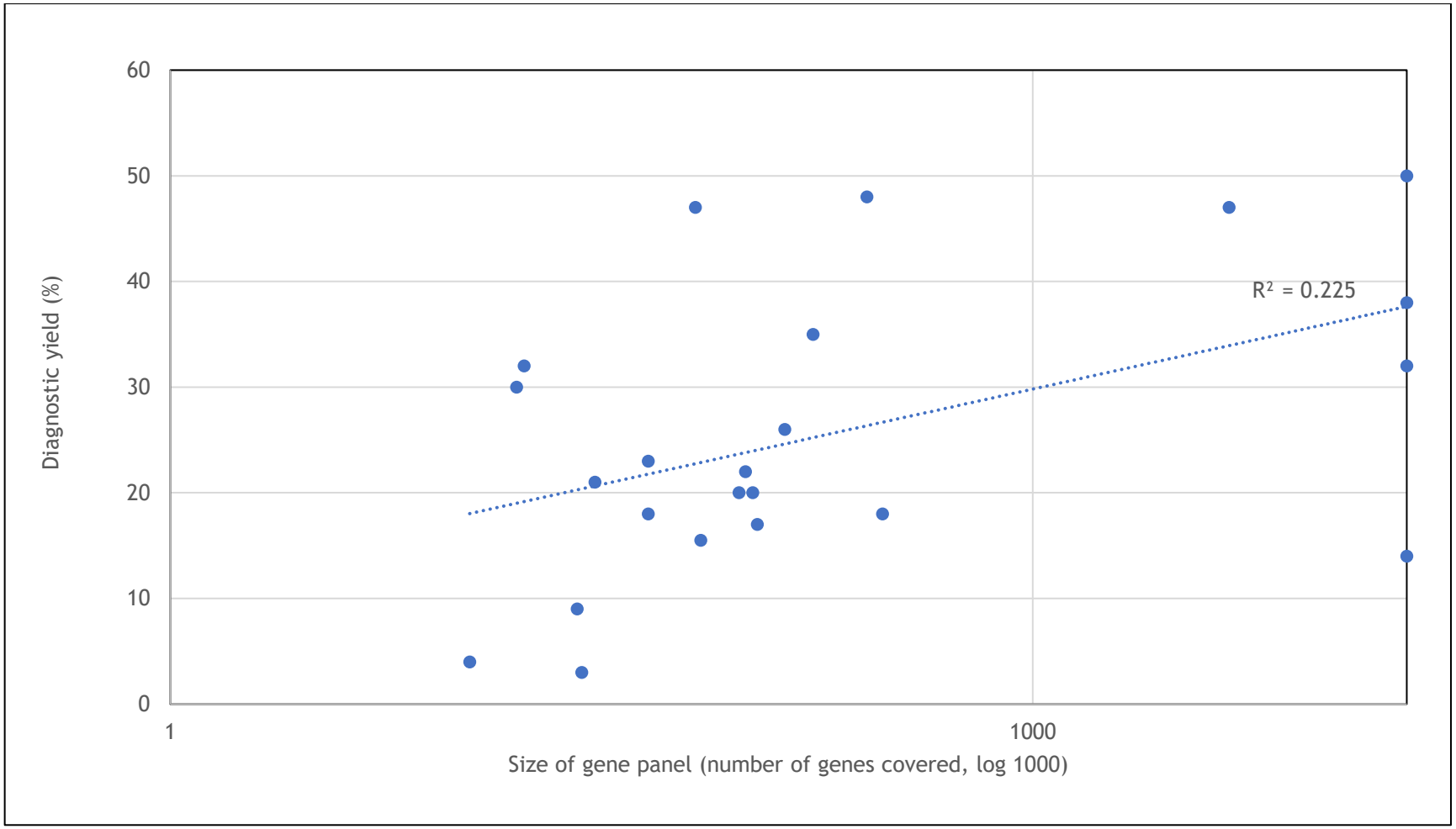


Figure 3.3e: Relationship between diagnostic yield and size of panel in 24 epilepsy NGS studies

3.3.3 Discussion

In this chapter I have shown that there has been a progressive increase in the number of genes associated with epilepsy discovered each year. The first gene, *GCSH* was discovered in 1991. By 2017 there were more than 350. Ion channel genes constitute a minority of the total numerically (19%), but account for more than half of the diagnostic results from NGS panel testing (51%). Patients with epilepsy associated with pathogenic variants in genes involved in metabolic processes may be under-represented in NGS samples, since with these conditions there may be biochemical markers pointing towards a diagnosis. The same may be true for patients with variants in genes associated with growth and proliferation, since these patients may have diagnostic MRI scans precluding them from entry into diagnostic studies. To some extent the categorisation of genes into distinct functional groups is artificial, as will be discussed further in chapter 7. However, it is helpful when considering precision therapy, since evidence relating to ion channel and metabolic genes is strongest.

The diagnostic yield when NGS is applied to epilepsy patients depends on three things: i) the genes selected for investigation; ii) the patients selected for testing, in relation to age of onset, severity of disease, and extent of previous testing; iii) the current state of knowledge about the significance of variants identified.

This meta-analysis does not overcome these limitations, since panel sizes and patient selection have been highly variable between studies. Nonetheless, in tallying the cumulative number of diagnoses between 24 studies it becomes clear that the majority of currently achievable diagnoses lie in a relatively small number of genes. Some important genes will doubtless be underrepresented in this analysis either because patient cohorts selected have not focussed on phenotypes relating to those genes, or because prior testing of patients has excluded them from the testing platform used.

Diagnostic results in this analysis have been counted in a binary manner. The reality is less clear cut. The interpretation of results from NGS studies remains a challenge. As with all diagnostic tests, a balance must be struck between sensitivity and specificity. The information delivered by of large panel investigations is extensive, the human genome is highly variable between individuals, and every human being, healthy or otherwise, carries a large number of rare genetic variants, including variants in genes associated with disease. No single tool is reliable for predicting the pathogenicity of a genetic variant. Computer-based algorithms such as Polyphen (Adzhubei et al., 2010) and SIFT® (Kumar, Henikoff & Ng, 2009) use broad principles such as amino acid conservation, polarity, and pH change to predict the effect of genetic changes on protein function. These have been developed to assist variant interpretation but they have poor specificity (Flanagan, Patch & Ellard, 2010). Relying on the published literature for variant/disease association is complicated by the large number of false associations that have been claimed and published (Ioannidis, 2003).

The American College of Genetics and Genomic Medicine (ACGM) has produced guidelines on variant interpretation (Richards et al., 2015). Application of this guidance is not consistent (Amendola et al., 2016), relying heavily on interrogation of the published literature, public repositories of variant and phenotype data such as ClinVar (National Center for Biotechnology Information, 2018), and genomic datasets of healthy populations such as ExAC (Broad Institute Exome Aggregation Consortium, 2018) and gnomAD (Broad Institute gnomAD Browser, 2018). Even when applying the objective guidance of ACMG, specificity is dependent on the reliability of deposited/published data, and sensitivity depends on the extent to which any real associations have been published or deposited. Ongoing efforts to develop robust databases of genotypes and phenotypes, if successful, will help to overcome these limitations.

4. Systematic review of the existing evidence for genotype-driven precision therapy in epilepsy

4.1 Introduction

In chapter I will appraise the current evidence for precision therapy in single gene epilepsies.

The most rigorous way of assessing the effectiveness of any therapeutic intervention comes from the double blind randomised-controlled trial (RCT). A well-conducted RCT should be able to determine causality of any association, and full double blinding should overcome any bias that would result from placebo response.

RCTs must have large enough numbers of subjects in both therapy and control arms in order to demonstrate and quantify any significant therapeutic effect. In epilepsy there is a huge amount of aetiological and phenotypic heterogeneity, so this also means that a trial group will typically be heterogenous.

The majority of new anti-epileptic drugs (AEDs) that have been introduced to the market in the last 30 years have gained a licence by demonstrating in one or more RCTs that they are superior to placebo when added to existing therapy in patients whose seizures have proved resistant to previous therapeutic regimens. It must also be demonstrated that they do not cause an unacceptable rate, or severity, of adverse effects (Ferlazzo et al., 2017). While this substantial evidence base informs us that many anti-epileptic drugs are efficacious, at least insofar as they reduce seizure frequency when used as add-on therapy, it does not help clinicians to decide which AED to use in any specific clinical scenario, such as age of patient, clinical phenotype, whether or not there has been previous treatment, or genetic test results.

Attempts to answer this last question through RCTs have been few because the more specific the clinical scenario the more difficult it is to recruit sufficient numbers of subjects to demonstrate significance. The unblinded SANAD trials attempted to answer the question as to whether response to first AED differed between patients with focal and generalised epilepsy. These trials showed that in focal epilepsy, the best results, in relation to efficacy and toleration, were seen with Lamotrigine (Marson et al., 2007a), whereas in generalised epilepsy the best results were seen with Sodium Valproate (Marson et al., 2007b). In a double-blind RCT for initial treatment of childhood absence epilepsy, Ethosuximide gave the best results (Glauser et al., 2010). In initial treatment of West syndrome, an unblinded RCT demonstrated that combined treatment with corticosteroids and Vigabatrin controlled infantile spasms significantly more rapidly than corticosteroid treatment alone (O'Callaghan et al., 2017).

The small extent of RCT success in defined epilepsy syndromes, along with high profile anecdotal evidence of dramatic treatment responses in certain highly specific scenarios, such as the case of Charlotte Figi's response to Cannabidiol (Scientific American, 2017), has driven popular demand for a precision approach to epilepsy therapy (Berkovic et al., 2015). However the dilemma remains that the more precise you want treatment to be, the harder it is to find enough patients for trials to generate evidence.

Since any individual single-gene epilepsy is very rare, generation of an evidence base for genotype-driven therapy is particularly challenging. Various lower grade approaches to evidence generation have been taken and these will now be reviewed systematically.

4.2 Methods

I used a Medline literature search for publications reporting precision therapy in epilepsy, by using the following search terms:

([epilepsy] or [seizure]) and [gene]. Search date April 18th 2018; 8534 results

Abstracts of papers whose titles suggested made reference to therapy response in a specific genetic epilepsy were reviewed.

4.3 Results

Of 7250 results from the search, 268 abstracts were reviewed, and 43 papers describing precision therapy were included in the review. Eight distinct categories of evidence were identified. At the weakest end of this evidence base were case reports and case series. At the strongest end were RCTs. It must be born in mind that some approaches that have little supporting evidence may in fact be the most important, for example in the treatment of epilepsies associated with inherited metabolic diseases. In many of these cases, precision therapy is so biologically plausible that no clinical evidence beyond case reports has been sought.

4.3.1 Outstanding case reports and small case series

There have been numerous reports documenting a relatively unexpected treatment success in a genetically determined epilepsy. Such reports are hugely vulnerable to publication bias since authors are more inclined to publish results of successes than failures. Nonetheless the reports of successes, particularly if biologically plausible, may inform more robust projects such as clinical trials, as did the Charlotte Figi Cannabidiol story, which triggered an RCT of Cannabidiol in Dravet syndrome.

Remarkable treatment successes in small numbers of cases could easily be coincidental, or even related to factors other than the genetic cause of the epilepsy. In some genetic epilepsies a dramatic change in seizure frequency may reflect the natural history of the condition rather than a treatment response. For example, in *KCNQ2* encephalopathy, though the associated developmental impairment may be severe, seizures themselves are often self-limited. Seizure frequency will fluctuate in all patients, so it is important to consider the timing and persistence of reported seizure frequency changes.

Table 4.3.1: Precision-therapy evidence: case reports and small case series.

Paper	Gene (inheritance) Variant and phenotype	Therapy	Summary	Benefit claimed beyond seizures	Biological plausibility
(Mahajnah et al., 2016)	ALDH7A1 (AR) Biallelic variants cause pyridoxine dependency	Pyridoxine supplementation + Lysine restriction	Patient diagnosed with *ALDH7A1*-related pyridoxine dependency at 3 months of age. Commenced pyridoxine supplementation and Lysine restriction and has been seizure free until the age of 44 months	Yes. Normal development reported	*ALDH7A1* encodes antiquitin, deficiency of which leads to pyridoxine depletion and Lysine accumulation.
(Byers et al., 2016)	CACNA1A (AD) *De novo* S1373L variant causing early onset developmenal and epileptic encephalopathy (EODEE)	Lamotrigine	6 year old child with epileptic encephalopathy having experienced no benefit from 2 previous AEDs, had a "precipitous decrease in seizure frequency and severity", which persisted over one year	Yes. Improvement in activity level, interaction and alertness reported	Unknown
(Dilena et al., 2016)	STXBP1 (AD) *De novo* nonsense variant causing EODEE	Levetiracetam	1 month old baby with epileptic encephalopathy and multiple clonic seizures per day, resistant to Phenobarbital and Phenytoin became seizure free with normalised EEG after starting Levetiracetam	No. Patients had severe developmental delay at 33 months of age	Possible. Both syntaxin binding protein (product of *STXBP1*) and SV2A (the target of Levetiracetam) are involved in synaptic vesicle docking at the presynaptic membrane.
(Bearden et al., 2014)	KCNT1 (AD) *De* novo R426Q variant causing epilepsy of infancy with migrating focal seizures	Quinidine	2 year old child with drug-resistant focal epilepsy, having failed 9 previous AEDs, became seizure free after commencing Quinidine therapy	Yes. Improved head control, increased spontaneous movements and alertness, and spoke her first words	*KCNT1* encodes a neuronal potassium channel. Quinidine is a potassium channel blocker.
(Joshi et al., 2016)	*PIGA* (XLR) Maternally inherited N179Y variant causing EODEE	Ketogenic Diet	2 brothers with epilepsy resistant to multiple AEDs achieved sustained seizure freedom on the Ketogenic Diet	Yes. Some developmental progress reported.	Unknown
(Foster et al., 2017)	*SCN2A* (AD) *De novo* missense variants causing EODEE	Mexiletine	2 unrelated cases with epilepsy resistant to multiple AEDs achieved improved seizure control on Mexiletine	No. Severe developmental impairment reported in both cases	*SCN2A* encodes a neuronal sodium channel. Mexiletine is a sodium channel blocker.
(Higurashi et al.,	PCDH19 (XLRF)	Intermittent	5 cases. Shorter seizure cluster	No	Unknown

2015)	Epilepsy with cluster seizures and developmental delay	corticosteroids	duration was reported after single dose IV methylprednisolone on 16 occasions between 5 patients.		
(Boerma et al., 2016)	*SCN8A* (AD) *De novo* missense variants causing early onset epileptic encephalopathy	Phenytoin	4 cases with drug-resistant epilepsy. 3/4 became seizure free following introduction of Phenytoin. In 2 cases seizures recurred on withdrawal of Phenytoin.	Yes. 2/4 reported to have developmental improvement	*SCN8A* encodes a neuronal sodium channel. Phenytoin is a sodium channel blocker.
Abbreviations – AR – autosomal recessive, AD – autosomal dominant, XLR – X-linked recessive, XLRF – X-linked restricted to females					

4.3.2 Larger case series

Larger case series that report treatment response provide more robust evidence than isolated case reports since at least they demonstrate effects that are reproducible. It is often a challenge to gather a cohort of patients with an extremely rare disease, so these reports typically describe a group of patients that is quite heterogenous in phenotype severity, previous AED treatment, and seizure frequency. Data for these series is invariably collected retrospectively, so follow-up is non-standardised and seizure frequency data may be subjective. The clinical significance of positive results from treatment is difficult to quantify due to absence of controls. For example, where the use of Retigabine in *KCNQ2*-related epilepsy is reported, the seizure reduction rate of 36% (Millichap et al., 2016) may in fact be comparable to that which would be seen with a number of conventional AEDs. Where a significant proportion of cases experience a dramatic response in what is typically a drug-resistant epilepsy, as seen with Fenfluaramine in *SCN1A*-related epilepsy, case series data can be very informative (Ceulemans et al., 2012).

Table 4.3.2: Precision-therapy evidence: larger case series

Paper	Gene (inheritance) Phenotype	Therapy	Number of patients	Outcomes measured	Effect seen	Benefit claimed beyond seizures?	Biological plausibility
(Mercimek-Mahmutoglu et al., 2006)	*GAMT* (AR) Guanidinoacetate methyltransferase deficiency	Creatinine supplementation	27	Reported improvement in seizure control, by clinician	19/27 had improved seizure control	Movement disorder improved in most patients but intellectual disability remained unchanged	*GAMT* deficiency results in impaired cerebral creatinine synthesis
(Khaikin et al., 2018)	*GAMT* (AR) Guanidinoacetate methyltransferase deficiency	Creatinine and Ornithine supplementation	16	Seizure freedom, reported by clincian	10/16 became seizure - free	2/5: movement disorder reportedly improved 2/16 cognition reportedly improved	*GAMT* deficiency results in impaired cerebral creatinine synthesis
(Mills et al., 2014)	*PNPO* (AR) Pyridoxal-phosphate-dependent seizures	Pyridoxal-5-phosphate	10	Seizure freedom, reported by clinician	8/10 were seizure free within 3 days	Not clear. Most had developmental delay	*PNPO* causes deficiency of pyridoxal-5-phosphate
(Crespel et al., 2017)	*CSTB* (AR) Progressive myoclonus epilepsy (Unvericcht-Lundborg disease)	Perampanel	11	Seizure type and frequency, and any other symptoms, as reported by clinician	Of 5 patients with generalised tonic-clonic seizures, all became seizure free on Perampanel therapy	9 patients had “clear improvement in myoclonus”. 5 patients had improvements in mobility	Unknown
(Lim et al., 2017)	CDKL5 (X-linked) Presents with early childhood onset epilepsy and developmental delay	Ketogenic Diet	104	Reported perception of seizure reduction, by caregiver	59% reported seizure reduction. 8% reported seizure exacerbation. 36% had severe side-effects	Improvement in alertness in 18%.	Unknown
(Millichap et al., 2016)	*KCNQ2* (AD) Epileptic encephalopathy cases	Retigabine	11	Reported perception of seizure reduction, by caregiver	4/11 (36%) reported improvement in seizure control. 5/11 had urinary retention.	5/11 (45%) reported improvement in development or alertness	Retigabine is an opener of the same potassium channel that *KCNQ2* encodes
(Kass et al., 2016)	SLC2A1 (AD) Glut1-deficiency	Ketogenic Diet	82	Caregiver reported estimation of seizure	38/82 (46%) had seizure freedom, 66/83	84% reported that movements and/or	Ketogenic Diet provided the brain

	related epilepsy			reduction	(80%) had ≥90% seizure reduction, and 78 (95%) had ≥50% seizure reduction at latest follow up	cognition was much better	with an alternative energy source to glucose
(Ceulemans et al., 2012)	*SCN1A* (AD) Dravet syndrome	Fenfluramine	11	Clinician-reported seizure freedom.	8/11 (73%) had complete seizure freedom.	Not reported	Unknown
(Anagnostou et al., 2016)	*POLG1* (AR)	Avoidance of Sodium Valproate	43	Clinician-reported hepatotoxicity	22/43 (51%) patients with *POLG*-related Alpers syndrome developed Sodium Valproate-related hepatotoxicity.	N/A	Unknown

4.3.3 Retrospective comparative efficacy analysis

In these studies, clinical data from patients are typically collected from multiple centres and retrospective seizure response data is collated. As with all retrospective case series, response data is rarely objective and may rely purely on a vaguely recalled clinical impression. With retrospective comparative analysis all therapies are analysed on an essentially equal footing, so there is less ascertainment bias than with case series. However, there is likely to be recall bias in favour of treatments most recently tried, since these will have had less time to fail. Some AEDs are far more popular than others and it is difficult to determine from these studies the utility of a treatment that is used in a small number of patients, even if the response data is good in those patients for whom it was used. The critical impact that timing of data acquisition can have on results is illustrated in the study on *CDKL5* by Müller et al. (Müller et al., 2016) which reported a 3-month response rate to Vigabatrin of 8/25 (32%) and a 12-month response rate of 1/25 (4%). Though these studies rarely quantify the effect of any given therapy, they can give a sense of which treatment may be relatively more useful or more harmful. The best evidence that sodium channel blocking medications frequently exacerbate seizures in *SCN1A*-related epilepsy comes from this type of study (Brunklaus et al., 2013), though this has recently been backed up by animal studies (Hawkins et al., 2017).

Table 4.3.3: Precision-therapy evidence: retrospective comparative efficacy analysis

Paper	Gene (inheritance)	Number of patients	Outcomes measured	Effect seen	Biological plausibility
(Ebrahimi-Fakhari et al., 2015)	PRRT2 (AD)	24	Seizure freedom, reported by clinician	23/24 (96%) cases became seizure free on treatment with **Carbamazepine**	Unknown
(Brunklaus et al., 2012)	*SCN1A* (AD)	60	Seizure exacerbation, reported by clinician	In 36/60 (60%) there was a history of seizure exacerbation with **Carbamazepine** treatment In 26/60 (43%) there was a history of seizure exacerbation with **Lamotrigine** treatment	*SCN1A* is preferentially expressed in inhibitory interneurons so blockade of these channels may exacerbate neuronal hyperexcitability
(Lotte et al., 2017)	*PCDH19* (XLRF)	58	Seizure frequency reduction by ≥50% at 3 months after commencing treatment, reported by clinician	For treatments taken by more than 20 patients, the greatest response rates were seen with **Clobazam** (68%) **Sodium Valproate** (44%) and **Phenobarbital** (40%)	Unknown
(Müller et al. 2016)	*CDKL5* (XLR)	39	Seizure frequency reduction at 12 months after commencing treatment, reported by clinician	For treatments taken by more than 20 patients, the greatest response rates were seen with **Sodium Valproate** (9%) and **Lamotrigine** (8%)	Unknown
(Pisano et al., 2015)	*KCNQ2* (AD)	15	Seizure freedom at 2 weeks after commencing treatment, reported by clinician	6/15 (40%) of cases were seizure free on **Carbamazepine** treatment 5/15 (33%) were seizure free on **Phenytoin** treatment	Reduced sodium influx is likely to stabilise neuronal cell membranes that have reduced potassium efflux due to *KCNQ2* loss-of-function
(Herbst et al., 2016)	PAFAH1B (AD)	22	Perception of benefit, reported by clinician and caregiver	All 9 families whose child had been treated with **Lamotrigine** reported it to be beneficial as did 7/8 clinicians 15/17 caregivers reported that **Sodium Valproate** was beneficial as did 11/12 clinicians	Unknown
(Wolff et al., 2017)	*SCN2A* (AD)	201	Seizure freedom, reported by	In cases with seizure onset before 3	Early onset cases of *SCN2A*-

			clinician (timing not specified)	months of age 12/19 (63%) patients treated with sodium channel blocking medications were seizure free, compared with 6/86 (7%) of patients not treated with sodium channel blocking medications In cases with seizure onset after 3 months of age 2/39 (5%) patients treated with sodium channel blocking medications were seizure free compared with 12/47 (26%) of patients not treated with sodium channel blocking medications	related epilepsy are likely to be the result of gain-of-function variants Late onset cases are likely to be the result of loss-of-function variants
(Johannesen et al., 2018)	SLC6A1 (AD)	34	Seizure freedom, reported by clinician (timing not specified)	10/15 (67%) of patients treated with **Sodium Valproate** became seizure free	*SLC6A1* encodes a cerebral GABA-transporter Sodium Valproate potentiates GABAergic neurotransmission

Abbreviations – GABA – Gamma-amino-butyric acid

4.3.4 Single cell models

For ion channel-related epilepsies single-cell models have become the gold standard for defining the functional effects of a genetic variant and proving pathogenicity. The most commonly used models involve transfecting cell lines (for example human embryonic kidney cells or *Xenopus laevis* oocytes) with the variant being investigated, and then using the patch clamp technique (Neher & Sakmann, 1976) to measure the properties of ion currents compared with those in wild-type cells (Shalaby et al., 1997). In the same models, potential therapeutic agents can be introduced *in vitro* to investigate whether they normalise any electrophysiologic changes associated with specific genetic variants. In order to translate this back into practice, medications that are successful *in vitro*, if already licensed for use, may then be tried *in vivo*, typically in a small number of cases. At least as a proof-of-concept this approach has been shown to yield useful information, as exemplified by *GRIN2A, GRIN2D, KCNT1* and *SCN8A*. Different missense variants in the same gene can have divergent effects on ion channel function, so the translatability of one single cell-model success to other cases with different variants in the same gene is uncertain. The extent to which a single cell model is able to represent the functional effect of complex neuronal circuits also has to be questioned.

Table 4.3.4: Precision-therapy evidence: single cell models +/- translation to clinic

Paper	Gene (inheritance) Variant and phenotype	Therapy/ies	Model	Effect seen in humans	Benefit beyond seizures?	Biological plausibility
(Pierson et al., 2014)	GRIN2A (AD) *De novo* L812M variant Patient with severe drug-resistant epilepsy with onset at 2 months and severe developmental delay	Memantine	***Xenopus laevis* oocyte model** in which the L812M variant was transfected. Memantine inhibited excessive NMDA receptor activity.	Yes Trial of Memantine in the patient resulted in a seizure reduction from an average of 11.1 episodes per week to 3.3	No Cognitive ability remained unchanged	*GRIN2A* encodes an NMDA receptor subunit and Memantine is an NMDA receptor antagonist
(Li et al., 2016)	GRIN2D (AD) *De novo* V667I variant in 2 unrelated patients with infantile onset drug-resistant epilepsy and developmental delay	Memantine Ketamine Magnesium	***Xenopus laevis* oocyte model** The V667I variant was transfected Memantine Ketamine and Magnesium inhibited excessive NMDAr activity	Yes Trial of Memantine resulted in mild to moderate improvement in seizure burden in both patients	Yes Improvement in development reported	*GRIN2D* encodes an NMDA receptor subunit and Memantine is an NMDA receptor antagonist
(Milligan et al., 2014)	*KCNT1* (AD) 7 different disease associated *KCNT1* mutations from patients with either epilepsy of infancy with migrating focal seizures or autosomal dominant nocturnal frontal lobe epilepsy	Quinidine	Xenopus laevis oocyte model in which the 7 variants were transfected Gain-of-function properties associated with the variants were normalised by Quinidine	Not tried	N/A	*KCNT1* encodes a neuronal potassium channel Quinidine is a potassium channel blocker
(Barker et al., 2016)	SCN8A *De novo* I1327V variant associated with EODEE	Phenytoin	Human dorsal root anglion ND7/23 cells transfected with the I1327V variant Gain-of-function properties associated with the variant were normalised by Phenytoin	Not tried	N/A	*SCN8A* encodes a neuronal sodium channel Phenytoin is a sodium channel blocker

Abbreviations - NMDAr – N-Methyl-D-Aspartate receptor

4.3.5 Whole organism models

Whole organism models more accurately reflect both the complex effects of genetic variants on the brain and may also represent clinical phenotypes, though in fact it seems that many animal models of human genetic epilepsies do not have seizures. In two very rare recessively inherited epilepsies – *ATAD1* and *STRADA* – successful treatment of a genetically modified mouse in which both functional copies of a gene have been removed has been followed by successful treatment in humans (Ahrens-Nicklas et al., 2017; Parker et al., 2013). This approach to precision treatment is highly resource intensive and may be difficult to apply to conditions where an animal model either does not exist, or does not express the desired phenotype.

Table 4.3.5: Precision therapy evidence: whole organism models +/- translation to clinic

Paper	Gene (inheritace) Variant and phenotype	Therapy	Model and effect	Effect seen in humans	Benefit beyond seizures?	Biological plausibility
(Ahrens-Nicklas et al., 2017)	*ATAD1* (AR) Patients with biallelic truncating variants present with neonatal onset progressive extreme hypertonia encephalopathy and seizures (reported in a consanguineous family with homozygous nonsense variants)	**Perampanel** (AMPA receptor antagonist)	Knockout mouse model Administration of Perampanel reversed behavioural effects, normalised brain MRI abnormalities prevented seizures and prolonged survival	2 patients with homozygous nonsense started on Perampanel Effect on seizures not reported but in both cases EEG normalised and other AEDs were weaned	Yes Both cases had temporary improvement in tone	*ATAD1* regulates AMPA receptor-dependent synaptic plasticity (Zhang et al., 2012)
(Parker et al., 2013)	STRADA (AR) Patients with biallelic truncating variants present with PMSE (polyhydramnios megalencephaly symptomatic epilepsy) syndrome	**Rapamycin (**mTOR inhibitor)	Knockout mouse model Rapamyin rescued aberrant cortical lamination and heterotopia	5 patients with *STRADA*-related PMSE treated with Rapamycin One case reducing from 180 seizures per year to seizure free No seizure exacerbation and no adverse effects reported in any cases	Yes Improvement in receptive language	*STRADA* encodes an upstream inhibitor of mTOR (Parker et al., 2013)
(Marguet et al., 2015)	*KCNQ2* (AD)	**Bumetanide** (NKCC1 antagonist)	Mutant mouse model Transient treatment with Bumetanide during neonatal period restored adult wildtype behaviour	Not tested	N/A	*NKCC1* blockade is likely to stabilise neuronal cell membranes that have reduced potassium efflux due to *KCNQ2* loss of function
(Hawkins et al., 2017)	*SCN1A* (AD)	Clobazam	Heterozygous knockout mouse model Screening of 9 AEDs in a mouse model of Dravet syndrome Clobazam shown to be most effective	Not in this study but Clobazam is a frequently used treatment in Dravet syndrome	N/A	*SCN1A* loss-of-function is thought to disproportionately affect inhibitory GABAergic neurones

Abbreviations – AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; mTOR – mammalian target of Rapamycin

4.3.6 High throughput screening

High-throughput screening for medication response in either a whole organism model or single cell model of genetic epilepsy offers a system for identifying potentially therapeutic compounds in a largely hypothesis-free manner. In a zebrafish model of *SCN1A*-related epilepsy 320 food and drug administration (FDA) approved compounds were screened for effect on zebrafish seizures and behaviour. Surprisingly, the most efficacious medication was an antihistamine called Clemizole (Baraban, Dinday & Hortopan, 2013). If a trial were to demonstrate efficacy of Clemizole in humans this could be brought to market relatively rapidly since it is already an approved drug.

Table 4.3.6: Precision-therapy evidence: high throughput screening

Paper	Gene (inheritance)	Model	Results	Effect seen in humans	Biological plausibility
(Baraban, Dinday & Hortopan, 2013)	SCN1A (AD)	**Zebrafish mutant model** of *SCN1A* in which the fish have spontaneous seizures Screening of 320 compounds for effect on zebrafish seizures	18/350 compounds significantly inhibited spontaneous seizures The largest effect was seen with **Clemizole** (an antihistamine)	Not tried	Unknown
(Atkin et al., 2018)	S*CN8A* (AD)	**Human Embryonic Kidney cells** expressing the disease-associated R1871Q variant demonstrated a leftward shift in sodium channel activation 90 compounds screened for effect on channel function	**Amitryptypline**, **Carvedilol**, **Nivladipine** and **Carbamazepine** all demonstrated concentration-dependent inhibition of sodium currents	Not tried	Carbamazepine is a sodium channel blocker

4.3.7 Open-label trials

There are situations where, for either practicality or safety and monitoring reasons, blinded trials cannot be performed and an open label approach is used, as with the SANAD trials. Often such trials still have a control group, but often one which is given either a different dose of the same treatment, or an alternative treatment. Placebo effect is less of a concern where the objective is to compare two treatments for which there is no pre-conceived idea as to which is likely to be more efficacious.

Table 4.3.7: Precision-therapy evidence: open label trials

Paper	Gene Phenotype	Therapy	Number of patients	Methodology	Results	Biological plausibility
(Smith-Hicks et al., 2017)	*MECP2* (AD) Rett syndrome	**Dextramethorphan** (NMDA receptor antagonist)	38	Patients randomised to 3 different doses of Dextramethorphan Seizure frequency at 6 months compared with baseline Formal neuropsychology assessments carried out at baseline and 6 months	Statistically significant dose-dependent reduction in seizures receptive language and behaviour	Patients with Rett syndrome demonstrate high levels of CSF glutamate (NMDAr agonist) (Lappalainen & Riikonen, 1996)
(Krueger et al., 2013)	TSC1/TSC1 (AD) Tuberous Sclerosis Complex	Everolimus (mTOR inhibitor)	20	Open label uncontrolled trial Seizure frequency over 12 week treatment period compared with 4 week baseline	12/20 (60%) had a ≥50% reduction in seizure frequency Statistically significant improvements in questionnaire measurements for child behaviour and quality of life	*TSC1* and *TSC2* negatively regular mTOR Everolimus is an mTOR inhibitor
(Hess et al., 2016)	TSC1/TSC1 (AD) Tuberous Sclerosis Complex	Cannabidiol	18	Open label uncontrolled trial Seizure frequency at 3 months compared with 1 month baseline	9/18 (50%) had a ≥50% reduction in seizure frequency 7/18 (38% experienced seizure aggravation) 13/18 (72%) had reported cognitive or behavioural improvements on treatment	Unknown

4.3.8 Double blind RCTs

Gold standard clinical evidence will always rely on double-blind RCTs. In genetic epilepsy there have been four to date. In Dravet syndrome, add on Stiripentol was shown to be significantly more efficacious than placebo though it is not known how many of these patients had an *SCN1A* variant (Chiron et al., 2000). In a larger RCT of Cannabidiol in which 93% of participants had a pathogenic *SCN1A* variant, a more modest response rate was seen in the intervention arm and a more significant placebo response was observed, though the difference was still significant (Devinsky et al., 2017). In tuberous sclerosis complex (TSC), caused by variants in the *TSC1* or *TSC2* genes, the mTOR inhibitor Everolimus has been shown in RCTs to be effective for treating subependymal giant cell astrocytoma (SEGA) (Krueger et al., 2010) and renal angiomyolipomata (AML) (Bissler et al., 2013) associated with TSC. The EXIST-3 trial demonstrated that in patients with TSC and drug-resistant epilepsy Everolimus was significantly more efficacious than placebo at reducing seizure frequency (French et al., 2016). This study neatly provided a proof-of-principal that a focus on treating the underlying cause can be effective in controlling seizures. Whilst EXIST-3 was not able to assess any impact of Everolimus on non-seizure aspects of TSC, a previous open label trial involving 20 patients aged 2-21 years treated with Everolimus over 12 weeks demonstrated significant improvements in validated measures of behaviour (Nisonger Child Behavior Rating Form) and quality of life (Quality of Life in Childhood Epilepsy questionnaire, QOLCE) (Krueger et al., 2013) (reported in table 4.3.8).

Mullen et al.'s crossover RCT of Quinidine in *KCNT1*-related epilepsy demonstrated that even with very small numbers of participants and short-term seizure measurements, trials can be sufficiently powered to generate evidence, provided baseline seizure frequency is high enough. Despite some encouraging evidence for the use of Quinidine in *KCNT1*-related epilepsy from case reports (Bearden et al., 2014) and single cell models (Milligan et al., 2014), the more robust approach of the RCT demonstrated that, at least in an adult and teenager group with

autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), there was no detectable benefit from Quinidine treatment (Mullen et al., 2018).

Table 4.3.8: Precision-therapy evidence: double blind RCTs

Paper	Gene Phenotype	Therapy	Number of patients	Methodology	Results	Biological plausibility
(Chiron et al., 2000)	*SCN1A**(AD) Dravet syndrome	Striripentol	41 (21 Stiripentol 21 placebo)	Placebo-controlled RCT of add on Stiripentol to existing therapy Seizure frequency at 2 months compared with baseline	71% of patients in the Stiripentol group had a ≥50% reduction in seizure frequency compared with 5% in the placebo group ($p < 0.05$)	Unknown
(Devinsky et al., 2017)	SCN1A ** (AD) Dravet syndrome	Cannabidiol	120 (61 Cannabidiol 59 placebo)	Placebo-controlled RCT of add on Cannabidiol to existing therapy Convulsive seizure frequency over 14 week treatment period compared with 4 week baseline	Median frequency of convulsive seizures per month reduced from 12.4 to 5.9 (39%) in the CBD group compared with 14.9 to 14.1 (13%) in the placebo group ($p = 0.01$) Caregiver Global Impression of Change was significantly improved in the Cannabidiol group compared with the placebo group (62% v 34%, $p<0.05$)	Possible cannabinoids shown to target sodium channels (Patel et al., 2016)
(Mullen et al., 2018)	*KCNT1* (AD) Autosomal dominant nocturnal frontal lobe epilepsy	Quinidine	6 (crossover trial)	Order-randomized blinded placebo-controlled, crossover trial Seizure frequency on continuous video-EEG monitoring measured	No difference in seizure frequency observed between intervention period and placebo period 2 cases developed prolonged QT interval on ECG	N/A
(French et al., 2016)	*TSC1* and *TSC2* (AD) Tuberous Sclerosis Complex	Everolimus	366 (130 high dose 117 low dose 119 placebo)	Random and double- blinded assignment to low dose Everolimus high dose Everolimus, or placebo Seizure frequency during 12 week maintenance period compared with baseline	40% of patients in the high dose group, 28% in the low dose group, and 15% in the placebo group experienced a ≥50% seizure reduction ($p < 0.05$)	Everolimus is an mTOR inhibitor *TSC1* and *TSC2* negatively regulate mTOR

* Inclusion in this trial was based on a clinical diagnosis of Dravet syndrome. The number of cases with *SCN1A* mutations is not known

** Inclusion in this trial was based on a clinical diagnosis of Dravet syndrome. 111/120 (93%) had pathogenic *SCN1A* variants

4.3.9 Discussion

Whilst the RCT is the gold standard for generating clinical evidence, it has not often been applied to the therapy in specific genetic epilepsies. Moreover, the small number of trials that have been conducted have had significant limitations. All single-gene epilepsies are extremely rare recruitment to genotype-specific RCTs will necessarily be challenging. In order to produce useful evidence with small numbers of participants, these participants need to have a consistently high frequency of seizures at baseline. It follows that participants in such RCTs typically have drug-resistant epilepsy and consequently the only question that is typically answered is whether a treatment is efficacious as an add-on therapy. A treatment that is shown to be efficacious as add-on therapy may not have the same benefit if used as a first line treatment. In both the Stiripentol and Cannabidiol trials for Dravet Syndrome, it is possible that some of the clinical effect seen in the intervention group was through drug interactions resulting in higher levels of Clobazam (Luszczki et al., 2010) and Clobazam metabolites respectively (Gaston et al., 2017). Another limitation of the RCTs done so far is that they have lacked control groups without the genetic diagnosis, so it is not possible to know whether any anti-epileptic effect is specific to the genotype or a more general anti-epileptic effect. Cannabidiol in fact appears to be more efficacious in a group of drug-resistant patients without *SCN1A* variants than it was in the Dravet syndrome trial, so its effect is unlikely to be specific to *SCN1A* (Devinsky et al., 2018).

The non-RCT evidence presented here also has major limitations, the most prominent of which are publication bias and ascertainment bias. Case reports and case series can be useful in terms of guiding efforts to generate more definitive evidence but must be used with great caution as evidence to guide treatment in themselves.

Though the advent of NGS has heralded a substantial increase in the proportion of patients for whom a precise genetic cause can be identified, optimism that this will translate to more effective precision therapy approaches has not yet been

realised. In Dravet syndrome, establishment of the clinical phenotype appears to be as strong a predictor of Stiripentol response as the identification of an *SCN1A* mutation (Cho et al., 2018). Clinical factors such as seizure type and age of onset may override the importance of genetic aetiology in relation to the utility of specific therapies.

The idea that identifying a genetic cause in itself is sufficient to guide treatment approach is likely to be over simplistic. Examples of cases that buck the trend of established evidence for a particular therapy include patients with *SCN1A*-related epilepsy who appear to benefit from sodium channel blocking treatments (Dalic et al., 2015, Takaori et al., 2017).

Variables beyond the genetic diagnosis itself that are likely to influence treatment include the precise functional effects of a variant, the age of the patient at the time of treatment, and the impact of genetic modifiers. In *SCN2A*-related epilepsy, response to sodium channel blocking medications appears to be dependent on predicted functional effect of the *SCN2A* variant. Patients with gain-of-function variants benefit from these therapies and patients with loss-of-function variants do not. Age of seizure onset is a strong predictor of which functional class of variant a patient has (Wolff et al., 2017). In *KCNT1*-related epilepsy response to Quinidine appears to depend on the age of the patient at the time of treatment, with most treatment successes being in the younger group (Abdelnour et al., 2018). Age-dependent treatment response is not surprising since many epilepsy-associated genes have a highly age-dependent expression profile. In *KCNQ2*-related epilepsy, mouse model evidence suggests that the ideal therapeutic window for treatment would appear to be the very early neonatal period (Marguet et al., 2015).

When considering genetic modifiers, genetic influences on susceptibility to adverse drug reactions (Amstutz et al., 2014), as well as determinants of drug absorption, distribution, action, metabolism and elimination, are all relevant (Chen et al., 2014; Shaheen et al., 2014; Löscher et al., 2009; Saygi et al., 2014; Thompson, Kahlig & George, 2011). To fully understand any relationship between genotype

and therapy response, a truly comprehensive approach to genotyping must be considered, taking into account all of the above variables. Because all these variables make an individual genotype unique, ultimately the best evidence may not come from RCTs but from longitudinal studies. If such studies are to be of any value, it is critical that clinical and genetic data are gathered objectively, comprehensively, and that this data is shared between groups in order to generate large datasets.

5. Prospective study of selected gene testing in a Scottish population-based cohort of children < 3 years with seizures

5.1 Introduction

As discussed in section 3.4, existing studies of genetic testing in epilepsy are limited in what they can tell us about the epidemiology of the monogenic epilepsies, since the cohorts studied have typically been selected via a referral process, rather than obtained from a population-based sample. Patients selected for inclusion in these studies may not represent the complete spectrum of epilepsies observed in the population due to conscious or unconscious biases of referrers, who may expect that those with more severe disease, early onset, or additional comorbidity to be more likely to have an identifiable genetic cause. Conversely, such cohorts may be depleted of patients with more common genetic causes of epilepsy due to such diagnoses having already been made through single gene (Sanger) sequencing. If we want to understand how patients may benefit from genetically stratified therapy we must first understand the epidemiology of the monogenic epilepsies.

There have been previous attempts to estimate the incidence of two monogenic epilepsies: *SCN1A* and *SLC2A1*.

Pathogenic *SCN1A* variants are associated with a spectrum of childhood-onset epilepsies and febrile-seizure disorders. At the severe end of this spectrum is a phenotype called Dravet syndrome. Though there remains to be an international consensus on an absolute clinical definition of Dravet syndrome, it is generally accepted that the following are essential components of the diagnosis: i) onset of febrile or afebrile seizures in the first 12 months of life; ii) developmental slowing or regression following seizure-onset; iii) progression to a drug-resistant epilepsy, with multiple seizure types (Wirrell et al., 2017). In 80% of Dravet syndrome cases, there is an associated pathogenic variant in *SCN1A* (Depienne et al., 2009; Zuberi

et al., 2011). Hence the clinical condition has verged on becoming synonymous with the gene.

There have been two attempts to determine the incidence of *SCN1A*-related Dravet syndrome. Wu et al. reviewed the electronic notes of 125,547 infants born at Kaiser Permanente northern California from 2007-2010. Records of all those who had had two or more seizures before the age of 12 months *and* who were prescribed anti-epileptic medication by the age of 24 months were reviewed. Objective criteria for a diagnosis of Dravet syndrome were used and defined as follows: i) normal or near-normal cognitive and motor development before the onset of seizures; ii) seizure semiology consisting of myoclonic, hemiclonic, or generalised tonic-clonic seizures; iii) At least two seizures lasting longer than 10 minutes; iv) failure to respond to first-line anti-epileptic medication; v) continued seizures after two years of age. From this cohort, the authors identified eight children who satisfied these diagnostic criteria for Dravet syndrome, six of whom had an associated *SCN1A* variant (the other two did undergo *SCN1A* gene analysis but were not found to have a causative variant). They estimated the incidence of Dravet syndrome to be 1 per 15,700 live births, and the incidence for *SCN1A* related Dravet syndrome to be 1 per 20,900 live births (Wu et al., 2015).

The main limitation of this study relates to the clinical definition of Dravet syndrome, which has not been based on any international consensus opinion. *SCN1A*-related epilepsies present a spectrum of epilepsies which have multifaceted components, including age of seizure onset, seizure type(s), seizure duration, and developmental comorbidity. This encapsulates the challenge for epidemiological study of monogenic epilepsies. At the mild end of the *SCN1A*-related epilepsy spectrum there are individuals who only have febrile seizures and who develop no associated comorbidities. At some undetermined point on the spectrum, the phenotype is deemed severe enough to merit the label “Dravet syndrome.” Patients with the “full house” of features may be easy to label as Dravet syndrome, but what about those who develop all the typical clinical features, but happen to present at little later than usual, say at 13 months of age? Or what

about those who achieve good seizure control, for example with the early application of the promising new medication Fenfluramine (Ceulemans et al., 2012), but still develop the associated comorbidities?

It with these complexities in mind that the epidemiological study reported in this chapter of the thesis has made every attempt not to be constrained by phenotype selection. The objective here is to identify every individual within a defined population presenting with epilepsy or complex febrile seizures and to ensure that as many as possible get genetic testing. In that way it should be possible to define the true incidence, and associated phenotypic spectrum of, each monogenic epilepsy.

The incidence of *SCN1A*-related Dravet syndrome has been estimated in Denmark to be 1 per 22,000 live births (Allan, Helle & Møller, 2015). Semi- objective clinical criteria were used to define those who satisfied a diagnosis of Dravet syndrome: i) seizure onset before 12 months; ii) "mainly" fever triggered and "often" prolonged [inverted commas added]; iii) later occurrence of other seizure types including focal seizures, myoclonic seizures, atypical absences, and tonic-clonic seizures; iv) normal motor an cognitive development prior to seizure onset; v) subsequent plateauing or regression of skills. In addition to the same limitations in relation to clinical definition as identified in the Wu study, this study from Denmark is vulnerable to selection bias. All patients were identified from the single national tertiary epilepsy centre, making the assumption that all patients with this severity of epilepsy would be referred to that centre. The study in this chapter aims to overcome such bias by recruiting from 24 different paediatric departments across Scotland, not just tertiary centres. Similar, single tertiary centre approaches have been used to estimate the incidence of Glut1 deficiency syndrome, which is caused by pathogenic variants in the *SLC2A1* gene. The quoted figures are 1 per 90,000 live births in Queensland Australia (Coman et al., 2006), and 1 per 88,000 live births in Denmark (Larsen et al., 2015b). Selection bias is likely to be a bigger issue in relation to *SCL2A1* than in relation to *SCN1A*, since this is more recently

described, and the full phenotypic spectrum of the disorder is therefore less likely to have been fully revealed.

In addition to describing the epidemiology and clinical spectrum of the monogenic seizure disorders of early childhood, a secondary objective of this chapter is to determine in what proportion of all children presenting with epilepsy before their third birthday an underlying aetiology can be identified: including structural, metabolic, infectious, and immune causes. Thanks to advances in genetic, neuroimaging, and immunological investigations – coupled with an increased awareness among clinicians that these causes exist and can be investigated for – it is becoming increasingly possible to ascribe a precise aetiological cause to epilepsy in cases that would have previously been designated "idiopathic" or "cryptogenic." Berg et al. reviewed the records of 775 children who were diagnosed with epilepsy before their third birthday. They were able to identify an aetiology in 227 patients (29.3%). These patients were identified from 17 epilepsy centres in the United States, but the sample was not population-based and the extent to which they were investigated for aetiology was limited – for example 448 of the patients did not undergo any form of genetic testing (Berg et al., 2017). With a more proactive approach to genetic testing, this chapter will investigate how much higher an aetiological yield can be obtained.

5.2 Methods

5.2.1 Cohorts

In this study I involved two overlapping cohorts. Both cohorts only included children who presented prior to their third birthday, and both cohorts only included those who presented between May 8th 2014 and May 7th 2017. The first cohort was a Scotland-wide cohort and the second cohort was a West of Scotland cohort, including only patients residing in the following Health Board regions: Ayrshire and Arran, Dumfries and Galloway, Forth Valley, Greater Glasgow and Clyde, and Lanarkshire. The majority of patients in Cohort 1 were identified prospectively through recruitment to the Genetic and Autoimmune Childhood Epilepsy (GACE) study (Symonds et al., 2019). The primary aim of the GACE study was to determine the diagnostic and clinical utility of genetic and autoantibody testing in patients with early childhood onset epilepsy of undetermined cause.

Patients in the Cohort 2 were retrospectively identified through reviewing the case notes of all children who were investigated using electroencephalography (EEG) between January 1st 2014 to December 31st 2017 in the EEG departments serving the West of Scotland (Royal Hospital for Children [RHC] Glasgow, Crosshouse Hospital Kilmarnock, and Forth Valley Royal Hospital Larbert), n = 1174.

Population and birth rate denominators for these cohorts were obtained from the National Records of Scotland, birth time series data (National Records of Scotland, 2018b), and 2011 census data (National Records of Scotland, 2018a).

Table 5.2a: Summary of Cohort 1 and Cohort 2

	Cohort 1	**Cohort 2**
Inclusion criteria	Presentation before the day of the third birthday and Presentation between May 8th 2014 and May 7th 2017 and Any one the following: • 2nd afebrile unprovoked seizure • 2nd prolonged (>10 minute) febrile seizure • 1st episode of febrile or afebrile status epilepticus (>30 minutes) • Cluster of two or more febrile or afebrile seizures within a 24-hour period	
Exclusion criteria	An aetiology that would explain seizures/epilepsy already established by the time of presentation, or through neuroimaging or biochemical testing, shortly after presentation	Not living in one of the following Health Board areas: Ayrshire and Arran, Dumfries and Galloway, Forth Valley, Greater Glasgow and Clyde, Lanarkshire
Case identification	Recruited to the GACE study and/or Referred for genetic testing through the national epilepsy genetic service	In Cohort 1 and/or EEG done at RHC Glasgow, Crosshouse or Forth Valley hospital between 01.01.2014 and 31.12.2017 and < 4 years at the time of EEG
Number of cases	322	313
Cases in both cohorts	194	

5.2.2 Cohort 1: Methodology

5.2.2.1 Introduction

The Genetic and Autoimmune Childhood Epilepsy (GACE) study is a Scotland-wide multicentre prospective cohort study. The aim of the study is to identify all cases of new onset epilepsy without a cause already known at presentation and then to determine the diagnostic and clinical utility of genetic and anti-neuronal antibody testing in this group. All families recruited were offered genetic testing on a 104-gene panel of epilepsy associated genes, and antibody testing for 10 antineuronal antibodies. Cases presenting with atypical febrile seizures (detailed in the

inclusion criteria above) or with either a single episode of afebrile status epilepticus or a single cluster of afebrile seizures within a 24-hour period were also eligible for inclusion. The rationale for including these additional cases was that it is recognised that some genetic epilepsies, most notably *SCN1A*-related epilepsy, present first with febrile seizures before the onset of unprovoked seizures (Brunklaus et al., 2012), and we did not wish to delay testing in such cases.

5.2.2.2 Recruitment

The GACE study was opened simultaneously in 11 of the 15 NHS Health Boards in Scotland (Figure 5.2a). Within those regions, participants could be recruited at all NHS health care facilities where it was anticipated that children presenting with seizures would be seen and assessed. This included four tertiary paediatric hospitals, 10 district general hospitals with paediatric inpatient services, and 10 district general hospitals with community based paediatric facilities (see Figure 5.2b). Though the study was not opened in NHS Orkney, NHS Shetland and NHS Western Isles, patients residing within these health board areas could be recruited to the study either following transfer to a secondary or tertiary paediatric unit, or on visiting outpatient assessment by a paediatric neurologist – a paediatric neurologist from Dundee does a regular clinic in both Orkney and Shetland. The Golden Jubilee Health Board does not provide either acute or outpatient paediatric services.

Patients who were not recruited to the GACE study but who were referred for diagnostic genetic testing at the Scottish Genetic Epilepsy Service at the West of Scotland Regional Genetics Service were also included in Cohort 1. The Scottish Genetic Epilepsy service is nationally funded via the NHS Scotland National Services Division (NSD) (NHS Scotland, 2018). NSD services are free for all NHS Boards in Scotland to access and use. The Genetic Epilepsy Service is the only laboratory that provides diagnostic testing for sequence variants in epilepsy-associated genes, so it is anticipated that few if any Scottish patients with epilepsy would receive a genetic diagnosis of a single gene cause of epilepsy elsewhere.

In order to facilitate maximum recruitment to the GACE study, a link clinician was identified in each Health Board. Each link clinician was sent a weekly reminder email about the study. All link clinicians were either paediatric neurologists or paediatricians with expertise in epilepsy. All link clinicians were active participants in the Scottish Paediatric Epilepsy Network (SPEN) (Scottish Paediatric Epilepsy Network, 2018), a Managed Clinical Network that is also supported through NSD. MCNs are defined as "linked groups of health professionals and organisations from primary, secondary and tertiary care, working in a co-ordinated manner, unconstrained by existing professional and Health Board boundaries, to ensure equitable provision of high quality clinically effective services" (NHS Education for Scotland, 2018). SPEN organises regular educational and training events for clinicians involved in the management of childhood epilepsy and supports the development of standards of care for children with epilepsy in Scotland. The GACE study was actively promoted at each SPEN meeting and in SPEN newsletters.

There are eight EEG departments in Scotland that perform paediatric EEG (in Inverness, Aberdeen, Dundee, Kirkcaldy, Larbert, Edinburgh, Glasgow, and Kilmarnock). A contact was identified in each of these EEG departments, and this person was sent a weekly reminder email about the study.

There are 17 paediatric epilepsy specialist nurses in Scotland. Each was sent a weekly reminder about the study.

Inclusion and exclusion criteria for Cohort 1 are detailed in table 5.2a.

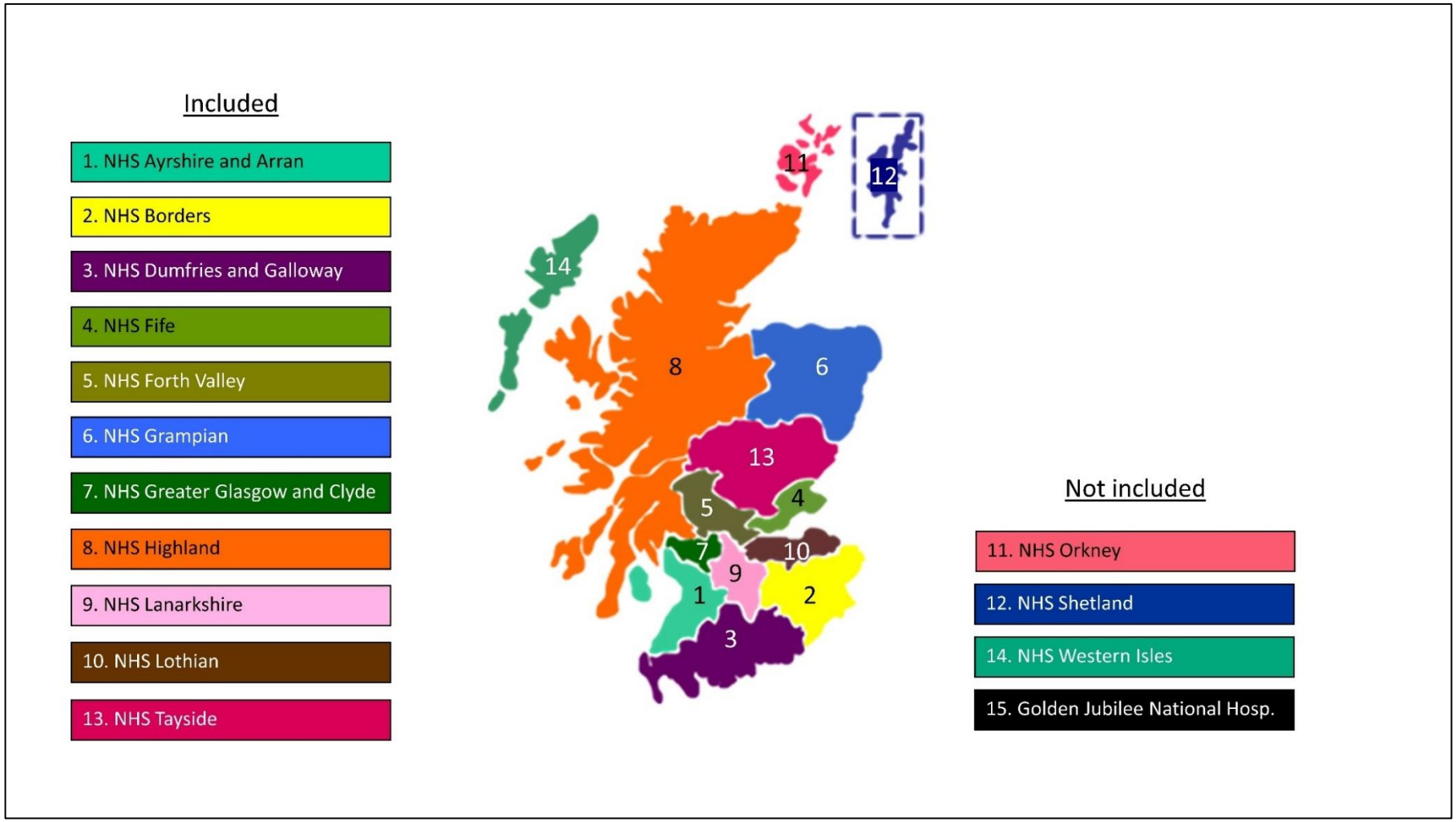


Figure 5.2a: Health Boards in Scotland

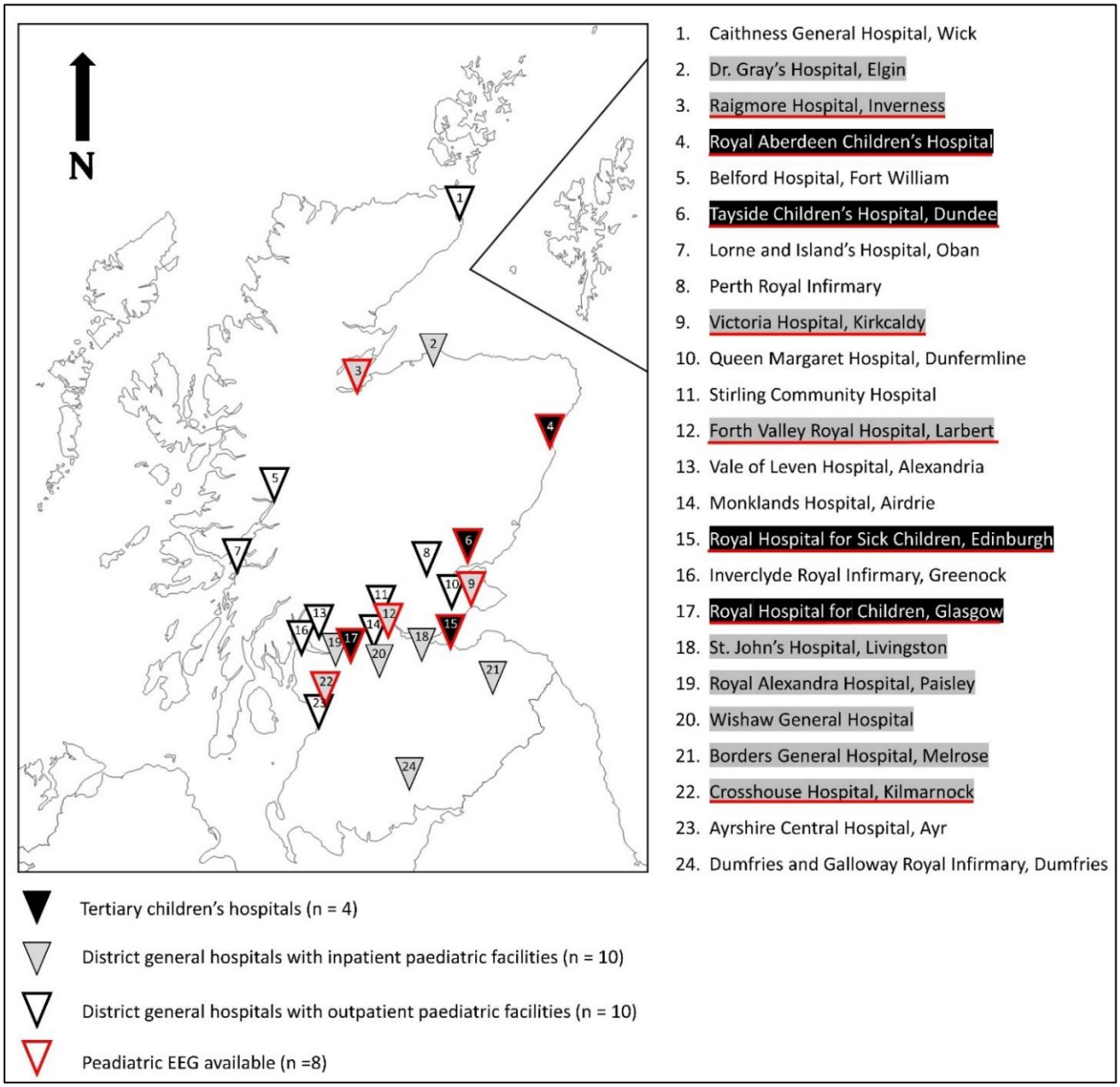


Figure 5.2b: Recruitment sites for Cohort 1

5.2.2.3 Consent

The carers of participants recruited to the GACE study were asked to give informed consent for the following:

- Genetic testing on a 104-gene epilepsy panel at the West of Scotland Regional Genetics Service in Glasgow
- Immune testing for 10 autoantibodies at the Nuffield Department of Clinical Neurosciences at the University of Oxford
- To be sent a series of questionnaires two months after recruitment and a second set 12 months' after
- For the research team to discuss the participant's clinical case with their hospital doctor or GP, and examine electronic and paper medical records
- For the participant's paediatrician to be sent follow-up questionnaires for a clinical update, and to enquire about the impact of genetic testing

The Parent Information Sheet and Parent Consent Form for the study are supplied in Appendix 1 and 2 respectively.

5.2.2.4 Ethical approval

The GACE study was approved by the West of Scotland Research Ethics Service (WoSRES) on December 11th 2013 (REC number 13/WS/0299, IRAS ID 135019).

A separate ethical approval for a genetic epilepsy research database was granted by WoSRES in 2012, and was updated on January 23rd 2017 (REC number 16/WS/0203, IRAS ID 214468). This approval specifically permits the analysis of anonymised clinical and genetic data from patients referred to the National Genetic Epilepsy Service and therefore allows cases not included in the GACE study to be included in Cohort 1.

5.2.2.5 Initial clinical evaluation

At the time of recruitment to the GACE study, the recruiting clinician was asked to complete a clinical detail proforma (Appendix 3). Details of age of onset, seizure types, relevant comorbidities, EEG findings, neuroimaging findings, family history and prior investigations were gathered for each patient. Within two months of recruitment parents of participants were sent standardised questionnaires, including the Adaptive Behaviour Assessment Tool II (ABAS II) questionnaire, which is a validated measure of adaptive behaviour in early childhood (Floyd et al., 2015). It was hoped that the ABAS-II would provide a more sensitive and objective measure of any early developmental comorbidity that clinician impression alone. Parents were also sent standardised questionnaires to assess Health Related Quality of Life (ELDQOL or Peds-QL) and parental adjustment (Parent Stress Index and Strengths and Difficulties Questionnaire).

For cases not recruited to the GACE study who are included in Cohort 1, a different clinical details proforma was completed (Appendix 4). This was a requirement before genetic testing was activated. Parental questionnaires were not sent out to these families.

5.2.2.6 Follow-up clinical evaluation

Six months after each participant's genetic report was issued, the recruiting clinician was sent a clinical follow-up questionnaire (Appendix 5), along with a questionnaire asking them about the utility of genetic testing (Appendix 6). The clinical follow-up questionnaire asked for many of the same details as the initial clinical details proforma, recognising that since cases were recruited to the GACE study early in their presentation, further clinical features may have become apparent since recruitment.

12 months after recruitment, provided genetic results had been fed back to families, parents were asked to complete a second set of questionnaires which was identical to the first set.

5.2.2.7 Case review

The clinical details proformas of all cases recruited to the GACE study were reviewed by a panel of three paediatric neurologists, to ensure that each case satisfied the inclusion criteria, that clinical details were sufficiently complete, and to determine whether accelerated testing of any specific genes was indicated.

5.2.2.8 Testing procedure and variant interpretation

For all cases in Cohort 1, clinicians requesting genetic testing were asked to obtain a 4ml blood sample in an EDTA tube from each patient. This was sent to the Genetic Epilepsy Service for DNA extraction and sequencing.

At the bottom of the clinical details proforma (Appendix 3) recruiting clinicians were asked if they wanted accelerated Sanger sequencing of one or more of 10 genes to be carried out. For some of these genes dosage studies in the form of Multiplex Ligation Probe Amplification (MLPA) was carried out in tandem with Sanger sequencing (Appendix 7). Sanger sequencing could be carried out significantly more quickly than gene panel testing, MLPA can detect copy number losses and exonic deletions that would otherwise be missed on panel testing.

In cases where no single gene testing was requested and cases where single gene testing did not return a diagnostic result, samples were tested on an Illumina MiSeq custom designed panel of 104 genes associated with epilepsy (Appendix 8).

Variants identified were classified in accordance with the UK Association for Clinical Genetic Science (ACGS) guidelines (Association for Clinical Genetic Science, 2017) with reference to the American College of Genetics and Genomics (ACGM) guidelines on variant interpretation (Richards et al., 2015). According to these guidelines variants are classified as 1 – benign, 2 – likely benign, 3 – uncertain significance, 4 - likely pathogenic, and 5 – pathogenic. Any variants classified as 3, 4, or 5 were brought to a multidisciplinary discussion (MDD) involving paediatric neurologists, clinical geneticists and clinical scientists. At the

MDD, variant and clinical details were reviewed and a decision on final interpretation made. Outcomes from the MDD depend on the strength of the evidence supporting pathogenicity of the variant, inheritance pattern, and clinical phenotype information (see table 5.2b).

Table 5.2b: Workflow for genetic variants identified

Outcome from genetic testing	Workflow
No class 3, 4 or 5 variants identified	Case not taken to MDD. Negative report issued. No parental samples requested.
Single heterozygous class 3, 4 or 5 variant identified in a gene associated with recessively-inherited disease	Case taken to MDD. If *in silico* evidence for pathogenicity sufficiently strong and clinical phenotype in keeping with the published literature, consideration of further testing (e.g. array CGH) to look for a deletion affecting the other allele. Otherwise negative report issued and no parental samples requested.
Homozygous class 3 variants identified in a gene associated with recessively-inherited disease	Case taken to MDD. If *in silico* evidence for pathogenicity sufficiently strong and clinical phenotype in keeping with the published literature then upgrading variant to class 4 considered. Parental samples requested to inform recurrence risk.
Two or more heterozygous variants identified in a gene associated with recessively-inherited disease, at least one of which is a class 3 variant.	Case taken to MDD. Parental samples requested to determine whether the variants are in *cis* or *trans*. If variants are in *trans,* in silico evidence for pathogenicity sufficiently strong, and clinical phenotype in keeping with the published literature then upgrading variant(s) to class 4 considered.
Heterozygous class 3 variant identified in a gene associated with dominantly-inherited disease	Case taken to MDD. If in silico evidence for pathogenicity sufficiently strong and clinical phenotype in keeping with the published literature then parental samples requested. Following parental testing, if the variant has been inherited from a similarly affected parent, or has arisen *de novo* in a gene commonly associated with *de novo* presentation, upgrading variant to class 4 considered.
Hemizygous class 3 variant identified in a gene associated with X-linked recessive inheritance	Case taken to MDD. If in silico evidence for pathogenicity sufficiently strong and clinical phenotype in keeping with the published literature then parental samples requested. If the variant has been maternally inherited, upgrading variant to class 4 considered.
Heterozygous class 3 variant identified in a gene associated with X-linked dominant inheritance	Case taken to MDD. If in silico evidence for pathogenicity sufficiently strong and clinical phenotype in keeping with the published literature then parental samples requested. If the variant has arisen *de novo* or been inherited from a similarly affected mother, upgrading of variant to class 4 considered
Homozygous class 4 or 5 variants identified in a gene associated with recessively-inherited disease	Case taken to MDD. Reported as causative unless phenotype is highly atypical. Parental samples requested to inform recurrence risk
Two or more class 4 or 5 variants identified in a gene associated with recessively-inherited disease	Case taken to MDD. Parental samples requested to determine whether the variants are in *cis* or *trans*. If variants are in *trans*, variants reported as causative unless phenotype is highly atypical.

Heterozygous class 4 or 5 variant identified in a gene associated with dominantly inherited disease	Case taken to MDD. Reported as causative unless phenotype is highly atypical. Parental samples requested to inform recurrence risk.
Hemizygous class 4 or 5 variant identified in a gene associated with X-linked recessive inheritance	Case taken to MDD. Reported as causative unless phenotype is highly atypical. Parental samples requested to inform recurrence risk.
Heterozygous class 4 or 5 variant identified in a gene associated with X-linked dominant inheritance	Case taken to MDD. Reported as causative unless phenotype is highly atypical. Parental samples requested to inform recurrence risk.

All cases presenting at under four months of age who did not have a positive diagnosis on panel testing had MLPA for *KCNQ2* carried out.

For all patients, a final result from genetic testing was issued to the requesting clinician. Where variant interpretation was altered by the results of parental testing a second report was sent.

5.2.3 Cohort 2: Methodology

Because cases with established aetiology were excluded from the GACE study and would be unlikely to be referred for clinical genetic testing, analysis of Cohort 1 will be uninformative in telling us about the overall incidence of epilepsy in the under three years age group. Moreover, Cohort 1 cannot tell us the incidence of all genetic epilepsies in this group, since some genetic aetiologies may have been established prior to presentation with seizures, or would be diagnosed through means other than single gene or panel testing. Array CGH testing is carried out at regional laboratories in Aberdeen, Dundee, and Edinburgh as well as Glasgow.

For Cohort 2, patients were identified retrospectively through reviewing all referrals for EEG investigations at the Royal Hospital for Children (RHC) in Glasgow, Forth Valley Royal Hospital, and Crosshouse Hospital. Postcodes were reviewed and checked against the National Records of Scotland list of postcodes covered by each of the 2014 Health Boards to determine the Health Board of residence of each patient.

Departmental EEG registers of all patients who had undergone EEG investigation at RHC Glasgow, University Hospital Crosshouse and Forth Valley Royal Hospital were reviewed and filtered to include only those who were under the age of four years at the time of EEG and whose EEG was done between January 1st 2014 and December 31st 2017 inclusive. Medical notes of all of these cases were then reviewed to see if they satisfied the inclusion criteria in Table 5.2a.

The following minimum dataset was collected in each case (Table 5.2c):

Table 5.2c: Minimum dataset for Cohort 2

Data point	**Categorical options**
Is the patient also in **Cohort 1?**	Y N
Health Board region	Ayrshire and Arran, Dumfries and Galloway, Forth Valley Greater Glasgow and Clyde, Lanarkshire
Date of birth	dd/mm/yyyy
Eligible for Cohort 1?	Y N
Date of inclusion seizure	dd/mm/yyyy
Inclusion seizure category	Febrile status epilepticus (30 minutes or longer) Febrile seizure cluster (2 or more within 24 hours) 2nd prolonged (>10 minute) febrile seizure Afebrile status (30 minutes or longer) Unprovoked cluster of afebrile seizures (2 or more within 24 hours) Acute symptomatic afebrile seizures (record provoking factor) – single episode or recurrent 2nd unprovoked seizure (i.e. epilepsy diagnosed)
Inclusion seizure type	Generalised clonic or tonic clonic Absence Myoclonic Myoclonic absence Atonic Tonic Epileptic spasms Focal Unclassified
Were epileptic spasms diagnosed?	Y N
Date of onset of spasms	dd/mm/yyyy
Date of most recent follow-up	dd/mm/yyyy

Diagnosis at most recent follow-up	1. Hypoxic ischaemic encephalopathy (HIE) 1A. HIE – no seizures 1B. HIE – seizures resolved 1C. HIE – seizures continued beyond day 28 or patient remained on treatment 2. Febrile status 2A. Single episode of febrile status 2B. Recurrent episodes of febrile status 2C. Febrile status plus other febrile seizures 2D. Febrile status plus single afebrile seizure (status or otherwise)* 3. Febrile clusters 3A. Single cluster of febrile seizures (2 or more within 24 hours) 3B. Recurrent clusters of febrile seizures (2 or more within 24 hours) 3C. Single cluster of febrile seizures (2 or more within 24 hours) plus other febrile seizures 3D. Single cluster of febrile seizures (2 or more within 24 hours) plus a single afebrile seizure (status or otherwise)* * febrile status plus recurrent afebrile seizures should be recorded as epilepsy 4. Recurrent prolonged febrile seizures Recurrent prolonged febrile seizures (>10 minutes) 5. Afebrile status or cluster 5A. Single episode of afebrile status 5B. Single cluster of unprovoked seizures 6. Acute symptomatic seizures 6A. Single episode of acute symptomatic seizures (clear cause identified**, seizures resolved within 28 days of event and treatment stopped***) 6B. Recurrent episodes of acute symptomatic seizures ** e.g. neonatal stroke, hypoglycaemia, gastroenteritis, ADEM etc. *** If seizures persisted beyond 28 days or remained on treatment, record as epilepsy (7.) 7. Epilepsy Epilepsy (i.e. recurrent unprovoked seizures)
MRI brain done?	Y N
MRI brain date	dd/mm/yyyy
MRI brain findings	freetext
Structural aetiology identified?	Y N
Structural aetiology details	freetext
Metabolic aetiology identified	Y N
Metabolic aetiology details	freetext
Genetic aetiology identified?	Y N
Genetic aetiology details	freetext
Immune aetiology identified?	Y N
Immune aetiology details	freetext
Infectious aetiology identified?	Y N
Infectious aetiology details	freetext

Classification of epilepsy	freetext
Current treatment	Never had regular AED therapy Previous AED therapy, now off medication Still on first AED monotherapy On second AED monotherapy (first did not control) On second AED monotherapy (first not tolerated) On second AED monotherapy (had come of first then seizures recurred) AED polytherapy (two or more AEDs at the same time) Other - freetext
Drug-resistant epilepsy*? * defined as having moved onto 3rd AED for efficacy reasons or continuing to have at least one seizure in 6 months despite at least 6 months of treatment with a second AED	Y N
Current AEDs or other therapies	freetext
Current seizure frequency	None in the last 6 months < 1 per month: At least one seizure in the last 6 months but an average of fewer than one per month Monthly: Average of at least one per month, but fewer than one per week Weekly: Average of at least one per week, but fewer than one per day Daily: Average of one or more per day
Developmental status	No concerns Global developmental delay Other concerns

5.2.4 Whole Genome Sequencing

Patients from Cohort 2 who had a diagnosis of drug-resistant epilepsy (see row 27. in Table 5.2b) or epileptic spasms (see row 8 in Table 5.2b) and for whom no aetiology explaining epilepsy had been identified were offered Whole Genome Sequencing (WGS). Methods are described in section 6.1

5.3 Results

5.3.1 Cohort 1: Recruitment and estimation of under recruitment

338 patients were recruited to the GACE study, of whom 49 were excluded from Cohort 1. 24 were excluded because their recruitment seizure occurred before May 8th 2014, four were excluded because the recruitment seizure occurred after the 3rd birthday, 16 were excluded because an aetiology had already been identified prior to the recruitment seizure, three were excluded because all events were determined to be non-epileptic, and two were excluded because no seizure satisfied the criteria for eligibility.

An additional 33 eligible patients were identified through reviewing all 695 referrals for genetic testing at the National Genetic Epilepsy Service between April 1st 2014 and December 31st 2017. A total of 322 eligible patients were identified. DNA samples were obtained from 315 of these and tested in Glasgow (Figure 5.3a).

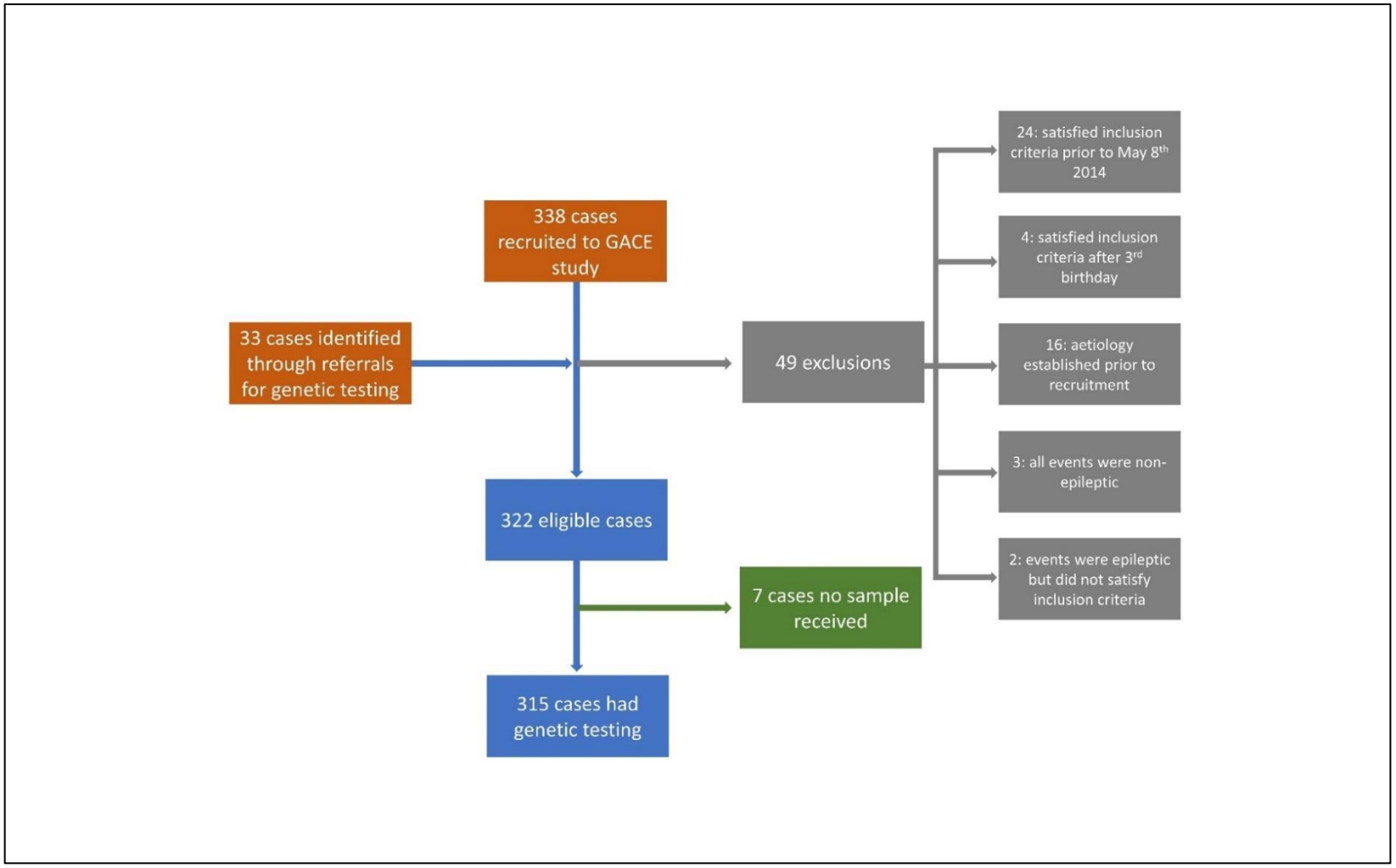


Figure 5.3a: Recruitment flowchart for Cohort 1

Because the study centre for the GACE study was in Glasgow it was expected that there would be a sampling bias, with a greater proportion of eligible cases recruited from the Greater Glasgow and Clyde Health Board than from other Health Boards. Total under 3 years population estimates for each Health Board region are based on 2011 census data (National Records of Scotland, 2018a) (see Table 5.3a).

Table 5.3a: Under 3 years of age population estimate by year (National Records of Scotland, 2018a)

Health Board	2011	2012	2013	2014	2015	2016	Mean	% of total
Ayrshire & Arran	11820	11710	11585	11159	10953	10971	11366	6.5
Borders	3366	3344	3456	3501	3387	3328	3397	2.0
Dumfries & Galloway	4570	4432	4266	4057	4005	3956	4214	2.4
Fife	12661	12556	12304	11916	11806	11643	12148	7.0
Forth Valley	9929	9868	9685	9532	9322	9248	9597	5.5
Grampian	19508	19523	19349	19208	19083	19079	19292	11.1
GG&C	38161	38794	39253	38516	37980	37643	38391	22.1
Highland	9928	9682	9535	9257	9075	9107	9431	5.4
Lanarkshire	22546	22336	22133	21514	21396	21211	21856	12.6
Lothian	29898	29664	29814	29159	28908	28855	29383	16.9
Orkney	658	640	630	587	580	558	609	0.4
Shetland	847	821	822	782	767	737	796	0.5
Tayside	12783	12828	12857	12626	12510	12217	12637	7.3
Western Isles	751	733	735	756	720	712	735	0.4

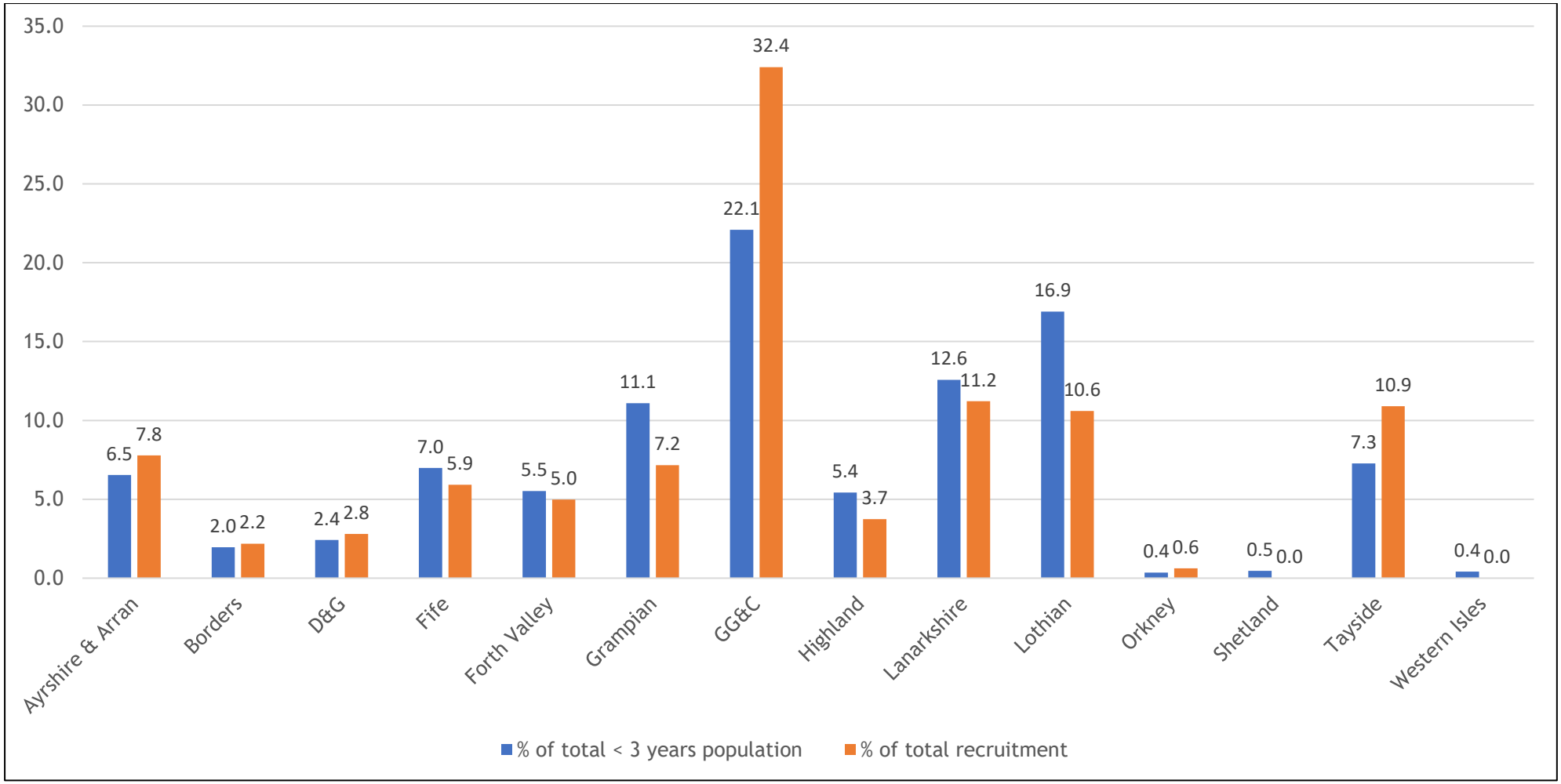


Figure 5.3b: Recruitment to Cohort 1 by Health Board population

Figure 5.3b compares the proportion of total patients in Cohort 1 with the proportion of < 3 years population in each Health Board. In the Greater Glasgow and Clyde and Tayside Health Boards the proportion of total cases recruited was significantly greater than the total proportion of the < 3 years population. If recruitment in all the other Health Boards had matched that of Greater Glasgow and Clyde and Tayside, additional 150 cases would have been expected, and would have made the total in Cohort 1 465. Figure 5.3c shows that recruitment to Cohort 1 was consistent throughout the study period, though there were fewer presentations with febrile seizures in the months May to July.

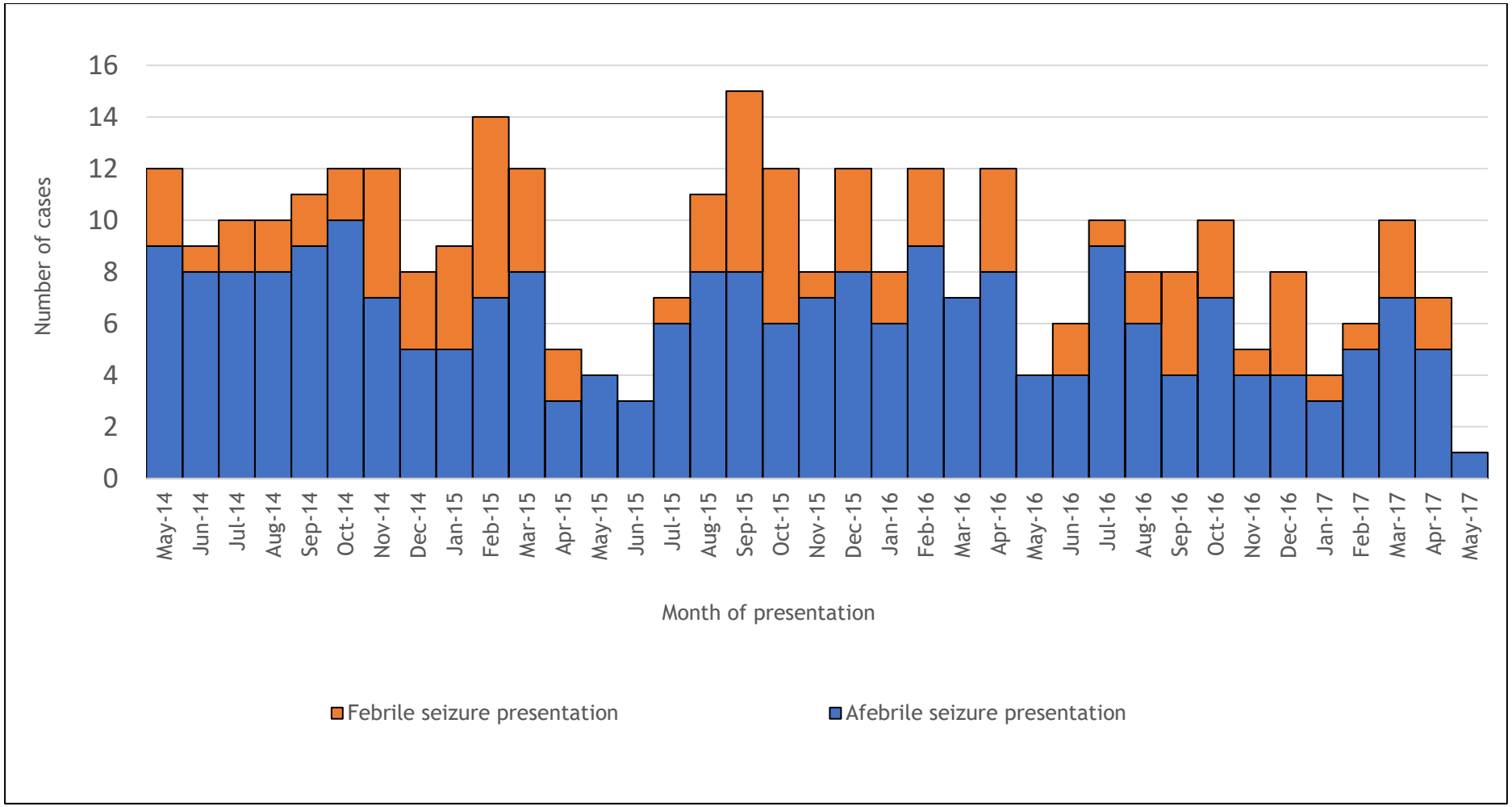


Figure 5.3c: Recruitment to Cohort 1 by month of presentation and type of presentation

5.3.2 Capture-recapture to estimate cases missing from Cohort 1

The total number of patients identified in Cohort 2 was 313, of whom 194 had been recruited to Cohort 1.

The majority of additional patients in Cohort 2 (83/119) picked up through review of EEG records were not eligible for Cohort 1, owing to the fact that an aetiology was established prior to, or immediately after, seizure presentation. However, 36 patients in Cohort 2 were found to be eligible for Cohort 1 but had not been recruited to the cohort. Conversely, there were 53 patients in Cohort 1 who were not also identified through reviewing EEG requests (they had not had an EEG during the period of January 1st 2014 to December 31st 2017). Figure 5.3c shows the overlap between patients in Cohort 1 and patients in Cohort 2 that were eligible for Cohort 1 but were not included.

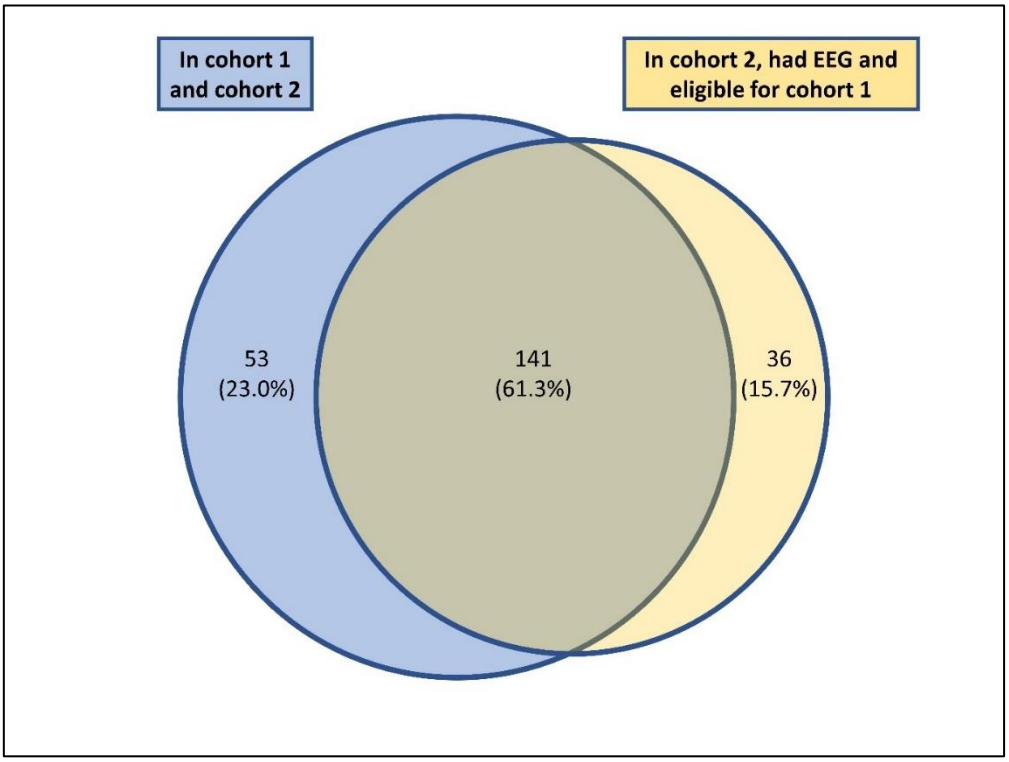


Figure 5.3d: West of Scotland patients eligible for inclusion in Cohort 1 according to whether they were recruited to the cohort, and whether they had been investigated with EEG between 01/01/2014 and 31/12/2017

Using a capture-recapture technique it is possible to estimate the number of additional "unidentified" patients that would have been eligible for Cohort 1 from the West of Scotland Health Boards. Because patients who had received a diagnosis of epilepsy were more likely to be investigated with EEG than those who had not received a diagnosis of epilepsy, cases have been divided into two groups: those diagnosed with epilepsy and those not diagnosed with epilepsy. Figures 5.3e and 5.3f represent the data in Figure 5.2d with cases divided into epilepsy and non-epilepsy groups.

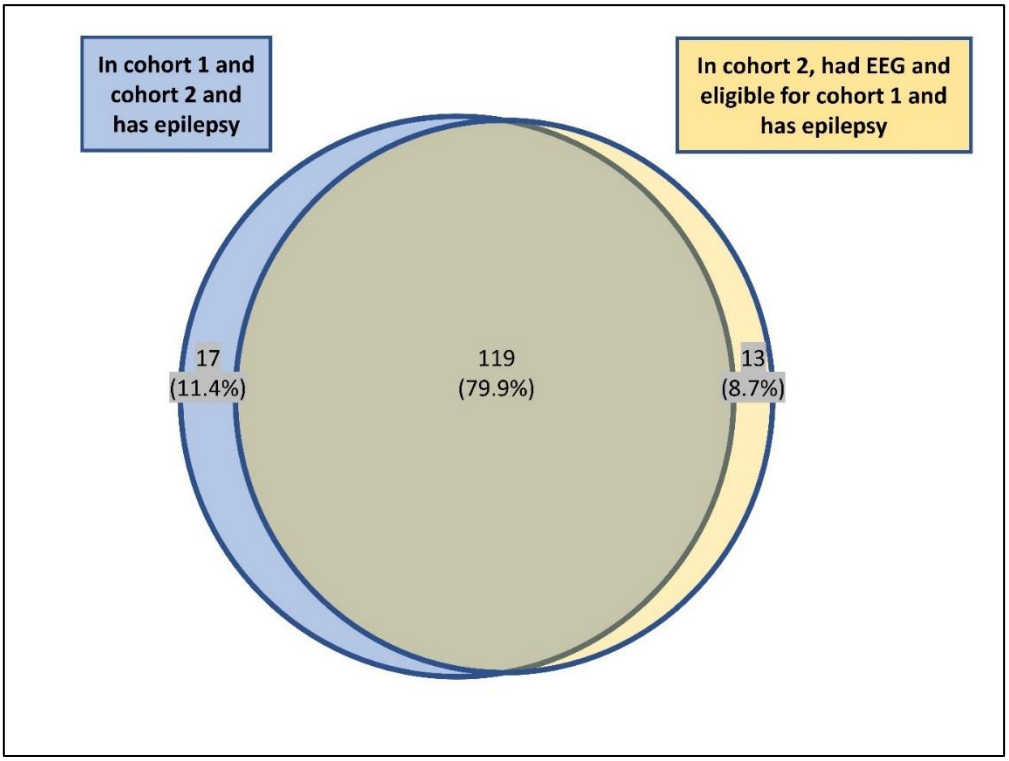


Figure 5.3e: West of Scotland patients <u>with epilepsy</u> eligible for inclusion in Cohort 1 according to whether they were included in the cohort, and whether they were investigated with EEG between 01/01/2014 and 31/12/2017

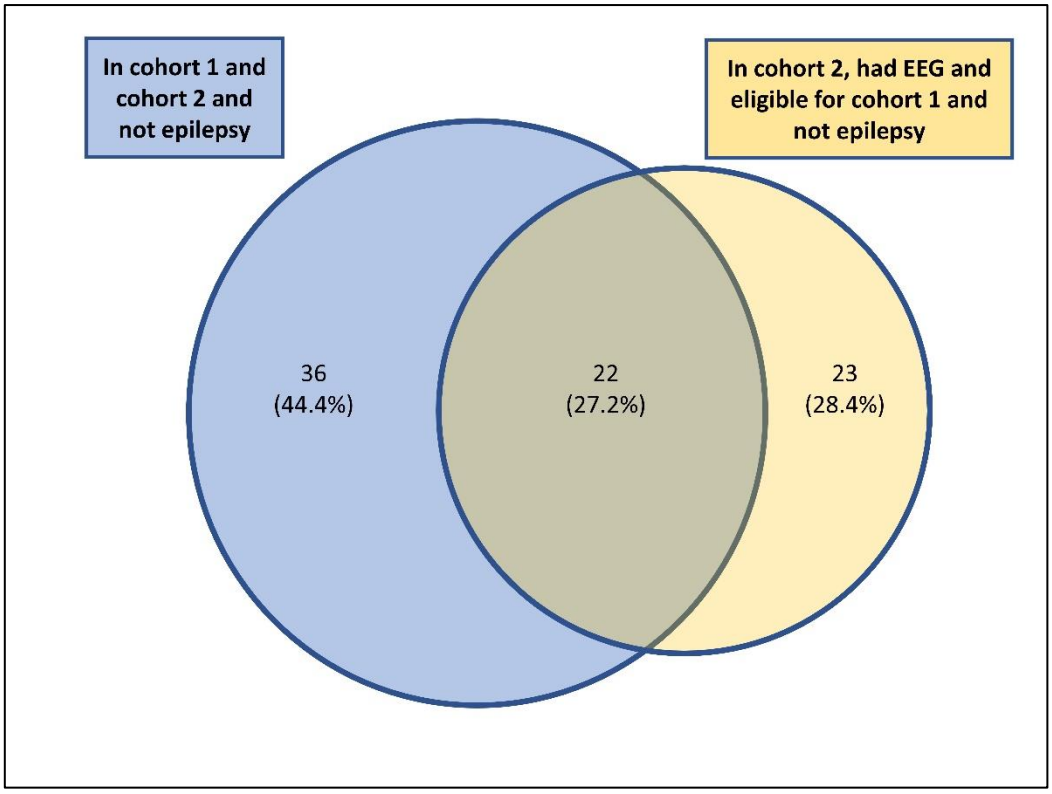


Figure 5.3f: West of Scotland cases without epilepsy eligible for inclusion in Cohort 1 according to whether they were included in the cohort, and whether they were investigated with EEG between 01/01/2014 and 31/12/2017

Figure 5.3e shows that of 136 patients with epilepsy who were recruited to Cohort 1 119 (87.5%) had been investigated with EEG, and that an additional 13 patients with epilepsy were identified through EEG review. The expected additional number of unrecruited epilepsy patients that had not been investigated with EEG would therefore be 2 (13/0.875 – 13), making the expected total number of West of Scotland epilepsy patients eligible for Cohort 1 151.

Figure 5.3f shows that of 58 patients without epilepsy who were recruited to Cohort 1 22 (37.9%) had been investigated with EEG, and that an additional 23 patients were identified through EEG review. The expected additional number of unidentified non-epilepsy patients that had not been investigated with EEG would therefore be 38 (23/0.379– 23), making the expected total number of West of Scotland non-epilepsy patients eligible for Cohort 1 119.

Adding these figures up, there was an estimated under ascertainment of patients in the West of Scotland of 28% (76 patients). The expected total number of West of Scotland patients eligible for Cohort 1 is estimated to be 270 whilst the actual number included was just under three quarters of this figure, 194. Given that the West of Scotland Health Boards comprise 49.13% of the < 3 years population, and assuming that presentations with seizures are equally distributed geographically per head of the population, the expected total number of patients eligible for Cohort 1 nationally would be 550 (270/0.4913), whereas only 58% of this number were actually included in Cohort 1 (322).

5.3.3 Single gene diagnoses made in Cohort 1

315 patients in Cohort 1 underwent genetic testing. 183 patients had single gene sequencing of one or more genes (Sanger +/- MLPA) initially. Of these 22 (13.6%) had a diagnostic result. The 162 patients for whom single gene sequencing was requested without a diagnostic result, and the 133 for whom no single gene testing was requested, were tested on the 104 gene panel. Of the 295 patients tested on the panel, 50 (16.9%) had a diagnostic result. Panel diagnosis rates were marginally higher in the group that had not had prior single gene sequencing compared with those who had (19.5% v 14.8%) though this was not statistically significant (Fishers exact, p = 0.35). 32/50 (64%) diagnoses made on panel testing involved genes for which a single gene test was available in Glasgow, but had not been specifically requested. 21 patients had MLPA requested for one or more single genes following a negative panel result, and two of these resulted in a diagnostic finding (both in the *KCNQ2* gene). Overall, 74/315 (23.5%) patients in Cohort 1 had a diagnostic result from Sanger, MLPA, or panel testing.

Figure 5.3g shows the diagnostic results from Cohort 1, per gene. Diagnostic results were made in 20 different genes, and 55/74 (74%) of diagnoses were in the most frequently implicated eight genes. The shaded bars represent those genes for which there is some published evidence to support the concept that knowledge of

that genetic cause may inform treatment choice (Chapter 4). On this basis 62/74 (84%) of genetic diagnoses made had potential treatment implications.

Using the mean birth rate in Scotland (Table 5.3b) between 2011 and 2016 as a denominator for the birth rate in this cohort, the incidence of the more common single gene epilepsies can be estimated (Table 5.3d).

Table 5.3b: Births in Scotland 2011-2016 (National Records of Scotland, 2018b)

Year	Births	Male births	Female births
2011	58,590	30,111	28,479
2012	58,027	29,713	28,314
2013	56,014	28,828	27,186
2014	56,725	29,056	27,669
2015	55,098	28,354	26,744
2016	54,488	28,236	26,252
Mean	56,490	29,050	27,440

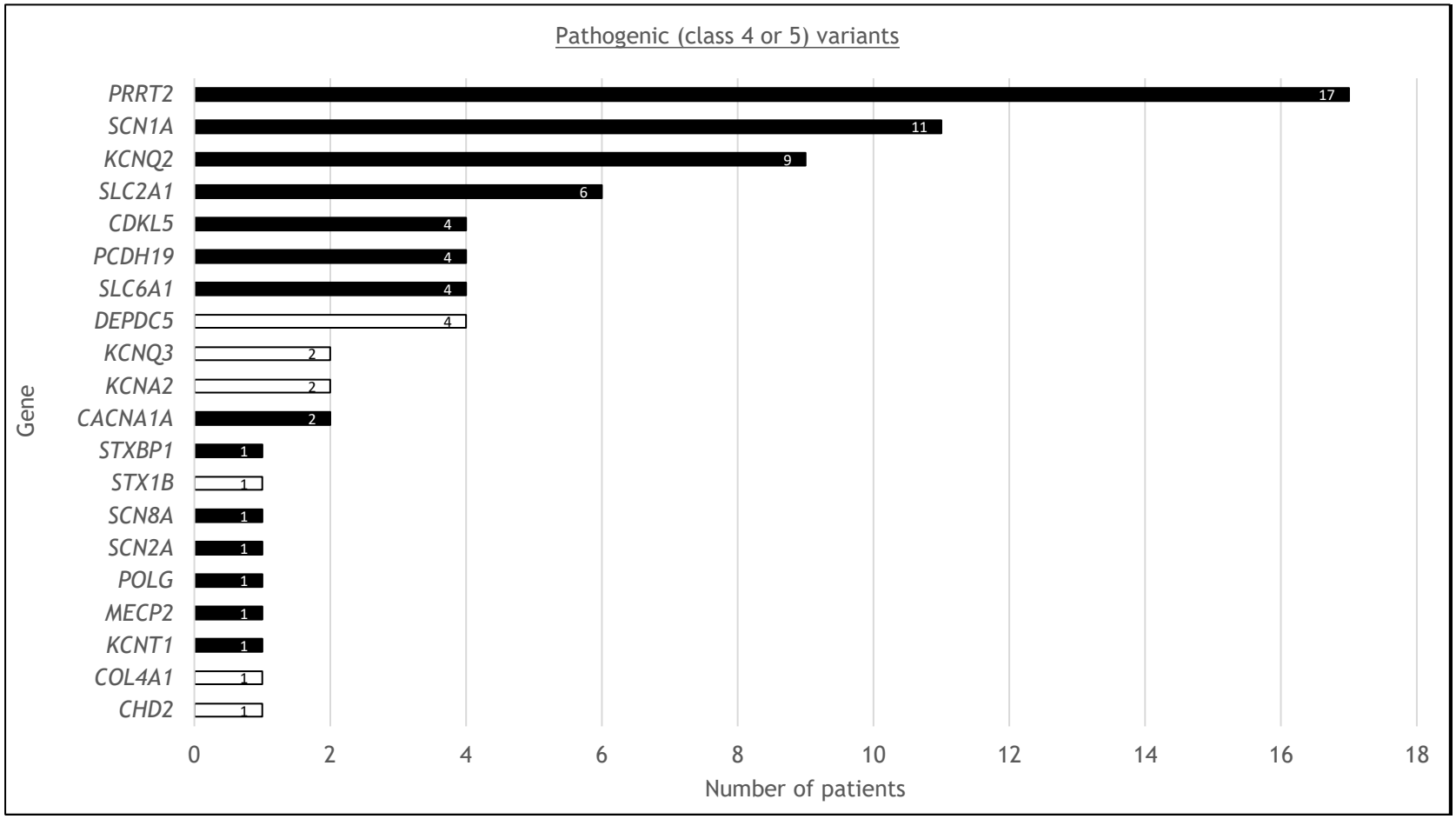


Figure 5.3g: Genes in which diagnostic results were achieved from Cohort 1; shaded bars represent genes for which there is published evidence to support a specific treatment approach (Chapter 4)

Table 5.3c: Minimum incidence estimates for the more common monogenic seizure disorders of early childhood; incidence figures rounded up to three significant figures

Genetic cause	Number of patients in Cohort 1	Estimated minimum incidence	95% Confidence intervals (Poisson distribution)
PRRT2	17	1 per 9,970 live births	1 per 6,300 to 1 per 17,200
SCN1A	11	1 per 15,500 live births	1 per 8,630 to 1 per31,000
KCNQ2	9	1 per 18,900 live births	1 per 9,940 to 1 per 41,300
SLC2A1	6	1 per 28,300 live births	1 per 13,000 to 1 per 77,100
CDKL5	4	1 per 42,400 live births	1 per 16,600 to 1 per 156,000
PCDH19	4	1 per 20,600 live born females	1 per 8,040 to 1 per 1 per 75,600
SLC6A1	4	1 per 42,400 live births	1 per 16,600 to 1 per 156,000
DEPDC5	4	1 per 42,400 live births	1 per 16,600 to 1 per 156,000

With 74 genetic diagnoses made in total, the estimated minimum incidence of all monogenic seizure disorders presenting before the age of three years is 1 per 2,290 live births (95% confidence intervals: 1 per 1,830 to 1 per 2,930). By the European definition of rare disease (affecting fewer than one in 2000 individuals) these genetic seizure disorders would still qualify as rare (Rare Diseases Europe, 2018).

5.3.4 Associations between clinical features of presentation and genetic diagnosis

Table 5.3d: Associations between clinical features of presentation and genetic diagnosis using Fisher's exact test in Cohort 1

	N (%) with genetic cause	Two-tailed Fisher's exact probability	Odds ratio (95% confidence intervals)
Total cohort	74/315 (23.5%)		
Age at presenting seizure			
< 6 months	34/86 (40.0%)	p<0.001*	3.1 (1.8-5.4)
6-12 months	19/76 (25.0%)	p>0.05	1.1 (0.6-2.0)
12-24 months	16/108 (14.8%)	p<0.01 ψ	0.4 (0.2-0.8)
24-36 months	5/52 (9.6%)	p<0.005 ψ	0.2 (0.1-0.6)
Presenting seizure			
Febrile generalised	1/37 (2.7%)	p<0.005 ψ	0.1 (0.0-0.6)
Febrile focal	2/9 (22.2%)	p>0.05	0.9 (0.2-4.6)
Febrile status	6/45 (13.3%)	p>0.05	0.5 (0.2-1.1)
Afebrile focal	37/97 (38.1%)	p<0.001*	3.3 (1.9-5.7)
Afebrile generalised (all, excluding spasms) ¥	20/90 (22.2%)	p>0.05	1.0 (0.6-1.7)
Afebrile generalised GTCS	10/48 (20.8%)	p>0.05	0.9 (0.4-1.9)
Afebrile generalised myoclonic	3/19 (15.8%)	p>0.05	0.6 (0.2-2.3)
Afebrile generalised tonic	3/8 (37.5%)	p>0.05	2.1 (0.5-9.2)
Afebrile generalised atonic	1/4 (25.0%)	p>0.05	1.2 (0.1-11.3)
Afebrile generalised absence	3/11 (27.3%)	p>0.05	1.3 (0.3-5.1)
Afebrile status	2/17 (11.8%)	p>0.05	0.9 (0.1-2.0)
Infantile spasms	3/21 (14.3%)	p>0.05	0.6 (0.2-2.0)
Development, at recruitment			
Normal	47/213 (22.1%)	p>0.05	0.7 (0.4-1.3)
Not normal	27/102 (26.5%)	p>0.05	1.4 (0.8-2.4)
* Significant positive association; ψ Significant negative association **¥** Combined group of all afebrile generalised seizures			

Significant associations with genetic diagnosis in this cohort were: presentation at age under six months, and presentation with afebrile focal seizures. Older ages of presentation were associated with progressively reduced odds of genetic diagnosis. Interestingly the presence of abnormal development at presentation was not associated with genetic diagnosis. The absence of association here may reflect that a high proportion of patients with genetic diagnoses presented at a young age, and it is possible that developmental impairments had not become apparent at that stage. However, of 86 patients presenting before the age of six months, 25 (29.1%) did have developmental concerns highlighted which is a slightly higher proportion than in the total cohort (26.5%). Of those presenting before the age of six months who had developmental concerns at the time of recruitment, 11 (44%) had a genetic cause identified, so even in the early onset group, identification of developmental concerns did not associate with the finding of a genetic cause. Specific types of presentation appeared to be associated with particular genetic causes (Figure 5.3e): For example 33% of children presenting between 4 months of age and 12 months of age with afebrile focal seizures had a diagnostic *PRRT2* variant.

5.3.4.1 Multivariate model

Since age at presentation and type of presenting were associated with genetic aetiology in the univariate model, I put these variables into a multivariate model. Age at presentation was divided into three groups (< 6months, 6-12 months, and > 12 months) and presenting seizure was divided into three groups (afebrile focal, afebrile generalised, and febrile). Both factors remained significant in the multivariate model. The model explained 15% of the variance (Nagelkerke R^2 = 0.15).

Table 5.3d-2: Binary logistic regression analysis for whether age at presentation and type of presenting seizure is associated with genetic diagnosis in Cohort 1 (Hosmer and Lemeshow)

	p value	OR (95% confidence intervals)
Age at presentation		
12-36 months (reference)	0.002	Reference category
6-12 months	0.021	2.3 (1.1-4.7)
< 6 months	0.001	3.1 (1.6-6.1)
Presenting seizure		
Febrile (reference)	0.003	Reference category
Afebrile generalised	0.214	1.7 (0.7-3.7)
Afebrile focal	0.002	3.7 (1.6-8.4)

Table 5.3e: High yield phenotype groups for genetic diagnosis in Cohort 1

Presentation	Afebrile focal seizures ≤4 months	Afebrile focal seizures ≥4 months and <12 months	Febrile or afebrile status epilepticus <24 months	Afebrile generalised seizures ≥9 months and <24 months
N	40	27	53	38
Genetic diagnosis	*KCNQ2* 9 (23%)	*PRRT2* 9 (33%)	*SCN1A* 4 (8%)	*SLC2A1* 5 (13%)
	SCN1A 3 (8%)	*SCN1A* 3 (11%)	*DEPDC5* 1 (2%)	CACNA1A 1 (2%)
	KCNQ3 2 (5%)	*KCNQ2* 2 (7%)	*KCNA2* 1 (2%)	*CHD2* 1 (2%)
	CDKL5 2 (5%)	PCDH19 1 (4%)	*POLG1* 1 (2%)	*KCNA1* 1 (2%)
	COL4A1 1 (2%)	SLC2A1 1 (4%)	*PRRT2* 1 (2%)	*PRRT2* 1 (2%)
	KCNT1 1 (2%)			*SCN1A* 1 (2%)
	PRRT1 1 (2%)			
	SCN2A 1 (2%)			
	STXBP1 1 (2%)			
TOTAL	21 (53%)	15 (59%)	9 (15%)	10 (26%)

5.3.5 Phenotypic spectrum of the monogenic seizure disorders

For the genes in which there were four or more diagnostic results I calculated the mean, median, mode(s), and standard deviation of the age of presentation (table 5.3d). Assuming a roughly normal distribution for age of presentation for each of monogenic seizure disorder, I extrapolated from this data to produce illustrative probabilistic distribution curves, where the x axis is the age of presentation and the y axis is a probability of a diagnosis involving that gene given presentation at age x (Figure 5.3h). The peak of each curve represents the mode, the slope is dependent on the standard deviation, and the curve has been skewed in order that the mean of the curve approximates the mean from the real data. Each curve has been scaled so that the area under the curve is equal to the total probability of a finding a diagnostic result in the gene from Cohort 1 (e.g. the area under the *KCNQ2* curve equals 9/315 = 0.0286 and the area under the *PRRT2* curve equals 17/315 = 0.540). Figure 5.3h demonstrates that up to the age of four months, the most likely genetic diagnosis in a child presenting with seizures is *KCNQ2*-related. At all ages beyond four months the most likely diagnosis is *PRRT2*. These data are based on hypothetical extensions from the small number of cases associated with each genetic diagnosis in Cohort 1, and larger samples would be required to validate this model.

Table 5.3f: Distribution of age of presentation for the eight most common monogenic seizure disorders in Cohort 1

Gene	N	Age of presentation (months)				
		Range	Median	Mode(s)	Mean	σ
PRRT2	17	3-20	6	5	7.56	3.97
SCN1A	11	1.5-25	6.5	4/7/11	7.75	7.24
KCNQ2	9	0.1-4	0.59	0.16/1/4	1.43	1.62
SLC2A1	6	11-19	14	12	14.67	3.44
CDKL5	4	0.49-6	1.15	N/A	2.20	2.58
PCDH19	4	6-18	12	N/A	12.00	4.97
SLC6A1	4	14-31	21	N/A	21.75	7.41
DEPDC5	4	2.5-26	19.5	N/A	16.86	10.73

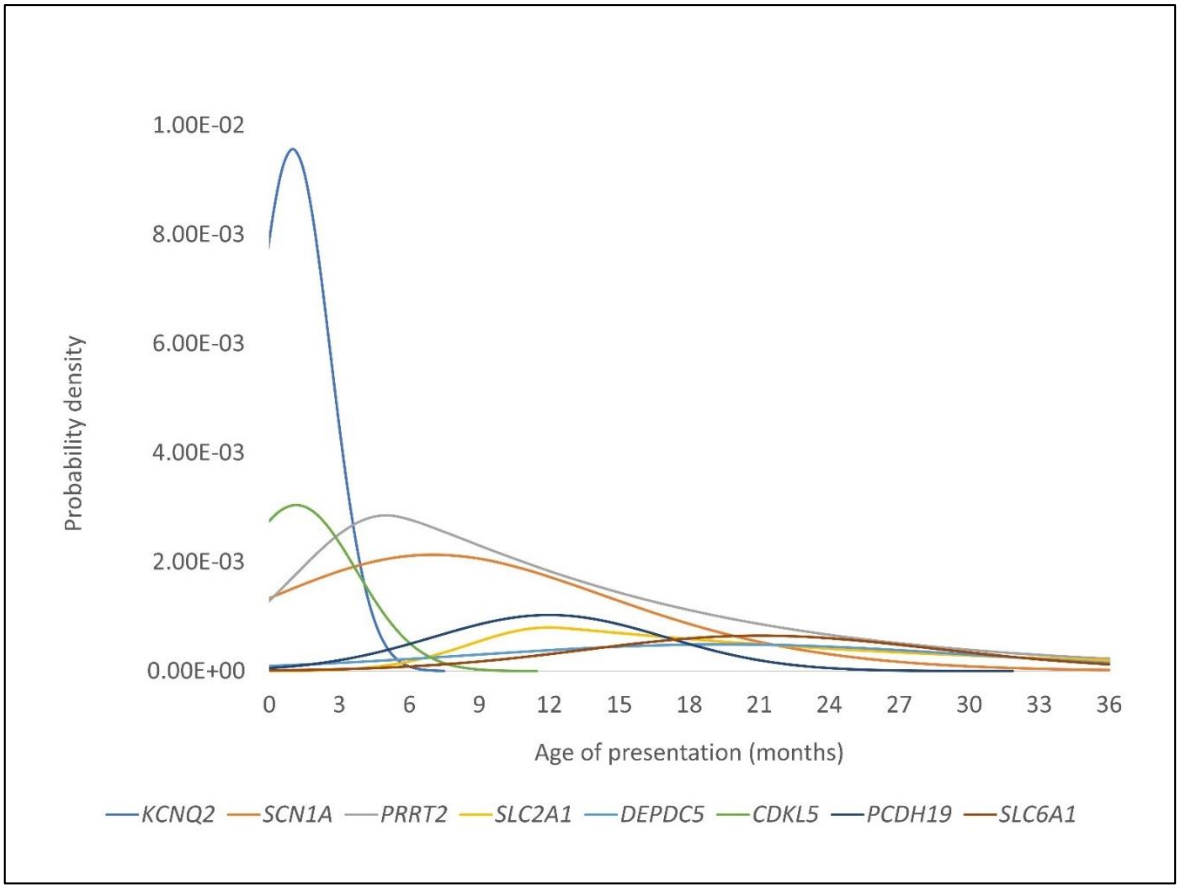


Figure 5.3h: Hypothetical distributions of age of presentation for seven monogenic epilepsies, based on extrapolated data from Cohort 1

5.3.5.1 PRRT2

PRRT2 encodes a protein called *proline-rich transmembrane protein 2*. *PRRT2* is highly expressed in brain. The transmembrane protein is believed to play a role in coupling Ca^{2+} sensing to synaptic vesicle fusion (Valente et al., 2016). *PRRT2* was first associated with disease in 2011. Chen et al. reported three different frameshift mutations among eight Han Chinese families with a dominantly-inherited non-epileptic movement disorder, Paroxysmal Kinesigenic Dyskinesia (PKD) (Chen et al., 2011).

A variety of clinical phenotypes have since been associated with heterozygous *PRRT2* variants, including PKD, self-limited familial and non-familial infantile seizures, febrile seizures and febrile seizures plus, hemiplegic migraine, migraine with aura, migraine without aura, and a phenotype termed Infantile Convulsions Choreoathetosis (ICCA) syndrome which is characterised by the presence of both infantile epilepsy and PKD in the same individual. Self-limited infantile seizures is the most common phenotype observed (Ebrahimi-Fakhari et al., 2015). There is no established relationship between genotype and phenotype, and often different phenotypes are observed within *PRRT2*-affected families. The majority of disease-associated variants observed are frameshift truncating variants. This observation, along with experimental evidence showing that human lymphoblastoid cell lines carrying heterozygous *PRRT2* frameshift variants undergo nonsense-medicated mRNA decay (Wu et al., 2014), supports the hypothesis that the primary mechanism in *PRRT2*-related disease involves haploinsufficiency.

80%-90% of *PRRT2*-related disease is associated with the recurrent frameshift variant (c.649dup, p.Arg217Profs*8) accounting for 78.5% of published cases (Ebrahimi-Fakhari et al., 2015).

There have been no previous incidence estimates for *PRRT2*-related disease.

Table 5.3g: *PRRT2* gene summary from literature

Chromosome locus	**16p11.2**
Phenotype observed in microdeletion syndrome?	Yes
pLI Score~	0.30
Brain expression ratio*	5.47
Localisation within brain	Localises to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor complexes (Schwenk et al., 2012)
Proposed gene function	Coupling Ca^{2+} sensing to synaptic vesicle fusion (Valente et al., 2016)
Proposed genetic mechanism of pathogenicity	Haploinsufficiency
Yield in epilepsy NGS literature (Chapter 3)	67/13063 (0.5%), Rank: 7
Previous incidence estimates	None
Previous clinical spectrum descriptions	38.7% PKD 14.3% ICCA 41.7% Self-limited infantile seizures 5.3% other phenotypes (Ebrahimi-Fakhari et al., 2015)
Previous age of onset descriptions	Data form 401 cases. Mean onset was 6.0 months (Ebrahimi-Fakhari et al., 2015)
Previous penetrance estimates	61% for PKD phenotype. Complete for PKD or infantile convulsions (van Vliet et al., 2012)
Evidence for specific treatment	Retrospective uncontrolled clinician-reported subjective treatment response analysis. N = 64. 63/64 (99%) of patients with Paroxysmal Kinesigenic Dyskinesia treated with Carbamazepine had a good response (Huang et al., 2015). Retrospective uncontrolled clinician-reported subjective treatment response analysis. N = 24. 23/24 (96%) experienced complete seizure control on Carbamazepine treatment (Ebrahimi-Fakhari et al., 2015).

~ From the Exome Aggregation Consortium (ExAC) dataset (Broad Institute Exome Aggregation Consortium, 2018)

* From the GTEx Portal data, based on adult post-mortem tissue expression profiles (Aguet et al., 2017)

Table 5.3h: Patients with *PRRT2* variants from Cohort 1

Yield	17/315 (5.4%), Rank: 1												
Case ID	Age of presentation	Presenting seizure	Dev. delay at presentation > follow-up	Age of follow-up	Epilepsy diagnosis	Seizure types observed	Current seizure frequency	Current treatment approach	Current treatment	Effective treatments	Variant	Inheritance	Family history
339	3 months	Febrile generalised status	No > No	11 months	Self-limited familial infantile epilepsy	Feb-status Afeb-GTCS Afeb-focal	None in 6 months	Still receiving first AED monotherapy	LEV	LEV	c.649dup, p.Arg217Profs*8	Unknown	Father, mother, paternal uncle and maternal uncle all had febrile seizures
344	4 months	Afebrile focal	No > Unknown	N/A – moved abroad	Unknown	Afeb-focal	Unknown	Unknown	Unknown	LEV	c.649dup, p.Arg217Profs*8	Unknown	Mother and 7 of her relatives had infantile seizures
323	4 months	Afebrile tonic	No > No	11 months	Self-limited infantile epilepsy	Afeb-tonic	None in 6 months	Still receiving first AED monotherapy	PB	PB	c.649dup, p.Arg217Profs*8	De novo	None
200	5 months	Afebrile focal	No > No	30 months	Self-limited familial infantile epilepsy	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	CBZ	CBZ	c.649dup, p.Arg217Profs*8	Paternal	Father had infantile seizures; paternal aunt had childhood epilepsy and hemiplegic migraine
82	5 months	Afebrile focal	No > Mild	23 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	CBZ	c.564delG, p.Ala189Leufs*40	Unknown	None
92	5 months	Afebrile focal	No > No	32 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	CBZ	c.649dup, p.Arg217Profs*8	Maternal	Mother has PKD
182	5 months	Afebrile focal	No > No	14 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	LEV	LEV	c.649dup, p.Arg217Profs*8	Maternal	Mother had febrile seizures. Maternal cousin had infantile seizures (case 13). Maternal uncle had self-limited infantile seizures
281	5 months	Febrile focal	No > No	19 months	ICCA	Feb-focal Spasms	None in 6 months	Previously received AED therapy – now off medication	None	VGB	c.896A>C, p.Gln299Pro	Paternal	Father had a seizure in his 30s
172	7 months	Afebrile focal	No > No	39 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	CBZ	CBZ	c.649dup, p.Arg217Profs*8	Unknown	None
244	7 months	Afebrile myoclonic	No > No	Not recorded	Self-limited infantile epilepsy	Afeb-Myo	None in 6 months	Still receiving first AED monotherapy	VPA	VPA	c.649dup, p.Arg217Profs*8	Unknown	Paternal cousin had infantile seizures

193	7 months	Afebrile focal	No > No	31 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	CBZ	CBZ	c.649dup, p.Arg217Profs*8	Paternal	Two paternal cousins had infantile seizures
271	8 months	Afebrile focal	No > No	18 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	CBZ	CBZ	c.649dup, p.Arg217Profs*8	Maternal	Mother has JME
88	9 months	Afebrile focal	No > No	48 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	LEV	c.649dup, p.Arg217Profs*8	Paternal	Paternal cousin had infantile seizures (case 7). Paternal uncle had self-limited infantile seizures. Paternal aunt had febrile seizures.
334	9 months	Afebrile focal	No > No	19 months	Self-limited familial infantile epilepsy	Afeb-focal Afeb-GTCS	None in 6 months	Still receiving first AED monotherapy	LEV	LEV	c.942G>C, p.Lys314Asn	Paternal	Identical twin had recurrent febrile seizures
117	10 months	Afebrile focal	No > No	36 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	VPA	c.649dup, p.Arg217Profs*8	Unknown	None
202	11 months	Afebrile focal	No > No	34 months	Self-limited infantile epilepsy	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	LEV	LEV	c.649dup, p.Arg217Profs*8	Maternal	None
77	20 months	Afebrile focal	Severe > Severe	55 months	Unclassified focal epilepsy	Afeb-focal	None in 6 months	Receiving a different AED monotherapy to the first one tried – initial therapy not effective	CBZ (Prev. tried LEV)	CBZ	c.649dup, p.Arg217Profs*8	De novo	None
Abbreviations used: GTCS – generalised tonic clonic seizure(s). Myo – myoclonic, Afeb – afebrile, Feb – febrile. AED – Anti-epileptic drug. LEV – Levetiracetam, PB – Phenobarbital. CBZ – Carbamazepine, VGB – Vigabatrin, VPA – Sodium Valproate, ICCA –Infantile Convulsions Choreoathetosis, PKD – Paroxysmal Kinesigenic Dyskinesia, JME – Juvenile Myoclonic Epilepsy,													

Table 5.3i: Summary of patients with *PRRT2* variants in Cohort 1

Median age of presentation	6 months
Development	Normal at presentation: 16/17 (94%) Normal at latest follow-up: 15/17 (88%)
Presenting seizure type	Afebrile focal: 13/17 (76%) Afebrile tonic: 1/17 (6%) Afebrile myoclonic: 1/17 (6%) Febrile status: 1/17 (6%) Febrile focal: 1/17 (6%)
Seizure types observed	Afebrile focal: 14/17 (82%) Afebrile GTCS: 2/17 (12%) Afebrile myoclonic: 1/17 (6%) Afebrile tonic: 1/17 (6%) Afebrile spasms: 1/17 (6%) Febrile status: 1/17 (6%) Febrile focal: 1/17 (6%)
Latest diagnosis	Self-limited infantile epilepsy: 14/17 (82%) ICCA: 1/17 (6%) Unclassified or unknown: 2/17 (12%)
Current seizure frequency	None in 6 months: 16/17 (94%) Unknown: 1/17 (6%)
Current treatment approach	First AED monotherapy: 10/17 (59%) Off medication: 5/17 (29%) Monotherapy changed due to poor efficacy: 1/17 (6%) Unknown: 1 (6%)
Effective treatments used	Carbamazepine: 7/17 (41%) Levetiracetam: 6/17 (35%) Sodium Valproate: 2/17 (12%) Vigabatrin: 1/17 (6%) Phenobarbitone: 1/17 (6%)
Variant type	Frameshift: 15/17 (88%) Missense: 2/17 (12%)
Variant inheritance	From affected parent: 5/17 (29%) From unaffected parent with family history: 3/17 (18%) From unaffected parent without family history 1/17 (6%): Undetermined but with strong family history: 3/17 (18%) Undetermined, no family history: 3/17 (18%) *De novo*: 2/17 (12%)

In this cohort, 15/17 (88%) of pathogenic *PRRT2* variants were frameshift variants and two were missense. In 14/17 (82%) cases the pathogenic variant was the recurrent c.649dupC variant which is similar to that reported in previous cohorts (Ebrahimi-Fakhari et al., 2015). In nine cases parental samples were tested: with the variant *de novo* in two and inherited in seven. In three cases where the variant was inherited, it was inherited from an unaffected parent, but in two of those cases other family members on the transmitting parent's side were affected. This suggests that the penetrance of *PRRT2* is not complete, as has been previously suggested (van Vliet et al., 2012).

A relatively consistent picture of *PRRT2*-related epilepsy as a disorder emerged. This disorder is characterised by onset of focal seizures in the first two years of life, with good short-term seizure outcome.

Presenting seizure was an afebrile focal seizure in 12/17 cases (76%) and 11/17 (65%) had afebrile focal seizures only.

15/17 patients had normal development at latest follow-up; one had mild delay, and one had severe delay. A beneficial therapy was reported in all cases: In seven this was Carbamazepine, in six it was Levetiracetam and in two it was Sodium Valproate. From this data it is difficult to come to any conclusions about whether any specific medication is associated with a positive response in *PRRT2*-related epilepsy since all patients, despite receiving a variety of treatments, had been seizure-free for at least 6 months at the most recent follow-up. This is likely to reflect the natural history of the condition. In only one case was AED therapy changed due to lack of efficacy (case 77), where initial therapy with Levetiracetam was ineffective so treatment was changed to Carbamazepine, with good response. None of these patients received AED polytherapy or developed drug-resistant epilepsy.

The estimated incidence of *PRRT2*-related seizures from this cohort is 1 per 9,970 live births. This is likely to be an underestimate since some patients with mild phenotypes will not have met inclusion criteria. For example, the identical twin of case 334 was found to have the same *PRRT2* variant, but because she only had febrile seizures she was not eligible for inclusion in the cohort. Despite being the most frequently observed seizure-associated gene in Cohort 1, *PRRT2* variants have much more rarely observed in the previous NGS studies (Chapter 3). A number of reasons could explain the relatively high yield for *PRRT2* variants in Cohort 1 compared with previous studies. First, because the seizure phenotype is often mild and self-limited, patients with *PRRT2*-associated phenotypes may not be considered ideal candidates for NGS since families and clinicians may not be invested in finding a cause. Second, *PRRT2*-related seizures may present in the

context of an established family history of PKD, in which case the *PRRT2* cause may already be known, clinicians may have been directed towards single gene *PRRT2* sequencing or a movement disorder gene panel. Third, since it was first established as a PKD gene rather than an epilepsy gene, *PRRT2* was not included in some of the smaller panels.

Patients with 16p11.2 microdeletion syndrome present with similar clinical features to those with *PRRT2* loss of function variants, but are more likely to have additional clinical features, including intellectual disability, skin manifestations, and hypotonia. In the largest study of 16p11.2 deletion carriers, involving 136 individuals, 27% had unprovoked seizures and 7% had febrile seizures (Steinman et al., 2016). Finally, since >80% of *PRRT2* variants in this cohort were inherited, it is possible that there is a population-specific effect of *PRRT2*-related disorders in Scotland.

5.3.5.2 SCN1A

SCN1A encodes the neuronal voltage-gated sodium channel $Na_v1.1$. Opening of this channel leads to rapid efflux of sodium from neuronal cells and brings about depolarisation associated with axonal signal propagation. Experimental studies in mice and expression studies un humans suggest that $Na_v1.1$ preferentially localises to inhibitory interneurons (Ogiwara et al., 2007;), leading to the hypothesis that loss of $Na_v1.1$ function leads to decreased excitability of inhibitory neurones, and thereby contributes to an overall network hyperexcitability. *De novo* pathogenic variants in *SCN1A* are typically associated with Dravet syndrome, a severe epilepsy that typically presents with prolonged febrile seizures in infancy and evolves to a drug-resistant polymorphic epilepsy with associated developmental impairment (Claes et al., 2001). Inherited variants are often associated with milder phenotypes including Genetic Epilepsy with Febrile Seizures Plus (GEFS+) (Escayg et al., 2000), and non-epilepsy neurological phenotypes including Familial Hemiplegic Migraine (FHM) (Dichgans et al., 2005). Patients with *de novo* variants and relatively mild phenotypes are reported (Myers et al., 2017b) and so too have

severely affected individuals within families where other members have had mild phenotypes (Nabbout et al., 2003).

The incidence of *SCN1A*-related Dravet syndrome has previously been estimated in Northern California (Wu et al., 2015). The authors interrogated the case notes of all infants born at Kaiser Peramanente Northern California from 2007 to 2010 to identify those with a clinical picture of Dravet syndrome. They identified eight infants, six of whom had a *de novo* pathogenic *SCN1A* variant. This was from a population base of 125,547 births so they estimated the incidence of *SCN1A-related* Dravet syndrome to be 1 per 20,900 live births. A similar incidence figure of 1 per 22,000 has been quoted in Denmark, though this study was based on the assumption that all cases of *SCN1A*-related Dravet syndrome would have been seen at a single centre (Allan, Helle & Møller, 2015).

Table 5.3j: *SCN1A* gene summary from the literature

Chromosome locus	**2q24.3**
Phenotype observed in microdeletion syndrome?	Yes. 2q24.3 deletion, often involving other sodium channel genes *SCN2A, SCN3A, SCN7A,* and *SCN9A* is associated with a severe epilepsy and developmental disorder (Lim et al., 2015)
pLI Score~	1.0
Brain expression ratio*	80.1
Localisation within brain	Preferentially localises to the axons of inhibitory interneurons (Ogiwara et al., 2007)
Proposed gene function	Encodes a neuronal voltage-gated sodium channel, opening of the channel leads to depolarisation during the action potential
Proposed genetic mechanism of pathogenicity	Haploinsufficiency
Yield in epilepsy NGS literature (Chapter 3)	423/13063 = 3.2%, Rank: 1
Previous incidence estimates	Estimated incidence of SCN1A positive-Dravet syndrome in California: 1 per 20,900 live births (Wu et al., 2015) Estimated incidence of SCN1A positive-Dravet syndrome in in Denmark: 1 per 22,000 live births (Allan, Helle & Møller, 2015)
Previous clinical spectrum descriptions	87% Dravet syndrome 5% Febrile seizures plus 7% other epilepsies (Zuberi et al., 2011) Also associated with a non-epilepsy phenotype, familial hemiplegic migraine (Dichgans M et al., 2005)
Previous age of onset descriptions	Median 6.5 months (Harkin et al., 2007) Median 6 months (Brunklaus et al., 2012)
Previous penetrance estimates	Incomplete penetrance in familial cases reported (Mhanni et al., 2011)
Evidence for specific treatment	RCT evidence to support use of Cannabidiol to treat seizures in Dravet syndrome (Devinsky et al., 2017) RCT evidence to support use of Stiripentol to treat seizures in Dravet syndrome Chiron 2000 (Chiron et al., 2000) Retrospective data suggests avoidance of Carbamazpeine and Lamotrigine (Brunklaus et al., 2012)

~ From the Exome Aggregation Consortium (ExAC) dataset (The Broad Institute Exome Aggregation Consortium, 2018)

* From the GTEx Portal data, based on adult post-mortem tissue expression profiles (Aguet et al., 2017)

Table 5.3k: Patients with *SCN1A* variants from Cohort 1

Yield	11/315 (3.5%), Rank: 2												
Case ID	Age of presentation	Presenting seizure	Dev. delay at presentation > follow-up	Age of follow-up	Epilepsy diagnosis	Seizure types observed	Current seizure frequency	Current treatment approach	Current treatment	Effective treatments	Variant	Inheritance	Family history
135	1.5 months	Febrile focal status	No > Moderate	37 months	Dravet syndrome	Afeb-GTCS Afeb-tonic Afeb-Myo Afeb-AA Feb-status	Daily	Receiving AED polytherapy	Stiripentol, Fenfluramine, CLB, LEV, ESM	Fenfluramine	c.2875T>G, p.Cys959Gly	De novo	None
212	3 months	Afebrile focal	Mild > Mild	19 months	Dravet syndrome	Afeb-focal	< 1 per month	Receiving AED polytherapy	VPA, CLB, Stiripentol	Stiripentol	c.4055T>G, p.Leu1352Arg	De novo	None
58	4 months	Afebrile focal	Mild > Moderate	37 months	Dravet syndrome	Afeb-focal Afeb-GTCS Feb-focal	Monthly	Receiving AED polytherapy	CBZ and VPA	CBZ	c.4441G>A, p.Val1481Ile	De novo	None
218	4 months	Afebrile focal	Mild > Mild	21 months	Dravet syndrome	Afeb-status Afeb-GTCS Afeb-Myo Afeb-focal	Monthly	Receiving AED polytherapy	VPA, Nitrazepam, Stiripentol	PB	c.2290delG, p.Val764Leufs*2	De novo	None
290	6 months	Febrile generalised status	No > No	20 months	Dravet syndrome	Afeb-Myo Afeb-focal Feb-status	Daily	Receiving AED polytherapy	VPA, CLB, Stiripentol	Stiripentol	c.4933C>T, p.Arg1645*	De novo	None
90	7 months	Afebrile focal	No > No	35 months	Febrile seizures plus	Afeb-focal Afeb-GTCS	< 1 per month	Receiving a different AED monotherapy to the first one tried – initial therapy not tolerated	LMT (prev. tried LEV)	None reported	c.5171C>T, p.Ala1724Val	De novo	None
123	7 months	Febrile focal	No > Moderate	44 months	Dravet syndrome	Afeb-GTCS Afeb-Myo Afeb-AA Afeb-atonic Feb-GTCS	Weekly	Receiving AED polytherapy	VPA and CLB	Stiripentol	c.4822G>T, p.Asp1608Tyr	De novo	None
65	11 months	Afebrile absence	No > Moderate	23 months	Dravet syndrome	Afeb-absence Afeb-GTCS	Monthly	Receiving AED polytherapy	VPA and CLB	None reported	c.2619G>C, p.Trp873Cys	De novo	None
180	19 months	Afebrile focal status	Mild > Mild	32 months	Dravet syndrome	Afeb-Myo Afeb-GTCS Afeb-focal Afeb-status Feb-status Feb-GTCS	Monthly	Receiving AED polytherapy	VPA, CLB, Stiripentol	VPA	c.1663-9A>G	Not maternal	None
317	19 months	Febrile focal status	No > No	29 months	Recurrent prolonged febrile seizures	Feb-status Feb-GTCS	< 1 per month	Never had regular AED	None	N/A	c.4091T>C, p.Met1364Thr	Unknown	None
5	25 months	Febrile unknown	No > No	40 months	Recurrent febrile seizures	Feb-GTCS	None in 6 months	Never had regular AED	None	N/A	c.2176+2T>C	Paternal	Father had febrile seizures

Abbreviations used: GTCS – generalised tonic clonic seizure(s). Myo – myoclonic, AA – Atypical absence, Afeb – afebrile, Feb – febrile. GEFS+ - Genetic epilepsy with febrile seizures plus; AED – Anti-epileptic drug. LEV – Levetiracetam, CBZ – Carbamazepine, VPA – Sodium Valproate, CLB – clobazam, PB – Phenobarbitone, ESM – Ethosuximide, LMT – Lamotrigine

Table 5.3l: Summary of patients with *SCN1A* variants in Cohort 1

Median age of presentation	**6.5 months**
Development	Normal at presentation: 7/11 (64%) Normal at latest follow-up: 4/11 (36%)
Presenting seizure type	Afebrile: 6/11 (55%) Afebrile focal: 5/11 (45%) Afebrile focal status: 1/11 (9%) Afebrile absence 1/11 (9%) Febrile: 5/11 (45%) Febrile focal: 3/11 (27%) Febrile focal status: 2/11 (18%) Febrile generalised status: 1/11 (9%) Febrile unknown: 1/11 (9%) Status: 4/11 (36%)
Seizure types observed	Afebrile GTCS: 7/11 (64%) Afebrile focal: 6/11 (55%) Afebrile myoclonic: 5/11 (45%) Afebrile atypical absence: 2/11 (18%) Afebrile tonic: 1/11 (9%) Afebrile atonic: 1/11 (9%) Afebrile status: 2/11 (18%) Febrile status: 3/11 (27%) Febrile GTCS: 3/11 (27%) Febrile focal: 1/11 (9%) Febrile seizures at any time: 7/11 (64%) Status at any time: 5/11 (45%) Multiple seizure types observed: 8/11 (73%)
Latest diagnosis	Dravet syndrome: 8/11 (73%) Febrile seizures or febrile seizures plus: 3/11 (27%)
Current seizure frequency	Seizures at least once per day: 2/11 (18%) Seizure at least once per week: 3/11 (27%) Seizures at least once per month: 7/11 (64%) < 1 seizure per month: 3/11 (27%) None in last 6 months: 1/11 (9%)
Current treatment approach	AED polytherapy: 8/11 (73%) Second AED monotherapy (initial treatment not tolerated): 1/11 (9%) Never had a regular AED: 2/11 (18%)
Effective treatments used	Stiripentol: 3/11 (27%) Carbamazepine: 1/11 (9%) Sodium Valproate: 1/11 (9%) Phenobarbitone: 1/11 (9%)
Variant type	Missense: 7/11 (64%) Frameshift/nonsense/splice-site: 4/11 (36%)
Variant inheritance	*De novo:* 8/11 (73%) From affected parent: 1/11 (9%) Unknown: 2/11 (18%)

Among the 11 patients with a pathogenic *SCN1A* variant identified in Cohort 1, eight had been diagnosed with Dravet syndrome at the time that the follow-up questionnaire was completed, making the estimated incidence of *SCN1A*-related Dravet syndrome in this population 1 per 21,200 live births – a very similar figure

to that obtained by Wu et al. and Bayat et al. All of the patients diagnosed with Dravet syndrome had a *de novo SCN1A* variant apart from one patient (case 180) for whom the variant was not maternally-inherited, but a paternal sample was not available. Dravet syndrome has been characterised as a severe drug-resistant epilepsy which typically presents initially with febrile seizures, before progressing to a severe drug-resistant epilepsy with frequent seizures of multiple types. Developmental stagnation or regression is seen from the second year of life and is hypothesised to be partly due to uncontrolled seizure activity (Brunklaus et al., 2012).

Cross-sectional data from patients with Dravet syndrome suggests that by the end of the second year of life, 40% of patients have normal development, 30% have mild learning disability, 20% have moderate learning disability, and 10 have severe learning disability (Brunklaus et al., 2012). At most recent follow-up, at a median age of 32 months (range 19 to 44) 36% of patients with *SCN1A* variants in Cohort 1 had normal development, which would be consistent with this literature.

Seizure control in this group was good compared with historical cohorts. Though just one patient was seizure-free, only 3/11 (27%) of these patients were having seizures at least once per week. Due to small numbers it is difficult to determine whether seizure control was related to early initiation of specific treatment in the group. All three patients who were experiencing seizures at least once per week were on highly specific treatments for Dravet syndrome (two Stiripentol, and one Fenfluramine).

5.3.5.3 KCNQ2

KCNQ2 encodes the neuronal voltage-gated potassium channel, $K_v7.1$. This is a slowly activating and deactivating potassium conductance that plays a critical role in determining the subthreshold electroexcitability of neurons as well as the responsiveness to synaptic inputs. Variants in *KCNQ2* were first described in families with self-limited familial neonatal seizures (Singh et al., 1998) but have

since also been associated with severe early-onset epileptic encephalopathy (Dedek et al., 2003) and with myokymia (Dedek et al., 2001) – a non-epileptic movement disorder. Milder phenotypes are associated with truncating variants (Soldovieri et al., 2014; Claes et al., 2004) and whole gene deletions (Heron et al., 2007; Kurahashi H et al., 2009) and more severe phenotypes are associated with missense variants (Kato et al., 2013; Olson et al., 2017; Weckhuysen et al., 2012). *In vitro* functional studies have demonstrated that missense *KCNQ2* variants associated with severe phenotypes exhibit gain of function properties (Millichap et al., 2017) suggesting that both loss-of-function and gain-of-function may play a role in *KCNQ2*-related seizures, with perhaps gain-of-function variants having a more detrimental consequence.

Table 5.3m: *KCNQ2* gene summary from the literature

Chromosome locus	**20q.13.33**
Phenotype observed in microdeletion syndrome?	Yes – neonatal seizures often observed in 20q13.3 deletion syndrome (Akihisa et al., 2015)
pLI Score~	1.0
Brain expression ratio*	30.2
Localisation within brain	High expression in hippocampus, temporal cortex, cerebellar cortex, and medulla (Kanaumi et al., 2008)
Proposed gene function	Slowly activating and deactivating neuronal potassium channel.
Proposed genetic mechanism of pathogenicity	Both gain-of-function and loss-of-function mechanism likely
Yield in epilepsy NGS literature (Chapter 3)	231/13063 (1.8%), Rank: 2
Previous incidence estimates	None
Previous clinical spectrum descriptions	No unselected cohorts published. Can present as self-limited neonatal seizures or as a severe neonatal-onset epileptic encephalopathy. Also associated with a non-epilepsy phenotype, peripheral nerve hyperexcitability (Wuttke et al., 2007)
Previous age of onset descriptions	Mean 1.8 days in encephalopathy cases (Olson et al., 2017; Millichap et al., 2016) First week of life in all familial cases (Soldovieri et al., 2014)
Previous penetrance estimates	Complete penetrance in all families reported, though phenotype can be variable (de Haan et al., 2006; Hewson, Puka & Mercimek-Mahmutoglu, 2017)
Evidence for specific treatment	Retrospective uncontrolled clinician report of seizure-freedom. N = 15. 6/15 (40%) of cases with *KCNQ2* encephalopathy achieved seizure-freedom within two weeks of commencing Carbamazepine. Retrospective uncontrolled clinician report of seizure-freedom. N = 15. 5/15 (33%) of cases with *KCNQ2* encephalopathy achieved seizure-freedom within two weeks of commencing Phenytoin (Pisano et al., 2015).

~ From the Exome Aggregation Consortium (ExAC) dataset (The Broad Institute Exome Aggregation Consortium, 2018)

* From the GTEx Portal data, based on adult post-mortem tissue expression profiles (Aguet et al., 2017)

Table 5.2n: Patients with *KCNQ2* variants from Cohort 1

Yield	9/315 (2.9%), Rank: 3												
Case ID	Age of presentation	Presenting seizure	Dev. delay at presentation > follow-up	Age of follow-up	Epilepsy diagnosis	Seizure types observed	Current seizure frequency	Current treatment approach	Current treatment	Effective treatments	Variant	Inheritance	Family history
336	3 days	Afebrile focal	No > No	12 months	Self-limited neonatal seizures	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	CBZ	Whole gene deletion	Unknown	None
302	5 days	Afebrile focal	No > No	13 months	Self-limited familial neonatal seizures	Afeb-focal	None in 6 months	Still receiving first AED monotherapy	PB	PB	c.1918delC, p.Leu640fs	Unknown	Mother has history of neonatal seizures
293	5 days	Afebrile focal	No > No	12 months	Self-limited familial neonatal seizures	Afeb-focal	None in 6 months	Never had a regular AED	None	N/A	Duplication of Exons 2-12	Paternal	Father has history of neonatal seizures
279	9 days	Afebrile focal	No > No	13 months	Self-limited familial neonatal seizures	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	CBZ	Duplication of Exons 2-12	Maternal	Mother has history of neonatal seizures
338	19 days	Afebrile focal	No > No	14 months	Self-limited familial neonatal seizures	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	CBZ	c.1874_1880del, p.Lys625Argfs*15	Unknown	Paternal cousin had neonatal seizures
294	1 month	Afebrile focal	Moderate > Moderate	13 months	Early infantile-onset developmental and epileptic encephalopathy	Afeb-focal Afeb-tonic	None in 6 months	Receiving a different AED monotherapy to the first one tried – initial therapy not effective	CBZ	CBZ	c.1657C>T, p.Arg553Trp	De novo	None
46	3 months	Afebrile focal	No > Mild	40 months	Unclassified focal epilepsy	Afeb-focal	Daily seizures	Receiving AED polytherapy	VPA and CBZ	CBZ	c.1116C>G, p.Tyr372*	De novo	None
177	4 months	Afebrile focal	Severe > Profound	31 months	Early infantile-onset developmental and epileptic encephalopathy	Afeb-focal	None in 6 months	Receiving a different AED monotherapy to the first one tried – initial therapy not effective	CBZ	CBZ	Whole gene deletion	Paternal	Father has history of neonatal seizures
326	4 months	Afebrile focal	No > No	17 months	Self-limited infantile seizures	Afeb-focal	None in 6 months	Previously received AED therapy – now off medication	None	CBZ	c.1106_1118+6del TGCCCATGTACAG GTACCG, p.Pro370Argfs*15	Unknown	None
Abbreviations used: GTCS – generalised tonic-clonic; Afeb – afebrile, Feb-febrile, AED – anti-epileptic drug, CBZ – Carbamazepine, VPA – Sodium Valproate, PB - Phenobarbitone													

Table 5.3o: Summary of patients with *KCNQ2* variants in Cohort 1

Median age of presentation	**18 days**
Development	Normal at presentation: 7/9 (78%) Normal at latest follow-up: 7/9 (78%)
Presenting seizure type	Afebrile focal: 9/9 (100%)
Seizure types observed	Afebrile focal: 9/9 (100%) Afebrile tonic: 1/9 (11%)
Latest diagnosis	Self-limited neonatal seizures: 5/9 (56%) Early infantile-onset developmental and epileptic encephalopathy: 2/9 (22%) Unclassified focal epilepsy 1/9 (11%) Self-limited infantile seizures: 1/9 (11%)
Current seizure frequency	None: 8/9 (89%) At least one seizure per day: 1/9 (11%)
Current treatment approach	Off medication: 4/9 (44%) Monotherapy changed due to poor efficacy: 2/9 (22%) First AED monotherapy: 1/9 (11%) Never had AED treatment: 1/9 (11%) AED polytherapy: 1/9 (11%)
Effective treatments used	Carbamazepine: 7/9 (78%) Phenobarbitone: 1/9 (11%)
Variant type	Frameshift/nonsense/splice-site: 4/9 (44%) Whole gene deletion: 2/9 (22%) Duplication of exons 2-12: 2/9 (22%) Missense: 1/9 (11%)
Variant inheritance	From affected parent: 3/9 (33%) *De novo*: 2/9 (22%) Unknown: 4/9 (44%)

The literature would suggest that it is very rare for *KCNQ2*-related epilepsy to present beyond the neonatal period (Zhang et al., 2017; Millichap et al., 2016; Olson et al., 2017; Millichap et al., 2017; Allen et al., 2014). In this cohort 4/9 cases presented at one month or over, perhaps suggesting an ascertainment bias in the literature. The findings within this *KCNQ2* cohort are at also odds with the literature which has indicated that inherited variants and missense variants are associated with more severe phenotypes. The most severely affected individual (case 177) – the only one with severe or profound cognitive impairment – had an inherited gene deletion from his father who had a personal and family history of self-limited familial neonatal seizures. The only case who developed drug-resistant epilepsy (case 46) had a *de novo* nonsense variant. All patients with *KCNQ2* variants presented with focal seizures, and only one patient (case 294) developed any other seizure type (tonic seizures). Seizure outcomes in this group of patients

were good, with 8/9 patients seizure-free for at least six months at the time of most recent follow-up.

5.3.5.4 SCL2A1

SLC2A1 encodes GLUT1, the major glucose transporter in brain, placenta and erythrocytes. Dysfunction of blood-to-brain glucose transport was initially postulated as the cause of a disorder identified in three unrelated patients who presented with early infantile onset drug-resistant myoclonic seizures, developmental delay, microcephaly, and low cerebrospinal fluid (CSF) but normal serum glucose levels (de Vivo et al., 1991). Subsequently, *de novo* loss of function variants (one whole gene deletion and two nonsense variants) of the *SLC2A1* gene were identified in these three patients (Seidner et al., 1998).

Subsequent reports have significantly expanded the phenotype associated with *SLC2A1* variants to include a broad range of clinical presentations, including various paroxysmal and static movement disorders – including dystonia, exercise-induced dystonia, ataxia, spasticity, and chorea – (Hully et al., 2015; Suls et al., 2008). The majority of affected individuals have epileptic seizures before the age of three years, yet seizure types and outcomes vary, as do developmental and cognitive profiles of affected individuals (Hully et al., 2015). Despite this wide phenotypic variability, a consistent finding is that affected individuals have a low CSF to plasma glucose ratio (de Giorgis & Veggiotti, 2013).

The finding that patients with GLUT1 deficiency (Glut1-D) often present with early childhood-onset absence or myoclonic seizures has prompted some groups to screen for *SLC2A1* variants in patients presenting with these seizure types. Larsen et al. screened 50 children presenting with absence seizures between six months and six years and identified pathogenic *SLC2A1* variants in five of these (10%). Those with *SLC2A1* variants presented at a wide range of ages, between three weeks and five years (Larsen et al., 2015b). Arsov et al. screened 89 patients with early onset absence seizures (before four years of age) and identified 11 (12%) with

pathogenic *SCL2A1* variants (Arsov et al., 2012). Mullen et al. screened 84 children with myoclonic-atonic seizures and identified four with pathogenic *SLC2A1* variants (5%) (Mullen et al., 2011). In the Larsen, Arsov and Mullen papers, more detailed description of the phenotypes reveals that the majority of affected patients do in fact have additional features, typically developmental delay and ataxia, though these may be subtle.

More severe Glut1-D phenotypes are observed with truncating variants than with missense variants, but no other genotype-phenotype correlations have been described (Hully et al., 2015; de Giorgis & Veggiotti, 2013).

Table 5.3p: *SLC2A1* gene summary from the literature

Chromosome locus	**1p34.2**
Phenotype observed in microdeletion syndrome?	Yes – features of Glut1-D observed in patients with deletions involving *SLC2A1* (Vermeer et al., 2007)
pLI Score~	0.94
Brain expression ratio*	1.0
Localisation within brain	Choroid plexus, ependyma, and glia (Vannucci et al., 1998)
Proposed gene function	Actively transports glucose across the blood-brain barrier
Proposed genetic mechanism of pathogenicity	Haploinsufficiency
Yield in epilepsy NGS literature (Chapter 3)	52/13063 (0.4%), Rank: 10
Previous incidence estimates	Estimated as 1 per 90,000 live births in Queensland, Australia (Coman David et al., 2006) and 1 per 83,000 in Denmark (Larsen et al., 2015b)
Previous clinical spectrum descriptions	No unselected cohorts published. Associated phenotypes include infantile onset epileptic encephalopathy (de Vivo et al., 1991; Seidner et al., 1998), early onset absence epilepsy (Agostinelli et al., 2013), epilepsy with myoclonic-atonic seizures (Agostinelli et al., 2013), generalised epilepsy with comorbid movement disorder (Larsen et al., 2015b), and exercise-induced dystonia without epileptic seizures (Schneider et al., 2009).
Previous age of onset descriptions	Range 3 months to 8 years (Hully et al., 2015; Larsen et al., 2015b)
Previous penetrance estimates	None. Occasional families with incomplete penetrance reported (Striano et al., 2012)
Evidence for specific treatment	Retrospective uncontrolled family-reported subjective treatment response analysis. N = 82. 38/82 (46%) had seizure-freedom, 66/83 (80%) had ≥90% seizure-reduction, and 78 (95%) had ≥50% seizure-reduction at latest follow up (range 1 month-20 years, median 5.5 years) (Kass et al., 2016).

~ From the Exome Aggregation Consortium (ExAC) dataset (The Broad Institute Exome Aggregation Consortium, 2018)

* From the GTEx Portal data, based on adult post-mortem tissue expression profiles (Aguet et al., 2017)

Table 5.3q: Patients with *SLC2A1* variants from Cohort 1

Yield	6/315 (1.9%), Rank: 4													
Case ID	Age of presentation	Presenting seizure	Dev. delay at presentation > follow-up	Age of follow-up	Epilepsy diagnosis	Seizure types observed	Additional features	Current seizure frequency	Current treatment approach	Current treatment	Effective treatments	Variant	Inheritance	Family history
119	11 months	Afebrile focal	No > Mild	42 months	Unclassified generalised and focal epilepsy	Afeb-focal Afeb-GTCS Afeb-atonic	Mild gait ataxia	None in 6 months	Receiving AED polytherapy and Ketogenic Diet	LEV, CBZ KD	Unknown – KD just started	c.497_499delTCG, p.Val166del	De novo	None
167	12 months	Afebrile myoclonic	Mild > No	36 months	Myoclonic epilepsy	Afeb-myo	Mild gait ataxia	None in 6 months	Never had regular AED Ketogenic Diet	KD	KD	c.736_739delGAAG , p.Glu246fs	De novo	Paternal uncle has epilepsy
139	12 months	Afebrile absence	No > No	44 months	Epilepsy with myoclonic-atonic seizures	Afeb-absence Afeb-myo Afeb-atonic	Mild gait ataxia	None in 6 months	Receiving a different AED monotherapy to the first one tried – initial therapy not effective Ketogenic Diet	VPA KD	VPA KD	c.277C>T, p.Arg93Trp	Unknown - adopted	Biological mother has learning difficulties and history of epilepsy
87	16 months	Afebrile GTCS	Mild > Mild	50 months	Unclassified generalised epilepsy	Afeb-status Afeb-GTCS	Mild gait ataxia	< 1 per month	Never had regular AED Ketogenic Diet	KD	KD	c.940G>A, p.Gly314Ser	Paternal	Father has exercise-induced dystonia
50	18 months	Afebrile GTCS	Mild > No	57 months	Unclassified generalised epilepsy	Feb-GTCS Afeb-status Afeb-GTCS	Severe 4-limb dystonia (resolved on KD)	None in 6 months	Previously received AED therapy – now off medication Ketogenic Diet	KD	KD	c.505_507delCTC, p.Leu169del	De novo	None
156	19 months	Afebrile GTCS	No > Mild	41 months	Unclassified generalised epilepsy	Afeb-GTCS	Mild gait ataxia	None in 6 months	Never had regular AED Ketogenic Diet	KD	KD	c.929C>G, p.Thr310Ser	De novo	None
Abbreviations used: GTCS – generalised tonic-clonic; myo – myoclonic, Afeb – afebrile, Feb-febrile, AED – anti-epileptic drug, LEV – Levetiracetam, CBZ – Carbamazepine, VPA – Sodium Valproate, KD – Ketogenic Diet														

Table 5.3r: Summary of patients with *SCL2A1* variants in Cohort 1

Median age of presentation	**12 months**
Development	Normal at presentation: 3/6 (50%) Normal at latest follow-up: 3/6 (50%)
Presenting seizure type	Afebrile GTCS: 3/6 (50%) Afebrile myoclonic: 1/6 (17%) Afebrile absence: 1/6 (17%) Afebrile focal: 1/6 (17%)
Seizure types observed	Afebrile GTCS: 4/6 (67%) Afebrile status: 2/6 (33%) Afebrile myoclonic: 2/6 (33%) Afebrile atonic: 2/6 (33%) Afebrile absence: 1/6 (17%) Afebrile focal: 1/6 (17%) Febrile GTCS: 1/6 (17%)
Latest diagnosis	Unclassified generalised epilepsy: 3/6 (50%) Unclassified generalised and focal epilepsy: 1/6 (17%) Epilepsy with myoclonic-atonic seizures: 1/6 (17%) Myoclonic epilepsy: 1/6 (17%)
Current seizure frequency	None in last 6 months: 5/6 (83%) < 1 seizure per month: 1/6 (17%)
Current treatment approach	Never had regular AED, on Ketogenic Diet: 3/6 (50%) AED polytherapy and Ketogenic Diet: 1/6 (17%) Monotherapy changed due to poor efficacy and Ketogenic Diet: 1/6 (17%) Off medication, on Ketogenic Diet: 1/6 (17%)
Effective treatments used	Ketogenic Diet: 5/6 (83%) Sodium Valproate: 1/6 (17%)
Variant type	Missense: 3/6 (50%) In-frame deletion: 2/6 (33%) Frameshift: 1/6 (17%)
Variant inheritance	*De novo:* 4/6 (67%) From affected parent: 1/6 (17%) Unknown: 1/6 (17%)

Though there has been no previous large cohort of unselected children with seizures tested for variants in *SLC2A1,* a surprising finding in this cohort is that age of presentation appears to be later than suggested by the published literature. In the largest published series of *SLC2A1* cases, from France, 16/38 cases had epileptic seizures, and for those who had seizures, the median age of presentation with seizures was 6 months (range 1.5 to 60 months) (Hully et al., 2015). In Cohort 1 the median age of presentation was 12 months (range 11-19 months). Seizures in Glut1-D can present at a wide range of ages, so that fact that no early-infantile-onset cases were captured in Cohort 1 is likely to reflect chance and small

numbers. There has been a perception from previous case reports of Glut1-D that patients are likely to present with, or develop, absence seizures or myoclonic seizures. In Cohort 1 the most commonly observed seizure type was GTCS, and 4/6 (67%) patients had neither myoclonic nor absence seizures. Seizure-outcomes in this group were good, with 5/6 (83%) patients having had no seizures for six months or more at the time of their most recent follow-up. Short term developmental outcomes were also good with three patients having mild delay, and three having normal development at latest follow-up. In common with previous studies investigating *SLC2A1* variants and epilepsy, all the patients identified in Cohort 1 had a movement disorder as well as seizures (ataxia or dystonia), though in 5/6 patients this manifested just as just mild gait ataxia.

The incidence of Glut1-D has previously been estimated as 1 per 90,000 live births in Queensland (Coman et al., 2006) and 1 per 83,000 live births in Denmark (Larsen et al., 2015b). These estimates have been based upon the fact that both Queensland and Denmark had single centres for *SLC2A1* testing, but because there was no active recruitment strategy for these studies there are likely to be a number of undiagnosed cases within those populations. The data from Cohort 1 suggests that Glut1-D is far more common than these previous estimates (1 per 28,000 live births) and that in cases where Ketogenic Diet is initiated early, the short-term outcomes are good. 1 per 28,000 is likely to be a significant underestimate of the incidence of Glut1-D since previous studies have shown that it may present without seizures, or with seizures after the age of three years (Kass et al., 2016; Coman et al., 2006; Hully et al., 2015; de Giorgis & Veggiotti, 2013; Larsen et al., 2015b). From the small number of cases identified in Cohort 1 it is not possible to assess genotype-phenotype correlation, though it is worthy of note that the one case with a truncating variant (case 167) was seizure-free and had normal development at most recent assessment (36 months).

5.3.5.5 Other genetic diagnoses made in Cohort 1

Between them four genes - *PRRT2, SCN1A, KCNQ2*, and *SLC2A1* - account for 43/74 (58%) of single gene diagnoses made in Cohort 1. For the other single gene diagnoses made in Cohort 1, the numbers are too small to draw conclusions about the clinical spectrum of each, and the confidence intervals for incidence estimates are broad (Figure 5.3c). There were four cases each of *CDKL5*, *PCDH19*, *SLC6A1,* and *DEPDC5*. These genes appear third, sixth, equal 68th respectively, in the list of genes most commonly implicated in diagnoses made on epilepsy panels (Chapter 3). The incidence of *SLC6A1*-related epilepsy is likely to be an over-estimate since three of the four patients in Cohort 1 were siblings. The figures in Chapter 3 are likely to underestimate the relative yield of *SLC6A1* and *DEPDC5*-related epilepsy because these genes were not included in the largest gene panel study by Lindy et al. (Lindy et al., 2018).

5.3.5.6 Diagnosing genetic epilepsies with treatment implications

Figure 5.3g highlighted those genetic diagnoses made in Cohort 1 for which there was some clinical or experimental evidence to support a specific treatment approach, or "precision medicine." 85% (63/74) of genetic diagnoses fell into this category and 20% (63/315) of the entire cohort were diagnosed with a genetic cause that had potential treatment implications.

I looked at what proportion of patients in Cohort 1 had in fact been treated with a specific therapeutic approach as supported by the evidence presented in Chapter 4 (table 5.3s). This shows that although 63 patients in this cohort had diagnoses involving genes for which there was some support for a specific therapeutic approach, only 36 patients did in fact receive such an approach. There are likely to be a number of reasons why not all "eligible" patients received precision therapy. First, four of these patients never in fact received any anti-epileptic therapy, because seizures were febrile or self-limited. Second, empirical therapy may have been started prior to knowledge of genetic result, and if this was

effective and tolerated, there would have been no incentive for the clinician to change treatment strategy. Third, clinicians may not have been aware of the evidence base, or had judged it too weak, to inform their treatment decisions. Finally, the timing of follow-up data was variable, so some patients may have been yet to start one of the evidence-based treatments.

The "clinician reported benefit" from treatment used here is a crude and subjective measure of treatment efficacy. Clinician reports of relative therapeutic efficacy could be partially validated by asking for reports from parents as well. The most objective method of determining treatment benefit would be to ask parents to keep prospective diaries of seizure frequencies, though this would be time intense for families. Additional benefits of therapy could be measured though prospectively accrued developmental, behavioural and quality of life questionnaires, though for patients on serial monotherapies or polytherapy it would be difficult to tease out the relative effects of different therapies.

Table 5.3s: Patients treated with precision therapy in Cohort 1

Gene	Proposed precision treatment(s) (Chapter 4)	Number of patients in Cohort 1	Number of treated patients in Cohort 1	Number of treated patients currently on, or reported to have previously benefited from, precision therapy
PRRT2	Carbamazepine	17	17	7/17 (41%)
SCN1A	Stiripentol Cannabidiol Fenfluramine Avoidance of Carbamazepine and Lamotrigine	11	9	8/9 (89%) 5 Stiripentol, 1 Fenfluramine, 8 never treated with CBZ or LMT
KCNQ2	Carbamazepine	9	8	7/8 (88%)
SCL2A1	Ketogenic Diet	6	6	6/6 (100%)
CDKL5	Ketogenic Diet Sodium Valproate Lamotrigine	4	4	3/4 (75%) 2 Ketogenic diet, 1 VPA
PCDH19	Intermittent corticosteroids Clobazam	4	4	2/4 (50%) 1 Intermittent corticosteroids, 1 Clobazam
SLC6A1	Sodium Valproate	4	4	0/4
CACNA1A	Lamotrigine	2	1	0/1
MECP2	Dextramethorphan	1	1	0/1
POLG	Avoidance of Sodium Valproate	1	1	1/1 (100%)
SCN8A	Phenytoin	1	1	0/1
SCN2A	Carbamazepine, Lamotrigine or Phenytoin	1	1	1/1 (100%) CBZ
STXPB1	Levetiracetam	1	1	0/1
KCNT1	Quinidine	1	1	1/1 (100%)
TOTAL		63	59	36/59 (61%)

Abbreviations used: *CBZ – Carbamazepine; LMT – Lamotrigine; VPA – Sodium Valproate*

What table 5.3s does show is that a very high proportion of genetic diagnoses do involve genes for which at least attempts have been made to make a case for precision therapy. In order to see if the same was true for genetic diagnosis as a whole in epilepsy I looked back at the data in Figure 3.2b which shows the cumulative results from 24 NGS studies in epilepsy. Genes for which diagnosis may have therapeutic implications are shaded black – this includes all of the top 10 genes, and 1571/2219 (71%) of all diagnoses made. A ten gene epilepsy panel which included *SCN1A, KCNQ2, CDKL5, SCN2A, STXBP1, PCDH19, PRRT2, SCN8A, MECP2,* and *SLC2A1* would theoretically capture 62% of all diagnoses, and 91% of diagnoses with treatment implications.

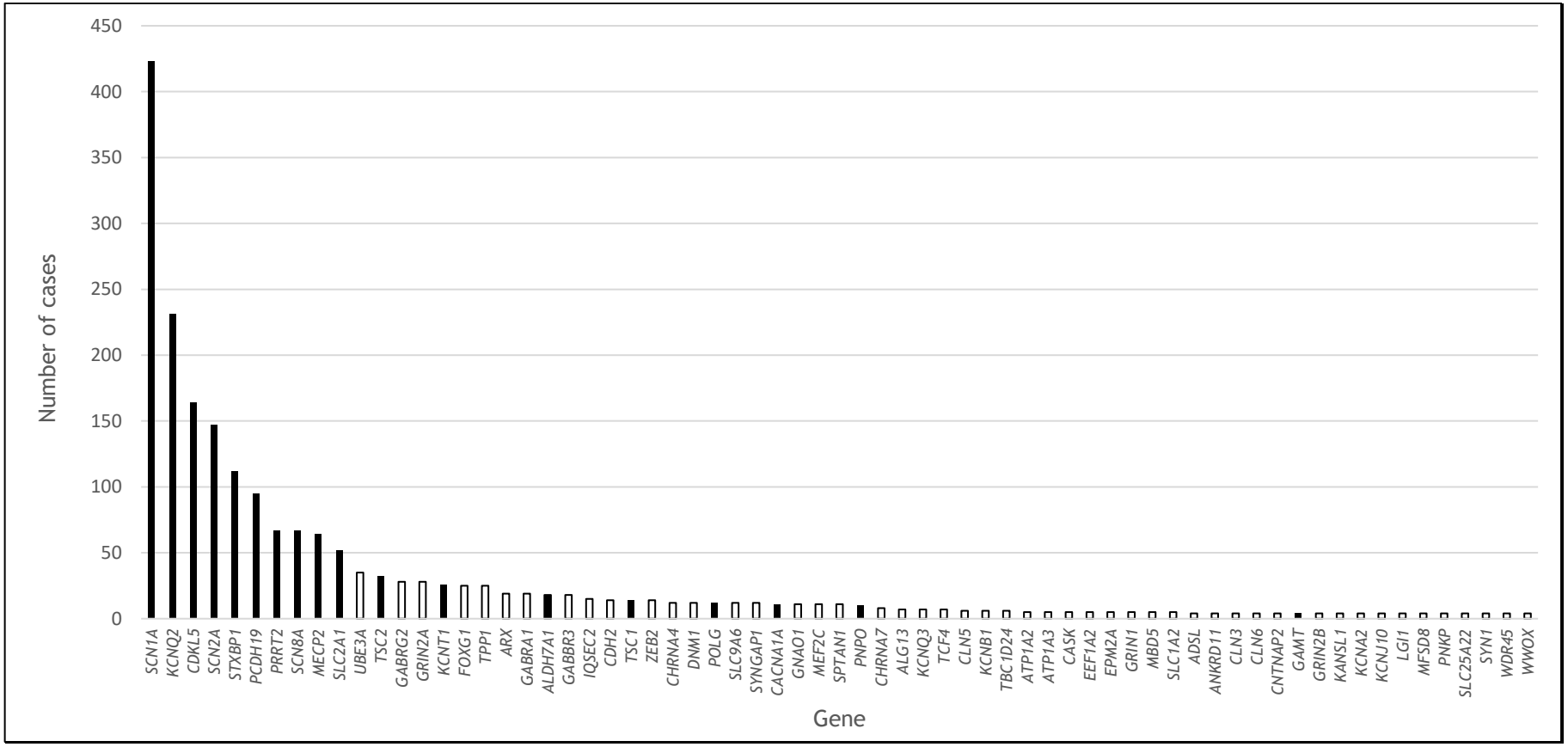


Figure 5.3i: Cumulative totals of genetic diagnoses made from 24 NGS studies (genes with 4 or more hits); Genes for which there is some evidence to support a precision approach are shaded black

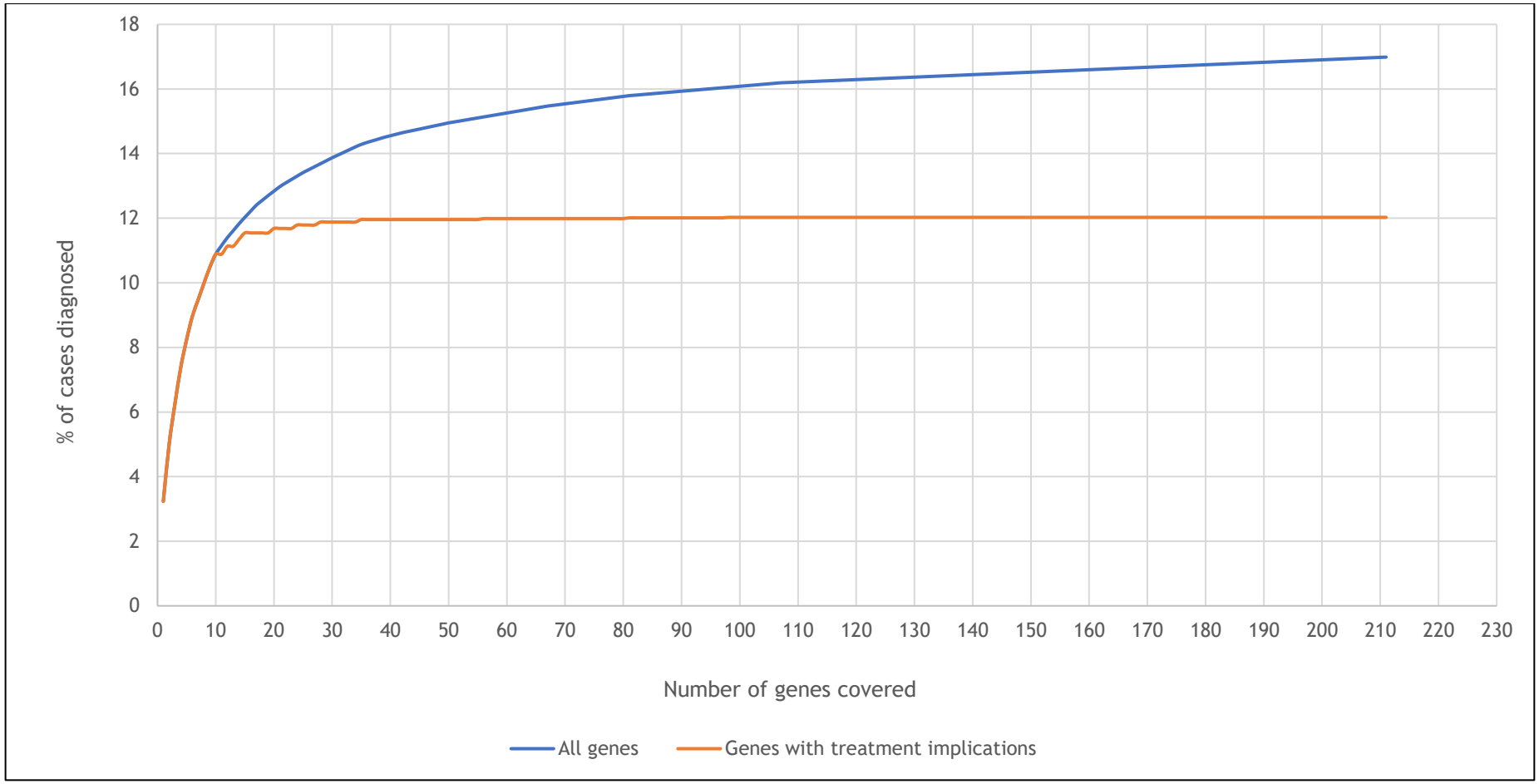


Figure 5.3j: Theoretical relationship between number of genes covered and % of cases diagnosable, based on data extrapolated from 24 NGS studies; a 10 gene panel would diagnose 62% of cases, and 91% of cases in which the genetic diagnosis had treatment implications

5.3.5.7 Limitations of the data from Cohort 1

Cohort 1 was a prospective national population-based cohort study in which the aim was to recruit all children presenting before the age of three years with seizure phenotypes ranging form the mild (a single cluster of afebrile seizures within 24 hours) to the severe (drug-resistant epilepsies). By having broad inclusion criteria this cohort was able to overcome the ascertainment biases inherent in previous NGS studies which have preferentially recruited patients with severe and/or pharmaco-resistant epilepsies. It is likely that because of the broad inclusion criteria, Cohort 1 has shown a higher yield for *PRRT2*-related epilepsy (a typically mild and self-limited condition) than previous NGS studies. The incidence of *SLC2A1*-related epilepsy is higher, and the phenotype milder, than has been suggested by previous studies, which is also likely to reflect ascertainment of milder cases that would not have been otherwise identified. Interestingly the cohort did not identify many mild *SCN1A* cases and did not show a greater incidence of Dravet syndrome than previous studies, suggesting that the established epidemiology and described clinical spectrum of *SCN1A*-related seizures is already quite accurate.

Despite having broader inclusion criteria than previous studies, Cohort 1 was still selective. Patients with very mild phenotypes such as single seizures, or recurrent short-duration febrile seizures were not included. Since these presentations may also have a genetic basis, it is possible that cohort 1 has underestimated the incidence of monogenic seizure disorders. Indeed, the twin sister of case 334 had the same *PPRT2* variant but was not included in cohort 1 because she did not meet eligibility criteria (all her seizures were febrile and <10 minutes). The cohort was also selective on age, and number of genetic epilepsies that were tested for in the study. Particularly, *SLC2A1, DEPDC5*, *POLG,* and *KCNT1* can present with seizures in later childhood and adulthood, so the incidences of these may have also been underestimated. In order to determine the true incidence of monogenic seizure disorders, a truly unselected birth cohort would have to be followed up for life, and whenever an individual from the cohort presented with a seizure of any type

they would be offered comprehensive genetic testing. Such as study would be costly and practically challenging.

Cohort 1 did not have complete recruitment, with capture-recapture methodology suggesting that an estimated eligible 229 cases were not recruited. This figure is based on the assumptions that the rate eligible presentations in the rest of Scotland would be equal to that in the West of Scotland Health Boards (Greater Glasgow and Clyde, Lanarkshire, Forth Valley, Ayrshire and Arran, Dumfries and Galloway), and that recruited patients and non-recruited patients were equally likely to have been investigated with EEG. These limitations notwithstanding, it is likely that patients with monogenic seizure disorders presented to health care but were not recruited to the cohort. It is also possible that patients presented to health care services outwith the Scottish NHS (to English services or to private providers) or to facilities in the three small Health Boards not included in the study (Western Isles, Orkney, Shetland). The limitation of potential under recruitment can be qualified by quoting the incidences as "minimum" incidences. However, because the numbers in each gene group are relatively small the confidence intervals for each incidence estimate are wide, as shown in table 5.3c. A study with a larger population, or over a longer time period would help narrow these confidence intervals and help define the incidence of some of the rarer monogenic seizure disorders.

Eligibility for inclusion was not based on year of birth but on age at presentation and date of presentation. This was a practical decision since it allowed 300 cases to be recruited in half of the time that it would have taken had a birth cohort approach been taken. The drawback of this approach is that it limited the accuracy of the denominator. Patients included in the cohort could have been born between May 9th 2011 and May 7th 2017. In the absence of a true denominator three times the mean number of births in Scotland between the years 2011 and 2016 inclusive has been used as a denominator. Since this birth rate remained relatively stable, it is unlikely to have significantly affected the accuracy of the incidence estimates.

Some of the data presented in this section is limited in its precision. For example, information on developmental status and at most recent follow-up is based on clinician-reported impression, which will be less accurate than formal developmental assessment. Though parents were given ABASII questionnaires which provide a proxy measure of child development, the low return rate on these has limited their application to analysis of the cohort. Impression of development may also change over time, but for practical reasons, follow-up data was not obtained at a standard age for all patients.

All patients in Cohort 1 were tested for pathogenic variants in 104 genes, yet this list of 104 does not represent all known epilepsy-associated genes, and it is possible that further diagnoses could have been made had a more extensive platform been used. Other genetic diagnoses, involving chromosomal microduplications and microdeletions may have been made had chromosomal microarray been part of the genetic testing protocol. Chromosomal microarray was not included as part of the testing protocol because this is a test that is was already available in the four regional genetics departments in Scotland. In reality some patients with chromosomal microdeletions and microduplications were in fact included in cohort 1 because their particular Copy Number Variant (CNV) was picked up through MLPA testing of a single gene (cases 336 177 with *KCNQ2* deletion and cases 293 and 279 with *KCNQ2* duplication). Other patients with CNVs may have never been recruited to Cohort 1 if this was known about before presentation with seizures, for example if the child had already been investigated for developmental delay. In order to understand the relative importance of single gene variants, CNVs and other aetiologies in the under three years group presenting with seizures another cohort, in which patients were not excluded on the basis of having an established aetiology, was required. This was Cohort 2.

5.3.6 Cohort 2: Epidemiology

Patients in Cohort 2 were identified through two independent routes: i) Being in cohort 1; and ii) Review the notes of all children who had an EEG done in NHS

Forth Valley, NHS Greater Glasgow and Clyde or NHS Ayrshire and Arran between January 1st 2014 and December 31st 2017, and who were younger than four years at the time of the EEG. The numbers identified in Cohort 2 have already been referred to in section 2.3.2 when estimating the number of missing cases from Cohort 1.

208 patients in Cohort 2 had been diagnosed with epilepsy by the time of their most recent follow-up. Of these, 136 had been included in Cohort 1, and 191 were identified through EEG records, with an overlap of 119.

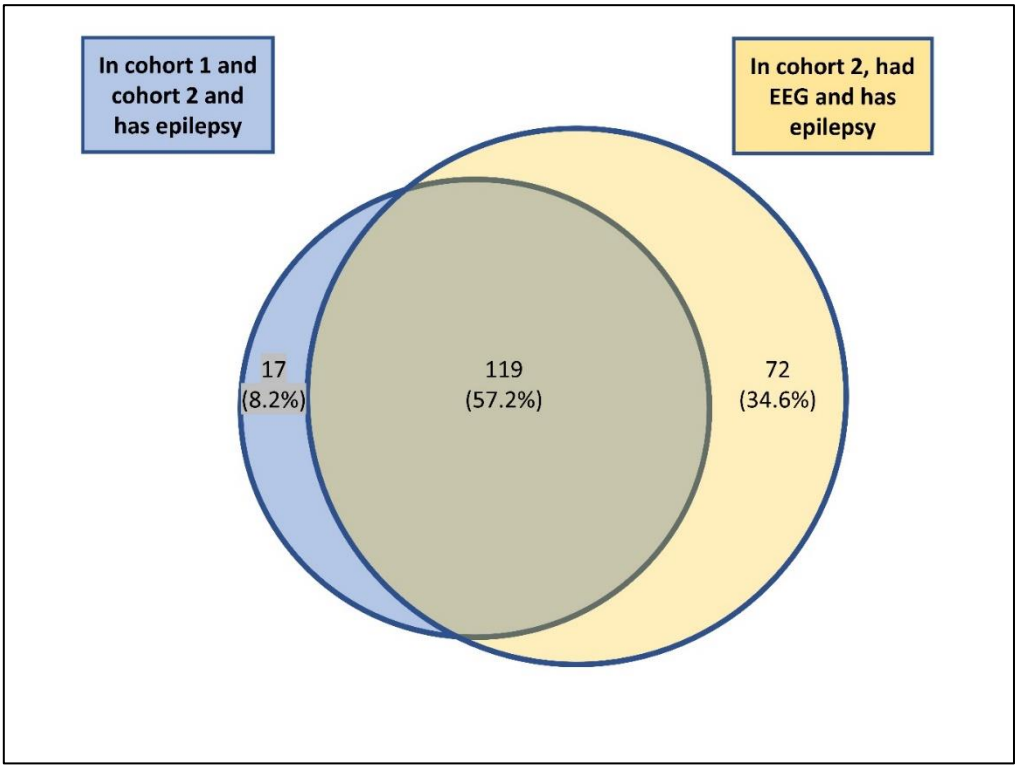


Figure 5.3k: Patients with a diagnosis of epilepsy in Cohort 2, according to whether they were in Cohort 1 and whether they were identified through EEG review

Figure 5.3k shows that of 136 patients with epilepsy who were recruited to Cohort 1. 119 (87.5%) had been investigated with EEG, and that an additional 72 patients with epilepsy were identified through EEG review. The expected additional number of patients with epilepsy that had not been investigated with EEG would therefore

be 10 (72/0.875 – 72), making the expected total number of West of Scotland patients with epilepsy 218.

Table 5.3t: Births in West of Scotland Health Boards, 2011-2016

Year	Total Births	A&A	D&G	Forth Valley	GG&C	Lanarkshire
2011	29,000	3,887	1,396	3,154	13,068	7,495
2012	28,558	3,701	1,390	3,254	13,098	7,115
2013	27,592	3,647	1,327	3,025	12,583	7,010
2014	27,880	3,571	1,286	3,114	12,788	7,121
2015	26,983	3,612	1,256	2,939	12,275	6,901
2016	26,598	3,495	1,318	2,887	12,082	6,826
Mean	**27,769**					

The mean number of births in West of Scotland Health Boards from 2011-2016 inclusive was 27,769, making the estimated denominator for this population 83,307 live births. The estimated incidence of epilepsy of onset before three years is therefore 1 per 383 live births. Wirrell et al. previously estimated the incidence of epilepsy of onset before 36 months of age to be 1 per 614 live births (Wirrell et al., 2012).

Incidence rates were higher at younger ages, as shown in figure 5.3l. Estimated incidence of epilepsy in the under 12 months age group in this cohort was 1 per 705 live births, which is significantly greater than previous population-based estimates from Minnesota (1 per 893) (Wirrell et al., 2011), Nova Scotia (Camfield et al., 1996) (1 per 848), Helsinki (Gaily et al., 2016) (1 per 807) and London (Eltze et al., 2013) (1 per 1,220).

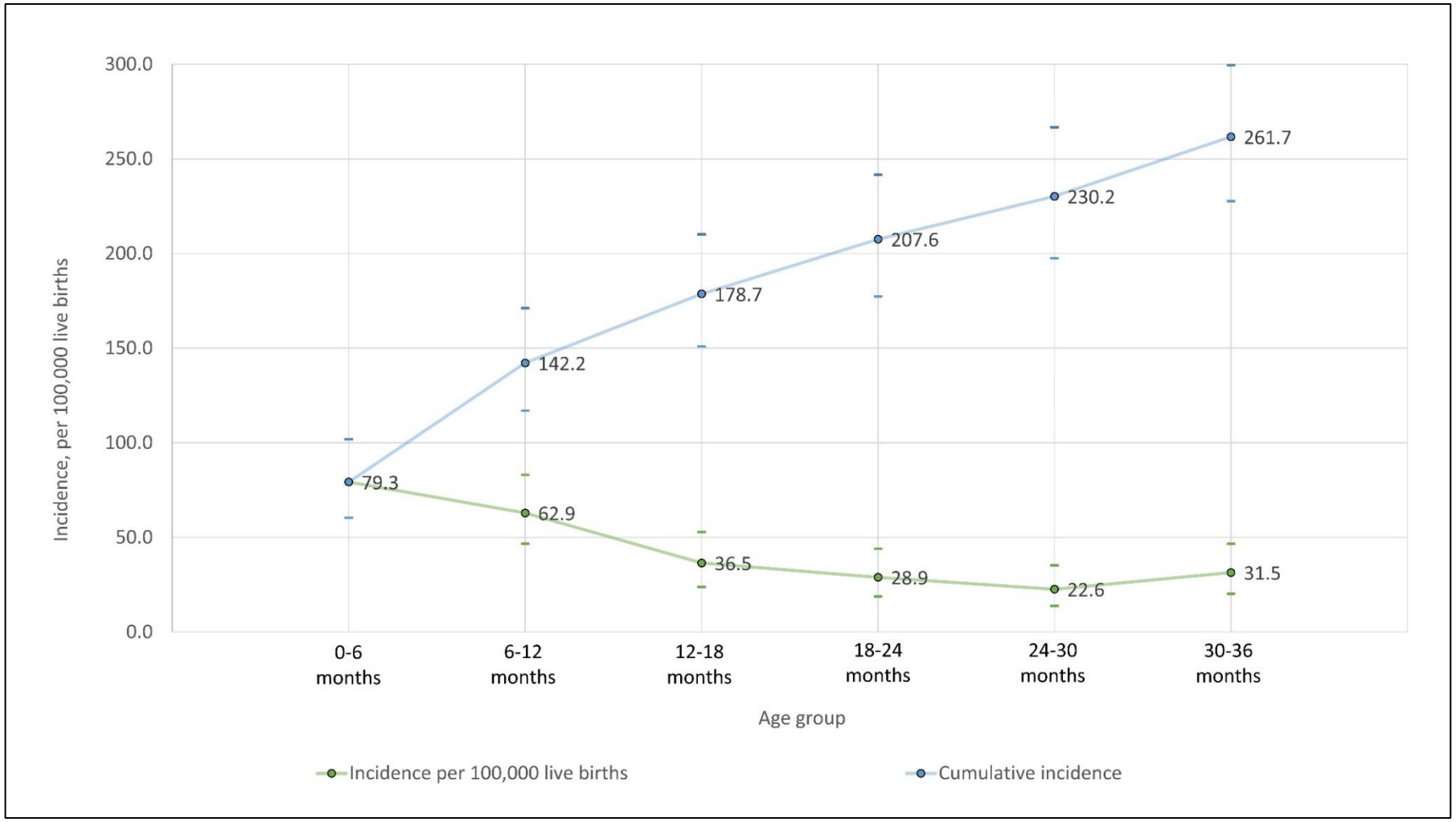


Figure 5.3l: Age-specific incidence of epilepsy in Scotland using data from Cohort 2, with 95% confidence intervals

5.3.7 Cohort 2: Presentation and investigation

54.3% of Cohort 2 presented in the first 12 months of life. 50.5% presented with focal seizures, 35.5% with generalised seizures, and 13.0% with infantile spasms.

At the time of most recent follow-up, 51% of the cohort had an aetiology identified. Investigations performed in order to determine aetiology included CT brain scan (49 patients), MRI brain scan (184 patients), karyotype (7 patients) chromosomal microarray (109 patients), Sanger sequencing and or MLPA for single genes (92 patients), and gene panel testing (151 patients). 11 patients in the cohort had no identified aetiology but had not undergone gene panel testing (Figure 5.3m). The diagnostic yields shown in Figure 5.3m reflects *a priori* diagnostic suspicion. Due to radiation risks, patients are only likely to undergo urgent CT when there is a strong clinical suspicion of a structural lesion. Similarly, single gene testing and microarray were only likely to be requested where the suspicion of a genetic diagnosis was relatively high, whereas gene panel testing was offered in all cases for whom aetiology had not been determined. In a hypothetical scenario in which neuroimaging but no genetic testing were available, an aetiological diagnosis could be made in 23.1% of patients. Chromosomal studies (karyotype and microarray) would increase diagnostic yield by 38%, and single gene studies would increase the yield by a further 77% (Figure 5.3n).

Table 5.3u: Descriptive data from Cohort 2

Age of presentation	**N**	**%**
< 6 months	63	30.3%
6-12 months	50	24.0%
12-18 months	27	13.0%
18-24 months	25	12.0%
24-30 months	16	7.7%
30-36 months	27	13.0%

Presenting seizure type	**N**	**%**
Focal	105	50.5%
GTCS	56	26.9%
Absence	5	2.4%
Atonic	2	1.0%
Myoclonic	8	3.8%
Myoclonic absence	1	0.5%
Spasms	27	13.0%
Tonic	2	1.0%
Unclassified	2	1.0%

Aetiology	**N**	**%**
Structural	27	13.0%
Genetic	52	25.0%
Trisomy	4	1.9%
Microdeletion or duplication	11	5.3%
Single gene variant	37	17.8%
Metabolic	2	1.0%
Aetiology in more than one group	25	12.0%
Unknown	102	49.0%

Antiepileptic therapy at latest follow-up	**N**	**%**
Never had a regular AED	32	15.4%
Off treatment	35	16.8%
First monotherapy	53	25.5%
Second third or fourth monotherapy	14	6.7%
Monotherapy having previously received polytherapy	5	2.4%
Polytherapy	62	29.8%
Deceased	6	2.9%
Unknown	1	0.5%

Seizure frequency at latest follow-up	**N**	**%**
None in 6 months	108	51.9%
< 1 per month	31	14.9%

Monthly	9	4.3%
Weekly	16	7.7%
Daily	31	14.9%
Deceased	6	2.9%
Unknown	7	3.4%

Seizure-frequency and treatment at latest follow-up	**Number**	**%**
Not seizure-free	87	41.8%
Seizure-free on therapy	48	23.1%
Seizure-free off therapy	60	28.8%
Deceased	6	2.9%
Unknown	7	3.4%

Development at latest follow-up	**Number**	**%**
Normal	95	45.7%
Global developmental delay	104	50.0%
Other concerns (ASD features, attention or behavioural difficulties)	8	3.8%
Unknown	1	0.5%

Epilepsy classification at latest follow-up	**N**	**%**
Unclassified focal epilepsy	71	34.1%
Unclassified focal and generalised epilepsy	11	5.3%
Unclassified generalised epilepsy	3	1.4%
Unclassified epilepsy	51	24.5%
West syndrome	15	7.2%
Infantile spasms (without hypsarryhthmia)	8	3.8%
Self-limited familial neonatal seizures	3	1.4%
Self-limited infantile seizures	12	5.8%
Early onset developmental and epileptic encephalopathy	6	2.9%
Epilepsy with myoclonic-atonic seizures	5	2.4%
Absences with eyelid myoclonia	1	0.5%
Alper's Huttenlocher syndrome	1	0.5%
Dravet syndrome	2	1.0%
Early onset absence epilepsy	4	1.9%
Epilepsy with GTCS only	1	0.5%
Epilepsy with myoclonic absences	1	0.5%
Familial focal epilepsy	1	0.5%
Febrile seizures plus	5	2.4%
Unclassified myoclonic epilepsy	4	1.9%
Myoclonic epilepsy of infancy	1	0.5%
Panayiotopoulos syndrome	1	0.5%
Unknown	1	0.5%

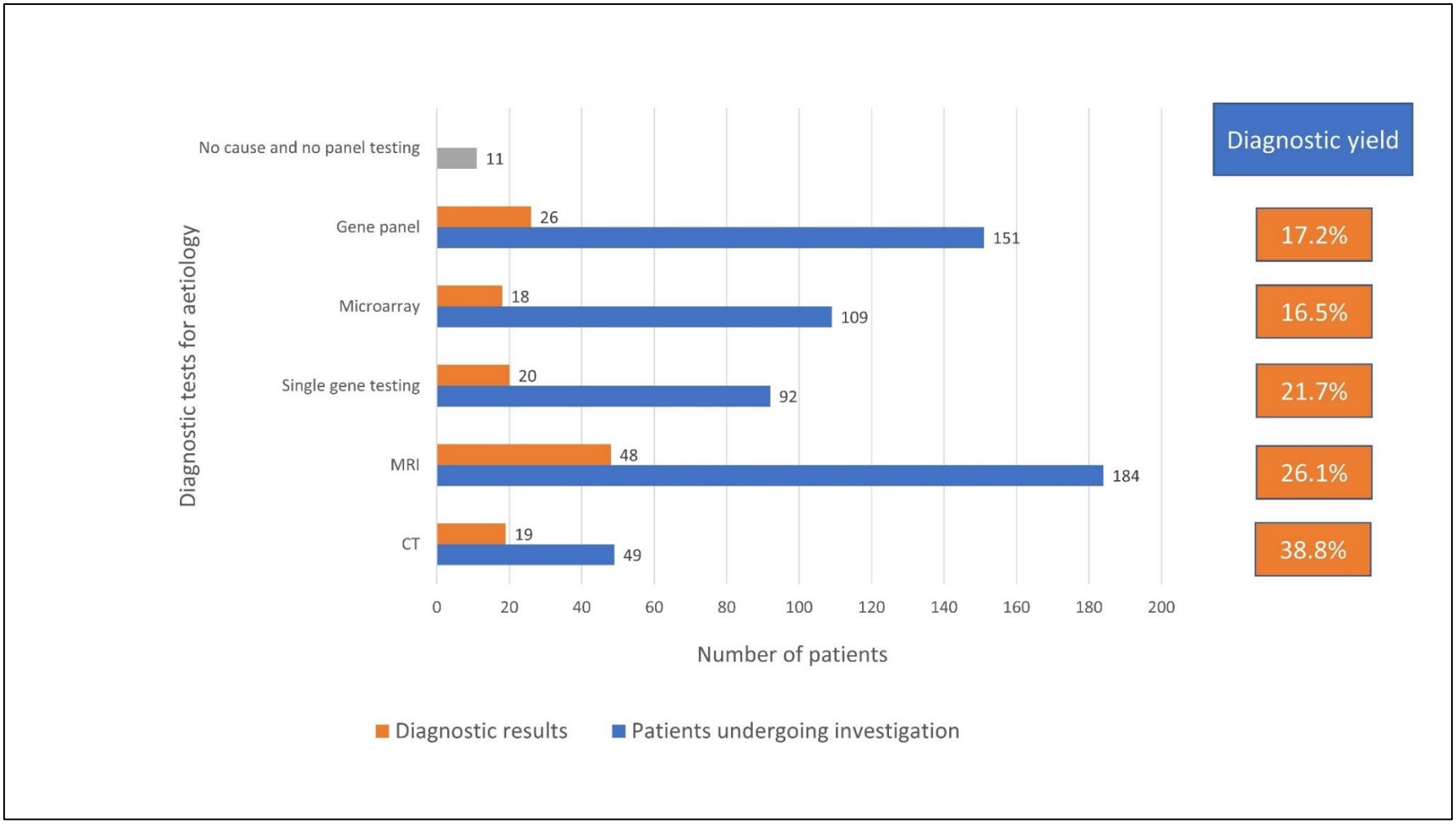


Figure 5.3m: Diagnostic investigations performed in Cohort 2

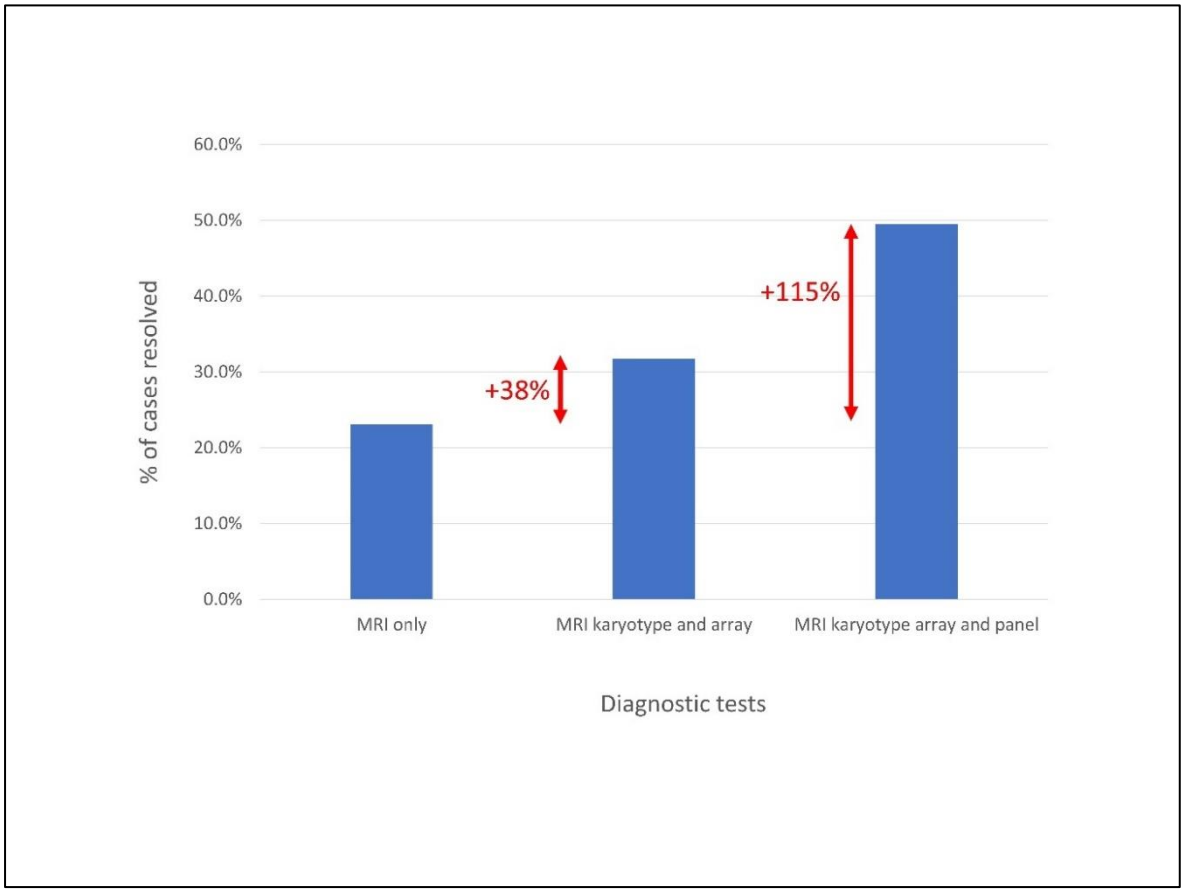


Figure 5.3n: Proportionate increase in diagnostic yield achieved from genetic testing in Cohort 2

5.3.8 Cohort 2: Aetiology

51% of the cohort had an aetiology identified. 69 (33%) had a genetic cause identified, 48 (23%) a structural cause, 8 (4%) a metabolic cause, and 2 (1%) and infectious cause. 21 patients (10%) had an aetiology that fell into more than one of these categories (Figure 2.3o). Of 102 patients with no aetiology determined 91 (89%) had been tested on the 104 gene panel and 84 (83%) had been investigated with MRI brain.

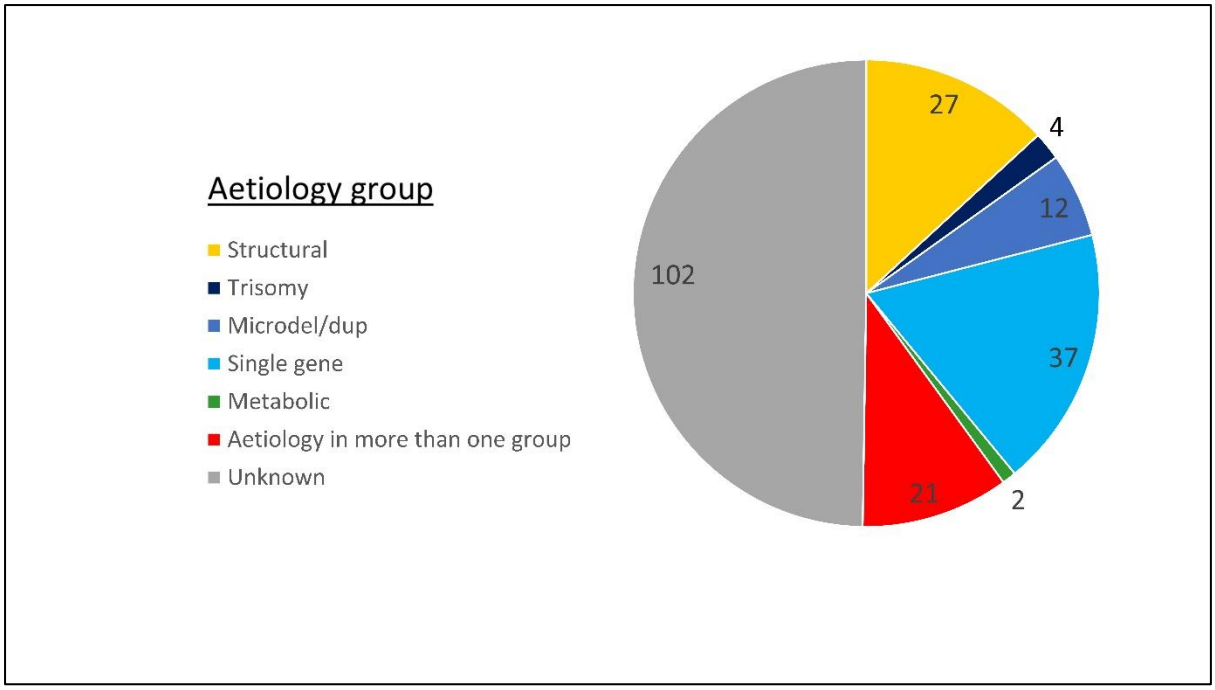


Figure 5.3o: Aetiological diagnoses made in Cohort 2

Table 5.3v: Aetiologies in Cohort 2, by category

Structural causes (n = 48)
Brain malformations (n = 18)
Cystic encephalomalacia (n =6)
Congenital hydrocephalus (n = 3)
Complex congenital brain malformation (n = 3)
Lissencephaly (n = 2)
Mesial temporal sclerosis (n = 2)
Aicardi syndrome (n =1)
Periventricular heterotopia (n = 1)
Bilateral perisylvian polymicrogyria (n = 1)
Perinatal hypoxic-ischaemic injury (n = 5)
Traumatic brain injury (n = 4)
Non-accidental (n = 2)
Accidental (n = 1)
Unknown (n = 1)
Vascular (n = 4)
Neonatal cerebral infarction (n = 2)
Haemorrhagic stroke (n= 2)
Subdural haemorrhage (n = 1)
Developmental cerebral venous anomaly (n = 1)
Tuberous sclerosis (n = 4)
Preterm with periventricular leukomalacia (n = 3)
Encephalitis (n = 2)

NMDA receptor encephalitis, following HSV (n = 1)
Cerebellitis due to lead toxicity (n = 1)

Hypoglycaemia-related cortical damage (n = 2)

Sturge-Weber syndrome (n = 2)

Periventricular leukomalacia from presumed antenatal insult (n = 1)

Metabolic causes (n = 8)

Neonatal hypoglycaemia (n = 2)

Hyperinsulinism (n = 1)

Glycine encephalopathy (n = 1)

Farber disease (n = 1)

Alpers-Huttenlocher disease (n = 1)

Lead toxicity (n =1)

Mitochondrial disease, suspected (n = 1)

Infectious causes (n = 2)

Herpes Simplex Virus encephalitis (n= 1)

Congenital parvovirus infection (n = 1)

Immune causes (n = 1)

NMDA receptor encephalitis, following HSV (n = 1)

Genetic causes (n = 69)

Trisomy (n = 7)

Trisomy 21 (n = 5)
Trisomy 13 (n = 1)
Trisomy 18 (n = 1)

Subchromosomal structural change (n = 16)

De novo 16p11.2 deletion (n = 3)
Angelman Syndrome (maternally-inherited 15q11.2-13.1 deletion)
De novo 16p13.11 deletion (n = 1)
De novo 17p13.3 deletion (Miller Dieker syndrome) (n = 1)
De novo 17q12 deletion (n = 1)
De novo 1p36 deletion (n = 1)
De novo 4p deletion (Wolff-Hirschhorn syndrome) (n = 1)
De novo 7q deletion (Williams syndrome) (n = 1)
De novo Xp22.31 deletion (n = 1)
De novo 16p.11.2 duplication (n = 1)
De novo 7p deletion and 7q duplication (n = 1)
De novo Idiodicentric chromosome 15 (n = 1)
De novo Marker chromosome 20 (n = 1)

Single gene variant (n = 46) (see figure 2.3p)

Table 5.3w: Aetiologies in Cohort 2 which fell into multiple categories

Metabolic and genetic diagnoses (n =2)

Farber disease due to homozygous *ASAH1* variants
Alpers-Huttenlocher disease due to compound heterozygous *POLG1* variants

Metabolic, structural and genetic diagnoses (n = 1)

Glycine encephalopathy due to compound heterozygous *AMT* variants, with associated lissencephaly

Metabolic and structural diagnoses (n = 3)

Hypoglycaemia-related cortical damage (n = 2)
Cerebellitis due to lead toxicity

Structural and genetic diagnoses (n = 13)

Trisomy 13 and complex brain malformation
Trisomy 18 and complex brain malformation
Trisomy 21 and perinatal hypoxic ischaemic event
Tuberous Sclerosis due to *TSC2* variant (n =3)
7p deletion and 7q duplication and perinatal hypoxic ischaemic event
16p13.11 deletion and MRI findings suggestive of Tuberous Sclerosis
1p36 deletion and bilateral perisylvian polymicrogyria
17p13.1 deletion and lissencephaly
KCNQ2 deletion and traumatic brain injury
KCNQ2 deletion and cystic encephalomalacia
Subdural haemorrhage and *MAF1* variant

Structural and infectious diagnoses (n = 1)

Congenital parvovirus and periventricular leukomalacia

Structural, infectious and immune diagnoses (n = 1)

Herpes simplex virus encephalitis complicated by NMDA receptor encephalitis

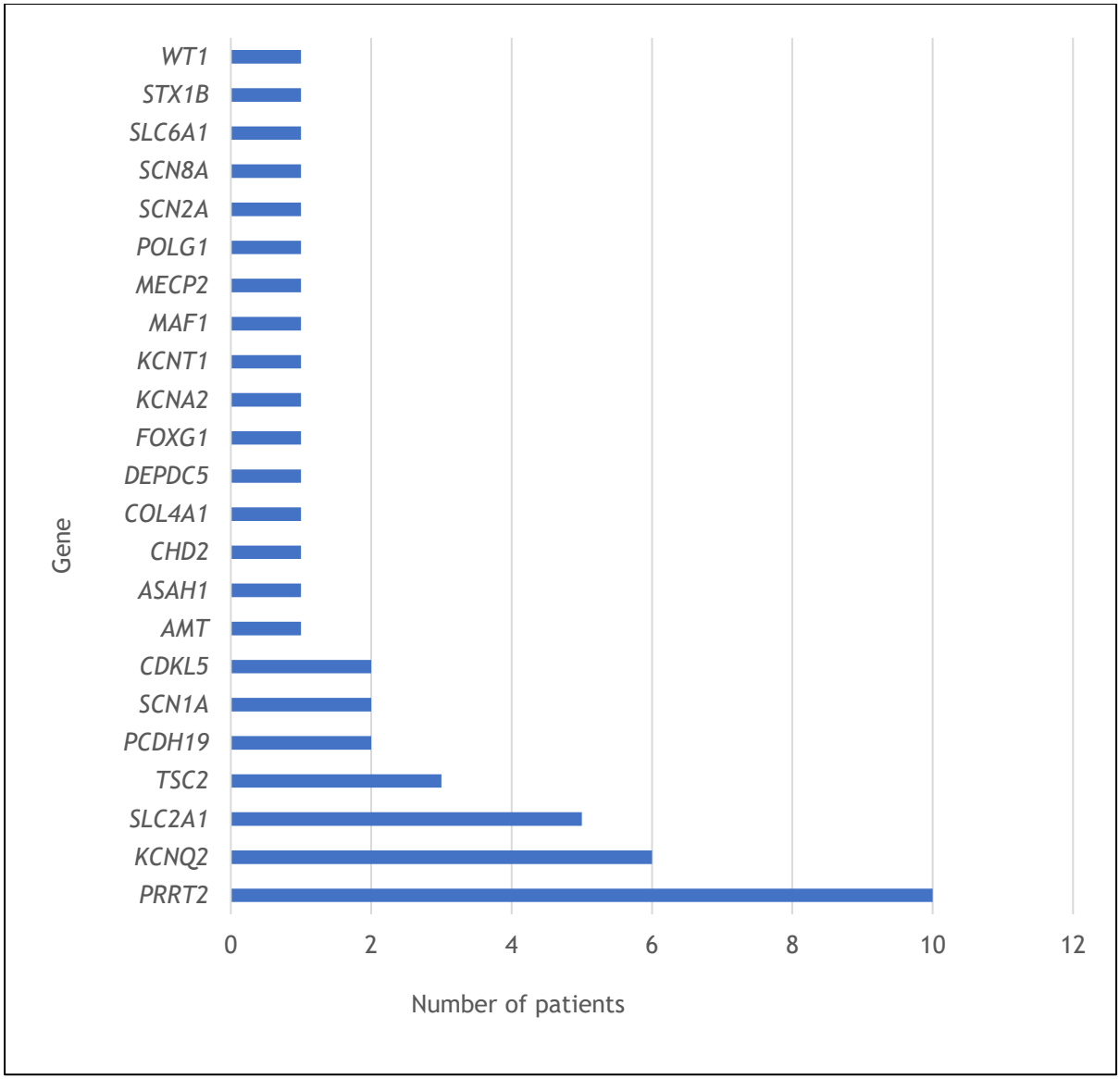


Figure 5.3p: Single gene diagnoses made in Cohort 2

As with Cohort 1, the majority of single gene diagnoses were clustered in a small number of genes, with 30/46 (65%) in the most frequently implicated seven genes. 7/16 (44%) genetic diagnoses that were identified in just a single patient were made not through the genetic epilepsy service but through specific single gene testing targeted due to phenotype (*AMT, ASAH1, COL4A1, FOXG1, MAF1, POLG1* and *WT1*). For example, a patient with glycine encephalopathy was tested for variants in the two most commonly implicated genes in this condition (*AMT* and *GLDC*).

Aetiology was more commonly identified in patients who presented at a young age. In the <12 months group 62% had an identified aetiology, falling to 40% in the 12-24 months group and to 37% in the 24-36 months group. The proportion of cases attributable to purely structural causes did not fall significantly with increasing age of presentation but the proportion attributable to genetic causes did (Figure 5.3q). However, mean age at presentation was no different between those with structural causes and those with genetic causes (11.7 months v 11.2 months, two-tailed t-test p>0.05).

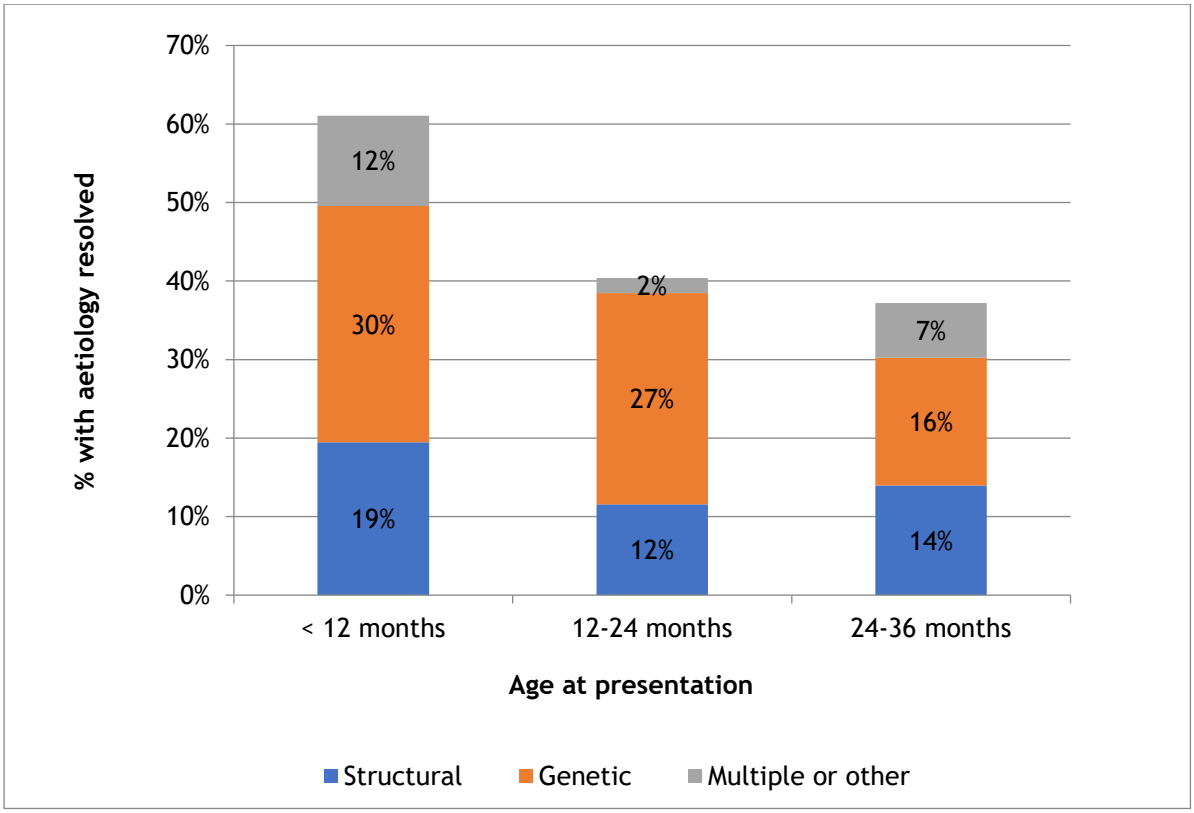


Figure 5.3q: Aetiological diagnoses in Cohort 2, by age of presentation

Table 5.3x: Associations between clinical features and aetiology in Cohort 2, using Fisher's Exact test

	N (%) with aetiology	Two-tailed Fisher's exact probability	Odds ratio (95% confidence intervals)
Total cohort	106/208 (50.1%)		
Age at presenting seizure			
<12 months	69/113 (61.1%)	< 0.005*	2.4 (1.4-4.3)
12-24 months	21/52 (40.3%)	> 0.05	0.6 (0.3-1.1)
24-36 months	16/43 (37.2%)	> 0.05	0.5 (0.2-1.0)
Presenting seizure			
Focal	59/105 (56.2%)	> 0.05	1.5 (0.9-2.6)
Generalised	26/74 (35.1%)	< 0.005 ψ	0.4 (0.2-0.7)
Spasms	19/27 (70.4%)	< 0.05*	2.6 (1.1-6.2)
Additional features			
Spasms present at any age	29/43 (67.4%)	< 0.05*	2.4 (1.2-4.8)
Outcomes at latest follow-up			
Seizure-free	50/108 (46.3%)	> 0.05	0.7 (0.4-1.2)
Drug-resistant epilepsy (DRE)	53/71 (74.6%)	< 0.005*	4.7 (2.5-8.8)
Global developmental delay (GDD)	70/104 (67.3%)	< 0.005*	3.9 (2.2-6.9)
DRE *and* GDD	44/59 (74.6%)	< 0.005*	4.1 (2.1-8.0)
Death	6/6 (100%)	< 0.05*	N/A

* Significant positive association; ψ Significant negative association

Table 5.3x shows factors that were associated with identifying an aetiology. The strongest associations were with early age of presentation (under 12 months), presentation with epileptic spasms, the development of drug-resistant epilepsy (DRE), and the presence of global developmental delay (GDD).

I then used a binary logistic regression model incorporating age of presentation (< 12 months, 12-24 months, 24-36 months), presenting seizure (focal, generalised, or spasms), DRE (present or absent), and GDD (present or absent). In this multivariate model, type of presenting seizure was no longer significant (Table 5.3x-2). The development of DRE had the strongest association with having aetiology identified. Overall the multivariate model explained >25% of the variance (Nagelkerke R^2 = 0.267) and was able to predict whether aetiology was identified or not with 61.3% sensitivity and 73.5% specificity (Table 5.3x-3).

Table 5.3x-2: Multivariate model for associations with identified aetiology in Cohort 2 (Hosmer and Lemeshow)

	p.	OR (95% confidence intervals)
Age at presentation		
24-36 months (reference)	0.036	N/A
12-24 months	0.476	1.4 (0.5-3.5)
< 12 months	0.017	2.8 (1.2-6.3)
Presenting seizure		
Generalised (reference)	0.158	N/A
Focal	0.055	1.9 (1.0-3.8)
Spasms	0.387	1.6 (0.5-5.0)
Outcomes		
GDD present	0.017	2.4 (1.2-4.8)
DRE present	0.002	3.2 (1.6-6.7)

Table 5.3x-3: Sensitivity and specificity of multivariate model for associations with aetiology in Cohort 2 (Hosmer and Lemeshow)

Observed		Predicted		
		Any cause		Percentage Correct
		Absent	Present	
Any aetiology identified	Absent	75	27	73.5
	Present	41	65	61.3
Overall Percentage				67.3

Previous population-based studies of epilepsy in early childhood have identified a higher proportion of structural causes compared with what was found in Cohort 2. In Gaily et al's. Finnish study 35% of those presenting at <12 months had a structural cause identified, compared with 33/113 (29%) in Cohort 2 (Gaily et al., 2016). In those presenting at under 24 months, Eltze et al. reported that a structural cause was identified in 35%, compared with 40/165 (24%) in Cohort 2 (Eltze et al., 2013). Since the overall incidences found in both the Gaily and Eltze studies were less than in Cohort 2, it is possible that their studies were affected by ascertainment bias, with patients with structural causes more likely to be identified and referred to the tertiary centres where the studies were based.

At most recent follow-up 136/208 (65%) had an unclassified epilepsy syndrome which is a significantly more than the number who did not have an established aetiology (49%), suggesting that in this age group aetiological classification of epilepsy may be more relevant than clinical "syndrome" classification.

5.3.9 Predictors of short-term outcomes in Cohort 2

The primary outcomes of interest in this cohort were: 1. the presence or absence or global developmental delay (GDD) and 2. the presence or absence of drug-resistant epilepsy (DRE). DRE was defined as ongoing seizures (at least one seizure in the last 6 months) despite two adequately trialled and tolerated AED regimens. I also looked at the outcome of seizure-freedom for >6 months at most recent follow-up.

Because follow-up data was not gathered at a consistent time point for all patients, it was important to test that duration and age of follow-up did not influence GDD and DRE outcomes. Table 5.3x shows that there were no differences in the mean duration of follow-up, nor in age at most recent follow-up, between those with and without GDD and DRE respectively.

Table 5.3y: Differences in mean duration of follow-up and age at follow-up between GDD and DRE groups in Cohort 2

	Mean age at most recent follow-up	**Mean duration of follow-up**
GDD present	39.8 months	25.9 months
GDD absent	38.6 months	23.4 months
Two-tailed t-test* for difference in means (GDD)	p >0.05	p >0.05
DRE present	39.2 months	27.0 months
DRE absent	39.2 months	23.4 months
Two-tailed t-test* for difference in means (DRE)	p >0.05	p >0.05
* Both samples were normally distributed		

Table 5.3z: Associations between clinical features, aetiology, and outcomes in Cohort 2, using Fisher's Exact test

Outcome	Seizure-free for > 6 months			GDD			DRE		
	N (%) with outcome	Two-tailed Fisher's exact probability	Odds ratio (95% confidence intervals)	N (%) with outcome	Two-tailed Fisher's exact probability	Odds ratio (95% confidence intervals)	N (%) with outcome	Two-tailed Fisher's exact probability	Odds ratio (95% confidence intervals)
Total cohort	108/201 (53.7%)			104/208 (50.0%)			71/201 (35.3%)		
Age at presenting seizure									
<12 months	60/107 (53.1%)	>0.05	0.8 (0.4-1.4)	60/113 (53.1%)	>0.05	1.3 (0.8-2.3)	44/107 (41.1%)	>0.05	1.7 (1.0-3.1)
12-24 months	31/52 (60.0%)	>0.05	1.4 (0.7-2.6)	22/52 (42.3%)	>0.05	0.7 (0.4-1.2)	15/52 (28.8%)	>0.05	0.7 (0.3-1.3)
24-36 months	17/42 (40.5%)	>0.05	1.0 (0.5-2.1)	22/43 (51.2%)	>0.05	1.1 (0.5-2.1)	12/42 (28.6%)	>0.05	0.7 (0.3-1.4)
Presenting seizure									
Focal	54/102 (52.3%)	>0.05	0.9 (0.5-1.6)	53/105 (50.5%)	>0.05	1.0 (0.6-1.8)	34/102 (33.3%)	>0.05	0.8 (0.4-1.4)
Generalised	38/72 (52.8%)	>0.05	0.9 (0.5-1.7)	27/76 (35.5%)	<0.005 ψ	0.4 (0.2-0.7)	24/72 (33.3%)	>0.05	0.9 (0.5-1.6)
GTCS	26/53 (49.1%)	>0.05	0.8 (0.4-1.5)	15/56 (26.8%)	<0.005 ψ	0.3 (0.1-0.5)	15/53 (28.3%)	>0.05	0.6 (0.3-1.3)
Other generalised seizure	12/19 (63.2%)	>0.05	1.5 (0.3-4.1)	12/20 (60.0%)	>0.05	1.6 (0.6-4.0)	9/19 (47.4%)	>0.05	1.7 (0.7-4.5)
Spasms	16/27 (59.3)	>0.05	1.3 (0.6-3.0)	24/27 (88.9%)	<0.005*	10.1 (2.9-34.8)	13/27 (48.1%)	>0.05	1.8 (0.8-4.2)
Aetiology									
Structural	24/46 (52.2%)	>0.05	0.9 (0.5-1.8)	34/48 (70.8%)	<0.005*	30.1 (1.6-6.3)	19/46 (41.3%)	>0.05	1.4 (0.7-2.7)
Genetic	33/69 (47.8%)	>0.05	0.7 (0.4-1.3)	45/69 (65.2%)	<0.005*	2.5 (1.4-4.6)	39/69 (56.5%)	<0.005*	4.1 (2.2-7.6)
Any cause	50/104 (48.1%)	>0.05	0.6 (0.4-1.1)	70/106 (66.0%)	<0.005*	3.9 (2.2-6.9)	53/104 (51.0%)	<0.005*	4.6 (2.4-8.7)
Outcome									
GDD	44/102 (43.1%)	<0.005 ψ	0.4 (0.2-0.7)				59/102 (57.8%)	<0.005*	9.9 (4.8-20.4)
DRE				59/71 (83.1%)	<0.005*	10.1 (4.9-20.6)			
* Significant positive association; ψ Significant negative association									

Table 5.3z supports the findings from previous studies that the most significant determinant of outcomes in childhood-onset epilepsy is the identification of an aetiology (Eltze et al., 2013; Gaily et al., 2016, Wirrell et al., 2012; O'Callaghan et al. 2017; Datta & Wirrell, 2000; Rantala & Ingalsuo, 1999). Identification of structural aetiology, genetic aetiology or any aetiology was associated with GDD. Identification of genetic aetiology, but not structural aetiology was associated with DRE.

Age of presentation was not significantly associated with any of the outcomes investigated, a finding which contrasts with the study by Wirrell et al. which reported that presentation before 12 months of age was associated with the development of drug-resistant seizures (OR 6.8, 95% CI 2.0-22.8) but is consistent with the findings of Rantala et al. who reported no difference in seizure-remission rates between children presenting at 0-12 months compared with those presenting at 12-24 months (Rantala & Ingalsuo, 1999).

Type of presenting seizure was associated with developmental outcome but not with epilepsy outcome. Children presenting with GTCS were significantly less likely to have GDD at most recent follow-up whilst those presenting with infantile spasms were significantly more likely to have GDD – a finding that is also consistent with previous studies (Gaily et al., 2016; Datta & Wirrell, 2000). The association between presentation infantile spasms on developmental outcome did not seem to be confounded by aetiology, since 8/8 (100%) patients with no identified aetiology had GDD, compared with 16/19 (84%) of those with an identified aetiology.

There was a strong association between the two outcomes themselves: DRE and GDD. This association did appear to be influenced by aetiology. 44/59 (75%) of patients with both DRE and GDD had an identified aetiology compared with 27/92 (29%) of those who had neither DRE nor GDD.

5.3.9.1 Multivariate models:

For prediction of GDD as an outcome, I used a multivariate binary logistic model with the following inputs: type of presenting seizure (focal, generalised, or spasms); aetiology (any identified or none identified); and DRE (present or absent). The strongest associations with GDD were presentation with epileptic spasms, and the presence of DRE. The model was able to predict >40% of the variance (Nagelkerke R^2 = 0.440) and predicted GDD with 72.1% sensitivity and 85.6% specificity (Table 5.3z-3)

Table 5.3z-2: Multivariate model for associations with GDD in Cohort 2 (Hosmer and Lemeshow)

	p.	**OR (95% confidence intervals)**
Age at presentation		
24-36 months (reference)	0.070	N/A
12-24 months	0.190	0.5 (0.2-1.4)
< 12 months	0.021	0.3 (0.1-0.9)
Presenting seizure		
Generalised (reference)	0.000	N/A
Focal	0.044	2.2 (1.0-4.6)
Spasms	0.000	22.2 (5.1-96.1)
Aetiology		
Any aetiology identified	0.020	2.4 (1.1-4.9)
Outcomes		
DRE present	0.000	9.9 (4.4-22.0)

Table 5.3z-3: Sensitivity and specificity of multivariate model for asscociation with GDD in Cohort 2 (Hosmer and Lemeshow)

Observed		Predicted		
		Development		Percentage Correct
		Absent	Present	
GDD	Absent	89	15	85.6
	Present	29	75	72.1
Overall Percentage				78.8

For prediction of DRE as an outcome, I used a multivariate model using just identification of a genetic aetiology (true or false) and GDD (present or absent) since these were the only significant predictors in univariate analysis. Both factors remained significant in the model (Table 5.3z-4). The model was able to predict

>35% of the variance (Nagelkerke R^2 = 0.367) and predicted DRE with 45.1% sensitivity and 90.5% specificity (Table 5.3z-5)

Table 5.3z-4: Multivariate model for associations with DRE in Cohort 2 (Hosmer and Lemeshow)

	p.	OR (95% confidence intervals)
Aetiology		
Genetic aetiology identified	0.000	3.7 (1.8-7.4)
Outcomes		
GDD present	0.000	9.1 (4.3-19.0)

Table 5.3z-5: Sensitivity and specificity of multivariate model for associations with DRE (Hosmer and Lemeshow)

Observed		Predicted		
		DRE		Percentage Correct
		Absent	Present	
DRE	Absent	124	13	90.5
	Present	39	32	45.1
Overall Percentage				75.0

5.3.10 Whole genome sequencing

Because the presence of epileptic spasms, and the development of DRE were but associated with a high chance of identifying an aetiology (Table 5.3x), and with adverse developmental outcome (Table 5.3z), cases with either of these two features who remained without a diagnosis were offered Whole Genome Sequencing (WGS).

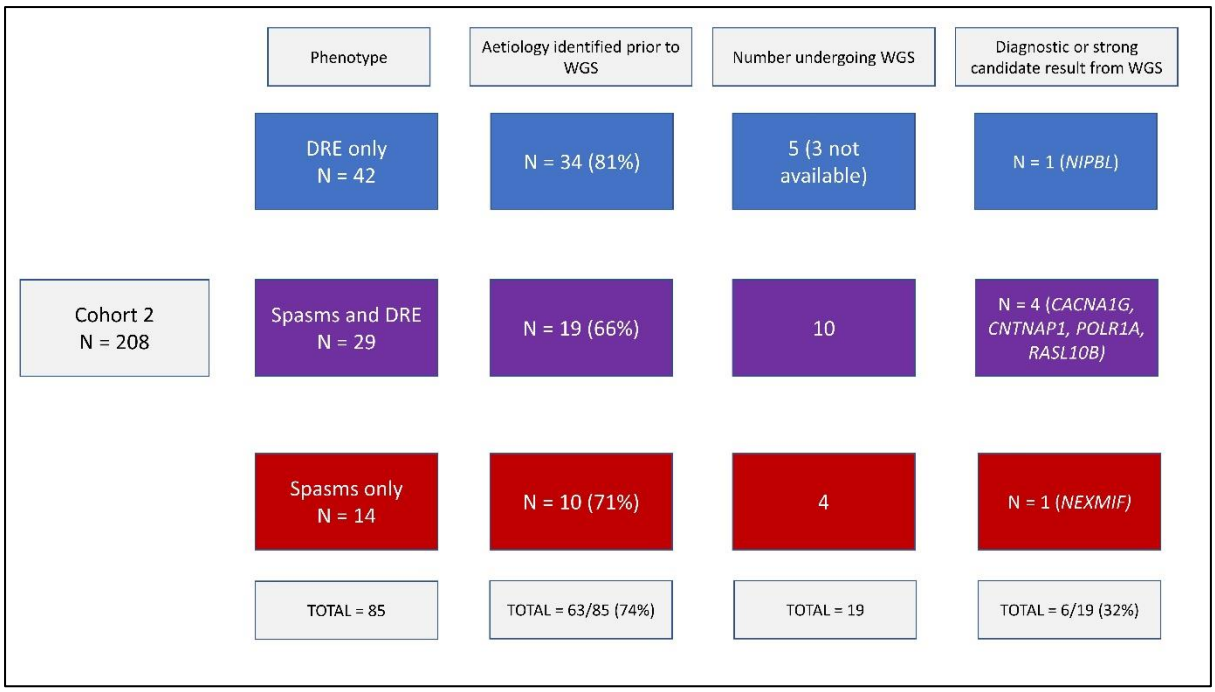


Figure 5.3r: Aetiological findings from patients in Cohort 2 with DRE and epileptic spasms, before and after WGS

In total 85 patients in the cohort had either DRE or infantile spasms. Of these the majority (74%) had an aetiology identified through neuroimaging or clinical genetic testing. Of the 22 patients who remained without and aetiological diagnosis, WGS was offered to all. Three families did not take up the offer of WGS and 19 underwent testing. For 6 patients a diagnostic or strong candidate genetic cause was identified through WGS. The methodology of testing, and results from WGS will be described and discussed in detail in chapter 6 of this thesis.

5.4 Discussion

In this chapter, I have described the establishment and follow-up two overlapping cohorts of children presenting with seizures in the first three years of life. Since these cohorts have been ascertained through independent mechanisms, it has been possible to use a capture-recapture technique to estimate how many patients may have been missed. Using this technique, alongside Scottish national birth record data, I have been able to calculate the incidence of epilepsy in the < 3 year age group as 1 per 383 live births. This is significantly more common than the previous estimate or 1 per 614 live births.

Because these cohorts were extensively investigated for aetiology, and particularly for single gene causes, this study has made it possible to estimate the incidence, and more objectively describe the phenotypic spectrum, of a number of single gene seizure disorders or early childhood, including *PRRT2*, *SCN1A*, *KCNQ2*, and *SCL2A1*. Apart from *SCN1A*-related epilepsy, all of these are more common than suggested by previous reports.

As well as enabling capture-recapture analysis, the development of Cohort 2 overcame some of the limitations of Cohort 1, which are discussed in detail in section 5.3.6. Perhaps most importantly, because Cohort 1 focused on patients for whom there was no immediately apparent aetiology it was depleted of patients with already established structural causes (e.g. neonatal stroke) and genetic causes (e.g. trisomy 21). Cohort 2 permitted the inclusion of such patients and therefore reflected more accurately the overall aetiologies involved in early childhood-onset epilepsy. Overall more than half of children presenting with epilepsy before their third birthday were found to have an aetiology. The majority of these aetiologies fell into “structural” or “genetic” groups.

So far all evidence relating to aetiology-specific therapy in epilepsy relates to patients with single gene causes, though it would be perfectly reasonable to carry out a trial of therapy in patients with other causes, such as trisomy 21 or one of

the more common microdeletion syndromes. It is well accepted that for epilepsies in which there is a well delineated structural cause such as focal cortical dysplasia, epilepsy surgery offers the best hope of seizure control (Wiebe et al., 2001). Only one patient from these cohorts had undergone epilepsy surgery by the time of most recent follow-up. This was a patient in Cohort 1 from Lothian who had an area of cortical dysplasia in the right occipital lobe. This region was resected and subsequently he has been seizure-free.

The largest aetiological group in both cohorts was the "monogenic" cause. Between one fifth and one quarter of the patients in these cohorts had such a single gene cause though this is likely to underestimate since the genetic testing was largely limited to a panel of 104 genes. With a larger platform further single gene diagnoses would be made, as demonstrated by the application of Whole Genome Sequencing (WGS) to selected patients within this cohort.

Looking at the potential scope for therapy to be guided specifically by the knowledge of a single gene cause, this is likely to be the case for a significant minority of children with early-onset seizure disorders. 23.0% (74/322) of Cohort 1 and 22.1% (46/208) of Cohort 2 had a single gene cause identified. Of the 74 single gene diagnoses made in Cohort 1 63 (85.1%) had potential treatment implications, and of the 46 single gene diagnoses made in Cohort 2 37 (80.4%) had potential treatment implications, based on evidence reviewed in chapter 4 of this thesis. These figures suggest that approximately 17-20% of all children diagnosed with epilepsy before their third birthday stand to benefit from a genetic diagnosis that may inform therapeutic choice. Since the incidence of epilepsy in the first three years of life is 1 per 383 live births, it can also be said that between 1 in 2,000 and 1 in 2,300 of all children born are likely to have a genetic abnormality causing epilepsy for which there is currently available some evidence for a specific treatment choice.

The significant limitations of the evidence in support of "gene-informed" treatment choices are discussed in section 4.4 of this thesis. It must be born in

mind however that when the clinician is faced with a patient presenting with frequent and intrusive seizures they will frequently come to the decision alongside families that they would like to start, or change, anti-epileptic therapy. The decision in this circumstance is therefore not one of "whether" to treat but of "what" specific therapy to use. In the absence of strong evidence to support a specific choice the clinician is faced with various options: i) reflect on their own previous experience of similar cases (phenotypically or aetiologically); ii) pick the therapy that they are most familiar with and which they feel is well-tolerated; iii) be guided by the side-effect profile of the medication and the concerns of the family in relation to these; iv) use the existing evidence from the literature, limited though it may be. In reality the best decision is likely to be informed by all of the above points. It was interesting to note that in Cohort 1 only 36/63 (57%) of patients with genetic diagnoses that had treatment implications had actually been treated with one of the therapies that the medical literature would suggest to be a good choice. As more evidence is generated and the results of studies disseminated, clinicians may be increasingly influenced by point iv).

By performing WGS in patients with drug-resistant seizures and/or infantile spasms who remained without an aetiological diagnosis after gene panel testing, I have shown that extremely rare genetic diagnoses continue to be made in these patients. The rarity of these diagnoses presents a significant challenge to those trying to progress precision medicine. To gather sufficient numbers of patients to perform well-powered cohort studies or therapeutic trials is likely to be a major challenge, though not unsurmountable with multi-centre collaboration.

An additional finding from Cohort 2 is that there is a strong association between identification of any aetiology and the presence of global developmental delay (GDD) and drug-resistant epilepsy (DRE) (table 5.3x). In a clinical context in which aetiological investigations, including imaging and genetic testing, are concluded rapidly, this data could potentially be used to reassure families. For example, of the 208 patients in Cohort 2, 97 had no identified cause. Of these 97, 28 (28.9%)

had GDD at most recent follow-up, 14 (14.4%) had DRE, and the majority (67, 69.1%) had neither GDD nor DRE.

There are a number of limitations of the data included in this chapter. Due to the large numbers in each cohort and the limited time available to gather phenotypic information (most collaborating clinicians contributed data in their own personal time), the depth of phenotype information has been limited and at times reduced to major categories. Increasing the resolution of phenotypic information may provide further insights into the nature of these epilepsies. For example, most of the patients in these cohorts had electroencephalogram (EEG) recordings but data from these have not been analysed. Similarly, the families in Cohort 1 were all sent the validated questionnaire Adaptive Behavioural Assessment Scale II (ABASII) as a measure of developmental progress. These data may have provided a greater depth of information that was obtained from the clinicians' assessments of development, but due to the relatively low return rate these haven't been included.

Analyses performed in section 5.3.9. were dependent on data obtained at most recent clinical follow-up, specifically focussing on whether GDD or DRE had been diagnosed by that time. The time point at which the latest clinical update was obtained for each patient varied from one year after initial presentation to four years after initial presentation. Longer term follow-up and reanalysis of all patients in these cohorts, ideally at consistent time points, would be invaluable. Some patients may go on to develop developmental comorbidity and/or drug-resistance despite having neither at the most recent follow-up.

The aim of this thesis is to investigate the scope for genetically-guided precision medicine in epilepsy and, since as discussed in chapter 1.3, seizure control may not be the most important aspect of their child's quality of life, long-term developmental and quality of life questionnaire data from these cohorts would add great value to this investigation, particularly if correlations between genetic diagnosis, therapeutic approach, and these outcomes can be investigated. In

SCN1A-related epilepsy it has previously been shown that early implementation of recommended therapy is associated with improved medium-term cognitive outcomes (Lange et al., 2018). Our research group aims to continue to follow-up these cohorts, gathering clinical and questionnaire data, and investigate for relationships between aetiology, treatment approach and long-term outcomes. The outcome of interest are seizure control, developmental comorbidity, and quality of life.

A major challenge for the progression of precision therapy in single gene epilepsies is the large number of different single gene causes, each of which may require a different treatment approach. The patients most in need of a precision approach are those whose seizures are not currently being controlled by current management. In Cohort 2 there were 71 patients identified with drug-resistant epilepsy, of whom 28 (39.4%) had a single gene cause identified. Among these 28 cases a total of 22 different genes were implicated. What this tells us is that most monogenic drug-resistant epilepsies of early childhood are extremely rare disorders.

6. Prospective study of Whole Genome Sequencing in a cohort of children with complex epilepsy from the West of Scotland

6.1 Introduction

As discussed in chapter 3, a great number of genes have been associated with epilepsy and there is currently no hint at a deceleration in epilepsy gene discovery. Data from Next Generation Sequencing (NGS) studies suggests that once the most frequently associated 50 genes have been excluded as cause, there remain still three times that number of known possible genetic causes (Figure 1.6d), with the likelihood of each one being extremely low (average probability of a diagnostic result = 0.01% per gene). With such a large number of extremely rare possible genetic causes, targeted testing using a gene panel approach, though useful, has limitations. It is therefore not surprising that the highest yields from NGS testing in epilepsy have come from approaches that have not restricted the number of genes tested. Three different approaches to untargeted sequencing are currently used: Clinical Exome or Mendeliome sequencing, Whole Exome Sequencing (WES), and Whole Genome Sequencing (WGS). The Mendeliome aims to capture all known disease-associated genes (n = c. 4,800), WES aims to capture all coding sequence, and WGS aims to capture all DNA, including intronic and intragenic regions. The precise limitations of each approach depend on the technology used, but broadly, the advantage of WES over Mendeliome is that it has the potential to identify variants in genes not hitherto associated with disease and the advantage of WGS over WES is that it may detect disease causing variants in intronic or intragenic regions, though many of these remain to be characterised. WGS also provides better coverage of the coding regions than WES (Belkadi et al., 2015).

In this chapter I will investigate the diagnostic utility of WGS in severe childhood-onset epilepsies and investigate how often diagnoses made using this technique have the potential to guide therapy choice.

6.2 Methods

6.2.1 Recruitment

Patients were recruited from the West of Scotland. Four key phenotypes were defined, and paediatric neurologists were asked to refer cases whom they thought were eligible and whose families would be interested in participating.

Potential participants were invited by letter (Appendix 9) enquiring about their interest in the study. Interested families were then contacted by telephone to offer them further information and a recruitment visit. Prior to recruitment visits families were sent Information Sheets and Consent Forms. Information was discussed, and consent was taken, at the recruitment visit.

6.2.2 Consent

Consent was taken for the following:

- Clinical history taking and examination
- Medical note review
- Contact with the patient's paediatrician/neurologist to discuss clinical aspects of the case
- Blood sample for DNA extraction in Glasgow
- Whole Genome Sequencing outwith Glasgow
- Sharing of anonymised clinical details with other research groups
- Future contact if additional information or biological samples required
- To be sent questionnaires about development, quality of life, and experience of genetic testing

Participants were offered to be informed of any relevant genetic findings that emerged from the research, and were advised that they would not be informed of

any genetic findings that were not of relevance to epilepsy – for example if variants associated with cancer susceptibility were identified.

Separate information sheets and consent forms were produced for Parents, Young People, Adult participants able to consent for themselves, and for Welfare Guardians of adult (over 16 years) participants unable to consent for themselves (see Appendix 10 and 11).

6.2.3 Ethical Approval

The study was approved by the Scotland A Research Ethics Committee on April 15th 2016 (REC number 16/SS/0054, Protocol number GN15NE178, IRAS ID 170749).

6.2.4 Clinical evaluation

I carried out detailed phenotyping in each case. Parents and child were offered a 90 minute appointment at the Glasgow Clinical Research Facility (CRF) during which structured history taking and physical examination took place (see Appendix 12). Three generation family history was taken from both parents (see section 3.2.5). Electronic and paper case notes were reviewed in each case for further clinical details and investigation results including EEG, MRI and previous genetic testing. Clinical and investigation finding were coded, where possible, using Human Phenotype Ontology (HPO) terms (Köhler et al., 2017). For an example phenotype summary see Appendix 13.

Participants were asked to complete five validated questionnaires and two unvalidated questionnaires asking about their expectations and experience of genetic testing (Table 3.2 and 14).

Table 6.2a: Questionnaires used in phenotyping patients recruited for whole genome sequencing

Questionnaires (and age groups) given at recruitment	Questionnaires given six months after test results communicated
ABAS II (Adaptive Behaviour Assessment Scale) < 5 years 5-21 years	Parent/Carer Experience of Genetic Testing * unvalidated (See Appendix 14)
SDQ (Strength and Difficulties Questionnaire) 2-4 years 4-17 years 17+ years	
PSI (Parental Stress Index)	
Peds QL (Pediatric Quality of Life) 2-4 years 5-7 years 8-12 years 13-18 years 19-25 years > 26 years	
ELDQOL (Epilepsy with Learning Disability Quality of Life)	
Parent/Carer Expectations of Genetic Testing * unvalidated (See Appendix 14)	

6.2.5 Family history scores

In order to have an objective measure of the strength of family history on both the maternal and paternal sides of the family, I devised a family history score. The score was attributed to each parent based on the presence of epilepsy and/or epilepsy-associated phenotypes in other family members on each side of the family. Where siblings of the patient were affected scores were applied to both parents equally.

Table 6.2b: Family history scores applied to each parent in the WGS study

Relative (to parent)	Phenotype	Score
Self	Same phenotype	80
	Epilepsy (different phenotype)	40
	Related disorder: learning disability, migraine, schizophrenia, autism, Asperger's, febrile seizures	24
(Another) child	Same phenotype	40
	Epilepsy (different phenotype)	20
	Related disorder: learning disability, migraine, schizophrenia, autism, Asperger's, febrile seizures	12
1st degree relative (parent, sibling)	Same phenotype	20
	Epilepsy (different phenotype)	10
	Related disorder: learning disability, migraine, schizophrenia, autism, Asperger's, febrile seizures	6
2nd degree relative (grandparent, uncle, aunt, niece or nephew)	Same phenotype	10
	Epilepsy (different phenotype)	5
	Related disorder: learning disability, migraine, schizophrenia, autism, Asperger's, febrile seizures	3

6.2.6 Pre-screening

Participants who had already been extensively investigated were preferentially contacted for recruitment to the study. Recruitment to the study began prior to the setting up of the 104 gene epilepsy panel in Glasgow, so not all cases had been pre-screened on the 104 gene panel prior to WGS. A note was made of which cases had been pre-screened on the panel so that the difference in yield between the pre-screened group and the not pre-screened group could be analysed.

6.2.7 Sequencing, filtering and analysis

DNA samples were obtained from the proband and both parents (where available) and tested using Illumina's HiSeq X Ten platform (Illumina, 2018). Mapping was performed using Stampy (Wellcome Trust Centre for Human Genetics, 2018). Data were provided from the sequencing laboratory in FASTQ format. Reeds were then mapped to make BAM files. Duplicates were removed and sequences were realigned around indels. Variants were called using the GATK best practice pipeline (DePristo et al., 2011), in reference to the GRCh37 human genome assembly. Variant files were saved in VCF format. Variants were filtered according to quality (IGV score > 100), rarity (heterozygous variants frequency in the ExAC database

(Broad Institute Exome Aggregation Consortium, 2018) of <0.0001 for heterozygous variants and <0.003 for biallelic variants) and predicted deleteriousness (Polyphen-2 (Adzhubei et al., 2010) score >0.45 for coding missense variants and PhyloP score (Pollard et al., 2009) >4 for non-coding variants). All variants that were predicated to lead to truncation of the gene product were included. Additional information gathered for each variant were the GTEx relative brain expression ratio (Aguet et al., 2017) and pLI score (Lek et al., 2016) for the gene involved. Variants which survived this filtering were imported onto an excel spreadsheet and were then reviewed manually. Variants of interest were imported into Alamut® software in order to review *in silico* predictions. *De novo* and biallelic variants considered first in all cases. Inherited variants were considered if inherited from a parent with a large family history score (>30). For male patients, X-linked maternally-inherited variants were considered. Literature-based reviews were undertaken to gather additional supporting evidence for gene candidacy, with particular note taken of genes that had previously been associated with neurodevelopmental disorders such as intellectual disability, autism, or schizophrenia.

In a parallel analysis, conducted by the bioinformatic team at UCB Pharma, phenotypic fatures coded for each patient using Human Phenotype Ontology (HPO) terms were uploaded onto the Spatientia™ platform and analysed using the Phenomizer tool which prioritises variants in genes that have been associated with closely matching phenotypes (Köhler et al., 2017). At the end of the study candidate variant identified by both proceses were discussed and a final list of best candidates was produced.

All variants that were considered strong candidates were confirmed by Sanger sequencing and discussed within a multidisciplinary team of professionals (Clinical scientists, Clinical Geneticists and Paediatric Neurologists) in order to determine further course of action required. Potential further courses of action were:

1. For findings considered diagnostic – report the finding and invite the family to clinic to discuss

2. For variants considered strong candidates – gather additional supporting evidence, for example:

- Any additional diagnostic tests from the patient that would support the genetic finding
- Use online gene-matchmaker systems such as Decipher (Firth et al., 2009), Gene Matcher (Sobreira et al., 2015), and Phenome Central (Buske et al., 2015) to contact other research groups who have identified patients with variants in the same gene
- Obtain additional DNA samples from other affected or unaffected family members

6.2.8 Defining novel candidate genes

A list of 359 genes previously associated with epilepsy was collated based on literature review (Chapter 3). Variants identified involving any of these genes were considered. Variants in genes previously implicated with other neurodevelopmental disorders such as intellectual disability, autism, schizophrenia or migraine were also considered. Because there is no online public library of such genes, these were reviewed and identified *ad hoc* using literature search and the Online Mendelian Inheritance in Man (OMIM) resource (Johns Hopkins University, Baltimore, 2018).

Additionally, novel candidate dominant epilepsy genes were selected from the filtered results based on GTEx ratio measure of relative brain expression, and pLI score of loss of function intolerance. In order to define sensitive and specific cut offs for GTEx and pLI values the 45 most commonly identified autosomal dominant genes (see chapter 3 and Appendix 15) were compared with the same values for all characterised human genes. Combinations of GTEx and pLI that would select just 5% of characterised genes were tested for their sensitivity to select genes associated with dominant epilepsy. The highest sensitivity (73.8%) was achieved using script which used a multiple of log pLI and GTEx ratio (see table 6.2c).

Variants in candidate genes were considered if the allele frequency was <0.0001 in the gnomAD database *and* either predicated to result in truncation or, for missense variants, had a Polyphen-2 score of >0.8.

Table 6.2c: Sensitivity of GTEx and pLI filters for selecting established dominant epilepsy genes

Cut off to achieve 95% specificity (only 5% of all genes selected)	Sensitivity (% of autosomal dominant epilepsy genes captured)
GTEx ratio > 7.9	22/45 (48.9%)
pLI > 0.9996	19/45 (42.2%)
pLI > 0.7 and GTEx > 1.7	26/45 (57.8%)
pLI > 0.8 and GTEx > 1.5	26/45 (57.8%)
pLI > 0.9 and GTEx > 1.2	27/45 (60.0%)
GTEx * $Log_2[0\text{-}Log_9(1\text{-}pLI)]$ > 2.0	32/45 (71.1%)

6.3 Results

6.3.1 Total recruitment, pre-screening and family testing

A total of 79 patients from 72 families were recruited – including four families with two affected children, and one family with three affected children.

Pre-screening, using the 104 gene epilepsy panel in Glasgow, was carried out for 44 patients from 40 families (56%).

In all cases DNA from the affected proband and both parents underwent WGS, apart from in one case where the proband, who presented with infantile spasms, had an extensive family history of focal epilepsy which appeared to follow a dominant inheritance pattern. In this family the proband and the most distantly-related affected relative, but not the proband's parents, were tested. In three of the families in which there was more than one affected sibling, just one of the siblings was tested with the other(s) sequenced later using the Sanger method to specifically look for the presence of any strong candidate variants.

6.3.2 Phenotypes

The four key phenotypes were defined following discussion with local paediatric neurologists about the phenotypes that were relatively common and were felt to have a likely genetic basis. These were:

1. Drug-resistant myoclonic epilepsy (DRM), n = 35

   Definition:

   - Onset of epilepsy with myoclonic seizures before the 6th birthday *and*
   - Ongoing myoclonic seizures despite adequate trials of two appropriately chosen and tolerated anti-epileptic therapy regimens

2. Infantile-onset developmental and epileptic encephalopathy (IODEE). N = 36

   Definition:

   - Onset of epilepsy before 18 months of age *and*
   - Developmental stagnation or regression reported in association with seizure onset

3. Landau-Kleffner Syndrome Spectrum (LKSS), n = 37

   Definition:

   - Onset of focal seizures before 10th birthday *and*
   - Comorbid autism diagnosis *or* significant sleep activation/electrical status epilepticus in slow wave sleep (ESES) reported on EEG

4. Comorbid paroxysmal movement disorder (PMD), n = 13

   Definition:

   - Epilepsy of onset before 10th birthday *and*
   - Comorbid paroxysmal non-epileptic movement disorder such as chorea, myoclonus, or dystonia

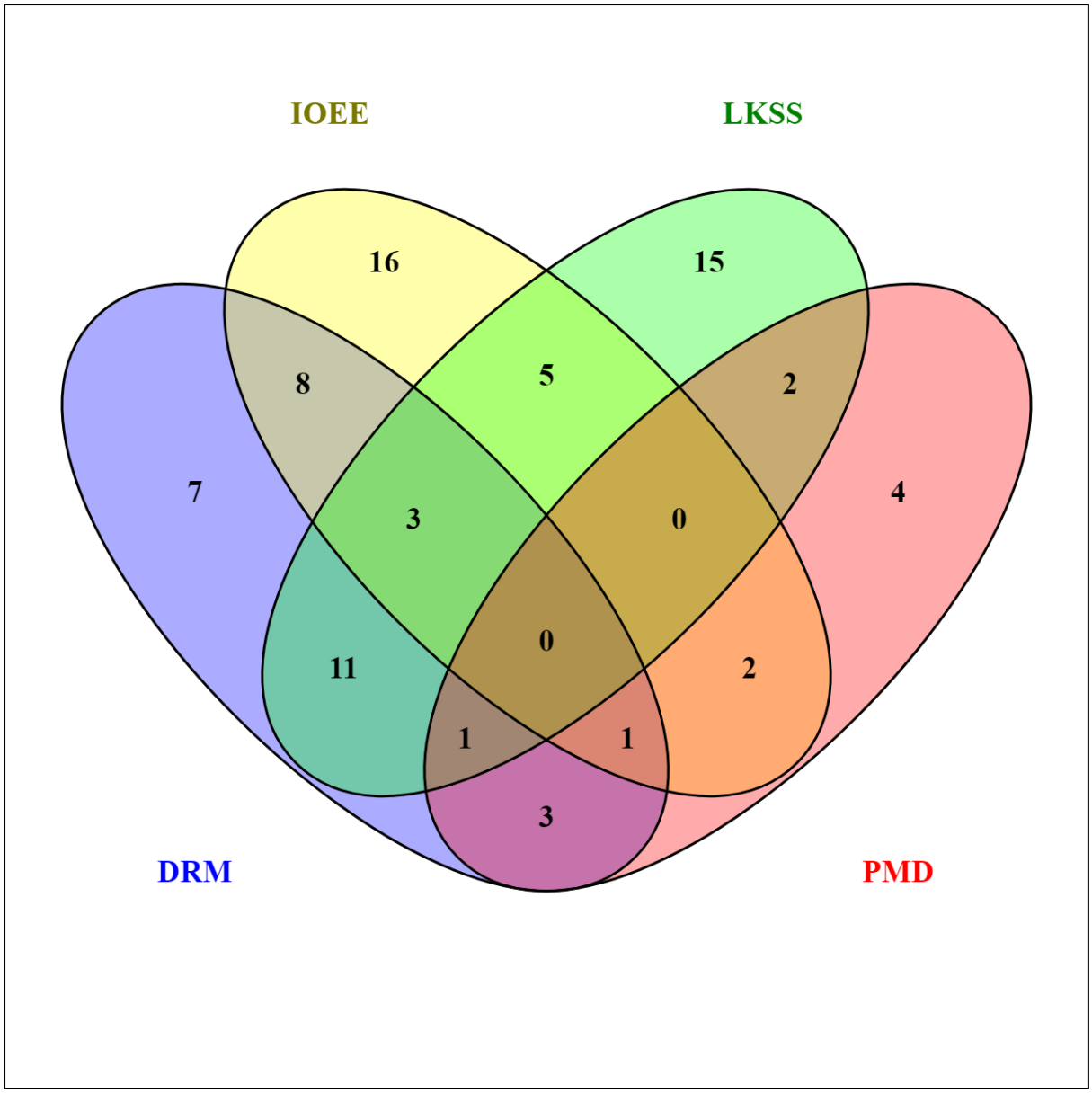


Figure 6.3a: Key phenotypes, and overlap between them; patients in the WGS study

As illustrated in figure 6.3a, there was significant overlap between the four key phenotype groups, with 47% of patients falling into more than one group.

A total of 47980 variants survived the filtering pipeline and were analysed on the excel spreadsheet, representing an average of 607 variants per individual. 76 variants were *de novo*.

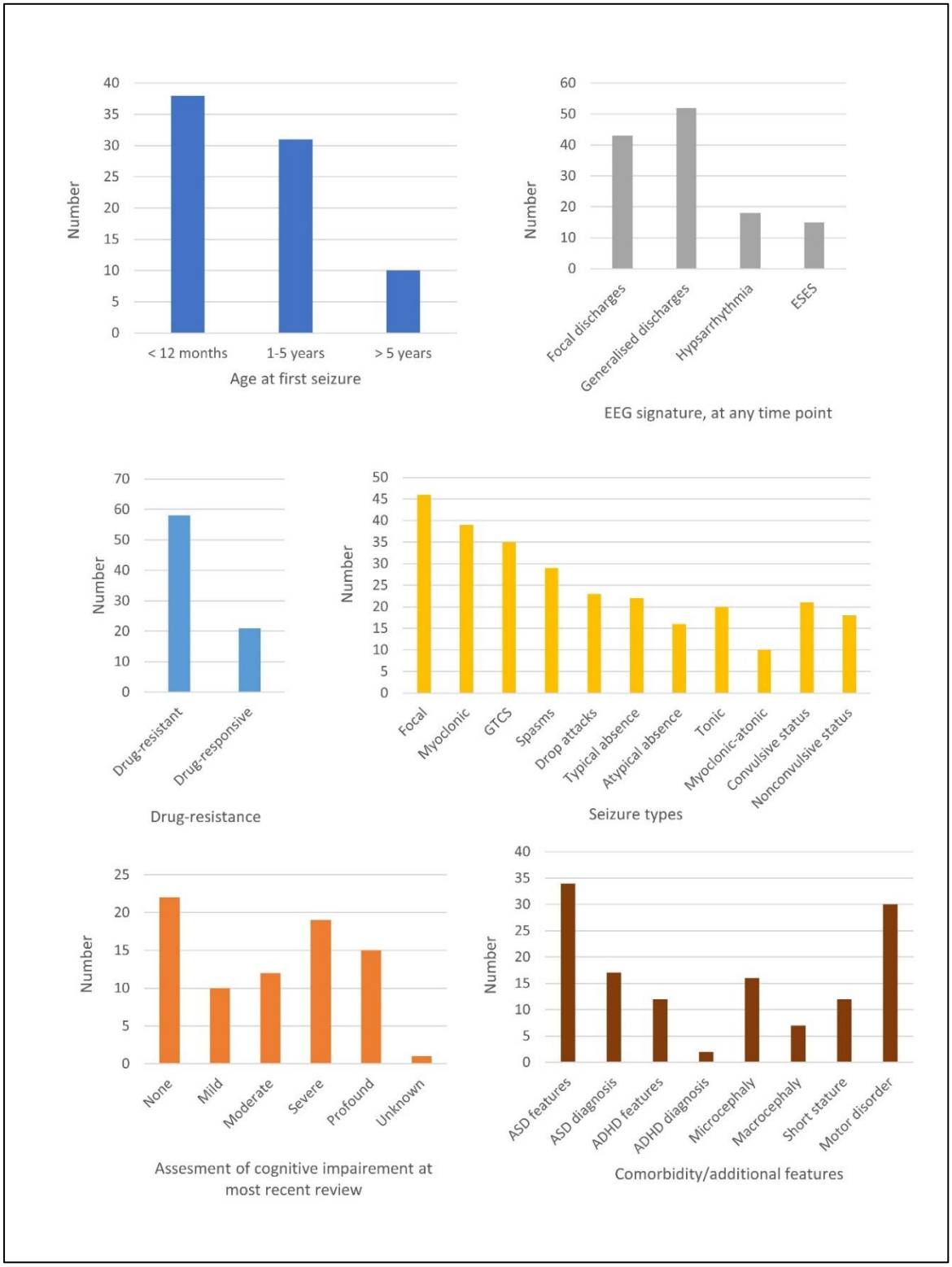


Figure 6.3b: Phenotypic features of the WGS cohort

6.3.3 Overall findings

Table 6.3: Diagnostic and candidate findings among the entire WGS cohort

Family	Patient	Phenotype	Pre-screened?	Diagnostic finding	Candidate finding
2	P-2	PMD	N		
4	P-4	DRM+LKSS	Y		
5	P-5	IODEE+PMD	N	*SCN8A (de novo)**	
7	P-7	LKSS	N		
11	P-11	LKSS	Y		
13	P-13	DRM+PMD	N		*TRIM46* (de novo)
14	P-14	DRM+LKSS	N		
16	P-16	LKSS	N		
22	P-22	IODEE+LKSS	N	*GNAO1 (de novo)*	
24	P-24	DRM+IODEE+PMD	Y		*POLR1A* (de novo)
25	P-25	DRM+LKSS	N		
29	P-29	DRM+IODEE	N	*SCL35A2 (de novo)*	
30	P-30	DRM	Y		
31	P-31	DRM+LKSS	N		
49	P-49	DRM+IODEE	N		
106	P-106	IODEE	Y		
115	P-115	LKSS	Y		
120	P-120	DRM+IODEE+LKSS	N		
137	P-137	IODEE	Y		
	B-137	IODEE	Y		
143	P-143	DRM+IODEE	N	*GABRA1 (de novo)**	
152	P-152	LKSS+PMD	N		
173	P-173	IODEE	Y		
183	P-183	DRM+IODEE+LKSS	Y		
185	P-185	IODEE	Y		
195	P-195	DRM	N	*SCN2A (de novo)**	
	B-195	DRM	N		
263	P-263	IODEE+PMD	Y	*SCN1A (de novo)**	
278	P-278	DRM	Y		*MAP2* (de novo)
309	P-309	IODEE	N		
321	P-321	IODEE	Y		
361	P-361	IODEE	Y		*TRIO* (maternal)
412	P-412	DRM+LKSS	N		
431	P-431	IODEE+LKSS	N	*ROGDI* (compound het.)	
437	P-437	LKSS	Y		
	B-437	LKSS	N		
463	P-463	DRM+IODEE	Y		
	B-463	IODEE	Y		
475	P-475	IODEE+LKSS	Y		
506	P-506	IODEE	N	*SMC1A (de novo)*	
527	P-527	DRM+PMD	Y		
	S-527	DRM+PMD	Y		
540	P-540	LKSS	Y		
570	P-570	DRM+LKSS+PMD	N		
597	P-597	DRM+LKSS	N		*MED13* (de novo)
621	P-621	IODEE	Y		
622	P-622	IODEE	Y		*RASL10B (de novo)*

699	P-699	IODEE+LKSS	Y		
722	P-722	IODEE	Y		*CACNA1G (de novo)*
755	P-755	DRM+IODEE	Y		
765	P-765	LKSS	N	*GRIN2A* (paternal)*	
	B-765	LKSS	N	*GRIN2A* (paternal)*	
	S-765	LKSS	N	*GRIN2A* (paternal)*	
772	P-772	DRM+IODEE	Y		*CNTNAP1* (compound het.)
773	P-773	LKSS	N		
780	P-780	DRM+LKSS	N		
818	P-818	DRM+LKSS	Y		
825	P-825	DRM+LKSS	N		
830	P-830	DRM+PMD	Y		
835	P-835	PMD	Y		
838	P-838	LKSS	Y		
847	P-847	DRM+IODEE+LKSS	Y		
913	P-913	IODEE+LKSS	N		
948	P-948	IODEE	Y	*NEXMIF (de novo)*	
951	P-951	PMD+LKSS	N		
955	P-955	DRM+IODEE	Y		
957	P-957	PMD	N		
958	P-958	DRM	N		*LRP8* (compound het.)
962	P-962	DRM	Y		*NIPBL (de novo)*
965	P-965	DRM	Y		
969	P-969	DRM+LKSS	N		
971	P-971	LKSS	Y		
976	P-976	LKSS	N	*STX1B* (maternal)*	
979	P-979	DRM+IODEE	Y		
981	P-981	IODEE	Y		
989	P-989	IODEE	Y		
991	P-991	LKSS	Y		
994	P-994	LKSS+PMD	Y		
999	P-999	IODEE	Y		

* = gene included in 104 gene panel

Overall, 13 patients from 11 families had a genetic finding that was considered diagnostic by the MDD. Another 10 patients from 10 families had a genetic finding that was considered a strong candidate, according to the criteria defined in section 6.2.8.

Families who had not undergone pre-screening with the 104 gene panel were more likely to have a *diagnostic* finding (9/31 = 19.4%) than those who had undergone pre-screening (2/41 = 4.8%) [Fisher's exact, p value = 0.007). This can be explained by the fact that 6/8 diagnostic results involved genes that were included in the

gene panel test. Families who had not undergone pre-screening were no more likely to have a *candidate* genetic cause (5/31 = 16.1%) than those who had been pre-screened (5/41 = 12.2%) [Fisher's exact p value = 0.74]. Associations between clinical features and genetic findings will be reported in section 6.3.6. In just 4/72 families (5.6%) was a genetic diagnosis with potential therapeutic implications made, and in all four cases this was a gene included on the 104 gene panel.

6.3.4 Diagnostic results from WGS

6.3.4.1 GABRA1

Female, born 2013. Phenotype: DRM+IODEE

P-143 was born at term plus 10 days by emergency Caesarean section for failure to progress. The pregnancy had been complicated by severe hyperemesis gravidarum during the first 20 weeks. Antenatal ultrasound scans and fetal movements has been normal. Birth weight was 3460g (+ 0.21 SD). No neonatal resuscitation was required.

There were no parental concerns about development until the age of two months. She had developed a social smile at six weeks of age. The first seizure occurred at aged two months. It was early in the morning and she was awake following a breast feed. Her eyes turned to the left and her head followed, and then she began making "clucking sounds" with her throat. The whole episode lasted 60-90 seconds. Very similar episodes continued to occur approximately twice per week. An EEG was performed at three months of age, during which a typical event was captured. This began with a focal epileptiform discharge in the right occipital area. This focal epileptiform discharge remained for about four minutes, before spreading to the left hemisphere. Only when the discharge spread to the left was there any detectable clinical change, which was head and eye deviation to the left, accompanied by facial flushing, and followed by a cry. Interictally there was

slowing and runs of high amplitude spikes, predominantly over the right occipital area.

From the age of two months onwards, parents had concerns about developmental progress. She seemed poorly responsive to both verbal and visual cues, and she developed a pervasive habit of stimulating her own vision by waving her left arm up and down in front of her eyes. She went on to develop several further seizure types including bilateral clonic seizures from aged 4.5 months, and both tonic seizures and myoclonic seizures from aged 1 year. Interictal EEGs continued to show multifocal and migrating epileptiform activity and her development plateaued. At the time of assessment she was 3 years old and she had profound cognitive impairment, with no independent mobility, no expressive or receptive communication and no meaningful use of her hands. She had central hypotonia, and cerebral visual impairment. Both height and head circumference were within normal range for age.

Beneficial treatments reported by the family were the Ketogenic Diet, Lorazepam for prolonged seizures, and Levetiracetam for focal seizures. Carbamazepine and Clobazam were both thought to have aggravated seizures. At the time of assessment she was having daily atypical absence seizures, myoclonic seizures every two days, and focal seizures between one and eight times per month.

MRI brain scan at the age of 4 months demonstrated a slightly thin corpus callosum and delayed myelination. Repeat MRI brain at 13 months demonstrated a progression of myelination which was now within normal limits. 12 lead ECG demonstrated a prolonged QT interval of 480-490ms. She had an implantable cardiac loop recorder inserted at the age of 2.5 years. No arrhythmia has ever been captured on this.

There was no family history on either side of epilepsy, febrile seizures, developmental disorder, mental ill health, or migraine.

Table 6.3b: *GABRA1* variant in P-143

***GABRA1* gene details**	
Function	Neuronal GABA-A receptor, alpha 1 subunit
Relative brain expression (GTEx ratio)	1755
Missense constraint (ExAC)	z = 3.53
LoF constraint (ExAC)	pLI = 0.963866
Published cases of a similar phenotype	Yes (Johannesen et al., 2016; Kodera H. et al., 2016)
Frequency in previous NGS studies (Chapter 3)	0.015%. Rank 18 =
Variant details	
Inheritance	*De novo*
Genomic location	Chr5(GRCh37): g.161318039C>T
HGVSc.	NM_000806.5(GABRA1): c.839C>T
HGVSp.	p.Pro280Leu
Region of the protein affected	Second transmembrane segment (Johannesen et al., 2016)
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (score: 0.04)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	1.00 (HumDiv) 1.00 (HumVar)
MDD conclusion	Causative

WGS revealed a *de novo* missense variant in the *GABRA1* gene. This was a novel variant, not found in the gnomAD or UK10K databases, and not previously reported in association with disease.

GABRA1 encodes a subunit of the dominant CNS receptor for the inhibitory neurotransmitter, Gamma-Aminobutyric Acid (GABA). *De novo GABRA1* variants have previously been associated with severe infantile onset epilepsies (Johannesen et al., 2016; Kodera et al., 2016), and since all *in silico* tools suggested pathogenicity, the MDD came to the conclusion that this variant was causative of P-143's phenotype. The spectrum of epilepsies that have been associated with *GABRA1* variants is broad, ranging from drug-responsive generalised epilepsies with onset in later childhood with good developmental outcomes, to severe neonatal onset epilepsies. No genotype-phenotype relationships have been identified. Only two of the previously reported cases presented at an earlier age than P-143 (Kodera et al., 2016). Interestingly P-143 had a second *de novo* frameshift variant in a gene with a high pLI score (*SNX13*) which encodes a regulator of G-protein signalling (Zheng et al., 2001), though due to relatively low brain expression this did not reach candidacy criteria for a novel epilepsy gene.

Table 6.3c: *SNX13* variant in P-143

***SNX13* gene details**	
Function	Regulator of G-protein signalling (Zheng et al., 2001).
Relative brain expression (GTEx ratio)	0.729
Missense constraint (ExAC)	z = 1.55
LoF constraint (ExAC)	pLI = 0.985322
Published cases of a similar phenotype	No
Cases in public databases	Yes. One case from the DDD study with a *de novo* missense variant (phenotype: abnormality of nervous system)
Variant details	
Inheritance	*De novo*
Genomic location	Chr7(GRCh37): g.17915341_17915344del
HGVSc.	NM_001350862.1(SNX13):c.510_513del
HGVSp.	p.Val171Serfs*8
Population frequency	Not in gnomAD or UK10K
MDD conclusion	Uncertain significance

6.3.4.2 SCN2A

Female, born 2003. Phenotype: DRM

P-195 was born by normal delivery at term + 12 days following an uncomplicated pregnancy, with normal antenatal scans. Birth weight was 3500g (+0.3 SD). No neonatal resuscitation was required. Developmental concerns were evident prior to epilepsy onset in that she never rolled over or crawled. At the age of two years she had a few single words. She presented with a cluster of seven bilateral tonic-clonic seizures in a 24 hour period at 22 months of age. During these episodes her eyes rolled back, all four limbs stiffened and then began jerking for less than one minute. Within five weeks of that cluster of seizures she was having hundreds of absence seizures per day. These were characterised by loss of contact, accompanied by clicking sounds of the tongue and fluttering of the eyelids. Further seizure types that developed were focal motor seizures which involved right foot clonus, myoclonic seizures, atonic drop seizures, tonic seizures, episodes of non-convulsive status, and epileptic spasms which where untypically late in onset at 13 years. These were the predominant seizure type at most recent follow-up with more than 30 episodes per day. She has clusters of bilateral clonic seizures approximately every 6 months. EEG at the age of two years demonstrated irregular

bisynchronous slow spike and wave activity. MRI brain was normal at the age of 2 years and again at the age of 7 years. The most effective antiepileptic treatment she had used was the Ketogenic Diet which was associated with a dramatic reduction in the frequency of absence seizures.

There was a clear regression in language skills after onset of epilepsy. At the age of 14 years she communicates through single words and gestures. Other areas or development were delayed. She started walking at 3.5 years of age. From the age of 5 years she developed ritualistic and repetitive behaviours. She has short stature (- 2.07 SD) but a normal head circumference (+ 0.35 SD).

P-195 has a brother (B-195), who was also recruited to this study. He presented at three years of age with a simple febrile convulsion. From the age of 3.5 years he had frequent myoclonic and atonic seizures (up to five per day) and from the age of four years he experienced clusters of brief bilateral clonic seizures two to three times per week. Seizures were resistant to therapy with Sodium Valproate, Nitrazepam and Clobazam, but responded to a combination of Levetiracetam and the Ketogenic Diet. His myoclonic and atonic seizures remitted at the age of five years of age, and the bilateral clonic seizures stopped at six years of age. At the time of assessment he had been seizure-free and off medication for four years and had normal cognitive ability. His EEGs showed frequent irregular bisynchronous polyspike and wave activity, which became more prominent during sleep. His height was 173cm at 16 years (+0.07 SD) and head circumference was 57.0cm (+ 0.3 SD). He had pectus excavatum and had been investigated for possible Marfan syndrome. The father of both P-195 and B-195 had a single febrile convulsion as a child and has macrocephaly (OFC 62.5cm) but normal height (173cm).

Table 6.3d: *SCN2A* variant in P-195

***SCN2A* gene details**	
Function	Neuronal voltage-gated sodium channel subunit, type alpha 2 ($Na_v1.2$)
Relative brain expression (GTEx ratio)	61.279
Missense constraint (ExAC)	z = 6.58
LoF constraint (ExAC)	pLI = 1.000000
Published cases of a similar phenotype	Yes (Wolff et al., 2017)
Frequency in previous NGS studies (Chapter 3)	1.13%. Rank: 4
Variant details	
Inheritance	*De novo*
Genomic location	Chr2(GRCh37): g.166245588A>T
HGVSc.	NM_021007.2(SCN2A): c.5272A>T
HGVSp.	p.Ser1758Cys
Region of the protein affected (Uniprot)	Segment 6 of repeat IV (transmembrane)
Population frequency	Not in UK10K or gnomAD
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	1.00 (HumDiv) 0.999 (HumVar)
MDD conclusion	Causative of the phenotype

Because P-195 and B-195 both had a history of drug-resistant epilepsy, there was an expectation that they would have a shared genetic cause. However, the most likely candidate variant in this family was a *de novo* variant in *SCN2A* in patient P-195 which was not shared by her brother.

SCN2A encodes the α_2 subunit of the neuronal voltage-gated sodium channel. *SCN2A* variants have been associated with a variety of epilepsy phenotypes, including patients with very similar phenotypes to P-195 - epilepsy onset at 1-3 years of age, late-onset epileptic spasms, drug-resistant myoclonic seizures, non-convulsive status epilepticus, bisynchronous slow spike and wave on EEG, and associated severe cognitive impairment (Wolff et al., 2017). In the largest published series of cases, age of seizure onset ranged from one day to over eight years. Patients who presented in the first three months of life were significantly more likely to have missense variants whilst those presenting after three months were significantly more likely to have truncating variants (Wolff et al., 2017).

The explanation for B-195's epilepsy remained undetermined. Because the father had a history of febrile convulsion, paternally-inherited candidate variants were considered. There were no variants in epilepsy-associated genes. There were

variants in two novel candidate genes, the most interesting of which was thought to be *CHD4* (Table 6.3e). *De novo m*issense variants in *CHD4* have been associated with a neurodevelopmental disorder called Sifrim-Hitz-Weiss syndrome, with 12 individuals described in two publications. Affected individuals presented with developmental delay, facial dysmorphism, macrocephaly, and congenital cardiac defects (Sifrim et al., 2016; Weiss et al., 2016). None of these features were prominent in family 195, though it was noted that the father had macrocephaly. Neither epilepsy nor febrile seizures were reported features in the published cases. Interestingly the Epi4k study identified a patient with Lennox-Gastaut syndrome who had *de novo* variants in both *SCN2A* and *CHD4* (Allen et al., 2013).

Table 6.3e: *CHD4* variant in family 195 (present in both P-195 and B-195)

***CHD4* gene details**	
Function	Encodes a chromodomain-containing protein that catalyzes ATP-dependent chromatin remodelling as a core component of the nucleosome remodelling and histone deacetylase repressor complex, which is involved in epigenetic regulation of gene transcription, DNA repair, and cell cycle progression (Sifrim et al., 2016; Weiss et al., 2016).
Relative brain expression (GTEx ratio)	0.636
Missense constraint (ExAC)	z = 7.05
LoF constraint (ExAC)	pLI = 1.000000
Published phenotype	Developmental delay, dysmorphism, macrocephaly, congenital cardiac defects (Sifrim et al., 2016; Weiss et al., 2016).
Frequency in previous NGS studies (Chapter 3)	None
Variant details	
Inheritance	Paternal (father had a history of febrile seizure)
Genomic location	Chr12(GRCh37): g.6709784T>A
HGVSc.	NM_001297553.1(CHD4) c.958A>T
HGVSp.	p.Asn320Tyr
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	0.981 (HumDiv) 0.312 (HumVar)
MDD conclusion	Candidate variant for familial phenotype

6.3.4.3 SCN8A

Male, born 2014. Phenotype: PMD+IODEE

P-5 was born by SVD at term following an uncomplicated pregnancy. Parents had no concerns until the age of 3.5 months when he "curled up and went blue." He was taken to his local paediatric unit where he was witnessed to have recurrent tonic seizures with accompanying desaturation and cyanosis. He subsequently developed infantile spasms and focal motor seizures. Through seizures were never captured on EEG, the inter ictal record demonstrated frequent spike and wave and polyspike and wave activity over both posterior occipital regions. MRI brain revealed delayed myelination, and abnormal signal in the globi pallidi, midbrain, pons and medulla. MR spectroscopy was normal. Following onset of seizures he had developmental stagnation. He developed a generalised choreoathetoid movement disorder and laryngomalacia. Seizure control was never achieved but the frequency of seizures was felt to reduce following introduction of Triple Bromide treatment. He died suddenly at the age of 22 months, with probable SUDEP as the cause. There was no family history of epilepsy but a maternal great uncle had had three sons who had all died in infancy of a condition which involved hydrocephalus.

Table 6.3f: *SCN8A* variant in P-5

***SCN8A* gene details**	
Function	Neuronal voltage-gated sodium channel subunit, type alpha 8 ($Na_v1.6$)
Relative brain expression (GTEx ratio)	7.270
Missense constraint (ExAC)	z = 7.71
LoF constraint (ExAC)	pLI = 1.000000
Published cases of a similar phenotype	Yes (Larsen et al., 2015a)
Frequency in previous NGS studies (Chapter 3)	0.51%. Rank: 7=
Variant details	
Inheritance	*De novo*
Genomic location	Chr12(GRCh37): g.52159459G>A
HGVSc.	NM_014191.3(SCN8A): c.2549G>A
HGVSp.	p.Arg850Gln
Region of the protein affected (Uniprot)	Segment 4 of repeat II (transmembrane)
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value:1)
Polyphen 2	1.00 (HumDiv) 0.999 (HumVar)
MDD conclusion	Causative of the phenotype

P-5 was found to have a *de novo* variant in *SCN8A* which was felt to explain his phenotype.

SCN8A encodes the α_8 subunit of the neuronal voltage-gated sodium channel. *De novo SCN8A* variants have been reported in association with early onset epileptic encephalopathy with a prominent movement disorder described. Disease-associated variants cluster in the transmembrane-encoding regions (Larsen et al., 2015a). Only missense variants have been described and *in vitro* functional studies have suggested that disease-associated variants confer gain-of-function properties on the $Na_V1.6$ channel (Barker et al., 2016). It has therefore been suggested that patients with *SCN8A* variants may respond better to sodium channel blocking medications than other classes of anti-epileptic drug, though evidence to support this remains anecdotal (Boerma et al., 2016). P-5 was never tried on a sodium channel blocking AED, and he died prior to his WGS result.

6.3.4.4 STX1B

Male, born 2007. Phenotype: LKSS

P-976 was born by forceps delivery following induction of labour at 38 weeks. His mother had gestational diabetes and had been on insulin since the 28th week. Antenatal scans had been normal and there had been no concerns about fetal movements. His birth weight was 3200g (-0.59 SD). There were no parental concerns about childhood development in the first 15 months of life. He walked at 14 months, and he had several single words with meaning at 12 months.

At 15 months of age he had a febrile convulsion. The family had taken him to the GP because of a temperature of 39.8 degrees. In the clinic room his eyes suddenly rolled back and all four limbs began jerking for about five minutes. 150 minutes after this first event he had a second convulsion of a similar nature. From that point onwards he had daily episodes in which his face would grimace, the right side of his mouth would lift, his eyelids would flutter, his pupils would dilate, and he would be unresponsive.

Following the febrile convulsion at 15 months there was an abrupt change in his developmental trajectory. He stopped speaking altogether for three months and made slow progress thereafter. At the age of nine years he can communicate with single words only. He also has difficulty with motor skills and is unsteady on his feet. He is unable to read or write. His cognitive ability has been assessed as severe learning disability. He was diagnosed with autism at the age of three years, and he has major difficulty with attention and concentration. MRI brain scan at the age of 3 years was normal. EEG shows right-sided and bilateral spike and slow wave and polyspike and slow wave discharges during sleep. Current seizure frequency is 3-4 per month, and the family feel that seizure-control has benefitted from the combination of Perampanel, Clobazam and Triple Bromide.

P-976's father has 5 female cousins. Each of those cousins has a son with epilepsy. The paternal grandmother was thought to have undiagnosed autism. P-976's mother has a history of a single febrile convulsion.

Table 6.3g: *STX1B* variant in P-976

***STX1B* gene details**	
Function	Pre-synaptic receptor for transport vesicles (Schubert et al., 2014)
Relative brain expression (GTEx ratio)	39.95
Missense constraint (ExAC)	z = 3.63
LoF constraint (ExAC)	pLI = 0.944475
Published cases of a similar phenotype	Yes (Schubert et al., 2014; Vlaskamp et al., 2016)
Frequency in previous NGS studies (Chapter 3)	0.015%. Rank: 82=
Variant details	
Inheritance	Maternal (mother had a history of febrile seizure)
Genomic location	Chr16(GRCh37): g.31012515C>A
HGVSc.	NM_052874.4(STX1B): c.106-1G>T (Essential splice-site interference)
HGVSp.	N/A
Region of the protein affected (Uniprot)	N/A
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Contributory to the phenotype

P-976 had a maternally inherited essential splice-site variant in *STX1B*. *STX1B* encodes a pre-synaptic protein which has a potential role in neurotransmitter release (Schubert et al., 2014). Variants in *STX1B* have been described in families

with dominantly-inherited epilepsy and/or febrile seizures. Penetrance of *STX1B* for phenotype appears to be variable within families, with some affected individuals having febrile seizures only, and others having drug-resistant epilepsy with comorbid intellectual disability (Schubert et al., 2014). The predicted effect of the *STX1B* variant in case P-976 was complete abolishment of the natural splice-site. Since truncating variants in this gene are heavily constrained in ExAC but have been observed in families with fever-sensitive epilepsies, the MDT was confident that the *STX1B* variant was relevant in this family. The reason for the disparity in phenotype between mother and son was not clear. Interestingly there was a paternally-inherited variant in the candidate gene *UNC13A* which also encodes a pre-synaptic protein with a role in synaptic vesicle priming (Rossner et al., 2004). Due to the absence of any established association with human disease, the significance of this variant was uncertain.

Table 6.3h: *UNC13A* variant in P-976

***UNC13A* gene details**	
Function	Essential role in synaptic vesicle priming (from rodent studies) (Rossner et al., 2004)
Relative brain expression (GTEx ratio)	17.08
Missense constraint (ExAC)	z = 5.89
LoF constraint (ExAC)	pLI = 0.999987
Published cases of a similar phenotype	No
Cases in public databases	No
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	Paternal (extensive family history of childhood onset epilepsy)
Genomic location	Chr19(GRCh37): g.17756625G>C
HGVSc.	NM_001080421.2(UNC13A): c.2214C>G
HGVSp.	p.Ile738Met
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value:1)
Polyphen 2	1.00 (HumDiv) 0.999 (HumVar)
MDD conclusion	Candidate modifier variant

6.3.4.5 GNAO1

Female, born 1997. Phenotype: IODEE + LKSS

P-22 was born by SVD at terms following an uncomplicated pregnancy. Birth weight was 3500g (+0.3 SD). Her parents had no concerns until the age of nine weeks when she began having prominent startle reactions in response to sudden noises. These were subsequently diagnosed as startle-induced reflex tonic seizures. Her whole body would become tense, her eyes would roll back, and her fists would clench. Ictal EEG performed at the time demonstrated rhythmic build-up of moderate voltage 4-5 per second theta activity over the left temporal area, spreading to all areas and becoming high voltage regular fast spiking. Inter ictal EEG showed left temporal sharp waves. Seizures remained resistant to medication until the age of four months when she was started on Sodium Valproate. She then became seizure-free until the age of four years when she began having generalised myoclonic seizures. From the age of 10 years she has had focal motor seizures arising from sleep, mostly in the early hours of the morning, and often triggered by noise. During a seizure her legs will stiffen with her toes pointing in plantar flexion. She will grab a pillow and appear frightened. She is usually responsive during events and can sometimes converse. She is currently having an average of two seizures per night and is taking three AEDs: Oxcarbazepine, Clobazam, and Sulthiame. Development has been considerably delayed. She has a diagnosis of autism. At the age of 20 years she cannot read or write and she mostly communicates with single words. She walks with a stooped gait. Her height and head circumference are within normal range. MRI brain scan was normal at the age of 13 years. There is no family history of epilepsy or any other neurodevelopmental disorders.

Table 6.3i: *GNAO1* variant in P-22

***GNAO1* gene details**	
Function	G-protein-coupled receptor subunit (Feng et al., 2017).
Relative brain expression (GTEx ratio)	13.888
Missense constraint (ExAC)	z = 3.49
LoF constraint (ExAC)	pLI = 0.984288
Published cases of a similar phenotype	Yes (Nakamura et al., 2013)
Frequency in previous NGS studies (Chapter 3)	0.084%. Rank: 31=
Variant details	
Inheritance	*De novo*
Genomic location	Chr16(GRCh37): g.56385384A>G
HGVSc.	NM_020988.2(GNAO1): c.812A>G
HGVSp.	p.Lys271Arg
Region of the protein affected (Uniprot)	Nucleotide binding site
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	0.891 (HumDiv) 0.869 (HumVar)
MDD conclusion	Causative of the phenotype

A *de novo* variant was found in *GNAO1*. *GNAO1* encodes a subunit of highly brain expressed G-protein coupled receptor which is believed to play a role in mediation of signals at multiple neuroreceptor sites (Feng et al., 2017). *De novo* variants in this gene have been associated with early-onset developmental and epileptic encephalopathy (DEE) (Nakamura et al., 2013) and with an apparently distinct developmental disorder characterised by developmental delay, infrequent epileptic seizures, and a paroxysmal choreoathetoid movement disorder (Marcé-Grau et al., 2016; Saitsu et al., 2016; Kulkarni et al., 2015; Ananth et al., 2016). There is an apparent clustering of the DEE-associated variants at codons 40-45, 174-203, and 270-279, though exactly what function these domains represent has not been delineated. The variant in P-22, codon 271, was between two other published DEE-associated variants (Myers et al.,2016).

6.3.4.6 GRIN2A

Male, born 2009. Phenotype: LKSS

P-765 was born by SVD at term following an uncomplicated pregnancy. Birth weight was 4700g (+ 2.4 SD). He was admitted to the Special Care Baby Unit for 48 hours

because of tachypnoea but there were no other neonatal concerns. His mother had no concerns about his early development. By the age of 22 months he was able to walk independently and communicate with single words. By the age of four years he had fluent speech and was fully toilet trained.

At the age of five years during a diarrhoea and vomiting illness he had a bilateral clonic seizure during his sleep. There was associated hypersalivation. The following morning his mother noticed an abrupt change. He became completely non-verbal and could no longer follow simple instructions. He was admitted to hospital for investigation. MRI brain scan was normal but EEG demonstrated frequent multifocal spike wave and polyspike wave complexes which became continuous during sleep, consistent with a diagnosis of Epileptic Encephalopathy with Continuous Spike Waves During Slow Wave Sleep (ESES). He was treated with a course of high dose steroids which resulted in normalisation of the EEG, and a return of his previous language skills. Since then he has had multiple episodes of focal seizures, occurring in clusters and associated with abrupt change in language skills and behaviour. Therapeutic trials of Clobazam, Sodium Valproate, Ketamine, and Sulthiame were not effective at preventing these clusters.

P-765 has an older sister (S-765) and an older brother (B-765). B-765 had global developmental delay and demonstrated autistic features from the age of 18 months. He was diagnosed with autism at the age of four years. At the age of nine years he began having focal seizure characterised by behavioural arrest, facial twitching and drooling. Seizures were drug-resistant and came in clusters. His EEG demonstrated continuous right sided centrotemporal discharges seen during wakefulness and a clear deterioration during sleep to continuous generalised spike wave and polyspike wave complexes (Figure 6.3c).

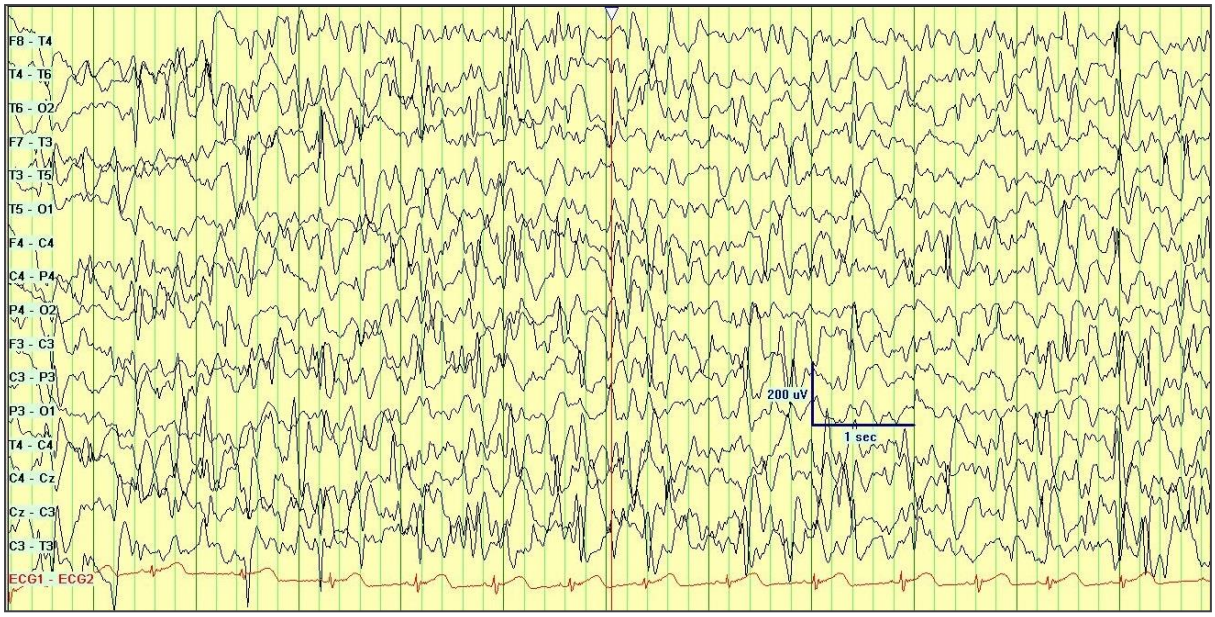


Figure 6.3c: Sleep EEG from P-765, demonstrating continuous spike wave without clinical accompaniment

S-765 was thought to have normal development until the age of two years, at which point her mother felt that she "suddenly withdrew into herself" and stopped all forms of imaginative play. She was diagnosed with autism at the age of five years. She began having focal seizures at the age of nine years. Seizures have been controlled with Sodium Valproate and Sulthiame. The father of all three children has learning disability and a possible history of language regression in early childhood, but no history of seizures.

Table 6.3j: *GRIN2A* variant in family 765 (Present in P-765, S-765 and B-765)

***GRIN2A* gene details**	
Function	NMDA receptor subunit, 2A
Relative brain expression (GTEx ratio)	16.58
Missense constraint (ExAC)	z = 3.80
LoF constraint (ExAC)	pLI = 0.998045
Published cases of a similar phenotype	Yes (Carvill et al., 2013; Endele et al., 2010; Lesca et al., 2013)
Frequency in previous NGS studies (Chapter 3)	0.21%. Rank: 13=
Variant details	
Inheritance	Paternal
Genomic location	Chr16(GRCh37): g.9934590A>C
HGVSc.	NM_001134407.1(GRIN2A): c.1565T>G
HGVSp.	p.Val522Gly
Region of the protein affected	Agonist binding domain S1 (Swanger et al., 2016)
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	0.999 (HumDiv) 0.999 (HumVar)
MDD conclusion	Causative of the familial phenotype

P-765 inherited a variant in *GRIN2A* from his father. Sanger sequencing confirmed that S-765 and B-765 also carried the variant. The phenotype observed in this family is consistent with previously published cases of *GRIN2A*-related epilepsy, a condition which is characterised by the coexistence of focal epileptic seizures, abnormal language development, and a predisposition to ESES (Carvill et al., 2013; Endele et al., 2010; Lesca et al., 2013).

GRIN2A encodes a key subunit of the excitatory glutamatergic NMDA receptor. Disease-associated variants in *GRIN2A* are usually missense, and cluster in the regions of the gene that encode the agonist binding domains and transmembrane regions of the receptor. *In vitro* functional studies show that epilepsy-associated missense variants in *GRIN2A* can be both gain-of-function and loss-of-function (Swanger et al., 2016). Pierson et al. demonstrated that in a *Xenopus ooyte* cell model, the epilepsy-associated Leu825Met variant resulted in increased agonist potency, and that this could be corrected with the NMDAr antagonist Memantine. They then treated a patient with [*GRIN2A*] Leu825Met-associated epilepsy with Memantine and reported a significant reduction in seizure frequency (Pierson et

al., 2014). In P-765 we trialled therapy with the NMDAr antagonist Ketamine but did not observe a reduction in seizure frequency.

6.3.4.7 SLC35A2

Female, born 2009. Phenotype: DRM + IODEE

P-29 was born by SVD following induction of labour at term +13 days. There were no antenatal or neonatal complications. Birth weight was 3710g (+ 0.74 SD). There were no parental concerns until six weeks of age when she had a seizure involving clonic jerking of all four limbs, lasting about 60 seconds. Four hours later she had a second seizure of the same nature, followed by several more over the next few hours. For the next two weeks she remained a hospital inpatient, during which time seizure control was not achieved despite multiple AEDs. At eight weeks of age she began having typical infantile spasms. EEG demonstrated hypsarrhythmia. Spasms, occurring in clusters were resistant to treatment, and continued until the age of two years. Further seizure types included myoclonic seizures, tonic seizures, and absence seizures from two years, and frequent periods of non-convulsive status epilepticus from four years. Seizures have been resistant to therapeutic trials of multiple AEDs, and surgical interventions in the form of Vagus Nerve Stimulation (VNS) and corpus callosotomy.

Development has been severely impaired. At the age of eight years she has truncal hypotonia, is non-ambulant and non-verbal. She can hold a bottle with both hands but has no pincer grasp. She has microcephaly (OFC 49.0cm, -2.85 SD) but MRI shows her brain to be structurally normal. The only family history is that a maternal third cousin also had a severe developmental and epileptic encephalopathy (P-321 in this study).

Table 6.3k: *SLC35A2* variant in P-29

***SLC35A2* gene details**	
Function	UDP-galactose transporter. Transfers nucleotide sugars from cytosol to Golgi apparatus for glycosylation (Miura et al., 1996)
Relative brain expression (GTEx ratio)	0.546
Missense constraint (ExAC)	z = 2.33
LoF constraint (ExAC)	pLI = 0.780526
Published cases of a similar phenotype	Yes (Kodera et al., 2013; Ng et al., 2013)
Frequency in previous NGS studies (Chapter 3)	0.015%. Rank: 82=
Variant details	
Inheritance	De novo
Genomic location	ChrX(GRCh37): g.48762569del
HGVSc.	NM_005660.2(SLC35A2): c.617del
HGVSp.	p.Val206Alafs*143
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Causative of the phenotype

A *de novo* frameshift variant was found in the X-linked gene *SLC35A2*. Truncating and missense variants in this gene have been previously reported in females with severe neurodevelopmental disorders (Kodera et al., 2013; Ng et al., 2013). Since truncating variants are not observed in males, hemizygosity for truncation is thought to be non-viable. *SLC35A2* encodes a UDP-galactose transporter involved in the glycosylation pathway, so this has been considered a Congenital Disorder of Glycosylation (CDG), though the typical biochemical signature of this group of disorders – abnormal transferrin isoforms – is often absent (Ng et al., 2013).

Epilepsy is a frequently observed feature of most CDGs (Fiumara et al., 2016) and is present in 12/13 (92%) of published *SLC35A2*-CDG cases. Epileptic spasms are the most frequently observed seizure type (Allen et al., 2014; Bosch et al., 2016; Kimizu et al., 2017; Lopes et al., 2016; Dorre et al., 2015; Evers et al., 2017). >70% of cases are reported to have microcephaly and >60% have hypotonia – both present in P-29. Variable dysmorphism and skeletal abnormalities are also frequently reported, but were not present in P-29. Following consultation with a leading expert in CDGs, P-29 is starting a diet with D-galactose supplementation. This treatment has not previously been tried in *SLC35A2*-realted CDG, but has been shown to be tolerated and effective at normalising biochemical markers of disease in another genetic CDG, PGM1-CDG (Wong et al., 2017)

6.3.4.8 ROGDI

Female, born 2011. Phenotype: IODEE + LKSS

P-431 was born by SVD at 36 weeks gestation following induction of labour for cholestasis of pregnancy. Antenatal ultrasound scans and fetal movements had been normal. Birth weight was 3200g (+ 1.28 SD) and there were no neonatal complications. There were parental concerns about her behaviour from about four months of age. She did not smile, laugh or interact with her parents. At eight months of age she was starting to sit independently. Her first seizure occurred at 8.5 months of age. Her mother heard a scream in the middle of the night and found her in the cot blue and floppy with her eyes rolled back. There was no stiffness or jerking noted. A second episode occurred one hour later. On this occasion she had stiffening of the right arm and the right leg, her eyes were rolled up and to the right, she was making lip smacking noises and her lips were blue. There were a few brief jerks of the right arm and leg. The entire episode, as with all subsequent seizures, was between three and six minutes duration. Identical seizures occurred every hour for the next two days but settled one she was started on regular Phenytoin. The Phenytoin was reduced and her focal seizures returned but this time did not settle when Phenytoin was restarted. She then had a four-month period of frequent seizures. Seizures would occur in clusters of typically three seizures per hour with between one and six clusters per day. These were refractory to multiple medications (Sodium Valproate, Carbamazepine, and Nitrazepam) but eventually stopped when she was established on Levetiracetam. At the age of four years seizures recurred during a trial of Levetiracetam withdrawal and stopped again when Levetiracetam was re-introduced. EEGs demonstrated multifocal spike waves and sharp wave discharges. MRI brain was normal at the age of 9 months.

Her development is delayed, at the age of five years she can hold a pencil and scribble but not draw, she has about 50 single words, she has very little imaginative play and extremely challenging behaviour including violent head

banging. She was diagnosed with autism at the age of five years. She has short stature (101cm, -2.1 SD) and microcephaly (49.5cm, -2.5 SD). She has prominent yellow discolouration and abnormal shape of her teeth (Figure 6.3B).

There was no family history of epilepsy or any other neurodevelopmental disorders.

Table 6.3l: *ROGDI* variants in P-431

***ROGDI* gene details**	
Function	Unknown
Relative brain expression (GTEx ratio)	3.840
Missense constraint (ExAC)	z = -1.50
LoF constraint (ExAC)	pLI = 0.000000
Published cases of a similar phenotype	Yes (Schossig et al., 2012; Tucci et al., 2013)
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant 1 details	
Inheritance	Paternal
Genomic location	Chr16(GRCh37):g.4848186C>T
HGVSc.	NM_024589.2(ROGDI): c.532-1G>A (Essential splice-site interference)
HGVSp.	N/A
Population frequency	Not in gnomAD or UK10K
Previously reported	No
Variant 2 details	
Inheritance	Maternal
Genomic location	Chr16(GRCh37): g.4851551G>GC,
HGVSc.	NM_024589.2(ROGDI): c.153dup
HGVSp.	p.Thr52Hisfs*2
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Causative of the phenotype

P-431 had two variants in *ROGDI*, one inherited from each parent. Both variants are predicted to result in truncation, with the paternally inherited variant abolishing the splice site and the maternally-inherited variant resulting in frameshift. Biallelic truncating *ROGDI* variants have been previously identified as a cause of Kohlschütter-Tönz Syndrome (KTS) (Schossig et al., 2012), a condition first described in 1974 (Kohlschutter et al., 1974) characterised by epilepsy, intellectual disability, and yellow discolouration of the teeth with enamel hypoplasia. To date 16 patients with KTS due to *ROGDI* variants have been reported (Schossig et al., 2012; Tucci et al., 2013). All have epilepsy, with median age of seizure onset at 9 months (range 2-22) and yellow discolouration of the

teeth. Short stature and microcephaly are also reported. Treatment efficacy is not reported in many cases: two were reported to respond well to Levetiracetam, one to Sodium Valproate, one to Clobazam, and one to a combination of Phenobarbitone and Vigabatrin.

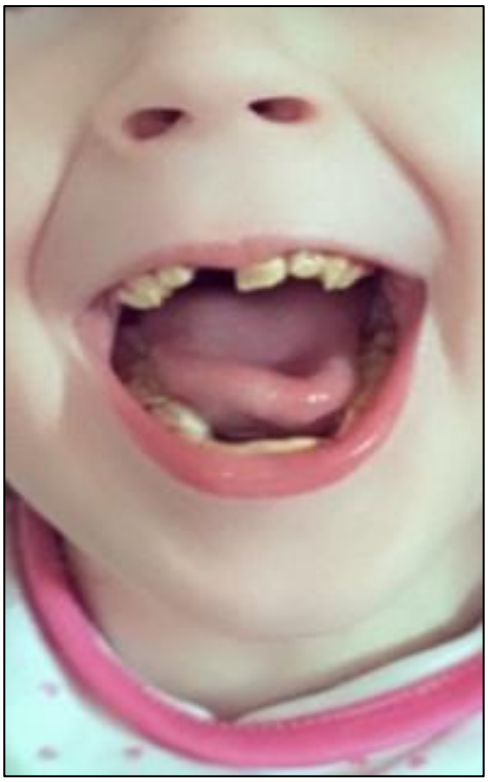


Figure 6.3d: P-431 teeth

6.3.4.9 SMC1A

Female, born 2000. Phenotype: IODEE

P-506 was born by elective caesarean section at 38 weeks gestation because of a previous caesarean section. Fetal movements and antenatal ultrasound scans were normal. Birth weight was 3600g (+ 0.51 SD) and there were no neonatal problems. Her parents first became concerned at five months of age because of poor head control. At about 5.5 months of age she had a seizure whilst she was with her mother at the hairdresser but this was not witnessed by the mother since she was out of the room at the time. The mother recalls her being very floppy following

the event. One week later she had a second event which was witnessed by the mother. All four limbs went stiff then began jerking, her eyes rolled backwards and her lips turned blue. Following that she began having epileptic spasms. This was her predominant seizure type until about 12 months of age. For the past ten years all of her seizures have been bilateral tonic clonic seizures. These have a striking clustering pattern. She will go for 8-12 weeks without any seizures at all, but then has 7-10 seizures per day for 48-72 hours. Interictal EEGs demonstrate multifocal sharp wave discharges. MRI brain at the age of 11 years demonstrated mild asymmetric posterior white matter volume loss. She has short stature (height 141.0cm, -3.9 SD) and microcephaly (48.0cm, -5.3 SD). She has profound cognitive impairment. She has never been ambulant, has never had any speech, nor any bowel or bladder control. She communicates through babbling noises, smiles, and laughs. She has cerebral visual impairment.

A maternal cousin has Asperger's syndrome but there is no other family history of note.

Table 6.3m: *SMC1A* variant in P-506

***SMC1A* gene details**	
Function	Forms part of the Cohesin complex, believed to play a major role in transcriptional regulation (Mannini et al., 2015)
Relative brain expression (GTEx ratio)	0.650
Missense constraint (ExAC)	z = 6.59
LoF constraint (ExAC)	pLI = 0.999988
Published cases of a similar phenotype	Yes (Lebrun et al., 2015; Goldstein et al., 2015; Jansen et al., 2016)
Frequency in previous NGS studies (Chapter 3)	0.0077%. Rank: 108=
Variant details	
Inheritance	*De novo*
Genomic location	ChrX(GRCh37):g.53421748G>A
HGVSc.	NM_006306.2(SMC1A): c.2923C>T
HGVSp.	p.Arg975*
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Causative of the phenotype

A *de novo* nonsense variant was found in *SMC1A* which was considered to be causative of the phenotype. *SMC1A*-related epilepsy will be discussed in more detail in chapter 7.

6.3.4.10 SCN1A

Male, born 2010. Phenotype: IODEE + PMD

P-263 was born by SVD at term weighing 3.6kg (+ 0.2 SD). There had been no concerns arising from routine fetal ultrasound scans and no concerns about fetal movements. He was born at a Community Midwifery Unit and discharged home on the day of his birth.

At 2 days of age, his mother noticed a subtle change in breathing pattern. The same thing happened on day 4 of life. On day 10 of life he had an episode where both his eyes simultaneously abducted, his arms both abducted at the shoulders and his colour turned blue. He was admitted to his local district general hospital for observation then transferred to the regional tertiary neonatal unit. Seizures were captured on EEG monitoring. These were characterised by extension of all four limbs, followed by a cry, then a run of twitching movements of the right side of his face. On EEG there was a run of bilateral fast activity followed by a marked bradycardia (Figure 6.3d).

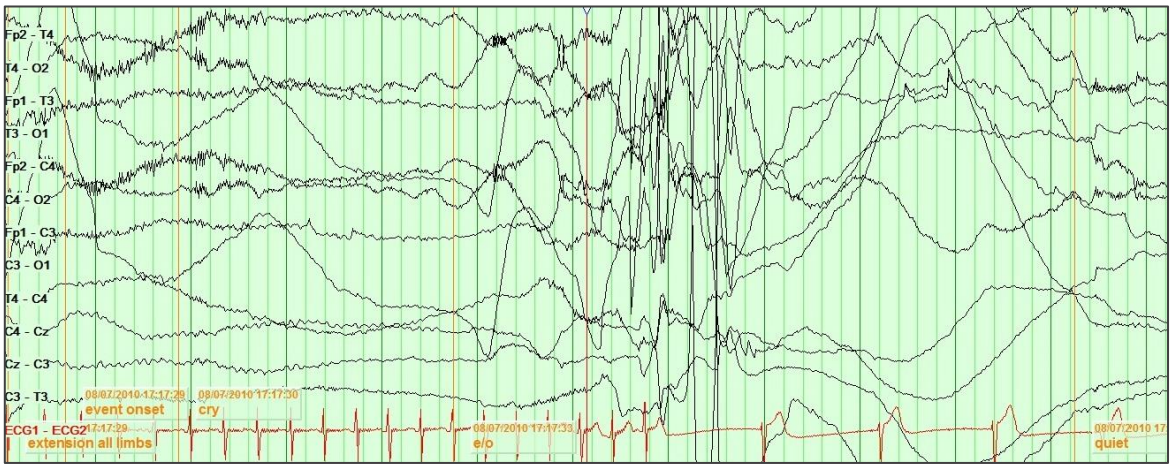


Figure 6.3e: EEG in P-263 capturing ictal bradycardia on ECG lead

MRI brain revealed linear T2 high signal within midbrain. He was started on Carbamazepine and his seizures stopped until 3 months of age when he had a focal

seizure following his second set of immunisations. He developed a stridor at 3 months of age and was referred for ENT evaluation. At 5 months of age he was found dead in his car seat when returning from an outpatient appointment.

Prior to his death there had been signs of developmental delay. He had poor head control and always held his hands in a clasped position. There were no concerns about his vision, he had a varied repertoire of babble, and responded to verbal cues.

Post mortem examination demonstrated metabolic injury (red cell change, shrunken nuclei and eosinophilic cytoplasm) involving the basal ganglia, dentate nucleus brainstem (midbrain, pons and medulla), and spinal cord (within anterior horn cells). These findings were thought to be suggestive of Leigh Syndrome. With this in mind a muscle biopsy was assessed for respiratory chain enzymes (normal), and genetic tests were preformed for *POLG1*, *MTATP6*, and *MTATP8* (all negative). Urine amino acids, plasma amino acids, and plasma organic acids were all also normal. There is no family history of epilepsy or neurodevelopmental disorders.

Table 6.3n: *SCN1A* variant in P-263

***SCN1A* gene details**	
Function	Neuronal voltage-gated sodium channel (type 1.1)
Relative brain expression (GTEx ratio)	80.1
Missense constraint (ExAC)	z = 5.61
LoF constraint (ExAC)	pLI = 1.00
Published cases of a similar phenotype	No
Frequency in previous NGS studies (Chapter 3)	423/13063 = 3.2% Rank: 1
Variant details	
Inheritance	*De novo*
Genomic location	Chr2(GRCh37):g.166852611 A>G
HGVSc.	NM_001165963.1 (SCN1A): c.4493T>C
HGVSp.	p.Ile1498Thr
Region of the protein affected	"IFMT" motif between repeat III and repeat IV (cytoplasmic loop)
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	0.999 (HumDiv) 0.968 (HumVar)
MDD conclusion	Causative of the phenotype

The *SCN1A* missense variant identified in P-263 was considered to be pathogenic at the MDD. Though not previously associated with disease in the literature, this variant was absent from healthy population databases and was predicated to be damaging by all *in silico* tools. A different amino acid substitution at the same residue has been recurrently identified in association with the rare *SCN1A*-related phenotype, familial hemiplegic migraine (FHM) (Weller et al., 2014; de Vries et al., 2013). A highly conserved four amino acid motif (IFMT, 1498-1501) in this loop of the $Na_v1.1$ protein between repeat II and repeat IV acts as a hinged lid that blocks the pore when the channel is in fast inactivated states (Catterall, 2014). It is hypothesised that variants in this region impair this blocking process and thereby confer gain-of-function properties on the ion channel. Gain-of-function properties of variants are observed in other FHM-associated *SCN1A* variants (Dhifallah et al., 2018). This contrasts with most epilepsy-associated *SCN1A* variants which result in loss-of-function (Escayg & Goldin, 2010). Further evidence in support of the p.Ile1498Thr variant conferring gain-of-function properties comes from reviewing the literature relating to variants affecting paralogous residues in other sodium channels. These are: *SCN2A* (p.Ile1488Asn), a mosaic variant associated with epilepsy and intellectual disability (Stosser et al., 2017); *SCN9A* (p.Ile1461Thr), a recurrent variant demonstrated experimentally to impair channel inactivation and associated with paroxysmal extreme pain disorder (Fertleman et al., 2006; Drenth & Waxman, 2007); *SCN8A* (p.Ile1479Met), presentation with drug-resistant epilepsy from 4 months of age (Larsen et al., 2015a) ; and *SCN5A* (p.Ile1485Val), associated with Brugada syndrome (National Center for Biotechnology Information: Bethesda, 2018).

The role of *SCN1A* variants in childhood-onset epilepsies has been discussed in detail in chapter 5. Patients with *SCN1A*-related epilepsy typically present between 6 weeks of age and 25 months of age with a median age of presentation of around 6 months (Harkin et al., 2007; Brunklaus et al., 2012). Around half of patients present initially with febrile seizures, which are often prolonged. Most patients will progress to a severe drug-resistant epilepsy with comorbid cognitive difficulties and ataxia (Dravet syndrome). Developmental impairment is typically

not apparent before one year of age (Brunklaus et al., 2012). Atypical cases of *SCN1A* related epilepsy have been recently described. These patients differ from those with Dravet syndrome or GEFS+ in that they all present relatively early, before three months of age, and they develop an early onset hyperkinetic movement disorder and early profound developmental impairment (Sadleir et al., 2017). It is well-established that the diagnosis of Dravet syndrome, and the presence of a pathogenic *SCN1A* variant, is associated with early mortality, with half of deaths caused by SUDEP (Shmuely et al., 2016).

The presentation with seizures in the neonatal period seen in P-263 has not been reported before. Pathogenic *SCN1A* variants have been identified in patients without epilepsy who have had Sudden Unexpected Death in Infancy however (Brownstein et al., 2018).

In view of the atypical phenotype it was worth looking for any potential genetic modifiers in P-263. A second *de novo* variant was found in *CHN1* gene (p. Leu84Ser), though due to a relatively low pLI score this gene was not considered a candidate for epilepsy. *CHN1* variants have been associated with dominantly-inherited Duane retraction syndrome (Chan et al., 2011; Miyake et al., 2008).

6.3.4.11 NEXMIF

Male, born 2015. Phenotype: IODEE

P-948 was born by SVD at 35 weeks gestation with a birth weight of 2.23kg (- 1.51 SD). Head circumference at birth was 30.0cm (- 2.38 SD). On the first day of life he had an apnoea and was found to be hypotonic. He was treated for early-onset sepsis, though blood cultures were negative. Due to a weak suck he required nasogastric feeding for the first three weeks of life.

At 4 months of age he presented with his first seizures. These were characterised by apnoea and desaturation but little in the way of motor features. No events were captured, but interictal EEG demonstrated multifocal epileptiform discharges. He

was started on Levetiracetam and his seizures stopped. He remained seizure-free until the age of 14 months. He then began having clusters of events on wakening in which his eyes rolled back, his arms flexed, and his legs stiffened, lasting just under one second. These were felt to be best described as epileptic spasms. Levetiracetam was restarted and these events ceased. EEG was not available until three days after restarting Levetiracetam. No events were captured on EEG at that time but the interictal EEG showed low to moderate amplitude mixed frequency background of theta and delta activity and rhythmical 4-5Hz theta activity intermittently over the posterior regions in brief runs. This activity became more marked and widespread during drowsiness.

He has global developmental delay. At 37 months of age he is non-ambulant and has global hyptonia. He mobilises by bottom shuffling and rolling and can sit without support. He has cerebral visual impairment and severe gastro-oesophageal reflux. He is non-verbal and does not respond to his own name or any other verbal cues.

He has microcephaly (OFC 45.3cm, -2.78 SD) and short stature (height 79.5cm, -4.08 SD).

MRI brain was normal at 7 months of age. His mother has a history of mild learning disability.

Table 6.3o: *NEXMIF* variant in P-948

***NEXMIF* gene details**	
Function	Involved in neurite outgrowth by regulating cell-cell adhesion via the N-cadherin signalling pathway (Van Maldergem et al., 2013)
Relative brain expression (GTEx ratio)	3.08
Missense constraint (ExAC)	z = 0.55
LoF constraint (ExAC)	pLI = 0.95
Published cases of a similar phenotype	Van Maldergem 2013(Van Maldergem et al., 2013) Kuroda 2015 (Kuroda et al., 2015) de Lange 2016 (de Lange et al., 2016) Webster 2016 (Webster et al., 2017) Lorenzo 2018 (Lorenzo et al., 2018)
Frequency in previous NGS studies (Chapter 3)	3/13063 = 0.023%.
Variant details	
Inheritance	*De novo*
Genomic location	ChrX (GRCh37):g.73963510 G>T
HGVSc.	NM_001008537.2 (NEXMIF) c.882C>A
HGVSp.	p.Tyr294*
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Causative of the phenotype

P-948 had a *de novo* truncating variant in *NEXMIF*. Variants in this gene, which is located on the X chromosome, are associated with a neurodevelopmental disorder. 37 patients with *NEXMIF*-associated disorders have been reported in the literature. 17/37 (46%) are male. The majority of male patients have inherited duplications, translocations, or microdeletions involving all or part of *NEXMIF*, which have been inherited from unaffected mothers. All reported females have *de novo* variants, the majority of which are frameshift or nonsense variants (Van Maldergem et al., 2013; Kuroda et al., 2015; de Lange et al., 2016; Webster et al., 2017; Lorenzo et al., 2018). Only one male with a *de novo* truncating variant has been reported (Lorenzo et al., 2018).

24/37 (65%) patients have epileptic seizures and 19/37 (51%) are reported to have dysmorphic features, though these vary markedly between patients. Only 4 patients (11%) are reported to have microcephaly (Van Maldergem et al., 2013; Kuroda et al., 2015; de Lange et al., 2016; Webster et al., 2017; Lorenzo et al., 2018).

Knockdown of *NEXMIF* in rat cortex results in altered neuronal migration and dendritic growth (Van Maldergem et al., 2013; Gilbert & Man, 2016), but the specific function of *NEXMIF* is not understood.

6.3.5 Novel candidate epilepsy genes

In section 6.3.5 I will present the phenotypes and genotypes of those patients who had strong candidate genetic variants identified through WGS.

6.3.5.1 TRIO

Male, born 2000. Phenotype: IODEE.

P-361 was born by SVD at term, weighing 3100g (- 0.8 SD). His mother, who had a history of temporal lobe epilepsy, took Phenobarbitone throughout pregnancy but did not have any seizures. At birth it was noted that he had hypertelorism, which was in common with other family members. There were no neonatal complications. There were no parental concerns about development in infancy. He was crawling at nine months, and was using several single words at 13 months.

Shortly after MMR vaccination at 13 months, he began having epileptic spasms. These presented as clusters of episodes in which his head and arms would flex. He would have 10-20 spasms in a cluster and 5-10 clusters per day. Despite multiple therapeutic trials of AED treatment, the spasms were not controlled for five months. Spasms were eventually controlled when he was started on Vigabatrin. Once spasms were controlled he remained seizure-free. During the period of uncontrolled spasms, his family noticed a marked change in his development. He stopped talking and stopped taking an interest in his surroundings. He became irritable and slept a lot more than he had previously. EEG demonstrated bursts of high amplitude spike and wave and polyspike and wave activity over all areas at 1-2Hz as well as prolonged runs of generalised irregular slow spike/polyspike and wave. Once the spasms were controlled he began to gain skills again. He went on to attend mainstream school with learning support. He has difficulties with short-

term memory, attention and concentration. As a teenager he has been diagnosed with Asperger's syndrome.

There is an extensive family history of focal epilepsy and migraine with aura on his mother's side of the family, shown in Figure 6.3f and Table 6.3q.

Because of the extensive family history, variant filtering in family 361 represented a departure from the methodology used in the other families in this study. Three affected family members underwent WGS (GENIE-P-361, GENIE-G-361, and GENIE-E-361). Variants present in all three were further filtered on rarity (< 0.003 in gnomAD), and on whether they were either missense or truncating. A total of seven variants survived this filtering. DNA from subsequent family members, affected and not affected, was then sequenced using the Sanger method in order to isolate variants that were segregating with epilepsy within the family. Table 6.3p demonstrates the segregation of the seven candidate variants, with only the variant in *TRIO* demonstrating complete segregation among the first seven family members sequenced.

Table 6.3p: Candidate variant segregation in family 361

Family member	Affected (Y/N)	Gene and variant (Variant present Y/N)						
		BEGAIN V356L	*DYNC2H1* Frameshift	*EIF2D* M391V	*ITPKC* Y316N	*NPM1* Codon loss	*SREBF2* V369I	*TRIO* K2036N
P	Y	Y	Y	Y	Y	Y	Y	Y
E	Y	Y	Y	Y	Y	Y	Y	Y
G	Y	Y	Y	Y	Y	Y	Y	Y
C	N	Y	N	Y	Y	N	Y	N
D	N	Y	N	N	Y	Y	N	N
J	Y	Y	N	Y	Y	N	N	Y
R	Y	Y	N	Y	N	N	N	Y

Table 6.3q: Phenotypes and genotypes in family 361; colour coding Orange = genotype/phenotype present. Blue = genotype/phenotype absent

Family member	Current age (2018)	Phenotype	*TRIO* Genotype
A	18 years	No history of epilepsy or migraine	WT/WT
B	21 years	No history of epilepsy or migraine	Unknown
C	42 years	No history of epilepsy or migraine	WT/WT
D	47 years	No history of epilepsy or migraine	WT/WT
E	73 years	Focal seizures from aged 16 years. Intense déjà vu sensation and a burning rubber smell lasting 2-3 minutes, up to five times per day. Now controlled on Carbamazepine Interictal EEG (2008): Slow complexes occur over the left temporal area. These reverse in phase at the site of electrodes F7-T3 and can occur in the form of short runs MRI brain: normal	K2036N/WT
F	51 years	No history of epilepsy or migraine	WT/WT
G	45 years	Focal seizures from aged 13 years. Intense déjà vu sensation and a burning rubber. Currently seizure-free for 25 years and off medication Onset of migraine in her 40s. Begins with a "kaleidoscope" visual aura then progresses to severe headache. Takes regular Amitriptyline	K2036N/WT
H	70 years	No history of epilepsy or migraine	WT/WT
I	23 years	No history of epilepsy or migraine	Unknown
J	22 years	Recurrent episodes of absence status aged 12-13 years. Currently seizure-free off medication for 7 years	K2036N/WT
K	Died aged 66 years	Focal seizures from aged 15 years. Intense déjà vu episodes. EEG demonstrated focal sharp waves in the right mid and posterior temporal regions.	K2036N/WT
L	48 years	Focal seizures from aged 16 years. Intense déjà vu sensation and olfactory aura.	K2036N/WT
M	45 years	Focal seizures from aged 15 years. Intense déjà vu sensation and olfactory aura of burning rubber and tomato soup, progressing to a "feeling like [her] whole head was slowly turning all the way round and [she] couldn't control it". Frequent seizures like this as well as others with secondary generalisation to bilateral clonic movements. Currently on Carbamazepine and Phenobarbitone and seizure-free for 12 years. EEG demonstrates left frontal-temporal sharp waves. MRI brain normal.	K2036N/WT
N	Unknown	No history of epilepsy or migraine	Unknown
O	21 years	No history of epilepsy or migraine	Unknown
P	18 years	Infantile spasms and abnormal EEG. Asperger's syndrome	K2036N/WT
Q	Unknown	No history of epilepsy or migraine	Unknown
R	50 years	4 convulsive seizures in her late 20s. Seizure-free for 20 years on Lamotrigine	K2036N/WT

S	16 years	Hemiplegic migraines with visual flashing and right arm weakness from aged 17 years	K2036N/WT
T	48 years	Migraine from aged 11 years. Visual aura of flickering lights rapidly followed by severe headache lasting 24 hours. At worst 3-4/month	K2036N/WT
U	24 years	No history of epilepsy or migraine	Unknown
V	40 years	No history of epilepsy or migraine	WT/WT
W	Died aged 72 years	Generalised tonic-clonic seizures from aged 15 years. None since aged 18 years.	K2036N/WT
X	44 years	Onset of intense déjà vu sensation in her mid-teens, occasionally accompanied by a feeling as if she is “about to pass out” and a “tingly” fleeing in one or other hand. No EEG data available.	K2036N/WT
Y	Unknown	No history of epilepsy or migraine	Unknown
Z	23 years	No history of epilepsy or migraine	Unknown
α	20 years	Febrile convulsion aged 2 years. Single unprovoked GTCS aged 4 years. Migraine with visual aura from aged 9 years. Attacks currently occur twice per year.	WT/WT
β	21 years	Severe migraine attacks once per week from aged 5 years. No aura. Vomiting ++ and bilateral frontal headache. On Pizotifen. Attacks currently occur twice per year.	WT/WT

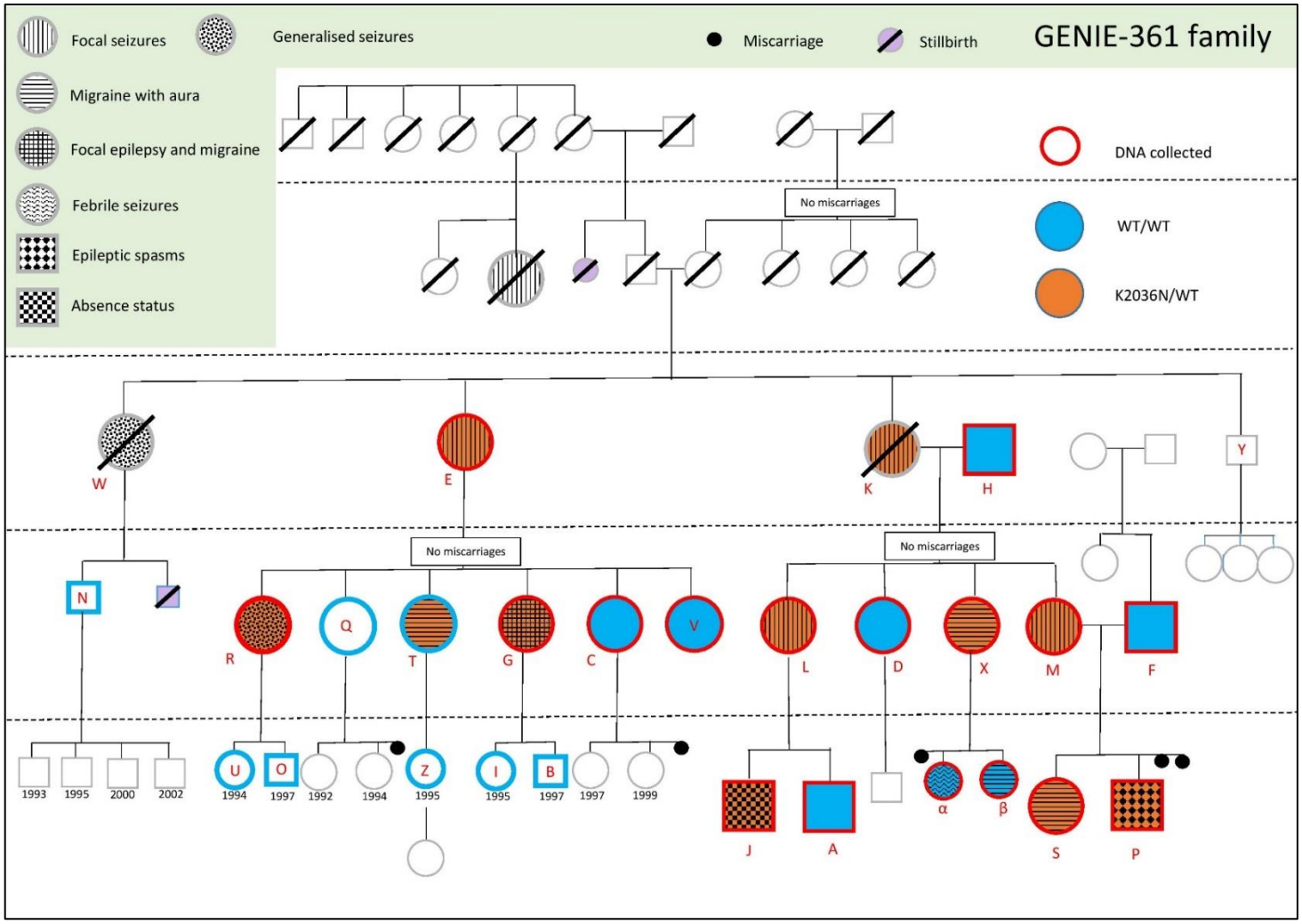


Figure 6.3f: Family 361 pedigree

Table 6.3r: *TRIO* variant in family 361

***TRIO* gene details**	
Function	Postulated to play a role in several signalling pathways that control cell proliferation (Ferraro et al., 2007).
Relative brain expression (GTEx ratio)	0.931
Missense constraint (ExAC)	z = 6.29
LoF constraint (ExAC)	pLI = 1.000000
Published cases of a similar phenotype	No, but is associated with intellectual disability (Pengelly et al., 2016)
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	Maternal in all affected individuals
Genomic location	Chr5(GRCh37): g.14477027G>C
HGVSc.	NM_007118.3(TRIO): c.6108G>C
HGVSp.	p.Lys2036Asn
Region of the protein affected	GEF2 domain (Katrancha et al., 2017)
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Tolerated (Score: 0.2)
Mutation Taster	Not available
Polyphen 2	0.863 (HumDiv) 0.565 (HumVar)
MDD conclusion	Strong candidate variant for the familial phenotype

TRIO encodes a Rho GTPase which has been shown in *Drosophila* to be involved in axon guidance and dendritic arborisation (Newsome et al., 2000; Iyer et al., 2012; Shivalkar; Giniger, 2012). Expression of *TRIO* in rat hippocampal cells is high in the early postnatal period but rapidly falls after birth, suggesting a role for *TRIO* in early neuronal development (Ba et al., 2016).

De novo variants in *TRIO* have been associated with intellectual disability, microcephaly, and subtle dysmorphic features (Ba et al., 2016; Pengelly et al., 2016) and with autism (Iossifov et al., 2014; Samocha et al., 2014). Two patients with epilepsy and *de novo TRIO* mutations have been reported in the literature. One was included in the series of six patients with intellectual disability by Pengelly et al. (Pengelly et al., 2016) – a nine year old female with developmental delay, gait ataxia and nocturnal tonic-clonic seizures (p.Asn1080Ile). The other was included in the Epi4K consortium paper of patients with Lennox-Gastaut Syndrome (p.Thr2945Met) (Allen et al., 2013). In the majority of published cases disease-associated *TRIO* variants are not associated with epilepsy. It is possible that different *TRIO* mutations have opposing functional effects.

Ba et al. investigated the functional consequence of *TRIO* inhibition (using Trio shRNA) in Pup hippocampal neurones and demonstrated significantly increased AMPAr-mediated but not NMDAr-mediated transmission compared with controls which would suggest that *TRIO* loss-of-function would predispose to reduced, not increased cortical excitability. The same finding was reproduced by Sadybekov et al. Using HEK293 cells they demonstrated that a truncating variant had identical functional consequences on AMPAr-mediated potentials as Trio shRNA (Sadybekov et al., 2017).

The variant in family 361 is predicted to disrupt an evolutionarily conserved Glu2033-Lys2036 hydrogen bond within the GEF2 domain of the protein, which might lead to destabilisation.

6.3.5.2 MED13

Male, born 2006. Phenotype: DRM + LKSS

P-597 was born by SVD at 35 weeks gestation following a pregnancy complicated by pre-eclampsia. Birth weight was 2600g (+ 0.25 SD). There were no neonatal complications. There were no concerns about his early childhood development. He began walking before his first birthday and had good speech by the age of four years. At the age of four years he had his first seizure. He was at nursery at the time. Nursery staff described whole body stiffening and four limb jerking, lasting several minutes. From then on he had frequent generalised myoclonic seizures, often causing him to fall. By the age of 4.5 years he was having hundreds of myoclonic and absence seizures per day, as well as up to 20 tonic-clonic convulsions. He lost ambulation and stopped learning and interacting. Seizures remained resistant to multiple therapeutic trials of AEDs, but dramatically responded to the Ketogenic Diet when introduced at the age of 5.5 years, which resulted in complete seizure freedom for eight months. He continues to have tonic seizures during sleep on most nights but all other seizure types have resolved. He stopped the Ketogenic Diet at the age of seven years but continues to take regular

Rufinamide and Topiramate. Since seizure control improved he has started regaining skills, and now attends main stream education with 1:1 learning support. EEGs have shown bilateral electrical status epilepticus in slow wave sleep (ESES), as well as frequent runs of bisynchronous spike/polyspike and wave activity during wakefulness, without clinical accompaniment. Serial MRI brain scans demonstrate a consistent area of abnormal cortical signal in the left occipital lobe. Since seizures and EEGs have always been consistent with a generalised epilepsy the significance of this is unclear. Height and head circumference are both within normal range. He has a younger half-brother to his father with language delay, but there is no other family history of developmental disorders.

Table 6.3s: *MED13* variant in P-597

***MED13* gene details**	
Function	Subunit of the large Mediator complex that functions with DNA-binding transcription factors and RNA polymerase II (Snijders Blok et al., 2018)
Relative brain expression (GTEx ratio)	0.591
Missense constraint (ExAC)	z = 1.18
LoF constraint (ExAC)	pLI = 1.000000
Published cases of a similar phenotype	No
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	*De novo*
Genomic location	Chr17(GRCh37): g.60088260G>T
HGVSc.	NM_005121.2(MED13) :c.1618C>A
HGVSp.	p.Pro540Thr
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Tolerated (Score: 1)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	0.999 (HumDiv) 0.915 (HumVar)
MDD conclusion	Strong candidate variant for the phenotype

Due to its high LoF constraint in ExAC, high Polyphen-2 score, and because it had arisen *de novo* this variant in *MED13* was considered a strong candidate as the cause of P-597's epilepsy. Two other patients with *de novo MED13* variants had been identified through the Deciphering Developmental Disorders (DDD) study with clinical and genotypic information deposited on the Decipher website (Firth et al., 2009), both of who had "abnormality of the nervous system" as a phenotypic feature. I made contact with the referrers of these cases and was subsequently put

in contact with a group in the Netherlands who were collecting genotypic and phenotypic data on patients with *de novo MED13* variants in order to characterise a new genetic syndrome. P-597 appears as patient H in their paper (Snijders Blok et al., 2018). 11 patients were described in total. Four had truncating variants, six had missense variants, and one had an in-frame deletion of a single amino acid. All 11 patients had cognitive impairment, ranging from borderline to moderate. Additional phenotypic features in this group were highly variable: two had Duane anomaly (a type of congenital strabismus), two had congenital cardiac anomalies, and three had hypotonia. Only P-597 had epilepsy.

The authors reported that the Pro540Thr variant identified in P-597 lies within a highly conserved linear motif and results in the high probability of formation of a Casein Kinase 1 phosphorylation motif. They hypothesised that this could lead to additional interaction with proteins containing forkhead domains (Dinkel et al., 2016).

6.3.5.3 POLR1A

Male, born 2013. Phenotype: DRM + IODEE + PMD

P-24 was born at term by planned caesarean section due to previous emergency caesarean section for placental abruption resulting in stillbirth. Birth weight was 2960g (- 1.08 SD). He was noted to be hypotonic from the first day of life and required supplementary nasogastric feeding for three weeks. Gross motor development was delayed. He first rolled over at 10 months and at the age of three years is currently unable to sit independently, has no pincer grasp, no expressive language and makes no response to verbal cues. His swallow is safe but his oral intake is poor so he receives supplemental nasogastric feeding. He has peripheral hypertonia in all four limbs and wears ankle food orthoses. He smiles and enjoys holding hands with others.

At the age of 12 months he began having clusters of epileptic spasms. Shortly after that he developed bilateral clonic seizures, generalised myoclonic seizures, and

absence seizures. He continued to have multiple seizures per day despite therapeutic trials of multiple AEDs. At the age of 3.5 years he was started on the Ketogenic Diet and the response was significant. He went from having >50 seizures per day to fewer than five.

As well as his epilepsy P-24 has episodes of whole body stiffening which have been captured on EEG and shown to be non-epileptic.

EEG during epileptic spasms demonstrated high amplitude bisynchronous spikes followed by suppression but not hypsarrhythmia. Background EEG is dominated by high voltage spike/sharp and slow waves. MRI brain at the age of 18 months demonstrated a symmetrical high T2 signal in the periventricular white matter around the posterior body and trigone of both lateral ventricles. MR spectroscopy was normal. He has short stature (86.7cm, -2.14 SD) and microcephaly (46.1cm, -2.32 SD). He has subtle dysmorphic features: hypertelorism, thin upper lip and carp-shaped mouth. His joints are hypermobile.

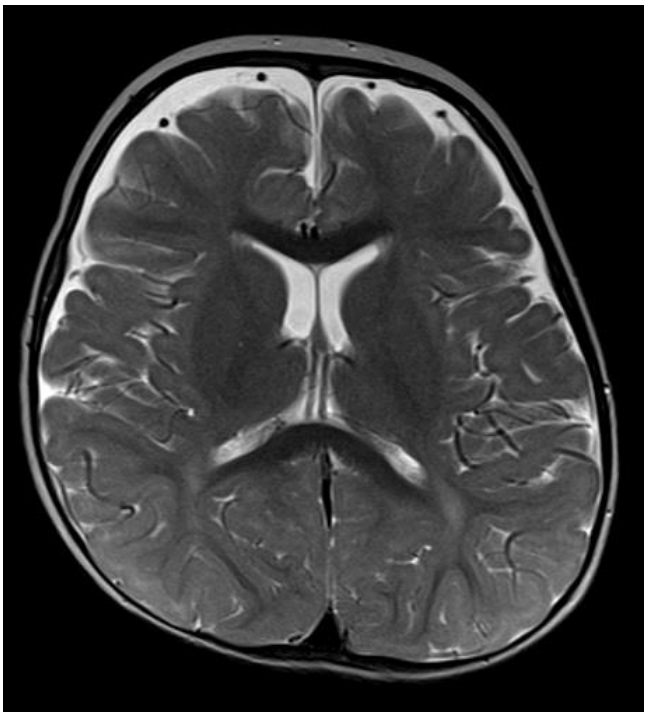


Figure 6.3d: T2-weighted axial MRI slice from P-24 showing high signal in the periventricular white matter

Table 6.3t: *POLR1A* variant in P-24

***POLR1A* gene details**	
Function	Catalyses DNA-dependent synthesis of ribosomal RNA (Weaver et al., 2015).
Relative brain expression (GTEx ratio)	0.668
Missense constraint (ExAC)	z = 3.00
LoF constraint (ExAC)	pLI = 0.999862
Published cases of a similar phenotype	No
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	*De novo*
Genomic location	Chr2(GRCh37): g.86257413C>A
HGVSc.	NM_015425.4(POLR1A): c.4685G>T
HGVSp.	p.Cys1562Phe
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Not available
Polyphen 2	0.999 (HumDiv) 0.977 (HumVar)
MDD conclusion	Strong candidate variant for the phenotype

A *de novo* missense variant in *POLR1A* was found. This was considered a strong candidate due to high Polyphen-2 score, absence from gnomAD, and significant constraint of variation in ExAC. However, *de novo* variants in *POLR1A* had already been published in association with an apparently quite different phenotype than that of P-24, involving severe mandibulofacial dysostosis and no epilepsy (Weaver et al., 2015). Using Gene Matcher (Sobreira et al., 2015). I was able to identify another patient in the USA with exactly the same variant as P-24, also arising *de novo*. This patient did not have acrofacial dysostosis, but presented with severe hypotonia in infancy, developed infantile spasms and had hypertelorism and flexion contractures of the limbs. That these two patients should have such similar phenotype, and one so different from other patients with *de novo POLR1A* variants is intriguing. *POLR1A* encodes a protein essential for ribosome biogenesis a ubiquitous process required by all cells in the body (Weaver et al., 2015). In theory distinct *POLR1A* missense alleles may uniquely alter lineage-specific translational regulation of protein expression, leading to tissue-specific phenotypes in affected individuals.

6.3.5.4 NIPBL

Female, born 2013. Phenotype: DRM

P-962 was born by SVD at term following an uncomplicated pregnancy with normal antenatal scans. Birth weight was 3500g (+ 0.3 SD). There were no parental concerns about development until prior to onset of seizures. At the age of 2.5 years she was in a supermarket café with her mother when both her arms stiffened, her eyes rolled back, her back extended and she became unresponsive. She remained like this for just over two minutes. She was then drowsy for a further 2.5 hours. Three days later she began having myoclonic jerks and four days after that she began having drop seizures. Tonic, atonic, and myoclonic seizures occurred every day for the next 21 months, despite trials of multiple AEDs, VNS, and the Ketogenic Diet. From the age of two years and nine months she also developed absence seizures, occurring 3-4 times per week. She first began having days without seizures at the age of four years and three months, which coincided with the parents starting her on a self-sourced Cannabinoid-based therapy. Though she continued to gain skills since onset of seizures, there was a slowing of her language development, reduced interaction with others, easy frustration, and clumsiness to her gait and fine motor skills. All of these improved when her seizure-control improved. She is due to start mainstream primary school. Formal neuropsychological assessment at the age of 3 years and 5 months using the Wechsler Preschool and Primary Scale of Intelligence – Third UK Edition (WPPSI-III) showed that her general intelligence scores fell within the average range.

EEGs demonstrate frequent generalised bursts of spike/polyspike wave interictally, and bisynchronous 2-3Hz spike wave activity during myoclonic seizures. MRI brain at the age of 2.5 years showed minimal ventricular asymmetry only.

At the age of 5 years she has short stature (height 98.4cm, - 2.56 SD) and microcephaly (OFC 49.0cm, -2.52 SD). There is no family history of epilepsy or other neurodevelopmental disorders.

Table 6.3u: *NIPBL* variant in P-962

***NIPBL* gene details**	
Function	Regulator of the cohesin complex (Watrin et al., 2006)
Relative brain expression (GTEx ratio)	0.521
Missense constraint (ExAC)	z = 5.04
LoF constraint (ExAC)	pLI = 1.000000
Published cases of a similar phenotype	One case reported with a *de novo NIPBL* variant epilepsy, and no features of Cornelia de Lange syndrome (Parenti et al., 2017)
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	*De novo*
Genomic location	Ch5 (GRCh37): g.37026336A>T
HGVSc.	NM_133433.3(NIPBL): c.5715A>T
HGVSp.	p.Lys1906Phe
Region of the protein affected	Between Heat Repeat 2 and Heat Repeat 3 (Selicorni et al., 2007)
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2	1.000 (HumDiv) 1.000 (HumVar)
MDD conclusion	Strong candidate variant for the phenotype

A *de novo* variant was found in *NIPBL*. Variants in this gene are associated with Cornelia de Lange syndrome (CdLS), a multisystem developmental disorder characterised by: microcephaly; pre- and postnatal growth restriction often associated with feeding difficulties and gastro-oesophageal reflux; characteristic facial appearance with fine arched eyebrows, synophrys, long philtrum, thin upper vermilion and low set posteriorly rotated ears; and variable presence of malformations (limb, cardiac, diaphragmatic, gastrointestinal and genitourinary) (Boyle et al., 2015). CdLS has been associated with variants in six genes (*NIPBL*, *SMC1A*, *SMC3*, *RAD21*, and *HDAC8*, and *BRD4*) all of which are structural components of, or regulators of the cohesin complex (Olley et al., 2018; Mannini et al., 2013) a structure which binds chromatin and plays a key role in transcriptional regulation (Mannini et al., 2015). Epilepsy prevalence in case series of CdLS ranges from 4% to 80%, but the epilepsy phenotype appears to be highly heterogenous (Pavlidis et al., 2014). In an Italian series of 62 patients with CdLS, 26 had *NIPBL* variants, of whom three had epilepsy (Selicorni et al., 2007). There is a single case in the literature of a patient with a *de novo NIPBL* variant (p.Ser2296Gly) and a non-CdLS phenotype. This is a German female of Turkish ancestry who presented

with clinical features of Coffin-Siris Syndrome (CSS), including coarse facies, frontal bossing, thick eyebrows, broad nasal tip, anteverted nares, wide mouth, thin upper lip, thick everted lower lip, and hypoplastic nails. This patient was reported to have seizures, but further details of the epilepsy phenotype were not described (Parenti et al., 2017). The only features of CSS or CdLS present in P-962 are short stature and microcephaly. Patients with variants in another CdLS-associated gene, *SMC1A* can present with severe epilepsy but no dysmorphisim (Symonds et al., 2017).

6.3.5.5 TRIM46

Male, born 2006. Phenotype: DRM and PMD

P-13 was born by SVD at term following an uncomplicated pregnancy. Birth weight was 3700g (+ 0.4 SD) and head circumference was 37.2cm at 12 days of age (+ 1.65 SD). His parents had no concerns about his early development.

He walked at 10 months, could ride a bike at three years, and could write his own name at four years. From the age of four years his language became less fluent and more disjointed, and from five years his rate of skill acquisition slowed down. Gradually he lost motor skills so that by the age of ten years he was unable to walk and could no longer use a pencil. His swallow became unsafe and he required salivary gland excision for excessive salivation.

His first seizure occurred at the age of seven years. His head turned to the left, and became locked in a tonic posture. His eyes were deviated upwards and to the left. The whole episode lasted 30-60 seconds. Subsequent seizures had the same semiology and duration. From the age of 10 years he developed generalised myoclonic seizures, which appeared to be triggered by bright lights. Seizures come in clusters. He can have months without any seizures then have several days of multiple seizures per day. He continues to have seizures despite AED polytherapy with Levetiracetam, Sodium Valproate, and Clobazam. Seizures appear to be frequently triggered by bright sunlight.

In addition to his seizures he also has an episodic non-epileptic movement disorder characterised by tremor and stiffening of the right leg. He has progressive pyramidal, extrapyramidal signs, and cerebellar ataxia.

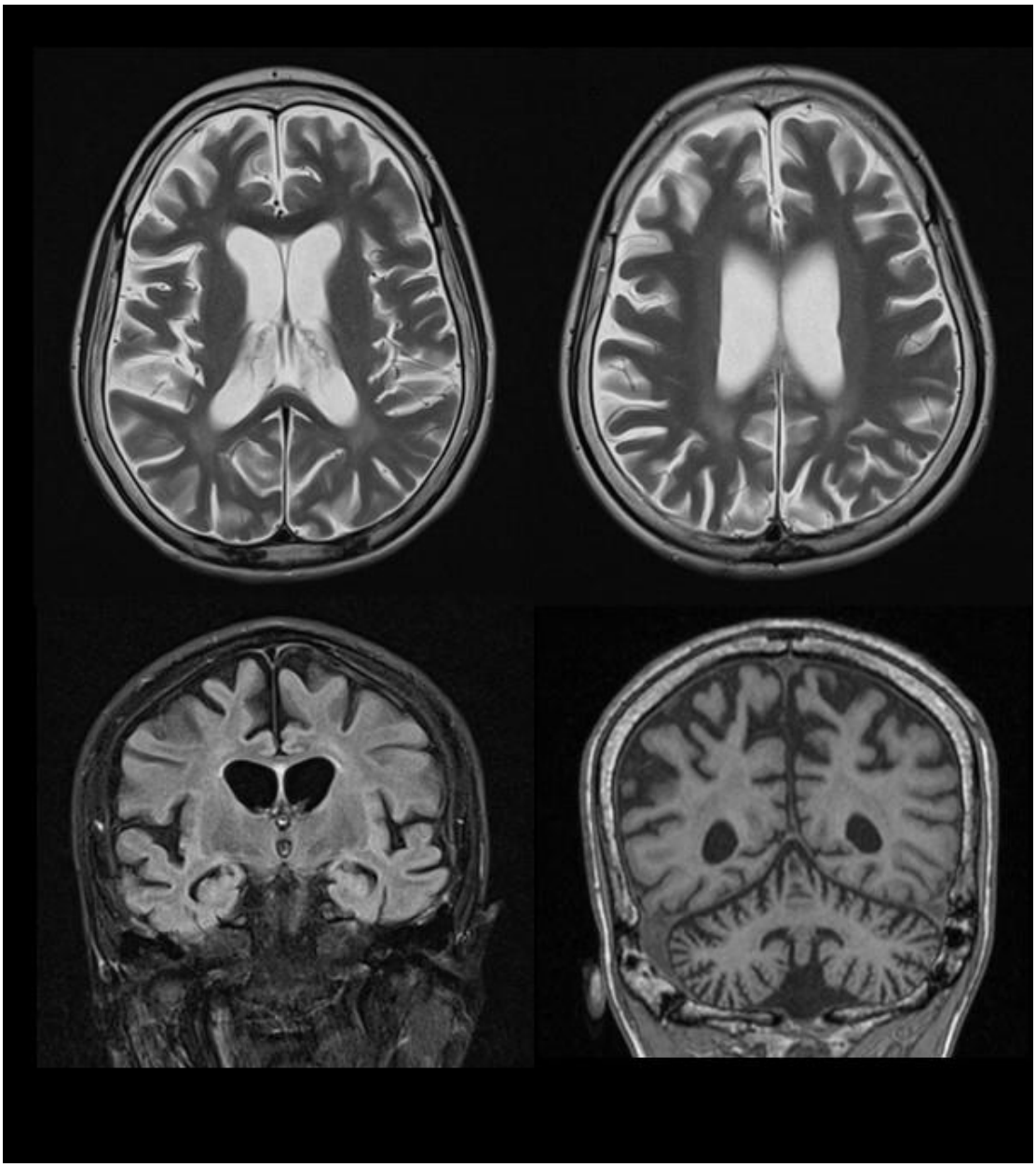


Figure 6.3d: MRI images from P-13 showing cerebral and cerebellar volume loss

EEGs have shown generalised abnormalities. Myoclonic seizures have been captured and are accompanied by generalised spike/polyspike and slow wave discharges. Interictally the EEG shows runs of bifrontal rhythmic slows, sometimes

with sharp wave accompaniment; and intermittent isolated bursts of generalised spike wave in the resting record and during photic stimulation. Visual Evoked Potentials (VEP), aged 11 years, had an amplitude at the upper range of normal. Electroretinogram (ERG) was present but at a subnormal amplitude.

MRI brain at seven years of age showed delayed myelination mild cerebral and cerebellar atrophy and increased peri-trigonal T2 signal. Repeat MRI at the age of 12 years demonstrated non-progression of the abnormal signal, but progressive atrophy of the deep white matter of the parietal lobes bilaterally.

At most recent assessment, aged 10 years, P-13 had normal height (141cm, -0.18 SD) but microcephaly (50.8cm, -2.39 SD). There was no family history of epilepsy or other neurodevelopmental disorders.

Table 6.3v: *TRIM46* variant in P-13

***TRIM46* gene details**	
Function	Required for neuronal polarity and axon specification (van Beuningen et al., 2015)
Relative brain expression (GTEx ratio)	8.421
Missense constraint (ExAC)	z = 3.87
LoF constraint (ExAC)	pLI = 0.998652
Published cases of a similar phenotype	No
Cases in public databases	No
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	*De novo*
Genomic location	Chr1(GRCh37): g.155152916C>T
HGVSc.	NM_001282379.1(TRIM46): c.1594C>T
HGVSp.	p.Gln532*
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Strong candidate variant for the phenotype

There was a *de novo* nonsense variant in *TRIM46*. This gene has high expression in brain compared with other tissues. TRIM46 protein localises to the proximal axon where it is involved in microtubule binding. Knockout of *TRIM46* results in disorganised microtubule binding (van Beuningen et al., 2015). No truncating variants in this gene are observed among the >60,000 individuals in the ExAC dataset. Recently antibodies to TRIM46 have been reported in three patients with small-cell lung cancer and paraneoplastic neurological syndromes (van

Coevorden-Hameete et al., 2017). Affected individuals presented with gait disturbance, focal seizures, cerebellar signs, and cognitive decline.

6.3.5.6 LRP8

Male, born 2002. Phenotype: DRM

P-958 was born by elective caesarean section at term following an uncomplicated pregnancy. His mother was taking the antidepressants Venflafaxine and Fluoxetine for the first 12 weeks of pregnancy. Birth weight was 4200g (+ 1.4 SD). There were no neonatal complications. His parents had no concerns about his early development. He crawled at 12 months, walked at 16 months, and could speak in sentences by the age of three years.

His first seizure was at 3.5 years of age. His mother heard a gurgling noise, and found him grinding his teeth and jaw, eyes rolled back, and unresponsive for two minutes. He went on to have frequent focal seizures, characterised by loss of contact, and eye deviation upwards and to the right. Occasionally seizures would progress to involve clonic jerking of all four limbs. He went on to develop multiple seizure types, including tonic-clonic seizures without focal onset, generalised myoclonic seizures, drop attacks, tonic seizures, typical absence seizures. At most recent assessment, aged 14 years, he was having 1-2 nocturnal bilateral clonic seizures per night, and focal seizures with right eye deviation every day. Seizures have been resistant to multiple AEDs. Introduction of the Ketogenic Diet was associated with cessation of drop attacks, and a reduction in frequency of myoclonic seizures. Vagal Nerve Stimulation was associated with reduced frequency of daytime seizures. When he was tried on Carbamazepine all seizure types became more frequent.

Onset of seizures was associated with a marked change in his developmental trajectory, and particularly with a change in his social communication skills. At the age of 14 years his drawing skills are the level of a seven year old. He communicates verbally but with the language skills of a five year old. He was

diagnosed with autism at the age of 10 years. EEGs have always been abnormal. When absence seizures have been captured EEG shows 1.5-2.5 per second slow spike and wave, which has been more pronounced over the left hemisphere at times. Interictal EEGs are poorly organised and slow. MRI brain at the age of four years was normal. Height and head circumference at the age of 14 years are within normal range.

There is no family history of epilepsy. His mother has a history of depression, his mother's father has autism spectrum disorder, and his mother's father's sister had schizophrenia.

Table 6.3v: *LRP8* variants in P-958

***LRP8* gene details**	
Function	Encodes the Reelin receptor, required for developmental layering of neurones within the cortex (Trommsdorff et al., 1999)
Relative brain expression (GTEx ratio)	2.353
Missense constraint (ExAC)	z = 2.77
LoF constraint (ExAC)	pLI = 0.999986
Published cases of a similar phenotype	No
Cases in public databases	No
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant 1 details	
Inheritance	Paternal
Genomic location	Chr1(GRCh37): g.53736706C>A
HGVSc.	NM_004631.4(LRP8):c.1246G>T
HGVSp.	p.Ala416Ser
Population frequency	0.00005772 in gnomAD. Not in UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2 (HumVar)	0.9992 (HumDiv) 0.998 (HumVar)
Variant 2 details	
Inheritance	Maternal
Genomic location	Chr1(GRCh37): g.53741411G>A
HGVSc.	NM_004631.4(LRP8):c.898C>T
HGVSp.	p.Arg300Cys
Population frequency	0.000004065 in gnomAD, Not in UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Disease causing (p-value: 1)
Polyphen 2 (HumVar)	0.887 (HumDiv) 0.849 (HumVar)
MDD conclusion	Strong candidate variants

Compound heterozygous variants missense were identified in *LRP8*. Based on GTEx ratio and pLI score this was considered a candidate gene. Both variants were considered candidates due to their rarity (>0.001 in gnomAD) and predicated deleteriousness (>0.8 Polyphen Score).

LRP8 encodes a cortical reelin receptor. Reelin is, encoded by *RELN*, is a large secreted glycoprotein which is produced by cells within the developing brain. Reelin activates a signalling pathway in postmitotic migrating neurons, which is essential for positioning of neurons within laminated nervous system parenchyma (Maha et al., 2007). Biallelic loss of function *RELN* variants are associated with lissencephaly in humans. Lissencephaly is a developmental cortical malformation syndrome resulting in a smooth appearance to the brain surface, and is associated with drug-resistant epilepsy and cognitive impairment (Hong et al., 2000). Additionally, heterozygous missense variants in *RELN* have been associated with dominantly-inherited familial lateral temporal lobe epilepsy (Dazzo et al., 2015). In a mouse model, biallelic knockout of *LRP8* does not result in a detectable phenotype, unless combined with knockout of *VLDLR* which encodes another cortical Reelin receptor. Knockout of both *LRP8* and *VLDLR* results in a clinical and neuropathological phenotype which mimicks that of the biallelic *RELN* knockout mouse (Trommsdorff et al., 1999).

6.3.5.7 CNTNAP1

Female, born 2014. Phenotype: DRM + IODEE

P-772 was born at 37 weeks by emergency caesarean section following induction of labour for polyhydramnios. Antenatal scans had been otherwise normal. Birth weight was 3130g (- 0.49 SD). She was in poor condition at birth requiring bag and mask ventilation for poor respiratory effort. She was intubated and ventilated from 20 minutes of age, due to severe respiratory distress. Her neonatal course was complicated by an episode of severe E. coli sepsis, pharyngomalacia, for which she had a tracheostomy at five weeks of age, and severe gastroesophageal reflux for

which she had gastrostomy and fundoplication at three months of age. She was discharged home from neonatal care at the age of six months. She has been able to self-ventilate via tracheostomy.

From birth she was noted to be hypotonic. She had an extended posture to her spine, a flexed posture to her wrists, and made very little spontaneous movement. She had bulbar and facial palsies bilaterally, exhibiting no facial movements and having no gag reflex. Facial features noted from early infancy were micrognathia, bilateral ptosis, and an upturned nose. She had investigations based on a suspicion of congenital myopathy. She had a normal creatine kinase level and negative genetic tests for spinal muscular atrophy, congenital myotonic dystrophy, and pyruvate dehydrogenase deficiency.

She began having epileptic spasms at two months of age. These involved symmetrical flexion at the elbows and eye deviation. The spasms were captured on EEG and were accompanied by bilateral suppressions followed by runs of right-sided sharp waves. The EEG background did not initially show hypsarrhythmia but progressively deteriorated over time and showed hypsarrhythmia by the age of three years. Epileptic spasms were resistant to multiple AEDs. She also developed generalised myoclonic seizures. Both Topiramate and Levetiracetam was associated with a reduction in seizure frequency.

Serial MRI brain scans at 12 days, one month, and two months, showed progressive cortical volume loss and delayed myelination of the cerebral and cerebellar hemispheres.

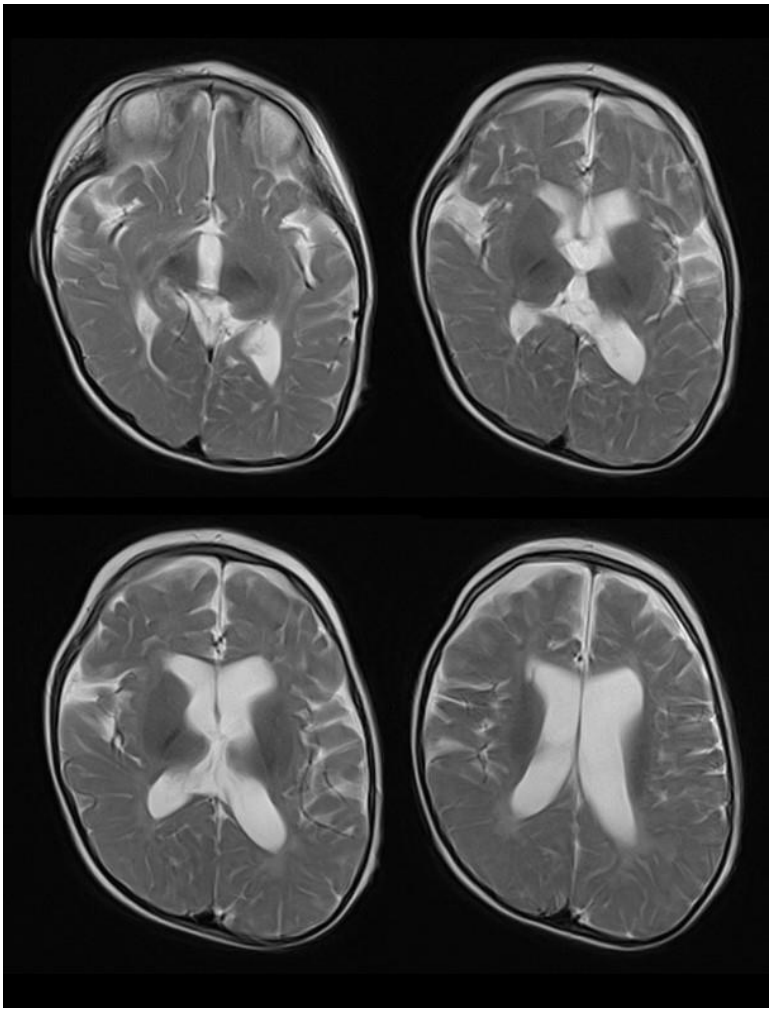


Figure 6.3e: MRI scan (aged 2 months) from P-772 showing cerebral atrophy and hypomyelination

At the age of four years she has profound developmental delay. She is unable to sit independently, unable to swallow, and has no verbal or non-verbal communication. She has cerebral visual impairment. Head circumference and height are within normal range. There is no family history of epilepsy or other neurodevelopmental disorders.

Table 6.3x: *CNTNAP1* variants in P-772

***CNTNAP1* gene details**	
Function	Component of complex at neuronal paranodal complex, required for high velocity nerve conduction (Bhat et al., 2001)
Relative brain expression (GTEx ratio)	4.184
Missense constraint (ExAC)	z = 4.50
LoF constraint (ExAC)	pLI = 0.004109
Published cases of a similar phenotype	Yes. Similar in other phenotypic features, but none previously reported with epilepsy (Low et al., 2018; Nizon et al., 2016; Laquérriere et al., 2014)
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant 1 details	
Inheritance	Paternal
Genomic location	Chr17 (GRCh37): g.40838099C>A
HGVSc.	NM_003632.2(CNTNAP1): c.840C>A
HGVSp.	p.Asp280Glu
Population frequency	0.00001624 in gnomAD. Not in UK10K
Previously reported	No
SIFT	Deleterious (Score: 0)
Mutation Taster	Polymorphism (p-value: 0.686)
Polyphen 2 (HumVar)	1.00
Splicing predications	Predicated new splice acceptor site
Variant 2 details	
Inheritance	Maternal
Genomic location	Chr17 (GRCh37): g. 40842225-229 GGTAA>G
HGVSc.	NM_003632.2(CNTNAP1) :c.1855+3_1855+6del
HGVSp.	N/A - intronic
Population frequency	Not in gnonmAD or UK10K
Previously reported	No
Splicing predictions	Predicted loss of splice donor site
MDD conclusion	Causative of the phenotype

Biallelic variants in *CNTNAP1* were identified, both of which were predicated to have an impact on splicing.

CNTNAP1 encodes caspr, an essential component of a multiprotein complex which locates to the nodes of Ranvier in myelinated nerve cells (Laquérriere et al., 2014). In a homozygous knockout mouse model, lack of caspr results in a phenotype of hypomotility, tremor, wide-based gait, and generalised motor paresis (Bhat et al., 2001). In humans, biallelic frameshift variants were first described by Laquerriere et al. They reported seven patients, from four unrelated families. Affected individuals present with severe congenital arthrogryposis and a pronounced motor neuropathy. All seven reported patients died in the first three months of life (Laquérriere et al., 2014). Nizon et al. subsequently described two

siblings with homozygous nonsense variants who had a very similar clinical picture but without arthrogryposis. Sural nerve biopsy demonstrated peripheral nerve hypomyelination (Nizon et al., 2016). Features common to all nine patients were polyhydramnios, severe neonatal hypotonia, facial diplegia, and absence of spontaneous swallowing or breathing.

Subsequently, a non-lethal form of recessive *CNTNAP1*-related disease was described by Low et al. Unlike the previously reported variants some of these were missense variants. All patients had a severe neurological phenotype, including orobulbar dysfunction, facial nerve weakness, vocal cord paresis, severe gastroeosophageal reflux, brain hypomyelination, and severe developmental delay. The patients with missense variants had a milder phenotype, with survival up to 15 years observed. The authors proposed that hypomorphic missense variants partially ameliorate the phenotype (Low et al., 2018).

The only reported patients with *CNTNAP1* variants and seizures are among seven patients from two families reported by Hengel et al. In one of these families, a large consanguineous Palestinian pedigree carrying a nonsense variant, two of three affected individuals survived to nine and 12 years old and had developed tonic-clonic seizures. In the second family, an Irish family in which affected individuals were compound heterozygous for one nonsense and one missense variant, one of two affected individuals developed clonic, brief tonic, and myoclonic seizures from the age of 10 weeks, and died at the age of four months (Hengel et al., 2017).

6.3.5.8 MAP2.

Male, born 2014. Phenotype: DRM.

P-278 was born by SVD at term weighing 4250g (+ 1.5 SD). There had been no concerns during the pregnancy, with normal routine fetal ultrasound and normal fetal movements reported. He was discharged home on day 1 of life. There were no concerns about his early infantile development, but by 12 months of age his mother had concerns about his motor development because he was unable to sit unsupported. He made developmental progress in other domains. By the age of 2 years he was able to scribble and had several single words, including “Daddy” “Hiya” and “Bye”. His started having seizures at the age of 22 months. These involved rapid jerks of both arms and both legs with rapid drops of the head. These occurred very frequently (up 100 per day). EEG during these events demonstrated bursts of bisynchronous frontally-predominant polyspike wave. He was started on Levetiracetam, which was associated with a reduction in seizure frequency to 20 per day, but was also associated with a change in mood and cessation of verbal communication. His treatment was changed to Sodium Valproate and he continued to have about 20 myoclonic seizures per day, but regained his words. From the age of 35 months he began having episodes every few weeks where he would become unresponsive for about 30-60 seconds, then giggle for a couple of seconds, then continue what he was doing.

MRI brain scan at the age of 30 months was normal. At the age of 4 years he has global developmental delay. He is unable to sit independently, he can transfer toys between hands and scribble with a pencil but not draw anything, he has single word language and appears to follow conversations. He is content and sociable and enjoys giving “High 5’s”. On examination OFC is 46.9cm (-2.67 SD) and height is 89.4cm (-1.56 SD). He has bilateral symmetrical lower limb hypertonia, with hyperreflexia and clonus. He has bilateral choroidoretinopathy.

Table 6.3y: *MAP2* variant in P-278

***MAP2* gene details**	
Function	Stimulates microtubule proliferation in dendrites (Fontaine-Lenoir et al., 2006)
Relative brain expression (GTEx ratio)	22.7
Missense constraint (ExAC)	z = 0.36
LoF constraint (ExAC)	pLI = 0.9999833
Published cases of a similar phenotype	None
Cases in public databases	4 in Decipher – all with abnormality of the nervous system
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	*De novo*
Genomic location	Chr2 (GRCh37): g.210565051 G>A
HGVSc.	NM_002374.3(MAP2): c.4573G>A
HGVSp.	p.Asp1525Asn
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (score 0.02)
Mutation Taster	Disease causing (prob: 0.997)
Polyphen 2	0.998 (HumDiv), 0.859 (HumVar)
MDD conclusion	Strong candidate variant

P-278 has a *de novo* variant in *MAP2*. *MAP2* is highly expressed in the neuronal dendrites and encodes a 280-kD protein, MAP2. *In vitro* experiments in rat brain samples demonstrate that MAP2 is a receptor for the neurosteroid pregnenolone. Decreasing *MAP2* expression results in loss of the stimulatory effects of pregnenolone on neurite extension (Fontaine-Lenoir et al., 2006). There are no published reports of human disease in association with variants in *MAP2*. However, I identified four patients from the Deciphering Developmental Disorders (DDD) study with *de novo* variants in this gene, all of whom had a phenotype of "abnormality of the nervous system" (Firth et al., 2009).

In addition to the *MAP2* variant, P-278 also has a maternally inherited nonsense variant (p.Arg581*) in *SPAST*, a gene associated with hereditary spastic paraparesis (HSP) (Hazan et al., 1999). Most HSP-related *SPAST* variants are truncating. The phenotype presents with progressive lower limb spasticity and weakness in the absence of other neurological signs or symptoms. First symptoms can present at any age between the first year of life and 77 years of age (mean = 28.7 years), and penetrance is not complete in that a minority mutation carriers, identified through family studies, are asymptomatic. Penetrance is lower in females (0.88) than in

males (0.94) (Parodi et al., 2018). It is possible that the lower limb hypertonia observed in P-278 is related to this *SPAST* variant, and that his mother is currently asymptomatic for this. It is unlikely that the other phenotypic features observed in P-278 are related to the *SPAST* variant since epilepsy, developmental delay, and choroidoretinopathy are not reported features of this condition, nor are they present in his mother.

6.3.5.9 RAS10LB

Male, born 2014. Phenotype: IODEE

P-621 was born by SVD at 39 weeks gestation. There were no concerns prior to birth in relation to fetal movements or ultrasound scans. Birth weight was 2500g (-1.99 SD) and head circumference was 29.3cm (-3.61 SD). At birth he was found to have an everted left ear and absent 12th ribs. Newborn hearing screening (auditory brainstem responses) demonstrated bilateral sensorineural hearing loss. He had some respiratory distress at birth and was treated with oxygen for 24 hours. He struggled to feed and required supplemental nasogastric feeding for the first 6 weeks of life.

He presented with epileptic spasms at the age of 17 months. These presented as clusters of 3-4 spasms once or twice per day. After 2 weeks of these events he had an EEG which demonstrated modified hypsarrhythmia (Figure 6.3h). He was started on Vigabatrin and the spasms stopped after 5 days. After 3 months of treatment the Vigabatrin was stopped. He remained seizure-free until the age of 25 months when he had a single cluster of spasms associated with intercurrent illness. MRI brain scans done at 1 year of age and 3 years of age demonstrated a structurally normal brain.

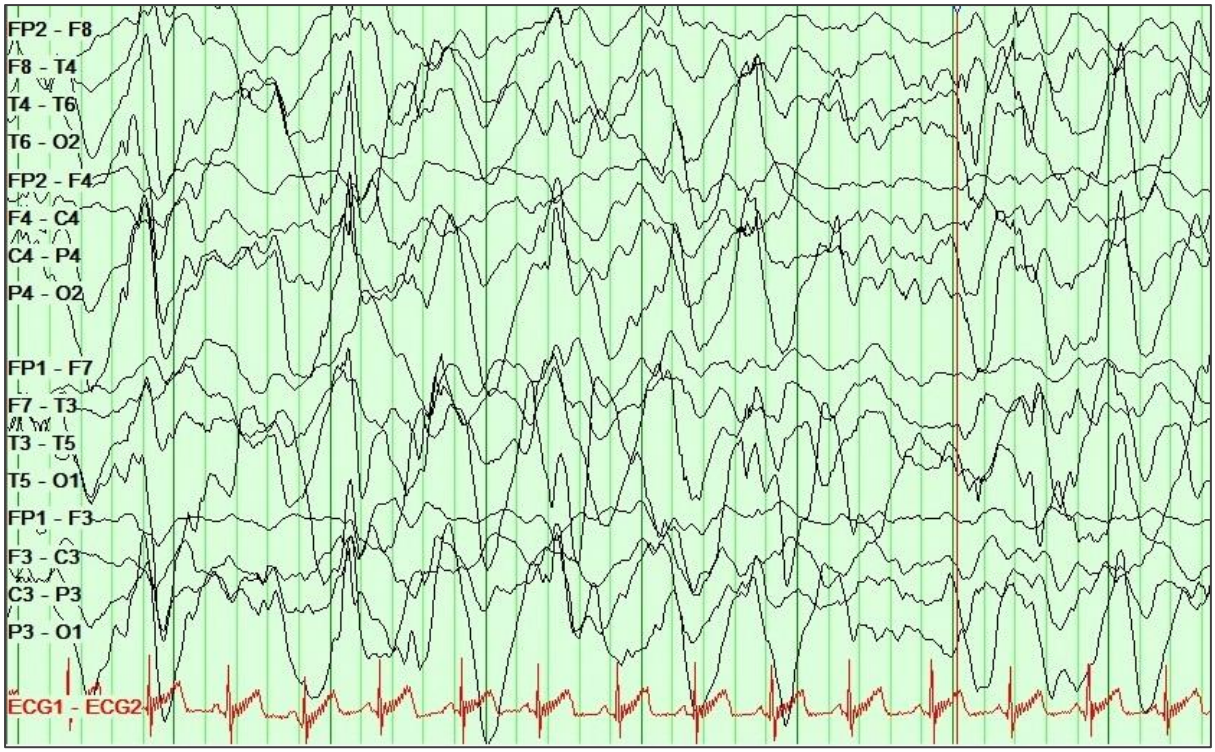


Figure 6.3h: EEG in P-621 showing modified hypsarrhythmia

At the age of 3 years he has global developmental delay. He has hypotonia, and has never been able to sit without support. He can hold a toy in a palmar grasp but cannot transfer toys or use a pincer grasp. He is non-verbal and responds only to his name. He enjoys music and makes attempts to sing. Head circumference is 42.5cm (-4.8 SD), height is 78.9cm (-3.98 SD), and weight is 10.1kg (-2.46 SD). There is no family history of epilepsy or other neurodevelopmental disorder.

Table 6.3z: *RASL10B* variant in P-621

***RASL10B* gene details**	
Function	Regulator of dense-core vesicle secretion. Biallelic knockout mice are viable and fertile but have small atrial cardiomyocytes and high blood pressure (Rybkin et al., 2007)
Relative brain expression (GTEx ratio)	5.52
Missense constraint (ExAC)	z = 3.14
LoF constraint (ExAC)	pLI = 0.03679261
Published cases of a similar phenotype	None
Cases in public databases	None
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	*De novo*
Genomic location	Chr17 (GRCh37): g. 34067458 C>T
HGVSc.	NM_033315.3 (RASL10B): c.247C>T
HGVSp.	p.Arg83Trp
Population frequency	Not in gnomAD or UK10K
Previously reported	No
SIFT	Deleterious (score: 0)
Mutation Taster	Disease causing (prob:1)
Polyphen 2	1.00 (HumDiv) 0.999 (HumVar)
MDD conclusion	Strong candidate variant

There was a *de novo* variant in *RASL10B,* a that has high brain expression and encodes the protein Ras-related protein 7, RRP7. RRP7 interacts with the C-terminal region of CAPS1, a regulator of vesicle secretion. The role of RRP7 in neurones has not been studied. In rat cardiomyocytes increased *RASL10B* expression results in elevated levels of atrial natriuretic peptide (Rybkin et al., 2007).

6.3.5.10 CACNA1G

Male, born 2016. Phenotype: IODEE

P-722 was born by SVD at term weighing 3410g (-0.18 SD) and with a head circumference of 35.5cm (+0.37 SD). There were no antenatal concerns in relation to routine ultrasound scans or fetal movements. On day 2 of life he was reviewed for poor feeding and was found to have central hypotonia. He was admitted to the neonatal unit for feeding support and supplemental oxygen for 2 weeks. At 3 months of age he presented with clusters of seizures characterised by symmetrical

extensor spasms of the arms, eye opening and staring, going red in the face, and trembling at the jaw.

EEG during these demonstrated a build-up of rhythmic slow waves with intermixed irregular polyfocal spikes (Figure 6.3i). Seizures continued despite treatment with initially Vigabatrin then Carbamazepine, then Levetiracetam.

MRI brain at the age of 5 months demonstrated a structurally normal brain with some enlargement of the subarachnoid spaces.

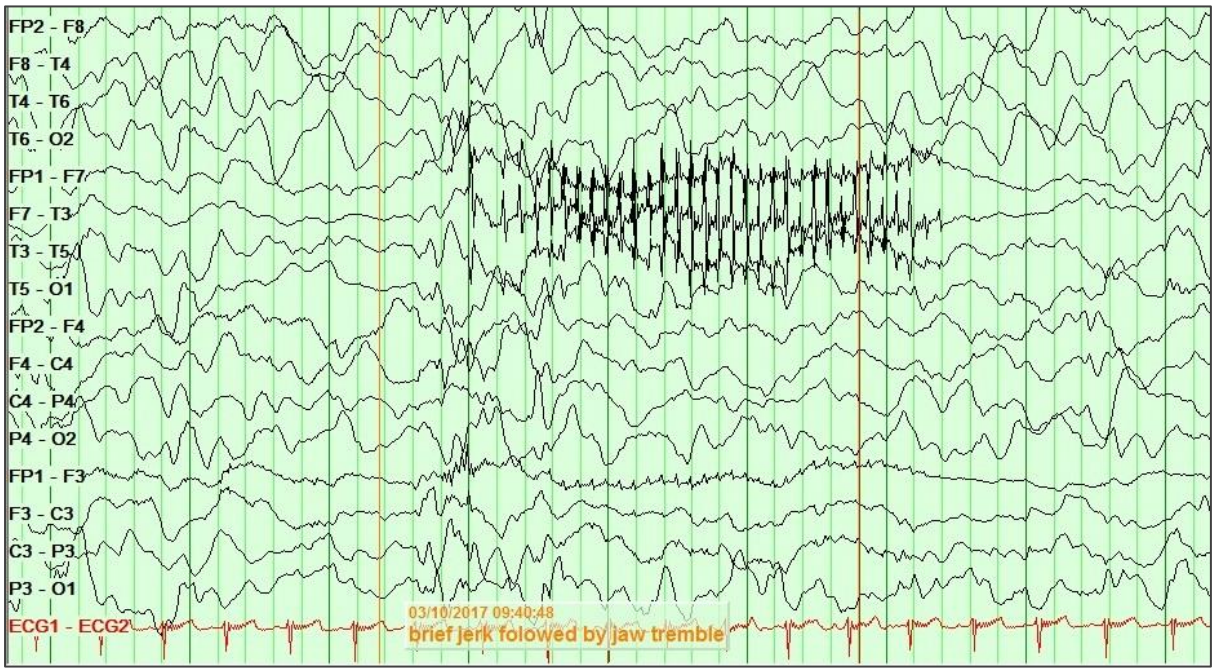


Figure 6.3i: Ictal EEG in P-722, showing irregular polyfocal spikes and muscle artefact of jaw trembling

At the age of 14 months he has global developmental delay and generalised hypotonia. He is unable to roll or sit. His hands come to the midline but he is unable to hold a toy. He responds to verbal cues with laughs and giggles, but has no words. His height is 72.4cm (-0.39 SD) and his head circumference is 45.2cm (-0.44 SD). There is no family history of epilepsy or other neurodevelopmental disorder.

Table 6.3α: *CACNA1G* variant in P-722

***CACNA1G* gene details**	
Function	Subunit of T-type voltage-gated calcium channel
Relative brain expression (GTEx ratio)	4.71
Missense constraint (ExAC)	z = 4.97
LoF constraint (ExAC)	pLI = 0.999997
Published cases of a similar phenotype	4 patients reported with *de novo* missense variants, presenting with cerebellar hypoplasia/atrophy, developmental delay, and hand/digital anomalies. 2/4 had neonatal-onset seizures (Chemin et al., 2018).
Cases in public databases	None
Frequency in previous NGS studies (Chapter 3)	Not reported
Variant details	
Inheritance	De novo
Genomic location	Chr17 (GRCh37): g. 48695666 C>T
HGVSc.	NM_018896.4 (CACNA1G): c. 5389C>T
HGVSp.	p.Arg1797*
Population frequency	Not in gnomAD or UK10K
Previously reported	No
MDD conclusion	Strong candidate variant for the phenotype

There was a *de novo* nonsense variant in the *CACNA1G* gene. This gene encodes a subunit of the neuronal T-type calcium channel. T-type channels are thought to be involved in pacemaker activity, low-threshold calcium spikes, neuronal oscillations and resonance, and rebound burst firing (Perez-Reyes et al., 1998). *De novo* missense variants in *CACNA1G* have been reported in 4 individuals presenting with childhood-onset cerebellar atrophy. All 4 had severe or profound intellectual disability and hypotonia. 2/4 presented with seizures in the first 2 weeks of life and were described as having an epileptic encephalopathy, whilst the other 2 had not had any seizures reported by the time of most recent follow up (8 years and 11.5 years). MRI scans in all 4 demonstrated cerebellar atrophy but with a normal cerebral cortex and normal brainstem. All 4 had anomalies involving the hands, including clinodactyly, short hands and feet, syndactyly, and broad thumbs. Functional characterisation of 2 of these missense variants was carried out by transfecting the variants into human embryonic kidney cells (HEK-293T) and using patch clamp current recording techniques. Both variants impaired channel inactivation, and effect that was fully-blocked by a selective T-type calcium channel blocker TTA-P2 (Chemin et al., 2018).

Truncating variants in *CACNA1G* have not been reported in association with neurological disease, but since these variants are heavily constrained in ExAC (pLI = 1.00), the nonsense variant identified in P-722 is a strong candidate cause. In contrast the published gain-of-function variants reported by Chemin et al. it is likely that calcium channel blocking medications, including the anti-epileptic drugs Topiramate, Ethosuximide, Zonisamide, and Acetazolamide, may exacerbate symptoms by further impairing calcium channel function, though this would have to be tested experimentally.

6.3.6 Associations between phenotypic features and the identification of diagnostic/strong candidate findings

6.3.6.1 Age of first seizure presentation

In order to assess whether age of first seizure presentation was associated with the finding of a diagnostic or candidate genetic variant, I performed two sample t-tests comparing the mean age of presentation between those families with diagnostic/candidate findings and those without. There were no significant differences between the groups.

Table 6.3Β: Differences between mean age at first seizure between groups with and without diagnostic/candidate variants in the WGS cohort (2-tailed t-test)

Group	Number	Mean age at first seizure	P-value
Diagnostic finding	11	11.2 months	0.17 (n.s.)
No diagnostic finding	61	22.4 months	
Diagnostic or candidate finding	21	17.2 months	0.44 (n.s.)
No diagnostic or candidate finding	51	22.1 months	

6.3.6.2 Family history scores

I wanted to test a hypothesis that patients without *de novo* causative or candidate variant would have higher family history scores than those with a *de novo* causative or candidate variant, since this would suggest that these patients had a more significant polygenic contribution to their epilepsy. The mean family history

score was higher in both the group without a *de novo* diagnostic variant and the group without a *de novo* diagnostic or candidate variant, but neither of these differences reached statistical significance. It is possible that this study was underpowered to detect significant differences. It would be interesting to repeat this analysis with a larger sample.

Table 6.3γ: Differences between mean Family History Scores between group with and without diagnostic/candidate variants in the WGS cohort (2-tailed t-test)

Group	Number	Mean Family History score	P-value
de novo Diagnostic finding	8	23	0.46 (n.s.)
No *de novo* diagnostic finding	64	28.2	
de novo Diagnostic or candidate finding	15	11.4	0.08 (n.s.)
No *de novo* diagnostic or candidate finding	57	31.1	

6.3.6.3 Phenotypic features

Table 6.3Δ reports the % of patients who had a diagnostic or candidate genetic variant according to the presence or absence of clinical features. The only clinical features that were significantly associated with the presence of either a diagnostic or a candidate variant were: i) the presence of moderate to profound cognitive impairment; and ii) the presence of microcephaly (>2 SD below the mean).

Table 6.3Δ: Associations between phenotypic features and diagnostic or candidate findings in the WGS cohort

Feature	Total number with feature	Number with diagnostic variant	% with diagnostic variant	p-value (Fisher's exact)	Number with diagnostic or candidate variant	% with diagnostic or candidate variant	p-value (Fisher's exact)
Seizure types							
Myoclonic	37	3	8.1%	>0.05	10	27.0%	>0.05
Generalised clonic/tonic-clonic	31	4	12.9%	>0.05	7	22.6%	>0.05
Focal	39	8	20.5%	>0.05	12	30.8%	>0.05
Drop	22	1	4.5%	>0.05	4	18.2%	>0.05
Absence	21	2	9.5%	>0.05	7	33.3%	>0.05
Atypical absence	16	2	12.5%	>0.05	5	31.2%	>0.05
Tonic	20	5	25.0%	>0.05	8	40.0%	>0.05
Spasms	27	5	18.5%	>0.05	10	37.0%	>0.05
Status	20	4	20.0%	>0.05	5	25.0%	>0.05
Cognitive impairment							
Mild-normal	29	0	0.0%	0.0072*	3	10.3%	<0.001*
Moderate-profound	42	10	23.8%	0.017*	17	40.5%	<0.001*
Unknown	1	1	100.0%	>0.05	1	100.0%	>0.05
Additional features							
Autistic features	31	6	19.3%	>0.05	8	25.8%	>0.05
Drug-resistant seizures	53	8	15.1%	>0.05	15	28.3%	>0.05
Microcephaly (<2 SD below mean)	16	4	25.0%	>0.05	9	56.3%	0.012*
Any abnormality on MRI scan	23	4	17.4%	>0.05	8	34.8%	>0.05
Any organ anomaly or deformity	15	3	20.0%	>0.05	5	33.3%	>0.05
Phenotype group							
DRM	33	3	9.1%	>0.05	10	30.3%	>0.05
IODEE	34	8	23.5%	>0.05	13	38.2%	>0.05
LKSS	34	4	11.8%	>0.05	5	14.7%	>0.05
PMD	12	2	16.7%	>0.05	2	16.7%	>0.05

6.4 Discussion

In this chapter I have investigated the utility of Whole Genome Sequencing (WGS) in a cohort of patients from 72 families. These patients presented with a broad range of epilepsy phenotypes, but all could be considered to fall into at least one of four phenotype categories, namely drug-resistant myoclonic epilepsy, infantile-onset developmental and epileptic encephalopathy, Landau-Kleffner spectrum, and comorbid paroxysmal movement disorder. Despite the WGS platform being significantly larger than the 104 gene panel described in chapter 5, the yield of diagnostic results from WGS was significantly lower than was obtained from panel testing in the unselected < 3 years cohorts. From WGS, 11/72 (15.3%) families had a diagnostic result. This relatively low yield almost certainly reflects that fact that this cohort had already been extensively investigated and was therefore depleted of patients with the more common genetic causes. When looking just at patients in this cohort who had not been pre-screened by testing on the 104 gene panel the yield of diagnostic results from WGS among was similar to the figures in chapter 5 at 19.4% (9/31), whilst among those who had been pre-screened the yield was just 4.8% (2/41). What this demonstrates is that once the more common genetic causes have been excluded, only a tiny minority of patients will have an identifiable genetic cause even when tested on the largest platform available. So what does explain these severe epilepsies with no identifiable cause despite WGS? There are a number of possibilities:

- The first possibility is that one of the candidate genetic causes identified (Table 6.3a) is in fact causative. Once you move into the realm of increasingly rare genetic diseases there is often little published literature on the phenotypic spectrum of any given condition. Thus it becomes increasingly challenging to make a judgement as to whether a genetic finding is truly “causative.” It was for this reason that the methodology for this chapter involved in depth multidisciplinary discussion with a panel of experts in clinical genetics, laboratory genetics, and epilepsy. Whether genetic findings were designated “diagnostic” or “candidate” came down to

the consensus opinion at each multidisciplinary discussion. For example, though the *CNTNAP1* variants identified in family 772 were considered "candidate" they were thought to be sufficiently strong to justify offering pre-natal testing to the family in future pregnancy, particularly since, as a recessive condition the chance of recurrence was not insignificant (25%).

- A second possibility is that there is genetic cause within the WGS data that has not been identified as a candidate because of the limitations of the methodology used in this study. The criteria for variant candidacy were necessarily restrictive in order to keep the number of candidate variants to a manageable number and to maintain specificity. For a variant to be considered as a candidate it had to be in a gene with a high brain expression ratio and a low tolerance for haploinsufficiency (i.e. a high pLI score). Furthermore, the variant itself had to be rare (found in less than 1 in 100,000 individuals), coding (within exons), and with either predicted loss of function effect, or with a strong prediction of deleteriousness according to Polyphen-2 (score >0.8). Finally, the variant had to be inherited in a manner in keeping with the family history (*de novo*, X-linked in a male, recessive, or inherited from an affected parent). Each of these filters may have inadvertently thrown out potentially causative variants. The gene filter, based on an algorithm using brain expression and tolerance of haploinsufficiency, used to select candidate genes was only 71.1% sensitive when tested against the 45 most commonly-implicated autosomal dominant epilepsy-associated genes. Examples of epilepsy-associated genes that are not selected by this filter include *KCNA2* and *KCNT1*, both of which have relatively low pLI scores and so are predicted to tolerate haploinsufficiency. The low pLI score is explained by the fact that epilepsy-associated variants in these genes are always missense variants with predicted gain-of-function properties (Barcia et al., 2012; Milligan et al., 2014; Lim et al., 2016; Syrbe et al., 2015). Other epilepsy-associated genes may have low pLI scores because they are associated with mild phenotypes and therefore deleterious variants have not been selected out of the population: examples include *PRRT2* and *CHRNA2*. Some other epilepsy-associated genes were missed by

the filter because they are ubiquitously expressed and therefore have a relatively low brain expression ratio (GTEx). Examples of this include *DYRK1A* and *SLC2A1*.

Moving onto genes associated with disorder that have recessive inheritance patterns, the chance that they would be filtered out increases markedly, principally because these genes tend to have low pLI scores: they tolerate loss of function of one allele and only cause disease when both alleles are affected. One of the most compelling diagnostic findings in this chapter were the recessive *ROGDI* variants identified in family 431. These were considered diagnostic since both variants were predicated to result in loss of function and because the phenotype of P-431, including amelogenesis imperfecta, was fully in keeping with the published cases. The *ROGDI* gene has a very low pLI score of $3.2x10^{-12}$, since carriers of truncating variants, like the parents of P-431, are expected to be healthy.

It is possible that intronic variants identified through WGS may have been causative. Intronic variants may lead to disease through a variety of mechanisms, the best understood of which are the disruption of transcription regulatory motifs, and the activation of non-canonical splice-sites, leading to pseudo exon inclusion (Vaz-Drago, Custódio & Carmo-Fonseca, 2017). Recently seven *de novo* deep intronic variants in *SCN1A* have been described in patients with Dravet syndrome. Using real time qualitive Polymerase Chain Reaction (RT-qPCR) the authors were able to demonstrate that these variants resulted in the inclusion of an additional "poison" exon to the *SCN1A* gene transcript which likely lead to loss of function of the allele (Carvill et al., 2018). I looked for possible causative intronic variants in the patients in this cohort, but there were no *de novo* intronic variants identified within established epilepsy-associated or neurodevelopmental disorder-associated genes.

Excluding variants on the basis that they were inherited from an unaffected parent may also have filtered out causative variants, since some epilepsy-associated genes are have incomplete or variable penetrance. For example, asymptomatic individuals carrying deleterious variants in *TSC1* or *TSC2* have

parented children with severe phenotypes of tuberous sclerosis (Staley, Vail & Thiele, 2011). In family 976 a maternally-inherited essential splice site variant in *STX1B* was identified. The proband had a severe focal epilepsy, whilst his mother had a history of a single febrile convulsion. Variable penetrance is well-described in this gene (Schubert et al., 2014).

Finally, all the tools used here to filter variants have their own specificity, sensitivity, and technical limitations. Alternative tools with the same objectives exist to the ones I have used, such as the Haploinsufficiency Index (HI) as an alternative to the pLI score (Firth et al., 2009); Combined Annotation Dependent Depletion (CADD) score (Rentzsch et al., 2019) and Provean (Choi & Chan 2015) as an alternatives to Polyphen-2; and Brain Cloud as an alternative to GTEx ratio (Colantuoni et al., 2011). In the absence of gold standard definitions of diagnostic results, it is very difficult to appraise the relative merits of these various tools.

- A third possibility is that there is a genetic cause not easily identified though standard WGS analysis, such as a copy number variant (CNV). Large CNVs were excluded in this cohort by ensuring that all patients had had array CGH testing, yet the technology used was only sensitive to a copy number variant size of 1 kilobase. It is possible to use tools to interrogate WGS data for small CNVs and this analysis is ongoing at present.
- A fourth possibility is that there is a non-Mendelian genetic cause such as oligogenic or polygenic inheritance, or epigenetic factors.
- A fifth possibility is that there is no genetic cause at all, and that some unidentified environmental factor explains the epilepsy, such as an infectious process, or environmentally triggered immune process.

In reality the aetiology for most patients with epilepsy will be a blend of the above possibilities. Every patient with epilepsy is likely to have, to varying and possibly unquantifiable degrees, some environmental and some non-Mendelian genetic contributors to their phenotype. Some will also have Mendelian genetic contributors as well, though it remains a minority in which such be identified. WGS does have the capacity to identify Mendelian causes after a large gene panel has

failed to do so, but with a relatively low yield. In this study I showed that two factors were associated with an increased likelihood of identification of a Mendelian genetic cause and these are the presence of microcephaly and the presence of moderate to profound cognitive impairment.

At present I have been unable to demonstrate that diagnostic results obtained though WGS *after* gene panel testing have direct implications for therapy choice, though this partly reflects the fact that the Glasgow 104 gene panel was designed to include as many "treatable" genetic epilepsies as possible. It is important to recognise however that there may be therapeutic value for families in connecting with other families affected by a rare genetic disorder.

In addition to the diagnostic results obtained through WGS, a number of promising new candidate epilepsy genes were identified: *POLR1A*, *MED13*, *TRIO*, *CACNA1G*, *CNTNAP1*, and *NIPBL* are all genes have been associated with neurodevelopmental disorders but typically without epilepsy as a prominent feature of the established phenotype. These findings lend support to the conceptualisation epilepsy as a *symptom* of multiple rare genetic brain disorders, rather than a *disease* in itself.

The candidate variants in *TRIM46*, *MAP2*, *RASL10B*, and *LRP8* are all in completely novel genes and now efforts are underway to identify more patients with potentially pathogenic variants in these genes through linking with other research groups.

7. Detailed phenotyping and therapy response in cohort of children with a newly-identified genetic epilepsy, *SMC1A*

7.1 Introduction

Genes that have been associated with epilepsy can be broadly divided into five groups, based on the function of the protein encoded:

- **Metabolic:** Genes encoding proteins involved in the transport and metabolism of intracellular small molecules, or mitochondrial function
- **Ion channel/G-protein:** Genes whose products form ion channels, ion channel accessory proteins, or G-proteins
- **Cell growth and proliferation:** Genes encoding regulators of cell growth and proliferation
- **Synaptic:** Genes encoding proteins with key synaptic functions (excluding ion channels and G-proteins)
- **Regulatory:** Genes thought to play a role in transcriptional regulation, translational regulation, or post-translational protein modification
- **Others:** Miscellaneous, or unknown function

7.1.1 Metabolic genes

An inherited metabolic disease (IMD) is a condition in which the body produces insufficient quantities of a key enzyme or cofactor that is involved in the transport or metabolism of one or more small molecules (e.g. sugars, amino acids, lipids) within the cell. The root cause of most IMDs is one or more pathogenic variant in the gene encoding the relevant enzyme. The majority of IMDs are recessively inherited since one working copy of a gene is usually sufficient to produce sufficient quantities of most enzymes. Because enzymes function within complex

and intersecting pathways, most IMD have both upstream and downstream effects. Symptoms relating to IMDs can be described in relation to three broad mechanisms: over accumulation of a toxic metabolite; insufficient production of a key substrate; and failure of energy supply. Epileptic seizures are common in patients with IMDs and all three of these mechanisms are likely to play a role in seizure predisposition in affected patients. Examples of each are given below:

7.1.1.1 Overaccumulation of a toxic metabolite:

e.g. Glycine encephalopathy, associated with recessively-inherited variants in genes (*GLDC*, *AMT, GCSH*) encoding components of the glycine cleavage complex. Results in accumulation of glycine within the CNS. Glycine is an agonist at excitatory NMDA receptors. Seizures observed in glycine encephalopathy are believed to be largely as a consequence of NMDA activation.

7.1.1.2 Insufficient supply of a key substrate:

e.g. Biotinidase deficiency, associated with recessively-inherited variants in *BTD* which encodes the biotinidase enzyme. Biotinidase releases biotin from protein in the diet. Biotin is a critical cofactor in a number of carboxylation reactions.

7.1.1.3 Failure of energy supply:

e.g. disorders of mitochondrial function, which may be associated with variants in nuclear DNA, or in mitochondrial DNA.

Distinction between these three mechanisms is not always clear cut. For example, in a patient with glucose 1 transporter deficiency (Glut1-D) this can be conceived of as both an insufficient supply of a key substrate (glucose to the CNS) and as a failure of energy supply. In many IMDs an single enzyme deficiency will result both overaccumulation of toxic metabolites and in insufficient supply of key substrates, and it is not always possible to tell which if these is the main factor driving a predisposition to seizures.

7.1.2 Ion channel and G-protein disorders

Ion channels and G-protein coupled receptors are transmembrane receptor proteins that are present in all cell membranes in the body and permit extracellular influences to transmit signals into the cell. Ion channels do this through opening pores which specifically allow charged ions to pass through the membrane, thereby causing a shift in charge. G-proteins act by triggering an intracellular cascade of interactions in response to extracellular binding of a ligand. Both ion channels and G-proteins have hundreds of subtypes which confer tissue and cell-specific functional properties. Some have critical roles within the CNS while others do not. Though ion channels and G-proteins are functionally quite different I have included them here in a single category because within the CNS, many neurotransmitters act as ligands at both types of receptor. For example, the GABA-A receptor is a chloride ion channel and the GABA-B receptor is a G-protein, but both receptors are agonised by GABA. CNS ion channels can be broadly divided into those that are triggered by electrical charge (voltage-gated) and those that are triggered by neurotransmitters (ligand-gated). Some ion channels have both properties. Voltage-gated ion channels are critical for the rapid transmission of electrical signals along neurones

Table 7.1a: Examples of ion channels and G-protein-coupled receptors associated with epilepsy

Type of transmembrane receptor	Gene	Receptor and function	Epilepsy phenotype(s)	Reference
Voltage-gated ion channel	SCN1A	Voltage-gated sodium channel. Allows membrane depolarisation during action potential	Dravet syndrome and fever sensitive seizures	(Claes et al., 2001)
Ligand-gated ion channel	GABRA1	GABA-sensitive chloride channel. GABA binding allows chloride entry into the neurone leading to hyperpolarisation and reduced excitability	Wide range of phenotypes associated: rom severe neonatal-onset developmental and epileptic encephalopathy to adult onset generalised epilepsies	(Johannesen et al., 2016)
G-protein coupled receptor	GABBR2	GABA-sensitive G-protein coupled receptor.	Infantile spasms	(Hamdan et al., 2017)

7.1.3 Growth and proliferation genes

Developmental organisation of the brain structure is a process that relies on multiple cellular and intracellular signalling pathways. Variants in many of the genes involved in these pathways have been associated with epilepsy. Some of the best characterised are those with roles in cortical development, a process which involves the migration of neurones from the centre of the brain to the outer cortex. Disruption of this process can lead to characteristic malformations which may be identified on neuroimaging. Examples include the *PAFAH1B1* (Saillour et al., 2009), *DCX* (Gleeson et al., 1998), and *TUBA1A* (Jansen et al., 2011). Variants in all these genes are associated with lissencephaly – a cerebral cortex with an absence of normal gyration – and epileptic seizures.

Variants in other genes may result in disruption of neuronal growth and proliferation at a later stage in development. The mTOR pathway is a ubiquitous intracellular signalling pathway which is linked to the insulin receptor. Disruption of the mTOR pathway has been associated with a wide variety of metabolic, neoplastic and developmental disorders. Certain proteins within the mTOR pathway appear to be specifically important in CNS tissue, and variants in genes encoding these proteins associate with epilepsy. Examples include hamartin and tuberin (encoded by *TSC1* and *TSC2* respectively) which are associated with tuberous sclerosis, and Dep-domain containing protein 5 (*DEPDC5)* which is associated with focal epilepsies, not all of which have associated neuroimaging abnormalities (Lal et al., 2014).

7.1.4 Synaptic genes

The fully developed human central nervous system has an estimated 100 billion neurones, and 1,000 trillion synapses, which equates to an average of 10,000 synapses per neurone. This extraordinary degree of connectivity is what gives the human brain a processing power that is unparalleled by even the most powerful of computers (Kunkel et al., 2014). In this context, it is not surprising that

orchestration of synaptic function is a highly complex process which is regulated by many genes, proteins and pathways. Ion channels and G-proteins are of critical importance at the synapse and these have been discussed. Other key functions of the synapse, including neurotransmitter release, synaptic vesicle recycling, and postsynaptic membrane organisation, underpin many genetic epilepsies. For example, docking and fusion of pre-synaptic vesicles is disrupted by variants in *STXBP1*, which encodes Syntaxin binding protein 1, and are associated with developmental and epileptic encephalopathy (Saitsu et al., 2008).

7.1.5 Regulatory genes

The final category of epilepsy-associated genes are those that are influence the expression of other genes through an effect on transcription, translation, or post-translational modification. Many of these are transcription factors which bind to DNA and control (activating or repressing) which genes are transcribed within a particular cell type (Spitz & Furlong, 2012). The most well-known epilepsy-associated transcription factor is MeCP2, a protein encoded by *MECP2*. MeCP2 can both activate and repress transcription. Variants in *MECP2* are associated with Rett Syndrome, an X-linked neurodevelopmental disorder characterised by cognitive impairment, drug-resistant seizures, and stereotypic hand wringing movements (Swanberg et al., 2009). Other epilepsy-associated transcription factors include *TCF4* (associated with Pitt-Hopkins syndrome) (Rosenfeld JA et al., 2009) and *ZEB2* (Mowat-Wilson syndrome) (Dastot-Le Moal et al., 2007).

The second major mechanism by which gene translation is regulated is through chromatin remodelling. Most of the time DNA is wrapped tightly around histone protein complexes to form chromatin. DNA must be exposed from the histone complex for transcription to take place. This is predominantly achieved through either covalent histone modification, or through ATP-dependent complexes which move, eject or restructure nucleosomes, the primary repeating unit of chromatin which consists of a segment of DNA sequence wrapped around eight histone cores (Saha, Wittmeyer & Cairns, 2006). Variants in genes encoding chromatin

remodelling proteins have been also been associated with epilepsy: Variants in *CHD2* are associated with a developmental and epileptic encephalopathy with marked photosensitivity (Galizia et al., 2015), and variants in *KANSL1*, are associated with Koolen de Vries syndrome, a multi-organ malformation syndrome in which half of affected patients have epilepsy (Koolen et al., 2012; Myers et al., 2017a).

7.1.6 Blurring of boundaries

Sections 7.1.1-5 present five categories of epilepsy-associated genes as if they are distinct and exclusive. Though this is helpful conceptually, the reality is no so clear cut. Some genes have multiple functions, some have functions that can be conceptualised in multiple ways, and others have not been fully characterised. Many IMDs, "ion channelopathies", and "synaptopathies" are likely to have an impact on neuronal growth and proliferation as evidenced the neuroimaging findings that can be associated with these conditions. At times the distinction between "metabolic" and "ion channel" becomes challenging. For example, *SLC6A1* encodes a transmembrane transporter of the small peptide GABA which removes GABA from the synaptic cleft. *SLC6A1* related epilepsy could be considered a metabolic disorder (disrupted transport of small molecules) or an ion channel disorder, since the direct consequence of excess GABA within the synaptic cleft is likely to be an over activation of GABA receptors.

The distinction between Ion Channel/G-protein disorders and Synapse-related disorders is particularly artificial since there is so much interaction between multiple ion channels, G-proteins and other synaptic proteins at every synapse. Nonetheless, because the ion channel disorders have been relatively well-characterised, and because most examples of genetically driven precision therapy in epilepsy relate to this group, it is helpful to consider these disorders separately from the other synaptopathies.

7.1.7 Precision therapy

In metabolic epilepsies, treatments can be targeted at the biochemical abnormality (e.g. replace the enzyme, restrict the diet, or supplement the deficient substrate). In ion channel disorders there is scope to modify neuronal ion channels with targeted therapy (e.g. sodium channel blocking drugs in *SCN8A*-related seizures). In disorders of growth and proliferation pathways can be modified therapeutically (e.g. mTOR suppression with Everolimus for tuberous sclerosis). Though there as yet no examples of success, it is conceivable that therapeutic agents could be developed to target specific synapse-related epilepsies once the molecular pathways involved have been characterised. For epilepsies relating to disorders of transcriptional regulation precision therapy presents a particular challenge since there are no cellular pathways to target. Even targeting the underlying cause (the genetic defect) through gene therapy has proved challenging since transcriptional regulation involves a complex and fine balance between promotion and repression. In *MECP2*-related Rett Syndrome gene therapy in mice has been hampered by the detrimental effects of over expression of *MECP2* (Sinnett & Gray, 2017). Perhaps a first step to precision therapy in the disorders of transcriptional regulation is to understand what the key downstream *regulated* genes are, what the impact of the disease is on these, and whether any targetable pathways are revealed.

7.1.8 SMC1A

In chapter 6 (section 6.4.3.9) I identified a *de novo* nonsense mutation in *SMC1A*. This gene encodes a structural component of the cohesin ring, which is thought to be involved in transcriptional regulation through binding to chromatin. Through gene matching systems I was able to identify another 15 patients with epilepsy and loss of function variants in this gene as well as nine published cases. The aim of this chapter is to describe the phenotype of this condition and explore the potential for precision therapy for patients with epilepsy associated with variants in this gene.

7.2 Methods

7.2.1 Case ascertainment

The Deciphering Developmental Disorders study (DDD) is a UK-wide multicentre project involving 23 recruitment centres which encompass all regional genetics departments within the UK NHS. Inclusion criteria for this study were any one of the following:

- Neurodevelopmental disorder
- Congenital anomalies
- Abnormal growth parameters (weight, height, head circumference)
- Dysmorphic features
- Unusual behavioural phenotype
- Genetic disorder for which the molecular basis is unknown

Most participants to DDD were recruited as "trios" (both patents and affected proband). Basic phenotype information was collected and coded in Human Phenotype Ontology (HPO) terms (Köhler et al., 2017). Genetic testing involved Whole Exome Sequencing, which was carried out at the Hinxton Sanger Institute in Cambridge (Firth et al., 2011).

Though personal correspondence with the DDD project leaders at the Sanger Institute I was given the contact details of eight clinical geneticists who had recruited patients to the DDD study in whom *de novo* truncating variants in the *SMC1A* gene had been identified among the first 4,293 families analysed (McRae et al., 2017). All of these patients were females with epilepsy. Because this was deemed to be a novel genotype-phenotype association I was granted a "Complementary Analysis Protocol (CAP)" which, within the overall protocol and ethical framework of the DDD study, permitted me to collect additional phenotype information directly from clinicians.

I published genotype and phenotype details of these patients in February 2017 (Symonds et al., 2017). Following this publication, I received personal correspondence about another six patients. I identified a further patient through my WGS study, described in section 6.3.4.9. There are also nine patients with *SMC1A* truncation variants published elsewhere (Helbig et al., 2016; Lebrun et al., 2015, Goldstein et al., 2015; Jansen et al., 2016; Hansen et al., 2013; Gorman et al., 2017).

7.2.2 Ethical approval

The Deciphering Developmental Disorders (DDD) study has United Kingdom Research Ethics Committee approval (10/H0305/83, granted by the Cambridge South REC).

7.2.3 Clinical information

I created a structured phenotype form, which was sent to both the clinical geneticist and the paediatric neurologist involved in each case (Appendix 16).

7.2.4 Genetic information

Original genetic reports were requested. *SMC1A* variants were described with reference to human genome build GRCh37 (Hg19) *SMC1A* transcript 0066306.3, which is the canonical transcript.

7.3 Results

7.3.1 Cases and ascertainment

25 patients were identified in total (16 new and nine published, see table 7.3a). Patients 1-8 were from the DDD study. As per the protocol of that study, the *SMC1A* variants in these cases were detected by trio Whole Exome Sequencing. Patient 9 was the affected sister of patient 8. She died at the age of 11 months and was tested, by Sanger method, for the variant present in her sister *post mortem*. Patient 11 was recruited to the Whole Genome Sequencing (WGS) study described in chapter 6 of this thesis, and patients 10, 12, 13, 14, 15, and 16 all had their *SMC1A* variants identified in different clinical or research laboratories.

7.3.2 Estimated incidence of *SMC1A*-related epilepsy

Analysing the first 4,293 families in the DDD study, the authors estimated that 42% of the cohort carried a disease causing *de novo* variant. They estimated that a *de novo* variant can be expected to result in a developmental disorder in between 1 in 213 and 1 in 448 births. Since eight patients with *SMC1A*-related epilepsy were among these 4,293 families, extrapolating from their estimates, the incidence of *SMC1A*-related epilepsy would be expected to be between 1 per 50,000 and 1 per 100,000 births.

7.3.3 Genetic findings

SMC1A encodes a 1,233 amino acid protein called smc1 (structural maintenance of chromosomes 1), which is a structural component of the cohesin ring (Rocques et al., 1995).

All 25 variants were novel, and all were predicated to result in truncation of the smc1 protein: 10 nonsense, 10 frameshift, and five splice interference. *De novo* status of the variant was demonstrated in 23/25 patients.

Table 7.3a: Summary of the phenotypic features of 16 new patients and the 9 published patients with *SMC1A* truncations

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
***SMC1A* variant**	c.1591C>T p.Gln531Ter Nonsense	c.3145C>T p.Arg1049Ter Nonsense	c.549G>A pGlu183Glu Splice interference	c.2197G>T p.Glu733Ter Nonsense	c.3326_3330delATGGC insC p.Asp1109AlafsTer102 Frameshift	c.2923C>T p.Arg975Ter Nonsense	c.511C>T p.Arg171Ter Nonsense	c.2477delA p.Asn826Thrfs*3 Frameshift
Inheritance of variant	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Method of testing	DDD study	DDD study	DDD study	DDD study	DDD study	DDD study	DDD study	DDD study
Age at completion of phenotype form	6 years	6 years	3 years	8 years	10 years	5 years	4 years	14 years
Birth OFC Z-score/ most recent OFC Z-score	-0.8/-3.5	-1.5/-4.5	-1.6/-0.8	-2.0/-2.5	-1.2/-2.0	Unknown/-3.0	Unknown/-2.0	Unknown/-2.0
Most recent height Z-score	-2.3	-2.6	0.06	-2.6	-4.5	-3.2	-2.5	-3.7
Tone	Hypotonia	Hypotonia	Axial hypotonia, peripheral hypertonia	Hypotonia	Hypotonia	Hypotonia	Hypotonia, with increased dynamic tone at ankles	Unknown
Cognitive ability	Moderate – severe impairment	Severe impairment	Severe impairment	Severe impairment	Severe impairment	Moderate – severe impairment	Moderate – severe impairment	Moderate – severe impairment
Gross motor development	Can run with unsteady gait	Unable to sit without support	Unable to sit without support	Non-ambulant	Unable to sit without support	Walking from 30 months	Can take a couple of steps with support	Walking from 2.5 years. Unsteady on feet with frequent falls, aged 7
Speech	Non-verbal. Smiles and makes hand gestures	Non-verbal.	Non-verbal	Non-verbal	Non-verbal	Lost speech aged 3 years following SE	Non-verbal. Coos, laughs, cries appropriately	Non-verbal. Communicates through signing
Autism	-	-	-	+	-	-	-	+
Age at first seizure	15 months	5-6 weeks	4 months	5 months	6 months	5 months	4 weeks	28 months
First seizure semiology	Cluster of GTCS	GTCS	Generalised tonic	Focal→bilateral clonic	Cluster of GTCS	Focal→bilateral tonic	Bilateral clonic	FS
Further seizure types	GTCS, hemiclonic, drop attacks, atypical absence	Focal	FS, CSE, focal, myoclonic, spasms, atypical absence	Focal, generalised tonic	GTCS, myoclonic, atypical absence, tonic, spasms, NCSE, reflex sensory	Focal→bilateral tonic focal→bilateral clonic	GTCS, hemiclonic	GTCS

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Seizure clusters?	+	-	+	+	+	+	+	+
Seizure freedom?	No	Yes, aged 5 years	No	Yes, aged 7 years	No	For 1 year then recurred	No	No
Therapies tried	PHT, VPA, LEV, LMT, CLB, TPM, CBZ	CBZ, VPA, TPM, CLB, RUF, LEV, GBP KD, VNS	GBP, AZA, CZP, LZP, VPA, LEV, Pyridoxine, STP	VPA, LMT, CBZ, TPM, LEV, PHT, CLB, PB	PB, CBZ, CLB, VPA, LMT, TPM, LEV, Pyridoxine, KD	CBZ, VPA, TPM, PB, LEV, KD	TPM, VPA, LEV, CBZ	PB, LEV, TPM, LMT, CBZ, CLB, VNS, KD
Beneficial therapies	VPA, PHT, CBZ	GBP (seizure-free)	STP	PB (seizure-free)	CLB, KD	LEV (seizure-free for 1 year), KD	None	KD
EEG	Independent left and right sided epileptiform abnormalities	Diffusely slow with sharp transients seen over both temporal regions	High amplitude background activity with epileptiform discharges over left and occasionally right hemisphere	Multifocal epileptic activity	Continuous high voltage waves without epileptiform discharges	High voltage slow background	Frequent multi-spike and slow right centro-temporal region	Right-sided spike and slow wave abnormalities
MRI(s)	Normal	Cerebral volume loss	Small hemorrhage along the posterior falx and tentorium	Normal	Small cavum septum vergae	Normal	Normal	Normal
Dysmorphisms and skeletal anomalies	Mild facial asymmetry with the right side of the face appearing fuller than the left; mild left ptosis; short, slightly up-turned nose; thin upper lip; shallow philtrum; small hands and feet	Bitemporal narrowing; upslanting palpebral fissures; thin, straight eyebrows; mildly posteriorly rotated ears; tapering of digits; short 5th fingers; short broad halluces; overlapping 2ndand 3rd toes	Hirsutism of the forehead, back, arms and upper lip; bitemporal narrowing with low anterior hairline; long eyelashes; downturned corner of mouth with midline groove of the lower lip	Small widely spaced teeth; deep-set eyes; downward sloping palpebral fissures; short, slightly up-turned nose; shallow philtrum; low-set posteriorly rotated ears; syndactyly of the 1st and 2nd toes	Deviation of both halluces at the metatarso-phalangeal joint; left 3rd finger camptodactyly; left 5th finger clinodactyly	Puffy eyes; right nasal deviation; small over-folded right pinna; short, slightly upturned nose; thin upper lip; shallow philtrum; clinodactyly of the middle toes of both feet; central incisor.	None	None
Malformations	None	Bilateral congenital hip dysplasia; bilateral talipes; bifid T8 vertebra	Cleft palate; multiple small cysts in kidneys	ASD; VSD	Bifid T6 vertebra; ASD; choanal atresia	Cleft palate	None	None

Patient	Patient 9	Patient 10	Patient 11	Patient 12	Patient 13	Patient 14	Patient 15	Patient 16
***SMC1A* variant**	c.2477delA p.Asn826Thrfs*3 Frameshift	c.3115C>T p.Gln1039Ter Nonsense	c.2923C>T p.Arg975Ter Nonsense	 Frameshift	c.615G>A p.Glu205Glu Splice interference	c.1495C>T p.Arg488Ter Nonsense	c.3305_3312del p. Asn1102ArgfsTer53 Frameshift	c.3321C>A p.Tyr1107Ter Nonsense
Inheritance of variant	De novo	De novo	De novo	De novo	De novo	Absent from mother	De novo	Unknown
Method of testing	Sanger testing for variant identified in sibling (Patient 8)	Clinical Exome Testing (Germany)	Whole Genome Sequencing (Chapter 6)	Cornelia de Lange gene panel (Edinburgh)	71 gene epilepsy panel (USA)	WGS (Ireland)	Clinical Exome Testing (India)	Clinical Exome Testing (UK)
Age at completion of phenotype form	Died aged 11 months	Died aged 9 years 2 months	16 years	7 years 6 months	10 years	4 years	2 years 7 months	25 years
Birth OFC Z-score/most recent OFC Z-score	-1.7/Unknown	-1.3/-6.3	Unknown/-5.3	-1.9/-7.8	Unknown/unknown (reportedly normal)	Unknown/-2.1	Unknown/-2.71	Unknown/Unknown
Most recent height Z-score	Unknown	-5.0	-3.59	-4.0	Unknow (reportedly normal)	Unknown	0.00	Unknown
Tone	Unknown	Neonatal hypotonia	Axial hypotonia, peripheral hypertonia	Hypotonia	Axial hypotonia, peripheral hypertonia	Axial hypotonia, peripheral hypertonia	Hypotonia	Normal
Cognitive ability	Profound impairment	Severe impairment	Profound impairment	Profound impairment	Severe impairment	Moderate – severe impairment	Profound impairment	Borderline impairment
Gross motor development	No head control	Unable to sit	Can sit independently Never able to walk	Unable to sit	Walked at 13 months. Ataxic gait	Walked at 3 years. Ambulant now. Ataxia	Ambulant. Ataxia	Normal development. Bilateral pes cavus
Speech	N/A	Non-verbal	Non-verbal	Non-verbal	Loss of all language at 18 months. Now non-verbal	Non-verbal	One word with meaning	Normal
Autism	-	-	-	-	+	-	+	-
Age at first seizure	< 1 month	2 months	5 months	3 weeks	13 months	3 years	10 months	12 years
First seizure semiology	Unknown	GTCS	GTCS	GTCS	Focal	Focal	Infantile spasms	Focal
Patient	**Patient 9**	**Patient 10**	**Patient 11**	**Patient 12**	**Patient 13**	**Patient 14**	**Patient 15**	**Patient 16**
Further seizure types	Unknown	Myoclonic, CSE	Spasms, atypical absence	Focal, myoclonic		GTCS, Atonic	Focal	GTCS, myoclonic, CSE

Seizure clusters?	-	-	+	+	+	-	-	+
Seizure freedom?	No	No	No	No	No	No	No	No
Therapies tried	Unknown	TPM, VPA, LEV, RUF	LEV, VPA, NZP	CBZ, VPA, PHT, LEV, TPM, LMT, PRP	VPA, LMT, ZNS, KD, VNS	OXC, TPM, VPA, LCS, CLB	VGB, VPA, ZNS, TPM, LEV, ACTH, KD	CLB, TPM, VPA, CBZ, LEV, PHT, LMT, LCS, PRP, PB
Beneficial therapies	Unknown	None	LEV, VPA	None	LEV, KD, VNS	VPA, LCS, CLB	ACTH, VGB	LEV, PHT
EEG	Unknown	Multifocal epileptogenic activity, more often over the right hemisphere	Focal frontotemporal sharp waves	Focal sharp waved independently on left and right	Left temporal epileptiform spike discharges, spreading to the right during a seizure	Focal epileptiform discharges	Bisynchronous bursts of spike-wave activity	Right anterior focal epileptiform discharges
MRI(s)	Semilobar holoprosencephaly	Thin abnormally shaped corpus callosum and minimal cerebral atrophy	Mild asymmetric posterior white matter volume loss	Normal	Normal	T2 hyperintensity in the posterior periventricular white matter without volume loss. Slightly immature myelination	Normal	Normal
Dysmorphisms and skeletal anomalies	Hypotelorism; small low-set posteriorly rotated ears; bilateral 5th finger clinodactyly; overlapping 4th and 5th fingers; left rockerbottom foot; hypoplastic nails of the 4th and 5th toes	Expressionless face, straight eyebrows; short, upturned nose; flattened midface; short philtrum; downturned corners of the mouth; small hands with tapering fingers	None	Low anterior hairline	None	None	Short neck, high nasal bridge, upturned nose, long eyelashes, flattened midface, shallow philtrum, small hands	None
Malformations	Partial anomalous pulmonary venous drainage	ASD; VSD	None	None	None	None	None	None

Paper and case	Hansen (Hansen et al., 2013)	Lebrun (Lebrun et al., 2015)	Goldstein 1 (Goldstein et al., 2015)	Goldstein 2 (Goldstein et al., 2015)	Jansen 1 (Jansen et al., 2016)	Jansen 2 (Jansen et al., 2016)	Helbig (Helbig et al., 2016)	Gorman 1 (Gorman et al., 2017)	Gorman 2 (Gorman et al., 2017)
SMC1A variant	c.1731G>A p.Asp577Asp Splice interference	c.1911+1G>T Splice interference	c.2853_2856delTCAG p.Ser951Argfs*12 Frameshift	c.3549_3552dupGGCC P.Ile1185Glyfs*23 Frameshift	c.2364del Asn788Lysfs*10 Frameshift	c.2421_2562del Leu808Argfs*6 Frameshift	c.3549_3552dupGGCC p.I1185Gfs*23 Frameshift	c.1114delG p.Val372Ter Nonsense	c.1113+1G?A Splice interference
Inheritance of variant	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo	De novo
Method of testing	323 gene epilepsy panel (Germany)	Whole Exome Sequencing (France)	Whole Exome Sequencing (USA)	Whole Exome Sequencing (USA)	Whole Genome Sequencing (Netherlands)	Whole Genome Sequencing (Netherlands)	Not reported	Whole Exome Sequencing (Ireland)	Whole Exome Sequencing (Ireland)
Current age (age at report)	11 years	7 years	4 years	3 years	46 years	14 years	Not reported	Died at 9 months	Died at 3 years
Birth OFC Z-score/most recent OFC Z-score	Unknown/-2.5	-3.9/-2.5	Unknown/-2.0	-1.0/0.0	Unknown/-2.5	Unknown/-1.7	Not reported	0.00/-2.5	Unknown/-2.5
Most recent height Z-score	-0.5	-2.0	Unknown	-0.05	-2.5	-2.0	Not reported	Not reported	Not reported
Tone	Not reported	Axial hypotonia, peripheral hypertonia	Axial hypotonia, peripheral hypertonia	Not reported	Axial hypotonia, peripheral hypertonia	Normal	Not reported	Profound hypotonia	Profound hypotonia
Cognitive ability	Impairment, but not qualified	Severe impairment	Severe impairment	Severe impairment	Severe impairment	Severe impairment	Not reported	N/A	Severe impairment
Gross motor development	Walked at 18 months	Delayed	Non-ambulant	Walked at 12 months	Never crawled or walked	Walked at 2y; suddenly stopped walking at 5 years	Not reported	No head control at 9 months	Non-ambulant
Speech	First words at 30 months	Non-verbal	Non-verbal	Non-verbal. Coos, interacts.	Non-verbal	Non-verbal	Not reported	N/A	Non-verbal
Autism	-	-	-	-	-	-	Not reported	N/A	N/A
Age at first seizure	3 months	< 1 month	4 months	17 months	9 months	2 years	Not reported	17 weeks	7 weeks
First seizure semiology	GTCS	Focal with eyelid myoclonia	Generalised tonic	Focal/atypical absence, GTCS	GTCS	GTCS	Not reported	Focal	Focal

Paper and case	Hansen (Hansen et al., 2013)	Lebrun (Lebrun et al., 2015)	Goldstein 1 (Goldstein et al., 2015)	Goldstein 2 (Goldstein et al., 2015)	Jansen 1 (Jansen et al., 2016)	Jansen 2 (Jansen et al., 2016)	Helbig (Helbig et al., 2016)	Gorman 1 (Gorman et al., 2017)	Gorman 2 (Gorman et al., 2017)
Further seizure types	Focal	Focal, spasms	Tonic, focal→bilateral clonic, CSE	Focal/atypical absence, GTCS	GTCS	GTCS	Not reported		Tonic, spasms, GTCS,
Seizure clusters?	+	-	+	+	+	+	Not reported	-	+
Seizure freedom?	Not reported	No	No	Yes	No	No	Not reported	No	No
Therapies tried	Not reported	Unknown	PB, LEV, CLB, TPM, CZP, VPA, KD	LEV, TPM, OXC, VPA, PB	PHT, PB, CBZ, VPA, VGB, CZP	LMT, TPM, CBZ, CLB, LEV, CZP, OXC, Carnitine, Pyridoxine, KD, Oestrogen	Not reported	VPA, CBZ, TPM, PHT, KD	Not reported
Beneficial therapies	Unknown	Unknown	None	None	None	Oestrogen	Not reported	None	None
EEG	Nonspecific monomorphic generalized slow-wave activity with rare spikes	Hypsarrhythmia (when presented with infantile spasms)	Multifocal epileptiform discharges	Generalised epileptiform activity or occipital abnormalities	“centrencephalic” epilepsy	Unknown	Not reported	Multifocal migrating epileptiform discharges	Slow background, left temporal epileptiform discharges
MRI(s)	Not reported	Thin corpus callosum	Mild symmetric T2 hyperintensities in the periatrial white matter on FLAIR sequencing with possible thickening of the insular cortex	Mild enlargement of extra-axial spaces; slight thinning of the corpus callosum. Temporal lobes prominent sulci and hippocampi round and somewhat smaller.	Slightly enlarged ventricles, hypotrophy cerebellar vermis	Mild periventricular white matter abnormalities	Not reported	Not reported	Not reported

Paper and case	Hansen (Hansen et al., 2013)	Lebrun (Lebrun et al., 2015)	Goldstein 1 (Goldstein et al., 2015)	Goldstein 2 (Goldstein et al., 2015)	Jansen 1 (Jansen et al., 2016)	Jansen 2 (Jansen et al., 2016)	Helbig (Helbig et al., 2016)	Gorman 1 (Gorman et al., 2017)	Gorman 2 (Gorman et al., 2017)
Dysmorphisms and skeletal anomalies	Round face with arched eyebrows; short nose; upslanting palpebral fissures; smoothed philtrum; mild retrognathia; crowded teeth; flattened midface; clinodactyly of the fifth fingers; camptodactyly of the fifth finger on the right	Mild synophrys; small hands; small feet; thin nose and upper lip; retrognathia; triangular face	Long eyelashes; short nose; hirsutism on legs and back	None	Low anterior hairline; flat midface; straight eyebrows with deep set eyes; small and elongated ears with a prominent anti-helix; long nose; short philtrum; eversion of the lower lip; disorganized dentition; small, narrow hands with tapering fingers; syndactyly of the 2nd and 3rd toes and a mildly broad first toe	Mild trigonocephaly; mildly up slanting palpebral fissures with blepharophimosis; posteriorly rotated Ears; full cheeks, full lips and short philtrum; hands small with slender fingers	Not reported	None	Small hands and feet; incomplete single palmar creases; big hallux bilaterally extended
Malformations	Diaphragmatic hernia	None	None	None	Cleft palate	None	Not reported	None	ASD

Abbreviations: GTCS – generalised tonic-clonic seizure; FS – Febrile Seizure; CSE – Convulsive Status Epilepticus; NCSE – Non-Convulsive Status Epilepticus; PHT – Phenytoin; VPA – Sodium Valproate; LEV – Levetiracetam; LMT – Lamotrigine; CLB – Clobazam; TPM – Topiramate; CBZ – Carbamazepine; RUF – Rufinamide; GBP – Gabapentin; ZNS – Zonisamide; KD – Ketogenic Diet; VNS – Vagal Nerve Stimulation; PB – Phenobarbitone; OXC – Oxcarbazepine; VGB – Vigabatrin; CZP – Clonazepam; ACTH – Adrenocorticotrophic hormone; OXC – Oxcarbazepine; LCS – Lacosamide; PRP – Perampanel; ASD - Atrial Septal Defect; VSD – Ventricular Septal Defect

7.3.4 Phenotype

7.3.4.1 Seizures

Patients presented with a variety of seizure types (generalised in 12, focal in nine, epileptic spasms in one, unknown in three). 17 patients developed multiple seizure types. Age of seizure onset ranged from the neonatal period to 12 years of age (median five months). Distribution of age at first seizure is shown in figure 7.3a. Patient 16, who presented at 12 years of age, was a clear outlier.

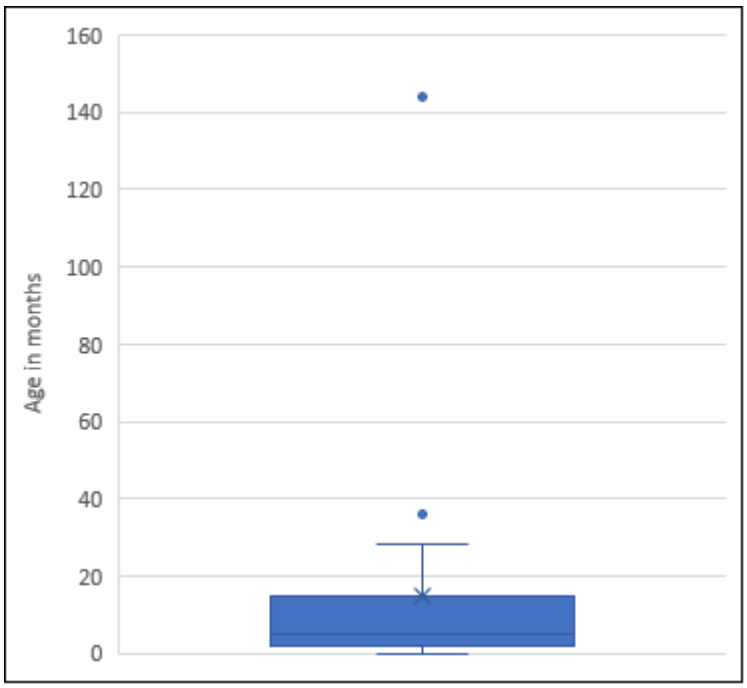


Figure 7.3a: Distribution of age at first seizure for patients with *SMC1A* truncations

Seizure types observed were: focal (n = 15), GTCS (n = 15), epileptic spasms (n = 6), atypical absence (n = 5), myoclonic (n = 6), tonic (n = 5), convulsive status epilepticus (n = 4) and drop/atonic (n = 3). Epileptic spasms, though the presenting seizure type in just one case, developed following other seizure types in four cases. This pattern is slightly unusual. For most children who develop epileptic spasms this is their first seizure type. Among 43 patients who had epileptic spasms in cohort 2 (chapter 5), for 27 (63%) spasms were the presenting seizure type.

Though there was wide variability in the seizure types observed in this cohort, a specific feature of the seizures that was noted as a common theme was the tendency for seizures to occur in clusters. For 16/22 patients for whom seizure characteristics were described a clustering pattern to seizures was reported. For some of the patients I was able to obtain a more detailed description of the seizure clustering:

- Patient 6: 3-10 focal-onset seizures occur over a 24 hour period every 10-21 days, with complete seizure-freedom between clusters.
- Patient 8: 15-25 GTCS occur over a 2-3 day period every 2-3 weeks, with complete seizure freedom between clusters. During the seizure clustering period she is unable to communicate or mobilise and she stops eating.
- Patient 11: 14-20 seizures over a 2-3 day period every 8-12 weeks, with complete seizure freedom between clusters.
- Patient 12: Clusters of focal-onset seizures with many per day over a 3-5 days period, with weeks or months of seizure-freedom between clusters.

Among the published cases too there are descriptions of seizure clustering:

- Hansen et al.: “The girl had generalized tonic–clonic seizures, occasionally occurring in impressive clusters lasting 24–48 h as well as clusters of seizures with secondary generalization” (Hansen et al., 2013).
- Goldstein et al., patient 1: “By 4 years of age, her seizure frequency consisted of clusters of 6-7 seizures every one to two weeks” (Goldstein et al., 2015).
- Jansen et al. Both patients reported to have “clusters of GTCS”
- Gorman et al. Both patients reported to have “clusters of focal seizures” (Gorman et al., 2017).

7.3.4.2 Electroencephalogram (EEG)

For 22 patients, EEG recordings, reports, or summaries were available. Though there was not a consistent EEG signature. Sixteen patients had at least one EEG demonstrating focal or multifocal epileptiform discharges. For two patients (patient 15 and the patient reported by Lebrun et al. the only EEG data provided was during an episode of infantile spasms, and this demonstrated generalised epileptiform abnormalities. Illustrative EEG recordings are shown in Figure 7.3b.

7.3.4.3 Development and cognition

Developmental impairment was reported in 24/25 patients for whom information was available. In all cases, apart from those with neonatal-onset seizures, concerns about development were present prior to presentation with seizures. In 21/24 cases development was assessed as being moderately, severely, or profoundly impaired. Specifically, verbal communication appeared to be affected, with 19/22 of the patients who survived to the age of usual speech acquisition having no verbal communication. Gross motor development was also affected in most cases: nine patients were non-ambulant.

For four patients I was able to gather evidence of developmental or cognitive regression, though this did not clearly relate to seizure onset. Patient 6 lost her ability to speak following an episode of convulsive status epilepticus at the age of three years, the second patient reported by Jansen et al. had an abrupt loss of ambulation at the age of five years. The parents of Patient 13 noticed a rapid decline in development from the age of 18 months. She had complete loss of language skills, having had a number of single words prior to that. Patient 16 is exceptional in that she presented at the age of 12 years and there had been no concerns about her early childhood development. She completed mainstream school education and went to university. She has clusters of GTCS once per month, often resulting in admissions to the Intensive Care Unit, and there has been a gradual decline in her cognitive functioning to borderline-low levels.

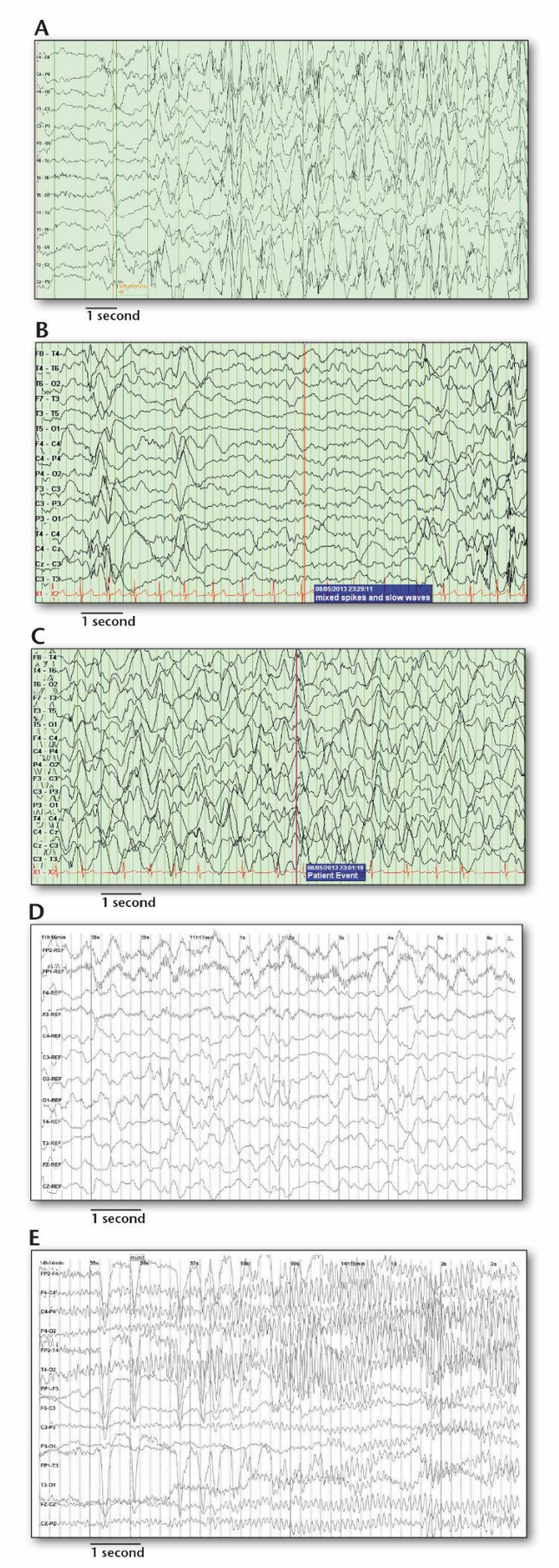


Figure 7.3b: Illustrative EEGs of patients with *SMC1A* truncations

(A) Inter ictal sleep EEG from Patient 1: showing generalised irregular spike/sharp and slow activity in sleep without any clinical accompaniment; (B) Inter ictal EEG from Patient 6: showing short bursts of mixed spike and slow waves with a bilateral but asymmetric distribution; (C) Ictal EEG from Patient 6 during a focal-onset seizure: showing generalized spike and slow wave activity; (D) Inter ictal EEG from Patient 10: showing irregular spike/sharp and slow wave activity, more prominent in the left temporal and occipital region; (E) Ictal EEG from Patient 10: showing right sided sharp waves and left sided slow waves.

7.3.4.4 Growth parameters (with reference to World Health Organisation [WHO] growth charts)

The overall picture is one of short stature and progressive microcephaly. The short stature observed was in proportion to head circumference (Figure 7.3c). Birth occipital-frontal circumference (OFC) measurement was available for ten patients. All were below the mean, though only 2/10 had a birth OFC of ≤-2 z-scores. Mean birth OFC was -1.8 z-scores (standard deviation [SD] 0.87). Most recent OFC was available for 20 patients and for 17/20 this was ≤-2 z-scores. Mean most recent OFC was -2.9 z-scores (SD 1.8), which was significantly lower than birth OFC (2 sample t-test, $p = 0.018$). Birth length was not available for any of the patients. Most recent height was available for 15 patients of whom 12 had a z-score ≤-2. Mean most recent height was -2.6 z scores (SD 1.5) and this was not significantly different from most recent OFC (2 sample t-test, $p>0.05$).

7.3.4.5 Additional features

Abnormalities of muscle tone were reported in 17/24 patients for whom information was available: Eight had a combination of axial hypotonia and peripheral hypertonia and nine had hypotonia only.

Dysmorphic features were reported in 16/24 patients for whom information was available. The features reported were variable. In no case was the appearance considered to be characteristic of CdLS. I reviewed the photographs of four patients with a consultant clinical geneticist (SJ) and we concluded that common features included a flattened mid-face, a short, upturned nose, and a shallow philtrum (Figure 7.3d).

10 patients had congenital anomalies, including five with cardiac defects, three with cleft palate, two with bifid vertebrae, and one each with bilateral talipes, cystic kidneys, choanal atresia, semilobar holoprosencephaly, and diaphragmatic hernia.

MRI brain reports were available for 21 patients, with nine reportedly normal. Patient 9 had semilobar holoprocencephaly. The other 11 abnormal scans demonstrated non-specific findings, including thin or abnormally shaped corpus callosum (n = 3), white matter signal change (n = 3) and cerebral volume loss (n = 7).

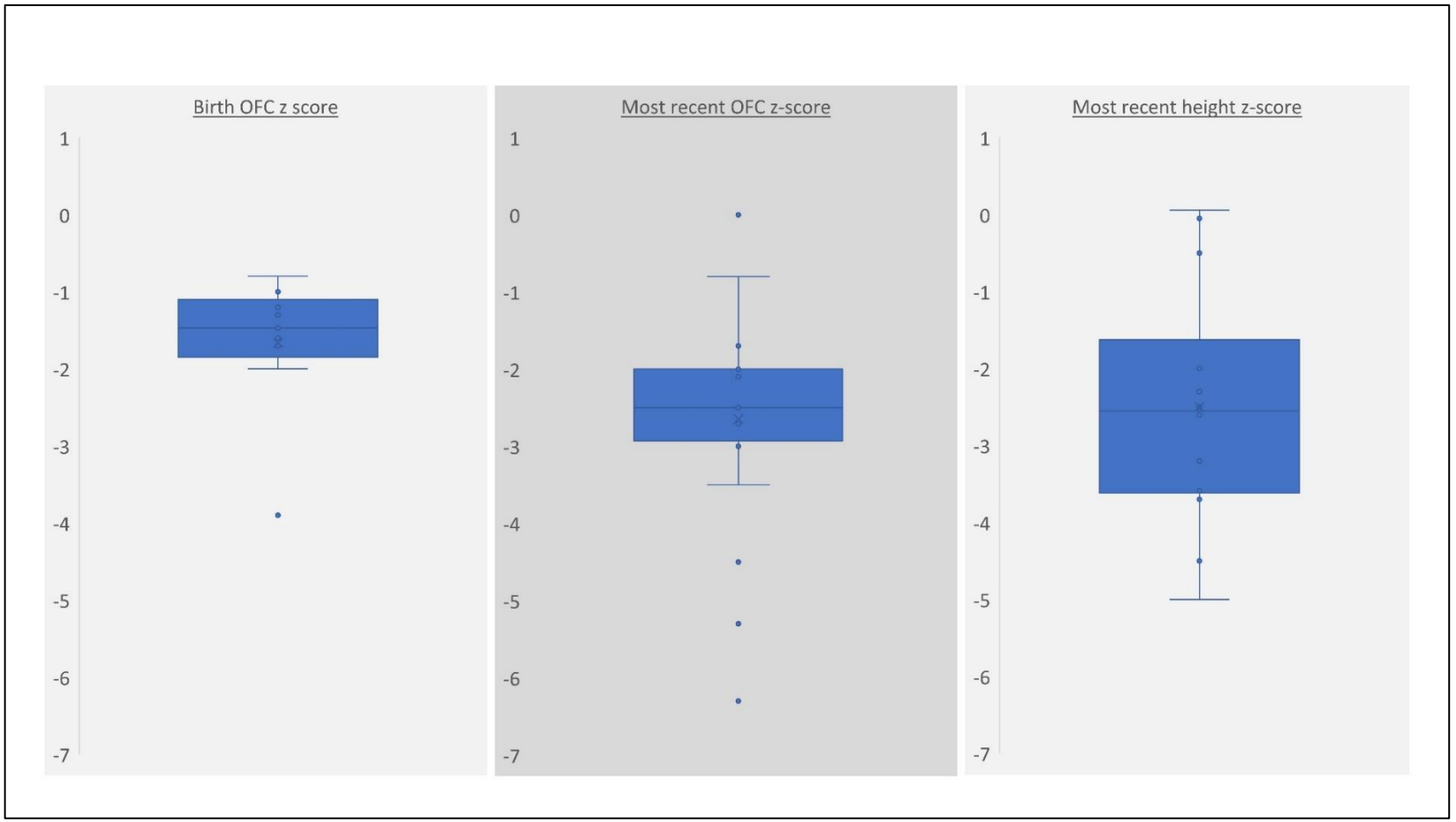


Figure 7.3c: Growth parameters of patients with *SMC1A* truncations

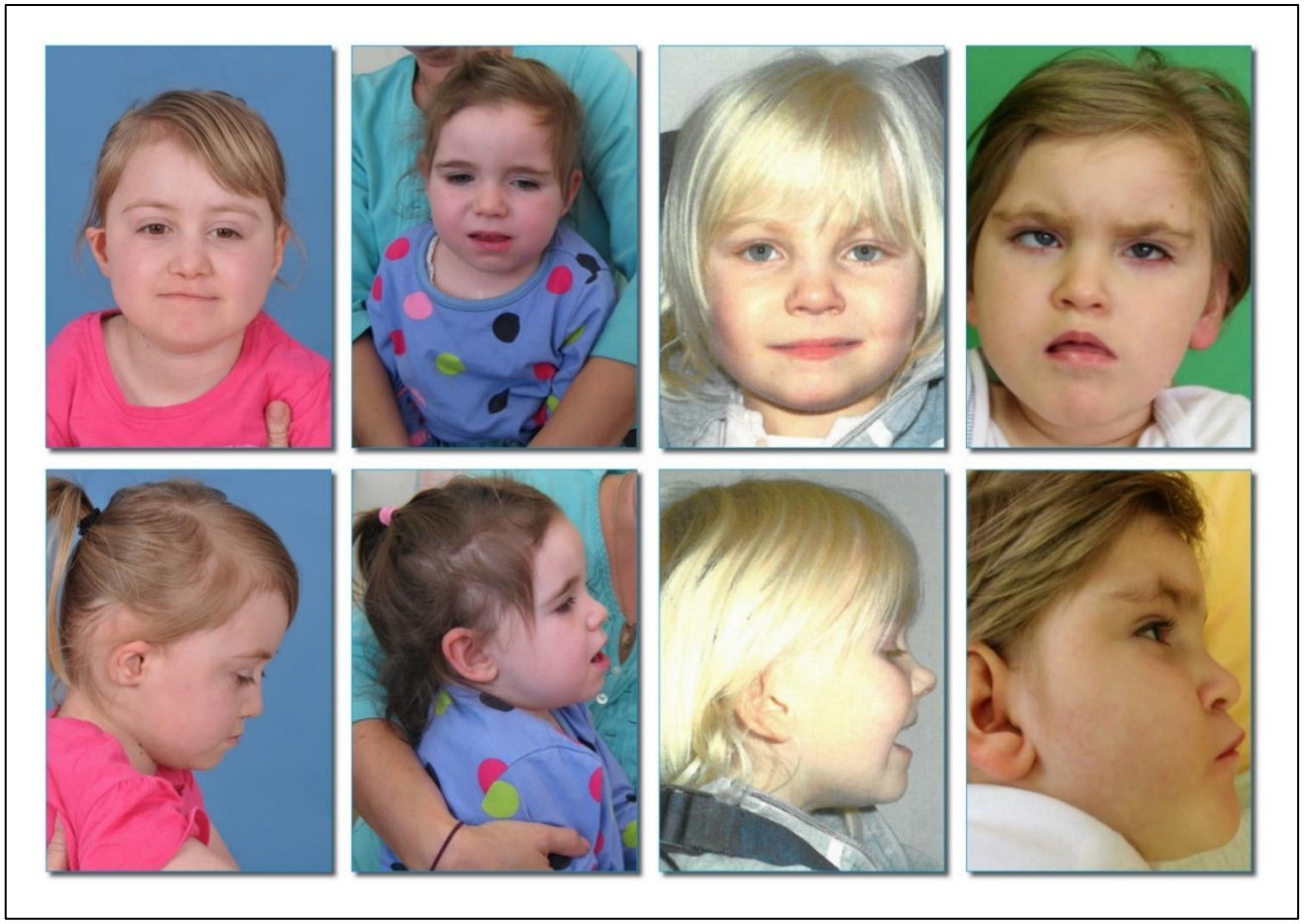


Figure 7.3d: Facial appearances of patients with *SMC1A* truncations; from left to right, patients 6, 4, 8 and 10. Figure as published appears in *Epilepsia* publication (Symonds et al., 2017) - republished here with permission of Wiley and Sons publishers. Written consent obtained from parents of all participants.

7.3.5 Therapy response

In the phenotype form clinicians were asked to report all medications or other therapies (such as Ketogenic Diet, Vagal Nerve Stimulation or surgery) used, and to highlight any that had been associated with a notable decrease in seizure frequency. These sections of the form were completed for 15/17 of the new patients. All 15 had drug-resistant epilepsy. Between the 15 patients 96 therapy trials had taken place (mean 6.4 per patient), 24 of which were reported to be successful.

Eight therapies were trialled in more than five patients (Figure 7.3e). Of these, the most successful were: Ketogenic Diet (4/6 responded), Sodium Valproate (5/13) and Levetiracetam (5/14). Topiramate was trialled in 12 patients and was successful in none of them. Trails of Carbamazepine, Clobazam and Lamotrigine were also undertaken in five or more patients without any reports of success.

The only therapy that was significantly more successful than other therapies was the Ketogenic Diet (Fisher's exact, $p = 0.043$, Odds ratio 6.3, 95% Confidence intervals 1.1-37.0).

Isolated cases of dramatic therapy responses were reported in the case of patient 2 (became seizure free after starting Gabapentin), patient 4 (became seizure-free after starting Phenobarbital), and Patient 6 (seizure-free for 12 months after starting Levetiracetam).

Among the nine patients reported in the literature, medication response was reported in five. All were had drug-resistant seizures. In contrast to the new cases, three patients had been treated with the Ketogenic Diet without apparent benefit. Among the five case reports only one successful treatment is described. The second patient reported by Jansen et al. was commenced on hormonal therapy with oestrogen, in view of the fact that her seizures occurred in clusters every four weeks. The authors reported that this therapy "appeared to have a positive effect

on seizure frequency and that seizure clusters by the age of 14, when still on oestrogen therapy, were occurring every eight weeks (Jansen et al., 2016)."

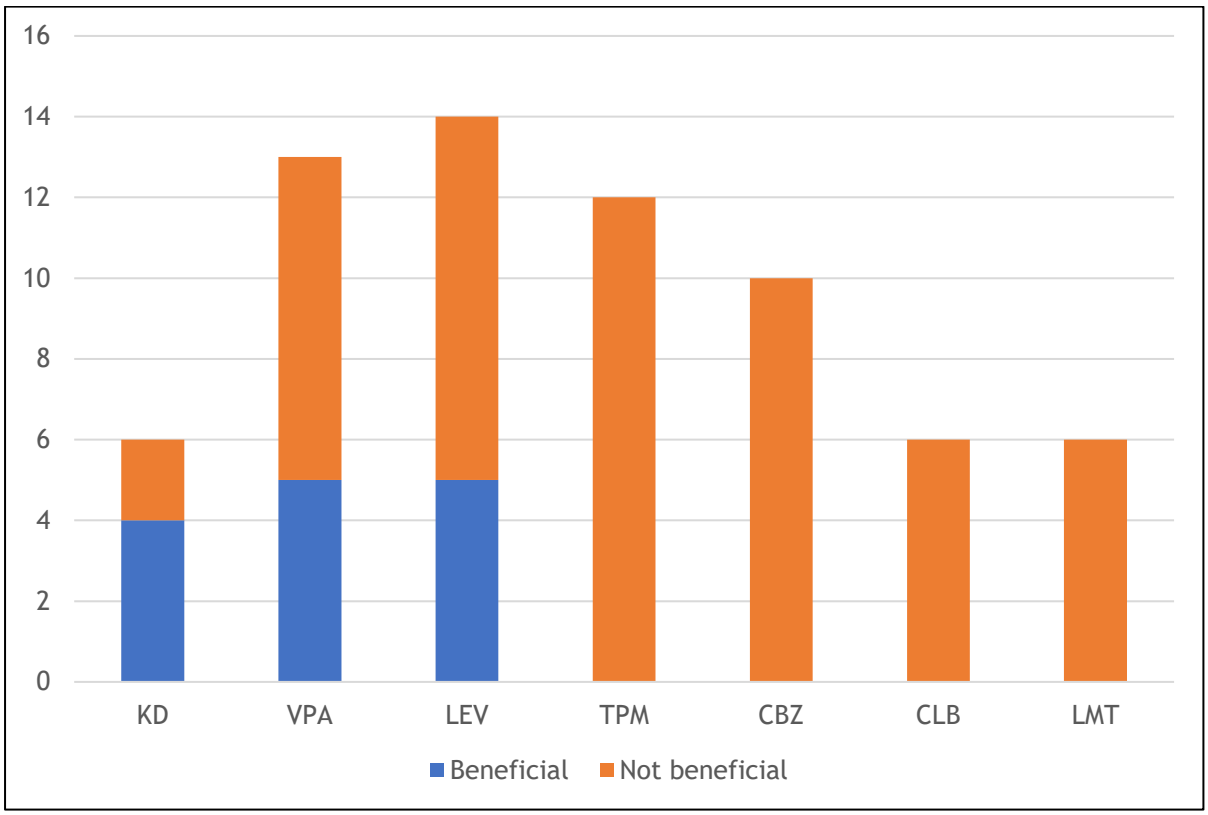


Figure 7.3e: Therapy trials applied to five or more patients with *SMC1A* truncation; Abbreviations: KD – Ketogenic Diet; VPA – Sodium Valproate; LEV – Levetiracetam; LMT – Lamotrigine; CLB – Clobazam; TPM – Topiramate; CBZ – Carbamazepine

Table 7.3b: Ketogenic Diet response in the six patients with *SMC1A* truncation for whom it was trialled

Patient	Ketogenic Diet response
2	Introduced aged 2 years, tried for 12 months then withdrawn, no reported benefit
5	Introduced aged 4 years. Allowed 2 AEDs (Clobazam and Phenobarbital) to be weaned
6	Introduced aged 3 years. Associated with decrease in seizure cluster frequency from once per 2 weeks to once per 6 months
8	Introduced aged 16 years. Associated with decrease in seizure cluster frequency from once per 2 weeks to once per 6 weeks
13	Introduced aged 3 years. Associated with improved seizure control and developmental progress
15	Used but no reported benefit

7.4 Discussion

7.4.1 A novel neurodevelopmental syndrome

Analysis of these 25 patients has allowed delineation of a distinct X-linked neurodevelopmental disorder associated with truncating variants in the *SMC1A* gene. This condition is restricted to females. Males with truncations may be non-viable. The evidence that these variants are causative of the phenotypes observed is strong: all the variants are predicted to result in truncation of the smc1 protein; truncating variants of *SMC1A* are not observed at all in the ExAC database (The Broad Institute Exome Aggregation Consortium, 2018); and there is a broad similarity between the phenotypes reported. The most notable features are drug-resistant epilepsy, progressive microcephaly, short stature, and tone abnormalities consisting of axial hypotonia with or without peripheral hypertonia. In several patients a notable pattern of seizure clustering is observed. Since eight of the patients were ascertained from the DDD study – which had broad inclusion criteria and in which only 24% of participants overall had seizures, and since no patients with *SMC1A* truncation variants who *did not* have drug-resistant epilepsy (DRE) were identified in the DDD study (Wright et al., 2015) – it can be concluded that the penetrance of *SMC1A* for a DRE phenotype is strong.

7.4.2 Cohesinopathy

SMC1A encodes the smc1 protein. Smc1 is a structural component of the cohesin complex (Musio et al., 2006). This is a multi-protein ring-shaped structure which is composed of four proteins: smc1, smc3, scc1, and sa. The cohesin complex binds to DNA and regulates its interaction with chromatin. The complex is believed to have diverse roles in cell division, transcriptional regulation and DNA repair (Horsfield, Print & Mönnich, 2012). Loading of cohesin onto DNA is dependent on a dimer composed of two proteins: nipbl and mau2. Off-loading of cohesin from DNA is carried out by walp and pds5, which open the interface between smc3 and rad21 (Horsfield, Print & Mönnich, 2012) (see Figure 7.4a and table 7.4a).

Of the four genes encoding structural components of the cohesin complex (*SMC1A, SMC3, RAD21* and *STAG1*) the first three are associated with Cornelia de Lange syndrome (CdLS), a multiorgan developmental syndrome first described in 1849 (Vrolick, 1849). Variants in *NIPBL* and *HDAC8*, which both encode important regulators of the cohesin complex, are also associated with CdLS. The final structural component of the cohesin complex, sa, is encoded by the *STAG1* gene. Variants in this gene have not been associated with CdLS, but with a separate neurodevelopmental disorder. Lehalle et al. reported 13 individuals with *de novo* heterozygous variants in, or deletions of, *STAG1*. All had intellectual disability, ranging from mild to severe. 11 patients had facial dysmorphism though this was not characteristic of CdLS. Five patients had epilepsy. Interestingly only two had microcephaly and only one had short stature. The epilepsy was not phenotyped in detail, but in at least two patients it was controlled with medication (Lehalle et al., 2017).

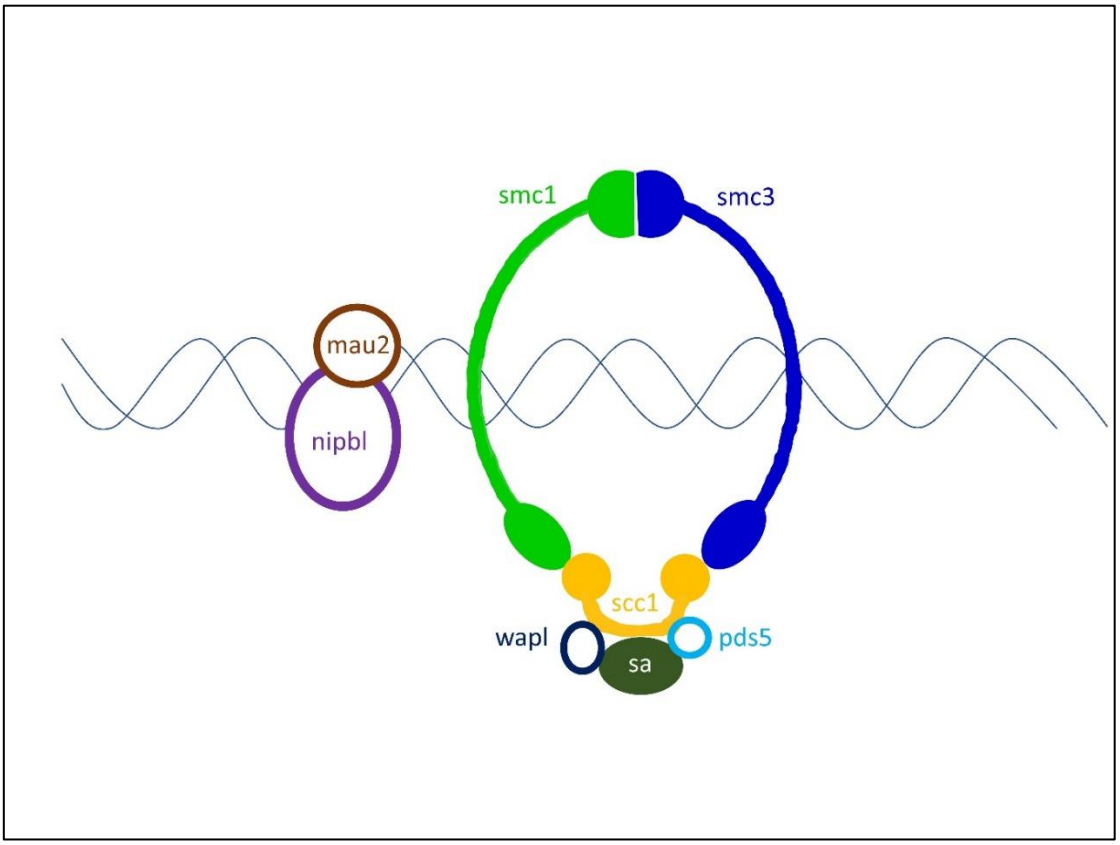


Figure 7.4a: Schematic of the cohesin complex and its regulators

Table 7.4a: Genes and proteins involved in the cohesin complex, and their associations with disease

Protein	Full name	Gene	Locus	Role	Association with disease
Smc1	Structural maintenance of chromosomes-1	*SMC1A*	Xp11.22	Structural component of cohesin ring	Heterozygous missense variants associated with CdLS (Musio et al., 2006)
Smc3	Structural maintenance of chromosomes-1	*SMC3*	10q25.2	Structural component of cohesin ring	Heterozygous missense variants associated with CdLS (Deardorff et al., 2006)
Scc1	Sister chromatid cohesion-1	*RAD21*	8q24.11	Structural component of cohesin ring	Heterozygous missense variants associated with CdLS (Deardorff et al., 2012)
Sa	Cohesin subunit 1a	*STAG1*	3q22.3	Structural component of cohesin ring	Heterozygous missense variants, truncating variants, and deletions associated with intellectual disability and epilepsy (Lehalle et al., 2017)
Wapl	Wings apart-like protein	*WAPL*	10.q23.2	Off-loads cohesin from chromatin	None
Pds5	Pds5 regulator of cohesion maintenance, 5a	*PDS5A*	4p14	Off-loads cohesin from chromatin	None
Nipbl	Nipped B-like	*NIPBL*	5p13.2	Loads cohesin onto chromatin	Heterozygous missense variants and truncating variants associated with CdLS (Krantz et al., 2004)
Mau2	Mau2 chromatic cohesion factor	*MAU2*	19p13.11	Loads cohesin onto chromatin	None
Hdac8	Histone deacetylase 8	*HDAC8*	Xq13.1	Involved in the recycling of cohesin	Heterozygous missense and truncating variants associated with CdLS (Deardorff et al., 2012)

7.4.3 Similarities and differences with Cornelia de Lange syndrome

Patients with CdLS have a characteristic facial appearance. Synophrys (single eyebrow) is considered an obligatory diagnostic criterion for the condition (Kline et al., 2007). Other typical facial features include short nose with anteverted nares, long philtrum, thin upper vermilion, high palate, and a small square chin (Boyle et

al., 2015). There is striking pre-and post-natal growth restriction, often associated with feeding difficulties and gastro-oesophageal reflux (Luzzani et al., 2003). Upper extremity malformations, ranging from small hands and/or digital anomalies to completely absent forearm(s) are observed in 27% of patients (Jackson et al., 1993). Congenital organ anomalies are common in these patients. 20-35% have congenital cardiac disease – most commonly ventricular septal defect, atrial septal defect, tetralogy of Fallot, and hypoplastic left heart syndrome – and 40% have structural renal tract anomalies (Boyle et al., 2015). CdLS related to variants in *SMC1A, SMC3* and *RAD21* is considered to be a milder form that that associated with variants in *NIPBL* or *HDAC8*. There are milder cognitive impairments, milder skeletal anomalies, and major organ anomalies are considered rare (Boyle et al., 2015; Mannini et al., 2013).

Dysmorphic features were observed in the majority of the patients reported here (15/23) but in none were they considered to be characteristic of CdLS. Only one patient had synophrys (Lebrun et al., 2015). 10/23 had minor upper limb malformations, which is similar to the proportion observed in CdLS series (Jackson et al., 1993). The most obvious similarity between the patients reported here and CdLS is the shot stature and proportionate microcephaly.

In case series of patients with CdLS between 4% and 80% have epilepsy. Epilepsy does not appear to be more common in *SMC1A*-associated CdLS compared with other cohesin complex genes, though the number of CdLS patients reported in which both genotype and epilepsy phenotype detail is described is small (Pavlidis et al., 2014; Musio et al., 2006; Deardorff et al., 2006; Pie et al., 2010; Borck et al., 2007). Focal seizures are the predominant seizure type in CdLS-associated epilepsy, and EEGs typically demonstrate focal or multifocal epileptiform abnormalities. In the majority of cases of CdLS-associated epilepsy seizure control is achieved (Pavlidis et al., 2014; Berney, Ireland & Burn, 1999; Verrotti et al., 2013).

In summary, female patients with *SMC1A* truncation have some phenotypic similarities with those with CdLS associated with *SMC1A* missense variants, including short stature, microcephaly, minor limb anomalies, and intellectual disability. However, this disorder is distinct from CdLS in that drug-resistant epilepsy is seen in all cases, the degree of developmental impairment is usually more severe, and the typical dysmorphic features of CdLS are absent.

7.4.4 Cohesin, transcriptional regulation and epilepsy

Evidence that cohesin plays a role in gene transcription comes largely from RNA sequencing (transcriptomic) experiments in which cohesin function is disrupted and differences in relative gene expression are compared between cases and controls (Dorsett, 2011). For example, in Drosophila, cleavage of scc1 (*RAD21)* resulted in reduced transcription of a specific subset of genes, including a number that are regulated by the steroid hormone ecdysone (Pauli et al., 2010), the receptor for which plays a role in postmitotic axon pruning (Schuldiner et al., 2008). Significant alterations in the transcriptome are also observed in knockout models of *NIPBL* and *SMC1A* (Dorsett, 2011).

Lui et al. generated lymphoblastoid cell lines (LCLs) from 16 patients with *NIPBL*-associated CdLS. They identified a unique profile of dysregulated gene expression which they were able to correlate with the severity of phenotype (Liu et al., 2009). Mannini et al. compared LCL transcriptome profiles between seven patients with CdLS-associated missense variants in *SMC1A* and four controls. They identified 571 differentially upregulated genes and 616 differentially downregulated genes (Mannini et al., 2015). The authors used automated analysis to identify patterns of differentially expressed genes. Pathways implicated included those involved in glucose and lipid synthesis, the NOTCH pathway which is essential for neuronal and cardiac development (Bray, 2006), and the EGF-EGFR (Epidermal Growth Factor [Receptor]) pathway which promotes cell proliferation, differentiation and migration (Yarden & Sliwkowski, 2001). These findings must be interpreted with caution due to low numbers of cases and controls (Conesa et al., 2016). The

transcriptome profiles of patients with truncating variants in *SMC1A* have not yet been investigated so it is not known how these may relate to those of patients with *SMC1A* missense variants.

7.4.5 Therapy response

Analysis of therapy response was limited by data precision and small numbers. An ideal study would prospectively study objective measures of response to therapy such as proportionate decrease in seizure or seizure cluster frequency. It would also be more precise to ascertain exact doses and timings of drug therapies, as well as specific details of carbohydrate ratios for those treated with the Ketogenic Diet, in order to verify that therapeutic doses were used. Due to the retrospective and multicentre nature of this study it was practically not possible to acquire such precision of data and I have relied on clinicians' general impressions. Despite these limitations, it is worth noting that the three most efficacious therapies in this group of patients were ones that are considered to have diverse or unclear mechanisms of action: Ketogenic Diet (Bough & Rho, 2007), Sodium Valproate and Levetiracetam (White, Smith & Wilcox, 2007). Conversely poor efficacy was reported for therapies with narrow mechanisms of action: Topiramate, Carbamazepine, Clobazam, and Lamotrigine (Bough & Rho, 2007).

Among 14 patients with *SMC1A* truncation the Ketogenic Diet was significantly more efficacious for seizure treatment than other therapies combined. 4/6 patients who were trialled on the Ketogenic Diet were considered to have benefited. Despite over a century of application to treating seizures, the anticonvulsant mechanism(s) of action of the Ketogenic Diet remain unknown. Postulated theories include a direct anticonvulsant effect of ketone bodies through agonist action at GABA-receptors; an impact of glucose restriction on neuronal proliferation, synaptogenesis and/or neuronal membrane stability; metabolic downregulation of the mTOR (Mammalian target of Rapamycin) pathway (McDaniel et al., 2011); membrane stabilising effects of polyunsaturated fatty acids;

improved energy delivery to neurones as a result of a high fat diet; and a modulating effect of the diet on noradrenergic neurotransmission.

The potential therapeutic impact of the Ketogenic Diet through action on cellular growth and proliferation is of particular note. It is a therapy that appears to be particularly effective in patients with mTOR over activation due to Tuberous Sclerosis (Kossoff et al., 2005). Evidence from transcriptome studies suggests that *SMC1A* dysfunction may have an impact on pathways driving cellular proliferation, including NOTCH and EGF-EGFR. NOTCH signalling is implicated in diverse pathologies (neurological, cardiovascular, endocrine, malignant). In a mouse model of NOTCH inhibition, survival was reduced as a result of cardiovascular disease and mTOR was upregulated. Administration of the Ketogenic Diet was associated with improved survival (Jabs et al., 2018).

7.4.6 Future directions

Further advance of this field would benefit from the collection of phenotype and medication response data on a larger cohort of patients with *SMC1A* truncation-related epilepsy. For medication response data to be reliable it should be collected prospectively and objectively, with seizure diaries and medication doses/dietary regimens documented precisely. If a sufficiently large cohort of patients were gathered, a randomised controlled trial of therapy could be considered, with the most promising candidate therapy being the Ketogenic Diet. Since there is mechanistic and phenotypic overlap between *SMC1A*-truncation related epilepsy, *SMC1A*-missense variant-associated CdLS with seizures, and other cohesinopathies in which epilepsy is a symptom, there would be value in more precisely delineating the epilepsy phenotype and therapy response. It is worth noting that patient P-962 in chapter 6 has a *de novo* missense variant in *NIPBL*. She had drug-resistant epilepsy, microcephaly and short stature. She was treated with the Ketogenic Diet but was not deemed to have a significant response. In her case the family felt that the most effective treatment was self-sourced Cannabinoid therapy.

It may be possible to gain a better understanding of the mechanisms of pathogenesis in *SMC1A*-trunaction related epilepsy by conducting transcriptome analysis in these patients, comparing patterns of gene expression with both *SMC1A*-CdLS patients and with healthy controls. Consideration should be given to the unusual clustering pattern of seizures observed in many of these patients, and to the reported benefit of hormonal therapy with oestrogen in one of the published cases. It is possible that the transcriptional regulation effect of *SMC1A* has a cyclical and hormone-dependent element.

7.5 Conclusion

In this chapter I have discussed five broad mechanisms through which damaging genetic variants can lead to epilepsy. Though there is some degree of overlap between these groups they provide a useful conceptual framework, particularly when considering potential approaches to precision therapy. A significant proportion of monogenic epilepsies can be considered as disorders of transcriptional regulation. These conditions are particularly challenging to the concept of precision therapy since precise targetable biological pathways are difficult to delineate. An example of a genetic epilepsy related to transcriptional dysregulation is that caused by *de novo* truncating variants in the *SMC1A* gene. Careful phenotyping of 25 of these patients has revealed a distinct neurodevelopmental disorder in which non-epilepsy features such as growth parameters, tone, and developmental profile are perhaps more characterising than seizure-related features. All of these patients have drug-resistant epilepsy so there is arguably a pressing need to discover more specific treatments for this condition. The most promising treatment used to date appears to be the Ketogenic Diet. Transcriptome analysis may provide further insights into the biological mechanisms through which disorders of transcriptional regulation lead to as epileptic seizures and may help to guide efforts to develop precision approaches to therapy.

8. Analysis of the relationship between response to Cannabidiol and specific nature of the gene variant in a cohort of children with *SCN1A*-related Dravet Syndrome

8.1 Introduction

8.1.1 Cannabinoids and precision medicine

The idea that Cannabinoids may have anti-convulsant properties is not new. The oldest documented application of the Cannabis-based therapy for epileptic seizures is considered to be inscribed on Sumerian tablets dated to 2000 years BC (Pereira, 1846). Various Arabic texts from between the ninth and 12th centuries provide evidence that Cannabis was administered for its anti-seizure properties (Russo, 2017). The first reference to the application of Cannabis to the treatment of epilepsy in Western medicine is an 1843 case report by Sir William O'Shaughnessy, an Irish physician in service to the British Crown in India. He reported in the *Provinical Medical Journal* the case of a 40-day old girl who presented with up to 30 seizures per day. He administered Cannabis tinctures until her seizures stopped. Subsequently she remained seizure-free and in good health (O'Shaughnessy, 1843). Despite the longevity of its use, until recently the only therapeutic trials of Cannabinoid-based preparations in the treatment of epilepsy involved extremely small numbers of patients. A 2014 Cochrane review concluded that due to low quality of evidence, no "reliable conclusions [could] be drawn regarding the efficacy of Cannabinoids as a treatment for epilepsy."

A recent resurgence of interest in Cannabinoids as epilepsy therapy has been driven by a high profile case reports in which patients with highly drug-resistant and severe epilepsies have apparently demonstrated a dramatic response to Cannabinoid-based therapy (Meacher & Clegg, 2016). The case of Charlotte Figi, a five-year old girl in Colorado was featured in a CNN documentary in 2013.

Charlotte had initially presented with status epilepticus at three months of age. She went on to develop multiple seizure types (tonic, tonic-clonic, and myoclonic) and to demonstrate developmental regression. Her seizures were resistant to at least eight anti-epileptic drugs as well as the Ketogenic Diet. She was diagnosed with Dravet syndrome, and had a *de novo* pathogenic variant in *SCN1A* (c.2791C>T, p.R931C). Before Charlotte was prescribed Cannabinoid therapy she was experiencing 50 generalised tonic-clonic seizures (GTCS) per day. After three months of treatment there had been a 90% reduction in GTCS frequency. At 20 months she was having just 2-3 GTCS per month. The parents also reported improvements in sleep, alertness, behaviour and interaction (Maa & Figi, 2014). Following the profile raised by the CNN documentary, many other parents of children with Dravet syndrome acquired Cannabinoid treatment for their children, with the majority reporting positive results in terms of both seizure control and other factors such as alertness, mood and sleep (Porte & Jacobson, 2013).

8.1.2 Cannabinoid pharmacology

More than 500 different chemical compounds have been isolated from the Cannabis plant (*Cannibis sativa*). The most abundant are Cannabinoids, of which there are more than 100. Cannabinoids have a 21-carbon skeleton, and they act at G-protein coupled "Cannabinoid" receptors in the brain, of which there are many subtypes. Different Cannabinoids have different neuropharmacological properties owing to their specific affinity for the different Cannabinoid receptors (Friedman & Devinsky, 2015). The most abundant and well-studied of the Cannabinoids are Δ9-Tetrahydrocannabinol (Δ9-THC) and Cannabidiol (CBD). Δ9-THC is a potent agonist at CBD1 and CBD2 receptors, which are predominantly found at presynaptic terminals. Δ9-THC is believed to be the principal psychoactive component of Cannabis. The subjective "high" associated with cannabis can be reversed by CBD1 receptor blockade (Huestis et al., 2001). In animal models of epilepsy, Δ9-THC demonstrates both anti-convulsant and pro-convulsant effects (Rosenberg et al., 2015). In contrast, CBD is a non-psychoactive Cannabinoid and has anti-convulsant effects in the majority of animal models (Rosenberg et al., 2015). CBD has low

affinity at CBD1 and CBD2 receptors (Thomas et al., 1998). It is an agonist at transient receptor potential (TRP) channels, serotonin receptors, and glycine receptors. It is an antagonist at GPR55 cannabinoid receptors (Rosenberg et al., 2015). Of these various actions the effect on GPR55 receptors has generated specific interest. GPR55 agonism results in increased intracellular Ca^{2+} levels and vesicular release probability at excitatory hippocampal synapses, which is opposed by CBD (Sylantyev et al., 2013).

The pharmacology of CBD is complex, multi-faceted, and incompletely understood. Nonetheless, owing to both theoretical and animal model evidence that is supportive of an anticonvulsant mechanism in absence of a psychoactive effect, the recent focus on generating evidence for Cannabinoids in the treatment of epilepsy has focused on high CBD, low Δ9-THC preparations. The majority of published successful case reports have been in relation to such preparations (Maa & Figi, 2014; Porter & Jacobson, 2013).

Unfortunately, in regions where Cannabis production and supply is not regulated, it is easy for patients and families to access preparations whose Cannabinoid content is unquantified and potentially inconsistent from one batch to another. In regions where Cannabis is unregulated as a recreational drug there are usually no barriers to Cannabinoid production for therapeutic purposes. As a result pharmaceutical companies are now marketing Cannabinoid preparations without ever having generated a robust evidence base to support their efficacy and tolerability (Lough, 2015).

8.1.3 Towards an evidence base

Recent efforts to generate an evidence base to support the use of Cannabinoids in the treatment of epilepsy have focussed on CBD.

In 2016 Devinsky et al. published the first open label uncontrolled study of purified CBD for epilepsy. They recruited 214 children with drug-resistant epilepsies, of

whom 33 had Dravet syndrome. Patients were given CBD titrated to a maximum dose of 25mg/kg per day or 50mg/kg per day depending on study site. 79% of patients experienced adverse effects, the most common of which were somnolence (25%), decreased appetite (19%), and diarrhoea (19%). 3% discontinued treatment because of adverse effects. The primary efficacy endpoint was reduction in the number of motor seizures per month compared to baseline and there was no significant difference (Devinsky et al., 2016).

In 2017 the first randomised-controlled trial (RCT) of CBD in epilepsy was published. Owing to the previous impressive anecdotes, the patient cohort selected was patients with Dravet Syndrome. 120 children were recruited, of whom 61 were randomised to CBD (20mg/kg per day) and 59 to placebo. Double blinding methodology was used. The primary outcome measure was percentage reduction in convulsive seizure frequency between four-week baseline and 14-week treatment period (including two weeks of dose escalation to 20mg/kg per day and 12 weeks maintenance). There was a significant difference between the groups. The median frequency of convulsive seizures per month reduced from 12.4 to 5.9 (38.9%) in the CBD group, compared with 14.9 to 14.1 (13.3%) in the placebo group. ($p = 0.01$). 5% of the CBD group became seizure-free compared with one of the placebo group ($p=0.08$). The adverse effect profile was similar to that observed in the open label study (Devinsky et al., 2017).

CBD has subsequently undergone two double blind RCT trials in another form of childhood onset epilepsy, Lennox-Gastaut Syndrome (LGS). Here statistically significant effects were reported. Drop seizures are common in LGS so in these trials efficacy was measured in terms of reduction in drop seizure frequency between baseline and treatment period. In the first trial (GWPCARE3, $n = 225$) patients were randomised to high dose CBD (20mg/kg per day) low dose CBD (10mg/kg per day) or placebo. Median drop in drop seizure frequency was 17.2% in the placebo group, compared with 41.9% in the high dose CBD group ($p=0.005$) and 37.2% in the low dose CBD group ($p=0.002$) (Devinsky et al., 2018). In the second trial (GWPCARE4, $n = 171$), patients were randomised to either 20mg/kg per day

CBD or placebo, and median reduction in drop seizure frequency was 43.9% versus 21.8% ($p = 0.0135$) (Thiele et al., 2018).

The success of CBD in LGS, which is an aetiologically heterogenous epilepsy, suggests that the drug, rather than having a specific effect on patients with Dravet Syndrome and *SCN1A* variants, may have broad anti-epileptic properties. Nonetheless, in view of the compelling anecdotal reports of extreme treatment success in some patients with Dravet Syndrome, a closer look at CBD response is warranted. It is possible that CBD response depends on the nature and location of the *SCN1A* variant.

8.2 Methods

8.2.1 Participants

The subjects in this study were all patients with Dravet Syndrome who were recruited to the multicentre double-blind RCT of CBD versus placebo (GWPCARE1) which involved 23 centres in the United States and Europe. For the trial, inclusion was dependent on a clinical diagnosis of Dravet Syndrome as determined by their clinician. To be included in the trial, patients had to be aged between two and 18 years, they had to have ongoing seizures despite treatment with antiepileptic medication, and any current anti-epileptic medication doses had to been unchanged for four weeks prior to trial commencement. Seizure diaries were kept during a four-week baseline period. Patients were excluded if they had fewer than four convulsive seizures during the four-week baseline period.

8.2.2 Procedures

Informed consent was obtained from parents or legal guardians. Caregivers were trained to record daily seizure activity. Participants were then assigned on a 1:1 ratio to receive either CBD, with the dose escalated to 20mg/kg/day over 14 days, or placebo. Daily total was administered in two divided doses. Treatment and seizure diary recoding continued for 14 weeks.

8.2.3 Trial outcomes

The primary outcome measure in the trial was median reduction in convulsive seizure frequency between the four-week baseline period and the 14-week treatment period. Safety data were also obtained. Overall trial outcomes were published in the New England Journal of Medicine in 2017 (Devinsky et al., 2017).

8.2.4 *SCN1A* variant information

Though 120 participants completed the GWPCARE1 trial, only patients with confirmed pathogenic variants in the coding sequence of *SCN1A* were included in this analysis. Variant information was either provided by the recruiting clinician or was obtained through private testing organised by the trial's funder's, GW Pharma®. I reviewed the variant details in each case and only included those variants were either published cases in association with Dravet Syndrome, or had high pathogenicity scores on all *in silico* tools used: Align, Polyphen-2, SIFT, and Mutation Taster. For missense variants, variant location was classified according to the functional domain of the sodium channel affected by the variant.

Table 8.2a: Definition of the functional regions of the $Na_v1.1$ channel; Greyed rows indicate regions in which no pathogenic variants were identified.

Code	Region
C	Cytoplasmic loop between transmembrane segments, or at the C-terminus or N-terminus of the protein
S1	Transmembrane segment 1
S1-S2	Extracellular loop between transmembrane segment 1 and transmembrane segment 2
S2	Transmembrane segment 2
S2-S3	Cytosplasmic loop between transmembrane segment 2 and transmembrane segment 3
S3	Transmembrane segment 3
S3-S4	Extracellular loop between transmembrane segment 3 and transmembrane segment 4
S4	Transmembrane segment 4 (voltage-sensing unit)
S4-S5	Cytosplasmic loop between transmembrane segment 4 and transmembrane segment 5
S5	Transmembrane segment 5
S5-pore	Extracellular loop between transmembrane segment 5 and the pore of the channel
pore	Channel pore region
Pore-S6	Extracellular loop between the pore of the channel and transmembrane segment 6
S6	Transmembrane segment 6

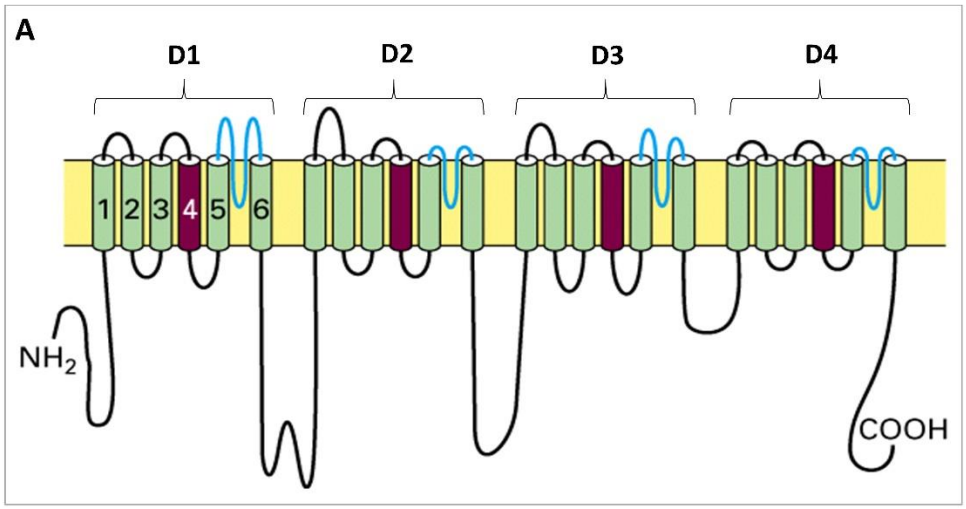
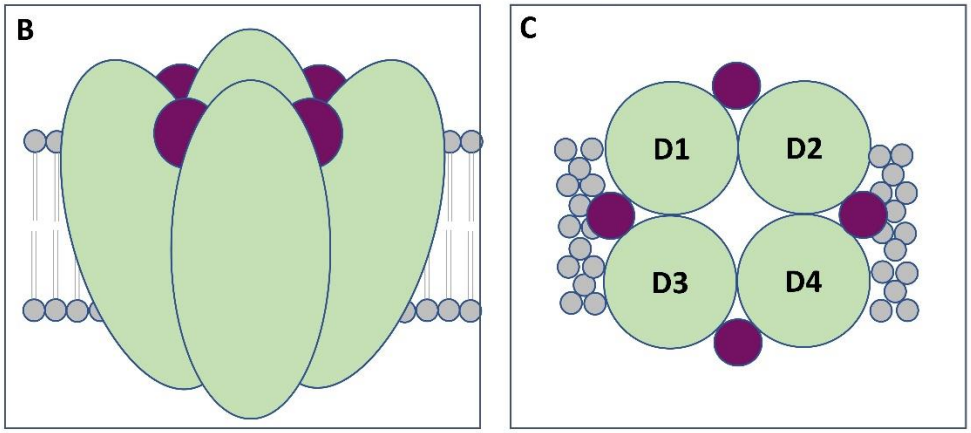


Figure 8.2a. Schematic of the voltage-gated sodium channel: A: momomer; B: transverse; C: axial. Voltage-sensor region coloured purple (no. 4 in A)



8.2.5 Analysis

Participants were divided into two groups: those with truncating variants, and those with missense variants. Within these groups, differences in response to CBD versus placebo in terms of the primary outcome measure were quantified. The Mann-Whitney-U test was applied to determine any significant difference in response between groups. For those patients with missense variants a waterfall

plot of CBD response and a separate waterfall plot of placebo response was produced to see if this highlighted any regions of interest in terms of CBD response. Subsequently, missense variants were further divided into two groups for analysis, again using the Mann-Whitney-U test. These two group were: variants affecting regions between S5 and S6 inclusive (highlighted yellow in table 8.2a); and missense variants elsewhere. For intellectual property reasons GW pharma were unable to hand over the individual patient-response data form the study to me. Statisticians working for GW pharma performed these analyses, though the subgroups were defined by me.

8.3 Results

8.3.1 Exclusions

Of the 120 participants recruited to the RCT, 111 were found to have a pathogenic or likely pathogenic variant in *SCN1A* and were included in these analyses. Of the nine participants excluded, two had benign or likely benign variants in *SCN1A*, two had undergone *SCN1A* testing and were negative, one had unclear information on *SCN1A* variant status, and four had unknown *SCN1A* status.

8.3.2 Truncation versus missense

61 participants had a truncating *SCN1A* variant and 50 had a missense variant.

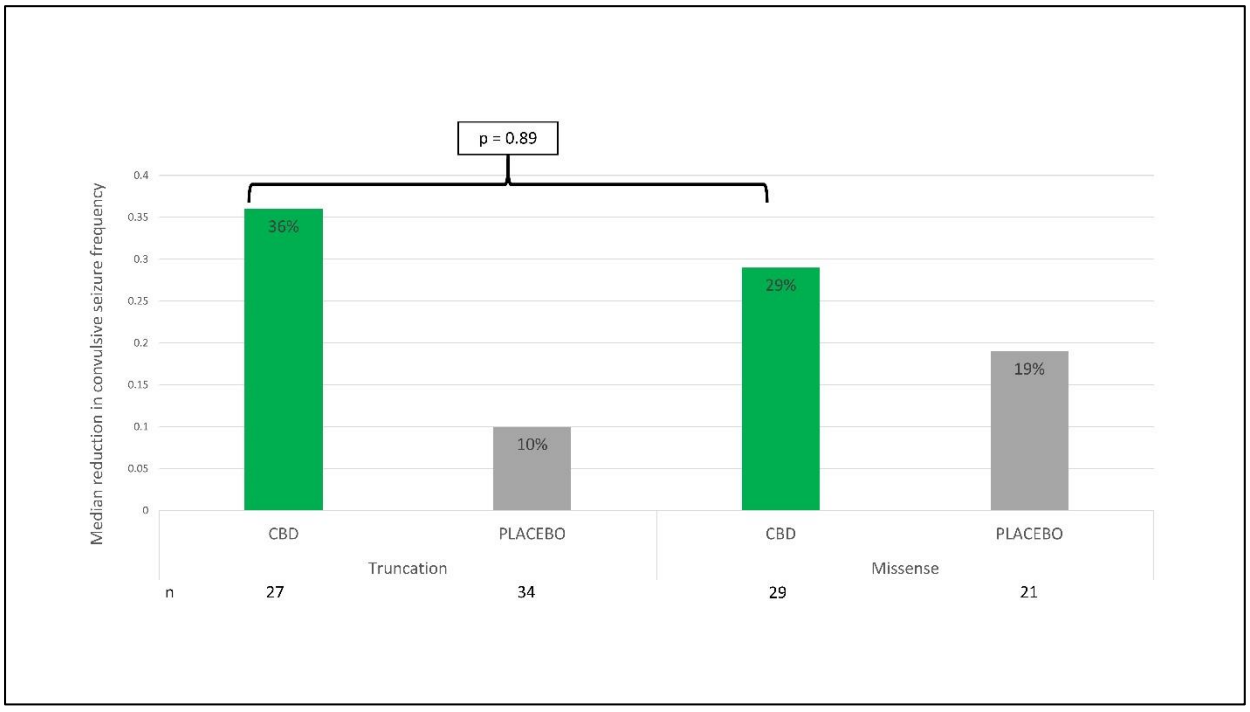


Figure 8.3a: Median reduction in convulsive seizure frequency in patients randomised to CBD and placebo; comparison between those with truncation variants and those with missense variants in *SCN1A*

There was no significant difference in CBD treatment effect between patients with a truncation or missense variant for percent reduction in convulsive seizures (p=0.89); for any of the convulsive seizure responder thresholds of ≥25% (p=0.96), ≥50% (p=0.60), or ≥75% (p=0.41); or for percent reduction in total seizures (p=0.59).

8.3.3 Differences in response by missense domain location

Step one of this process was to produce a "waterfall plot" of CBD and placebo response with each the missense domain location annotated for each participant. Observation of the plots could then be used to identify any trends.

Looking at figures 8.3b and 8.3c it appeared that participants with SCN1A variants in the S5-S6 region who were randomised to CBD were concentrated at the positive response end of the plot, and none of these patients had any significant increase in convulsive seizure frequency. In contrast, those who with missense variants in this region who were randomised to placebo were more evenly spread throughout the plot, with seven of these patients reporting an increase in convulsive seizure frequency. In order to test for significance of this potential interaction I performed a Mann-Whitney-U test on the data, with missense variants divided into "S5-S6" and "non S5-S6" categories (figure 8.3d). Though there appears to be a striking differential in CBD response between these groups, the numbers were small, and this did not reach statistical significance (p = 0.242).

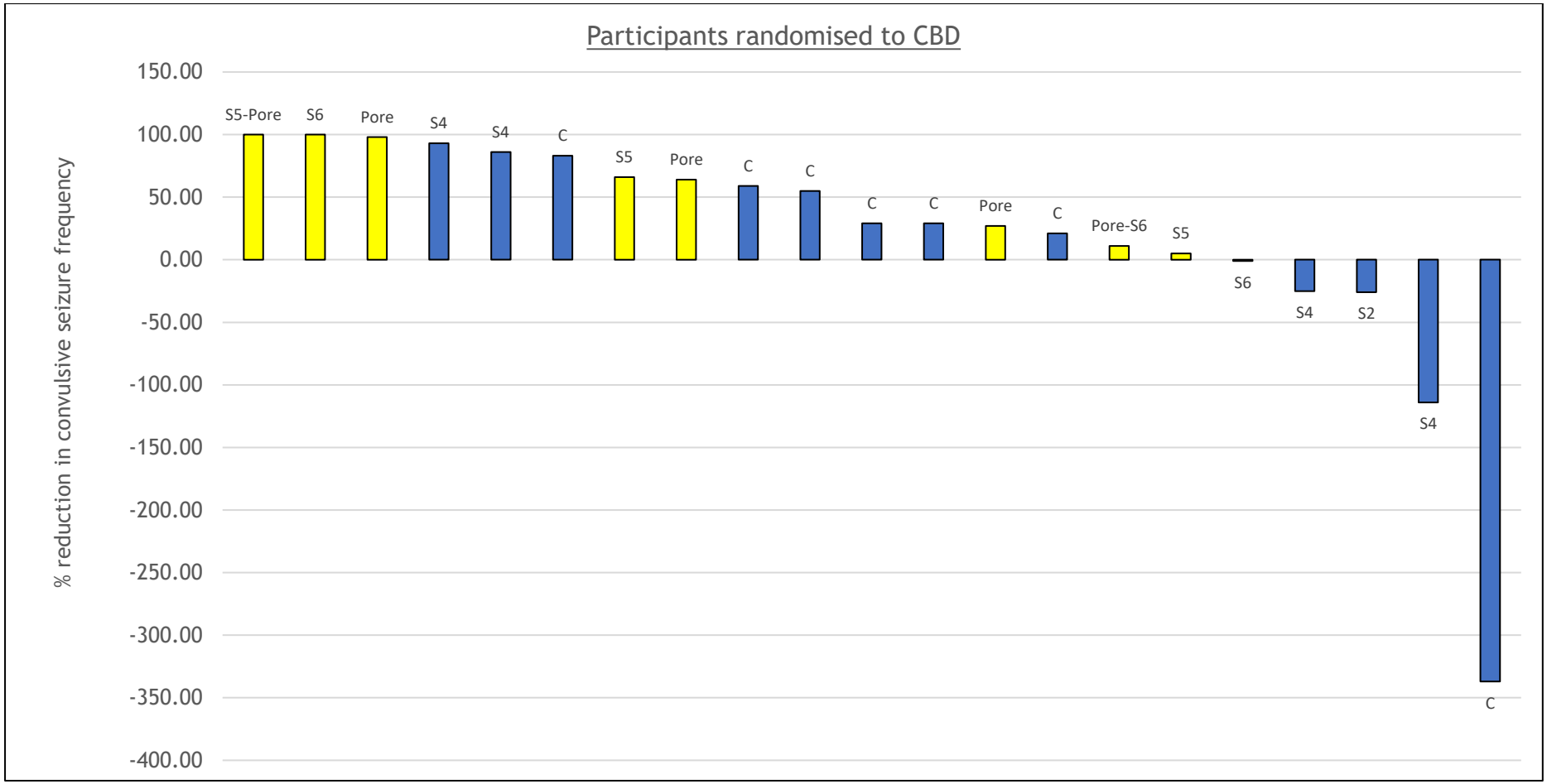


Figure 8.3b: Waterfall plot of CBD response, with missense domain location annotated; each column represents an individual participant; a negative y axis value indicates that a seizure frequency increase occurred; yellow columns indicate participants with variants in the S5-S6 region

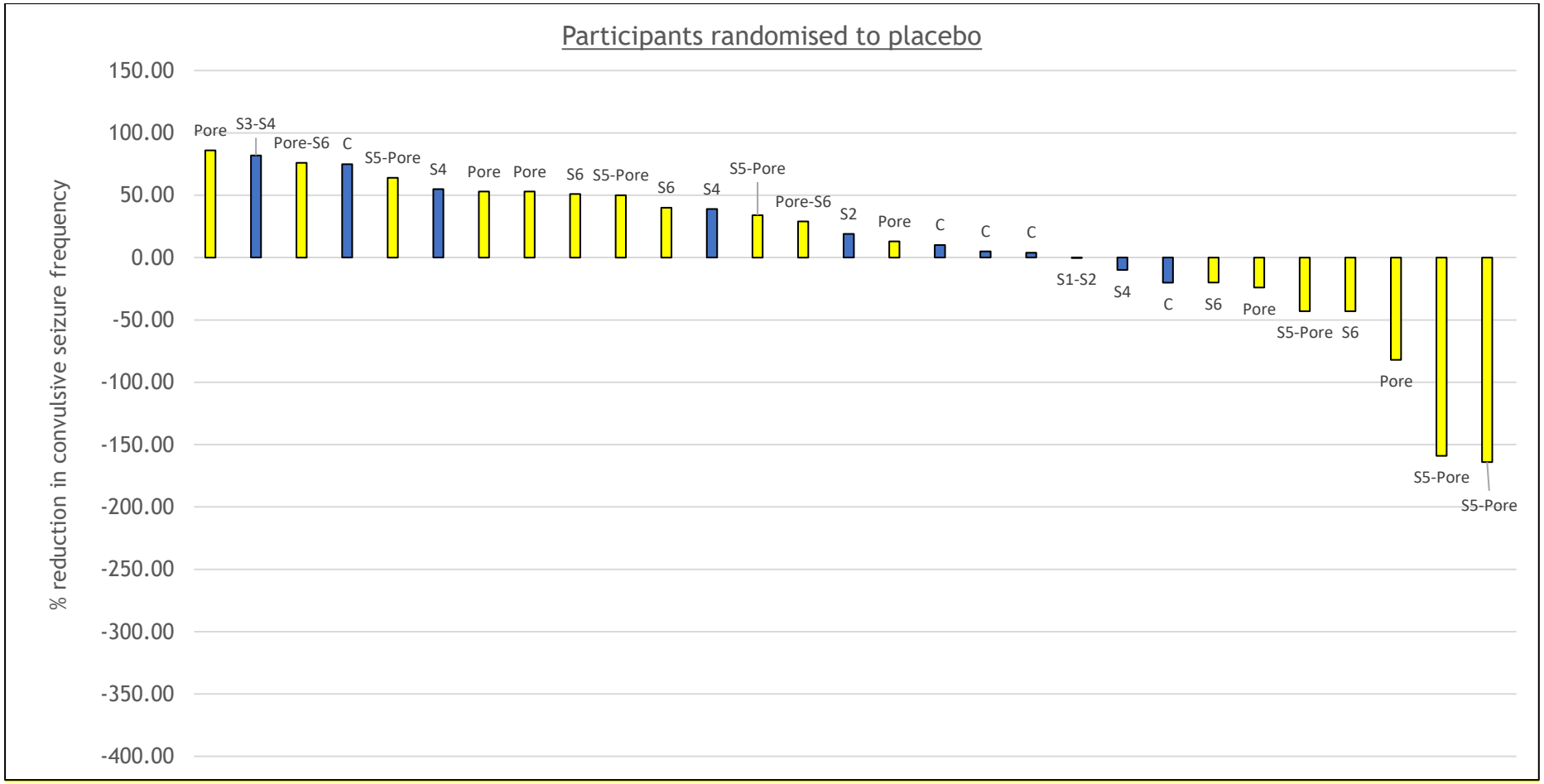


Figure 8.3c: Waterfall plot of placebo response, with missense domain location annotated; each column represents an individual participant; a negative y axis value indicates that seizure frequency increase occurred; yellow columns indicate participants with variants in the S5-S6 region

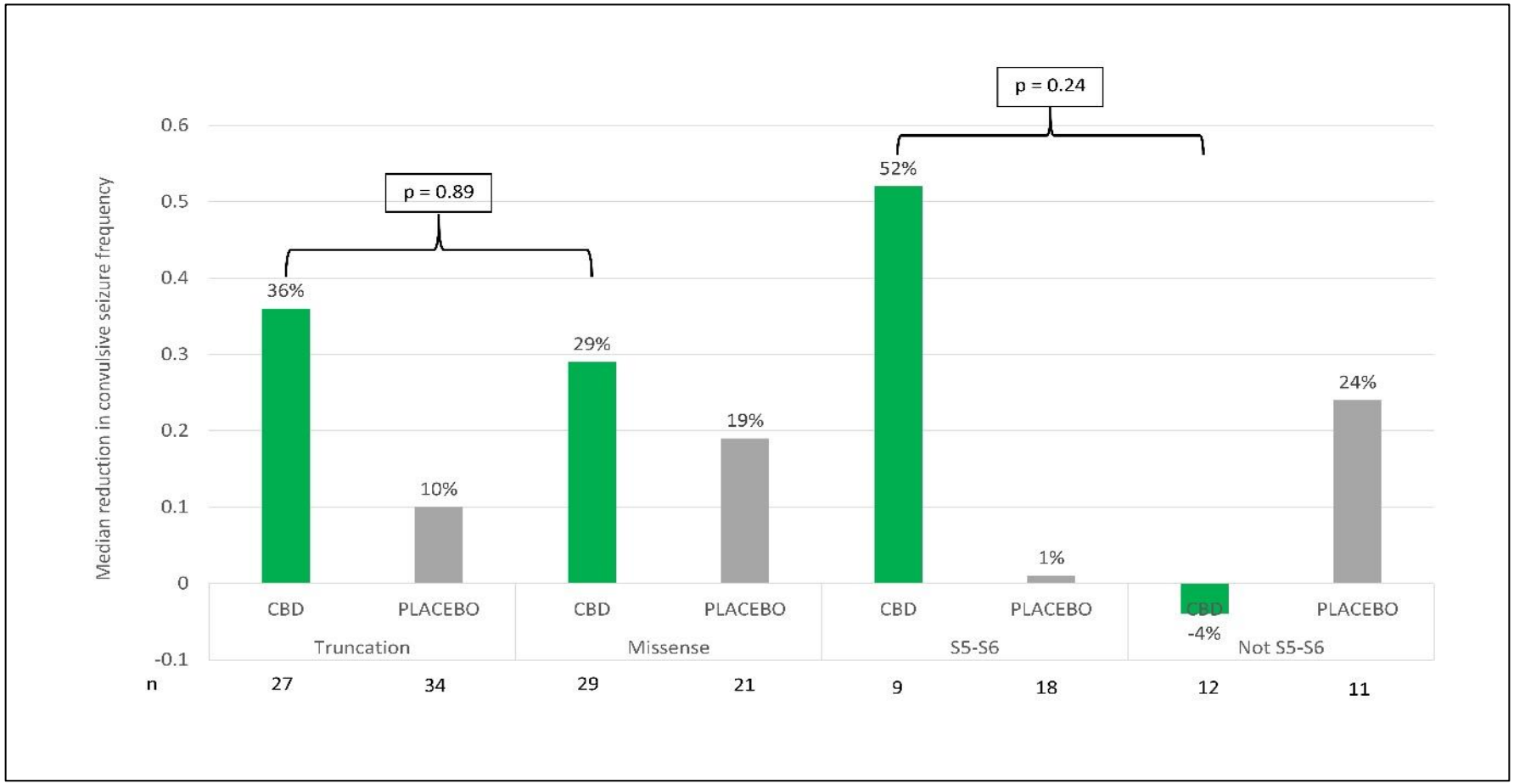


Figure 8.3d: Median reduction in convulsive seizure frequency in patients randomised to CBD and placebo and missense variants in *SCN1A*; comparison of those with S5-S6 variants and those with missense variants elsewhere; truncation and missense data also plotted on this chart

8.4 Discussion

In this chapter, taking data from a randomised-controlled trial, I have attempted the investigate whether variant type (truncation or missense), or variant missense location, are associated with response to Cannabidiol therapy in *SCN1A*-related Dravet syndrome.

One of the potential limitations of taking an approach to epilepsy precision therapy that focuses solely on primary genetic cause, as in chapter 4 of this thesis, is that within each genetic causal group there may be significant heterogeneity of treatment response. Therapy response, like other aspects of phenotype, will depend not only on the causal gene, but on the nature and effect of the causal variant, and on other factors such as genetic modifiers, epigenetic changes, and environmental influences.

It is broadly accepted that in Dravet syndrome, sodium channel blocking medications are best avoided. The potential for these medications to exacerbate seizures in patients with Dravet syndrome was described before the genetic basis for Dravet syndrome was understood (Dravet, 1978). The most commonly prescribed sodium channel blocking medications are Carbamazepine and Lamotrigine. A retrospective review of a large cohort of patients with Dravet syndrome (n = 241) showed that 60% (36/60) of patients treated with Carbamazepine, and 43% (26/60) of patients treated with Lamotrigine, experienced seizure exacerbation whilst taking these mediations (Brunklaus et al., 2012). As well as exacerbating seizures, taking these medications may have an impact on cognitive outcome. de Lange et al. studied 164 Dutch patients with Dravet syndrome and found a significant association between duration of sodium channel blocking therapy use and long-term cognitive outcome (de Lange et al., 2018). Since *SCN1A* is preferentially expressed in inhibitory interneurons (Ogiwara et al., 2007), and since the majority of Dravet syndrome associated *SCN1A* variants appear to result in loss of function, it has been hypothesised that an epileptogenic predisposition in this disease is as a result of reduced inhibitory GABA-ergic

transmission, and that further sodium channel blockade is likely to exacerbate this tendency (Catterall, Kalume & Oakley, 2010).

Contrary to this theory, there appear to be a minority of patients with *SCN1A*-related Dravet syndrome who benefit from sodium channel blocking medication. In their report of three patients with *SCN1A*-related Dravet syndrome who benefited from Lamotrigine, and who experienced deterioration when this mediation was temporarily withdrawn, all three had a missense variant rather than a truncation variant in the gene, prompting the hypothesis that there may be a variant specific effect to this response (Dalic et al., 2015). It is difficult to draw firm conclusions from a small case series like this, since so many uncontrolled factors have the potential to influence medication response, and because in a retrospective and unblinded model subjective factors may come into play when reporting treatment benefit. In contrast, the randomised controlled trial offers an excellent model to investigate the potential role of genetic variant type on treatment response.

In this trial of CBD in Dravet syndrome, 93% of participants had a causative *SCN1A* variant; all had a baseline seizure frequency above a specific threshold; all kept prospective seizure diaries in order to objectively report treatment responses; and all those treated with CBD or placebo received the same dosing schedule whilst all other medications remained unchanged.

Overall median convulsive seizure reduction in the CBD group was 39%, compared with 13% in the placebo group. In the group with truncation variants who were randomised to CBD, median convulsive seizure reduction was 36%, and in those with missense variants it was 29%. Hence there is no evidence to suggest that CBD response depends on variant type. Analysis within the missense variant group showed that median convulsive seizure reduction was 52% in the group with variants in the S5-S6 region, whilst the group with missense variants outwith this region experienced a 4% *increase* in convulsive seizure frequency on CBD treatment. Though this difference appears striking it must be considered with caution for the following reasons: it is based on small numbers; it is not

statistically significant; and these groupings of missense variants were not based on an *a priori* hypothesis but on empirical review of the response data. To determine whether there is any basis to this finding would require testing this hypothesis on another RCT group, ideally with larger numbers of participants. The GWPCARE2 study, enrolled 200 participants with Dravet syndrome to an RCT of CBD versus placebo, and would have provided an opportunity to test the hypothesis generated from the present study. Our research group were initially given permission by GW Pharma® to analyse the data from GWPCARE2. Following presentation of my preliminary analysis to GW Pharma the decision was made to not to proceed analysis of the second cohort.

8.4.1 Limitations of this study:

CBD is arguably not the best medication to investigate for variant specific response in *SCN1A*-related epilepsy, since it is not known to have any direct action on the $Na_V1.1$ sodium channel. It would be interesting to perform similar analysis on patients receiving sodium channel modulating therapy, but since no such RCT has been performed, this is not a possibility. CBD appears to have multiple neuropharmacological actions. There is some *in vitro* evidence, from a single cell model using human embryonic kidney cells, of a modulating effect on $Na_V1.6$ sodium channels, though the clinical significance of this remains to be explored (Patel et al., 2016). In the absence scientific grounds upon which to base a hypothesis, *SCN1A* variants were divided into crude groupings of truncation versus missense variants. Since most Dravet syndrome associated missense variants are believed to act via a loss-of-function mechanism, it is perhaps not surprising that no significant differences in response were observed between these groups. Division of missense variants was also based crudely – on location in relation to the pore forming region of the channel. A more comprehensive evaluation could have considered more precise details of variant location, or even the functional effects of each missense variant. A major barrier to the latter approach is that the majority of disease-associated *SCN1A* missense variants have not been functionally characterised, and of those that have, methodologies have varied between studies.

Of course, the more precision with which one desires to investigate relationships between variant characteristics and treatment response, the larger numbers one will need in any study to achieve sufficient power. It must also be borne in mind that non-pathogenic variants in *SCN1A* may prove equally as important as pathogenic ones when it comes to medication response, as too may variants in other genes, such as those involved in the transport and metabolism of the study drug. From a pharmacogenetic perspective particular focus has been on the rs3812718*AA (IVS5 G>A/c.603-91 G>A) polymorphism of *SCN1A*, which is found in approximately 10% of the population, and is associated with resistance to sodium channel blocking medications across a spectrum of epilepsies (Yip et al., 2013).

8.4.2 Conclusions:

In this chapter I have demonstrated how RCT data can be harnessed to investigate for relationships between causal variants and therapy response in monogenic epilepsies. Due to its relatively high incidence, and its strong association with a single genetic cause, Dravet syndrome provides a particularly good model for this approach. Though no statistically significant relationships were identified, there was a suggestion that patients with missense variants around the pore forming region of the channel may derive particular benefit from CBD. Without access to GWPCARE2 data I was unable to undertake further scientific evaluation of this observation. Pharmacogenetic research is rapidly developing and it is likely that the discipline would benefit from open access to data from pharmaceutical trials in genetic epilepsies.

9. General Discussion

9.1 The research question

The question this thesis asks is: "what is the potential impact of genotype-driven precision therapy for children with epilepsy." This question was something I wanted to explore because during my clinical training I had become aware that clinicians treating children with epilepsy were being presented with rapidly increasing access to genetic testing technologies, enabling them more easily to identify specific genetic aetiologies for these patients and their families. Inevitably, when a family is informed that a genetic cause for their epilepsy has been identified, further questions will be asked: What does this mean? What can we now expect in the future? What are the implications for other family members and/or future children? Families may also ask this: Does this mean there is a specific treatment that should be given? In the case of the condition caused by pathogenic variants in the *SLC2A1* gene, Glut-1 deficiency syndrome (Glut1-D), most paediatric neurologists would agree that the answer here is "yes" (Kossoff et al., 2018). *SLC21A* encodes the principal active transporter of glucose into the human brain. Variants that impair the function of this transporter result in relative deficiency of cerebral glucose and are associated with a constellation of symptoms as a result, including seizures, dystonia, cognitive impairment, and microcephaly (Pearson et al., 2013). Use of the Ketogenic Diet provides the brain with an alternative energy source, bypassing the need for cerebral glucose and overcoming many of the symptoms (Kass et al., 2016). One of the most clinically significant findings arising from this thesis is that the incidence of Glut1-D is more than twice that which has previously been reported (Coman et al., 2006, Larsen et al., 2015b) and that initial symptoms of the condition can be mild and variable (chapter 5). Early introduction of the Ketogenic Diet in the seven patients identified in the Scottish cohort appeared to result in better short-term epilepsy and developmental outcomes than observed in historical controls (Akman et al., 2016). This combination of poor predictability of Glut1-D from early clinical features, alongside good outcomes associated with early therapeutic intervention, makes a

good case for screening for this genetic cause in children presenting with seizures in early childhood. That said, in the absence of a randomised trial, the evidence supporting the use of Ketogenic Diet leaves this a grade C recommendation. A similar scenario plays out across many of the genetic epilepsies. Very few randomised trials of therapy have been performed in defined genetic epilepsies and therefore clinicians resort to low level evidence, or making logical decisions based on what is known about the mechanism of the genetic cause, when deciding on a therapy.

9.1.1 Sub-questions of the research question

In order to understand the potential impact of precision medicine in childhood onset epilepsy, a number of questions need to be answered along the way. First and foremost of these questions is:

9.1.1.1 "How common is childhood-onset epilepsy?"

Through establishing a population-based cohort of children presenting with epilepsy before their third birthday I have shown that epilepsy of onset in these early childhood years is significantly more common than previously estimated (Wirrell et al., 2012) at 1 per 383 live births (chapter 5).

The next logical question is:

9.1.1.2 "How often can we find a monogenic cause of childhood-onset epilepsy?"

Performing a meta-analysis of the published literature on gene panel testing in epilepsy (chapter 3), the answer to this is that diagnostic yield varies markedly between 4% and 50%, but the overall diagnostic rate is 17%. The reason for such variability largely relates to heterogeneity of study populations and of testing protocols. Due to this heterogeneity it is very difficult to draw any conclusions about the epidemiology of individual monogenic epilepsies. Nonetheless is becomes clear that the majority of currently achievable genetic diagnoses are

concentrated in a relatively small number of genes, with *SCN1A, KCNQ2, CDKL5, SCN2A, STXBP1*, and *PCDH19* between them accounting for more than 50% of diagnostic results. In order to overcome the limitations of the meta-analysis in relation to population heterogeneity I analysed the results of gene panel testing in a population-based cohort of children presenting with seizures who were only selected on the basis of age, being under three years at the time of presentation (chapter 5). Using a 104 gene panel, overall diagnostic yield of monogenic seizure disorders was 23.5%. Representing monogenic causes relative to other aetiologies it becomes clear that this is the single largest aetiological category, at least in those presenting before the age of 3 years. In line with the results of the meta-analysis the majority of single gene diagnoses were concentrated in a small number of genes, with 74% of diagnoses involving just eight genes, *PRRT2*, *SCN1A, KCNQ2, SLC2A1, CDKL5, DEPDC5, PCDH19,* and *SLC6A1*. The clear limitation of this epidemiological data is that it tells us nothing about the incidence of genetic epilepsies in those presenting beyond the age of three years. It is likely, though difficult to quantify, that the majority of single gene epilepsies have been captured despite this restriction. This is because there is a marked drop off in yield from genetic testing when age of presentation extends beyond six months, as well as a marked drop in incidence of epilepsy itself, until incidence rises again as age reaches >60 years.

The final key question of the sequence is this:

9.1.1.3 "How often could making single gene diagnosis influence therapeutic approach?"

In chapter 4 I performed a systematic review of the evidence to support precision approaches in the monogenic epilepsies. Only four trials could be considered as grade I evidence, with the majority of evidence being grade III. Nonetheless, assuming all levels of evidence could have the potential to influence a clinician's therapy choice, I was able to estimate that this was the case 85% of genetic diagnoses made in the Scottish cohort, and 71% of the diagnoses made in the meta-

analysis (chapter 3). Combining these findings with the epidemiology from chapter 5 I have shown that for every 2,000 children born, one is likely to develop a monogenic epilepsy for which there is some supportive evidence for a specific therapeutic approach. To put this figure in context, 1 in 130 people born is expected to develop epilepsy at some point in their life (Fiest et al., 2017).

9.2 Common monogenetic epilepsies, and beyond

The meta-analysis of next generation sequencing studies (chapter 3) and population based cohort study (chapter 5) both demonstrated that the majority of currently diagnosable genetic epilepsies are accounted for by a small number of genes. Using the population-based approach I have been able to define the incidence of several of these for the first time, with *PRRT2*-associated epilepsy being the most common, followed by *SCN1A*, *KCNQ2* and *SLC2A1*. Having these incidence figures in the public domain will be valuable for research teams designing observational and interventional studies in the future. Beyond the common monogenic causes there is a long tail of exceedingly rare genetic epilepsies, with 104/210 genes being implicated on just one occasion among 13,063 patients included in the meta-analysis, giving a diagnostic yield of 0.007% per gene. My experience from application of Whole Genome Sequencing technology in this thesis (chapter 6) echoes this. Just one of the diagnoses made among those patients who had been pre-screened on the Glasgow panel of 104 genes implicated a gene that had also been implicated among the 13,063 patients in the meta-analysis (*NEXMIF*, 3 diagnoses). An interesting aspect of these infrequently implicated genes is that many are also implicated in other neurodevelopmental disorders, such as autism and intellectual disability. Some of these monogenic disorders may in fact be more commonly observed in patients without epilepsy than those who have seizures. This observation lends support to the arguments of those who think it best to consider epilepsy as a symptom of an underlying brain disease, rather than a disease entity in itself (Reynolds & Rodin, 2009). Genetic brain disorders that are extremely rare pose a challenge to those trying to progress precision therapy initiatives for the obvious reason that it is difficult to gather a

cohort of patients if those patients are very infrequently encountered. In chapter 7 I have demonstrated how this can be done. Rare genetic brain disorders in which epilepsy is a symptom that may or may not be present, alongside other developmental problems, pose and an additional challenge: they demonstrate that an epileptic predisposition is not a necessary component of that genetic brain disorder and therefore imply that any anti-epileptic treatment approach is unlikely to be aetiology-specific. If it is possible to have the same brain disorder and not have seizures, it has to be questioned whether treating the seizures can be expected to improve the brain disorder.

9.3 Challenges encountered in this thesis

9.3.1 When is an aetiology causative?

An unexpected challenge encountered in developing this research was that of determining when exactly an identified aetiology could be considered sufficient to explain a patient's epilepsy and be deemed causative. This was of critical importance considering many of the analyses undertaken were done on the basis of a binomial system of cause identified/cause *not* identified. This challenge of thresholds of causation manifested in several of the studies undertaken, and partly reflects the binary nature in which clinical medicine tends to operate when it comes to determining aetiology.

9.3.2 Variant interpretation

In the population-based cohorts reported in chapter 5, genetic variants were interpreted and an objective manner. Variants identified from gene panel testing were discussed within an experienced multi-disciplinary team of clinicians and scientists, and this team was assisted by being able to reference the UK Association of Clinical Genetic Science (ACGS) guidelines for variant interpretation (Association for Clinical Genetic Science, 2017). This task was made significantly easier than, for example, interpretation of Whole Genome Sequencing results,

because the gene panel of 104 epilepsy-associated genes only included genes that had been well studied, whose mechanisms were reasonably well-understood, and whose associated phenotypes were well-described. Nonetheless subjective judgement was still an essential component of variant interpretation, particularly since the ACGS guidelines emphasise that variants must always be considered in the context of phenotype. In neurodevelopmental disorders phenotype is often a multifaceted complex of symptoms that may be difficult to objectify. When it came to interpretation of many of the variants identified though Whole Genome Sequencing, taking an objective approach was significantly more difficult since many of the genes implicated had very little clinical literature published on them.

9.3.3 Multiple aetiology

12% of patients in cohort 2 of chapter 5 were deemed to have an aetiology that fell into more than one of the broad categories of genetic, structural, metabolic, infectious, immune. Some such patients had a single aetiology, but one that could be considered to belong to more than one group. For example, tuberous sclerosis is a genetic disorder invariably associated with structural brain malformations. For others there was a genetic disorder or infectious component present which had predisposed them to a secondary event. For example one patient in this study had both trisomy 21 and a hypoxic ischaemic injury, and another had Herpes Simplex Virus encephalitis followed by anti-NMDA receptor autoimmune encephalitis. For others the combination of two aetiologies could be considered as coincidental, for example a patient had both a pathogenic *KCNQ2* variant and accidental head trauma.

In reality, all patients with epilepsy will have multiple aetiological factors contributing to their condition. When one single factor appears to dominate the picture we ascribe causation, though this is process inevitably involves some degree of subjective judgement. Figure 9a illustrates this concept of multiple aetiological factors contributing to an overall phenotype. In this figure I have divided aetiological contributors to phenotype into four groups: environmental

factors, for example head trauma or exposure to infection; common genetic variants; rare genetic variants; and extremely rare genetic variants. Within each of these groups there will be factors that we are currently able to identify and understand and there will be others that remain concealed, so each aetiology group has been divided into explained and unexplained groups. We know from large twin studies that epilepsy is approximately 60% heritable, but that most of this heritability is currently unexplained (Manolio et al., 2009; Kjeldsen et al., 2005; Speed et al., 2012), thus the largest proportion of this figure is occupied by *unexplained* common and rare genetic variants. Here common genetic variants could be considered those that have a population frequency of >1% and rare genetic variants could be considered those that have a population frequency of between 0.01% and 1%, though these distinctions are somewhat arbitrary. Evidence that common genetic variants contribute to epilepsy heritability comes from genome wide association studies (Hibbar et al., 2014; Stefffens et al., 2012; Speed et al., 2014; Tan & Berkovic, 2010) whilst evidence that rare genetic variants contribute comes from burden analysis studies (Allen et al., 2017). Importantly, neither a common genetic variant nor a rare genetic variant is likely to explain the presence of an epilepsy phenotype on its own, but must act in combination with other genetic and environmental risk factors.

The concept of the monogenic epilepsy arises when a single genetic variant, or a pair of recessively inherited variants, is thought sufficient to explain an epilepsy phenotype alone. Invariably, such highly penetrant variants are extremely rare, present <0.01% of the population. This explains why the monogenic epilepsies are individually extremely rare. Such variants will never explain all aspects of a phenotype since there will be genetic and environmental modifiers which explain why two patients with an identical genetic cause can sometimes present quite different phenotypes. Nonetheless, conceptually, there is a threshold where a sufficient proportion of the phenotype is felt to be explained by the variant(s), that it is ascribed as causative. This conceptual threshold is marked A. in figure 9a. We know that monogenic causes are identified more often in severe epilepsies than they are in mild epilepsies, and this is illustrated in the lower portion of

figure 9a. Here mild epilepsies might be those in which seizure control is achieved and cognitive comorbidities are absent and severe epilepsies might be considered those in which there is therapy resistance and/or cognitive comorbidity. In much the same way there is a threshold point at which an environmental factor will be deemed to be sufficient on its own to have caused a person's epilepsy (point B. in figure 9a.). Here again there will genetic modifiers of the phenotype. Occasionally a patient will have both a monogenic and an environmental factor, each of which would be considered sufficient on its own to have caused their epilepsy. This scenario is illustrated as point C. in figure 9a.

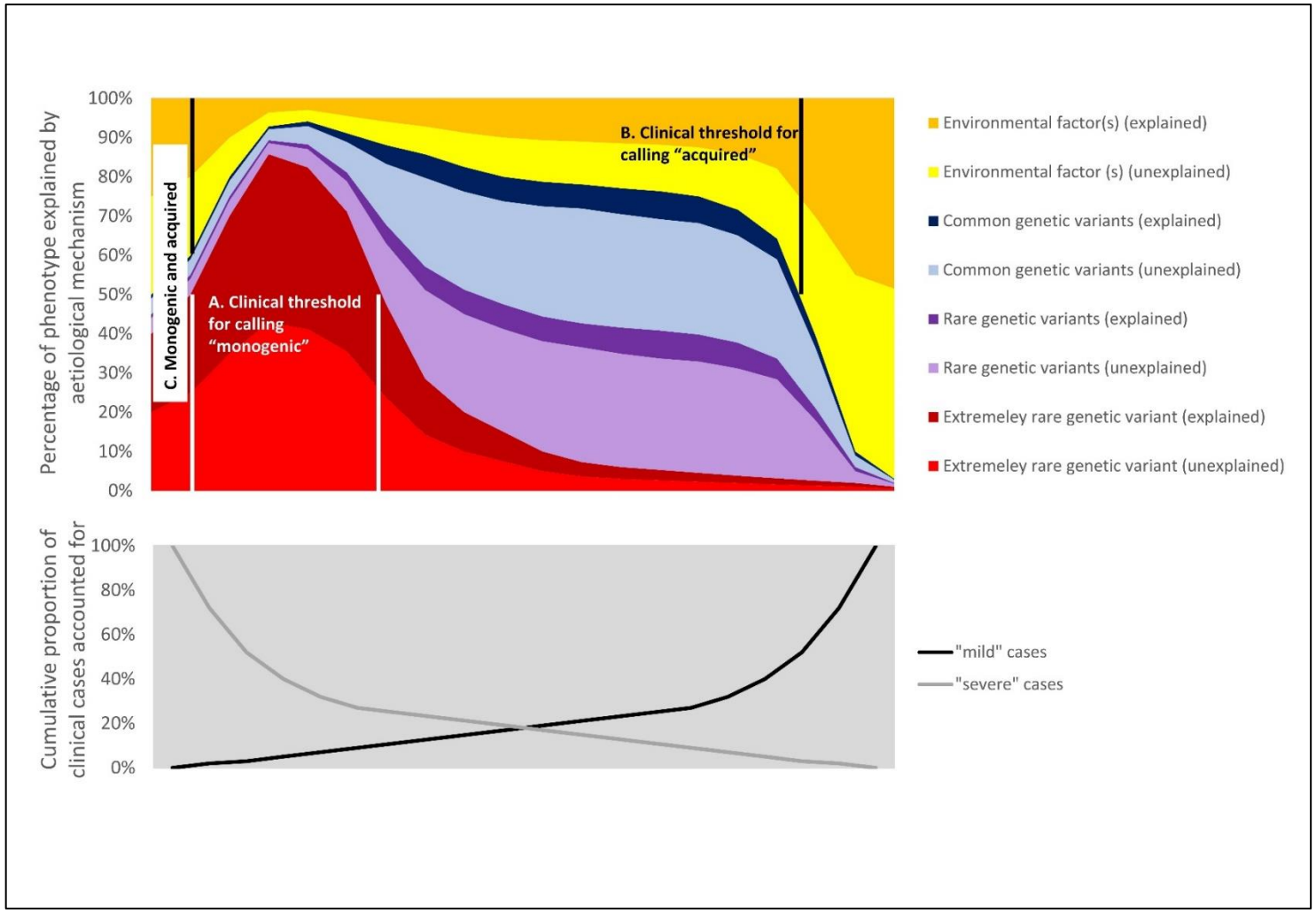


Figure 9a: Illustration of the concepts of multiple aetiologies and causation thresholds in epilepsy

9.4 Capturing complexity and moving forward

As figure 9a. illustrates, epilepsy aetiology is complex and multi-faceted in all cases. It is therefore perhaps not surprising that it has been difficult to find strong evidence to support the notion that knowledge of specific genetic cause meaningfully informs therapeutic decisions. Even the gold standard of evidence-base medicine, the double blind randomised controlled trial (RCT) has significant limitations when it comes to answering the question posed by this thesis. Though the RCT of Cannabidiol in Dravet syndrome demonstrated efficacy and tolerability of this therapeutic compound when used as add-on therapy for patients with drug-resistant seizures, this key question remains. Was the response to CBD in any way related to the genetic cause? Response to CBD varied markedly between patients with causative *SCN1A* variants in this RCT, yet an investigation into whether this in any way related to the nature of the *SCN1A* variant was unrevealing (chapter 8 of this thesis).

In vitro models of human epilepsies in which disease-associated genetic variants are introduced, physiological parameters measured, and response to medications investigated are an attractive model for precision therapy, and are often promoted as such in the titles of scientific publications. Unfortunately these models miss much of the complexity of the human brain, both in terms of genetic background and neuronal circuits which are made up of trillions interconnected pathways of excitatory and inhibitory circuits. Successful *in vitro* models have almost exclusively been restricted to ion channel disorders (Symonds, Zuberi & Johnson, 2017), and these are likely to comprise just half of the patients with monogenic epilepsy (chapter 3). In order to progress this field models that incorporate both genetic background and complex neuronal circuitry will be required. Thanks to the rapidly progressing technologies of induced pluripotent stem cells (iPSCs) (Okita et al., 2011) and CRISPR/CAS gene editing (Sánchez-Rivera &Jacks, 2015) progress is being made on this front though there have been no translational results so far (Tidball et al., 2017).

9.5 Final reflections on this PhD project

Through designing and conducting the six studies which make up this thesis I have developed a number of transferrable research skills, including those involved in systematic review and meta-analysis; in primary epidemiological research; and in the acquisition and analysis of large genomic datasets. Given the rapid advance of the genetic-epilepsy field, the specific skills I have gained in genetic analysis and interpretation are likely to prove valuable to me in the future in both clinical and research settings. The professional links which I have been able to build between Oxford and Glasgow on this front will hopefully endure and continue to be fruitful.

The highest impact aspect of this research is likely to be that described in chapter 5. Here, using a prospective population-based epidemiological cohort study design, I have been able to describe the incidence and phenotypic spectrum of a number of early childhood-onset single gene epilepsies for the first time. This aspect of the project would not have been successful without the good will and cooperation of many clinicians throughout Scotland, most of whom contributed to this research in their own personal time. Coordination of the research effort, and maintenance of motivation was strongly facilitated by the Scottish Paediatric Epilepsy Network. Acquisition of clinical data was made relatively easy by development of functional fully-computerised health records, many of which only came online within the past five years. Through my experience over the last five years, I have come to fully appreciate that Scotland offers real opportunities when it comes to this type of epidemiological research, and it is on this front that I would like to focus further research efforts in future.

Given the number of variables, it seems unlikely that many questions in relation to precision medicine in epilepsy will be answered by traditional randomised controlled trials (RCTs). Conversely some important answers may well come from good quality prospectively gathered data, if this approach can be refined and scaled up sufficiently. Where RCT data may come in useful is in relation to some of the more common genetic epilepsies, such as *SCN1A*. As demonstrated in chapter

8, progress in this area may be hindered unless a framework for transparency of genomic data is developed. To some extent the whole concept of precision medicine is contrary to the objectives of the pharmaceutical industry.

Gaining a greater understanding of some of the rarer genetic epilepsies requires the casting of a much wider net than would be possible for either an RCT, or a single nation epidemiological study. The syndrome of *SMC1A*-truncation, described in chapter 7, is estimated to affect between 1 per 50,000 and 1 per 100,000 births. In circumstances such as these, multi-centre collaboration, facilitated by gene-matching software systems can prove valuable. Since publication of the manuscript "Heterozygous truncation mutations of the *SMC1A* gene cause a severe early onset epilepsy with cluster seizures in females: Detailed phenotyping of 10 new cases" (Symonds et al., 2017) I have been contacted by a number of clinicians and researchers, and through this have been able to further develop my understanding of this extremely rare condition.

Through attendance and presentation of my data at a number of international conferences I have been able to disseminate the findings from this thesis. Such meetings have also provided opportunities for me to network and collaborate with clinicians and researchers in other centres. Without such collaboration knowledge of the very rare genetic epilepsies cannot be progressed.

Finally, a core component of my time during this project was spent directly interacting with the families of children with early-onset epilepsy, in order to acquire the comprehensive history necessary to understand phenotype in detail. The narratives of many of these families are presented in the results section of chapter 6. At the time of their involvement in this project, many of these families were undertaking a frightening journey, with unpredictable turns at every corner, and multiple challenges facing them far beyond just "seizure control." Participating in a research study on top of these challenges often added more uncertainty to this journey, yet all of these families welcomed me into their lives and passed on their stories to me freely and gracefully. History taking remains the

bedrock of clinical medicine, and these experiences have no doubt helped develop me as a clinician. Despite all the technical genetic detail, this thesis remains grounded in the narratives of these families.

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11. Appendices

Appendix 1: GACE study parent information sheet

Appendix 2: GACE study parent consent form

Appendix 3: GACE study referral proforma

Appendix 4: West of Scotland epilepsy genetic testing request form

Appendix 5: GACE study clinical follow-up questionnaire

Appendix 6: GACE study clinical utility questionnaire

Appendix 7: Sanger and MLPA genes in the Glasgow genetic epilepsy service

Appendix 8: Glasgow 104 gene epilepsy panel

Appendix 9: WGS study participant invitation letter

Appendix 10: WGS study parent information sheet

Appendix 11: WGS study parent consent form

Appendix 12: WGS study blank history taking form

Appendix 13: Example phenotype summary from WGS study

Appendix 14: WGS study questionnaires

Appendix 15: 45 most commonly-implicated autosomal dominant epilepsy-associated genes, with pLI scores, mis-Z scores, and GTEx ratios

Appendix 16: *SMC1A* study clinical details proforma