Phenotyping paroxysmal conditions to empower genetic research

Doctor of Philosophy (PhD) Thesis

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2012

To Naomi and the twins…

Summary

I describe the process of preparing cohorts of individuals with two paediatric onset paroxysmal disorders – hyperekplexia and juvenile myoclonic epilepsy – for second generation sequencing. This involves: i) listening to the individual; ii) identifying subgroups; iii) using non-core features to create subgroups; iv) and assessing the importance of copy number variation.

Using focus groups and an interpretative phenomenological approach clinicians and people with epilepsy produced 398 questions focused on epilepsy treatment. The most important themes for the professionals were – treatment programmes for non-epileptic attack disorder and concerns about side effects *in utero*. For patients cognitive drug side effects and managing the consequences of drug side effects were most important.

Studying ninety-seven individuals with hyperekplexia confirmed that all gene-positive cases present in the neonatal period and that clonazepam is the treatment of choice (95% found it efficacious). Patients with *SLC6A5* and *GLRB* mutations were more likely to have developmental delay (RR1.5 $p<0.01$; RR1.9 $p<0.03$) than those with *GLRA1* mutations; 92% of *GLRB* cases reported a mild to severe delay in speech acquisition.

Juvenile myoclonic epilepsy is challenging to subdivide based on seizure and EEG features. The neuropsychological profile of a limited number of patients (39) was examined in great detail including tests of IQ (WAIS), memory (TYM, WMS), executive function (BADS, DKEFS), affect (HADS). TYM was as sensitive as a full WMS for identifying cognitive errors and the zoo map and key search tests were performed particularly poorly. Personality profiling (EPQ-BV) identifies the cohort as having high levels of neurotic and introvert traits.

Three atypical 'hyperekplexia' cases had alternative diagnoses suggested by copy number analysis. The juvenile myoclonic epilepsy patients had an 8% frequency of recognised pathogenic CNVs – but no recurrent variants were identified. A number of non-epilepsy related findings were identified including a potentially preventable cause of SUDEP.

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This work has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

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Acknowledgments

I want to first offer my whole-hearted thanks for the tireless support and mentorship offered to me by Professor Mark Rees and Professor Phil Smith. I have been provided with limitless good humour, opportunity and optimism. They have challenged me to become both a better clinician and an academic. My mistakes are my own but any successes I have had can be attributed to their generous supervision. I hold you in the highest of respect and wish only to keep working alongside you both to finish the work we have started.

*

The DUETs project had many 'parents' and Sir Iain Chalmers and Professor David Chadwick provided essential advice, but the project would not have been possible without the enthusiasm of Dr Carrie Hammond. Carrie was also a founding figure in the patient research group, the family study and the hyperekplexia collection. I remain indebted to her not only for these works but also for her friendship.

I am very grateful to Professor Tony Marson and Dr Graeme Sills from the University of Liverpool for making me welcome as part of the ReJuMEC collaboration and for their continued assistance with our *Epilepsy Research-UK* project grant. In particular Professor Gus Baker and his research student Jordana Walsh have provided invaluable assistance with regards to the analysis of the psychometric testing. Here I must also offer my thanks to Professor Rodger Wood of Swansea University who provided me with the environment to learn how to administer these tests.

I have had the pleasure of working in a larger team and have had many colleagues who have directly and indirectly contributed towards the work that makes up this thesis. In particular Dr Ann Johnston and Dr Owen Pickrell have provided both camaraderie and clinical acumen - the success of the family study is down to you. I certainly would not have had as much fun during my time in research without you. The JME collection needed a wider network for recruitment and Nigel Kitkatt, Dr Khalid Hamandi, Vicki Myson, Malisa Pierri, Dr Frances Gibbon and Dr Cathy White all referred cases. Dr Kate Everett and Dr Paul Timmings were very welcoming in their invitations to analyse their precious JME cohorts. I value our

collaborations and your opinions on JME. Finally, when I needed a helping hand Carla Church, Zoë Abbot, Sarah Dawes and Deirdre O'Sullivan offered their practical support.

I am aware that I joined a mature hyperekplexia project – already the subject of Dr SeoKyung Chung's Doctoral thesis. Her team of research assistants and PhD students have produced an unrivalled supply of variants for me to study and no one has worked harder for hyperekplexia than she has. The samples themselves and the clinical information were provided by the worldwide network of paediatricians, clinical geneticists and neurologists. I want to specifically thank Professor Amira Masri and Professor Fred Andermann for their crucial support. Our closest collaborator however is Professor Rob Harvey with whom we have published most of our hyperekplexia discoveries. Rob's indefatigability has driven our projects on, culminating in our *Action Medical Research* Award. I also want to acknowledge my appreciation of Fiona Togneri and Dominic McMullan at the West Midlands Regional Genetics Laboratory for teaching me how to analyse copy number variation and for their technical assistance with samples.

Dr Rose Thompson has been a constant source of inspiration and I am proud of the work we have put in together on Digital Storytelling and our *Epilepsy Action Diamond Award*. She also provided a number of pertinent suggestions regarding the complex ethics of exome sequencing. Dr Cheney Drew is one of my finest friends and over the last two years, closest colleagues. She has rescued me from near disaster on a number of occasions but is too professional to keep count of them. She is not just a fine scientist in her own right, but a superb co-ordinator and a confidante: thank-you Cheney.

A translational thesis is not possible without a large supporting cast. Central amongst this then should be the patients and their families who have volunteered so much, whilst being promised so little. I only hope that I can one day repay your altruism and generosity. In particular I want to thank Andy Latham, the founder of the Hyperekplexia Society, who has taught me much and inspires many.

*

Every genetics project has to acknowledge the impact of the family on the individual. And so last but not least I want to acknowledge my gratitude for the unconditional support

provided to me by my wife, Naomi, and my family – particularly my parents. Naomi's involvement has also been practical - providing support for the DUETs focus group meetings, as a colleague for meeting hyperekplexia patients, and as a proof reader for this thesis. You have provided the sagest advice, but more often you indulged me by just listening. Your role has also been an emotional one too – I remain astounded at your unwavering support. The completion of this work marks the start of a new chapter together for us.

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Abbreviations and acronyms

Wherever possible the abbreviations have been explained in the first instance of their use.

ABNAS	AB Neuropsychological Assessment Schedule
AD	Auditory Delayed
AED	Anti-epileptic drug
AFS	Atypical febrile seizures
AI	Auditory Immediate
AiW	Alice in Wonderland syndrome
ARD	Auditory recall delayed
ATP	Adenosine triphosphate
BADS	Behavioural Assessment of Dysexecutive Syndrome
BBC	British Broadcasting Company
BECCTS	Benign Epilepsy of Childhood with Centro Temporal Spikes (also BECTS)
BMJ	British Medical Journal
BNT	Boston Naming Test
bd	bis in die (twice daily)
bp	basepairs
BWS	Beckwith-Wiedermann syndrome
CA1	cornu ammonis 1 – one of the main histological divisions of the hippocampus
CAE	Childhood absence epilepsy
CEGAT	Center for Genomics and Transcriptomics
CGH	Competitive Genomic Hybridisation
Ch	Chromosome
ChAS	Chromosome Analysis Suite
CLB	Clobazam

CNS	Central nervous system
CNV	Copy number variation
COPD	Chronic obstructive pulmonary disorder
CPAP	Continuous positive airway pressure
CWIT	Colour word interference test
DKEFS	Delis-Kaplan Executive Function System
DNA	Deoxyribonucleic acid
DECIPHER	Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources
DEX-O	Dysexecutive questionnaire (completed by Other)
DEX-S	Dysexecutive questionnaire (completed by Self)
DGV	Database of Genomic Variants
DMD	Duchenne muscular dystrophy
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders (IV)
DUETs	Database of the Uncertainties of the Effects of Treatments
DQ	Development quotient
EEG	Electroencephalogram
EPQ-BV	Eysenck Personality Questionnaire Brief Version
EU	European
FAS	Verbal fluency test (the three trials are for words beginning with F, A and S)
FS	Febrile seizure
FS+	Febrile seizures plus
FSIQ	Full scale IQ
GABA	Gamma-amino-butyric-acid
GEFS+	Genetic (or generalised) epilepsy with febrile seizures plus
GLRA1	Alpha one subunit of the glycine receptor

GLRB	Beta subunit of the glycine receptor
GlyR	Glycine receptor
GlyT2	Glycine transporter 2
GGE	Genetic generalised epilepsy
GM	General Memory
GP	General Practitioner
GTCS	Generalised tonic clonic seizure
GWAS	Genome wide association study
HADS	Hospital Anxiety & Depression Scale
HbA1c	Glycosylated haemoglobin
HD	High density
Hz	Hertz
ICR	Imprinting control region
IES	Impact of Epilepsy Scale
IGE	Idiopathic generalised epilepsy
ILAE	International League Against Epilepsy
IM	Immediate memory
IQ	Intelligence Quotient
ISCA	International Standards for Cytogenomic Arrays
IVF	*in vitro* fertilisation
JAE	Juvenile absence epilepsy
JME	Juvenile myoclonic epilepsy
kbp	kilo basepairs
LD	Learning difficulty
LEV	Levetiracetam
LOH	Loss of heterozygosity

M[number]	[ordinal] Membrane-spanning domain
mane	In the morning
mg	milligram
MND	Motor neurone disease
MRI	Magnetic resonance imaging
nAChR	nicotinic acetylcholine receptor
NCBI	National Center for Biotechnology Information
NG	Nasogastric
NGS	Next generation sequencing
NHS	National Health Service
NISCHR	National institute for Social Care and Health Research
NMDA	N-methyl-D-aspartic acid
nocte	At night
ns	Not significant
NZ	New Zealand
OMIM	Online Mendelian inheritance in Man
PCR	Polymerase chain reaction
PEPD	Paroxysmal extreme pain disorder
PO	Perceptual Organisation
PPR	Photoparoxysmal response
PS	Processing Speed
ReJUMEC	MRC funded Refractory Juvenile Myoclonic Epilepsy Cohort collection
RDG	Research Development Group
RNA	Ribonucleic acid
SCN1A	Sodium channel alpha one subunit gene
SD	Standard deviation

SE	Standard error
	Solute carrier family 6, member 5 (codes for GlyT2)
SMEI	Severe myoclonic epilepsy of infancy
SNP	Single nucleotide polymorphism
SPSS	Statistical Package for the Social Sciences
SRS	Silver-Russell syndrome
STAI	State–Trait Anxiety Inventory
Std	Standard
SUDEP	Sudden unexpected death in epilepsy
tds	ter die sumendum (three times a day)
TLE	Temporal lobe epilepsy
TMT	Trail making test
TRESK	TWIK-related spinal cord K^+ channel
TYM	Test your memory
UCSC	University of California, Santa Cruz
VAL	Sodium valproate
VC	Verbal comprehension
VD	Verbal Delayed
VI	Verbal Immediate
WAIS	Wechsler Adult Intelligence Scale
WCST	Wisconsin card sorting test
WERN	Wales Epilepsy Research Network
WES	Whole exome sequencing
WORD	Wales Office of Research and Development
WMRGL	West Midlands Regional Genetics Laboratories
WMS	Wechsler Memory Scale

WM	Working Memory

Structural Overview

Phenotyping paroxysmal conditions to empower genetic research

The era when genetic discoveries could be made by bright individuals in small 'cottage industry' laboratories has probably passed. The advent of second generation genetic technologies has brought unrivalled opportunity but has also only elucidated the complexity of most neurological disorders. The consequence of this is that genetic research is team work, with each researcher contributing expertise from their own domain – and in time learning how best to tessellate their work with that of their colleagues. The projects that comprise this thesis therefore never forget their clinical origins but also rotate through a variety of methods with the ultimate goal of empowering genetic research.

It is therefore – unapologetically – that I will first focus on the first step of translational research, 'the individual and involvement.' Within chapter three and the connected discussion of the data are the approaches I have made to improve engagement, to evaluate the research priorities of people with epilepsy and those of clinicians, and I touch upon methods of capturing narrative. These qualitative approaches are too often overlooked. The epilepsy story (as discussed in detail below) is primarily an oral history and a failure to fully appreciate the way a person communicates their symptoms will bias all future analyses of those data.

Chapters four and five describe the clinical features of two genetic disorders: hyperekplexia and juvenile myoclonic epilepsy (JME) respectively. The success of this description for the rarer disorder parallels the success of unravelling the genetic causes here too. This is predominantly because hyperekplexia is thought to be a monogenetic disorder where upon JME has a more complex polygenic cause. Chapter six asks that if novel methods of case definition are needed for JME – can neuropsychology be an accurate tool? Endophenotypes identified from analysis of executive function or personality would be helpful in unravelling the heterogeneity of JME. Chapter seven takes-up the challenge offered in the title. If we have used condition appropriate phenotypic description methods, can this help us select the correct cases for genetic analysis and make the most accurate analysis of the often

complicated results we receive? In this case I present data from copy number experiments of both hyperekplexia and JME.

In expanding the field of knowledge we but increase the horizon of ignorance

Henry Miller

The Wisdom of the Heart, 1947

Chapter One

Introduction

1.1 Introduction

A thesis of the scope described in the structural overview necessitates a detailed introduction to the themes and a lengthy materials and methods section. I will first start by describing epilepsy in a way that may explain why we have not managed to make the breakthrough into the genetic causes of the epilepsies in a way that was once predicted. The description of 'the intermittent trait' is relevant when considering hyperekplexia too. The stigma and sociological consequences discussed within are relevant for the next section which introduces the themes of the individual, involvement and translational research. This section also discusses the importance of evidence based research and asking the 'correct' questions.

The third section describes hyperekplexia starting from the position that the majority of clinicians would not be familiar with this condition. Hyperekplexia and JME both share a number of clinical features- including unremarkable neuroimaging, a characteristic age of onset, a good response to medication and 'electric shock speed' motor jerks. JME is described next – focussing on the heterogeneity and the breadth of the disorder. After considering neuropsychological features the introduction considers JME in families. There then follows a detailed description of the current medical genetics landscape, specifically how technology driven 'next generation' sequencing is changing what we thought was possible. After describing what is known about JME genetics I describe the current state of play with regards to copy number variation in the epilepsies.

1.1.1 The Epilepsies

The International League Against Epilepsy (ILAE) has unwittingly confused casual students of epilepsy by producing and then revising their system for classifying both the epilepsies and their component symptom – the seizures. This (allied to personal preference) has unnecessarily increased the vocabulary used to describe epilepsy. I hope this in part excuses my use of both the suggested correct term 'genetic generalised epilepsies' and the previously accepted term 'idiopathic generalised epilepsies' throughout this manuscript. Lay usage of terms (particularly 'grand mal' which is deemed to be synonymous with generalised tonic clonic seizures (GTCS) and 'petit mal' (which can mean just about anything up to and including a GTCS) abound. Against this backdrop the ILAE define a seizure as 'the manifestation(s) of epileptic (excessive and/or hypersynchronous), usually self-limited activity of neurons in the brain.' I have never liked this definition as it is self-referential (an epileptic seizure is a seizure which is epileptic). Furthermore (as described in detail below) any attempt to define epilepsy as a collection of seizures is flawed. Epilepsy does not come alone; the neuropsychological deficits, mood disorders and stigma that go hand-in-hand with many epilepsies are excluded by a definition of epilepsy as the propensity towards unprovoked seizures.

Epilepsy - the propensity towards seizures – is a syndrome of symptoms (the seizures) of which the causes are legion. In that manner it may considered as comparable to pain, and our services as like chronic pain clinics. After eliminating the risk that the cause of the seizure may be fatal or progressive (or not be a seizure at all) we aim to remove the symptom (the seizure) with tablets rather than address the root cause in most cases. Although marketed for specific indications – broadly all the drugs work on all the epilepsies – and equally up to a fifth of people continue to have seizures despite adequate treatment, often inexplicably. Like pain the seizures do not come alone – and depression, anxiety and suicidality are all greatly increased for people with epilepsy. The anxiety is particularly socially disabling and understandable – as the condition is paroxysmal. It is all too easy to associate leaving the house with the embarrassment of the seizure in Tescos. The fear and loneliness of waking up, cold, confused and amnesic on the bathroom floor erodes the confidence of people who live alone. Yet seizures for most people are infrequent – perhaps

occurring once or twice a month; this means that 90% of days could be seizure free and the majority of time is spent awake, conscious and well – yet the threat of another attack prevails.

The medications are often complex (very few once daily regimens) and mostly the agents have been identified serendipitously. They all have side effects which limit use and many provoke drowsiness or cognitive problems – particularly in the elderly. This is unfortunate and unwanted as the seizures themselves (particularly in the temporal lobe) and the underlying structural lesion (potentially) both can cause and exacerbate memory difficulties. These memory lapses make drug errors likely and both over and under dosing can aggravate clarity of thinking.

Epilepsy is common (307,000-460,000 in the UK) but poorly understood by the general public and some people working within healthcare. Many GPs admit that manipulation of drug doses is outside of their expertise – despite sodium valproate and carbamazepine (the two most prescribed of medications) having been around in the UK since 1965. This lack of familiarity is hampered by the prevalent negative misconceptions that are held about 'the epileptic' and 'suffering from fits'. Associations within and outside the UK with the devil, witchcraft, mental feebleness and misfortune add to the stigma that persisted throughout the twentieth century. Doctors were guilty of (unintentionally) giving unhelpful advice. My Grandad's brother married a woman with epilepsy: because of the shame she only confessed to this after their wedding – fearing rejection. They never had children – her doctor had informed her that the shock of intercourse would trigger a seizure and it was best avoided. I cannot blame her for her concealment, I am certain that limited disclosure continues as people attempt to avoid the social barriers created by driving ineligibility or joining certain professions.

Epilepsy is common (prevalence of 0.5 to 0.75% of the UK) however the lack of a celebrity champion is a sign of the perceived stigma attached to the diagnosis. I could name famous people with Alzheimer's, multiple sclerosis, Parkinson's and motor neurone disease, conditions that all have a poorer prognosis than epilepsy – but fewer than a handful of people who are happy to discuss 'my life with epilepsy.' Epilepsy's intermittent nature lends

itself to concealment. Epilepsy is common – and common throughout the span of life from paediatrics to geriatrics; and all the GPs, obstetricians, emergency medics and psychiatrists in between. Some epilepsies, such as JME are thought to be lifelong and so drug decisions made at ten or twelve may have a bearing on fertility and foetal health, weight gain or loss, or bone thinning and falls.

Epilepsy is categorised as a chronic disease. However it doesn't have much in common with COPD, diabetes or atrial fibrillation. There is no 'test' for epilepsy in the way that an HbA1c, spirometry reading or ECG may define a diagnosis. As a result misdiagnosis rates, even in specialist centres, approaches a fifth – and the error trends towards over rather than under diagnosing events as seizures. The epilepsy 'tests' – the MRI brain and EEG, could both be completely normal and yet sit quite comfortably with a definite epilepsy diagnosis. The physical examination (although important) is quite secondary to listening to the proband and the witness describe the event. The witness is essential, as a period of unconsciousness is indescribable – however – there remains a great deal of information to be gained from the proband about events surrounding the seizure; this is often difficult to explain such as déjà vu, an epigastric aura, an absence, a myoclonic jerk. How can anyone take an accurate history in a second language, or from someone with learning difficulties? When does a day-dream become an absence, a twitch become a jerk or an epigastric aura become a sense of anxiety? I wonder how people taking a history in a language with a more limited descriptive vocabulary than English cope. Having the time to listen carefully to what they say and how they say it helps differentiate epilepsy from vasovagal syncope, cardiac arrhythmia, psychogenic non-epileptic attacks and anxiety-attacks (Reuber *et al*. 2009). The majority of people attending a 'first seizure' service will not have epilepsy and so when planning the number of specialists needed, relying on the prevalence of epilepsy alone will lead to a shortage of epileptologists. Epilepsy is different, and recognising this for service provision is essential. However the skills needed to care for people with epilepsy are not the exclusive domain of tertiary referral centres, nor dusty old neurologists. Co-ordinating clinical care should be a priority. When asked, patients prioritise improving the public's understanding of epilepsy and tackling treatable co-morbidities.

1.1.2 Episodic or intermittent traits

Epilepsy is phenomenally resistant to accurate subcategorisation. The events are brief (lasting two or three minutes); by definition the person experiencing the event is unconscious or has altered awareness (except for very brief seizures like myoclonic jerks); epileptic events are very difficult to describe even without altered consciousness; there is often pre-ictal amnesia; witnesses are often distressed and detailed recall of the event is poor. Yet a description of these events (in tandem with age of onset, frequency and response to mediation) is our best tool. Childhood epilepsies are often aided by EEG correlations – however adults may have a normal (falsely negative) inter-ictal trace. Furthermore the broad brush strokes of focal (single ictus – with or without secondary spread) versus generalised (occurring in many brain regions at inception) may not stand up to challenging by new technology. Are generalised epilepsies just focal epilepsies with ultra-rapid spread? Do they originate in deeper structures that do not produce a reliable EEG trace in humans – such as thalamic and subthalamic structures? Even the focal versus generalised definition matters less when it comes to medication (broadly all trialled as an adjunct in focal epilepsy but may work in all epilepsies).

Paroxysmal conditions – by their very nature - can be difficult to describe: the symptoms are episodic and therefore have to be either provoked or described by a witness. This is in contrast to fixed or progressive impairments seen in conditions such as Parkinson's or Alzheimer's disease. Migraine is paroxysmal, but yet is well described by those who experience it because it does not alter consciousness, it can occur in adults and it does not induce a strong behavioural change. The disorders I have chosen to study however are much more challenging to describe.

Indeed, correctly defining 'the condition of interest' chosen is again problematic. The ILAE definitions of epilepsy are at best a compromise – and are designed for both research and clinical practice. There is a great degree of variation even within tightly-defined clinical syndromes and are the familial variants of epilepsy similar to those observed in the population? We will find – like the psychiatrists have – that our present understanding of epilepsy and seizure classification will be challenged when presented with the genetic

causality (Craddock and Owen 2005, Craddock and Owen 2010). As a metaphor – who would have believed that the nearest genetic 'cousin' to the whale – is the hippopotamus? Similarly, work looking at small chromosomal losses of genetic information (microdeletions) or copy-number variation (CNVs) suggests common risk-factors for a whole range of neurological conditions from idiopathic generalised epilepsy, autism and bipolar disorder. To overcome this murky gene pool the numbers involved need to be very large indeed for genome wide association studies (GWAS); this then has implications because epilepsy is not as well funded as cancer, diabetes or even other neurological disorders. An alternative is to extensively phenotype smaller numbers of people with epilepsy and look at more than seizure types and age of onset.

1.2 Involvement

The central person in a genotype-phenotype study is the patient; this focus can be lost all too easily. The divorce between the research volunteer, the sample and their data is perpetuated by current research ethics trends which prioritise anonymisation of data and 'protection' of the volunteer from researchers. Examples of this include the difficulty in recruiting patients for studies (cooling off periods needed, written information must be posted and digested) and the difficulties in re-contacting patients for follow-up or secondary data. This results in a false separation of the person - from their clinical story and from their research data.

People with symptoms are motivated to take part in research for a variety of reasons. However, they are separated from their psycho-social context (or at best these 'quality of life' issues are coded and scaled) and become a data point. Their blood sample – extracted through a very human interaction (the patter about being needle phobic, 'just a sharp scratch' is automatic to every clinician) is spun down, separated out or teased into a volume of DNA. Ironically DNA- the data-rich double helixes, Watson and Crick's compendium of complexity - are stripped of the wealth of personal data that could augment the collection. The DNA sample becomes an anonymous frozen Eppendorff. Amongst a large team, or when performing specialist laboratory science it would be the norm for those who are handling the sample to never meet the person behind the sample; indeed it may be mandated that they never do.

This thesis is focussed around paroxysmal disorders and specifically juvenile myoclonic epilepsy and hyperekplexia. In hyperekplexia the tonic spasms and startles are first identified in infancy and so the individual cannot relate their symptoms. The parents may be prevented from doing so through health anxieties, unfamiliarity with these new events and a lack of vocabulary to describe these complex hyperkinetic events. This is very important because although it is difficult to relate the details of these paroxysmal events, this narrative is exactly what the clinician is reliant upon to provide certainty and accuracy of diagnosis.

This paradox in particular is why I have chosen to focus in chapter three on capturing the patient's experiences. I hope to demonstrate that by using a variety of qualitative and quantitative methods I have learned to help the individual and their family tell the very best paroxysmal event history that they can; and I have learned to hear and assimilate this information to provide the most accurate diagnosis that one could be expected to provide. I accept (and embrace) the inherent doubt implicit in transforming a verbal report into a clinical entity and do not shy away from reproducing this doubt in later chapters regarding phenotyping. During the data analysis of the hyperekplexia cases (chapter four) I have had to reply on the reports of others; I learned how best to produce standardised data sets. In contrast, chapter six (JME neuropsychology) is almost entirely focussed around clinical and psychological data that I have extracted. Alongside the coded information regarding depression on the HADS score, I have field notes documenting their concentration, some of their spontaneous conversation, whether they lacked confidence, whether they responded to my reassurance.

1.2.1 Bench to bedside and back again

I have had patient encounters in people's homes as a field researcher for the family project (chapter five) including arriving one day to discover a family member having a non-epileptic attack, *"Thank God you've arrived!"* they said…. However I am in a privileged position in that I have been at the metaphorical and literal bedside, the bench and the bedside again for the people that I have met with JME. Many I met in an NHS clinic, met for many hours of psychometric testing following a number of phone calls and then I left with their data. I think this responsibility has motivated me to protect their data as responsibly as possible – both by being involved in writing and securing ethics amendments and securing a new ethical agreement – but also in deciding how best to use very powerful second sequencing techniques (chapter seven). This is of great importance because as a clinical-academic I will also meet these volunteers as they return to NHS clinics, some of whom will be given genetic counselling as an eventual consequence of my research findings. The risks of returning incomplete, uncertain or incomprehensible data to the clinical domain is one that I am very much aware of regarding newer genetic testing. Through involvement in patient groups for epilepsy and hyperekplexia (below) I have been able to hear first-hand how much

they value and respect research and particularly genetics research – but also how easily research outputs can be misconstrued.

In chapter three I describe a number of collaborative projects which I have undertaken with patients as partners rather than participants. The outputs of these projects are reproduced in full in the appendixes. I will describe my involvement in the Wales Epilepsy Research Network (WERN) patient Research and Development Group (RDG) as a novel example of involvement; this is patient exposure outside of the clinical setting, and without the support of the NHS infrastructure. One of the priorities that the RDG stated for itself was to improve community knowledge about epilepsy and seizures and reduce stigma. As an example of a venture that aims to do this I will then discuss the 'Digital Narratives' project. This online storytelling compendium also has the function of collecting first person data, but keeping the person and personality intact.

Patient stories are more conventionally told in print media. To illustrate where I have managed to do this as collaboration with the person with epilepsy, I have included two stories of artists with JME, with whom we wrote and published an article focusing on artists with epilepsy in appendix B. I also assisted a shy school teacher from Cardiff to discuss what it is like to live with drug-resistant JME; a lifelong condition – which immediately produces challenges for drug choices – particularly regarding pregnancy. It remains unusual to name patients in case reports, but this was essential for this BMJ article (appendix B). Promoting Nicola to the 'headline' to the author's list, genuinely keeps her at the centre of her story. Nicola was also not only a participant in the psychology and genetics study presented in chapters six and seven (her data are not identifiable by this disclosure) but she was a volunteer in our project which aimed to identify research priorities. This process is described in full – as are the motivations behind the database of uncertainties of the effects of treatments (DUETs). Involvement is not purely face to face and so I discuss the Hyperekplexia Society patient group (run from *facebook*) and the challenges and opportunities of social media for patient partners in the conclusion to this work.

1.2.2 Database of the Uncertainties of the Effectiveness of Treatments (DUETs)

How research topics are chosen and prioritised is unclear. Pharmaceutical sponsored projects may be selected to show an advantage of their product (Tallon *et al*. 2000, Garattini and Chalmers 2009) and independent topics may be chosen for investigation because the project is of a size that can be completed given the time and money available (Scadding 2006). Grant committees rightly ask applicants to consider the views of patient groups when submitting an application, but this is difficult without knowledge of exactly *what is uncertain* and which topics are patient priorities. Do funded research questions address the priorities of people with epilepsy (Oliver and Gray, 2006)? Do they even reflect the uncertainties prioritised by clinicians?

1.2.3 James Lind Alliance and uncertainty

When prescribing for certain groups, e.g. people with epilepsy and learning disability, we are often working in an evidence vacuum, extrapolating from relevant studies of related patient groups. Our comfort with uncertainty and our acceptance of this 'grey area' has unwittingly promoted a climate where an immeasurable amount of harm has been perpetrated because we fail to identify the uncertainties which actually *have* sufficient evidence to in fact be certainties; for example, steroids in head injury, (CRASH Trial collaborators, 2004) caffeine for neonates (Schmidt *et al*. 2007) or prophylactic anti-arrhythmia agents after myocardial infarction (Furberg, 1983). The James Lind Initiative aims to promote the "identification of the most important gaps in knowledge about the effects of treatments". To achieve this they have created a database of uncertainties of treatments (DUETs). This growing database is published on-line at NHS Evidence (www.library.nhs.uk/DUETs). Working in collaboration with this project we aimed to help people personally affected by epilepsy and its treatment (patients, carers, professionals) to create and prioritise treatment uncertainties. The DUETs project is published as: 'Identifying and prioritising epilepsy treatment uncertainties' (Thomas *et al.* 2010a, Appendix A).

1.3 Hyperekplexia

Hyperekplexia literally means exaggerated surprise or shock from the Greek word έκπληξη; εχπληξις being a theatrical device used in Greek tragedies to challenge (or shock) the audience with juxtaposing themes (Grassi 1983). Hyperekplexia is a rare neurogenetic synaptopathy with both dominant and recessive inheritance. The full phenotype of hyperekplexia has been a challenge to describe because it affects infants predominantly and because it is a very rare condition. This results in cases being described in isolation as 'case reports' and there has never been an attempt to systematically collect cases and describe their features (outside of large multiplex families). I have had unrivalled access to the clinical custodians and genetic analysis of kindreds with a known genetic cause for their hyperekplexia. In this chapter I will describe what was known about hyperekplexia when I joined the group; and then in chapter four how the process of capturing a uniform clinical dataset on referred cases permitted phenotypic analysis and the first genotype-phenotype study in hyperekplexia.

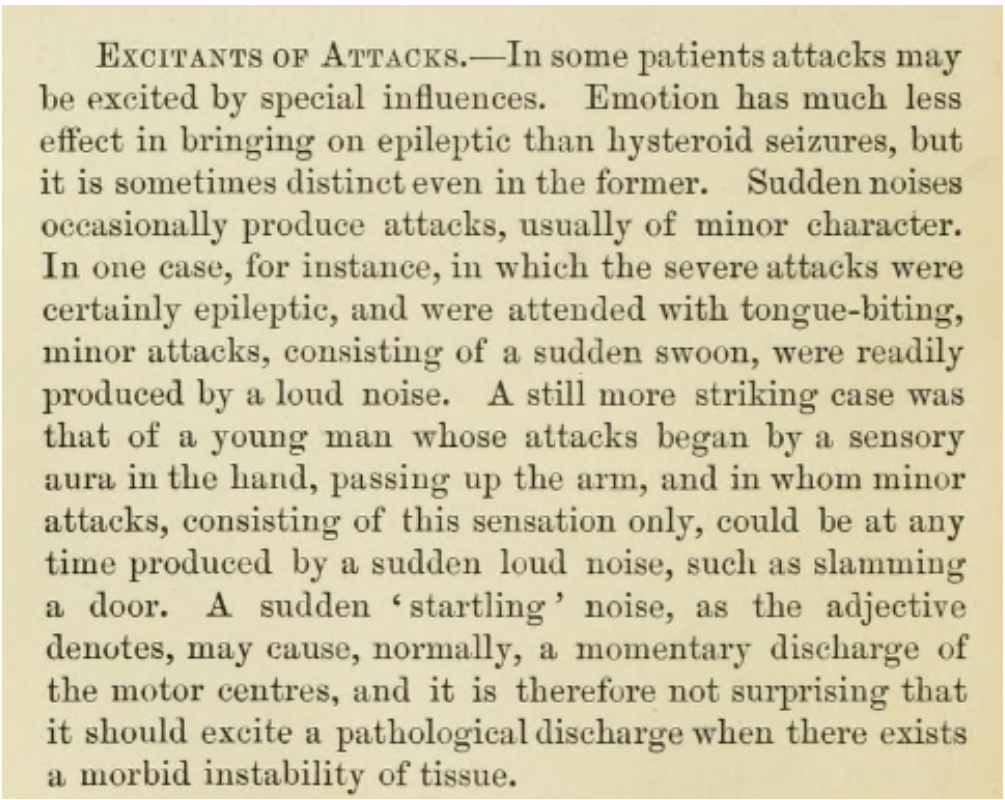
1.3.1 The Hyperekplexias

Hyperekplexia is classically characterised by pronounced startle responses to tactile or acoustic stimuli, hypertonia and episodic neonatal apnoea. The hypertonia may be predominantly truncal, attenuated during sleep and less prominent after a year of age. Although primarily seen as a neurogenetic syndrome with both familial and sporadic cases, acquired hyperekplexia is also recognised. Hyperekplexia certainly appears to be a rare disorder but the incidence is as yet unknown. It is very probable that a lack of familiarity with this condition leads to under-reporting and misdiagnosis. This is evident when a positive gene test for a neonate leads on to delayed positive tests for older symptomatic family members. Despite the classical features being present from birth (and in some reports exaggerated startle responses felt in utero) the majority of definite diagnoses are made, retrospectively, after infancy. Once appreciated the pattern appears to be relatively straightforward to recognise which may explain why hereditary hyperekplexia was described so accurately prior to gene testing, but only as late as forty years ago. Corroborating the importance of pattern recognition, the majority of requests for genetic testing come from a small number of tertiary centres.

1.3.2 History

One of the hazards of describing a very rare condition is that it may be named by many authors separately. Therefore for hyperekplexia we have the original unnamed descriptions (below), startle disease, hyper*ex*plexia and stiff-baby syndrome as now obsolete synonyms.

Inherited hyperekplexia has been seen in many communities but it was in continental Europe that it was first described. In addition to the descriptions of Kirstein and Silfverskiold in 1958 and Kok and Bruyn in 1962, the first description may have been by Thomas Mann in Doctor Faustus (1947): *"But to return to eccentrics of Kaisersaschern there was for instance a man of indeterminate age, who at any sudden shout would feel compelled to perform a kind of jerky dance with knees pulled high, and making a sad and ugly face, he would smile, as if to apologize to the urchins after him in yowling pursuit."* (Rot, 2004). If nothing else this describes a paroxysmal auditory stimulus triggered motor disorder that persists into adulthood – and the consequences of shouting at the 'eccentric' of Kaisersaschern was well known to the urchins who teased him so. Mann's description is not unlike that of Gowers (figure 1.1 below).



EXCITANTS OF ATTACKS.—In some patients attacks may be excited by special influences. Emotion has much less effect in bringing on epileptic than hysteroid seizures, but it is sometimes distinct even in the former. Sudden noises occasionally produce attacks, usually of minor character. In one case, for instance, in which the severe attacks were certainly epileptic, and were attended with tongue-biting, minor attacks, consisting of a sudden swoon, were readily produced by a loud noise. A still more striking case was that of a young man whose attacks began by a sensory aura in the hand, passing up the arm, and in whom minor attacks, consisting of this sensation only, could be at any time produced by a sudden loud noise, such as slamming a door. A sudden 'startling' noise, as the adjective denotes, may cause, normally, a momentary discharge of the motor centres, and it is therefore not surprising that it should excite a pathological discharge when there exists a morbid instability of tissue.

Figure 1.1 (previous page) Extract taken from *Epilepsy and the Chronic Convulsive Disorders* (1881) by Sir William Gowers. Gowers describes two cases of auditory-stimulus provokes reflex epilepsies. Or perhaps he is describing a hyperekplexia startle?

The initial families identified and studied in Western Europe and America were multiplex pedigrees with an apparent autosomal dominant inheritance (Kok and Bruyn, 1962). This was a sensible strategy for differentiating between genuine hyperekplexia and mimics: family members were more likely to have the condition, whereas in the population this may be diluted out with anxiety mimics, epilepsy or rarer syndromal diagnoses. This strategy was justified in the discovery that mutations in the glycine receptor gene co-segregated with symptoms in these large families (Shiang *et al.* 1993).

However since the original Caucasian descriptions there has been an ethnic shift away from Western Europe and North America as the 'hot bed' of hyperekplexia. Jordanian and Turkish populations recognise and diagnose a great number of familial and sporadic cases. Some of this will be in part to founder affects which have been described in some Turkish populations; (Becker *et al.* 2006) some will be due to cultural practices of marrying within ethnic and religious subgroups. This cultural pressure is much more pronounced in isolated communities such as migrants. Turkish cases in Germany, Pakistani cases in the UK and Caucasians transplanted to Commonwealth countries are all more common descriptions than 'indigenous' sporadic hyperekplexia.

1.3.3 Startle and hypertonia

Hyperekplexia is characterised by hypertonia that is predominantly seen in the trunk or lower limbs and easily-provoked startle responses to tactile or acoustic stimuli (Harvey *et al.,* 2008, Koning-Tijssen and Rees 2009). The generalised stiffness can be prominent enough to permit infants to be rotated between the vertical and horizontal plane with barely a change in posture (Suhren *et al.* 1966).

Startle is defined as a sudden involuntary movement caused primarily by surprise (Brown, 2002, Matsumoto *et al.* 1992). These startle attacks can be prolonged, with many minutes of axial rigidity. The threshold for a normal startle response can be lowered by anxiety states –

either longstanding such as generalised anxiety disorder or post-traumatic stress disorder, or by situational events (e.g. being alone in the house on your own in the dark). The startle response in hyperekplexia is very slow to habituate meaning that adults with this condition report jumping to each firework's bang on Guy Fawkes's night as if they had never heard a rocket explode before. Even being prepared for the stimulus is not sufficient to over-ride this strong startle response. The startle therefore is said to be 'exaggerated' in that it is easier to produce, however it is thought to represent a normal physiological startle, perhaps with increased gain (Bakker *et al.* 2006). The tactile stimuli can be particularly disabling, with everyday actions such as putting on a coat often enough to provoke an attack. Although not fully explored, affected individuals do report exaggerated hypnagogic jerks which are not triggered by stimuli (Kirstein and Silfverskiold, 1958).

The afferent and efferent pathways of the startle reflex in hyperekplexia are the same as the normal startle reflex (Bakker *et al.* 2006). However, the motor responses are larger and electromyographic burst durations of the orbicularis oculi and sternocleidomastoid muscles are longer than seen in controls; the latencies of the startle reflexes are also abnormally short. In addition there is a suggestion of increased autonomic responses measured with the psychogalvanic response. In contrast the pathophysiology of hypertonia in hyperekplexia has been studied less extensively.

As a consequence of the hypertonia, it is not uncommon for children to be diagnosed as having a gait disorder before the startle episodes are uncovered and the correct diagnosis is made. Once ambulant, falls can be frequently triggered by auditory stimuli: only a mild startle is needed to provoke a fall. The combination of axial hypertonia and exaggerated startle often results in falls that cause facial injury. Patients repeatedly tell of when the other school children discover that it only takes the slam of a desk to trigger a 'falling tree' like attack in their classmate. We are aware of two people with hyperekplexia who were previously thought to have a 'neurotic phobia' of hard surfaces – particularly concrete – because as children they were reluctant to walk, fearing injurious falls and stumbles. Another patient described the hazards of wearing high heel shoes as each heel strike on a solid surface risks provoking an auditory triggered attack.

It is almost certainly a rare condition with fewer than a fifty published cases confirmed via genetic testing since the first gene of effect was described in 1993 (Shiang *et al.* 1993). In addition to anxiety-induced startle, culturally-specific triggered startle conditions are recognised, such as the 'jumping Frenchmen of Maine' and 'Latah'. However, it is not clear whether these conditions represent a variant of hyperekplexia, phenocopies or neuropsychiatric disorders. Surprisingly, for such a clinically recognisable condition, classical (congenital) hyperekplexia was first described in Europe as late as 1958 by Kirstein and Silfverskiold, with key features seen in a three generation family, in addition to sudden falls and exaggerated hypnagogic jerks. Papers in the 1960s (Kok and Bruyn 1962; Suhren *et al.* 1966; Gastaut and Villeneuve, 1967) linked additional features to this unidentified hereditary condition, including epilepsy in some affected family members and attenuation of the startle response with alcohol or phenobarbitone. It is now recognised that periodic limb movements in sleep and a characteristic head-retraction response completes the phenotype (Bakker *et al.* 2009b). Essential diagnostic criteria include the presence of symptoms soon after birth, which are often maximal in the first year of life.

A later onset of symptoms should prompt investigations for an acquired cause - often pontine injury (Bakker *et al.* 2006, Davies *et al.* 2010) or rarely, anti-GlyR antibodies (Hutchinson *et al.* 2008, Piotrowicz *et al.* 2011, Turner *et al.* 2011, Schmidt *et al.* 2010). This is an important distinction, since treatment for classic hyperekplexia is based on medication to combat hypertonia and startle rather than true disease modification. Unlike the genetic form these autoimmune cases were described as having “mild bilateral ptosis, bilateral partial horizontal gaze palsies, mild left lower motor neuron facial weakness” in addition to the hypertonia and triggered startle which are seen in hyperekplexia. They defined this constellation as PERM (progressive encephalomyelitis with rigidity and myoclonus). Symptomatic hyperekplexia can be provoked by a number of acquired causes including brain stem or pontine trauma or demyelination. In animals the caudal brainstem appears to be the root of the auditory startle reflex. In support of this, glycine receptors have been demonstrated to be present at high levels in the basal ganglia, brainstem and spinal cord.

1.3.4 Additional features

As already stated, hyperekplexia presents in the neonatal period – and occasionally has been described as 'recurrent hiccups' by mothers before birth (Leventer *et al.* 1995). Unusually for a genetic neurological condition – it remits rather than progresses over time. The axial hypertonia seen in hyperekplexia may predispose to an increased frequency of hernias seen in affected infants (Suhren *et al.* 1966).

The final motor symptom of hyperekplexia is that of prolonged apnoea attacks (Kurczynski, 1983) These are cyanotic spells of up to two or three minutes that are again thought to be triggered by external stimuli. They are seen in the perinatal period and in the first years of life before slowly attenuating. It is this symptom primarily that defines why hyperekplexia cannot be seen as a benign condition – despite the predominantly favourable prognosis. These apnoea attacks appear to be a triggered extension of the hypertonia and hypertonic 'seizures' which splint the abdominal muscles and completely prevent respiration. Infants quickly lose colour, desaturate and it may be two to three minutes before spontaneous respiration resumes. On very rare occasions infants may experience upwards of twenty or thirty apnoea attacks a day. Present advice is to time the period of apnoea and offer supplementary oxygen as best as possible without triggering further startle by unnecessary tactile stimulation. If needed, parents can be taught the Vigevano manoeuvre: this process of forced flexion of the head and legs towards the trunk can result in the termination of hypertonic attacks. The description was *"during a long seizure, one of us intervened in an attempt to counteract the hypertonia: the patient was held by the head and legs and forcibly flexed towards the trunk. The seizure ceased immediately. The procedure was repeated several times and always proved effective." (Vigevano et al. 1989).* It has also had varying success in abating tonic attacks. The procedure is particularly useful as it can be taught to parents and can help provide non-pharmacological control of symptoms.

1.3.5 Progression

These symptoms are maximal in the first years of life and improve with age. Some adults who are still symptomatic also report that alcohol can help subdue their symptoms. Alcohol sensitivity therefore makes hyperekplexia (like essential tremor and social phobia) a risk factor for alcohol dependency. Unusually for a genetic syndrome classic hyperekplexia is associated with normal brain imaging and no skeletal or facial dysmorphism. It appears to

be a pure neurological disorder with no sequelae in any other system – other than consequences of the attacks themselves such as hernias. Occasionally comorbid epilepsy is suspected – but because of the high misdiagnosis rate of startle and hypertonic attacks with epileptic seizures, it has always been difficult to clearly describe just how frequently they may co-exist.

1.3.6 Genetics of hyperekplexia

Hyperekplexia is a rare neurogenetic synaptopathy with both dominant and recessive inheritance (figure 1.2). Hyperekplexia is predominantly caused by mutations in the genes encoding the postsynaptic inhibitory GlyR subunits of α1 (*GLRA1*), β (*GLRB* (Rees *et al.* 2002)) and the cognate neuronal-specific, presynaptic glycine transporter GlyT2 (*SLC6A5* (Rees *et al.* 2006)). There is rare (less than 1% of cases), but recognised genetic heterogeneity via association with mutations in gephyrin (*GPHN*; (Rees *et al.* 2003)), and collybistin (*ARHGEF9;* (Harvey *et al.* 2004)). The phenotypes associated with these mutations are more devastating: deletions in *GPHN* are also associated with molybdenum co-factor deficiency, characterised by untreatable seizures and neonatal death, whilst chromosomal re-arrangements in *ARHGEF9* give rise to a diverse range of symptoms including profound intellectual disability (Harvey *et al.* 2008).

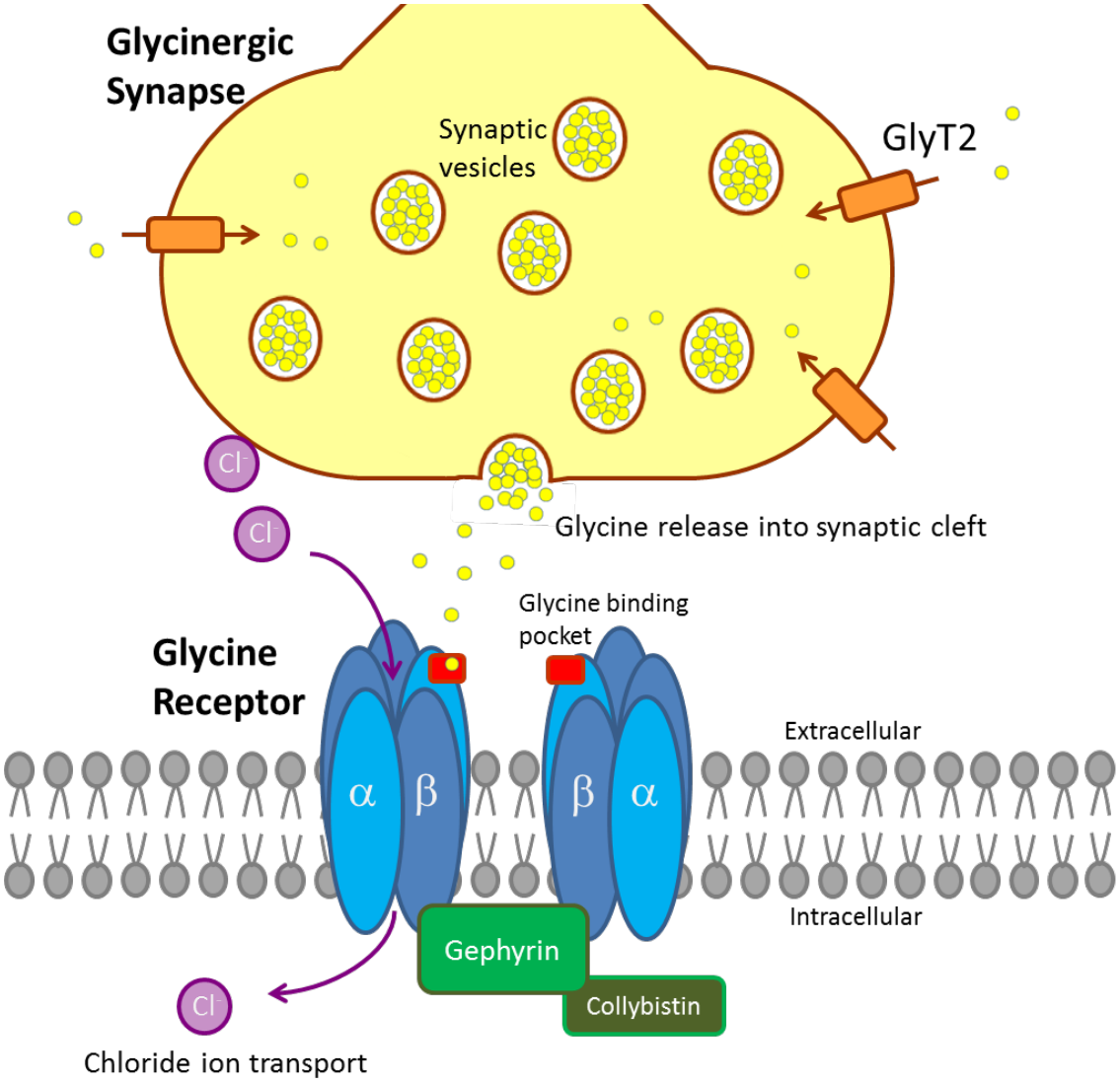


Figure 1.2 Cartoon illustrating the glycinergic synaptic machinery. It is neither to scale nor comprehensive but illustrates i) the structural role of *GLRB* compared to *GLRA1* and the presynaptic location of GlyT2.

GlyRs are heteropentameric (2α1:3β) inhibitory ligand-gated chloride ion channels (LGICs) that facilitate fast responses predominantly in the brainstem and spinal cord. GlyT2 is a sodium and chloride dependent transporter, which is involved in the re-uptake of glycine from the synaptic cleft into glycinergic neurones, thereby maintaining the pool of glycine for presynaptic vesicular replenishment. In hyperekplexia associated with *GLRA1* variants, all deletion and nonsense mutations are associated with recessive inheritance, whereas missense mutations can exert either a dominant or recessive pattern depending on the location of the mutation in the polypeptide (Chung *et al.* 2010b). Hyperekplexia has some notable firsts: 1) The description of mutations in *GLRA1* by Shiang and co-workers in 1993 was the first channelopathy associated with LGICs, 2) mutations in *SLC6A5* (Rees *et al.* 2006)

defined the first neurological disorder linked to a defect in presynaptic transporter for a classical fast-acting neurotransmitter and 3) the first demonstration of a gain-of-function, tonic activation of the GlyR channel as a novel mechanism of disease in LGICs (Chung *et al.* 2010b). Similar phenotypes in mice and cattle have been described with mutations in GlyR α1, β and GlyT2 genes (Harvey *et al.* 2008). These have helped to guide the choice of candidates for human gene screening.

Gene	Location	Protein	OMIM[2]	Associated human disease
Glycine receptors				
GLRA1	5q33.1	GlyR α1	138491	Hyperekplexia
GLRB	4q32.1	GlyR β	138492	Hyperekplexia
Receptor clustering proteins				
GPHN	14q23.3	Gephyrin	603930	MOCO deficiency, hyperekplexia, leukaemia
ARHGEF9	Xq11.1	Collybistin	300429	X-linked mental retardation, seizures, anxiety, hyperactivity, facial dysmorphism, sensitivity to thermal pain, hyperekplexia
Glycine transporters				
SLC6A5	11p15.1	GlyT2	604159	Hyperekplexia

Table 1.1 The genes of hyperekplexia

1.3.7 Treatment

The mainstay of treatment has been clonazepam, an allosteric potentiator of GABAA receptors which is efficacious for both patients with *GLRA1* and *SLC6A5* mutations (Andermann *et al*. 1980, Ryan *et al.* 1992, Bakker *et al*. 2009a). Although potentially treatable, hyperekplexia is not necessarily a benign condition: there have been reports of an increased incidence of sudden infant deaths in hyperekplexia families and startle attacks when ambulant (combined with a variable hypertonic spastic gait) can lead to sudden falls and subsequent head injuries and fractures (Giacoia and Ryan, 1994). It remains unclear whether the sudden deaths are all attributable to hypertonic/apnoeic attacks, or whether the children who died prematurely had even inherited the familial mutation(s). As one of

the global reference centres for hyperekplexia diagnosis and genetics, we wanted to ascertain whether there may be genotype-phenotype correlations in hyperekplexia.

1.4 Juvenile myoclonic epilepsy

1.4.1 History

Lund in 1975 was the first to use the term 'juvenile myoclonic epilepsy' in print. JME was recognised in the nineteenth century as a distinct epilepsy subtype and formally described in the modern era by Dieter Janz. The 'impulsive petit mal' (so named because 'impulsio' is Latin for shock) was both a homage to Herpin's description (1867) and a recognition that the jerks are not myoclonic as they do not represent contractions of isolated muscles, rather the forceful movement of entire limbs (figure 1.3). Despite his etymological care the name failed to adhere itself to the syndrome which may represent bias against the original articles (written in German): North-American epileptologists were slow to recognise and adopt this new diagnosis. Instead it was 'rediscovered' by both East and West coast epileptologists working in America (Asconape and Penry 1984; Delgado-Escueta and Enrile-Bacsal 1984). Despite this Janz will always remain intimately associated with JME as a testament to the novel and intensive research where he and Christian (his electrophysiologist) described a cohort of 47 patients in 1957.

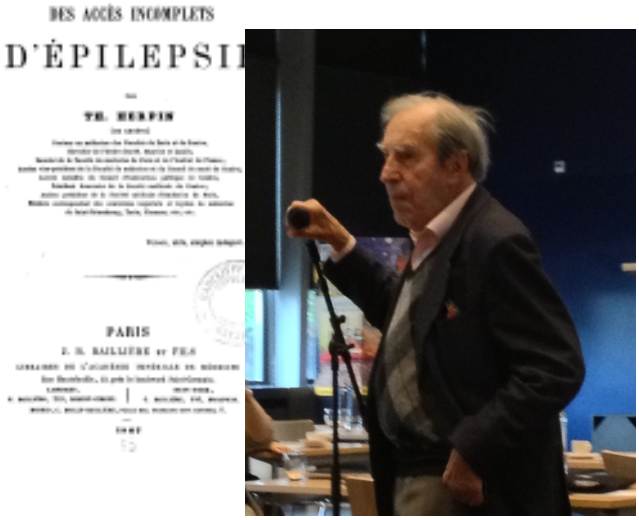

DES ACCÈS INCOMPLETS
D'ÉPILEPSI
TH. HERPIN
PARIS

Figure 1.3 Herpin's *Des Acce`s Incomplets d'Epilepsie* (left) and Professor Dieter Janz addressing the International Conference of JME, the Hague, October 2012 at the age of 92 (right).

If JME is genetic and common– why was it recognised so late in human history? JME is intimately associated with the factors which precipitate it - namely sleep deprivation. The descriptions by Theodore Herpin (1799–1865), in *Des Acce`s Incomplets d'Epilepsie*, published posthumously in 1867, appear to entirely precede John Hughlings Jackson's treatise on epilepsy. Herpin described the wide varieties of the manifestations of nonconvulsive epileptic seizures (Edie 2002). The introduction of electric lights soon after (and the ability to live like an owl rather than a lark) is said to explain the predominance of JME in the developed world and it's 'appearance' in the late 19th Century. A year after Janz' description it was also independently described by two Uruguayan neurologists Castells and Mendilaharsu (1958) as *'La epilepsia mioclonica bilateral y consciente'* – or the 'bilateral myoclonic epilepsy with retained awareness'. The Swiss claim however that Samuel Auguste Tissot, (1728 – 1797) of Lausanne was the first to describe an epilepsy very much like JME in his work *'Traité de l'épilepsie.'* Tissot worked under the sobriquet of "the physician of princes and the prince of physicians" but is best remembered for his works on masturbation.

1.4.2 JME phenotypes

The importance of genes in the causation of epilepsies could not be more clearly stated than in the most recent ILAE revision of the nomenclature for seizure types and epilepsy syndromes (Berg *et al.* 2010). Idiopathic as a term meaning broadly 'something that occurs spontaneously' is not too poor a synonym for a genetic epilepsy. However idiopathic also has a co-meaning- 'something with an obscure or unclear origin'; this cannot unerringly describe the state of our knowledge regarding the 'idiopathic' epilepsies. Of the generalised genetic epilepsies (GGE previously IGEs) juvenile myoclonic epilepsy (JME) has been estimated to have the highest likelihood of having a genetic cause (Kinirons *et al.* 2008). JME can cluster in families (sometimes occurring with other absence epilepsies, sometimes with more heterogeneous family phenotypes such as GEFS+),(Thomas *et al*. 2012) there is a slight preponderance of maternally-inherited cases and an increased chance of developing JME is seen in twin studies (Pal *et al.* 2006). Identifying myoclonic seizures (the ubiquitous seizure in JME) is important as myoclonic seizures in families show a concordance distinct from GGEs with absence seizure alone (Winawer *et al.* 2003, Winawer *et al.* 2005). JME was

chosen for the focus of my study because it is predominantly genetic epilepsy that had for the most part defied effective scrutiny.

Of course other potential exemplar 'genetic epilepsies' exist. The catastrophic childhood epilepsies (often associated with learning difficulties) are clearly strong models of primarily genetic epilepsy syndromes. They are predominantly rare on a population basis, relatively uniform in their presentation, occur early in life and there is strong evidence for the epileptic encephalopathies that they appear to be caused by *de novo* mutations or inheritance from mosaic parents. This is in contrast with JME which is a common epilepsy syndrome (for 5-11% of all epilepsies and up to 26% of the GGEs), where homogeneity of presentation has not been confirmed, seizure-onset can be as late as the 20s, and the familial link is strong. Furthermore, twenty years of scrutiny has not identified a single gene that is important on a population level for JME (Rees 2010; Rees 2007).

This is not to say that the genes identified have not brought us closer to understanding this complex condition – for example *EFHC1* (EF-hand domain (C-terminal) containing 1) may disrupt migration of post-mitotic neurons and tantalisingly hints at JME as a disorder of impaired neuronal development. It is simply that candidate gene screening has not identified a convincing high frequency genetic association. The heterogeneity and the sample size needed have prevented an adequately powered genome-wide association study. Those genes that have been described are restricted to isolated families (so called private mutations) and do not appear to have an effect across unrelated cases. A multinational cohort of JME families did identifying a major susceptibility locus at 15q13-14 via linkage analysis (Elmslie *et al.* 1997, Elmslie *et al.* 1996 Taske *et al.* 2002) however, despite the efforts of large collaborations we are currently many years away from routine gene testing for JME in clinical practice. The candidate gene approach has been likened to searching for a 'needle in a haystack'; if the majority of cases are answered by private mutations then maybe we are searching for many, many needles. It is therefore from two complementary directions that we are working towards better understanding JME and through this understanding make a valuable translation of scientific findings into clinical practice. The first of these is a critical clinical analysis of what we call JME to enable stringent case identification and classification of JME; the second is harnessing the power of

cutting edge genetic tools which enable small numbers of patients to be studied in great depth.

1.4.3 JME variability

Clinically JME is recognised as an electroclinical syndrome under the IGE/GGE umbrella mainly characterised by seizure types; age of seizure onset; EEG pattern; and response to medication. One would also expect unremarkable standard neuroimaging, predominantly early morning myoclonus with a benign progression, a lifelong liability for seizures and seizures triggered by photo-stimulation, sleep deprivation and illness or stress. Some authors will only make a diagnosis of JME in the context of a normal IQ. However, there is a remarkable degree of variability between individuals – so much so that some authors have made the case that there may be a spectrum of 'juvenile myoclonic epilepsies'. An attempt by seizure type alone to sub classify these epilepsies – for example by the age of onset of absence seizures (discussed below) - is fraught with difficulty as absence seizures are not an essential seizure type for a diagnosis of JME.

Age of Onset and Seizure Type There is significant variation in the age of onset of absence seizures particularly – with many individuals seemingly having a true childhood absence epilepsy (CAE) or juvenile absence epilepsy (JAE) phenotype before evolving into JME – whereupon others have their first absence seizures after their myoclonus begins (typically in the teenage years). Furthermore epileptic myoclonus is the *sine non qua* of JME but as increasingly more women are advised to avoid sodium valproate, how do we classify myoclonus that appears to be brought on by lamotrigine or carbamazepine therapy? There is also variety seen within myoclonic seizures experienced. Some have exclusively early morning attacks, some only ever have upper limb jerking – others have leg and head involvement. When positive and negative jerks are included in the equation it is no wonder that facial injury is reported so much more frequently in JME compared to other epilepsies (Thomas *et al.* 2009). If the age of absence onset is crucial in defining subtypes (Martınez-Juarez *et al*. 2006) then we need to pay greater heed to the major gene of effect for early onset CAE (up to 12% of cases) *SLC2A1* and other major genes as they emerge (Suls *et al.* 2009).

EEG pattern The EEG has the potential to add diagnostic doubt rather than clarity with up to a third of people demonstrating inter-ictal EEG characteristics that would be in keeping with a focal onset of seizures (Jayalakshmi *et al.* 2010, Usui *et al.* 2005). This is more remarkable when you consider this proportion is very similar to those who report absence seizures at all. Do this third of people have a similar but unrelated disorder or is a mix of focal and generalised activity typical for JME?

Response to medication The response to sodium valproate although generally excellent is by no means uniform (up to 80% become seizure free) and the degree by which myoclonus is exacerbated by lamotrigine or carbamazepine depends on the individual. Although valproate is uncontroversially the treatment of choice for young men with JME the best agent is not clear for women of child bearing age (Nicolson and Marson, 2010). This heterogeneity in drug response to second line agents goes against the homogeneity of JME.

Unremarkable imaging Although standard clinical magnetic resonance imaging does not reveal an abnormality on visual inspection we can no longer say that imaging is normal in JME (Anderson and Hamandi 2011). A range of advanced techniques including PET, structural MRI, diffusion tensor imaging (DTI) and magnetic resonance spectroscopy reveal evidence of predominantly frontal lobe and thalamic changes at the group level. Changes in microstructure connectivity in the mesial frontal region (measured using functional MRI and DTI) are postulated to be the crux for triggering motor seizures (Vollmar *et al.* 2012).

Neuronal development disorder? Some authors ask whether cortical developmental abnormalities could underpin JME (de Nijs *et al.* 2012). Specifically, this is proposed after identifying a role for the *EFHC1* gene – a gene linked to JME phenotypes. *EFHC1* is a microtubule-associated protein involved in the regulation of cell division. *EFHC1* impairment in the developing rat neocortex causes a marked disruption of radial migration, with defects in the radial glia scaffold organization and in the locomotion of post-mitotic neurons.

Benign epilepsies Not only have cognitive defects in JME been long established (below) but these defects have been correlated with the imaging abnormalities mentioned previously. A

recent DTI study of 25 people with JME (versus matched controls) demonstrated widespread disturbance of microstructural white matter integrity in the frontal lobe and corpus callosum that interconnects frontal cortices (Kim *et al*. 2012). This was taken as further support of the theory of thalamofrontal network disconnection syndrome in JME.

Life-long seizures Even the dictum that JME is a lifelong condition and that seizure recurrence should be expected following drug withdrawal has been challenged with long-term studies. For example Delgado-Escueta and colleagues (1984) noted that only 12/43 relapsed at two years following valproate withdrawal. This was corroborated by a study of 23 people (17 female) from Canada where 11 discontinued treatment and six remained seizure free, 3 had myoclonus only and two had infrequent seizures (Camfield, Camfield 2009). JME is thought to be an epilepsy syndrome with little in the way of serious complications but the Camfield paper added further clinical heterogeneity as eight of the 23 had an episode of convulsive status epilepticus. This pattern of a varied long-term outcome was further described recently in a review of 31 people (Geithner *et al.* 2012). Here, only two thirds achieved true seizure freedom. Nine patients attempted to discontinue drug treatment – six of these were successful (mean duration of seizure free follow up was 19 years). This study concludes (and I would agree) that this is once more very strong evidence against the homogeneity of JME.

1.4.4 Are there true neuropsychological traits in JME?

If the above clinical features are variable and cannot be relied upon to identify a 'true JME' is there a role for neuropsychological or cognitive trait analysis? In Janz & Christian's seminal paper of 1957 personality was a key part of the description, alongside information on: characteristics of minor seizures; rhythmicity; age of onset; prevalence; aetiology; heredity; course; triggering factors; nosology; EEG; treatment; prognosis; differential diagnosis; pathophysiology and constitution of the patients. From the onset a JME personality was proposed *"characterized by unsteadiness, lack of discipline, hedonism and an indifference to their disease…most were of average intellectual ability, none was extraordinarily gifted… They often appear self-assured and bragging, the girls and women coquettish and seducing, but can also act decidedly mistrustfully and be, timid, frightened and inhibited. … Their mood changes rapidly and frequently. This makes their contact both*

charming and difficult. .. They are easy to encourage and discourage, they are gullible and unreliable." Allowing for the change in use of language since 1957 this description still seems stark. Clearly if thalamofrontal circuitry disconnection is an important feature of JME pathogenesis then the above quote could be describing executive function impairment – but the concept of an 'epileptic personality' let alone a JME personality remains highly controversial.

The GGEs have not been as aggressively investigated by psychological researchers as temporal lobe epilepsy, where surgical treatment has focussed attention. The first studies into JME, which were mainly retrospective, identified a "neurosis of character" seen in JME patients more frequently than other idiopathic epilepsies (Bech *et al*. 1976, Lund *et al.* 1976). However, a number of centres have investigated large samples (up to fifty individuals) using a variety of comparison groups. The majority of studies have recruited patients taking a sodium valproate preparation, however Kim *et al.*'s study of 27 people is notable for the inclusion of only unmedicated persons. Of course inter-ictal epileptiform discharges would be more likely in an untreated patient arm and reduced attention and concentration would be expected – resulting in low scores on tests such as digit span.

1.4.5 Executive function

Janz' initial description would suggest that executive function would be impaired in JME or that symptoms in keeping with a borderline personality disorder could be expected. A study of forty patients with JME (and twenty-two controls) using the temperament dimension of the temperament and character inventory demonstrated much greater levels of novelty seeking behaviour in people with JME. In addition those with poorer seizure control had greater novelty seeking and impulsive behaviour (Moschetta *et al.* 2011). The distribution of epileptiform activity seen over frontocentral regions in JME corroborates with poorer executive function: however test results are heterogeneous within each study - some patients have marked deficits, others none. In particular mental flexibility and concept formation-abstract reasoning are abnormal, even when compared to people with temporal lobe epilepsy (Devinsky *et al.* 1997). Levav and colleagues (2002) ascertained that unaffected family members had poorer scores for attention – a pertinent finding as relatives would not have any psychomotor retardation from antiepileptic medications. In contrast,

people with JME and their siblings were indistinguishable from controls on tests of visuospatial ability such as the Rey Complex Figure task (Iqbal *et al.* 2009). Neuropsychology (where available) remains an attractive focus for trait analysis as it is inexpensive (compared to advanced imaging), acceptable to patients and can help reveal clinically useful psychopathology. Gelisse *et al.* (2001), using DSM-IV, criteria evaluated the prevalence and types of psychiatric disorders in patients with JME. They found a high frequency of personality disorder (14%), mostly borderline personality disorder. This study was corroborated by Araujo-Filho et al. (2006) who evaluated 100 patients with JME using the Structured Clinical Interview for DSM Disorders I and II (SCID-I and SCID-II) as diagnostic instruments, identifying that 20% had personality disorders, especially Cluster B personality disorders.

What is currently missing from the published literature is a truly comprehensive study of people with JME. Too few studies have looked to ascertain a full scale IQ and all too often only one or two tests of executive function are employed and these are frustratingly replicated (Stroop, trails) rather than novel tests utilised and described. It is important to also be able to describe the affective burden in the cohort and to begin to look at personality subtypes.

1.5 JME families

Many patients in clinic know that they have a family history of epilepsy or febrile seizures. They are aware that even if epilepsy seems to run in their family, that it can look different in each family members and that– even if both mum and dad have had seizures, it doesn't mean that their children will have epilepsy. Genetic counselling for families with epilepsy, however, is a very rare service and giving specific risks and details is often impossible. This will change in my lifetime with access to routine personal genome analysis; providing we can learn to interpret the results that the process yields.

Often individual differences are genetic differences. We are comfortable understanding that height and eye colour are complex phenotypes (not all or nothing) – but epilepsy must be

one of the ultimate challenges. Why do some people get drug side effects at relatively low doses? Why do some people get rare drug side effects at all? Why do some people get frequent seizures- and others very few? Why is sleep a trigger for some people and the menstrual cycle for others? Why do some women have foetal malformations when taking medications in pregnancy but most don't? Why are some people more likely to develop epilepsy following head trauma? We are slowly chipping away at conditions that were thought to be 'psychological' such as fainting, stammering or panic attacks (Kang *et al.* 2010) – and describing the genes that underpin these conditions. In order to begin to understand what this may mean for seizures we at WERN have been investigating the genetics of familial epilepsy.

1.5.1 Family study

We have been working in Wales to identify and recruit families with epilepsy and have recruited over ninety families from England and Wales and (Johnston *et al.* 2010). This has been a labour intensive process: perhaps only a fifth of families eligible for research consent to take part. This figure is much higher when you meet the individuals 'face to face' in clinic. We then visit the family (and extended family) in their own homes, consent them into research and take blood samples for DNA extraction. This process has permitted us to find out so much more about a family than their inherited material; such as how the implications of a result may impact on family dynamics. We are obliged to forge long relationships with each family to permit them to engage fully in research. So far we have had no families withdraw from our project once we have taken their samples- but some have been 'cancelled' while we were on our way to their homes. That is their prerogative and we make sure that they understand what a genetic finding may (or may not) mean for their family. There remains a great degree of stigma and indeed guilt regarding familial epilepsy, which can be brought into sharp focus when people are invited to be participants in research (Hammond *et al.* 2010).

1.5.2 GEFS+

Generalised epilepsy with febrile seizures plus (GEFS+) was first proposed as a diagnostic entity in 1997 (Scheffer *et al.* 1997). The families broadly have idiopathic generalised

epilepsy but with a predominance of febrile and febrile seizures plus (FS+); indeed febrile seizure are considered the prototype seizure in GEFS+. Additionally, young children with catastrophic epilepsy syndromes (such as Dravet syndrome, known first described as the severe myoclonic epilepsy of infancy (SMEI)) occur more frequently among GEFS+ families than expected by chance. Family members with specific electroclinical syndromes (such as childhood absence epilepsy or juvenile myoclonic epilepsy) would be unusual, but are seen infrequently. However, the occurrence of occasional family members with focal epilepsies has led to the suggestion that the GEFS+ acronym better describes *genetic* epilepsy with febrile seizures plus. GEFS+ is now widely recognised and is a core diagnosis seen in studies of families with epilepsy.

Berkovic and Sheffer's original GEFS+ description defined a GEFS+ 'spectrum', ranging from individuals with simple febrile seizures and FS+ up to SMEI. The generalised epilepsy phenotypes included absence seizures, myoclonic seizures, and more rarely atonic and myoclonic-astatic seizures. It was also suggested that the pedigree showed an autosomal dominant pattern with incomplete penetrance (Scheffer *et al.* 1997). The gene mutations thus far described in association with GEFS+ have coded for ion channels: private mutations in *SCN1A, SCN1B* and *GABRG2* genes occur in 10% of families with the GEFS+ phenotype (Escayg *et al.* 2000, Wallace *et al.* 1998). Although the genetic causes of most epilepsies (including GEFS+) have yet to be elucidated it is still widely believed that most epilepsies have some genetic component (Tan *et al.* 2004). Siblings of an individual with confirmed seizures have an approximately fivefold risk of developing epilepsy (compared to a background risk of 0.75%), depending on the age of onset of seizures. A monozygotic twin with an affected twin has between 37% and 80% risk of developing epilepsy, compared to between 3% and 32% for a dizygotic twin (Kjeldsen *et al.* 2003). This also implies an environmental aspect to the aetiology; in the case of GEFS+ this may be the infantile illness needed to provoke typical and FS+. Mutations in the sodium channel may also lead to an increased risk of febrile convulsions which itself increases the risk of developing epilepsy (Hirose *et al*. 2000). This would certainly seem logical, as sodium channel defects are known to causes disorders influenced by temperature, such as paramyotonia congenita (McClatchey *et al.* 1992).

More than six hundred *SCN1A* mutations have been described (www.scn1a.info/, Lossin 2009) missense mutations being the most frequent. Frameshift and nonsense mutations are associated with the more deleterious epilepsies, such as SMEI (Escayg *et al*. 2000, Wallace *et al.* 1998, Kanai *et al.* 2004). The mutations are spread throughout the gene but occur most frequently outside the pore-forming region. Initially, the GEFS+ family phenotype was tightly defined, but the borders of both epilepsy within GEFS+ families and the epilepsy caused by *SCN1A* mutations are increasingly blurred. GEFS+ families from the UK have not been previously systematically described and analysed; despite the original family described being Anglo-Australian (Scheffer et al. 1997). I present our experiences and the clinical description of our GEFS+ families.

1.6 JME genetics

So what has been achieved thus far? The genetic mutations that underlie complex but rare disorders have been well described – such as Angelman's, Dravet's or Rett's syndromes. Even so the relationships can be complex: some children with mutations in 'Rett's' genes will have different phenotypes; some children with Dravet's won't have sodium channel mutations; the same sodium channel mutation may cause febrile seizures in a child, but Dravet's syndrome in their sibling. This mismatch persists because of our previous – hypothesis driven – process. Hypotheses are useful: predicting that you'll find a result, then discovering it is scientifically powerful. However in this 'needle in the haystack' process – it means you can only find what you set out to find. For a while all the epilepsy gene mutations were found in genes which code for channels: this was in keeping with our idea of what might cause epilepsy and in keeping with prior findings. Possible genetic results have been limited by our current knowledge and our imagination.

The next generation of research does not require a candidate gene to be selected: sequencing of the exome (all the coding genes) or the whole genome allows for a search for genes that can throw up unexpected results. Whole genome analysis will permit the discovery of non-channel coding genes – and more usefully multiple genes of effect. The challenges however then become how best to sift through the vast amounts of information provided and then to prove that the genetic changes really do cause epilepsy.

1.6.1 The Juvenile Myoclonic Epilepsies

Accepting that there is variability within the current definition of JME that is clinically important (response to drugs, prognosis) – where does the phenotypic homogeneity lie? There have been a number of attempts to subcategorise JME based on clinical and seizure characteristics (Geithner *et al.* 2012, Martınez-Juarez *et al.* 2006). One classification created four groups (the number is arbitrary but the clinical reasoning sound): namely classic JME (72%); CAE evolving to JME (18%); JME with adolescent absence (7%); JME with astatic seizures (3%) (Martınez-Juarez *et al.* 2006). A creditable 257 individuals were identified – predominantly from California and Latin America and were exclusively those with a family history of JME. It is unclear whether familial JME is similar to sporadic JME and of course the

majority of individuals with JME do *not* report a family history of epilepsy. This admirable study clearly delineates the relationship between variability of absence seizure history and JME prognosis – but did not publish sub-classifications based on other criteria.

Sixty five sequential patients seen in a Brazilian epilepsy clinic were also stratified (Guaranha *et al.* 2011). In addition to seizure type, seizure triggers and psychiatric comorbidity (as estimated using the State–Trait Anxiety Inventory (STAI) psychiatric interview and use of the Schedule Clinical Interview for DSM-IV, Axis I (SCID-I) and Axis II (SCID-II) questionnaires) were investigated. A good prognosis (seizure free on medication after five years) was associated with a later onset of seizures, a smaller likelihood of having a personality disorder (4% vs. 25%) and a lower score on the STAI traits scale. A poor prognosis was associated with reflex traits, a longer epilepsy duration, a combination of all three seizure types and discharges seen on the base line video-EEG. This level of multifactorial complexity may obfuscate clinical researchers' efforts to tidily create a number of neat subcategories for JME. The first strategy will be to dissect out a trait associated with a large gene effect – such as photosensitivity. This has been the successful approach in Rolandic Epilepsy (Benign Epilepsy of childhood with Centro Temporal Spikes (BECCTS)). Researchers identified that the EEG trait segregated with the Elongator Protein Complex 4 (*ELP4*) (Strug *et al.* 2009). They have then gone further to describe the subtle neurocognitive features of children with this epilepsy (Smith *et al.* 2012).

Other relatively unexplored ways to sub classify JME (or the JMEs) are by better understanding the diurnal variation so clear in early morning myoclonus and the paucity of sleep related generalised tonic clonic seizures; the exacerbation of seizures by sleep deprivation, stress and menstruation; and non-photosensitive reflex tendencies. JME, however-defined, is unlikely to be monogenetic and gene mutations may be distinct from channelopathies and therefore provide insights into the neurobiology of seizure onset.

1.6.2 Next generation sequencing

In recent years, next generation sequencing (NGS) has transformed the way in which disease causative genes are identified in both the research and clinical diagnostic domains. NGS is a

new technique that exploits the massively-parallel sequencing capabilities of next-generation platforms to rapidly identify rare variants in the entire genome (whole-genome sequencing) or only in the 1% of the genome that codes for proteins (whole-exome sequencing). The value of whole genome/exome sequencing has been demonstrated in the publishing explosion seen in the last three years which has identified genes associated with conditions as diverse as common cancers to rarer disorders such as Charcot-Marie-Tooth neuropathy, Kabuki syndrome and Miller syndrome (appendix A). Tight endophenotyping (sub-categorisation) is essential to make the most of the power of next generation sequencing. The cornerstone of our work is the deep scrutiny of phenotype - using strict and novel criteria.

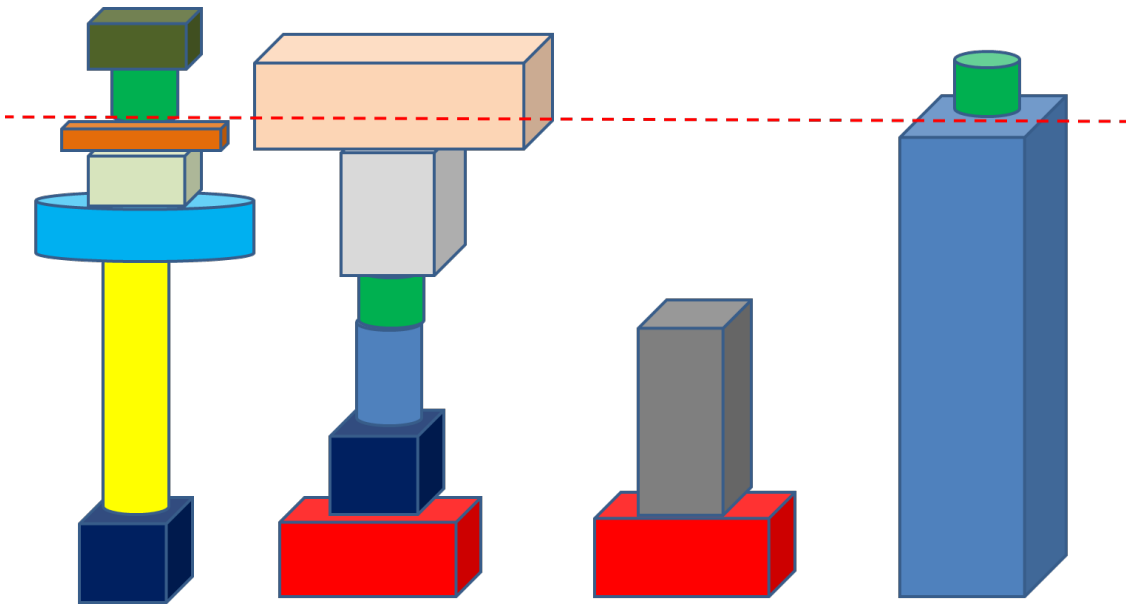


Figure 1.4 A cartoon describing genetic causality. The first and second columns represent two disorders with a polygenic cause. Although some rare variants are shared (in this case the navy box) the majority of the shapes are different. This could either represent the variety of rare variants needed to breach the imaginary threshold for pathogenicity (the red line) in two different disorders that share some heritability such as IGE and bipolar disorder – or two similar disorders (two JMEs). The third column represents an unaffected relative. They share a gene in common with their relative (red box – column two) but do not have sufficient variants to cause a phenotype. They may however share an indirect link to the condition of interest – a so called 'endophenotype'. The fourth column represents a disorder such as hyperekplexia where one variant is so dominant that even if there are additional genes that predispose to the disorder having a single mutation causes the phenotype and so it behaves like a monogenetic disorder.

1.6.3 Traditional and Modern Methods

The traditional attempts at genetic studies have concentrated on candidate gene analyses by either screening previously-identified disease associated genes or identifying a novel gene based on linkage or other hypothesis-led instincts. *Sanger* sequencing to find mutations in candidate genes is a powerful tool, however, it is time-consuming and can be a very costly method for genetically-heterogeneous groups such as adults with epilepsy. Also ascertaining pathogenicity of novel variants can be notoriously difficult – even with the ion-channel genes that have predominated the early discoveries in epilepsy genetics (Chung *et al*. 2010). More recently, it has been noted that the human genome contains a number of missense and deleterious nonsense mutations that do not appear to have any phenotypic effect..Alternatives to single gene screening involved studying families with multiply-affected family members and identifying shared areas of homogeneity. Six chromosomal loci are currently genetically linked to JME: chromosomes 6p12 (Bai *et al.* 2002, *Serratosa et al.* 1996), 6p21.3 (Greenberg *et al*. 2000), 15q14 (Elmslie *et al.* 1997, Whitehouse *et al.* 1993), 5q (Cossette *et al.* 2002) 16p13 and 7q32 (Pinto *et al.* 2005). Of these chromosome loci, two potentially disease-causing mutations, namely, *GABRA1* in 5q34-q35 (Cossette *et al.* 2002) and Myoclonin/*EFHC1* in 6p12 (Suzuki *et al.* 2004) have been associated with JME. *EFHC1* specifically has a growing body of functional work to support its role in cell division and neuroblast migration. Two genes with putative association include *BRD2, RING3* in 6p21 (Pal *et al.* 2003) and connexin 36 in 15q14 (Mas *et al.* 2004).

Although linkage-analysis has provided invaluable insights into the underlying genetic causes of many disorders, there are inherent difficulties including the ascertainment of large multiply-affected families, the ambiguity of large linkage interval regions and the amount of work trawling through interval candidate genes or further fine mapping. One of the ways to overcome the complex heterogeneity of epilepsy genetics is the simultaneous screening of multiple epilepsy genes by using epilepsy-specific panels (targeted NGS). A recent study presented approximately 50% mutation detection rate (16 out of 33 patients) by utilising a sequencing panel containing 256 genes relevant to epilepsy phenotypes (Lemke *et al.* 2012). However, a persuasive argument can be made in favour of the use of direct exomic sequencing in place of hypothesis-based gene panels. The advantage of whole-exome sequencing is that by remaining agnostic you can identify genes in families

and systems that either have no current known function, or have been previously and erroneously discounted, or suggest a pathogenicity which had not previously been described. NGS studies identify causative genes that would not have been selected using traditional methods and can remain hypothesis-free (Corbett *et al.* 2010, Chen *et al.* 2011). Although the gene panels currently present a marginally better quality of coverage over exomic sequencing (for the selected genes), it would be inevitable, given progress in the field, to adopt exome sequencing or even the whole-genome sequencing as the diagnostic tool of choice in the near future (Dixon-Salazar *et al.* 2012).

1.6.4 Copy number variation in GGEs

Both genome and exome sequencing can identify variants that change the coding regions of proteins including the unmasking of missense or nonsense single-base substitutions, or small insertions or deletions (indels) (Lupski *et al.* 2010, Ng *et al.* 2010). However CNV analysis is used in preparation for second generation sequencing and increasingly as a front line tool for the investigation of individuals with learning disability. Copy number variation is a form of structural variation that results from usually having one extra or one too few copies of a portion of DNA often many kilobases in length. A CNV is traditionally defined as over a kilobase and can be many megabases in size. They can be both inherited and occur spontaneously during early development (*de novo*). CNVs can be deletions, insertions, translocations or duplications. Certain genomic areas appear to be susceptible to CNVs, such as regions with low copy repeats.

Chromosomal rearrangements such as CNVs have been increasingly recognised underlying neurological disorders with a complex genetic inheritance, such as epilepsy, schizophrenia and autism (Sebat *et al.* 2007, International Schizophrenia Consortium *et al.* 2008, Talkowski *et al.* 2012). Currently, CGH-array is considered as a powerful method to detect CNVs which are seen disproportionately in the idiopathic generalised epilepsies (de Kovel *et al.* 2010, Mefford *et al.* 2010). This technique has made its way into clinical practice although the coverage is not universal and it is only routinely used in major academic centres.

Array CGH (described in detail in materials and methods) has been used by a number of authors to identify copy number variations in adults with generalised genetic epilepsies. There is a great variation in phenotypic expression too - here I present the CNVs published in GGE and related epilepsies.

Mefford *et al.* (2010)

Technique: Array CGH

135,000 probes (mean marker distance 38kb) but with a higher-density customised array design to cover known CNVs (mean marker distance 2.5kb). 5 or more consecutive probes needed

Sample Size: 517 cases, 2493 controls

Phenotype: GGE and SFE

Findings: 3% have 15q11.2, 15q13.3 or 16p13.11 CNVs.

189 cases had JME: 8 had frequently occurring CNVs (4.2%), 9 having other CNVs (4.8%). In contrast absence epilepsy saw 5/94 frequently occurring CNVs (5.3%) and 5/94 others; IGE with GTCS only saw no frequently occurring CNVS (33 cases) and 2 atypical ones (6.1%); and unclassified IGE saw 2/63 (3.2%) and 4 atypical ones (6.3%).

The JME CNVs were at 1q21.1; 6q12; 7q11.22; 8q21-q22; 9p21.3; 13q31.1; 14q24.2; 15q13.3; 15q13.3; 15q13.3; 15q13.3; 16p11.2; 16p13.11; 16p13.11; 16p13.11; 17p11.2; 18q11.2; and 18q11.2.

Galizia *et al.* (2012)

Technique: Array CGH

135,000 probes, mean distance 13kb, 3 consecutive probes needed

Sample Size: 82

Phenotype: Adults with drug resistant epilepsy and complex co-morbidities – 63% had LD; 37% with comorbid psychiatric disorders in London

Findings: 12 CNVs thought to be pathogenic and a further 69 of unknown significance reported: 15q11.2-q13.1; 1p36.33-p36.32; 9p24.3-p24.2; 15q11.1-q13.1; 16p13.11; 6q22.31-q22.22; 4q16.3-p12; 15q13.2-q13.3; Xp22.23-q28 and 16p11.2; 15q11.2-q13.2; 16p13.11; 7q35.

Striano *et al*. (2012)

Technique: Array CGH

44,000 probes, mean distance 43kb, 8 consecutive probes needed

Sample Size: 279 with epilepsy, 265 with LD, 246 controls

Phenotype: Prospective patients with epilepsy in Italy

Findings: 10 pathogenic CNVs (6q26-q27; 8p23.3-p23.1; 8p23.1-p21.2; 9q34.3; 15 q11-q13.1; 15q24.1-q24.3; 16p13.11; 22q13.31-q13.33; 22q13.32-q13.33; Xp22.31) and a further 18 novel ones (2p12; 2q11.2; 4q31.23; 6q16.1; 6q22.2 ; 6q22.31; 7q36.3; 14q32.33; 9p21.2; 9q13-q21.13; 10p12.33-p12.31; 12q24.33; 15q26.1; 17q24.3; 19p13.3; 19q13.41-q13.43; 19q13.43; Xp11.3).

CNVs in patients were more likely to be gene rich (particularly over 10 genes) and larger than a megabase. Epilepsy was not associated with rare CNVs (neuropsychiatric features and LD were).

Of those with GGE they were 16p13.11 deletion (?JME no LD) and 6q22.31 duplication (IGE), 9p21.2 duplication (?CAE), 12q24.33 duplication (IGE), 17q24.3 duplication (?CAE) and Xp11.3 duplication (?JME).

Jiang *et al.* (2012)

Technique: Affymetrix SNP microarray 5.0

Targeting 15q11.2 – 100 probes per CNV

Sample Size: 198 with CAE, 400 controls

Phenotype: CAE in China

Findings: Three 15q11.2 and one 15q13 – none in controls

1.6.5 Bioinformatic Analysis

CNV analysis identifies many variants that will need validation, replication and segregation analysis. Validation involves the confirmation of the location of the breakpoints as well as cross-referencing the CNV with established gene variation databases; the segregation analysis focuses on the genotype / phenotype trend in family members of each case with a focus on the flow of variants with affection status. Wherever possible researchers have

been keen to utilise computational software or molecular modelling pipelines to predict the effect of single points of variation on protein structures by comparing wild-type with variant genotypes for any given gene candidate (Mullins *et al.* 2010). However when trying to assess the clinical consequences of a contiguous gene disorder there are fewer complementary tools that can be utilised: many CNVs are private and therefore their consequences unique. The endpoint of this bioinformatic pipeline is convergent genetic, database and modelling data that would then pass onto an *in vitro* testing strategy – the methods employed dependant on the biological context of the newly-identified candidate gene(s).

Before we can begin to harness the power of second generation sequencing techniques we must first reduce JME down to lacunae of homogeneity – using increasingly more sophisticated phenotyping tools. The current technological advances in gene sequencing have been used to dramatic effect to identify single gene causes in rare syndromes and identify risk variants in malignancies. Filtering the variety of the human exome or genome down into a handful of biologically plausible candidates now relies on a pipeline of bio-statistics, software and functional analyses. It is simply unacceptable to return uncertain findings to the clinical domain and therefore it is crucial that pathogenicity is as fully determined as possible before families receive genetic counselling and test results.

Chapter Two

Materials and Methods

Materials and Methods

This chapter details the methodologies used to investigate the questions and hypotheses set out in the introduction – namely to best describe the features of hyperekplexia and JME to enable genetic examination. The translational nature of this thesis is such that a number of methods were employed and they are explained in the order that the chapters appear. Additional material is provided in the appendices which may also aid in the understanding of the results chapter (appendices B-F).

2.1 Involvement

Two anecdotes are explored to augment the description of the DUETs research: the patient RDG and digital storytelling projects. The first of these is funded through WERN which itself is a directly funded research network.

2.1.1 WERN

Wales (unlike England) has a funded research network to coordinate epilepsy projects – alongside networks for more well-supported health condition themes such as cancer and heart disease. The Wales epilepsy research network (WERN) is a thematic network, funded initially through the Wales office of research and development (WORD) 2005 to 2009 and then through WORD's reincarnation as NISCHR (National Institute for Social Care and Health Research). Our shared goal is to reduce the morbidity of epilepsy in Wales and to work towards fewer seizures, fewer side effects and fewer "wasted lives" (All Party Parliamentary Group on Epilepsy, 2007). WERN is not positioned as a rival to any of the existing epilepsy charity groups – rather we have built strong relationships with groups such as Epilepsy Action, Epilepsy Wales and Epilepsy Bereaved. This mutual support has been invaluable in launching the network from concept to conceit in under five years. WERN is again the funder of the family study (explored below with regards to chapter five).

2.1.2 RDG Creation

Dr Carrie Hammond (WERN's first co-ordinator) was tasked with founding the RDG and decided to make it patient focussed from the very start. In addition to the interested parties

that she and other clinicians from the network had met through research projects, she contacted *Involving People* to find volunteers. *Involving People* is a support service funded by NISCHR to help researchers engage appropriately with interested lay individuals. Alongside recruiting and matching projects with participants *Involving People* provides training for people to be able to make the most of their interaction with researchers – to enable genuine patient involvement. Having identified a group of people who had an interest in epilepsy – we asked for a volunteer chairperson and wanted them to circulate ideas that would go on to form their rules of engagement. The decisions made, including – face to face meetings every three months, sub-groups to work on specific projects, email circulation lists and token clinical representation on their group - were largely patient initiated. A patient led group is a very progressive, unusual initiative; similar groups may have just made do with a single 'professional' patient.

When you consider the difference between someone with learning difficulties, someone seizure free following surgery, someone with a familial epilepsy syndrome and a carer for a child with epilepsy and a neurodegenerative syndrome it becomes clear that a single representative would not suffice. The present RDG (as of November 2012) has a variety of backgrounds represented: parents of a young adult who passed away unexpectedly during a seizure, a woman with lifelong epilepsy from a family with epilepsy, a gentleman who is seizure free following surgery, a carer for people with epilepsy, someone with both mild learning difficulties and subsequent epilepsy. The establishment of the patient RDG has been a new experience for WERN and for the patients, carers and service users involved; primarily because there were no other similar examples to learn from. I hope that our experiences may stimulate other groups to consider such a panel of experts as a vital part of health and social research.

2.1.3 Digital Storytelling

My introduction to digital storytelling was through Lynne Thomas (*Involving People* Officer). She had previously worked in the medium – which has a strong history in Wales. The BBC in partnership with Cardiff University ran an award-winning Digital Storytelling project called Capture Wales. BBC Capture Wales ran monthly workshops from 2001 - February 2008,

facilitating people in the making of their digital stories. There is a permanent archive accessible via www.bbc.co.uk/wales/arts/yourvideo/queries/capturewales.shtml

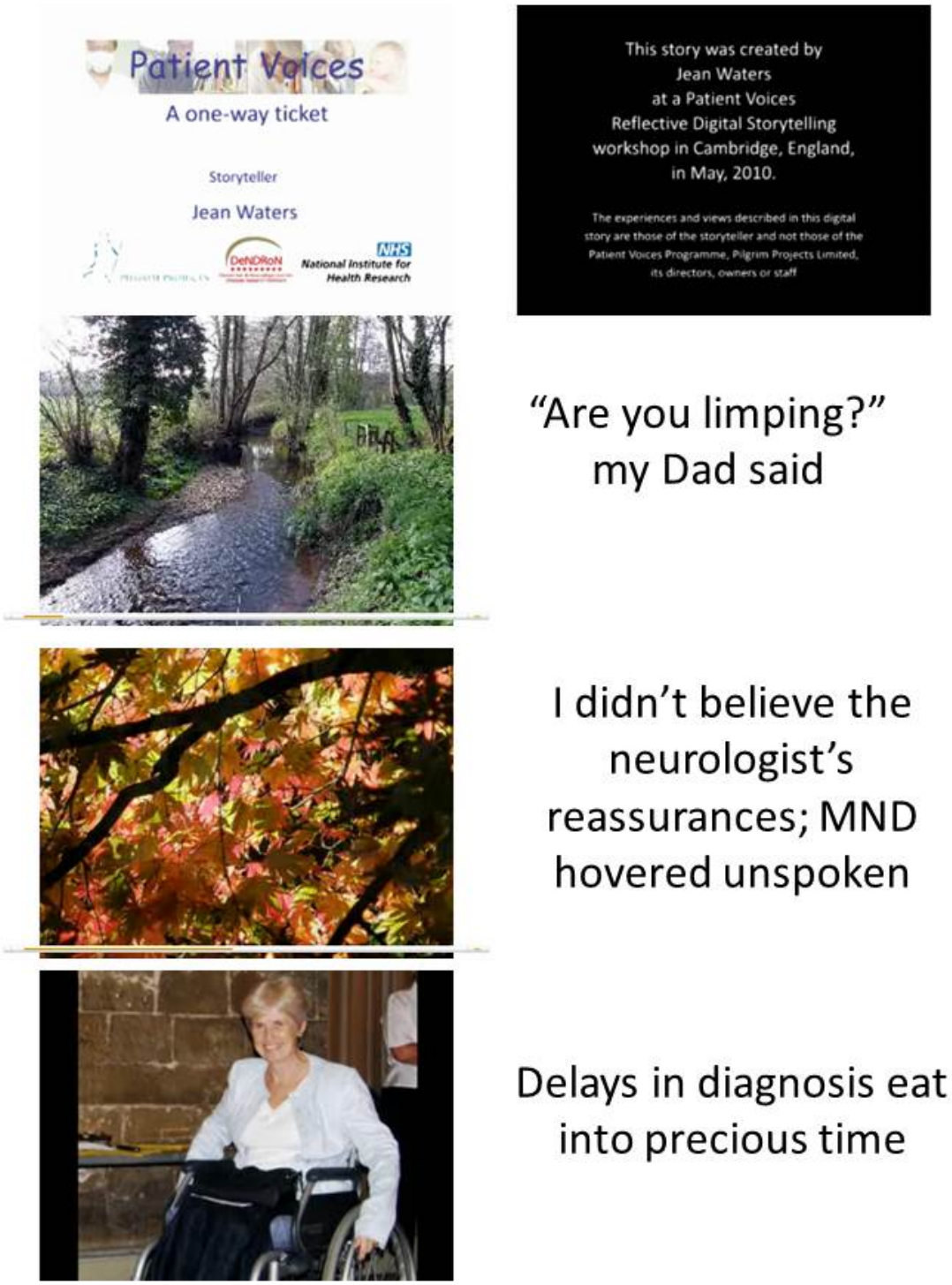


Figure 2.1 An example of a Digital Story. Dendron sponsored video of a clinician talking about her slow process of being diagnosed with motor neurone disease. Her quotes are taken out of context and placed alongside the pictures selected.

Digital story telling is a method of producing short emotive films using inexpensive digital cameras, editing software and notebook computers: the resulting story is typically comprised of a series of pictures or photographs set to an audio narrative that tells a personal story. Practitioners working in Cardiff have successfully used the technique with a wide variety of people, including people from a number of professions, children, people with learning disabilities and people who are computer illiterate. (Meadows 2003) These stories have been used previously with great success in a number of fields including medical education (Sandars *et al.* 2008, Sandars 2009) cancer genetics and palliative care. We think that digital story telling is an important and compelling medium that would be highly appropriate for helping people with epilepsy tell their journey and to inform others.

Digital story telling is an emotionally powerful method of communicating personal experiences. An audio track lasting three or so minutes is complemented by a series of pictures or photographs. When telling their story, people commonly choose to use personal and family photographs to complement the audio narrative about their health (please see www.cancergeneticsstorybank.co.uk and www.patientvoices.org.uk for examples; Hawkes, 2010; Herxheimer *et al.* 2000). The production of stories involves the story teller giving a fairly long and free flowing interview with a member of the research team. The interview is then edited down to capture the essence of the story in a two/three minute piece. The digital storyteller has full editorial control over the video and creating the piece is a collaborative effort, so the storyteller retains control over how they are represented. The personal pieces are emotionally relevant and often have a profound effect on those viewing them.

2.1.4 Starting the Epilepsy Story Collection

Alongside my colleague Dr Rose Thompson we were successful in winning an *Epilepsy Action Diamond Award* grant to create a permanent digital educational resource: an epilepsy story collection. We employed a professional team to help us found the collection – teaching us how to interview people efficiently and how to use the editing software. We have created five full stories thus far – enough to host on a WERN website, for people with epilepsy in the UK and indeed the English speaking world to see. We plan to augment these with short instructional talks such as ‘what to expect from a brain scan’ or ‘how to make the most of

your clinic appointment'. Epilepsy has a myriad of causes and journeys. Some videos will be important to some viewers only (epilepsy and pregnancy, pre-conception counselling, breastfeeding with seizures) and others may strike a universal chord. We would like support to found this repository and then will seek continuation funding to build this as a permanent educational resource.

2.1.5 DUETs

2.1.5.1 Ethics

This study involves patients and professionals as partners in a consultancy about research and as such no one is considered a participant in research (National Research Ethics Service, 2009).

2.1.5.2 Meetings

The qualitative study was based on the successful consultations in rheumatoid arthritis (Carr *et al.* 2003). We arranged five separate focus groups: three with patients and carers and two with clinicians. Patients were invited from a variety of sources: clinic appointments, previous involvement in local projects, and membership of local charity groups. Involvement was voluntary and unpaid; meetings lasted 90 to 120 minutes. No-one who expressed an interest in attending a meeting was discouraged from doing so. Participants were invited to try to ensure a balance of adult and paediatric, oligoepilepsy and refractory epilepsy, pre- and post-surgical candidates, people with learning disability, parents and children, carers and support workers (table 2.1). Many later became members of the RDG (above).

The focus groups were asked to identify questions that addressed treatment uncertainties (we excluded questions that exclusively addressed diagnosis, complaints about services or the natural history of epilepsy). When facilitating the meetings we attempted to involve everyone in offering questions and for participants to try to consider all groups and all treatments. So as not to discourage participation we did not attempt to answer any of the questions posed, or suggest that the answers may be known during the group meetings.

Each participant was then asked to rank the questions created in their meeting only, so that we could identify the most important potential research questions.

Epilepsy	**Professionals** (n=16)	**Patients and carers** (n=25)	
Sex	Male (8)	**Sex**	Male (8)
Role	Adult neurology consultant (4) Paediatric neurology consultant (2) Learning disability consultant (1) Neurology registrar (3) General Practioner (1) Epilepsy nurse specialist (4) Genetic counsellor (1) Dietician (1)	**Role**	Patients (19) Carers (6)
		Mean Age	Patients (46.6) Carers (50.8)

Table 2.1 Focus group participants – demographics

2.1.5.3 Analysis

Using an interpretive phenomenological approach to analysis, we used not only the text of the question produced but also our knowledge of who asked the question and the context in which it was created to better understand what was being asked. Blinded to the ranking, I worked with Dr Carrie Hammond to group the questions into themes so that we could compare professionals' and patients' questions both in content, number and relative ranking. Some sub-categories involving special groups (e.g. people with learning disability or children) were teased out from the main themes for comparison. The themes were developed by Dr Hammond and myself both individually and in group work until agreement was reached that the themes identified were both all-encompassing and useful. After adjusting for the number of questions produced by each meeting, a mean rank for each was produced and standardised as a value between 1 and 100; the lower the standardised rank score, the more important its rating. In order to allow us to test the significance of the differences between focus groups or themes we examined the most highly ranked questions. We identified, for every participant, the number of questions from each theme that appeared in the top quartile. The Mann Whitney U test, using asymptotic significance,

was then performed to identify differences between the focus groups, or between the themes, with statistical significance taken as $p<0.05$. We identified both differences between the two groups and the similarities: the shared priorities. Finally, a database of unique uncertainties was produced for publication in the UK DUETs database. www.library.nhs.uk/duets/ Epilepsy remains the only neurological disorder entered into NHS Evidence's data repository.

Data maps were created to visually depict the architecture of the thematic hierarchy. The full map is shown in figure 3.4 and an excerpt figure 3.5 is blown up for greater legibility.

2.2 Hyperekplexia

The analysis of the hyperekplexia cases are primarily through samples recruited to Professor Mark Rees' research group at Swansea University.

2.2.1 Genotype Phenotype

2.2.1.1 Identification of cases

Cases are passively recruited on a prospective basis for enrolment into research at Swansea University. An extensive network of clinical geneticists, paediatric and adult neurologists has developed since our group first began accepting samples in the 1990s. The geographical bias of the network favours the UK, however we expect to receive samples from North Western Europe, the Iberian peninsula, Turkey, Jordan, India and Australia. On occasion we are contacted by people with a clinical diagnosis of hyperekplexia (or a personal suspicion that this diagnosis answers their symptoms best). As discussed in chapter one we openly engage in participant involvement but must be certain to 1) offer the self-referral the same degree of genetic counselling and support as someone who has been referred through traditional routes; 2) put the case under the same level of phenotypic scrutiny (described below).

2.2.1.2 Entry Criteria

Deciding which patient samples to screen can be challenging. If entry criteria are too narrow then the phenotype is never expanded, too broad and the service can be overwhelmed and yet not produce valuable results. Hyperekplexia is almost certainly a rare condition, but of unknown prevalence and the true phenotype is complicated by cases which report association with a co-morbid conditions such as sudden infant death ((Giacoia and Ryan, 1994), epilepsy (Lerman-Sagie *et al.* 2004), abdominal herniae (Eppright and Mayhew, 2007), learning difficulties, (Andermann *et al.* 1980, Gastaut and Villeneuve, 1967) or eye movement disorder (Al-Owain *et al.* 2012).

When to suspect hyperekplexia

Essential features

Auditory or tactile startle episodes which do not habituate.

These startle attacks be present from the perinatal period.

Supportive features

Hypertonia and triggered hypertonic attacks

Apnoea attacks in the first few years of life

Developmental delay (in some)

Clonazepam sensitivity (in most)

Normal MRI, no dysmorphism

Features that are unhelpful

Although genetic – a family history is not important. Most cases are recessive.

Later onset triggered startle – an alternate diagnosis may be possible.

Figure 2.2 When to suspect hyperekplexia – A summary of the clinical criteria I have used to enter a case into the research project

The early descriptions of hyperekplexia focussed on the shared clinical features (stiffness, startle and falls), the hereditary nature of the condition and the degree of phenotypic variability (which were qualified as 'major' and 'minor' variants). Suhren *et al.* 1966 and (Andermann *et al.* 1980) were both convinced of the authenticity of the minor phenotype. The minor form "quantitatively different (that is, the movements were more violent than normal [startle])"; the minor form did not include the "generalized muscular stiffness with loss of voluntary postural control causing them to fall". The major and minor forms appeared in the same families – and the hypothesis was that an autosomal dominant gene was responsible (they were correct – it was *GLRA1* in these families) – but that it would be responsible for minor startle (in this instance, this was not to be proved correct).

Flow chart demonstrating clinical genetic screening pipeline.

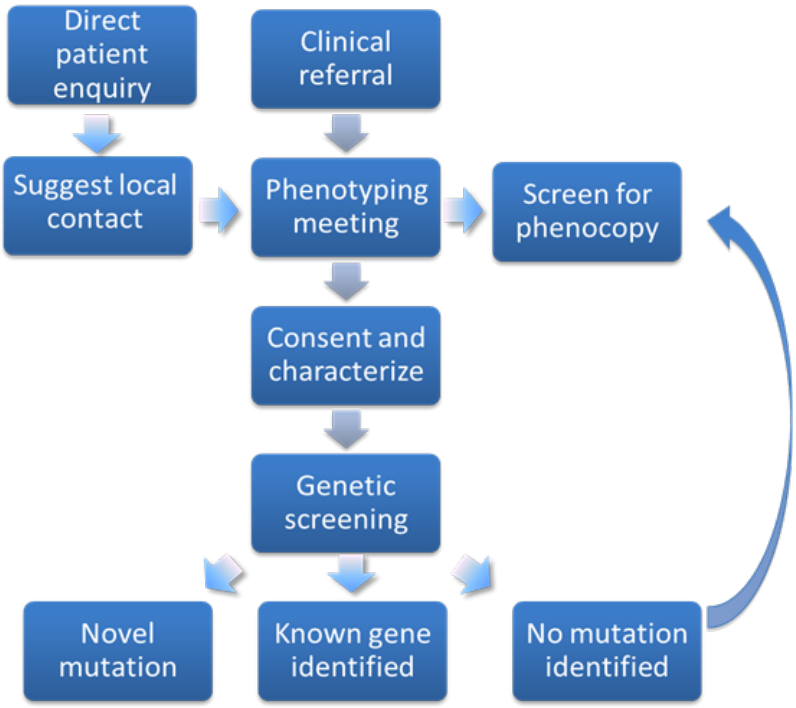


Figure 2.3 Cartoon demonstrating our screening pipeline

2.2.1.3 The Spectre of Phenocopy

Hyperekplexia is (not unsurprisingly) most commonly confused with a seizure disorder. However, during startle episodes clear consciousness is retained and there is no EEG correlate to either the startle or hypertonic posturing. False positive results are reported, as of course the exaggerated limb jerking will provide a great degree of artefact on the EEG trace and improvement is often seen with benzodiazepines and less frequently with anti-epileptic drug therapy. I therefore set out to formalise the scrutiny of new cases (described in full in (Davies *et al.* 2010), Appendix A).

Paroxysmal extreme pain disorder (PEPD) (Fertleman *et al.* 2007) PEPD (previously known as familial rectal pain syndrome) is an autosomal dominant condition recently shown to be a sodium channelopathy involving *SCN9A*.
Similarities Onset in the neonatal period or infancy; persists throughout life. Dramatic syncope with bradycardia and sometimes asystole are common. Tonic attacks are triggered by factors such as defecation, cold wind, eating, and emotion

Differences Autonomic manifestations predominate initially, with skin flushing in all and harlequin colour change. Later attacks of excruciating deep burning pain often in the rectum, ocular, or jaw.
Acquired Hyperekplexia (Hutchinson *et al*. 2008) Sub-acute anti-glycine receptor antibody mediated condition that responds to immunosuppression and plasma exchange.
Similarities Features include truncal rigidity, muscle spasms, brainstem signs, and stimulus induced startle.
Differences Features not present from early life. Immunosuppression clearly efficacious. Apnoea attacks described rarely. May relapse.
Crisponi syndrome (Crisponi *et al.* 2007) An autosomal recessive syndrome initially described in 12 different families in Southern Sardinia; c*aused by mutations in the CRLF1 gene.*
Similarities This disorder is evident at birth. Marked muscular contraction of the facial muscles in response to tactile stimuli or during crying; the contractions slowly disappear as the infant calms. Rarely generalised seizures. Mild psychomotor delay in some. Low GABA levels in CSF have been described.
Differences Abundant salivation simulating a tetanic spasm. Neck muscle hypertonia with a tendency to opisthotonus. Facial anomalies such as large face, chubby cheeks, broad nose with anteverted nostrils, and long philtrum. Bilateral camptodactyly. Hyperthermia.
The Jumping Frenchmen of Maine / Latah syndrome (Andermann *et al,* 1980) Culturally bound neuropsychiatric syndromes thought to be an anxiety / somatisation disorder but the true cause is unknown.
Similarities Excessive response to startle
Differences Echopraxia (involuntary repetition of another's words or actions) and echolalia (repetitive vocalisations – such as those seen in Tourette's syndrome).
Startle Epilepsy Startle epilepsy is a reflex epileptic seizure precipitated by a sudden stimulus; most patients are young and have infantile cerebral hemiplegia.
Similarities Surprising stimuli induce motor reactions – consciousness can be preserved in seizures.
Differences Neuro-imaging will almost certainly be abnormal. EEG often helpful
Stiff Person Syndrome Progressive axial stiffness and intermittent spasms mainly evoked by unexpected stimuli; associated with anti-GAD antibodies in CSF.
Similarities Stimulus induced hypertonia, startles and falls. Hypertonia can preferentially affect lower-limbs.

Differences Stiffness / hypertonia is much more prolonged than the paroxysmal attacks seen in hyperekplexia.
Tourette's syndrome Motor and vocal tics, associated with an exaggerated startle reflex, behaviour change and stereotypy.
Similarities Startle response; symptoms precipitate by stressors.
Differences Vocalisations and obsessive / compulsive behaviours. Motor tics can be complex and appear semi-purposeful.
Symptomatic Startle and Myoclonus (Bakker *et al,* 2006)
Neuropsychiatric Anxiety states including generalised anxiety disorder Post-traumatic stress disorder
Cerebral Children with cerebral palsy Post-traumatic or hypoxic encephalopathy Para neoplastic syndromes Multiple sclerosis
Brainstem – particularly pontine pathology Brainstem infarct , haemorrhage or encephalopathy Posterior fossa malformations Medulla compression Multiple system atrophy
Strychnine and tetanus toxicity

Table 2.2 Hyperekplexia Mimics – table of the common and uncommon hyperekplexia mimics

2.2.1.4 Clinical proforma and interviews

Since 1994 we have received referrals for 230 families where a clinical diagnosis of hyperekplexia has been suspected. Genetic testing, on a research basis has occurred initially at Cardiff University, UK, then Auckland University, NZ and now at the Institute of Life Science at Swansea University, UK. I contacted all referring clinicians of patients with a positive genetic diagnosis using a standard proforma to establish both a minimum quality dataset and to acquire a clinical update on the individual cases (figure 2.4). The proformas were designed to include details of known co-morbidities (such as neonatal apnoeas and seizures) and theoretically possible complications (such as anxiety or deafness). All cases with confirmed GlyRα1, *GLRB* and *SLC6A5* mutations were included and the referring

clinicians, or whoever had taken over the patient's care, were asked to complete the proformas as comprehensively as possible. I also cross-checked with the original referrals and clinic letters when available; spoke to clinicians, parents or probands via email (where appropriate) and on rare occasions arranged to see the individuals in clinic.

In addition I performed a clinic letters review of the 'gene negative cases', and together with Dr Naomi Thomas (paediatric colleague) we critically analysed the clinical information provided. In some cases more recent contact provided an alternative diagnosis; in others they later turned out to have *GLRB* mutations on re-analysis. It was possible to perform a more detailed analysis of the most recently collected gene-negative cases using the proforma collected data.

Wales Epilepsy Research Network WERN

Hyperekplexia Clinical Information

Professor Mark Rees - Swansea University, Wales

Swansea University Prifysgol Abertawe

Surname _____
First name _____
DOB (D D / M M / YYYY)
Sex (M / F)
Ethnicity _____
Prematurity XX /40

Other affected relatives

Any sudden infant deaths in the family?

Medical History (other medical problems)

Investigations (Y/N details overleaf)
EEG / EMG
ECG
MRI
Antibody tests
Genetic testing

Clinical Features (Present? Variability, pattern)
Tone Y/N
Response to nose tap Y/N
Startle response Y/N
Seizures Y/N
Falls Y/N
Diurnal variation Y/N

Additional or atypical features? (please circle)

Epilepsy	Arrhythmias
Autonomic abnormalities	Apnoea attacks
Developmental delay	Metabolic deficiency
Anxiety	Dysphormism
Learning Difficulty	Hypersensitivity to pain
Dystonia	Autism
Visual problems	Hearing difficulties
Myopathy	Hernias

Any previous diagnoses?
Epilepsy Y/N _____
Arrhythmias Y/N _____
Others _____

Medication history (Tried? Y/N and what was the initial result? Did the effect wear off over time?)
Diazepam Y/N _____
Clonazepam Y/N _____
Other benzodiazepines Y/N _____
Sodium Valproate Y/N _____
Carbamazepine Y/N _____
Others Y/N _____

Please return to: Carrie Hammond, WERN Coordinator, Institute of Life Sciences, Swansea University, Singleton Park, Swansea, SA2 8PP, United Kingdom
c.l.hammond@swansea.ac.uk ☎(+44)1792 602310

Figure 2.4 (previous page) Clinical proforma I designed to produce a standardised minimum dataset

Once the data were received we analysed the data by comparing GlyRα1, *GLRB* and *SLC6A5* mutations, dominant versus recessive or compound heterozygous inheritance and sporadic versus familial cases. When comparing characteristics I used one individual from each family for comparison which we denote as 'families'. The chi-squared test was used, using Yate's correction when needed, and statistical significance was taken as a probability less than p=0.05.

For variants that are not found in the unaffected population we use a range of functional analyses for estimating pathogenicity. Structural modelling of GlyR α1 and GlyT2 wild type and variant proteins was performed using a homology modelling pipeline (http://membraneproteins.swan.ac.uk/modelling/), assembled with the Biskit structural bioinformatics platform (Grünberg *et al.* 2007), which scans the entire Protein Data Bank for candidate homologues, as described in (Chung *et al.* 2010b).

2.2.2 Ethnicities

The hyperekplexia phenotype was initially described in large northern European families and it was thought that inheritance was solely dominant. It became clear when gene positive and negative families were described that not every family showed this inheritance pattern and recessive inheritance is now recognised as important. Can a better understanding of the case's background help us target our resources at likely points of variation? I contacted all referring clinicians of patients with a positive genetic diagnosis using a standard proforma to establish both a standardised quality dataset and to acquire a clinical update on the individual cases. Ethnicity data was also cross checked against original referral letters and clinic letters.

In order to compare our data regarding ethnicity and the proportion of *GLRA1* cases, we performed a review of the literature from 1993 when the first genetically proven cases were published (Ryan *et al.* 1992; Shiang *et al.* 1993). PubMed was searched using the terms

'hyperekplexia', 'hyperexplexia', 'startle', '*GLRA1*'. Additional articles were identified using Scopus the citation database and by contacting authors still active in the field. Where information was not available from the papers, we contacted the authors directly. To compare patterns of gene mutations we have arbitrarily used six groupings of ethnicity – Caucasian, Asian, Arabic, Turkish Israeli/Jewish and Afro-American. The Chi squared test with Yate's correction was used.

2.3 JME clinical

A number of cohorts are described in this chapter. As part of the *Epilepsy Research-UK* grant that I lead, it was important to scrutinise and integrate (as much as possible) the clinical cohorts held by collaborators. I present in chapter five the data from St George's collection of JME families and ReJuMEC's (primarily Liverpool University's) collection of individuals with JME. I also draw comparison with a collection made by Dr Paul Timmings in Cardiff in the 1990s that I helped collect outcome data on.

2.3.1 St George's Hospital

Dr Kate Everett is the custodian of a multinational cohort of families and sib pairs that have been collected in the late 1990s and early 2000s. The sample contains detailed clinical descriptions alongside stored DNA with broad research consent. They have many types of idiopathic generalised epilepsy – predominantly electro-clinical syndromes (childhood absence, juvenile absence, juvenile myoclonic etc.). The consortium was initially led by Prof Mark Gardiner with financial support from pharmaceutical companies.

Data available were

1. Pedigrees (and I elected to study only those which still had DNA available)
2. Number of affected individuals
3. Country of origin
4. Age of onset for each affected individual
5. A frequency of seizures for
    i. GTCS
    ii. Myoclonic jerks
    iii. Absences
6. EEG description
7. Photoparoxysmal response

From this information I synthesised their likely epilepsy syndrome. The broad categories were JME, JAE, CAE, IGE, FS and Jeavon's syndrome. If the diagnosis was JME I also chose to

further describe it such as 'with prominent myoclonus' or 'older onset with negative myoclonus'.

2.3.2 Cardiff cohorts

The first sporadic JME cohort was recruited by Dr Paul Timmings when studying for his thesis in Cardiff in the 1990s. He prospectively recruited eighty patients with JME for a pharmacology project with special interest in the PPR. I was involved collecting follow-up data for Dr Timmings including identifying the original EEG reports which I present in chapter five. The data we have include name and hospital number, medication, EEG, PPR response and family history.

The neuropsychology cohort is described in detail below (page 60 onwards).

2.3.3 Analysis

Not every case had every attribute documented and therefore characteristics are presented as a percentage of those that I have data for. Analysis was primarily via the Chi squared test and using Yate's correction where indicated.

2.3.4 Borderline GEFS+

The analysis of individuals from the family study is only made possible with the support of a large field team. Dr Carrie Hammond and Dr Ann Johnston are responsible for the recruitment of the first sixty families into this project, before I joined the group. Eighty families were consented and recruited for future genetic analysis (MREC approval 05/MRE09/78). Families were referred by epilepsy clinicians or self-referred following advertisements placed in epilepsy charity magazines. All available family members were interviewed by neurologists with clinical experience with epilepsy (my colleague Dr Ann Johnston and I). The characteristics of individuals were ascertained via semi structured interview and the pedigrees charted. Focussed co-morbidity data were collected including conditions with a presumed channelopathy basis (migraine) and conditions associated with increased frequency of micro-deletions and insertions (learning difficulties).

Consensus as to whether the families met the original (1997) criteria for GEFS+ was achieved following debate within a team comprising two clinical research fellows, a professor of molecular genetics, a paediatric neurologist and a genetic counsellor. Families were divided into four groups: classical GEFS+, borderline GEFS+, unlikely to be GEFS+ (broadly unclassified epilepsies), and those with another specific familial epilepsy syndrome. The characteristics of the individuals with epilepsy from within these families were extracted for analysis and the chi-squared test (with Yate's correction when needed) was used. People were identified as having FS (simple febrile seizures), FS+ (febrile seizures plus; generalised or partial seizures that continue to occur rafter the age of five years, or in the absence of a very high temperature) (Scheffer *et al.* 1997) generalised tonic-clonic seizures (GTCS) as an adult (beyond aged 18 years) or other types of seizures. Focal epilepsy syndromes were confirmed by reviewing clinic letters.

2.4 JME Neuropsychology

This protocol aims to describe the neuropsychological profiles of people with drug resistant JME.

2.4.1 Protocol and study design

During the planning stage of the protocol I was made aware of Liverpool University's ReJuMEC project (proposal in appendix C). This was an MRC funded cohort collection aiming to recruit 200 individuals with drug refractory JME for use in future imaging, genetics and drug discovery programmes. As it was my intention to interview the same individuals – and I thought that their protocol design was very sensible – I elected to ensure that my protocol was very similar to theirs; and when possible co-recruit cases.

2.4.1.1 Original protocol

It was my intention to identify and recruit individuals with a broad definition of JME to ensure an ecological validity of the results. Previous studies had chosen to exclude people with significant psychological or psychiatry co-morbidities and those with a low IQ. It is not common practice for people in the UK to have an IQ test unless a clinical psychologist or physician has a suspicion of learning difficulties – there are many people with low average IQs who have never been tested and so exclusion of these people based on the bias of prior testing appeared arbitrary. Furthermore I was aware of people with a classical JME presentation in the context of low IQ and wanted to study this further. Similarly it is well known that people with epilepsy have a greater psychopathological burden and so excluding those with these conditions appeared to be in effect removing the neediest cases from the study. Professor Marson and the ReJuMEC investigators agreed and so a broad definition of JME remained.

Individuals were excluded however if they had a current or prior history of severe alcohol abuse. Although this dual pathology would be interesting to describe – alcohol intoxication effects both acute and chronic are major confounders of psychological function and the individuals may be too chaotic to engage with interviews.

2.4.1.2 Drug Refractory

JME was considered to be refractory when patients continue to experience myoclonic seizures (>1 per week) and/or absence seizures (>1 per week) and/or generalised tonic clonic seizures (>1 per month) despite on-going treatment with adequate doses of appropriate antiepileptic drugs. All patients must have had prior or current exposure to sodium valproate at a dose of at least 1,000mg per day and which must have failed to adequately control their seizures.

Individuals who have withdrawn from sodium valproate therapy due to intolerable adverse effects rather than a lack of efficacy will be specifically excluded, irrespective of maximum exposed dose. Care will be taken not to confuse this with pseudoresistance – i.e. non-compliance with prescribed medication and non-compliance with lifestyle factors known to provoke seizures. Each participant had a full neurological exam and a clinical history will be taken. I also enquired about family history, photosensitivity and medical and psychiatric comorbidities.

2.4.1.3 Consent, ethics and funding

Cases were contacted and consented into the WERN epilepsy-biobank (eBiobank). To collect neuropsychological data an amendment was needed which was approved in 2008. Informed consent was taken to collect biological samples (blood or saliva) for DNA extraction. Our consent form explicitly asks participants want they want to happen with their sample following resolution of the study (destroyed, returned or retained): all participants asked for it to be retained for use in future projects. Furthermore individuals were asked what their intentions were regarding the return of information following the analysis of their DNA. They had to state their wishes regarding receipt of genetic information potentially attaining to the epilepsy (90% wishes to be recontacted) or attaining to another disorder unrelated to their epilepsy (the proportion dropped to nearer 60%). A copy of the invitation letters, information sheet and the consent forms are in appendix D.

This study was not directly funded. My first two years' salary came from the St. David's Medical Foundation (Swansea University) and this in turn was partly from an unrestricted *UCB Pharma* grant. Following this I received my salary from the Welsh Assembly

Government via their research arm NISCHR (National Institute for Social Care and Health Research) which funded my Welsh Clinical Academic Training post by providing my salary to Swansea University (to date). I paid a nominative amount to contribute towards travel expenses and a meal allowance to participants (£20) which I financed personally. The cost of my training and the purchase of licences for the psychological tools, the test materials and the sample collection and processing costs were covered by WERN. My travel to conferences during this period was financed by a combination of personal contributions, WERN, the Cardiff Epilepsy Unit fund and awards from societies and charities; there was no pharmaceutical company sponsorship.

2.4.1.4 Identifying cases

Cases were identified by a number of methods: i) scrutinising the WERN databases in Cardiff and Swansea, ii) searching personally curated patient lists – such as those created to identify patients eligible for other studies; iii) prospective recruitment from paediatric transitional clinic and adult neurology services. Standard letters were written to potential participants requesting that if they were interested that they return a slip in a stamped addressed envelope and include their telephone number. All those who returned valid and current telephone numbers were contacted on at least three occasions (or until they replied) and those who did not return their slip stating that they did not want to take part in the study also received two more invitations – no closer than a month apart.

After speaking on the telephone to confirm eligibility – an appointment was made to meet for the clinical interview and psychological testing. It was agreed that psychological testing could be interrupted by early morning myoclonus and as such no tests could start before eleven in the morning. The interview and physical examination lasted just over an hour on average.

2.4.1.5 Case definition

As one of the outcomes was to identify the variability with the juvenile myoclonic epilepsies – a wide case definition was needed.

1. Consultant epileptologist diagnosis of juvenile myoclonic epilepsy

2. Age 16 to 65 – Although JME can be identified before the age of 16, as some of the psychological tests are validated only for those aged over 16 years.
3. Extreme drug or alcohol problems sufficient to prevent them completing psychological testing
4. First language English or Welsh
5. Unremarkable MR imaging
6. Any EEG pattern as long as it does not strongly suggest an alternative diagnosis

2.4.1.6 Clinical history

In an attempt to standardise the minimum data required for the clinical history a proforma was used with additional information annotated onto this form, a copy is included in appendix D. Questions were focused on a number of areas: i) demographics including handedness, educational attainment and driving status; ii) background medical and psychiatric history – specifically tailored to enquire about their eligibility for future drug studies; iii) information pertaining to the epilepsy itself; iv) potential physical consequences of drug refractory seizures such as injury and insomnia; v) a detailed drug history. All cases had to have details (particularly times and doses of medication) cross referenced with medical notes where available.

2.4.1.7 EEG and imaging

Each participant had to have EEG and imaging in keeping with a diagnosis of JME; this did not have to be specifically diagnostic however and an unremarkable EEG in the context of a compelling clinical picture was sufficient. Although ReJuMEC was funded to provide prospective 48-hour EEG analysis of each recruited participant, the premature dissolution of this funded project prevented any cases from South Wales having 48-hour EEG in this context.

2.4.2 Neuropsychological tests

A battery of standard neuropsychometric tests was performed. In all bar once case these were conducted on a single attendance. They comprised the Wechsler Adult Intelligence

Scale (WAIS III), Wechsler Memory Scale (WMS III), Boston Naming Test (BNT), Delis-Kaplan Executive Function System (D-KEFS) and three sub-tests of the BADS (Behavioural Assessment of Dysexecutive Syndrome). Complementing these are a number of user-completed tests - Hospital Anxiety & Depression Scale (HAD), the ABNAS (AB Neuropsychological Assessment Schedule), the Impact of Epilepsy Scale (IES), test your memory (TYM) and the Eysenck Personality Questionnaire Brief Version (EPQ-BV). These last questionnaires were sent out in advance of the interview for completion ahead of time – poor compliance with this request resulted in them also being distributed on the day and sent out following the interview.

This battery was designed to permit evaluation of intellectual ability, verbal and non-verbal memory, frontal lobe-mediated executive functions, language functioning, depression and generalised anxiety, and the psychosocial impact of a diagnosis of JME. It both included tests where previous authors had identified poorer tests scores and many which had not previously been used to study people with JME. All the tests are described in detail in appendix E.

2.4.3 Data Extraction and Statistical Analysis

Data Extraction

Tests were scored, tallied and entered in to two software programmes: WAIS-III, WMS-III Writer v1.1.1.1 (1999) (for WAIS and WMS data only) and PsychCorpCentre -D-KEFS Scoring Assistant v2.0.0 (2003) (for D-KEFS data). Once the raw and scaled scores were produced from these programmes they were entered into a custom made database and then manipulated using SPSS v16.0 and later v19.0. Graphs were drawn in both SPSS and Microsoft Excel (version 14.0.61235.5001).

2.4.3.1 Data Presentation

Clinical characteristics that were recorded and used in the analyses were age of onset, duration of epilepsy, family history, history of febrile seizure, photosensitivity, seizure type,

seizure frequency, number of AEDs, and AED type. Demographic characteristics that were recorded and used in the analyses were sex, age, years of education, WAIS full scale IQ index score, and employment status. Means and standard deviations were reported for data that met the normal distribution.

Data are presented both as histograms demonstrating the subtests' variability from the mean complete with SE bars and as a line plot of the subtest scores, demonstrating the variability from test to test. These are presented for WAIS, WMS and DKEFS data. The neuropsychological assessment administered to all the participants produced an age adjusted score for each cognitive domain assessed. The spread of these scores was determined by visual analysis of histograms, and consideration of the skew and standard error statistics.

2.4.3.2 Statistical Analysis

In order to compare the participants' scores to a healthy population, one sample t-tests were conducted based on the means and standard deviations given by the assessment manuals. The alpha level for these tests was set at $p < 0.01$. This significance level was chosen to reduce the risk of making Type I error, due to the amount of multiple comparisons being made. This was chosen in favour of using Bonferroni correction, as this would have given too conservative a value due to the number of inferential statistics conducted, and therefore would have increased the likelihood of making Type II error.

Means, standard deviations and standard errors were calculated. A one sample t-test was performed quasi-manually by this process.

I) Identify the number of samples (n), the control mean and the control SD
II) Calculate the sample mean
III) Calculate the sample standard error (control SD/square root of the control mean)
IV) Calculate the differences in means (sample mean – control mean)
V) Calculate the z statistic (difference in means / sample standard error)
VI) Calculate the number of degrees of freedom (n-1)
VII) If the z statistic is negative – turn it into a positive number

VIII) Use the Student's t-distribution function (z statistic, degrees of freedom, two-tailed)

The sub-test norms are ten (SD 3) and the index norms are 100 (SD 15) for the WAIS, WMS and D-KEFS subtest data and the metastatistic indices. The BADS subtests have published norms in the manual from control data: profile score 18.05 (SD 3.05), Key search mean 2.6 (SD 1.32), Rule shift mean 3.56 (SD 0.78), Zoo map mean 2.44 (SD 1.13) and Temporal Judgement mean 2.15 (SD 0.91).

The decision as whether to use a one tailed or two tailed t-test was a difficult one. Two tailed tests start with the hypothesis that the sample statistic has an equal chance of being both higher and lower than that of the control; that is to say in this example that people with JME would be equally likely to have statistically significantly higher FSIQ than lower. Although it in inconceivable that a randomly ascertained patient sample, taking medication, could out-perform controls – it is not inconceivable that the patient group were not randomly ascertained. If there was a healthy volunteer bias then this would tend towards normal, and perhaps supranormal FSIQ. Finally in the context of wanting to avoid accusations of multiple comparisons I elected to use a two tailed t test. The two tailed test returns a p statistic that is double the value of that of a one tailed test and therefore produces a more conservative estimate. This – in combination with the alpha statistic choice above – could contribute to missing genuine differences (type II error), but in context is more appropriate than identifying type I errors.

When studying correlations of one set of scores against another I used Pearson's R correlation coefficients. Pearson's was chosen as the scores fitted assumptions of normality. Post hoc t-tests were run for any variables that were highly correlated. T-tests were run for the variables full scale IQ, verbal IQ, auditory memory (immediate and delayed recall), and verbal inhibition.

2.4.3.3 HADS

Bjelland *et al.* (2002) identified that the optimal cut off for non-cancer medical patients was 8+ for both the anxiety and depression arms to provide the mean sensitivity 0.90, and mean specificity 0.78 for anxiety symptoms and mean sensitivity 0.83, and mean specificity 0.79

for depressive symptoms. Means in 1,792 community based adults have been reported as 6.14 (SD = 3.76, median = 6) for anxiety symptoms and 3.68 (SD = 3.07, median = 3) for depression. For the total scale, 9.82 (SD = 5.98, median = 9) was presented (Crawford *et al*. 2001). These data were produced from a UK sample consisting of 978 females. These data facilitated the use of a one sample t-test to analyse the results. Data are presented both as a histogram showing the anxiety and depression scores paired per patient and as a histogram showing the difference between these two scores. To compare the two sets of HADS results a two-tailed unpaired t-test was used.

2.4.3.4 ABNAS

Aldenkamp and Baker (1997) in reporting this tool published three sets of norms: mean of 19 (SD 15.2) for 53 people with seizure remission, mean 22.5 (SD 15.2) for 52 people with moderate seizure frequency and mean 18.9 (SD 17.2) for 37 with a high seizure frequency. This insinuates that the people with a high seizure frequency have fewer drug side effects – presumably as they are 'under treated' with anti-epileptic drugs. Later (Aldenkamp *et al.* 2002) reported a mean on 19.46 (SD 15.8) for a stratified population of 96 people with epilepsy (consisting of 55 well controlled people on monotherapy and 41 with poorer seizure control on polytherapy). I elected to use the more clinically applicable mean created by the larger sample size from the 2002 work and applied a one sample t-test.

In the absence of factor means I made the presumption that the total mean was spread equally across the six factors and scaled up or down appropriately depending on the number of questions that constitute each factor: five for fatigue and slowing, four for memory and concentration and three for memory and language. Acknowledging the imperfections of this I employed it to attempt to ascertain which of the factors played the greatest role in creating the JME sample's difference from the control mean. Data are also presented for total ABNAS scores as compared to the control mean (19.46) to identify the variation between the participants.

2.4.3.5 TYM

The controls for TYM published in the BMJ were age matched to people with Alzheimer's disease (mean score 46.6, SD 4.0). Their mean age was 65 and test performance dropped after the age of 70, with mean scores before 70 approximating to 47/50. I therefore elected to use the mean of 47.4 which was calculated by Brown and colleagues by administering TYM to 100 males and 100 females without memory impairment. This group had a mean age of sixty-one – almost double that of my JME group – however as stated the mean performance on this task did not significantly decline until after the age of seventy and so this is i) probably more representative and ii) still a conservative mean which would tend towards a Type II error. The TYM scores are presented as a histogram as variation about the control mean (47.4). To estimate how strongly TYM predicted results on standardised testing and subscales the Pearson product moment correlation coefficient (r) was calculated for each statistic with relation to the TYM score. This produced an r as a marker of correlation and the significance could also be quantified. The statistical significance of each r statistic was calculated quasi-manually using the following method.

I) Sample number (n) is calculated and therefore degrees of freedom (n-1)

II) t statistic = r x square root (n – 2) / square root (1 – r^2)

III) If the t statistic is minus is it converted into a positive number

IV) Two tailed t distribution function on the t statistic using the degrees of freedom produces p

Eighteen scales were chosen and so the correct Bonferroni correction was to 0.0028. Data will be presented both with r figures and p statistics with reference to both an alpha statistic of 0.01 and the above figure.

2.4.3.6 BNT

The normative means for the BNT were taken from (Nicholas *et al.* 1988). They reported a mean of 54.50 (SD 3.52) – which was also in keeping with a previous estimate from Van Gorp *et al*. 1986 (mean 54.31). The Nicholas *et al*. estimate was produced by interviewing

sixty health non-brain injured adults between the ages of 40 and 78 (mean age 56.1). A one sample t-test was employed.

2.4.3.7 DEX

The Thames Valley Test Company does not supply normative values for the DEX. Pa and colleagues (2009) reported that controls scored at a mean of 1.6 (out of a maximum of eight), SD 2.1, that amnestic mild cognitive patients scored 8.7 (SD 8.7) and those with dysexecutive mild cognitive impairment scored higher with a mean of 12.7 (SD 10.1). This figure for controls is a power of ten less than that published by Canali *et al.* for older 17 adults, mean 17.9 (SD 7.7); they did not report a figure for the 'others' questionnaire –only for self. Burgess and colleagues (1998) set the DEX self and other to 216 non-patient control participants (mean age 46.1). This study group were a heterogeneous number of patients, staff and volunteers. They presented a mean score for DEX-S of 21 (SD 10) and for DEX-O 17 (SD 11). As these correlate with Canali and have a biological validity – I elected to use these means. The scores are presented as a histogram ranked by DEX self score with the paired DEX other score alongside. To compare the two sets of DEX results a two-tailed unpaired t-test was used.

2.4.3.8 Identifying Subgroups

The WAIS / WMS writer software permits standardised analyses of variation in performance across indices. For WAIS these are VIQ and PIQ; Verbal comprehension (VC) and Perceptual Organisation (PO); VC and Working Memory (WM); PO and Processing Speed (PS); PO and WM; and WM and PS. For the WMS tests these were Auditory Immediate (AI) and Verbal Immediate (VI); AI and Auditory Delayed (AD); VI and Verbal Delayed (VD); AD and Auditory recall delayed (ARD); AD and VD; Immediate memory (IM) and General Memory (GM); IM and WM; GM and WM. These comparisons were the used to tease the cohort into subgroups that performed in a uniform way.

2.4.3.8.1 Verbal IQ versus Performance IQ

This comparison is one of the most robust because of the number of sub tests that comprise each factor. The VIQ and PIQ were compared to each other case by case using the "discrepancy score tables based on predicted-difference method" tables in Appendix B of the WAIS and WMS Technical Manual. This explains why they can only be given to the >0.05 level of significance. Data are expressed as a histogram with both VIQ and PIQ charted to demonstrate the variability of the two statistics and as a separate histogram which plots the difference in VIQ and PIQ scores complete with those which are statistically different at the <0.05 level.

2.4.3.8.2 Executive function Tests

BADS score were totalled across the four tests and scaled up (as four from six of the elements were employed) to create a statistic comparable to the standardised score. This – when age is taken into account – allows scores to be labelled 'impaired', 'borderline', 'low average', 'average', 'average', 'high average', superior', or 'very superior.' The scaled and subtest means and SDs are given above and taken from the technical manual.

2.4.3.8.3 Personality

Sato (2007) reports sex based norms and standard deviations for the EPQ-BV. Samples were analysed by sex using a one sample t-test comparing with these manual means. The means for females were 30.54 (SD 9.38) for neuroticism and 42.07 (SD 8.97) for extrovertism and for males were 26.93 (SD 9.96) and 42.58 (9.11) respectively. Samples were both analysed separately by sex and then together by combining the means proportionately (accurately) and the SDs (an estimate). This technique will be more accurate for extrovertism where the means and SDs between the sexes were more accurate.

Due to the number of multiple comparisons being made, and reduce the likelihood of making Type I error the significance level was set at $p< 0.01$. Bonferroni correction was not

applied as this would have given too conservative a value due to the number of inferential statistics conducted, and therefore would have increased the likelihood of making a Type II error. To compare the two sets of EPQ-BV results a two-tailed unpaired t-test was used.

2.4.3.8.4 BADS

BADS standardised score was used to break the sample into three groups: mean score (100) and up; score one SD below the mean (85) to below the mean (99); and scores one SD below the mean (84 and below). The three groups (one, two and three) were presented with their mean scores for each subgroup for all meaningful statistics. The Pearson product moment correlation coefficient (r) was calculated for each statistic with relation to the standardised BADS score. Pearson's was chosen as the scores fitted assumptions of normality.

Subgroups were identified using quantitative methods but were decided upon post-hoc. After analysing the full dataset I set about to develop a number of subgroups of the whole (two to five) based upon the existing dataset. As only some tests were answered by all participants I was limited to using WAIS, WMS, DKEFS and BADS indices as the primary means for subdivision. To demonstrate that the subdivisions were valid unpaired t-tests were performed analysing the series of scores of the individuals in each subgroup to demonstrate their difference. As before I have chosen an alpha statistic of <0.01 to suggest significance – however I also employed a formal Bonferroni correction also. These forty t tests would need a p value less than 0.00125 (which is 0.05 / 40). I have reported both those where the alpha statistic and where it is less than the corrected value.

2.5 Copy number variation

Chapter seven details the copy number variation in two main cohorts – hyperekplexia and JME and using two techniques, CGH array and SNP genotyping (Cooper and Mefford 2011). The cohort selection and these techniques are described in the chapter one (page 37) - as well as their methods used to analyse the data when they were received.

2.5.1 CGH array

As a pilot for the JME CNV analysis project 5 samples were submitted for CGH array analysis and then a further 24 were submitted to BlueGnome. Samples included not only those with epilepsy but some with hyperekplexia and hyperekplexia like syndromes. The hypothesis is that one or more copy number variants (CNVs) affecting unknown genes underlie unresolved cases of hyperekplexia and JME with additional features.

2.5.1.1 Technique

Comparative Genomic Hybridisation Array: Changes in genomic DNA resulting in a reduced or increased copy number were detected using array comparative genomic hybridisation (Array CGH; figure 2.5). This method was used to analyse 24 DNA samples from individuals who mostly do not have mutations in glycine receptor (*GLRA1*, *GLRB*) or transporter (*SLC6A5*) genes. A pilot sample of five hyperekplexia patients (results presented in chapter seven) was performed in collaboration with Dr Reinhard Ullmann (Max-Planck Institute for Molecular Genetics, Berlin), using the 400K Whole Human Genome CGH array (Agilent Technologies). The array contains 411,056 oligonucleotide probes with a median probe spacing of 5.3 kb. Comprehensive probe coverage is enhanced with emphasis on known genes, promoters, microRNAs, pseudo autosomal and telomeric regions. In pilot experiments, colleagues (Prof Rob Harvey, UCL) detected a known CNV - a 329 kb deletion encompassing exons 1-7 in the GlyR α1 subunit gene using this technique.

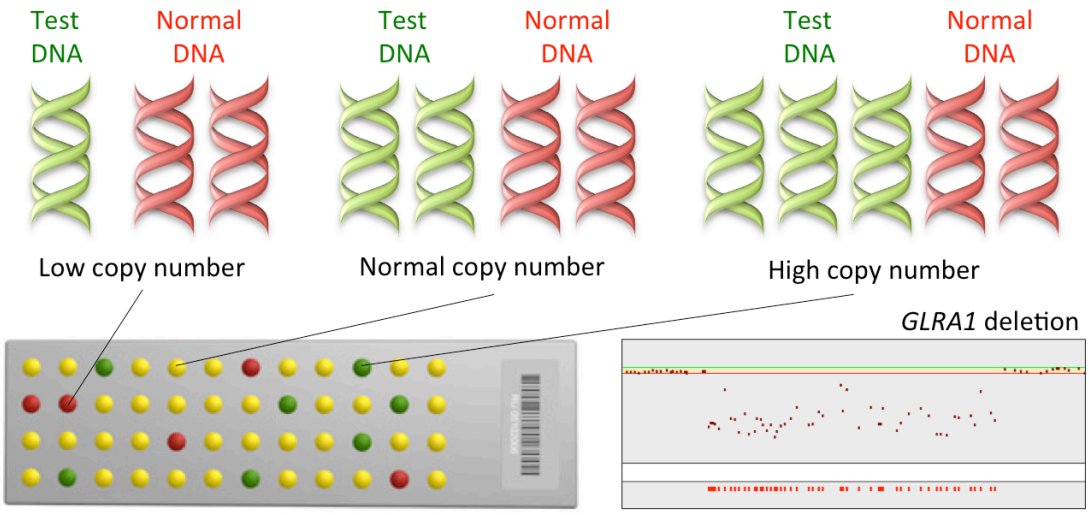


Figure 2.5 Array Comparative Genomic Hybridisation. Genomic DNA from a test sample and a reference sample are labelled using different fluorophores and hybridised to thousands of oligonucleotide probes derived from most of the known genes and non-coding regions of the genome, printed on a glass slide. The ratio of the fluorescence intensity of the test DNA to that of the reference DNA is then calculated, revealing copy number variants (CNVs) in a particular location in the genome. *Right lower panel*: a proof of principle, we detected a known deletion in the GlyR α1 subunit gene (*GLRA1*). Red and green lines correspond to log2 ratios -0.3 (loss) and 0.3 (gain), respectively. The *GLRA1* exons 1-7 deletion is indicated by a downward shift for several consecutive oligos. Array CGH predicted that the *GLRA1* deletion spanned human chromosome 5: 151,211,750-151,528,058 (Hg18) - defined by the positions of the first and last deleted oligonucleotides - in good agreement with the published deletion breakpoint.

2.5.1.2 Sample selection

Twenty four samples with mixed phenotypes were selected for a pilot study of CNV analysis using a CGH array technique. The samples S1 to S12 were chosen because they had atypical additional features (S1, S6) or they had deletion syndromes and could act as an internal control (S4, S7) or the clinical features were very atypical (S5). Samples F1 to F12 were part of a WORD funded family history of epilepsy project and CNV analysis was part of a pipeline of research which includes second generation sequencing (on-going).

Case	Sex	Basic Phenotype	Extended
S1	M	JME and LD	Noonan's syndrome
S2	F	JME and LD	
S3	M	Hyperekplexia and LD, gigantism	Known *GLRA1* variant
S4	M	Hyperekplexia LD	Known *GLRA1* deletion
S5	M	Atypical hyperekplexia	Possible seizures, early death
S6	M	IGE and LD	SUDEP
S7	M	Jeavon's syndrome	DiGeorge's
S8	F	JME and LD	
S9	M	IGE and LD	
S10	M	Atypical BECCTS and LD	
S11	F	GEFS+ and LD	
S12	M	LD and myoclonic epilepsy	
F1	F	Alice in Wonderland Syndrome	Migraine
F2	M	Alice in Wonderland Syndrome	Epilepsy and LD
F3	F	Alice in Wonderland Syndrome	
F4	F	IGE	
F5	F	IGE	
F6	F	IGE	
F7	F	Unaffected relative of F8	
F8	F	TLE	
F9	F	TLE	
F10	F	Unclassified epilepsy	Migraine
F11	M	Unclassified epilepsy	Migraine
F12	F	TLE	

Table 2.3 Samples and basic clinical descriptions of cases identified for CGH array project

The samples were loaded into *BlueFuse Multi* version 3.0 for analysis. The NCBI36 build is used by this programme. This software package provides limited CNV analysis and groups CNVs into three likely categories: pathogenic, unknown and benign. Initially the automated calls were analysed, then the unknown calls. The pipeline included i) identifying if the loci has been associated with epilepsy, then ii) identifying if the loci is associated with a CNV

syndrome, then iii) looking at genes of interest, iv) manually analysing the CNV to see if it is genuine and v) comparing with DGV is as described in detail below for SNP genotyping.

2.5.2 SNP Genotyping

Thirty four samples were submitted for high-resolution SNP genotyping to Molecular Cytogenetics and Microarray Diagnostics at the West Midlands Regional Genetics Laboratories, Birmingham. We chose to use an Affymetrix platform CytoScan HD. At the time of submission (Spring 2012) it provided the best gene level coverage of any commercially available array for copy number: coverage is in excess of 18,500 RefSeq genes with one marker per 3kb; non-gene (backbone) coverage was at 1 marker per 5kb. Over 12,000 OMIM genes are covered at a marker per 2 kb. In total across the genome 2.5 million markers are used constituting 750,000 known SNPs and 1.7 million non-polymorphic probes. Figure 2.6 provides more technical details.

2.5.2.1 Analysis

The data were analysed using Affymetrix custom software – *ChAS* (Chromosome Analysis Suite); this uses NCBI build GRCh37. Advantages of this graphical analysis tool are: that you can choose to focus analysis on specific regions of known significance; analyse the genome at different levels of resolution; customize and load your own annotations and regions for focused analysis; directly access external databases such as NCBI, UCSC Genome Browser, Ensembl, and OMIM.

Taking advantage of these functions I chose to compare our dataset against a number of known databases uploaded into ChAS as 'bed' files. I am grateful to West Midlands Regional Genetics Laboratories (WMRGL) Birmingham for giving me access to these files. The bed files are described below -

1. **HD calls from WMRGL**

This is a dataset of CNVs that have been created using the CytoScan HD method and analysed using *ChAS*. They have reached their in house criteria for a diagnostic laboratory to label them as probable pathogenic. This list is relatively short as WMRGL must be cautious

about how they choose to identify CNVs; any CNV from my dataset that corresponds to one of these 'calls' would need careful scrutiny.

2. **Cooper Warning regions**

This is a dataset of areas of likely significance across the genome. For example 22q deletions associated with DiGeorge syndrome could be overlooked as pathogenic as they will look like infrequently repeating deletions across all reference databases. The Cooper file highlights areas which are known to be frequently recurring but of clinical relevance.

3. **EU CNV control data**

This file contains the summary data from a large European collection of unaffected individuals all analysed using the CytoScan HD platform. This dataset when used in conjunction with DGV permits both 1) the identification of frequently recurring CNVs – likely to be benign; and 2) the identification of artefact brought about by the CytoScan method – which appears to produce novel CNVs – but are frequently seen in the EU data set and yet not in DGV.

4. **ISCA**

The International Standards for Cytogenomic Arrays (ISCA) file lists a large number of genes that are thought to contribute to intellectual disability, autism, and developmental delay.

5. **WERN**

We have included our cases as a dataset to enable us to quickly see if the CNVs identified are recurrent within our study population.

6. **HI (Haplotype Index) Scores**

This data file estimates the likely outcomes of homozygosity for a gene. This is a non-standard custom function provided by Cytoscan HD. The scores are scores by percent with scores over 40% being very unlikely; 40 to 20% being of moderate significance and under 20% deserving of careful consideration.

7. **Custom epilepsy datafile (appendix F)**

Together with Fiona Togneri (Clinical Scientist at WMRGL) we created a custom bed file for the *ChAS* platform. I identified a list of genes that I wanted to be able to identify quickly if they were affected by CNVs in the dataset. There were six areas that were included

1. All ion channel genes

   I chose to include all 237 ion channel genes identified and screened by Klassen *et al.* (2011) in their study of targeted NGS in people with epilepsy.

1. CNVs identified in studies of IGE
2. Linkage regions identified as likely to be important in IGE or JME

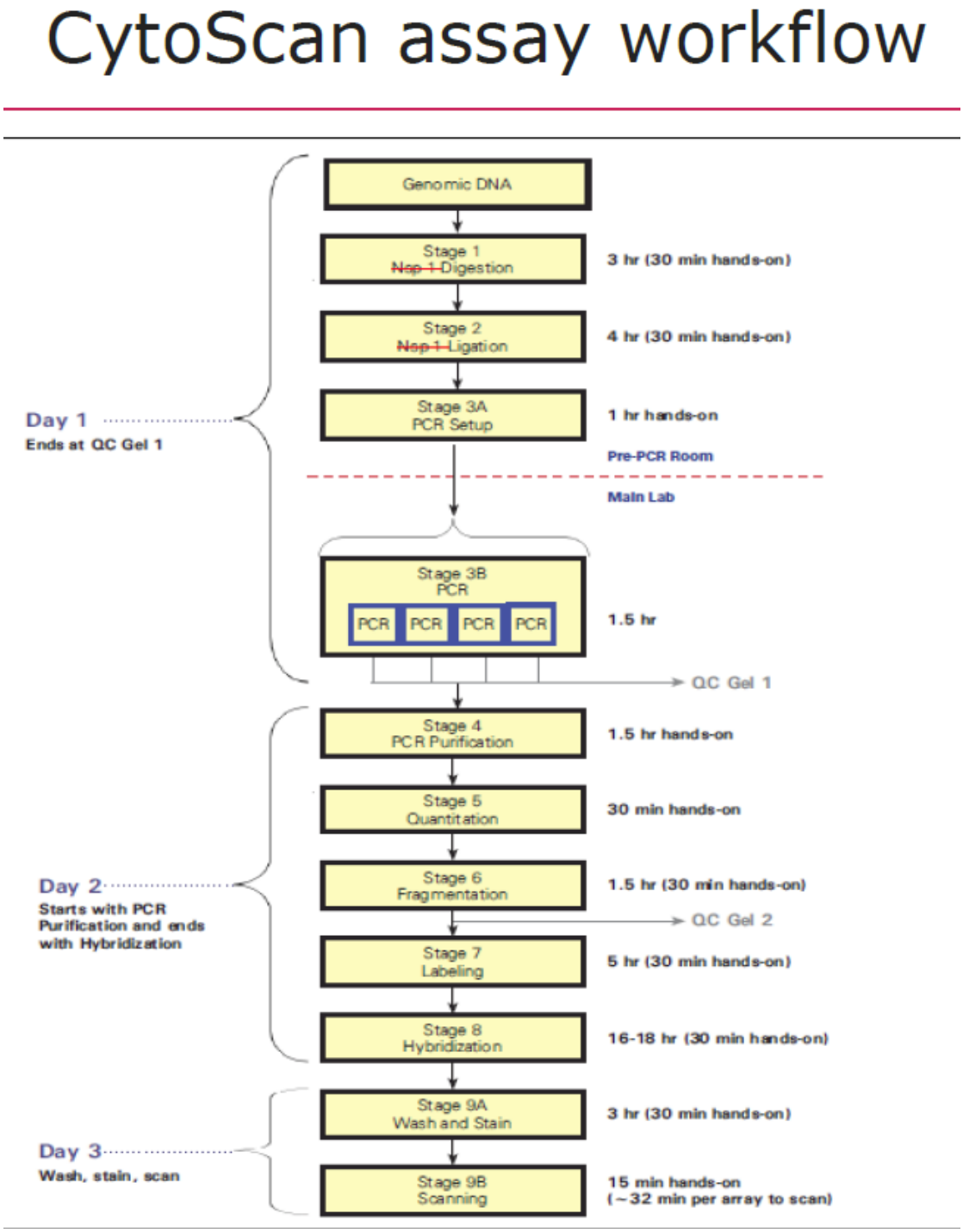


Figure 2.6 CytoScan HD workflow for SNP genotyping

3. A list of 143 genes identified as likely to be important in epileptic encephalopathy. These were taken from a number of sources such as the screens

offered by the commercial services of CEGAT (http://www.cegat.de/), and a systematic review of published papers in PubMed.

4. A list of rare variants identified by Heinzen *et al.* (2012) in the study of 118 individuals with IGE and genes that may be implicated in neighbouring pathways.

Using these guide files I analysed each of the thirty-four files using a number of the *ChAS* options to aid analysis.

1. OMIM genes

OMIM (Online Mendelian Inheritance in Man) described itself as "a comprehensive, authoritative, and timely compendium of human genes and genetic phenotypes." Including this annotation allows me to right click on a gene within a CNV to then jump to the database. Every gene within a CNV was checked in this way because OMIM is thought to contain updated information on all Mendelian disorders and over 12,000 genes and is particularly strong on the relationship between phenotype and genotype.

2. Genes

This marker brings up each transcript of a gene.

3. DGV

The Database of Genomic Variants (DGV) http://projects.tcag.ca/variation/ describes itself as a "curated catalogue of structural variation in the human genome". It defines structural variation as "genomic alterations that involve segments of DNA that are larger than a kilobase." However they have started to annotate indels in the 100bp-1kb range. The database is designed to include only the structural variation identified in healthy control samples.

2.5.2.2 Resolution

Each file was also analysed with the calculated copy number state, weighted Log2 Ratio and smooth signal shown. This permits one by eye to judge the validity of an automated call of a gain or loss. When appropriate these are shown in results (chapter seven). The standard high density resolution used by WMRGL is that 50 markers are needed for each gain or loss, and they limited themselves in describing only CNVs larger than a 100kbps. Recognising this a sensible standard for a diagnostic laboratory – but that also CytoScan HD permits

identification of 'micro CNVs' I elected to retain their definition for a CNV but analyse at a higher resolution: 50 markers and looking at CNVs as small as 1kbp.

2.5.2.3 Pipeline

Associated with Epilepsy

The first analysis was to see whether any CNVs previously described as associated with GGEs were present. To create this list I used

1. Information from published papers (mostly from supplementary files).
2. Information from meetings and conferences (this is quickly moving field and not everything is published yet).
3. Linkage areas previously described as associated with JME
4. Areas harbouring putative JME genes

The list of these CNVs and locations is given in appendix F.

2.5.2.4 Datafile

I used *ChAS* to create a datafile for each case listing

1. Automated copy number state (0, 1, 3, 4)
2. Type (Gain or loss)
3. Chromosome number and coordinates including the location of the cytoband
4. Size in kbp
5. Markers – both the absolute marker count and the mean distance between markers
6. An automated estimate of the 'confidence' of the call
7. The OMIM genes in the region
8. Whether DGV has seen similar variation in the normal population.

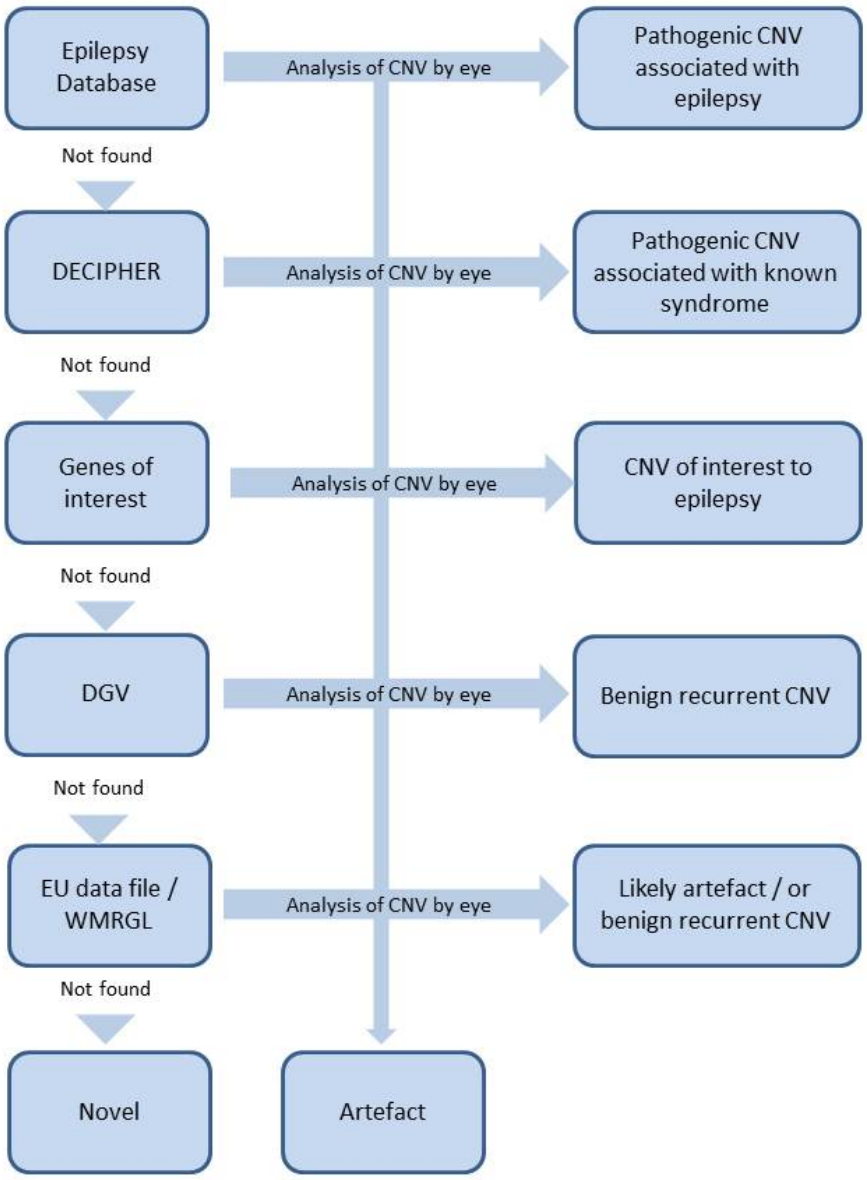


Figure 2.7 Diagram demonstrating the pipeline – Horizontal arrows illustrate 'Yes, the CNV was identified here', vertical arrows are negative

Chapter Three

Involvement

Involvement: Rediscovering the individual at the heart of genetic research

3.1 Introduction

Every genetics study must start at the bedside. *Kline* is the Greek for a bed or couch and from where we derive the term clinician – he or she who is at the bedside. This chapter describes processes of engagement. Without engagement a patient does not become a participant; and with true engagement they can become a partner in research.

In this chapter I give three examples of patient involvement in research. The first of these is as part of a novel advisory board, the second harnesses the power of individual's stories and the third compares the research priorities of patients with those of clinicians and researchers. I also describe he creation of a patient support group using a new media resource.

3.2 WERN Patient Research and Development group

The formation of the Wales epilepsy research network in 2004 – as a collaboration of clinicians, scientists and people with epilepsy forced us to address patient participation and engagement in research. The traditional model of patients being 'sampled' or 'experimented on' by researchers – without a clear pathway of later relating the study's findings back to them – is redundant. Creating a scheme from scratch has allowed us to trial a novel strategy for genuine patient partnership. I am aware that this model of collaboration may not necessarily be taken 'off the peg' for other research disciplines, however as an example or working together - rather than token consultation and as an example of genuine involvement it has many merits. I strongly feel that a panel of people, who can each reflect on their different experiences, provides a more representative and authoritative 'expert' view than the historical single 'professional patient' alternative. It therefore could be

adopted and adapted by many other disciplines where clinicians and patients need to communicate research ideas efficiently.

3.2.1 RDG Meetings

The dynamic of the patient RDG is unlike any other scientific meeting. The clinicians (normally numbering one or two) are outnumbered by the patients and carers (normally ten to sixteen): and the clinicians do not chair the meeting. There is an agenda, circulated via email the week before and the meetings last ninety to 120 minutes. It is not unusual for RDG members to relate how the issue being discussed (e.g. memory difficulties in epilepsy) affect them or their loved ones. There is a very open and welcoming attitude towards first person narrative: this is a key feature of the group. No one is expected to represent 'everyone with epilepsy', be a 'professional patient' nor even keep up to date with 'hot topics' in epilepsy. I am not entirely certain that the individual members even associate strongly with the mean (or median) epilepsy patient. However through balanced discussion the members are able to reflect on whether the issues discussed matter to them and to people like them. This individualised approach is highly motivating – and permits us to prioritise the projects that matter. It also produces a forum where every voice can be heard - it is not an intimidating place for a patient or carer to interject and help shape the conversation and the projects discussed.

3.2.2 Review Process

The benefits for the research community are in part achieved through the review process which is available to all WERN members. The patient RDG actively encourages WERN clinicians, scientists and health professionals to provide the group with an opportunity to review, comment upon and become involved in the development of research projects and grant applications. The RDG would prefer to see applications at an early stage to help shape the research question from conception – however understanding that researchers can work chaotically and to tight deadlines – has meant that the RDG has been able to return with helpful constructive support in as a little as three or four days for research grants. In this

way the Patient RDG members are helping to ensure that all WERN research activity is truly patient-focused.

The Patient RDG has established on what basis they will review research ideas and grant applications and what feedback they will be able to provide. They decided their rules of engagement, the criteria that they will judge applications on and how they will provide feedback. At the group's request they have asked active researchers to come and address the meeting discussing common difficulties that they have with projects as well as recent successes. Clinicians and researchers active in Wales have been quick to embrace the Patient RDG. Starting with Dr Khalid Hamandi (University Hospital of Wales, Cardiff) researchers have attended the sessions to bring the group up to date with news in their field of interest in general and of course to hear the views of the group.

3.2.3 RDG research projects

The patient RDG aims to identify and investigate methods of improving seizure control often outside of prescribed medications; ideas include diet, sleep-pattern, exercise and better education. Improved access to services and patient-lead service review are important themes for the group. Members of the Patient RDG are fully aware of the consequences and difficulties associated with a diagnosis of epilepsy; they are ideally placed to initiate research projects with a social focus in the WERN portfolio with assistance from the Health Professionals. For example, a novel idea to help reduce teeth, tongue and mouth injuries from sleep-related seizures was suggested by a RDG member last year. The patient member had found a great deal of benefit from sleeping with a mouth guard in place (originally designed to protect teeth following cosmetic dentistry). We aim to identify a number of outcomes – i) the frequency of tongue, teeth and lip injuries in people with sleep-related seizures, and ii) whether wearing a specially fitted gum-shield at night can reduced or prevent these painful and embarrassing injuries. We want to evaluate her suggestion – ultimately for patient benefit.

3.2.4 Reflection

Increasing awareness of epilepsy and the lives of people who have seizures was identified as a priority by the RDG. When we were invited by *Involving People* to take part in a 'digital story telling' project – there were many volunteers. Digital story telling is a form of video diary, where the narrative is personal – therefore feeling less claustrophobic than an interview (discussed in full below). The RDG members discussed the highs and lows of patient involvement in research. The stories were poignant, funny and informative. We learned that initially we may not have provided enough for support for individuals with epilepsy to cope with failed grant submissions and applications. Working as health professionals in the field you become aware that successes can be few and far between and prepare yourself for potential disappointment; the patient RDG members took these failures more personally. Now we are aware of this we have moved to provide independent support. Perhaps we were naive and should have predicted this as a consequence of improved patient involvement in research. Clinical colleagues have often asked who was offering *us* supervision and pastoral support – and we failed to ask this question of our fellow RDG members.

3.3 Social Media

It is impossible to ignore the utility of social media when trying to connect in a meaningful way with patients. However there is a lot of mistrust and many a pitfall surrounding these new opportunities. In light of my experiences with hyperekplexia and epilepsy whilst working on these projects I will discuss my experiences.

3.3.1 Facebook

In 2009 Andrew Latham and two contacts with hyperekplexia started a Facebook group – the Hyperekplexia Society. I advised Andrew at the time and helped promote it to our network of referring physicians, but wanted it to be patient founded, patient run and patient lead. I certainly did not want to be responsible for moderating messages on the forum – or set myself up as an advice guru. However I do watch the fora with interest, glean research questions from them and do post when people ask simple questions about genetic

testing or heritability. I make sure I'm very careful not to give specific medication advice, nor to try and diagnose someone who I have not met. Andy himself is very proud of the 230 members who make up the thriving society, and is also proud that his case was written up as 'An unusual case of Hyperekplexia' (Jungbluth *et al.* 2000). I was lucky enough to meet him as we spoke at the University Hospital of Wales, Cardiff. That talk was filmed and uploaded to the Hyperekplexia society site.

Stephanie Claire Smith

Hi everyone, It's Steph again! Sorry its been so long since I've been on the site. My little boy is nearly 19 months now but still cant walk or talk, he just babbles occasionally, and he can pull himself up to cruise a little bit (rarely) but mostly to climb onto the sofa. If i help him to stand/walk his legs go floppy, they go everywhere except straight and flat! ..and then he goes back to crawling - he doesn't even want to try! (his physio doesn't understand it she's been seeing him for months and done tests, she's baffled) He doesn't watch TV, he plays with his toys for a very short while, hes not very sociable with other babiesat all (...he's in his own little world) I'm getting worried about his development physically and socially :(its really getting to me as I have friends whos little ones are younger and already walking/running around, putting sentences together, playing together, eating all sorts/feeding themselves with a spoon, brushing their own teeth and all sorts! In a year's time he's suppose to be ready for pre-school but I cant see that happening at this rate :(I know people say development is different for each child but I can't help thinking its due to Hpx or Clonazepam? He's got Alot of catching up to do in a short amount of time, and I dont want to wait and hope, I'm wondering - Is there anything I can do to help him with his development? Thanks xx

Like · Comment · Follow post · 9 August at 21:57

Bonnie Fletcher Hi there Stephanie, Your son sounds so much like mine. Angus (22mths) didn't start walking until about 16-17mths and didnt even babble until 18mths. He too has regular physio, Ot and speech therapy. He still cannot use his cutlery or run. Although in the physio had suggested I get him into some child care to help him improve with his development. I decided to enrol him in as many activities as I could fit into week. So he now attends preschool musical play, child care, swimming lessons and play group. His improved out of sight. He walks, talks (babbles), and although he won't play with other children he will play along side them. I've got my fingers crossed for your little guy. I'm sure he will surprise you soon. Good luck :)

10 August at 03:17 via Mobile · Like · 2

Jessica Rivers Lear My son Landon is 16 months old and is not walking or talking either. But do not lose hope! The most wonderful thing about this site and network of people is that they all give me hope for Landon. After having been a part of the group for 1 yr now, I have noticed a trend . . . All kids with Hpx are delayed in their development but almost all people with Hpx grow up to be high-functioning citizens of society and live normal lives! My husband and I accepted the fact long ago that Landon will be delayed BUT he will catch up some day. Our hope is that all the hard work he is putting in now with his Speech, Vision, Occupational and Physical Therapists, as well as the play groups and preschool, will pay off when it really counts - when he is school age. Hang in there and don't lose hope! God has great plans for your little boy!

10 August at 15:03 · Like · 2

Jessica Rivers Lear Caurie Miner Putnam and Donna Spencer O'Neal's stories of their children's progress have been such a source of inspiration for me and my hopes for Landon. Look at how much

Figure 3.2 Hyperekplexia Society – Facebook (left)

This open forum fulfils many roles. Here a mother, Stephanie, is asking for advice, support and reassurance surrounding her son's slow neurological development. She receives five replies within two days (three shown). This is a very powerful tool for a person with a rare disorder.

It is also a rich qualitative dataset for a researcher or enthusiastic clinician. There are multiple parental reports of delayed development in hyperekplexia – a novel finding borne out in chapter three; but validated here.

The *facebook* group has also yielded research participants for colleagues in Switzerland and UCL for advanced neurophysiology projects.

I update the site with press releases from recently published articles in the field; our team is often showered with thanks when I do. This small community feel neglected and appreciate and support research efforts.

In addition to not over-stepping my physicianly duties – I also don't accept friend requests from patients on Facebook. This can sometimes seem mean spirited – not accepting Andy for example, or a patient who has done me a favour by speaking at a meeting for example. However the BMA have very clear advice regarding this *"Facebook friends a no-no for doctors; BMA warns doctors against interacting with patients on social networking sites to prevent blurring of professional boundaries"* (Guardian online, July 2011).

3.4 Capturing the individual's story

From the very first meeting of the patient RDG it became clear that the group prioritised greater communication with the lay public and an aim to reduce the stigma associated with epilepsy. The RDG members were very keen to reach a wide audience and tell 'their story'. We dismissed *YouTube* as a medium as it permits users to 'comment' on videos and we thought they might become a magnet for disparaging or unflattering messages. After a year or so we discovered digital storytelling.

Figure 3.2 (previous page) Screen grab of 'Capture Wales, BBC' - www.bbc.co.uk/wales/arts/yourvideo/queries/capturewales.shtml

Epilepsy is ideally suited to the digital storytelling technique as the condition is often lifelong, varies greatly from person to person and it can often be difficult to describe. Despite the great work of charities such as Epilepsy Action with helplines, for advice and specialist information - there is a role for hearing people's story 'directly' using digital story telling. I feel strongly that there is a need to counterbalance a number of unhelpful, insulting or inaccurate videos that have proliferated on *Youtube* - and where many people with newly diagnosed epilepsy may go to for information (Cossburn and Smith, 2007). There is also some evidence that the process of telling their story can be beneficial for the quality of life of individuals with chronic conditions such as asthma (Rich *et al*. 2006), and as such we envision this project as being empowering for the people who give us their stories, as well as those who are able to derive accurate information from them.

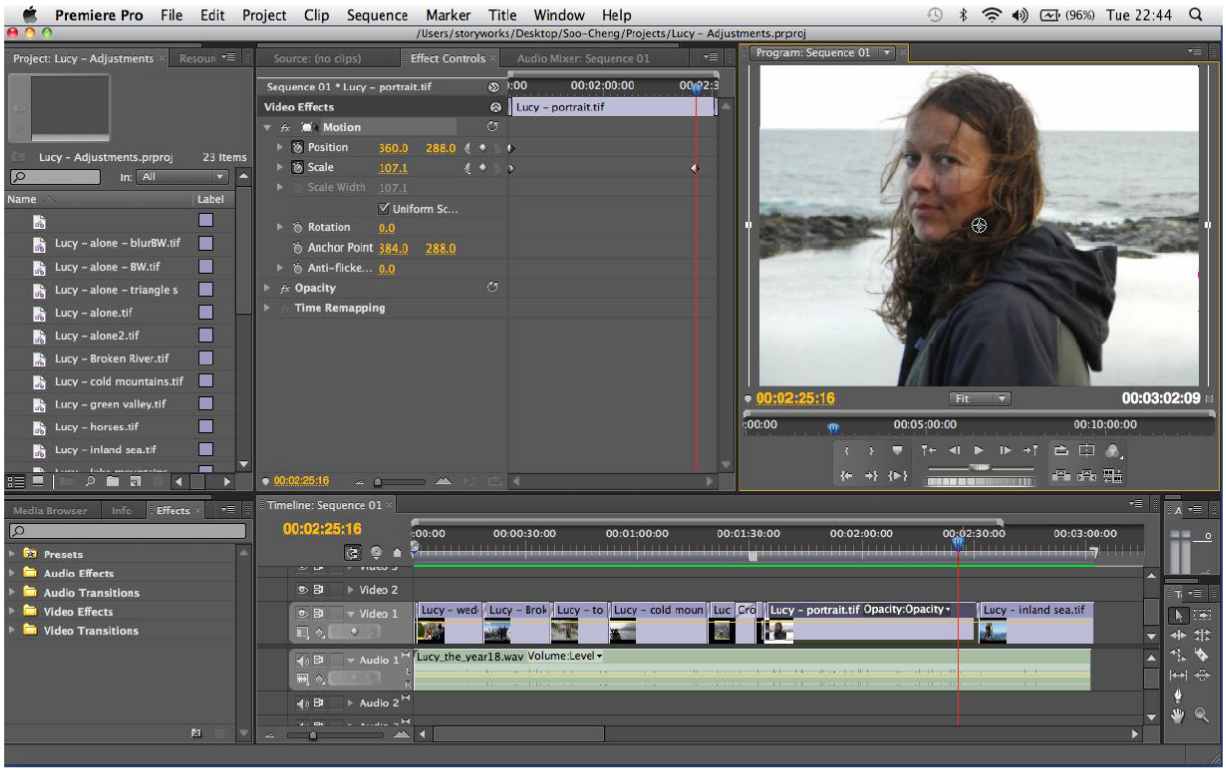


Figure 3.3 Screen capture of the editing software used to make our Digital Stories

Figure 3.4 Involving the participant at every stage frequently creates more personal and emotionally resonant videos than could be conceived by 'interviewing' someone. Here a wedding photograph is used to illustrate her story.

In addition to the patient stories which we continue to collate – we think the medium lends itself to specialists describing certain processes such as 'epilepsy surgery', 'investigations' and 'speaking to your doctor about pregnancy'. To fully inform patients and clinicians qualitative stories need to be enhanced with quantitative information to permit estimates about generalisability and scale. Our attempts to set a joint research agenda using clinicians, patients and scientists using modified focus group methodology; DUETS is such an example.

3.5 Database of the Uncertainties of the Effectiveness of Treatments (DUETs)

3.5.1 Introduction

Do research questions address the priorities of people with epilepsy? Do they even reflect the uncertainties prioritised by epilepsy professionals? Mapping this mismatch in rheumatoid and osteoarthritis demonstrated that the priority treatment outcome for

patients with rheumatoid arthritis was not pain reduction, but fatigue and sleep quality. Trials of new anti-epileptic drugs (AEDs) typically aim for a fifty percent reduction in seizure frequency to demonstrate efficacy. Is this a valid outcome for people with epilepsy? A failure to induce seizure cessation would still prevent someone from applying for a driving licence or entering certain professions. Indeed new AEDs are often an adjunct and therefore interactions and side-effects are likely. What would people with epilepsy say would be the best outcome of a new AED for someone with refractory epilepsy? Improved wakefulness, better sleep, less fatigue, improved mood, weight loss or seizure reductions? Grant committees rightly ask applicants to consider the views of patient groups when submitting an application, but without knowledge of prioritised treatment outcomes providing a comprehensive answer to this has been difficult. This would be the first attempt to ask people with epilepsy to identify and prioritise research outcomes in refractory epilepsy. The James Lind Alliance is curating a database of research questions posed by patients and professionals to encourage patient prioritised research.

3.5.2 Results

3.5.2.1 Thematic analysis

The thematic groupings used to cluster the questions generated and rated are given below - they can be found as the headings for figures 3.4 and 3.6.

3.5.2.1.1 Thematic groupings

- **Who should be treating epilepsy?**

- **Epilepsy Surgery**
  - Choice and pre-surgical considerations
  - Uncertainties following surgery

- **Prescribing Uncertainties**
  - Special groups – Children, older people and pregnancy

  - Financial Influence
  - On-going therapeutic monitoring
  - Uncertainties about drug action (such as drug interactions)
  - **Drug Side Effects**
    - Recognition and acknowledgement of side effects
    - Management of drug side effects
    - Specific side effects – mood, fertility, bone health, cognitive side effects and side effects in utero
    - Learning disability
  - **Acute treatments**
    - Oxygen and midazolam
    - Status and non-convulsive status epilepticus
  - **Drug Withdrawal**
    - Including withdrawal in non-epileptic attack disorder

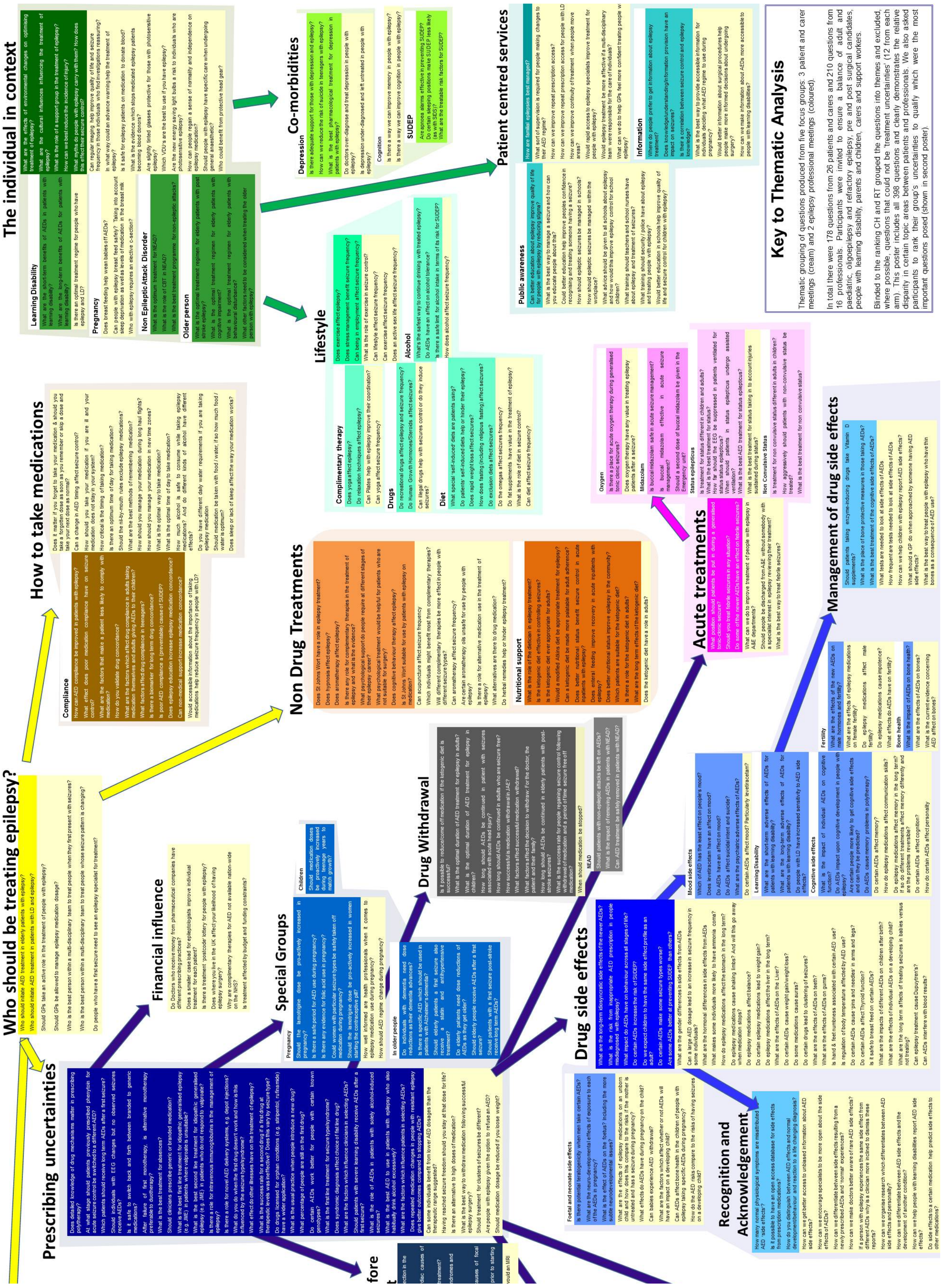
- **How best to take prescribed medications**
  - Compliance with prescribed medications

- **Non-drug treatments of epilepsy that are supervised by a professional**
  - Nutritional support

- **Considering the patient as an individual**
  - Special groups – Pregnancy, older people, learning disability
  - Non epileptic attack disorder
  - **The effect of lifestyle on seizures**
    - Drugs, alcohol and diet
    - Complimentary therapies and stress management
  - **Epilepsy co-morbidities**
    - Depression, cognitive problems and sudden unexplained death in epilepsy
  - **Information based epilepsy management**
    - Patient centred-services
    - Public awareness of seizure management

Next page – Figure 3.5 The hierarchical thematic map. This illustration shows all the unanswered questions flowing in themes from central themes. Each question is written out in full in the boxes underneath the theme name. The patients' questions are in black with an cream background – the clinicians' questions are on a coloured background – the colour of which helps to visualise the grouping of each theme and subtheme.

From the first question theme 'Who should be treating epilepsy?' comes themes about epilepsy surgery, prescribed drugs and non-drug treatments. From the second question theme 'the individual in context' are question themes such as concerns about lifestyle affecting seizure control and co-morbidities.


The individual in context
Co-morbidities
Patient centred services
Key to Thematic Analysis
How to take medications
Lifestyle
Who should be treating epilepsy?
Non Drug Treatments
Acute treatments
Management of drug side effects
Financial influence
Special Groups
Drug Withdrawal
Prescribing uncertainties
Drug side effects
Recognition and Acknowledgement

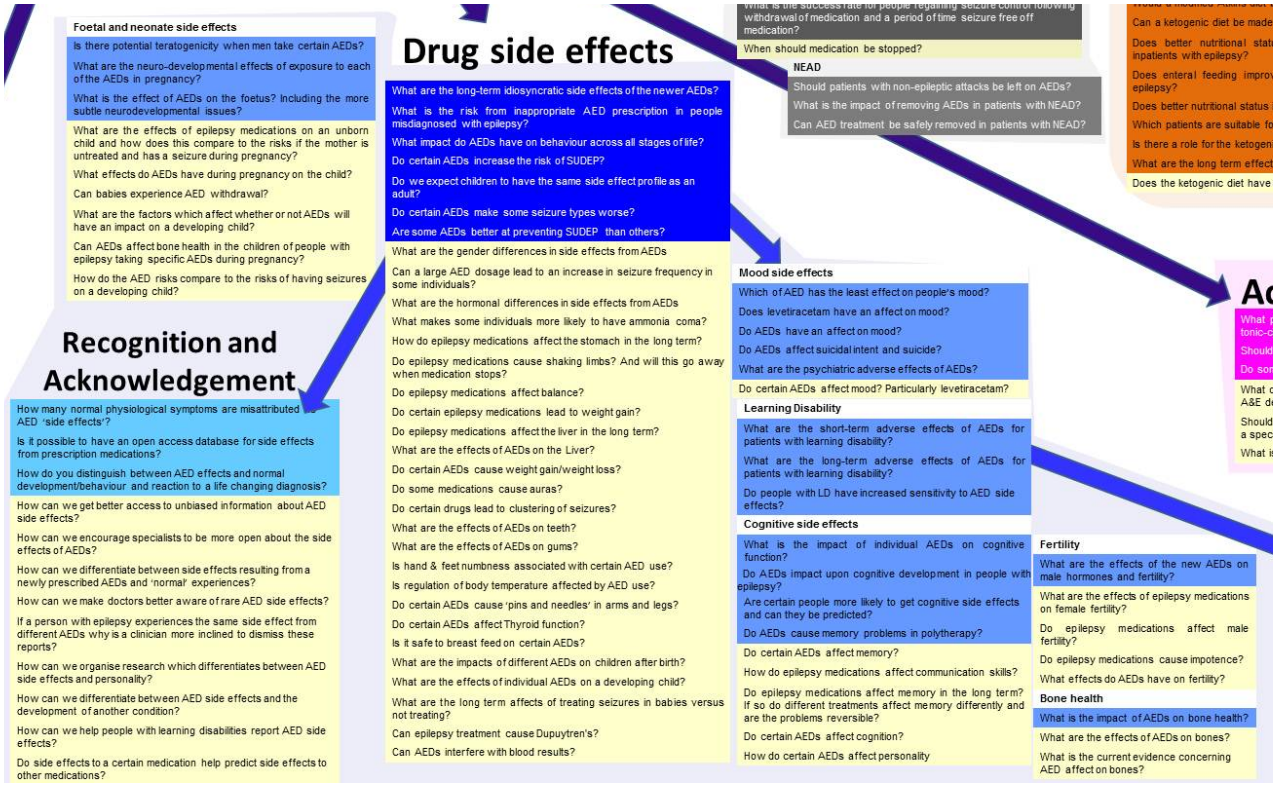


Figure 3.6 A blown up detail from figure 3.5 The professional (scientist and clinician) derived questions are given first; the colour of the boxes helps demark which thematic arc the questions had been deemed to sit under. The patient and carer questions are shown underneath (cream). This demarcation quickly allows one to see that surgical questions (top left of the main diagram) were predominantly asked by professionals yet drug side effects (below) are asked about by patients much more frequently.

What the above map (figure 3.6) does not identify is the relative importance of each theme and each question within the theme. Just because a participant asked the question – it does not mean that the groups thought it needed to be answered. Similarly just because it was only asked once – rather than rephrased a number of times – does not mean the question was deemed of less importance. I therefore created another pictorial scale to present the data (full map figure 3.7 and figures 3.8 to 3.13 are extracts taken from the main diagram). Questions first and to the right were posed by professionals, questions below and to the left by patients. The longer the line – the more important the question was deemed to be. The top 20 questions from each arm were imposed adjacent to the appropriate bar (which has been coloured according to the original thematic grouping). The mean ranked score is given above the histograms – the smaller the number – the more 'important' the questions are.


Who should be treating epilepsy?
The individual in context
Pregnancy
Older person
Non Epileptic Attack Disorder
Learning Disability
Co-morbidities
Depression
Cognition
SUDEP
Lifestyle
Alcohol
Patient centred services
Information
Public Awareness
How to take medications
Compliance
Non Drug Treatments
Complimentary therapy
Drugs
Diet
Nutritional support
Acute treatments
Oxygen
Midazolam
Status Epilepticus
Non convulsive status
Drug Withdrawal
Non Epileptic Attack Disorder
Drug side effects
Mood side effects
Learning Disability
Cognitive side effects
Side effects in utero
Management of drug side effects
Fertility
Bone health
Prescribing uncertainties
Pregnancy
Special Groups
Older people
Children
Financial influence
Recognition and Acknowledgement
Key to Ranking
After producing the database of questions in the five focus groups we asked the participants to rank the questions in terms of importance to them.
The numbers above each histogram shows the mean rank of the theme, a low score shows that most questions concerning that topic were ranked as important by the participants.
The histograms show the number of questions produced by all five groups in each theme. Light blue bars (left of centre) denote patient questions; dark blue (right of centre) questions from professionals. The longer the bar the higher the ranking from the group, i.e. a bar 100% long would have been top ranked by everyone in that group.
The top 20 questions from each arm have been included adjacent to the appropriate bar (which has been coloured according to the original grouping).

Previous page Figure 3.7 Light blue bars (left of centre) denote patient questions; dark blue (right of centre) questions from professionals. The longer the bar the higher the ranking from the group, i.e. a bar 100% long would have been top ranked by everyone in that group.

The histograms show the number of questions produced by all five groups in each theme. Drug Withdrawal (figure 3.8) contains ten professional questions with a mean ranked score of 33.7 against a single question of similar 'importance' for the patients. Two questions for the professionals were in the top 20 and both are alongside the histogram. Underneath this is a different looking graph – three questions of lesser importance (47.2); none from the patients and none in the top 20. To the right of these histograms is the pregnancy sub theme. Here again there are more questions from the professionals with a very low mean ranked score (14.2) and two the questions are in the top 20; surprisingly there is just a single question, lowly ranked at that, for the patients.

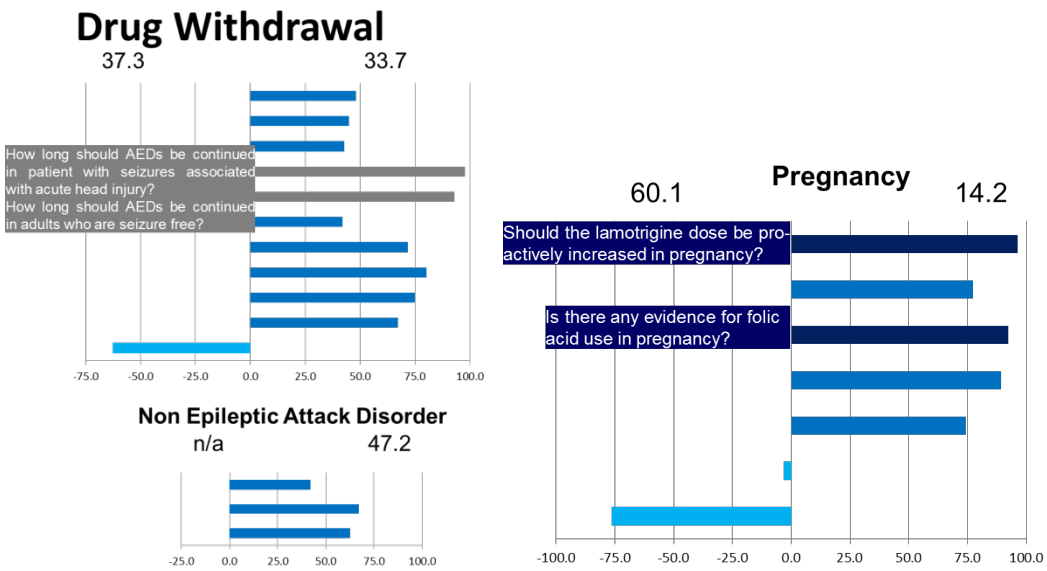


Figure 3.8 (left) Topmost - Drug Withdrawal theme which has more clinicians' questions including two which are top rated and NEAD (bottom) questions are exclusively posed by clinicians. **Figure 3.9** (right) Pregnancy questions again are dominated by clinicians' concerns.

In contrast the following histogram (figure 3.9) looked at questions relating to cognitive side effects. Both professionals and patients rated these questions as important; with the patients ranking it very highly indeed (mean ranked score 13.3, versus 31.5). Two of the professionals' questions were in the top 20 and three of the patients' questions (5 out of 9 questions in total).

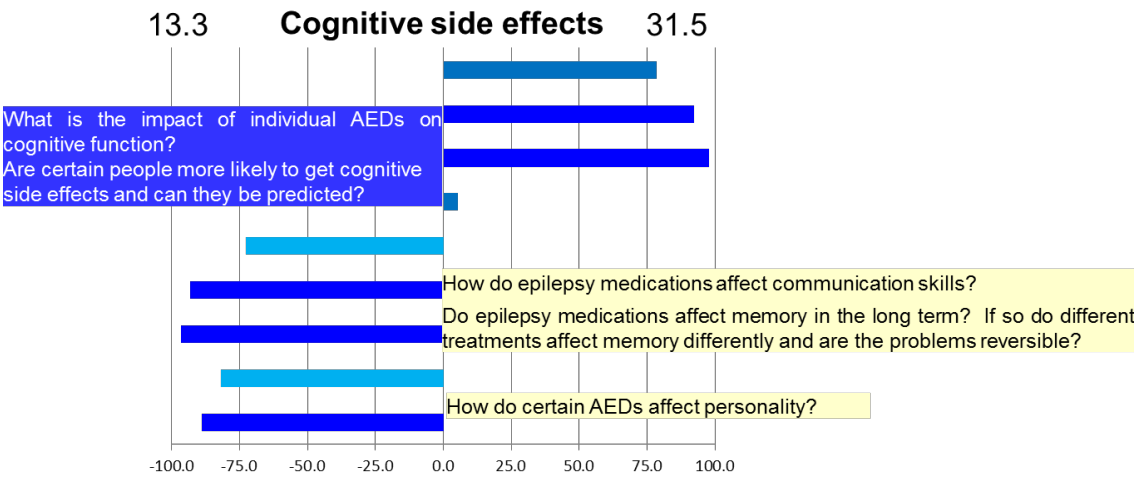


Figure 3.10 Theme regarding concerns about cognitive side effects of drugs; although two of the four clinicians' questions are top rated – three of the five patients' questions also are. This is clearly an area of high priority for both.

The first histogram looking at the subtheme 'co-morbidities' (figure 3.11, top) looks a little like 'drug withdrawal'; a bias of questions from the professionals and a mean rank score indicating importance, versus two questions from patients – but one of these was ranked in the top 20. Interestingly it is *'Do doctors over-diagnose and treat depression in people with epilepsy?'* which is qualitatively from the clinicians' questions were regarding how to identify depression – and how best to treat it. The cognition questions are separate from cognitive side effects – but rather focusing on general cognition in people with epilepsy. Only two questions were posed (both by patients) and one was highly ranked. In contrast there were three questions regarding sudden unexplained death in epilepsy (SUDEP) and all three were from professionals (figure 3.11).

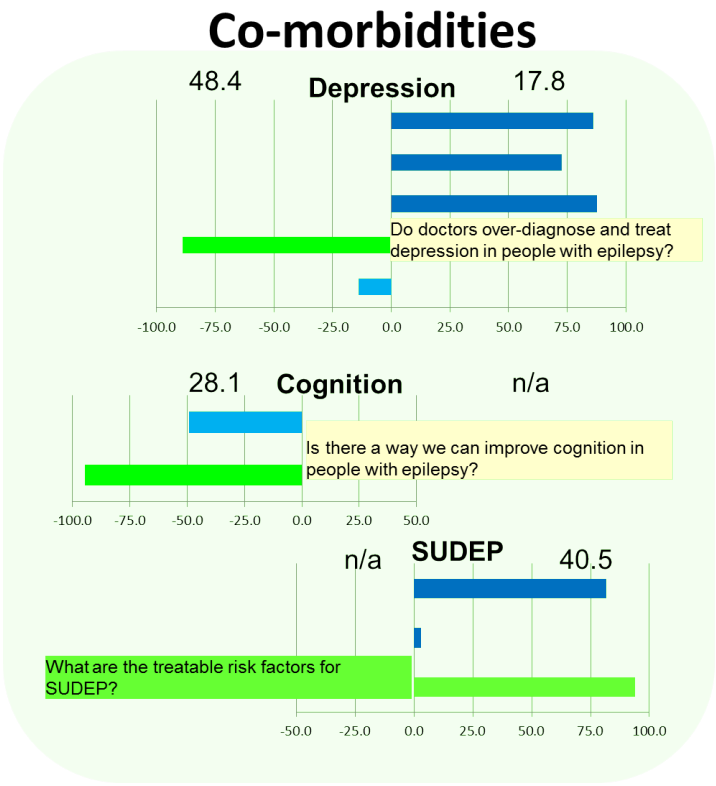


Figure 3.11 Theme regarding concerns about common co-morbidities and epilepsy (top), epilepsy and cognition (middle) and SUDEP (bottom)

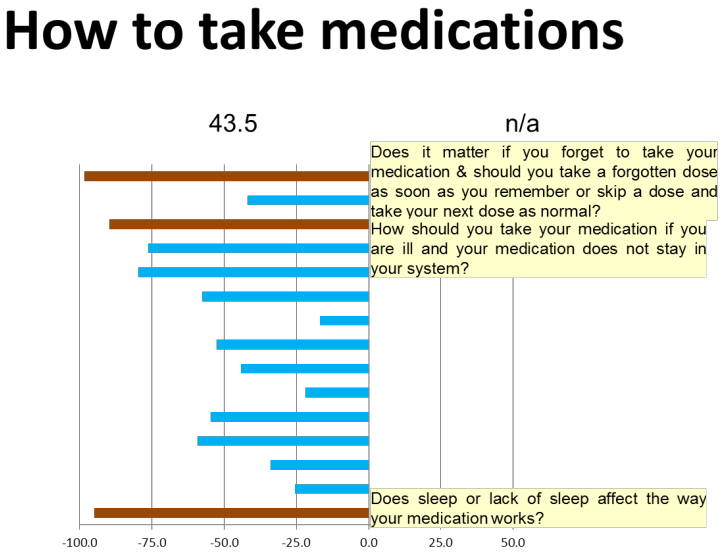


Figure 3.12 Theme regarding concerns about how to take the medications. This was one of the most disparate of themes – producing three top rated questions for patients.

In terms of contrast is the histogram which describes the fifteen questions asked about how best to take prescribed medication (figure 3.12); all were posed by patients and carers. In addition three of these questions were ranked in the top 20. This is one of the most lopsided and unequal of the histograms.

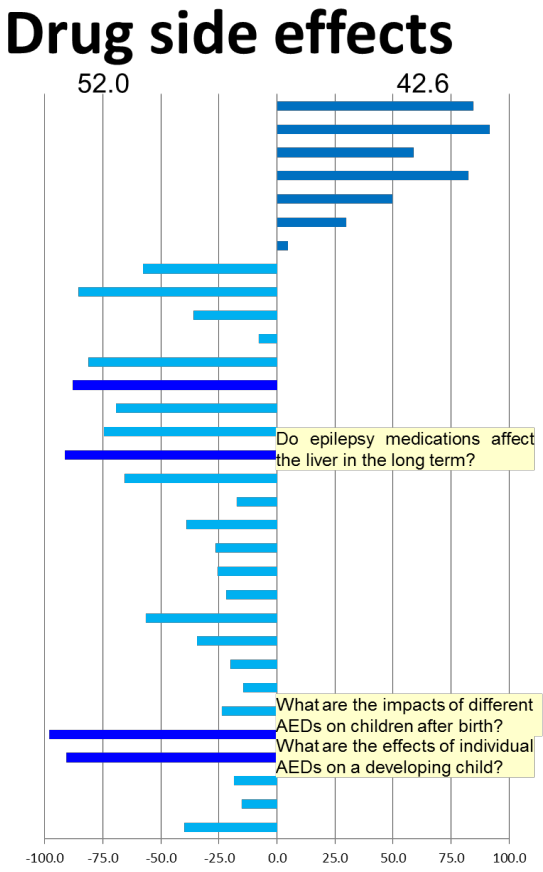


Figure 3.13 Theme regarding concerns about antiepileptic drug side effects. This is an example of a larger theme with a greater proportion of questions (particularly top 20 questions) posed and rated by patients.

The final histogram used as illustration is for 'drug side effects' (figure 3.13) – this is a thematic spine with many subgroups asking questions about specific side effects, or side effects in particular patient groups having been syphoned out into more homogeneous subthemes. Although there are three times as many questions posed by patients (inlcuding three in the top 20) many of the questions were ranked of as little or no importance (very short lines).

2.5.2.2 Statistical analysis

The meetings produced 188 questions from 25 patients and carers and 210 questions from 17 professionals. Despite the repetition of themes of research, only eleven identical questions were posed by both patients and clinicians. Figure 3.13 shows the top two questions prioritised by each group.

Highest ranked research questions

Both groups prioritised practical uncertainties affecting the greatest number of people ahead of esoteric questions or single issue, minority group uncertainties.

Professionals

"What are the neuro-developmental effects of exposure to each of the AEDs in pregnancy?"

"What do you do when the first drug doesn't work?"

"How is the choice of drug influenced by the seizure type/syndrome?"

Patients and carers

"Does it matter if you forget to take your medication?" i.e. should you take a forgotten dose as soon as you remember or skip a dose and take your next dose as normal?

"What training should teachers and school nurses have about epilepsy and treatment of seizures?"

Figure 3.14 The highest ranked research questions for each group

3.5.2.2.1 Professionals

The highest ranking themes were uncertainties concerning: individual differences in the treatment of non-epileptic attack disorder (mean rank 10.2, number of questions=3); side effects of antiepileptic drugs (AEDs); *in utero* exposure to AEDs (11.5, n=3); prescribing uncertainties in pregnancy (14.2, n=50); individual differences in the treatment of depression in epilepsy (17.8, n=3) and mood side effects (19.1, n=5). Figure 3.14 illustrates

key questions from the themes. Professionals rated uncertainties regarding prescribing (including prescribing for older people, children and in pregnancy) as more important than patients did ($p<0.0001$). In contrast drug side-effects were more important to patients and carers ($p<0.0001$).

3.5.2.2.2 Patients and carers

The highest ranked themes by patients and carers were: cognitive drug side effects (mean standardised rank 13.3, n=5); managing the consequences of any side effect from an AED (25.7, n=5); improving public awareness about the treatment of epilepsy and seizures through services (26.2, n=8) and non-medical treatment of cognitive problems (28.1, n=2). Patients rated information based epilepsy management (such as improving public awareness) consistently a higher priority than did clinicians ($p=0.001$). Figure 3.14 demonstrates the number of questions generated for each theme and figure 3.16 the differences in the wording of the questions each group posed.

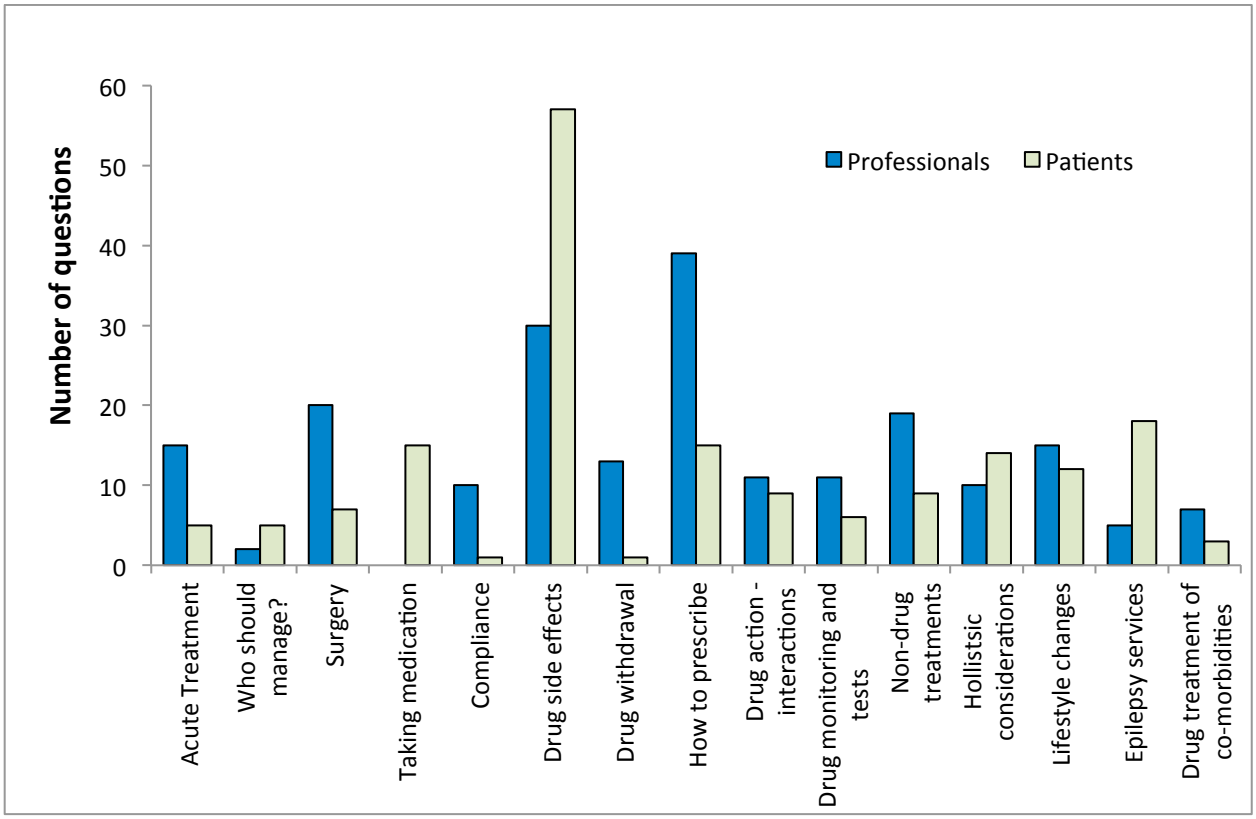


Figure 3.15 Graph showing the number of questions posed by each group under the thematic headings

Example questions from key themes

Professionals

Considering individual differences in the treatment of non-epileptic attack disorder

"What is the best treatment programme for non-epileptic attacks?"

Individual differences in the treatment of depression in epilepsy

"What is the optimum support those with depression and epilepsy?"

Patients and Carers

Managing the consequences of any side effect from an AED

"How frequently should we test to look at side effects of AEDs?"

Improving public awareness about the treatment of epilepsy and seizures through services

"Would rapid access to epilepsy specialists improve treatment for people with epilepsy?"

Shared priorities

Cognitive drug side effects

Professionals – "Are certain people more likely to get cognitive side effects and can they be predicted?"

Patients – "Do different epilepsy medications affect differently? Are the problems reversible?"

Improving epilepsy control by improving public awareness of epilepsy

Professionals – "Can better education about epilepsy improve quality of life for people with epilepsy by reducing stigma?"

Patients – "What advice should be given to all schools about epilepsy and how would this improve epilepsy control for school children?"

Figure 3.16 Differences between patient and clinician questions

3.5.2.2.3 Shared priorities

There was a great deal of consensus between the groups concerning the areas that were ranked as important to both. The most important shared priority was research on the cognitive side-effects of AEDs. Five of the top 12 shared priorities concern drug side-effects, two pregnancy and two mood disorders (table 3.1). When themes that concerned uncertainties around starting AEDs were compared to the questions posed about drug withdrawal, it was clear that commencing medication was more highly prioritised ($p<0.001$).

Shared prioritisation	**MSR**	**Professionals**		**Patients**	
		Number of Questions	Number in top quartile	Number of Questions	Number in top quartile
Cognitive drug side effects	21.4	**4**	3	**5**	4
Public awareness	25.4	**1**	0	**8**	3
Mood side effects	26.3	**5**	3	**1**	0
Prescribing in pregnancy	27.3	**5**	4	**2**	0
Treatment of depression as a co-morbidity	30.1	**3**	2	**2**	1
Drug withdrawal	34.1	**10**	2	**1**	0
Compliance	35.2	**10**	0	**2**	0
In utero side effects	35.9	**3**	2	**6**	2
Information and self-management	36.2	**3**	0	**3**	1
Recognition and acknowledgment of side effects	37.8	**3**	1	**9**	2
Management of side effects	37.9	**3**	0	**5**	4
Pre-surgical choices	38.3	**10**	4	**4**	2

Table 3.1 Table showing the top shared priorities of patients and clinicians. MSR – Mean Standardised ratio – the smaller the number the more important the theme, **Prof Qs** – number of questions in this theme from epilepsy-professionals, **Top Q** – number of questions about that theme which are ranked in the top quartile **Pt Qs** - number of questions in this theme from patients and carers.

3.5.3 DUETs Conclusion

The DUETs project proves that it is possible to use the same methodology to analyse the opinions of all people interested in epilepsy research and there is a common middle ground of 'shared prioritisation'. The patient preferences tend towards seeking a practical solution to both simple and complex questions; the clinicians (surprisingly) had more 'emotional' responses – prioritising special groups such as pregnant women and people with LD.

3.6 Summary

Choosing to perform *clinical* research often means accepting that there will be a greater number of variables beyond your control. However one factor within our control is engagement and ensuring that the people who volunteer are treated with respect - which includes listening to them. The research development group and the digital storytelling project are just small examples of this. The DUETs project puts people with a condition on equal footing with clinicians and researchers to attempt to identify what are shared research priorities. Interestingly patients are much more practical and pragmatic than may be expected; they prefer simple solutions but welcome investigation into more difficult areas too.

Chapter Four

Hyperekplexia

Genotype-phenotype correlations in Hyperekplexia

4.1 Introduction

With the exception of Professor John Stephenson, the study of hyperekplexia has been primarily by geneticists, pharmacologists and electrophysiologists since Professor Andermann's clinical descriptions in *Brain* (Andermann *et al.* 1980). What follows in this chapter is my attempt to redress the balance and augment the exquisite molecular genetics discoveries and the break-through in electrophysiology with clinical correlations. This is only possible because of the volume of cases that have been studied through Professor Rees' lab in Cardiff, then Auckland and now Swansea.

I first present the comparison between the phenotypes of individuals with *GLRA1, GLRB, SLC6A5* mutations and a cohort with no identified mutation. I also describe the audit which identified these gene negative cases and an analysis into how the ethnicity of the referral predicts the type of mutation seen and the inheritance pattern. Finally I describe in greater detail the presentations of individuals with known mutations (specifically *GLRB*).

4.2 Results

One hundred and ten cases positive for either *GLRA1, GLRB* or *SLC6A5* mutations were identified from our database (rare cases with digenic inheritance were excluded –pending further functional analyses) and we received proforma replies for ninety-four cases (86% return). A further 35 cases with no genetic cause were included for comparison. The cases with no proforma return were often referred over ten-years ago and the custodian clinicians (and in some cases, the institutions) were no longer clinically-active. The non-repliers were proportionate - four of the sixteen had *SLC6A5* mutations. Three additional cases were obtained from colleagues in Liege, Belgium (Jean-François Vanbellinghen). There were 61 cases with *GLRA1* mutations (14 dominant missense (23%), 24 recessive missense (39%), 23 recessive nonsense (38%)); 24 with *SLC6A5* mutations (8 recessive missense (33%), 14

recessive nonsense (58%) and 2 not confirmed (8%)); and 12 *GLRB* cases (1 dominant missense (8%), 2 recessive missense (17%), 9 recessive nonsense (75%)). The majority of the mutations have in-vitro validation of pathogenicity from previous functional studies (Rees *et al.,* 2002, Rees *et al.,* 2006, Chung *et al.,* 2010, Carta *et al.* 2011).

4.2.1 Phenotypic Characteristics

Every patient exhibited an exaggerated startle in response to tactile or auditory stimuli; whereas the pattern of hypertonia was surprisingly varied. All *GLRB* cases demonstrated clear hypertonia – compared with *GLRA1* (74%, $p<0.05$) and *SLC6A5* cases (84%, ns) with many cases explicitly having normal or reduced tone (table 4.1). Every gene positive case had symptoms from birth, indeed some exhibited startle *in utero* during the last trimester - in contrast 54% of the gene negatives had onset after the first month of life, often into childhood ($p<0.001$). Recurrent injurious falls were common (over 50% of the gene positive cases) in contrast to the gene negative group where it was seen in less than a quarter ($p<0.001$). Seizures were a definite feature in seven cases and probable in another five: all twelve cases were due to recessive mutations. This gives an estimate of the prevalence of epilepsy in hyperekplexia of between 7 and 12%. If an estimated population prevalence of epilepsy is taken as 0.75%: this represents an increased risk of epilepsy in hyperekplexia of 9 to 16 fold. No children were reported as having cardiac arrhythmias, autonomic abnormalities, metabolic deficiencies, or hearing difficulties. There were sporadic reports of ocular apraxia and congenital extraoccualar eye movement disorders (all *GLRB* cases). We received many reports of hyper salivation and relative failure to thrive. Oro-facial tactile stimulation of feeding appears to be a potent trigger for hypertonic attacks which can produce feeding difficulties.

Gene	Inheritance / Mutation	Startle	Hypertonia	Apnoeas	Falls	Delayed development	Epilepsy	Clonazepam	Neonatal onset
GLRA1	**Dominant Missense**	**14**	**10**	**4**	**9/13**	**3**	**0**	**6/7**	**14**
	n=14	100%	71%	29%	69%	21%	0%	86%	100%
GLRA1	**Recessive**	**47**	**35**	**22**	**30/45**	**21**	**3**	**29/30**	**47**
	n=47	100%	69%	38%	43%	41%	10%	97%	100%
	Missense	**24**	**19**	**17**	**14/22**	**11**	**2**	**13/14**	**24**
	n=24	100%	79%	71%	64%	46%	8%	93%	100%
	Nonsense	**23**	**16**	**5**	**9**	**10**	**1**	**16/16**	**23**
	n=23	100%	70%	22%	39%	43%	4%	100%	100%
GLRA1	**Total**	**61**	**45**	**26**	**39/58**	**24**	**3**	**35/37**	**61**
	n=61	100%	74%	43%	67%	39%	5%	95%	100%
SLC6A5	**Recessive**	**22**	**14/17**	**14/18**	**8/15**	**12/17**	**2**	**16/17**	**14**
	n=22	100%	82%	78%	53%	71%	14%	94%	100%
	Missense	**8**	**5/5**	**5/6**	**2/4**	**4/4**	**1**	**4/4**	**8**
	n=8	100%	100%	83%	50%	100%	13%	100%	100%
	Nonsense	**14**	**9/12**	**9/12**	**6/11**	**8/13**	**1**	**12/13**	**14**
	n=14	100%	75%	75%	55%	62%	7%	92%	100%
	Not confirmed	**2**	**2**	**2**	**0**	**2**	**0**	**2/2**	**2**
	n=2	100%	100%	100%	0%	100%	0%	100%	100%
SLC6A5	**Total**	**24**	**16/19**	**16/20**	**8/17**	**14/19**	**2**	**18/19**	**24**
	n=24	100%	84%	80%	47%	74%	14%	95%	100%
GLRB	**Dominant Missense**	**1**	**1**	**1**	**NA**	**NA**	**0**	**1**	**1**
GLRB	**Recessive**	**11**	**11**	**7**	**7**	**9**	**2**	**11**	**11**
	n=11	100%	100%	88%	58%	82%	18%	100%	100%
	Missense	**2**	**2**	**1**	**1**	**1**	**0**	**2**	**2**
	n=2	100%	100%	50%	50%	50%	0%	100%	100%
	Nonsense	**8**	**9**	**6**	**6**	**8**	**2**	**9**	**8**
	n=9	100%	100%	100%	67%	89%	25%	100%	100%
GLRB	**Total**	**12**	**12**	**8/9**	**7**	**9**	**2**	**12**	**12**
	n=12	100%	100%	89%	58%	82%	18%	100%	100%
No mutation		**30**	**24**	**8**	**8**	**12**	**9**	**NA**	**19/35**
	n=35	86%	69%	23%	23%	34%	26%		54%

Table 4.1 Hyperekplexia characteristics broken down by gene of effect, inheritance of gene and mode of action

4.2.1.1 Apnoeas and Learning Difficulties

Recurrent apnoea attacks are commonly seen in hyperekplexia ranging from a feature of a third of dominant *GLRA1* cases, to 80% of those with *SLC6A5* and 89% *GLRB* mutations ($p<0.004$, $p<0.03$). Delays in gross motor and speech acquisition were commonly reported – with a striking gene of effect pattern seen; 8/12 and 11/12 children with *GLRB* mutations had delays in speech acquisition (*GLRA1* vs. *SLC6A5* RR 1.5, $p<0.01$*, vs. GLRB* RR1.9, $p<0.02$); Global developmental delays were predominantly a feature of the recessive hyperekplexias – but again seen twice as frequently in *GLRB* and *SLC6A5* (where they were equivalent at over 80%) than in recessive *GLRA1*. The difference between this and the reported rate of 8% in dominant *GLRA1* (the first reported cases) is stark.

Twenty one kindreds had LD and recurrent apnoea attacks (nine with *SLC6A5, seven with GLRB* mutations), ten had LD without a prior history of apnoeas (all had *GLRA1* mutations, $p<0.001$ and all bar one were caused by recessive or compound heterozygous mutations). Patients with recessive or compound heterozygote mutations (independent of the gene involved) were also more likely to exhibit developmental delay or learning difficulties ($p<0.02$), 51% with recessive inheritance compared with 10% dominant.

4.2.1.2 Clonazepam and referral pattern

Sixty-five cases had been treated with clonazepam (67%) 96% of these had a symptomatic benefit from its use. As expected, dose-related sedating side-effects limited its utility in some cases. There was no pattern formed by looking at those who had a dramatic or equivocal response to clonazepam. Of the seventeen individuals with *SLC6A5* mutations fourteen had been tried on clonazepam and thirteen found a sufficient and sustainable benefit from this therapy; similarly all *GLRB* cases responded well to clonazepam (one to nitrazepam).

4.2.1.3 Gene negative cases

The thirty five cases without mutations in the three genes of effect had a number of similarities with the genetically proven cases: the majority had exaggerated startle (86%), were hypertonic (69%) and apnoeas and falls were seen albeit it a lower frequency than other cases (23% RR2.4 p<0.001, 23% RR2.7 p<0.001). Importantly just under half of cases reported that startle symptoms began after the first month of life; to date we have not identified a genetic answer for a later onset case (p<0.001).

4.2.1.4 Gene negative case audit

157 kindreds (sometimes single cases) were referred and a genetic cause was found in 51 families (figure 4.1) Nine kindreds were no longer thought to have hyperekplexia: probable symptomatic hyperekplexia (n=5), onset after infancy (n=3) and paroxysmal extreme pain disorder (n=1) accounted for these. Of the remaining families 66% had no identifiable genetic cause for their symptoms. We have identified a genetic cause in a minimum of 40% of cases submitted to the group (Thomas *et al*., 2010c). Since this analysis was performed and presented eight of the 'typical cases' have been further analysed and identified as having *GLRB* mutations.

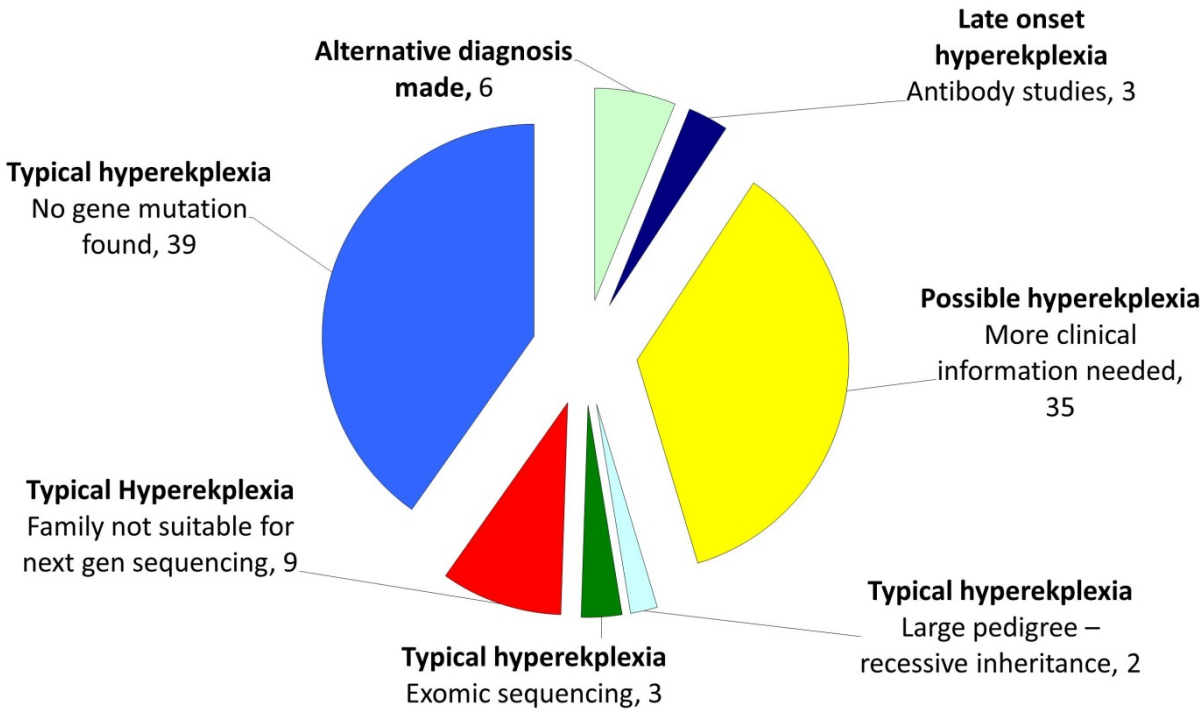


Figure 4.1 (previous page) Exploded pie chart describing the gene negative cases studied

4.2.2 Ethnic variation

The review of the literature identified 50 families with a genetic diagnosis of hyperekplexia (appendix G) and from this data set we were able to identify the ethnicity in 70% of families (n=35). Our research project yielded sixty further cases where the ethnicity was known (table 4.2).

	Source	Asian	Caucasian	Arabic	Turkish	Israeli /Jewish	African-American
del ex1-6	**Swansea**	0	1	0	6	0	0
	Literature	0	0	0	10	0	0
R271	**Swansea**	0	10	0	0	0	0
	Literature	2	4	0	0	0	1
Homozygous	**Swansea**	6	4	11	5	7	0
	Literature	0	4	1	1	0	0
Compound heterozygote	**Swansea**	0	4	0	2	0	0
	Literature	2	0	0	0	0	0
Non R271 dominant	**Swansea**	1	2	0	0	0	0
	Literature	3	5	0	1	0	0
Total	**Swansea**	7	17	11	11	7	0
	Literature	5	13	1	12	0	1
	Both	12	30	12	23	7	1

Table 4.2 Hyperekplexia ethnicities. This table shows the numbers of cases that harbour different types of *GLRA1* mutations by their ethnicity. The light blue lines come from our data and the white lines from the literature review I performed.

Deletions exons one to six Of the twenty-three cases that were identified as Turkish or Turkish Kurds, sixteen (70%) were homozygous for large deletions (exons one to seven). Homozygous deletions of exons one to seven were predominantly seen in people of Turkish or Turkish Kurd backgrounds (16/17, $p<0.00001$).

Dominant hyperekplexia Both Japanese families, 14/30 of Caucasian cases and a single African-American kindred had hyperekplexia due to mutation of an arginine residue at point 271. In contrast dominant hyperekplexia caused by mutations R271 has not yet been reported in people with Arabic, Israeli or Turkish ethnicities ($p<0.0005$). The non R271 dominant mutations followed a similar pattern; except that there were four Asian and a single Turkish case.

Homozygous mutations The majority of *GLRA1* hyperekplexia is caused by homozygous mutations (66%) - 20% are large deletions (above) and the remainder accounted for by private mutations, infrequently recurring. All of the twelve Arabic and all seven Israeli/Jewish cases were homozygous.

4.2.3 *GLRB* phenotypes

Before 2011 there was only a single case described with hyperekplexia caused by a *GLRB* mutation (Rees *et al*., 2002). Then in 2012 a large consanguineous Saudi family was described (Al-Owain *et al.*, 2012), accompanied by our two publications which confirm the importance of GLRB in hyperekplexia (James *et al.* 2012, Chung *et al*. 2012). The clinical vignettes that describe the fourteen new cases are given below.

Patient 1. L285R (heterozygote) James *et al.* 2012

He was born at term plus 11 days, however his weight and height were at the 3rd centile at delivery. Apnoeas seen within 40 minutes of birth and stiffness presented within the first hour of life. These features necessitated intubation and startle was prominent during this time. He has the nose-tap reflex. MR brain imaging was subtly abnormal – with mild

increase in signal seen on T2 sequences in the white matter and obvious dentate nuclei, however the basal ganglia had an unremarkable appearance.

At three months of age stereotyped but unusual dystonic posturing movements were seen (including an arm raising phenomenon which is followed by a period of generalised hypertonia during which voluntary movements are impossible); clinical suspicion was that this was hyperekplexia. At nine months of age his gross motor performance was at the 7month level but this had broadly normalised by 14 months; the dystonic movements were still prominent. At no stage were epileptic seizures a complication. Strabismus was observed at 14 months, but is no longer present. At last clinical contact he was nine years old and remained of small stature. He is still hypereflexic with cervical hypertonia prominent however exaggerated startling is less of a feature. He plays football well and is making good progress in school and socially.

Patient 1 harbours a two base missense mutation (c.920_921ΔinsGA) resulting in a L285R substitution in the second membrane-spanning domain (M2) of the GlyR β subunit. Since this change was found in the heterozygous state and neither parent carries the mutation, this mutation appears to be de novo. L285R results in the insertion of a positively-charged side chain into the pore-lining 9' position. Mutations at this site are known to destabilise the channel closed state and produce spontaneously active channels. This leaky channel is similar to the Y128C mechanism we described in *GLRA1* (Chung *et al.,* 2010).

Patient 2. W310C (homozygous) James *et al*. 2012

The patient is a 5-year old girl (born 2006) originating from Turkey, her parents are first cousins. According to the family the maternal brother and patient's parents had 'light symptoms' in early life. However the story of their early childhood has been lost over time and bound up within cultural restrictions surrounding such disclosure. Her older sister (untested) has clear symptoms and her younger brother born in 2010 (tested) is also affected. The parents currently do not have symptoms, but are both heterozygous for the mutation.

The index patient had neonatal hypertonia and irregular breathing necessitating CPAP treatment for several days. In addition she had episodes of bradycardia neonatally. She had an excessive startle reflex and consciousness was unaltered during startle episodes. She had a short period of generalised stiffness following the startle response during which voluntary movements were impossible. MR brain imaging was performed at 2 months of age showing periventricular bilateral cystic changes suspected as being periventricular leucomalacia. She is not dysmorphic and not currently of small stature, at only -1 SD below the mean.

At 4 months of age the child had screaming periods without clear loss of consciousness; she at the same time had spasms in the jaw and odd movements of her arms. At that time her EEG was normal without focal signs. At 16 months of age she had a pathological EEG with clear high amplitude spike and wave activity seen and occasional hypsarrythmia. She was treated with nitrazepam and both the seizures and EEG changes disappeared. She currently takes clonazepam and has no further symptoms. There is no gaze palsy nor eye movement disorders, nor any learning difficulties. There is no suggestion of autistic behaviour in the proband or in the family.

Patient 2 has a different missense mutation (c.G996T), resulting in a W310C substitution in the third membrane-spanning domain (M3) of the GlyR β subunit. At first glance, this mutation appears to show recessive inheritance, since both parents were heterozygous carriers. However, clinical assessments and functional data suggest an alternative mechanism of inheritance for this mutation. W310C was predicted to interfere with hydrophobic side chain stacking between M1, M2 and M3. We found that W310C had no effect on glycine sensitivity, but reduced maximal currents in α1β GlyRs in both homozygous (α1βW310C) and heterozygous (α1ββW310C) stoichiometries. Since mild startle symptoms were reported in W310C carriers, this may represent an example of incomplete dominance in hyperekplexia (i.e. a mutation that has an intermediate effect in heterozygous carriers), providing a potential genetic explanation for the 'minor' form of hyperekplexia. This *forme fruste* is predicted to be more likely in *GLRB* than *GLRA1* as the likelihood of a heterozygous carrier being able to synthesize fully wild-type GlyRs with a 2α1:3β stoichiometry when carrying a defective *GLRA1* allele is 1 in 4, versus 1 in 8 for a defective *GLRB* allele.

Case1 G229D, Del Ex 5 (compound heterozygote) (Rees et al., 2002)

Case 1 is a Caucasian male from North Western Europe (currently aged 15), there is no family history of exaggerated startle. His genotype and phenotype were previously described in 2002 (Rees *et al.*, 2002). His tone varied greatly with a tendency to become quite rigid particularly in his first six months; anxiety, excitement and touch increased tone and triggered the startle response. He was noted to have an abnormal 'staccato' cry. His development was not noticed to be delayed. After a year his hypertonia was only noticeable when agitated. His speech was slow in evolution – at four years he made phrases with abnormal pronunciation. Startle responses when tapped on the nose and lip were still present (although much reduced) at the age of six. He was prone to frequent injurious falls – as he could not put out his hands to break the impact as he fell. Clonazepam was started at the age of four months with good effect.

Case 2 R190X, Del S262 (compound heterozygote) Chung *et al.* 2012

Case2 is a Caucasian female from North Western Europe (currently aged 4), there is no family history of exaggerated startle. Her mother had noticed that the child would 'startle' in utero if she were to sneeze in late pregnancy. Birth was uneventful. Hypertonia and frequent symmetrical non-epileptic 'convulsions' were seen in her limbs from birth. The tonic attacks were associated with apnoeas. She appeared particularly sensitive to acoustic stimuli, but the hyperkinetic movements could also apparently occur spontaneously. Feeding was initially difficult and NG feeding was needed. Clonazepam was started at the age of one week with good effect; although perhaps hypotonia was an initial consequence of therapy.

Case 3a and b E24X (homozygous) Chung *et al*. 2012

Case3a is a Chinese female from China (currently aged 25), beyond her sister there is no family history of exaggerated startle. She was described originally in Shiang (Shiang *et al*., 1995) as a *GLRA1* negative 'sporadic' case. Birth was uneventful and abnormal 'myoclonic' movements were seen within 18 hours of delivery; these attacks were readily provoked by

touch or sounds – but appeared to occur spontaneously. Anti-epileptic drugs did not help these movements, however nitrazepam was efficacious (and indeed an ictal EEG demonstrated that these were not epileptiform in nature). Her motor and speech development was slow and her DQ (development quotient) was impaired at five years (a score of 59). Although she was not prone to falling, startle was readily induced at 7 years.

Case 3b is a Chinese female from Japan, and the sister of 3a (currently aged 24). There was mild birth asphyxia- however her symptoms at birth and throughout childhood were identical to her sister (3a). Clonazepam was used to control startle events with good effect. Her development again was slow and she had mild learning difficulties at the age of six.

Case 4 Del exons 1-8 (homozygous) Chung *et al.* 2012

Case4 is an Indian female from India (currently aged 6), there is no family history of exaggerated startle. Startle and jerking to nose tap were seen before two days of age, although the tone was very variable. Once ambulant (2 years onwards) falls were prominent which improved over time. There was both developmental delay (mild speech delay) and frank learning difficulties with mild autistic features. Of note is that she has a squint. Clonazepam was utilised with good effect.

Case 5 P169L (homozygous) Chung *et al.* 2012

Case 5 is a Turkish male (currently 3 years), there is no family history of exaggerated startle. Exaggerated jerky foetal movements were reported in the last trimester and the neonate had life-threating laryngospasm and could not be intubated until 47 minutes of age. The cognitive outcome is poor (Bayley Scales of Infant development II: 52), this is thought likely to be due to both the significant asphyxia and the hyperekplexia. The boy had again a laryngospasm at the age of 1 year and could not be intubated, when the mother stopped his clonazepam suddenly. Since that time his triggered startle episodes have been in abeyance with clonazepam at a dose of 0.05-0.07 mg/kg/d.

Case 6 Frameshift 19I (fs3X) (homozygous) Chung *et al.* 2012

Case6 is an Indian male from India (currently aged 4), there is no family history of exaggerated startle. He had both exaggerated startle, but a positive nose tap test. The child was noted to be frankly rigid and had both developmental delay and learning difficulties. Clonazepam along with other benzodiazepines were use with good effect.

Case 7. Splice site – loss of exon 5 (homozygous) Chung *et al.* 2012

Case 7 is a female from North Western Europe (currently aged 10); beyond her sister there is no family history of exaggerated startle. Triggered falls were a serious problem for her once she was walking and she took to wearing a helmet. Hypertonia was improved over time, although at the age of four she was running on tip toes. She has learning difficulties and needed support with speech and language at school. Of note she had an intermittent convergent squint (anisometropic hypermetropia). Clonazepam was utilised with good effect and was weaned from five years of age.

Case 8. Splice site – loss of exon 5 (homozygous) Chung *et al.* 2012

Case 8 is an Indian male from India (currently aged 3), there is no family history of exaggerated startle. He was born at term. His hypertonia was more mild – and he is not reported to have learning difficulties, although development was somewhat delayed. In addition to the non-epileptic startle he had very frequent generalised tonic-clonic seizures and jerks (at times – over 100 a day) which were proven with EEG to be epileptic in nature. Clonazepam was utilised with an initially good effect which waned over time; he also took sodium valproate for the epilepsy and levetiracetam which did not clearly demonstrate a benefit.

Case 9. Splice site – loss of exon 5 (homozygous) Chung *et al.* 2012

Case 9 is an Indian male from India (currently aged 15), there is no family history of exaggerated startle. He was born at 39 weeks and had events in the first week of life. His tone was increased particularly in his lower limbs. His development was delayed and he had mild learning difficulties. In addition to the non-epileptic startle he had generalised epileptic seizures which were treated satisfactorily with sodium valproate. Clonazepam was used to treat startle with an initially good effect which waned over time.

Case 10. Splice site – loss of exon 5 (homozygous) Chung *et al.* 2012

Case 10 is an Indian female from India (currently aged 7), there is no family history of exaggerated startle. In addition to the characteristic hypertonia, triggered startle events and positive nose tap test – she was prone to falls. Her development was noted to be delayed and she had a degree of learning difficulty. The hypertonia was probably a causative factor for her umbilical hernia. Clonazepam and sodium valproate were use with good effect.

Case 11. R450X (homozygous) Chung *et al*. 2012

Case 11 is an Arabic female from Jordan (currently aged 11), her brother died at 4 months due to aspiration and with a similar clinical presentation. Three cousins also died in the neonatal period, who were 'jittery' however they were not formally diagnosed. She had startle from birth but her motor and speech were delayed and she had learning difficulties with mild autistic features. Clonazepam and phenobarbital were utilised with good effect and but weaning at two and a half years exacerbated the tonic attacks and apnoeas.

Case 12. Y470C (heterozygous dominant) Chung *et al*. 2012

Case 12 is a Caucasian female from North Western Europe (currently aged 11 months), there is no family history of exaggerate startle. She was born at term and her mother felt exaggerated foetal movements from 30 weeks gestation. She began to have non-epileptic hypertonic seizures from 3 hours of age. These attacks were provoked by tactile stimuli and

were associated with apnoeas. Speech at appears to be delayed as she has no polysyllabic babble, but can only hum. Clonazepam was used with good effect; the dose needed to be split to avoid side effects and the dose needed to be increased to achieve the same effect over time. Phenytoin, sodium valproate and phenobarbitone had no demonstrable effect. There appears to have been an improvement however with levetiracetam. Interestingly this case was reported prematurely by Hussain *et al*. (2012) and erroneously reported as 'gene negative' while we were still analysing our results!

4.4 Conclusion

The *GLRB* cases summarised above represent not only the largest collection of hyperekplexia cases complied, but also the first collection of *GLRB* cases based on more than one kindred. Analysing the clinical evolution in section 4.2.3 provided the 'raw material' for the larger genotype-phenotypes analysis demonstrated 4.2.1. The inclusion of gene negative cases is unusual for a genotype-phenotype study but is crucial as we seek the cases to take forward for second generation sequencing.

4.4 Summary

Access to large volumes of cases has permitted me to identify that *GLRA1* is associated with a less malignant phenotype than S*LC6A5* and *GLRB*; that gene negative cases often don't present at birth; and that tone needn't always be increased in gene positive cases. The collection of *GLRB* clinical cases presented is by some margin the largest and confirms the homogeneity of this primarily monogenetic disorder. The next chapter in contrast demonstrates the variability inherent in polygenic JME.

Chapter Five

JME clinical descriptions

A clinical description of Juvenile Myoclonic Epilepsy; considering sporadic and familial cases

5.1 Introduction

Juvenile myoclonic epilepsy (JME) is an electroclinical syndrome described by Janz and Christian in the modern era and thought to be a genetic generalised epilepsy. It is characterised by myoclonic jerks (in all cases – and predominantly in the mornings), generalised tonic-clonic seizures from wakefulness, infrequent absences which only occur in a minority of cases, photosensitivity and cognition or praxis induced myoclonus also in a minority of cases. Standard magnetic resonance imaging is ubiquitously normal, sex incidence is near equal and seizures are first noticed between the ages of ten and twenty-five; peaking at puberty. Sleep deprivation is very clearly a strong provoking factor for seizures and often this technique is needed to elucidate the characteristic inter-ictal EEG patterns of 3.5- to 6-Hz spike-and-wave and multiple spike-and-wave complexes. Drug-induced myoclonus is a feature of treatment with sodium channel blocking drugs such as carbamazepine and phenytoin and the agent with the best chance of seizure remittance is sodium valproate. Drug resistance to valproate is reported in approximately a fifth of cases with an unknown proportion of these cases being secondary to lifestyle factors and non-concordance – so called pseudoresistance.

This chapter deals with the clinical description of cohorts of patients with JME and other idiopathic generalised epilepsies. Each cohort is attached to its own bias of selection and so it is only through analysing a number of collections can we hope to garner a better understanding of how JME presents and the clinical features of the syndrome. I will start by describing in detail the St George's familial epilepsy cohort, then in less detail a collection of sporadic cases of JME from the University Hospital of Wales. I will then describe the clinical features of the cases with neuropsychology (chapter six) before describing a novel subtype of a familial epilepsy – borderline GEFS+.

5.2 St George's Cohort of JME families

160 individuals from 54 families had clinical data available for analysis; in eight cases there was insufficient information to even make a tentative epilepsy syndrome / seizure description diagnosis.

Most of the families identified were small and the mean number of affected individuals per pedigree was 2.87, (median 2) with a range from 2 to 7. 98 individuals had a likely syndromic diagnosis in keeping with a broad definition of JME. For this analysis CAE evolving into JME was taken to be a subtype of JME. This represents 64.5% of the sample and the focus of the original collection which was to 'identify JME families'. It is therefore of interest that even within JME families 35% of affected individuals have an alternative epilepsy diagnosis. The other diagnoses were CAE (n=7, 4.6%), JAE (7, 4.6%), other IGEs (23, 15.1%), focal epilepsies (6, 3.9%), febrile seizures only (5, 3.3%) and a single seizure only (6, 3.9%). Therefore if the febrile convulsions and single seizures are excluded then 14% of the affected family members in JME families that did not have JME themselves, had a *focal* epilepsy syndrome.

Only three people with a 'JME' were also thought to have febrile convulsions and in one case their presentation was very atypical: they had prominent GTCS from sleep and an asymmetrical EEG. Although focal discharges are common in JME the myoclonus was not early morning either but before bedtime. Including one further case with IGE and febrile seizures this produces an estimate of 9.2% of people with an epilepsy phenotype within JME families having febrile seizures. This clearly does not equate to a prevalence of febrile seizures in 9% of people in JME families – as was previously stated the number of affected individuals in each pedigree was very small. CAE and JAE are both represented in JME families but perhaps at a much lower level than one might expect. In five cases CAE evolved into an adult epilepsy tendency and on three occasions this included myoclonic jerks and was therefore coded as a JME.

5.2.1 Familial JME

96 of the 98 people with JME had data for age of onset (mean 13.2 years, range 3 to 24 years). Figure 5.1 plots the distribution of the ages of onset. At first instance it appears to

show a normal distribution curve – with perhaps a leftwards skew to reflect the CAE evolving into JME cases. Does it in fact represent two normal curves? One with the peak at 8 years of age (CAE peak) and the second with a peak at 13 (JME peak). If this is the case then an argument could be made for a third – inexplicable peak evolving out of the 'JME peak' and itself peaking at 17 years. Does this instead represent late diagnosis of myoclonic jerks as an epileptic phenomenon?

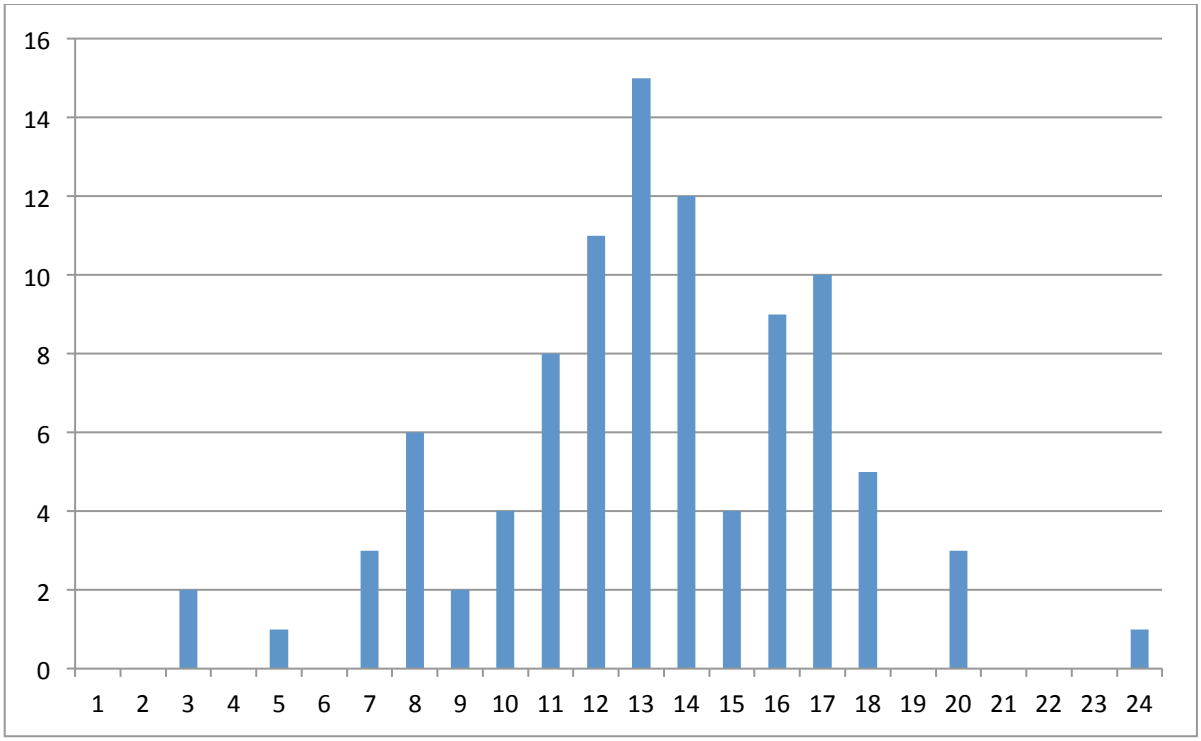


Figure 5.1 Frequency histogram of age of onset of the familial JME cases. The x axis is the age of first seizure and the y the number of cases with the age of onset.

All patients with JME reported myoclonic seizures. In twenty cases (20.4%) there was evidence of a negative myoclonus alongside the positive myoclonic jerks. These were predominantly in the upper limbs and caused people to lose tone and sag and drop objects rather than the positive myoclonus which may rapidly propel their arms apart – upwards and with a small degree of external rotation – causing objects (if held) to be flung. If the negative myoclonus occurred in the lower limbs it caused falls. Despite not being specifically enquired about, 4 people spontaneously reported falls as a core phenotype in their JME; all had negative myoclonic jerks. 34 people (34.7%) specifically mentioned a morning

preponderance of myoclonic jerks. Six of the fifteen people (40%) who noted that their GTCS were mostly in the mornings did not report a propensity towards morning myoclonus.

5.2.2 Absences and GTCS

Thirty six people described episodes which are in keeping with absence seizures. Of these. 4 out of 36 exclusively had their 'absence' associated with myoclonus and so these are not typical of absence seizures. This equates to a prevalence of absence seizures in familial JME of 32.7%. Of these, only nine (28% of those with absences) reported them to be daily at any stage and seven (22%) had them at least monthly but not as frequently as daily. Generalised tonic clonic seizures were reported in 86 (87.8%, 79 with frequency data) – but on the whole were not frequent. 48 (60.8%) had infrequent GTCS (never as frequently as monthly). No cases ever reported GTCS weekly or more frequently.

5.2.3 EEG

84 of the 98 provided EEG data for analysis (86%). Seventy of the cohort had information documented for activation responses such as the photoparoxysmal response (PPR) and hyperventilation. The activation response data are analysed on the presumption that they were performed on all individuals and 'no record' in this context is synonymous with no abnormality being identified. Forty people had a PPR in keeping with photosensitivity (47.6%) and a further seventeen more (20.2%) had a clear response to hyperventilation but no PPR response. In eleven cases (13.1%) the best EEG that could be provided yielded no abnormality. The traces were reported by a variety of clinicians and so the terminologies used to describe the abnormalities seen in the vast majority of tests are disparate. Despite this there was a great deal of support towards the median abnormalities being generalised spike and wave discharges at 3 to 5 hertz with polyspikes frequently seen (32% of cases, table 5.1).

Frequency (hz)	Number of reports	%
2	1	4
2-4	1	4
2.5-3	1	4
3	4	16
3-4	2	8
3-5	8	32
3-6	1	4
4	1	4
4-5	1	4
4-6	2	8
4-7	1	4
5-7	2	8

Table 5.1 EEG activity from 25 EEG which recoded the frequency of the epileptiform abnormalities.

5.2.4 JME subgroups

Martınez-Juarez *et al.'s* (2006) subdivision of JME in Latin American families in their Brain paper used four major groupings: classical JME, CAE evolving into JME, JME with adolescent absence and JME with astatic seizures. They defined CAE-JME as absence seizures starting at 11 or younger. They used the archaic term 'pyknoleptic' to describe the high frequency of CAE absences - and 'spanioleptic' to mean the less frequent absence seizures of teenage years. Therefore individuals in their subgroup of 'classic JME' were permitted to have infrequent absences but if this seizure type was frequent – they were deemed to have JME with absences. The table (5.2) below demonstrates how the St George's cohort would fare under these subheadings.

JME subtype	Latin American %	St George's cohort	%
Classic JME	72	82	83.6
CAE-JME	18	5	5.1
Absences and JME	7	6	6.1
Astatic seizures	3	5	5.1

Table 5.2 Comparison between the Martınez-Juarez (2006) JME subgroups and the St George's family data

5.2.4.1 'Classic JME'

With regards to the classic JME group they report a mean age of onset of 15.1 years and a range between 7 to 28 years. Whilst I agree that this is in keeping with JME several features are atypical enough that members of this 'classic' group would not be deemed to have JME by the forthcoming consensus guidelines. Firstly an age of onset between 10 and 25 is needed and secondly no attempt to look at IQ is made. In my 'classic group' (table 5.2) are nine who would be too young for this definition and two with learning difficulties (together comprising 11.2%). The mean age of onset in my cohort is 13.7 (range 7 to 24). 74/82 of this group have EEG data available and 32 (43%) are photosensitive – this is remarkably different to the 13% rate reported by Martınez-Juarez *et al*.

5.2.4.2 CAE to JME

The first thing to state is that the Martınez-Juarez analysis has a number of advantages over this one: their sample size is larger (257 individuals vs. 98) and their prospective collection model permits routine and standardise EEG in all individuals. It is therefore possible that the smaller number in the CAE-JME group seen here is an under ascertainment. However the scale of difference is such that it is likely to be a true difference. The 'CAE evolving to JME' group represented 46/257 of their group and 5/98 of ours, ($p<0.002$). This is balanced out therefore by the higher proportion of individuals classified as having 'classic JME'. This group representing 185/257 compared with 82/98 in our study ($p< 0.02$). They report an age of onset of 5 years (range 1 to 10) whereas ours is 6 years (3 to 10). 4/5 had abnormal EEGs and all of these four were also photosensitive – in stark contrast to the 22% reported by

Martınez-Juarez *et al*. Although this was a common group for them – they were all sourced from five families suggesting a bias from a small number of atypically presenting kindreds. I have the sex for 25 people: 18 are female (72%, 2.5F:1M); the Martınez-Juarez *et al*. ratio was 1.25F:1M.

5.2.4.3 JME with frequent adolescent onset absences

In this group I report a mean age of onset of 14 (range 11 to 17) whereas the Martınez-Juarez *et al*. team report an older age of onset (16 years) no doubt skewed by some very much older onset cases (range 11 to 32 years). They report that 15% of their subgroup were photosensitive whereas 2/6 (33%) of the St George's group was – and the remaining four all having a dramatic response to hyperventilation. I have the sex of three of this group (all female); the Martınez-Juarez *et al*. ratio was 2.6F:1M

5.2.4.4 Astatic seizures

As I have previously mentioned a minority of the St George's cohort reported dramatic and injurious falls (rather than retropulsion with jerks) – although 20% suffered from negative myoclonus. The Martınez-Juarez *et al*. age of onset here was 14.2 years (range 8 to 19) whereas ours is younger at 12.4 years (10 to 14). PPR was infrequently seen in their group (25%) and is positive in 20% of our cases. They say absences are rare – our series had them as a feature in 2/5. I have the sex for 4 people: 3 are female; the Martınez-Juarez *et al*. ratio was 1.2F:1M.

5.2.5 New groups

Although I laud the work that enabled the identification of these subgroups I have a number of concerns regarding their robustness. Firstly they have merely thinly sliced a few patients from the whole by identifying small subgroups such as those with astatic seizures and the occasional large family with CAE evolving to JME. Secondly they have left a large and heterogeneous miscellaneous group 'classic JME' which is so diverse that many within it

would not conform to the case definition in the forthcoming consensus guidelines. Thirdly each of the subgroups retain heterogeneity in terms of very wide ages of onset and response to photostimulation. I do not believe that they suit the St George's cohort any better than they suit the Martınez-Juarez *et al*. collection and therefore I suggest a few additional ways of subdividing the collection.

Photosensitive JME If the forty individuals with a clear PPR were taken to represent a subgroup then it has a broad age of onset including both the youngest (3) and oldest (24) ages of onset. Both early morning myoclonus and negative myoclonus are equally distributed between the PPR and no-PPR groups.

Age of onset The age of first seizure type (febrile convulsions excluded) is perhaps a more attractive method of creating subgroups. Arbitrarily subgrouping ages 3-7; 8 to 10; 11 to 15; 16 to 18; and 18 plus; produces some groups which appear useful. For example for six the of seven in the first group who had EEGs, all had prominent photosensitivity. They also all had absence seizures and all had GTCS. However it hangs together less well for the other age groups (table 5.3 below).

Age of onset	PPR	GTCS	Absences	Negative myoclonus	Morning pattern
3-7	100%	100%	100%	0%	17%
8-10	50%	83%	42%	17%	50%
11-15	50%	90%	16%	34%	36%
16-18	35%	67%	21%	17%	29%
18+	25%	100%	25%	0%	50%

Table 5.3 Basic clinical features of familial JME by age groups

There are however a number of patterns that emerge. Photosensitivity clearly is a more prevalent feature of younger onset JME and the frequency of this trait reduces as the age of onset of the first seizure increases. Similarly there is a sharp drop off in the frequency as absence seizures as age of onset increase. There is however the suspicion of a 'U' shaped curve with the lowest frequency seen at 11-15 and rising again a little towards older

adolescence. Negative myoclonus appears to be the exclusive feature of pubertal (classic) JME.

Negative Myoclonus The mean age of seizure onset for this group was 13.45 years (10 to 17) and only 2 (10%) had frequent absences – 25% reporting any absence seizures at all. A low proportion of this cohort (36.8%) had a PPR response on EEG and only 25% had a clear morning preponderance to their myoclonus. 17 (85%) had GTCS which were only frequent in 10%. This feature therefore does not identify a homogenous subgroup.

Learning difficulties Two of the 98 were identified as having LD. One had seizure onset at 8 years, the other at 14. Both had a morning predominance to their seizure type with one also having negative myoclonus. Both had GTCS and only one had a PPR (the same patient also having absence seizures). It is difficult to identify a subgroup with only two members.

5.2.6 Summary

Producing robust subgroups using seizure and EEG features alone is not straightforward for familial JME. It may be possible to use other non-seizure clinical features to aid subgrouping (chapter six) or it may be that the polygenic nature of JME means that the juvenile myoclonic epilepsies are as broad in their presentation as any electroclinical syndrome can be. It may however give a greater clue towards the genetic nature of JME. Genes that produce a broad neurological phenotype may include those like *EFHC1* that appear to affect neuronal migration, neuronal development or channel subunit genes such as *GABRA1* and *CACNA1* which may affect synaptic clustering of channels. Both of these mechanisms (or as likely both mechanisms concurrently) would produce a broad spectrum of cortical hyperexcitability.

5.3 Sporadic JME

5.3.1 Cardiff – 1990s

In contrast to the collection above I had access to a cohort of seventy-nine sequential patients with JME who presented to the University Hospital of Wales epilepsy clinic in the mid 1990s. Interestingly there is a sex bias to this cohort; 29 are male (1.6F, 1M). Although age of onset is not collected, EEG data and response to drugs are. 74 had an inter-ictal EEG recorded (94%) and of these 10 were normal records (13.5%) and a further five (6.8%) were abnormal but not sufficiently so to support a diagnosis of JME. There was no significant difference when comparing the proportion of people with familial JME and normal EEGs to those with sporadic JME and normal EEGs. 63 had their PPR response recorded: 38 had a clear positive response (60.3%), one was equivocal and only 24 had no response (38.1%). When compared to the family samples above (PPR in families 40/84 vs. sporadic 38/63) there is no significant difference.

Nine knew that they had a family history of epilepsy (11.4%), with 4 (5.1%) having other people with JME in their family. Reflecting the drug therapy of the time and the current understanding of JME, valproate therapy dominates. Of the 73 with drug information recorded 60 (82.2%) were on either valproate mono or polytherapy. 12 were taking two antiepileptic drugs (16.4%) and three (4.1%) had elected not to be on medication. This limited analysis therefore fails to identify greater variability between sporadic and familial JME than there exists within 'classic JME' for example.

5.3.2 Wales 2010s – psychology cohort

The thirty-nine individuals who were recruited to the psychology / ReJUMeC project were predominantly female (8 males) and right handed (all bar three). Figure 5.2 demonstrates the spread of ages at interview – mean age was 32. 4 years. Seven had been well controlled (or poorly advised) and had held a UK driving license at one stage in their life; none currently were eligible to drive under UK driving law. Of the 28 people who gave me their profession eight were unemployed (28.6%) but others were still working – such as two teachers, a police constable, a NHS manager and a civil servant. 22 (out of 37) reported a family history

of epilepsy (59.5%). Of these eight knew it was on their mother's side and seven knew it came from the father's side.

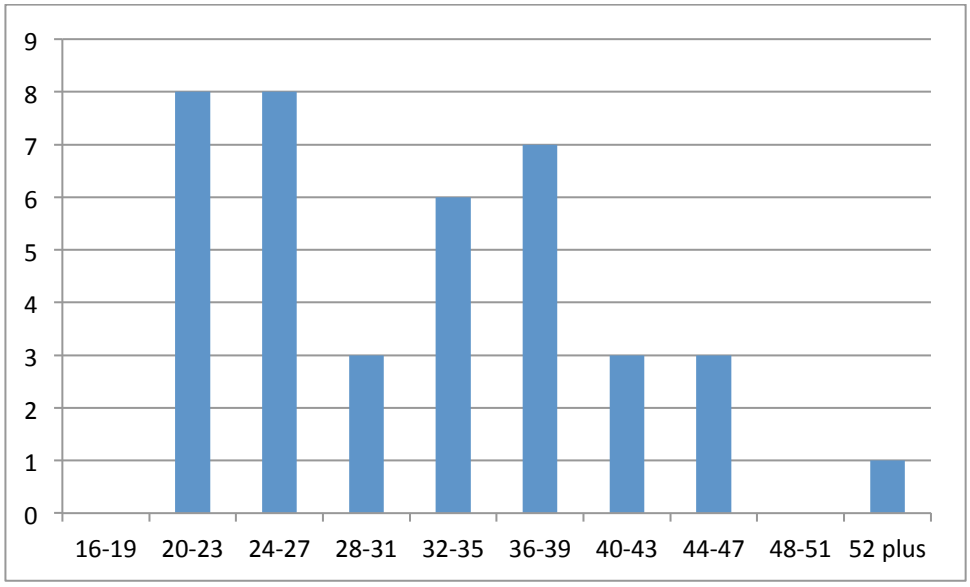


Figure 5.2 Age at time of interview of participants to psychology project.

Only two people reported a family history of febrile seizures (5.1%) and only one a personal history of febrile seizures. Seven people reported having had a psychiatric history to report – all were low mood disorders. The age of diagnosis was provided and ranged from e to 20 years of age (figure 5.3). The distribution of the participants'' age at diagnosis is shown in figure 5.3 Figure 5.3 initially appears to be a Gaussian curve (just as figure 5.1 did) however although they share a peak at 13 – they also suggest a second peak at 18 year of age. This could (again) be due to delayed diagnosis and recognition of jerks epileptic – or perhaps a subtype of JME worth teasing out. The mean age of diagnosis in this cohort is 14.1 years. People had had their seizures for between 3 and 54 years with the mean duration of epilepsy of 18.6 years.

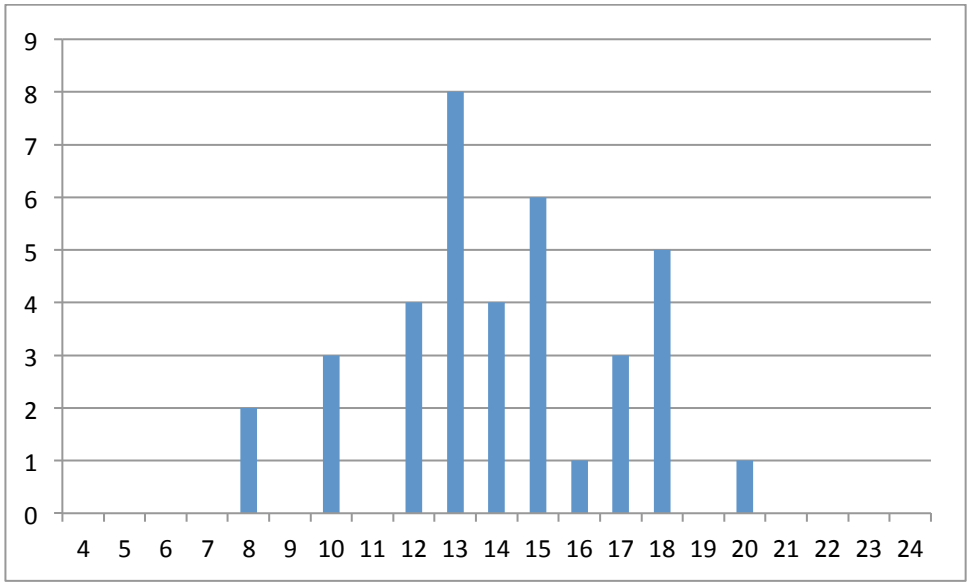


Figure 5.3 Histogram demonstrating the age of diagnosis of each participant

5.3.2.1 Seizure types

There was a high proportion of people with absence seizures within this cohort (70%) and 55% (whom provided a frequency of these) had very frequent absences at one stage (weekly or more). There appeared to be none with CAE that evolved into JME within this cohort. 95.6% of participants had experienced at least one GTCS. Mostly these were infrequent and the participants could remember each one individually. However one (JM22) claimed to be having up to ten a week at one stage and six more had GTCS monthly or more frequently at one stage (20% of those who had GTCS). When asked (without leading them) as to whether there was a time of day that was more likely for their GTCS only two spontaneously said 'mornings'. The mean age of first GTCS was 13.3 (range 8 to 18). This is just younger than the mean age of JME diagnosis which may represent the oft reported delay in diagnosis of JME or (as likely) the fact that with infrequent GTCS a single seizure would be insufficient to diagnose epilepsy let alone JME.

All participants reported myoclonus. Of the 24 who reported a preference for which time of day it was more prominent, 16 (66.7%) stated it was the mornings. The rest either reported no pattern or myoclonus worst in the evenings. The mean age of onset was 16 years (range 4 to 27 years). 39.3% reported a clinical history of photosensitivity – with some reporting

sensitivity to patterns also. Inquiry about negative myoclonus was incomplete in this cohort – however four spontaneously reported it and a further four reported falls that caused dental injury (six in total, 15.4% of the cohort). A further five had suffered a facial injury as a result of their seizures. Eight (20.5%) suffered from insomnia and thirteen (33.3%) reported that they experienced parasomnias. None admitted to significant alcohol or substance misuse.

5.3.2.2 Medication

There were a large number of AED combinations - but at the time of interview 74.4% were on monotherapy (figure 5.4). Thirteen of these were on sodium valproate alone (44.8% of all monotherapy prescriptions), seven were on lamotrigine (24%), five were levetiracetam (17.3%) and two took zonisamide monotherapy and two topiramate monotherapy (6.9%). Levetiracetam was a component of 80% of the polytherapy prescriptions and clobazam 60%.

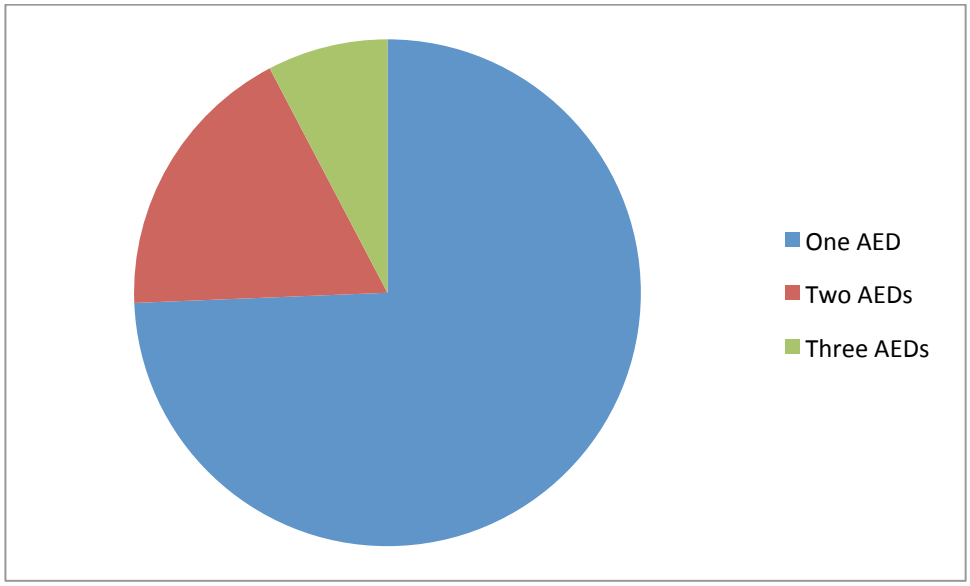


Figure 5.4 The majority of people interviewed were taking just one antiepileptic drug

Eight (20.5%) had previously tried carbamazepine – none were currently taking it. Eleven (28.2%) had tried lamotrigine without any success and a number reported myoclonic seizures were exacerbated with it; eleven others were still taking lamotrigine either singly or in combination. Six people had tried levetiracteam previously – one discontinued due to

drowsiness but found it efficacious for controlling the myoclonus. No one reported a hypersensitivity reaction to any anti-epileptic drugs.

5.4 GEFS+ and JME: The cuckoo in the nest

5.4.1 Introduction

As demonstrated above there is a great deal of heterogeneity within 'JME families' which identifies CAE, JAE and other IGEs as likely epilepsy syndromes in these families. The most well-known familial epilepsy syndrome, however, is called genetic epilepsy with febrile seizures plus. Although defined by occasional family members having Dravet syndrome and the preponderance of febrile seizures it demonstrates a great deal of heterogeneity. Below I describe the process of identifying GEFS+ families in Wales and the identification of a subgroup – borderline GEFS+. Of note is that borderline GEFS+ more frequently contains the 'cuckoo in the nest' in the form of a person with a defined electroclinical syndrome – such as JME.

5.4.2 Results

5.4.2.1 Characteristics of the families

Of the first 80 families, 29 were recruited via neurologists, 19 by paediatricians and 17 were self-referrals, seven from epilepsy nurse specialists, four through clinical databases, three from the learning disability service and one from clinical genetics. Fourteen families were classified as classical GEFS+ and ten as borderline (table 5.4.) In eight families, GEFS+ was considered unlikely and the epilepsy phenotype was unclassifiable; forty-eight had another specific syndromal diagnosis. Borderline GEFS+ families had a greater mix of epilepsy diagnoses (including electroclinical syndromes such as juvenile myoclonic epilepsy and focal epilepsies such as temporal lobe epilepsy) than those with definite GEFS+. Idiopathic generalised epilepsy comprised 80% of diagnoses that persisted into adulthood in definite

GEFS+, but only 60% for borderline families (p<0.01) – where there were twice as many adults with focal epilepsies.

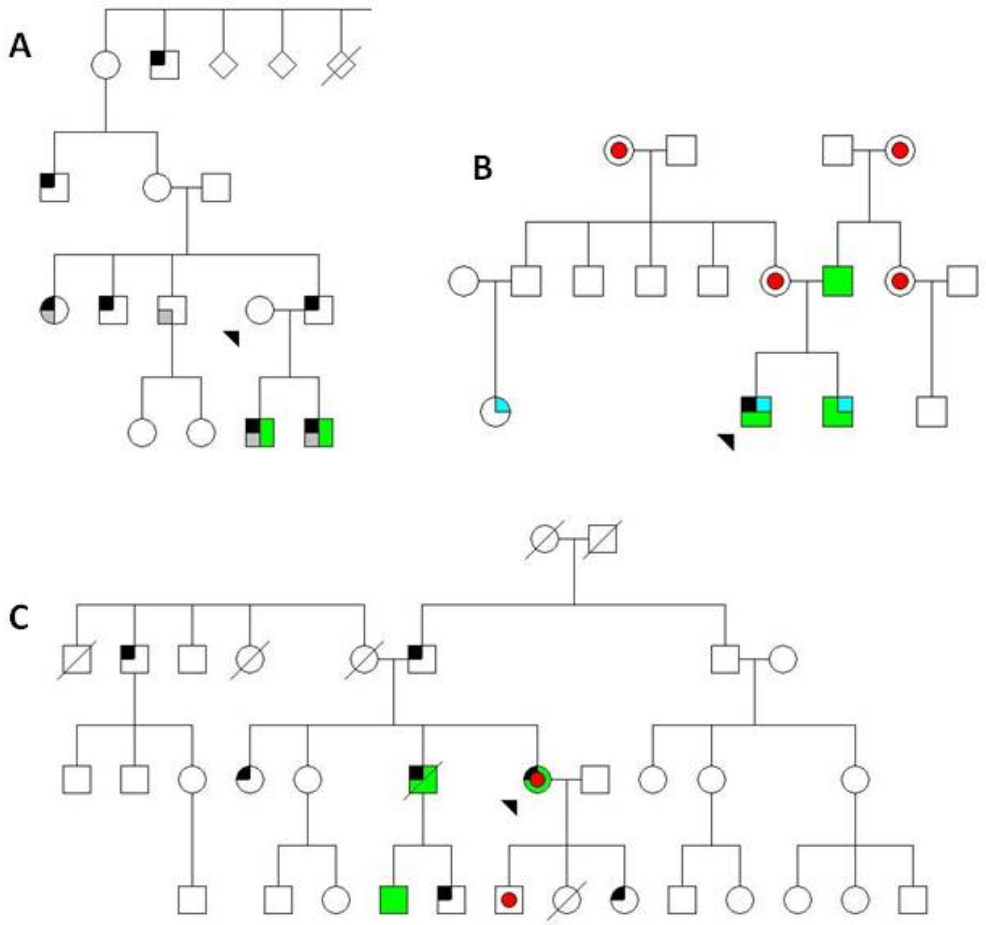


Figure 5.5: **GEFS+ Pedigrees.** Pedigree A GEFS+, B and C Borderline GEFS+. FS (top left quadrant black), AFS (Bottom left quadrant grey), GTCS as an adult (green), migraine (red central circle), learning difficulties (top right quadrant blue).

5.4.2.2 Individual characteristics

Classical GEFS+ families - 120 individuals had seizures: 77 had FS (64.2%) – of these 7 also had AFS (5.8%) and 18 later had adult GTCS (1.5%); 23 had AFS (19.2%) – five later going on to have adult GTCS (4.2%); 22.5% (27 people) had adult GTCS with no preceding febrile seizures.

Borderline GEFS+ families - 83 people reported seizures: 43 had FS (51.8%), three with both FA and AFS (3.6%) and 9 later having adult GTCS (10.8%); eleven people had AFS (13.3%) – three of whom later developed GTCS (3.6%); 27 people (32.5%) had adult onset GTCS only.

Unclassified familial epilepsy - 80 had seizures or epilepsy: 20 had FS (25%) – 3 (3.8%) had both FS and AFS and five later had adult GTCS (6.3%); thirteen has AFS (16.25%) - four of whom (5%) later had adult GTCS; 45 (56.3%) had adult generalised tonic-clonic seizures only.

	Classical	**Borderline**	**Unclassified**	**Other diagnoses**
FS	Y	Y	Y	Y/N
FS+	Y	Y	Y /N	N
Adult epilepsy - IGE	++	+	+/-	+/-
Characteristic seizure type	FS+	FS+	GTCS without prior FS	
Migraine	+	++	+	+
Electroclinical syndromes	+/-	+	+	++
Most common seizure type	FS	FS	GTCS	Other

Table 5.4 The characteristic seizure type is the most distinctive event that may help differentiate the subtype, whereupon the most common seizure is the most frequently occurring.

Febrile seizures were reported twice as often in classical GEFS+ or borderline GEFS+ families ($p<0.005$) than in those with unclassified epilepsies. Febrile seizures plus (FS+) were rare but occurred disproportionately in classical and borderline GEFS+ families, accounting for 24% and 19% of febrile seizures respectively. There was also a trend towards typical febrile seizures occurring earlier in GEFS+ families. No family included any member with SMEI or a similar epileptic encephalopathy. Thirty-five people had learning difficulties (thirty-three having a clinical diagnosis of epilepsy); there was no increased likelihood of learning

difficulty across any of the four diagnostic groups. The prevalence of migraine was 12%, similar to the population estimate. However, migraine prevalence was significantly higher in the borderline GEFS+ group, with 25% of those with epilepsy also having migraine. The migraine prevalence in the classical GEFS+ group was 8.2% (classical vs. borderline groups $p<0.01$) and was 7.1% in the unlikely GEFS+ group (borderline vs. unclassified $p<0.03$). The migraine described was mostly without aura, but migraine with aura and rarely hemiplegic migraine was also seen.

5.5 Summary

The heterogeneity within the clinical description in the introduction has been dealt with by the epilepsy community by producing an expert-led consensus guideline for the case definition of JME. This self-selecting group of experts met in a closed workshop in Avingnon last year and presented their unanimous definition to the 2012 International Conference on JME at The Hague. These guidelines are due to be published at the same time as this thesis. They include some uncontroversial statements i) must have myoclonic jerks, ii) neuroimaging is normal and not necessary for the diagnosis; however I would counsel further discussion on a number of points.

1. The personal belief of the panel of experts was that JME beings at puberty and true onset in the twenties is rare. Data presented above does not fully support this. It is telling that the consensus therefore agreed to a window as large as 10 to 25 years for ‘type II JME’. The age of onset histograms instead hint towards two or three populations with a peak at 8, a peak at 13 and perhaps a third at 18 years.

2. They insist that a diagnostic EEG is needed for the diagnosis. This I believe is biased against the UK and other health systems where it is more customary to treat after the second seizure – than wait for a diagnostic EEG. UK practice believes that seizures are unpleasant, that EEG has its faults in terms of ‘over diagnosis’ and that JME is primarily a clinical diagnosis. The counter argument is that an EEG within a week and a sleep deprived EEG a week after does not provide an undue delay and

perhaps reflects the greater personal experience that Continental epileptologists have in performing and interpreting EEG. In the St. George's cohort 13.1% had a normal EEG and 13.5% in the Cardiff collection from the 1990s.

3. They insist that a 'normal IQ' is needed - despite the panel of experts presenting a video-EEG case of JME in someone with learning difficulties. It is my belief that IQ is not formally tested by most epileptologists and that they are being overly optimistic when estimating their patients' IQ. Evidence provided in chapter six will clearly demonstrate that many people who went through standard UK schooling are performing at the level of people with learning difficulties. Within the JME families presented here 2% had 'classic JME' but also had LD.

4. The consensus guidelines have ended up with a 'JME I' or classic JME and a 'JME II' or a borderline JME. Unfortunately they have left room for a 'JME III' in all the UK patients who 'behave' like they have JME but do not meet their criteria. I do not believe that there are sufficient environmental, sociological nor genetic factors to expect UK patients to differ from those of North Western continental Europe. It is by studying the outliers and being aware of the limits of classifications that we can understand more about the epilepsies. It is therefore that I suggest a borderline GEFS+ familial phenotype recognised by migraine and individuals with electroclinical syndromes such as JME.

The following chapter will describe the neuropsychological data linked to the Welsh 2010s JME cohort described here and further discuss subcategorisation of JME.

Chapter Six

JME Neuropsychometry

The neuropsychological phenotypes of Juvenile Myoclonic Epilepsy

6.1 Introduction

Chapter four described the use of clinical characteristics do determine genotype-phenotype correlations in a predominantly monogenetic disorder and chapter five looked at their utility in a polygenetic disorder. Chapter six examines which clinical tools can be used to identify phenotypic differences in a polygenetic disorder. In this chapter I present the data from neuropsychological interviews of thirty-nine people with drug refractory JME. As well as presenting data from standard neuropsychological tests I present data from tests that are not commonly employed in epilepsy. I end this chapter by identifying subgroups within the cases presented.

6.1.1 Aims

1. Describe the cognitive, memory and dysexecutive function of people with drug refractory JME
2. Use neuropsychological tools that have not been previously utilised in the study of people with JME; demonstrate that they are sensitive enough to identify impairments. Specifically these are the BADS, elements of the D-KEFS, TYM and EPQ-BV.
3. Compare drug resistant JME to previously described cohorts of drug sensitive JME and other epilepsies in terms of cognition and executive function
4. Identify subgroups where they may exist

6.1.2 Hypotheses

1. JME does exist in the context of lower IQ: this might represent a robust subphenotype

2. JME is thought to be associated with executive function deficits; can this be purely explained by attentional deficits producing a more global pattern of deficits?
3. People with JME will score in the impaired range on the additional BADS and DKEFS scales
4. Test Your Memory can be used to identify people who score less well on the WAIS and WMS in people with epilepsy
5. Some people with epilepsy will score in the significantly impaired range on the TYM scale
6. There will be high levels of neuroticism on the EPQ; some will score highly for extrovertism
7. People with drug resistant JME will score less well on tests of cognition, memory and executive function than people with drug sensitive JME
8. JME is clinically heterogeneous; I predict there will be significant variation in neuropsychological performance between individuals

I will begin by discussing how the participants were recruited and then describe the results of the tests by i) comparison with standardised means and ii) comparison between the performance of the people studied. The clinical and demographic characteristics were included in the previous chapter where they were compared with data gleaned from other sources.

6.2 Recruitment

189 cases were identified from the database who were coded as i) JME and ii) seizures not yet under control. 35 names were identified by a neuroimaging project. An additional 16 cases were identified prospectively from paediatric and adult specialist epilepsy clinics. All were written to and there were 60 replies volunteering to take part in the project; one was found to be ineligible once she had attended but another ten then failed to reply, answer their phone, changed their number, or did not attend testing on two or more occasions. Of the 39 who did attend the psychological assessment there was a failure to attend without

previously cancelling the appointment rate of between 60 and 70%. This was not improved by a phone call to the participant the previous day – or on the morning of the testing. This pattern was reproduced when trying to request that questionnaires were posted back for scoring.

6.2.1 Interviews

The interviews were well tolerated by the participants. Only one volunteer (JM14) needed testing split over two sessions because of cognitive fatigue and a dip in concentration. The interviews were held in ideal environments, as planned, with not even a computer in the room for noise. Ample breaks were needed to retain concentration and as such the whole session on occasion lasted over five hours. Only one participant (JM25) had a seizure that I could recognise (a typical absence seizure) and no myoclonus was seen. JM25 was given time to recover from the seizure and her partner helped me know when to restart testing. I presented her with an optional test from the WMS that should be affected by processing speed and working memory deficits; she scored in the normal range and therefore I continued testing. Her scores before and after the absence were very similar.

6.3 Results

6.3.1 Descriptive statistics

To identify which statistics could be used to analyse the data it was important to see whether the results followed the normal distribution. The table below (table 6.1) provides not only the means, SE and SD but also the skewness statistic; the smaller the skewness statistic – the more normally distributed the data. Histograms of both PIQ (figure 6.1) and digit-symbol coding (figure 6.2) are shown to illustrate the differences between normally distributed and skewed sample data. The histogram in figure 6.3 shows a more typical rightwards skew on the auditory immediate test. A positive skewness statistic indicates that the tail on the right is longer than that on the left and as such the bulk of the values lie to the left of the mean.

	Range	Min	Max	Mean		Std. Deviation	Variance	Skewness	
				Statistic	Std. Error			Statistic	Std. Error
V IQ	69	65	134	96.21	2.695	16.8	283.325	.191	.378
P IQ	56	69	125	97.28	2.458	15.4	235.629	-.002	.378
FS IQ	67	64	131	96.21	2.618	16.4	267.378	-.193	.378
Auditory Immediate	77	65	142	99.43	2.265	14.1	200.200	.523	.378
Immediate Memory	59	69	128	96.53	2.165	13.5	182.887	.151	.378
Auditory Delayed	61	71	132	104.00	1.976	12.3	152.263	-.233	.378
Visual Delayed	61	68	129	95.07	2.014	12.4	154.237	.127	.383
Auditory Recognition Delayed	55	75	130	102.17	2.202	13.8	189.204	.301	.378
General Memory	54	70	124	100.26	2.020	12.5	155.172	-.117	.383
Working Memory - WMS	61	63	124	95.69	2.296	14.3	205.692	-.418	.378

Table 6.1. Descriptive statistics covering the major indices of the WAIS and WMS.

Comparing the median and mean to look for skewedness of data; all scaled scores (WMS and WAIS) were within 0.5 of a point of each other – except digit symbol coding, blocks, matrix, symbol search, verbal pairs 1, verbal pairs 2 and faces 2 which were within a point of each other. Coding however exhibited the most extreme difference of 1.36 (mean 8.36, median 7) and a skewness statistic of .730 – the histogram below demonstrates how poorly it fits the normal distribution. In general however all data were decided to sufficiently follow a normal distribution to permit this assumption for data analysis.

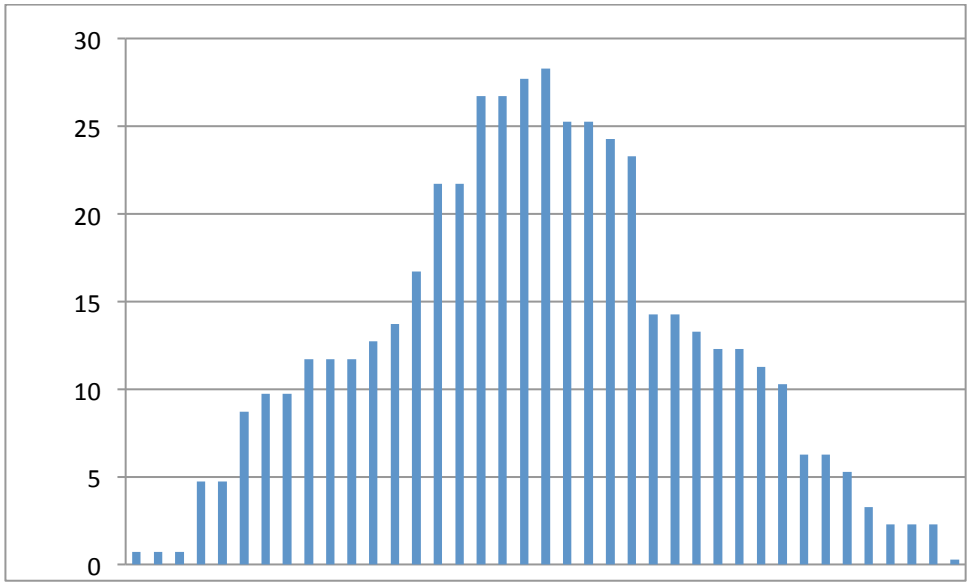


Figure 6.1. Performance IQ histogram –the smallest skewed statistic and therefore most normally distributed.

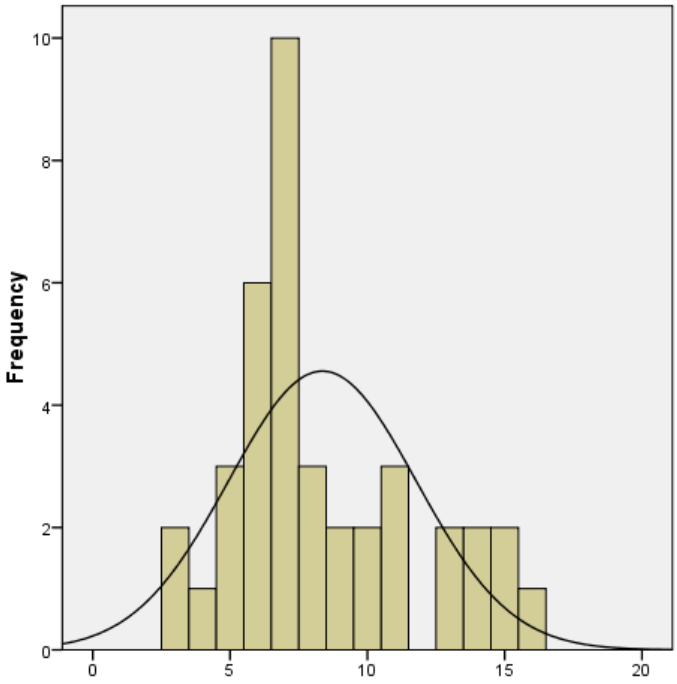


Figure 6.2. Digit symbol coding has an excess of people scoring between 5 and 10 on the scales score. It shows a clear rightwards skew.

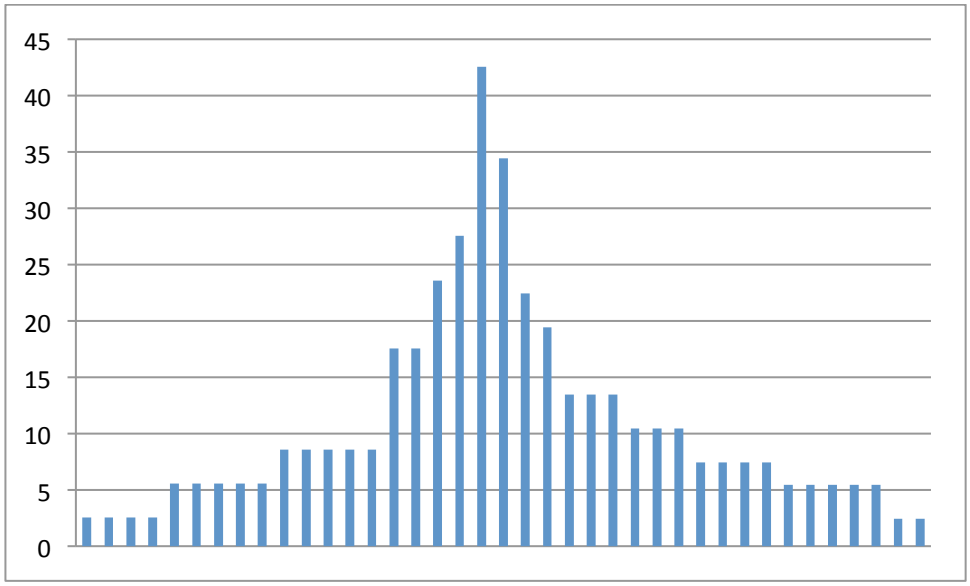


Figure 6.3. Auditory immediate Histogram demonstrating right skew of data (skewness statistic of .523; rightwards skew).

6.3.2 WAIS

	Minimum	Maximum	Mean	Std. Deviation	p=
Verbal IQ	65	134	96.21	16.8	0.1224
Performance IQ	69	125	97.28	15.4	0.2649
Full Scale IQ	64	131	96.21	16.4	0.1224
Working Memory	55	128	95.95	17.6	0.0999
Processing Speed	63	137	94.08	17.2	0.0217*

Table 6.2. Index scores, statistics and significance levels for the WAIS. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01.

Performance across the WAIS was generally strong with mean scaled scores between 8 and 11 for sub tests and between 94 and 98 for the metastatistics (table 6.2 and figure 6.4). This is as expected as JME has always been described as an epilepsy associated with normal learning abilities.

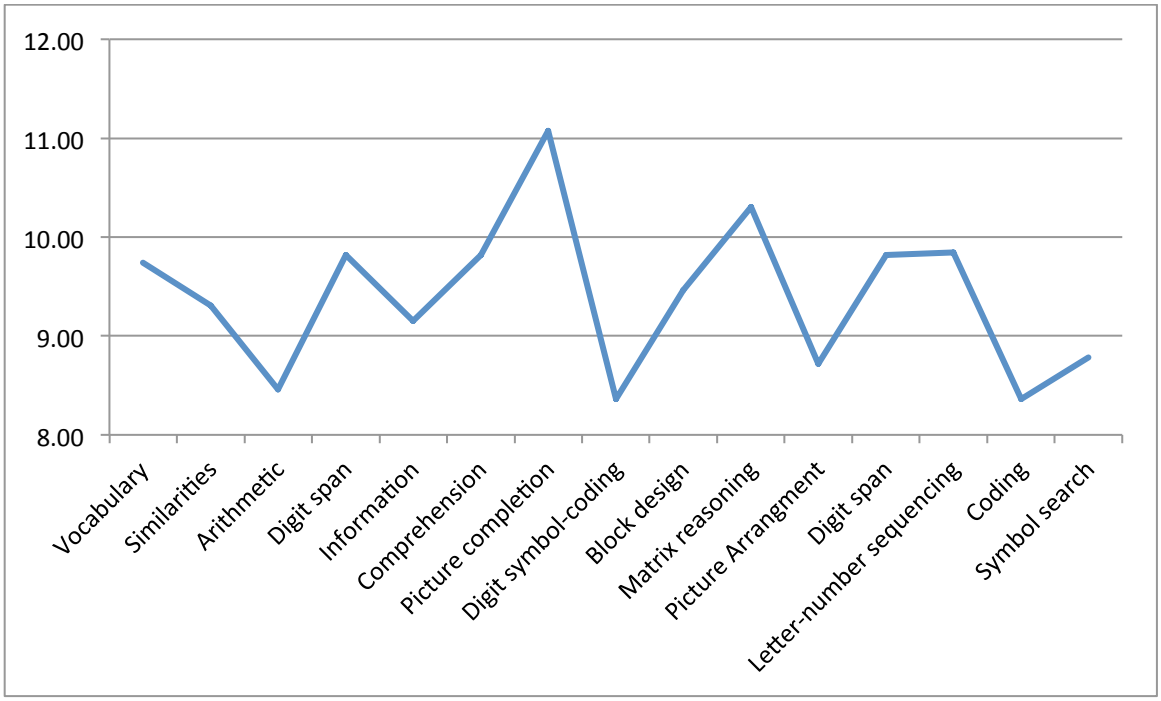


Figure 6.4 Scaled scores of the WAIS mostly falling below the mean score of ten. It can be clearly seen that some VIQ subtests (first six) and some PIQ subtests (next five) were answered well and that there was not a clear pattern of dominance in either the verbal or perceptual domains.

The table of index scores for the WAIS (table 6.2) are given above. None were significantly different from the normalised performance at the level of the alpha statistic, 0.01. Within the aggregated index scores however there were a number of subtests that were answered less well by people with JME. These included arithmetic (VIQ subtest, mean scaled score 8.46, $p<0.003$). This was apparent to me whilst performing the tests. Anecdotally participants had difficulty with auditory processing of the information. They either i) mis-heard the question, ii) could not immediately recall it, iii) repeated the question to themselves and in doing so mis-remembered the numbers or iv) spent so long trying to remember the numbers that they forgot the calculation that was needed to find the right answer. Most participants asked for a pen and paper (not permitted in this test).

The single most demonstrative metastatic is arguably the FSIQ; with GM perhaps a close second. The histogram below (figure 6.5) demonstrates the variation of FSIQ about the sample mean (96.2). Four people had a FSIQ one SD below the mean (JM35, JM24, JM31 and JM5) and a further four had FSIQs two SD below (JME29, JM14, JM3 and JM23). There were however a good number of high scoring individuals too: JM15, JM34 and JM9 all scored over one SD above the mean and JM18 scored above two SDs from the mean.

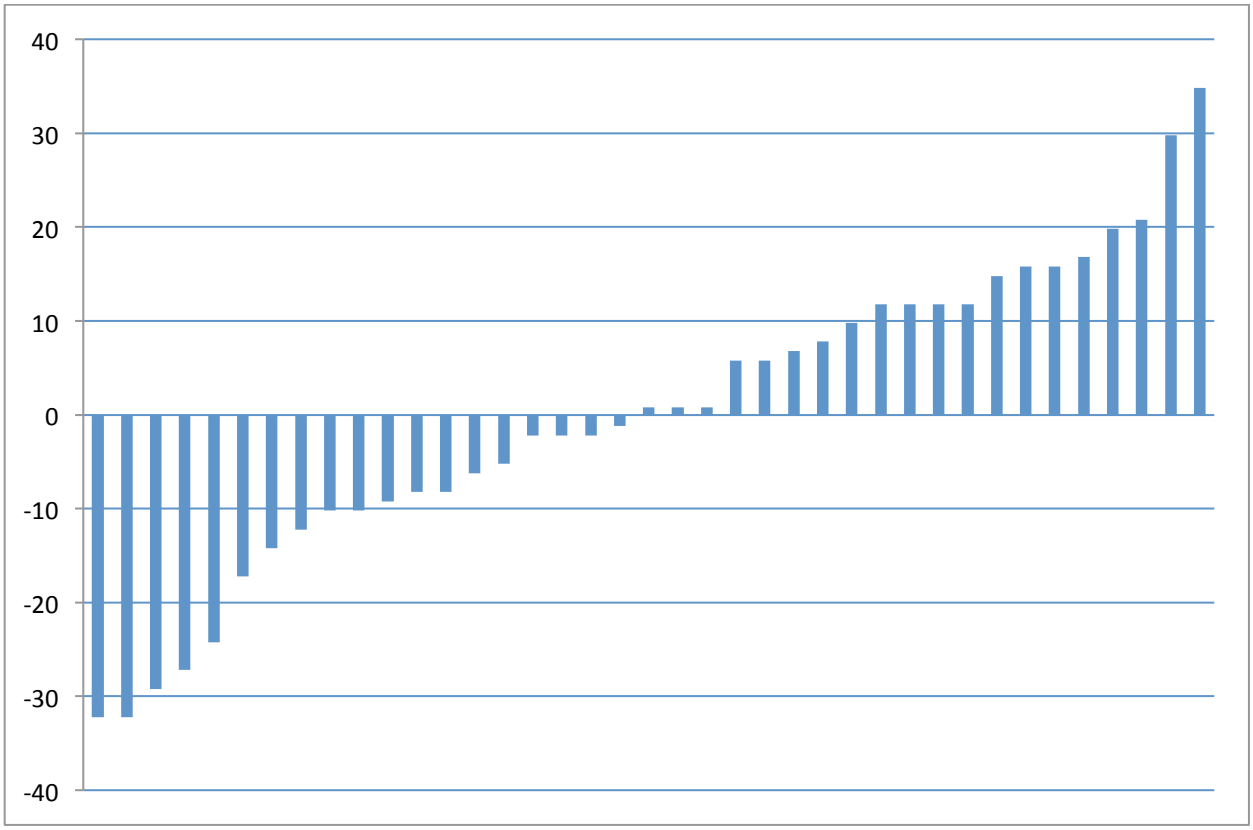


Figure 6.5 Histogram demonstrating variation from mean full scale IQ (96.2). Difference from mean Full Scale IQ (y axis – ordered by said difference, x axis is difference from FSIQ). The poorest performing participants are far left.

6.3.2.1 Verbal IQ

Table 6.3 demonstrates the subtests comprising VIQ and as discussed above, arithmetic was performed particularly poorly. Mathematics is considered to be a frontal lobe function and broadly comprises two areas: automatic (i.e. requires retrieval from long-term memory) and method-based (i.e. requires calculation) processing. The stark difference between the performance on arithmetic and the other VIQ tests suggests that this subtest is also scoring people on performance of an unrelated cognitive function. Table 6.3 also shows a common theme - that there are many people scoring in the supra-normal range (maximum scores of 17, 18) but also those scoring particularly poorly (minimum scores of 2 and 3).

	Minimum	Maximum	Mean	Std. Deviation	p=
Vocabulary	3	18	9.74	3.385	0.5966
Similarities	3	16	9.31	3.435	0.1577
Arithmetic	2	17	8.46	3.560	0.0028**
Digit Span	3	15	9.82	2.761	0.7108
Information	5	16	9.15	3.091	0.0862
Comprehension	3	17	9.82	3.698	0.7108

Table 6.3. Subtest scores for verbal IQ. . * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

6.3.2.2 Performance IQ

Within PIQ there were a number of sub scales that were answered less well by participants (figure 6.4, table 6.4). The first of these was digit-symbol coding: the mean scaled score was 8.36, $p<0.0015$. The second of these was picture arrangement (which did not quite reach significance at the alpha level); the mean scaled score was 8.72 and the $p<0.011$. Picture completion was scored a little better than average with a mean score of 11.08 and a $p<0.031$. This test did appear to be completed without error by the majority of participants on the day.

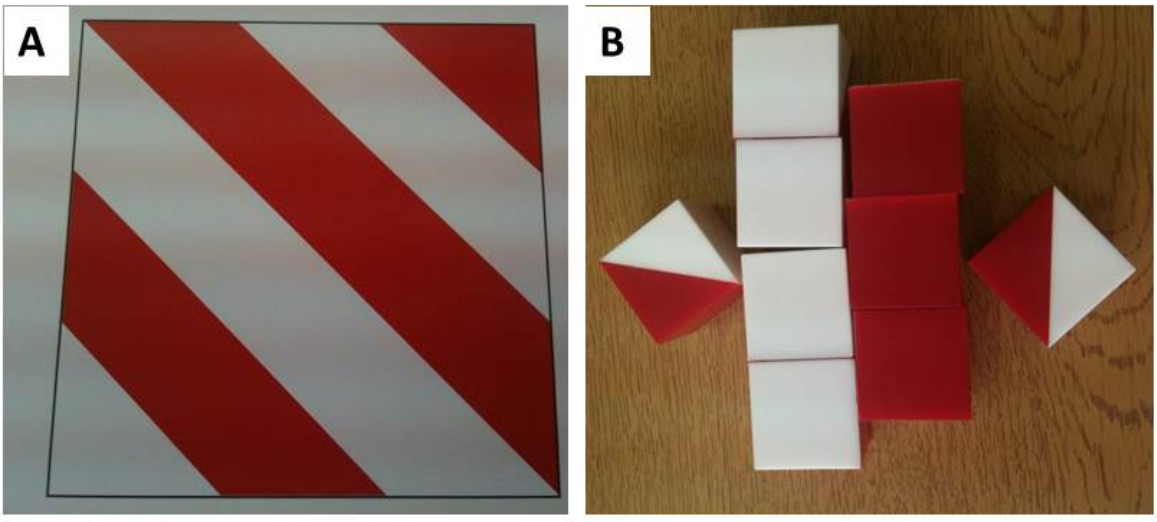


Figure 6.6 (previous page) **A:** photograph of stimulus from the block designs subtest of the WAIS. Participant is asked to recreate this figure using nine blocks. **B:** photograph of final attempt to replicate the stimulus. Participant knew it wasn't quite right but wasn't sure where the differences were or how to change their attempt to rectify this.

Across the cohort PIQ was better scored than VIQ (mean index scores of 96.21 vs. 97.28) but had a greater number of sub components that were performed poorly when compared to the scaled scores (table 6.4). Amongst the better performed subtests there were still a number of very poor performances (figure 6.6 – block design). Picture completion is the only subtest that is better performed by people with JME than the scaled scores (figure 6.3). This weakly significant finding I think is the fault of my test administration. It was always the first test performed and I should have been stricter about the time limits for providing a correct answer. Instead I may have been too lenient, using the 'straight forward' first test to reassure the participant and build rapport.

	Minimum	Maximum	Mean	Std. Deviation	p=
Picture completion	4	18	11.08	3.382	0.0309*
Digit Symbol Coding	3	16	8.36	3.414	0.0015**
Block design	3	14	9.46	2.713	0.2694
Matrix	4	16	10.31	3.130	0.5257
Picture Arrangement	4	16	8.72	2.685	0.0111*

Table 6.4. Subtest scores for performance IQ. . * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

The histogram in figure 6.7 displays the variation between VIQ and PIQ for each participant interviewed. Looking at the far right of the figure one can see a number of high VIQ or PIQ bars with very low corresponding scores for the other index. In contrast (far left) are those who performed badly on the WAIS and they appear to perform equally poorly on both VIQ and PIQ.

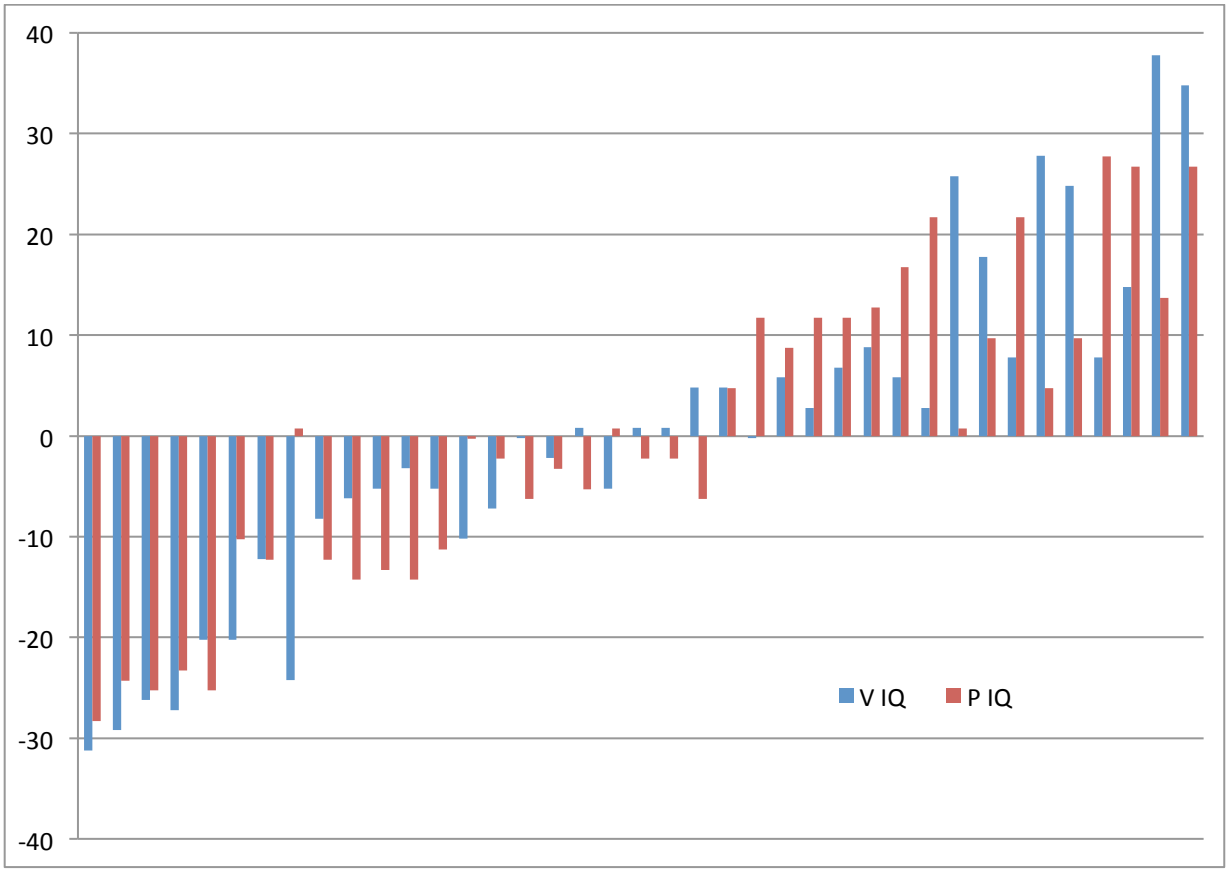


Figure 6.7 Histogram demonstrating variation from mean for both verbal IQ (VIQ – blue; 96.21) and performance IQ (PIQ, red – mean; 97.28). Difference from mean IQ (y axis – ordered by said difference, x axis). On a sample basis there was no difference between the PIQ and VIQ scores p=0.77) but there is a great deal of heterogeneity. A higher score on the VIQ did not necessarily predict a higher score on the PIQ. More on these differences is explained below under 'sub-groups'.

6.3.2.3 Processing Speed and Working Memory

	Minimum	Maximum	Mean	Std. Deviation	p=
Digital Span	3	15	9.82	2.761	0.7108
Letter Number Sequencing	2	17	9.85	3.391	0.7505
Symbol Search	2	17	9.56	3.164	0.0186*

Table 6.5. Subtest scores for processing speed and working memory subtests. . * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01

The lowest mean index score for any WAIS index was for processing speed (94.08). This did not quite reach significance from standardised performance. Working memory is considered to be a frontal lobe function. The range of results was between 63 and 137. Three

participants scored two SDs below the mean (JM14, JM3 and JM23); these three also had the lowest FSIQ scores. The ABNAS scale should identify people who had symptoms of neurotoxicity (presumably from AED side effects). The mean ABNAS score from this cohort was 29.12 and the mean score of those two SD below the mean at PS was 48. Of those scoring between one and two SDs below the mean at PS, (n=8) their mean ABNAS score was 41.6. The correlation between PS and ABNAS is quite strong; Pearson's r statistic of -0.5058 and a $p < 0.014$. This relationship is shown in figure 6.8 below. This suggests that people with JME with higher self-reported symptoms of neurotoxicity perform less well on tests of processing speed.

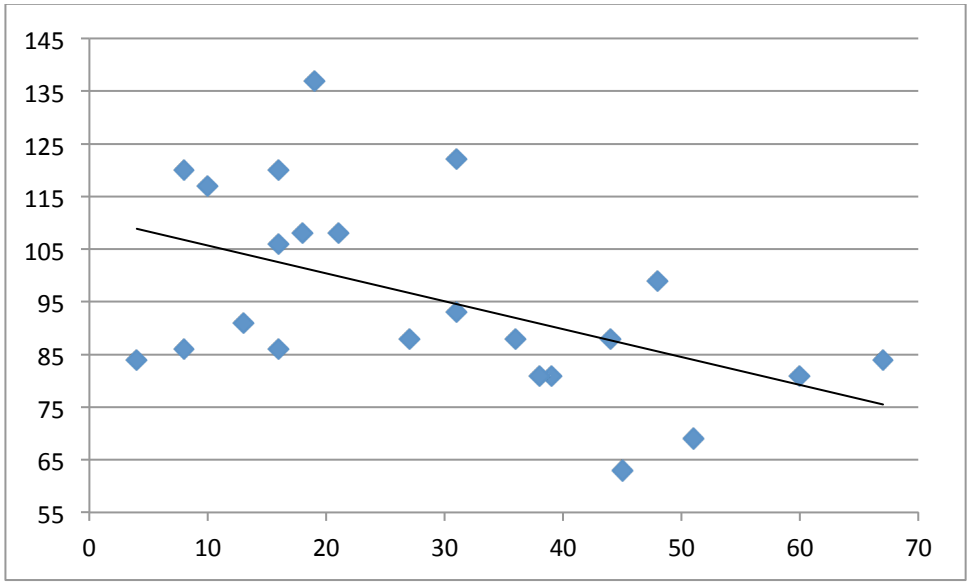


Figure 6.8 Relationship between PS and total ABNAS score. Histogram demonstrating the strong correlation between PS score (y axis) and the total ABNAS score (x axis).

The variability of medications, monotherapy and polytherapies, doses and doubt regarding compliance made analysing the PS and ABNAS results with regards to medication difficult and of doubtful significance. The three who scored least well were prescribed (at the time of testing) VAL 500mg tds; LEV 1500mg bd; and VAL 200mg tds, LEV 250mg bd, CLB 10mg tds. Therefore only one was on polytherapy and perhaps it could be argued was on a sub therapeutic dose of two agents (VAL and LEV).

6.3.3 WMS and Memory

The WMS and TYM scales will be considered together here although elements of TYM also attempt to test executive and nominative abilities. Surprisingly only the abbreviated test – TYM – was answered in a statistically different way from controls $p<0.007$. It appears to be more sensitive than the full WMS to identify memory deficits in JME. The range, means and SDs are given in table 6.6 below. Figure 6.9 demonstrates the variation in subtest scale results. Consistently, logical memory was performed better than faces, which was performed better than verbal paired associates, which scored better than family pictures; this was the pattern for both immediate and delayed testing (figures 6.9, 6.10). The poorest scored test was spatial span (tables 6.7, .8) with a mean scaled score of 8.67, ($p<0.009$).

	Minimum	Maximum	Mean	Std. Deviation	p=
Auditory Immediate	65	142	99.44	14.1	0.8156
Visual Immediate	65	127	94.79	12.6	0.0366*
Immediate Memory	69	128	96.54	13.5	0.1577
Auditory Delayed	71	132	104	12.3	0.1041
Visual Delayed	68	129	95.07	12.4	0.0504
Auditory Recall Delayed	75	130	102.17	13.8	0.3699
General Memory	70	124	100.26	12.5	0.9145
Working Memory	63	124	95.69	14.3	0.0809
TYM	34	50	45.44	4.272	0.0063**

Table 6.6. Subtest scores for WMS and TYM indices. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

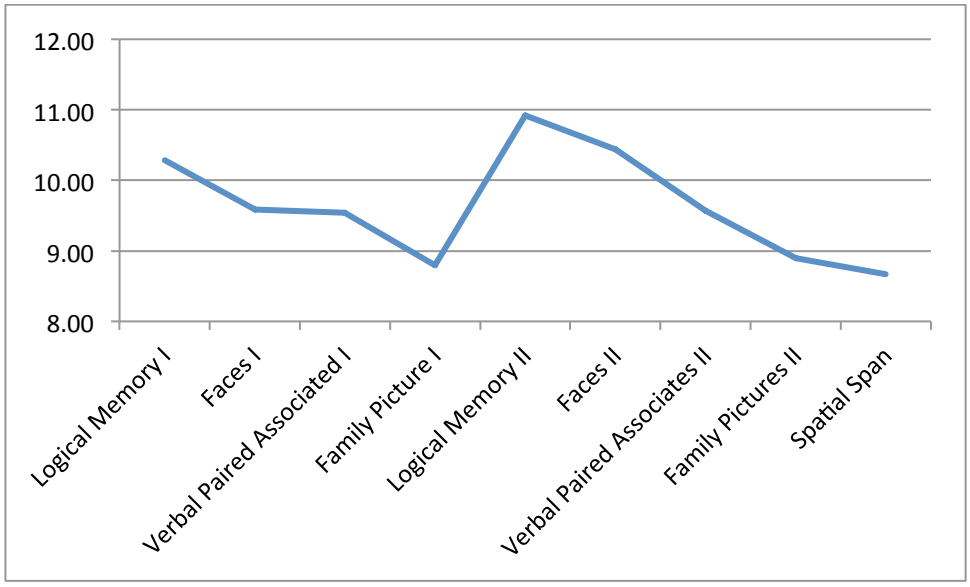


Figure 6.9. Variation in WMS subtest scaled scores.

Post hoc analysis of these data using a paired two-tailed t-test showed that the auditory WMS tests were significantly better answered during delayed testing than during immediate testing (compared to the control means): Logical memory I vs. Logical Memory II, ($p<0.0014$) and Verbal Paired Associates I vs. Verbal Paired Associates II, ($p<0.012$). Where upon the visually presented tests (faces and family pictures) did not show a significant difference ($p=0.94$, $p=0.28$).

6.3.3.1 Immediate Memory

	Minimum	Maximum	Mean	Std. Deviation	p=
Logical memory I	4	17	10.28	2.7	0.5606
Verbal Pairs I	4	16	9.59	2.8	0.3984
Faces I	4	17	9.54	2.7	0.3427
Family Pictures I	4	14	8.79	2.5	0.0165*
Spatial Span	3	13	8.67	2.7	0.0085**

Table 6.7 Subtest scores for immediate memory subtests * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

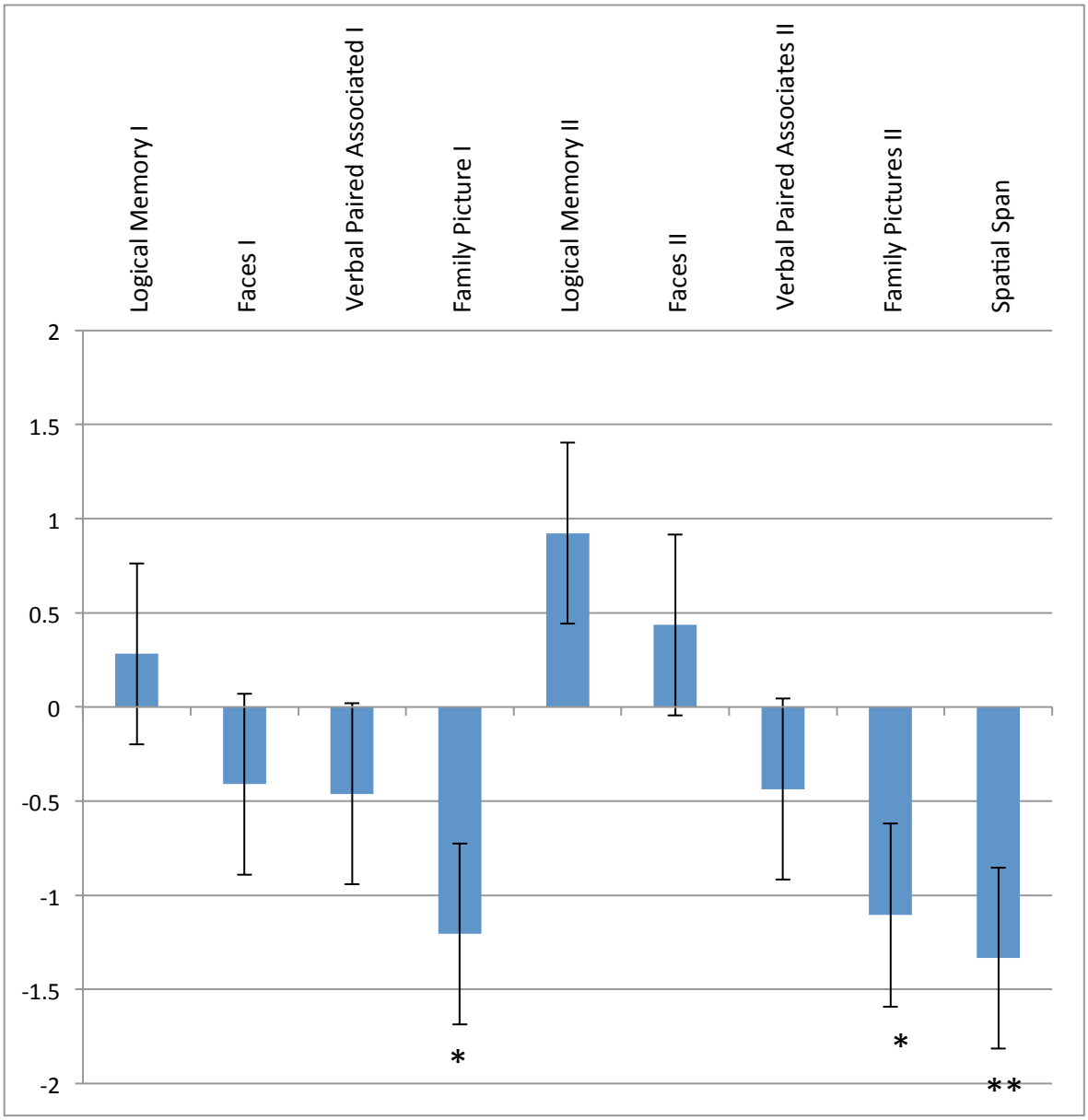


Figure 6.10. Histogram demonstrating variation in scaled scores from the mean for the WAIS. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

Logical memory was particularly well scored across the cohort – particularly logical memory II – the delayed memory test (figure 6.10). Family picture subtests were the most likely to be poorly performed by people with JME. This is a test of visual memory but then relies of verbal retrieval of the stimulus.

6.3.3.2 Delayed Memory

	Minimum	Maximum	Mean	Std. Deviation	p=
Logical memory II	5	17	10.92	2.7	0.0622
Verbal Pairs II	2	13	10.44	2.6	0.3699
Faces II	4	16	9.56	2.8	0.3699
Family Pictures II	4	13	8.89	2.5	0.0291*

Table 6.8. Subtest scores for delayed memory subtests. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

6.3.3.3 Test Your Memory

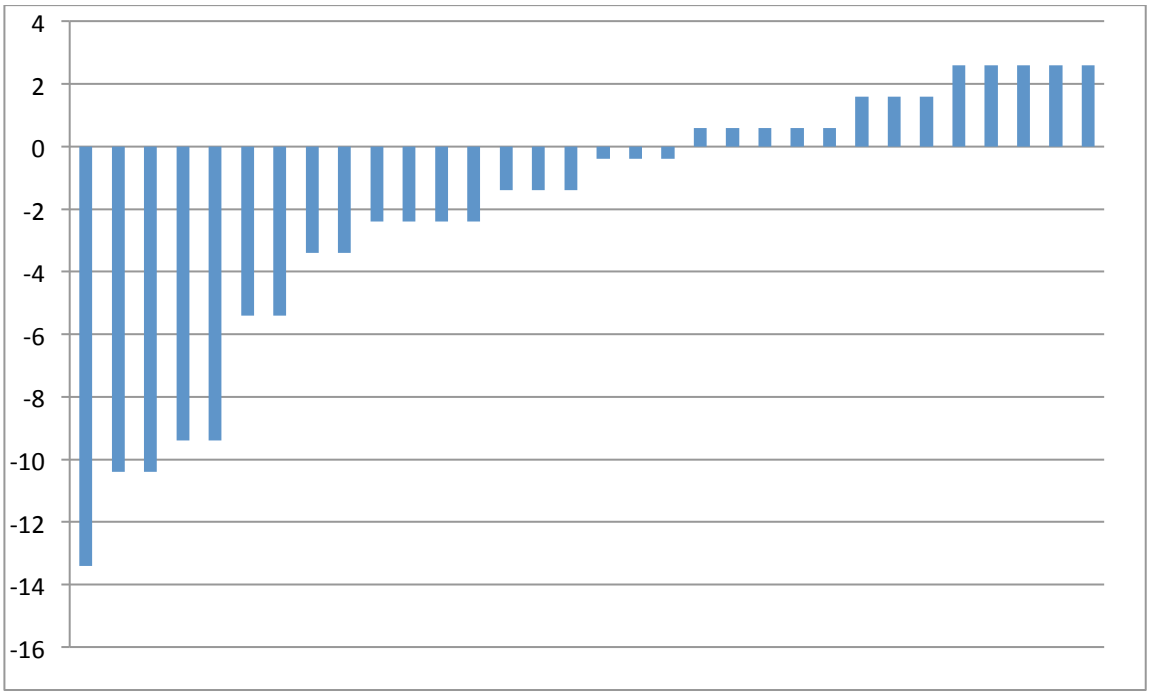


Figure 6.11. TYM score variation from the control mean (47.4) demonstrating significant skew towards poorer TYM scores.

Although TYM scores were generally high and clustered around the control mean and above (47.4) there was significant skew down towards poor scores (figure 6.11). Generally there was not a single component of TYM that was least well performed. Very few individuals admitted to needing support completing it; although a few admitted they used a calculator

for the arithmetic, used *Google* for the dates of World War One or the Prime Minister – or asked their parents! The visuospatial test (drawing a W from the dots and squares) was uniformly performed well and therefore may not be an adequate discriminator of ability in this cohort.

Although TYM scores only varied by sixteen points in total (minimum score 34) it was a good predictor of functioning on other cognitive tests. Of the eighteen scales compared with TYM it was a strong predictor on ten (r statistic greater than 0.5) and a weak predictor on a further six. The most strongly correlated scales were surprisingly the ABNAS, then then less surprisingly general memory, then full scale IQ, then working memory, then processing speed. There was a poorer correlation between TYM and BADS score and BNT performance. The poorest correlation however was with personality type – followed surprisingly by visual immediate memory. Personality type could not be proven to be correlated with TYM scores; this was hampered by the smaller number of people who had completed the EPQ-BV (n=21).

	r	p	
GM	0.636	0.0001	***
FSIQ	0.620	0.0001	***
VIQ	0.575	0.0002	***
PIQ	0.580	0.0001	***
WM - WAIS	0.609	0.0001	***
PS	0.609	0.0001	***
AI	0.530	0.0006	***
VI	0.340	0.0367	*
Immediate memory	0.538	0.0005	***
AD	0.580	0.0001	***
VD	0.391	0.0165	*
BNT	0.353	0.0344	*

ABNAS	-0.698	0.0001	***
EPQ Neuroticism	-0.277	0.2356	
EPQ Extrovertism	0.276	0.2375	
HADS Anxiety	-0.431	0.0275	*
HADS Depression	-0.508	0.0078	**
BADS	0.394	0.0156	*

Table 6.9. Correlation between TYM and other neuropsychological indices. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level; *** significance at the level of the Bonferroni correction.

6.3.4 D-KEFS

Four sub scales were administered in total from the D-KEFS; verbal fluency, colour word interference, the trails task and the proverbs test. Figure 6.12 demonstrates the mean performance across the subtests. Broadly it can be seen that participants scored near normally on verbal fluency and proverbs, but in the impaired range for CWIT and trails. However there was a degree of variability within the participants and between the components administered.

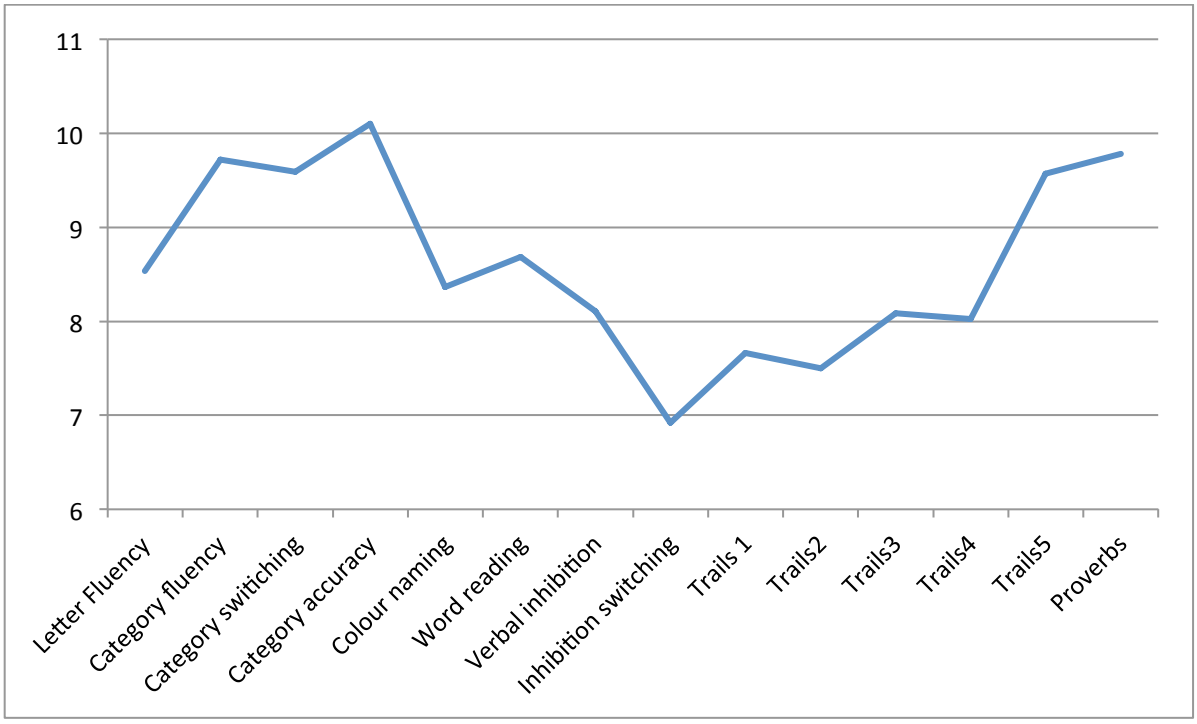


Figure 6.12 (previous page) Mean subtest scaled scores for the D-KEFS

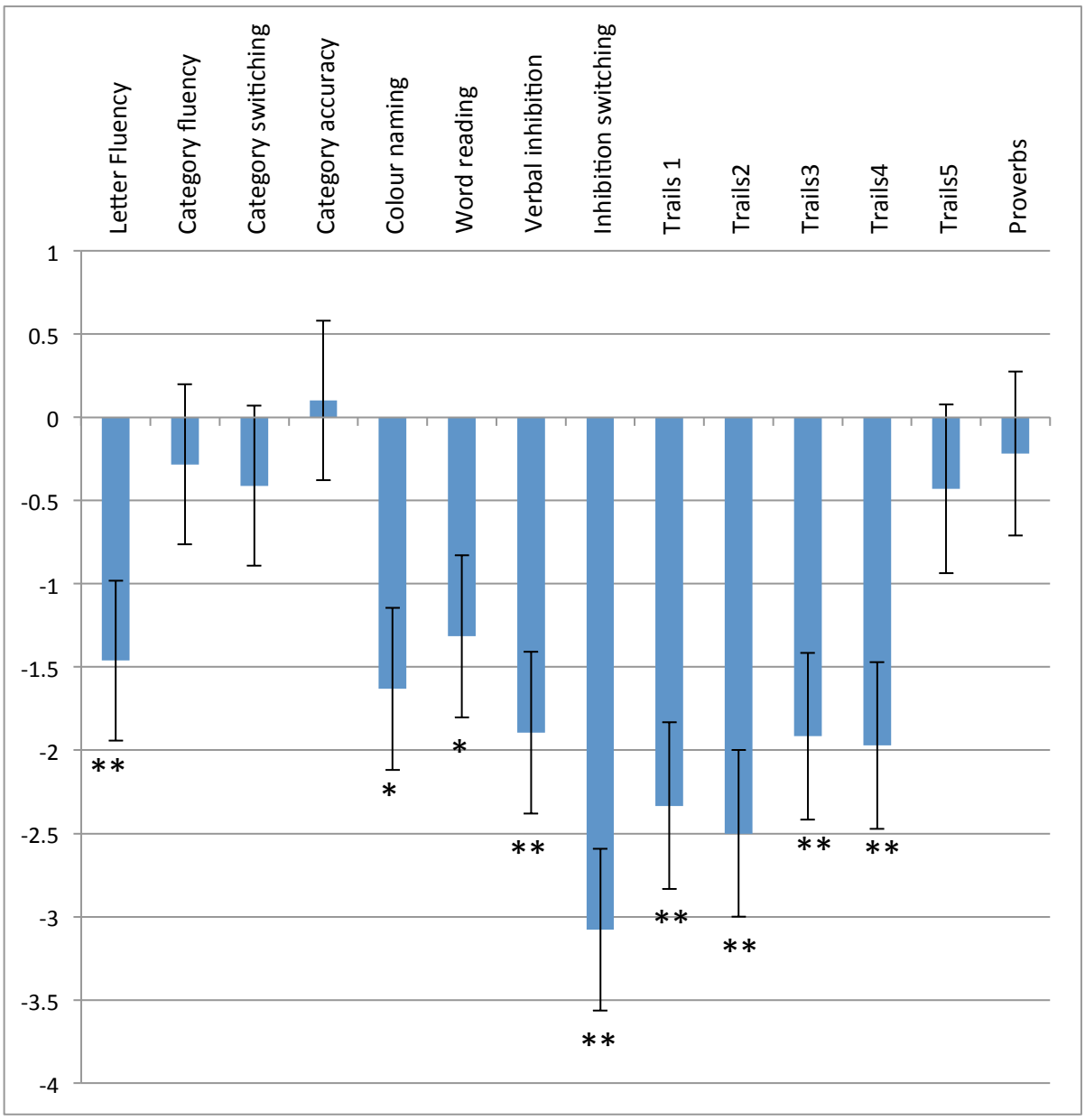


Figure 6.13. Difference in mean scaled score from the mean – shown with SE bars. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

6.3.4.1 Verbal Fluency

The first element of verbal fluency in the D-KEFS is a straight 'FAS' test. This was how it appeared during administration too. Despite coaxing and supporting the participants

through they fared poorly on the task of unstructured free thinking of words that begin with a certain letter. However when provided with categories they very quickly adopted a strategy that lead to higher score attainment; for animals this would be starting with domestic pets, then farm-yard animals and then wild creatures and critters (for example); for 'boys' the most common strategies employed would be to cycle through members of their family, or boys from their year in school. Category switching and accuracy was generally performed well a number of individuals (figure 6.13).

	Minimum	Maximum	Mean	Std. Deviation	p=
Letter Fluency	1	18	8.54	3.2	0.0042**
Category Fluency	3	22	9.72	4.8	0.5606
Category Switching	3	16	9.59	3.9	0.3984
Category Accuracy	3	17	10.10	3.7	0.8321

Table 6.10. Subtest scores for verbal fluency – D-KEFS. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

6.3.4.2 Colour Word Interference

Colour word interference sub tests were all significantly poorly answered by the study cohort (table 6.11). Each of the four sub components were scored in the impaired range by the mean scaled score. People with drug resistant JME scored more poorly on the trail making tasks (TMT) than controls. Number and letter sequencing should be answered fairly similarly - and both demonstrate highly significant differences from controls. The motor-trail (trail five) is the trail least demanding of executive function and it is therefore no surprise that there is no significant difference between sample performance and standardised means.

	Minimum	Maximum	Mean	Std. Deviation	p=
Colour Naming	1	12	8.37	2.8	0.0019**
Word Reading	1	14	8.68	3.3	0.0103*
Verbal Inhibition	1	15	8.11	4.4	0.0004***
Inhibition Switching	1	15	6.92	4.4	p<0.0001***

Table 6.11 Subtest scores for CWIT – D-KEFS. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

6.3.4.3 Trail Making Task

	Minimum	Maximum	Mean	Std. Deviation	p=
Visual Scanning	1	12	7.67	3.3	p<0.0001***
Number Sequencing	1	13	7.5	3.5	p<0.0001***
Letter Sequencing	1	13	8.08	3.3	0.0005***
Number-Letter Sequencing	1	13	8.03	3.6	0.0004***
Motor Speed	4	14	9.57	2.6	0.4039

Table 6.12. Subtest scores for trials – D-KEFS. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

The five trail subcomponents test different skills. The first is almost a screen for visual inattention and as such I did not expect it to be as poorly performed as trails 2, 3 and 4 (sequencing) which are clearly tests of executive function and processing speed; the number-letter sequencing test being the most complex of the sequencing trails. The fifth component is almost a pure test of processing speed and has no sequencing within it. The better performance here helps to exclude processing speed as a major contributor towards the poor performance on the sequencing tests.

6.3.4.4 Other Executive Function Tests

Table 6.13 below shows that proverbs was answered by the mean as well as controls. Six individuals scored in the impaired range (scaled score of five or below). The rank score also takes into account the multiple choice component of the test and here the mean rank was 59.7 (100 is full attainment, 1 is poorest). Here twelve individuals scored in the bottom quintile (twenty or below). Although on a cohort level adequate performance was upheld, the D-KEFS task could identify some who could not correctly interpret abstract sayings.

	Minimum	Maximum	Mean	Std. Deviation	p=
Proverbs	1	14	9.78	3.7	0.6637
BADS	20	129	87.92	22.2	p<0.0001***
DEX Self	3	56	24.06	13.6	0.0929
DEX Others	3	59	24.69	15.6	0.0008***

Table 6.13. Subtest scores for the reminding executive function tests * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level.

6.3.5 BADS

Four elements of the BADS were administered. Figure 6.14 below shows the mean points per question difference for each of the elements. Key search and zoo map both contributed more to the overall difference of the sample BADS score from the standardised mean than rule shift and temporal judgment did. Taken individually each test was not significantly different from the control means. However figure 6.15 clearly shows just how skewed the total BADS score was away from the standardised values: only 9/38 scored at the standardised value (100) or more. A discussion of those who scored one and two SDs below the mean follows in the subgroups section.

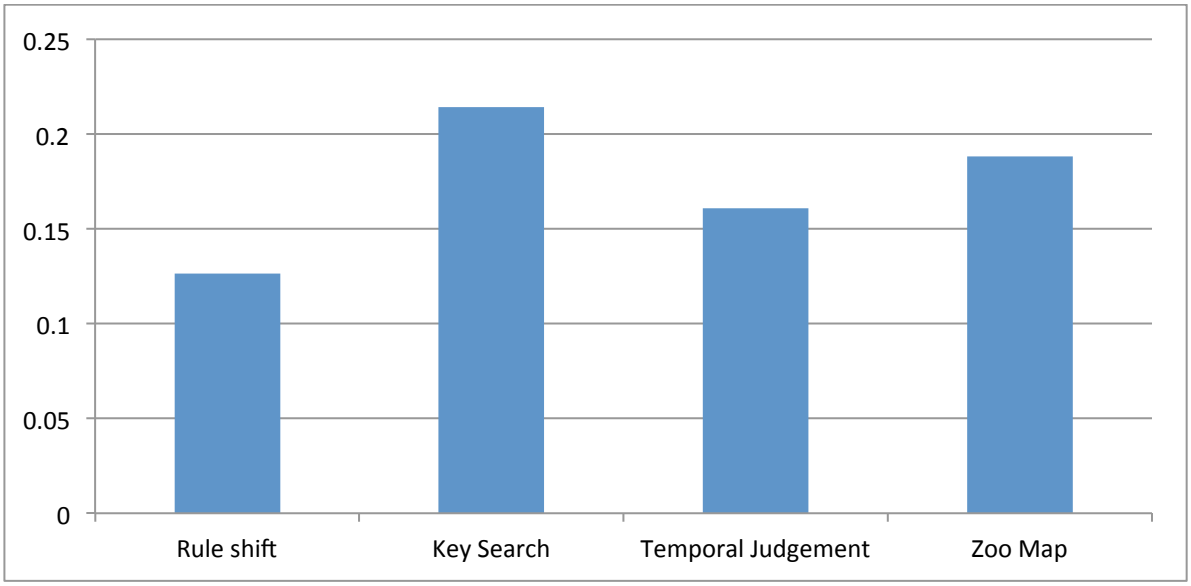


Figure 6.14 Mean points per question below the mean for BADS elements

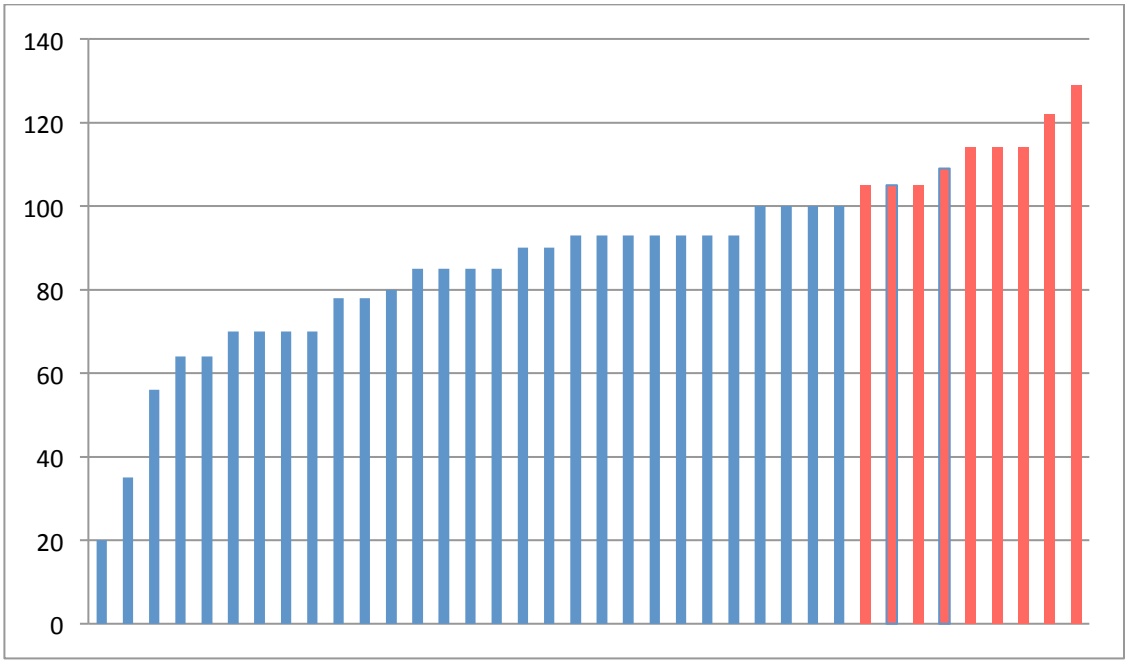


Figure 6.15 Standardised BADS score – bars in red represent individuals who have scored above average in this test.

The BADS subtests have a high degree of ecological validity and therefore some performances on these tests are quite dramatic - such as the key searches shown in figure 6.16.

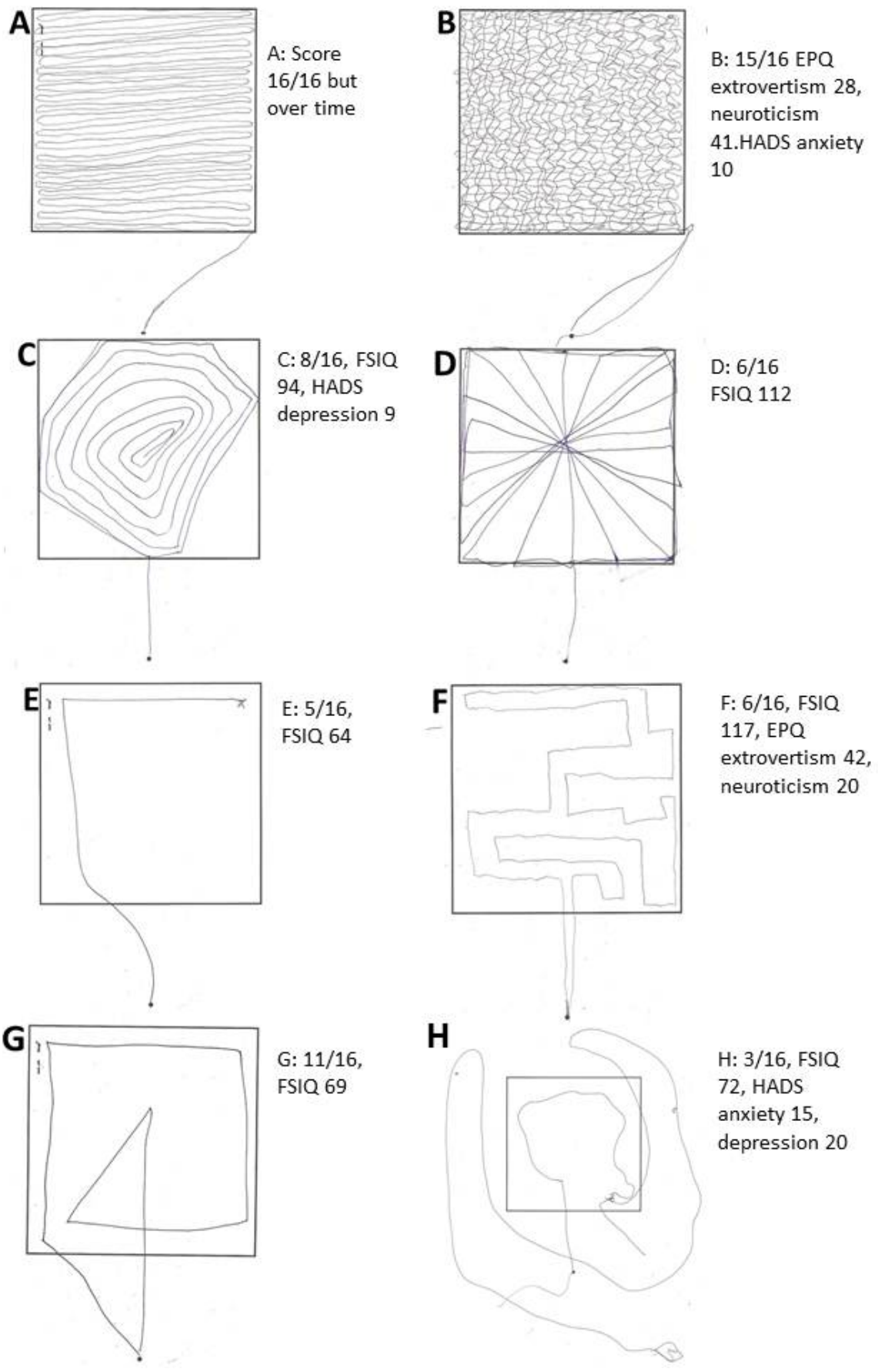


Figure 6.16 Key search test from the BADS was poorly performed across the cohort

6.3.5.1 Dysexecutive Questionnaire

People with drug resistant JME and their nearest friend or relative both scored the participant as higher than the mean on the DEX questionnaires. The self-assessment questionnaire provided to a close individual (DEX-other) showed a highly statistically significant difference from the control mean, but the DEX-self did not (table 6.13). The two scores however were well correlated (mean DEX-self 24.06, DEX-other 24.69) and not surprisingly not significantly different (p=0.78). This suggests that people with JME under report their difficulties with executive function, or have poor insight into their abilities (figure 6.17).

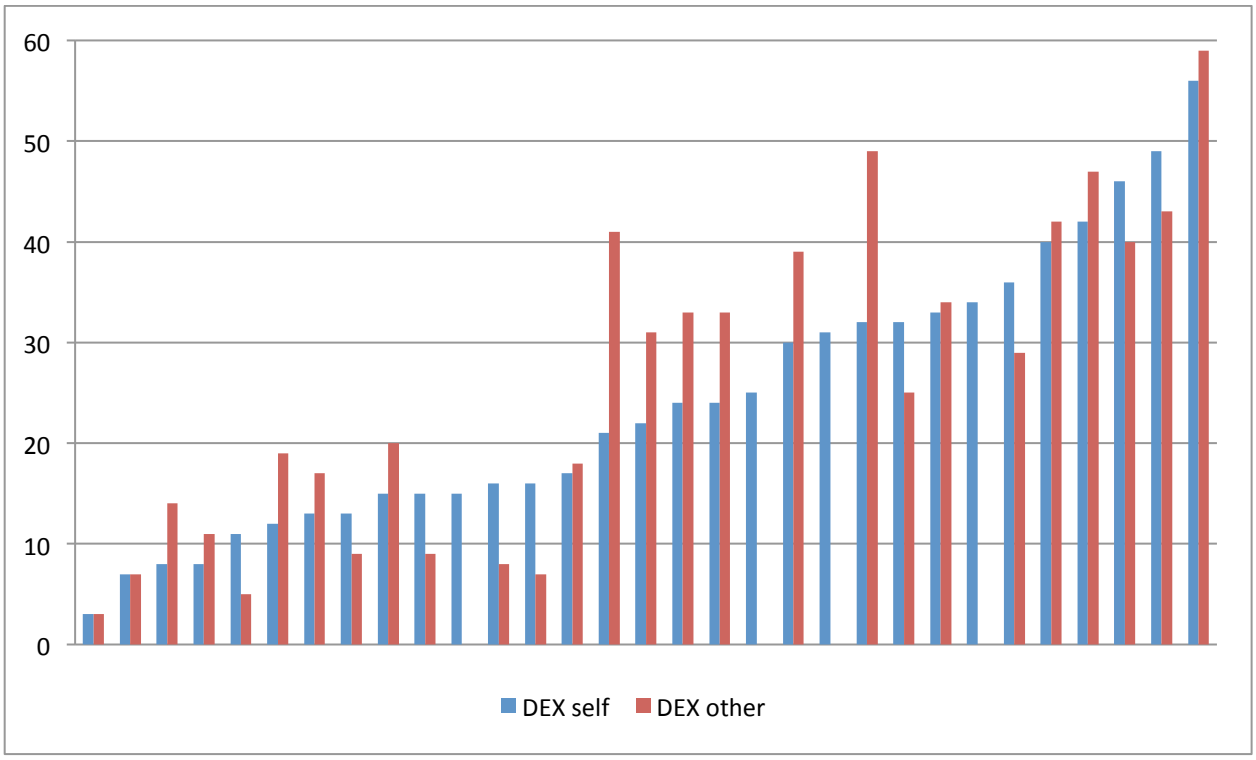


Figure 6.17. Comparison of DEX self (blue) and DEX other (red) scores ranked by DEX self score. Although the means are not different (24.06 self, 24.71 other) there are a number of individuals who score themselves midway on the scale whose significant other scored them much higher using the scale. That said there appears to be a medium to strong correlation between the two scores; confirmed by statistical analysis.

DEX-self was strongly correlated (r statistic greater than 0.5) with the number-letter sequencing trail task, the proverbs test of the D-KEFS and DEX-other score. It was also statistically significantly correlated with seven of the scales (table 6.14) including the BADS standardised score. The tests which did not best correlate are the Stroop like tests of the CWIT.

	r	p	
Letter Fluency	-0.3914	0.0265	*
Category fluency	-0.3559	0.0453	*
Category switching	-0.4918	0.0042	**
Category accuracy	-0.4744	0.0060	**
Colour naming	-0.2994	0.0957	
Word reading	-0.2945	0.1015	
Verbal inhibition	-0.3424	0.0548	
Inhibition switching	-0.1635	0.3710	
Trails one	-0.2790	0.1217	
Trails two	-0.4945	0.0039	**
Trails three	-0.4346	0.0128	*
Trails four	-0.5742	0.0006	***
Trails five	-0.4252	0.0151	*
Trails composite	-0.3912	0.0266	*
Proverbs	-0.5966	0.0003	***
DEX-other	0.8568	0.0001	***
BADS	-0.4987	0.0036	**

Table 6.14. DEX-S as a predictor of score on other tests of executive function. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level; *** significance at the 0.0029 level (level of the Bonferroni correction).

6.3.6 Additional Tests

The tests shown in table 6.15 are the EPQ-BV test of personality, the BNT (language), HADS scale for affective symptoms and ABNAS scale for neurotoxicity. They are all self-completed (except the BNT) and therefore test subjective symptoms. They will be discussed in turn.

	Minimum	Maximum	Mean	Std. Deviation	p=
EPQ-BV Neuroticism	18	54	35.5	11.4	0.0130*
EPQ-BV Extrovertism	14	48	31.6	10.1	0.0001***
Boston Naming Test	39	60	53.3	6.3	0.0006***
HADS Anxiety	3	17	10.11	3.9	p<0.0001***
HADS Depression	0	20	7.22	5.7	p<0.0001***
ABNAS	4	67	29.12	17.6	0.0054**

Table 6.15. 'Additional tests' – i.e. not the WAIS, WMS, BADS or D-KEFS. Total (raw) scores shown. * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level; *** significance at the 0.001 level.

6.3.6.1 Eysenck Personality Questionnaires

The mean scores for neuroticism and extrovertism used were 29.6 and 42.2 respectively. Therefore although the two EPQ-BV scores presented in table 6.15 appear similar they are in fact demonstrating two different findings; people with drug resistant JME were more neurotic and less extrovert than the control sample. Figure 6.18 shows the pattern of EPQ results. There is no significant difference between the score values (t-test, p=0.25), but there is a strong and significant inverse correlation (r=-0.639, p< 0.002); as EPQ-BV extrovertism scores drop, EPQ-BV neuroticism scores increase. Although the cohort are predominantly introverted and have high neuroticism scores there are a subset with a reverse pattern (far left of figure 6.18). This pattern is discussed further in subgroups below.

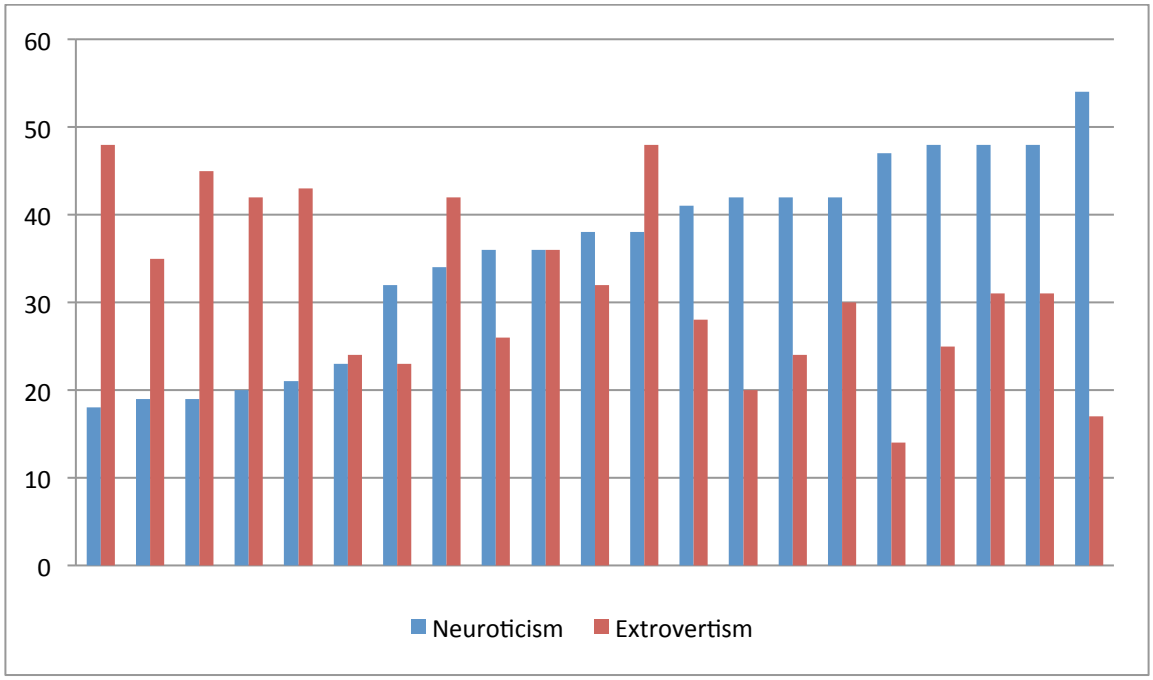


Figure 6.18 Histogram demonstrating total scores on the EPQ-BV questionnaire. Participants' data is paired and ranked by neuroticism score.

6.3.6.2 Boston Naming Test

The mean score of 52.3 on the BNT is significantly lower than the control mean (54.5) as shown in table 6.15. Figure 6.19 demonstrates the variability with all the negative bars (below the line to the left) being scores below the mean.

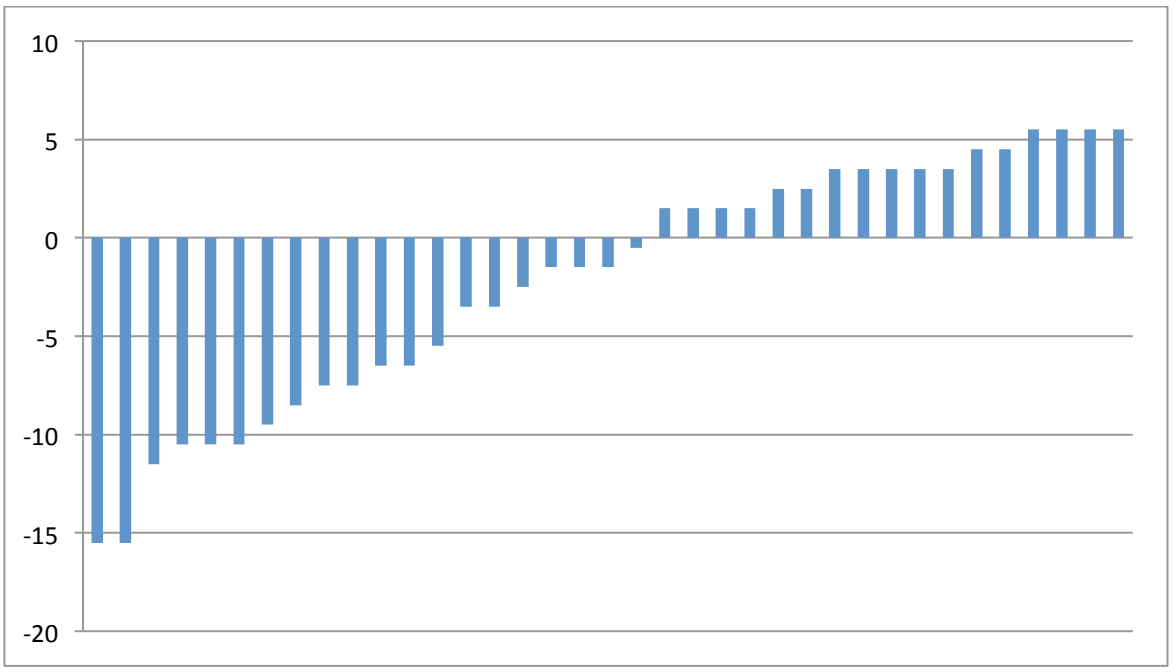


Figure 6.19 (previous page) Histogram demonstrating the variation from the mean BNT score (54.5) with poorer performances producing negative results (leftmost on graph).

6.3.6.3 ABNAS

ABNAS scores were significantly higher across the range than the control means (table 6.15); how this varied by ABNAS factor can be seen in figure 6.20. The control (explained in methods chapter) was not a true control group, but rather people with drug sensitive epilepsy and therefore these results are probably more significant. Across the factors it would appear that memory, followed by slowing and then fatigue are the areas that people with drug-resistant JME self-report difficulties in comparison with other people with epilepsy. Just as described above the variations in anti-epileptic drug preparations taken by such a small cohort prohibits a full analysis of the effects of drugs on ABNAS scores here. However the person (JM05) with the highest total ABNAS score was taking a relatively modest dose of zonisamide (25mg mane and 50mg nocte) only.

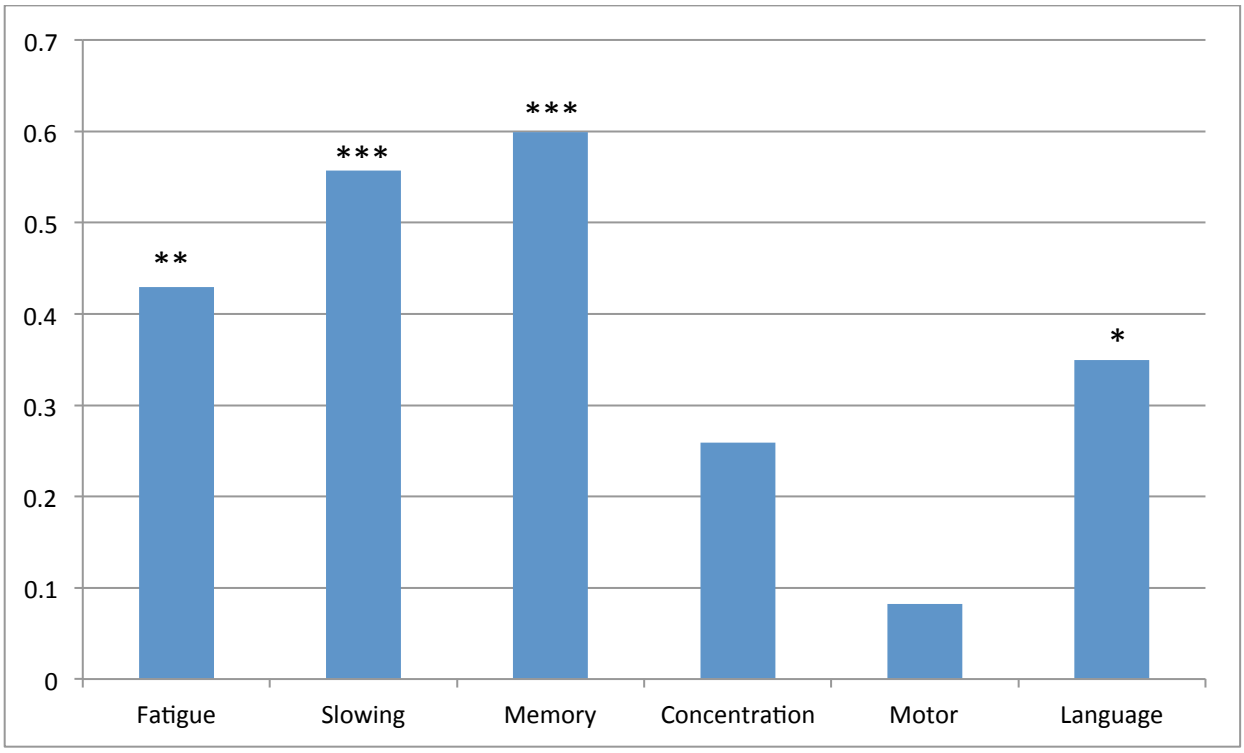


Figure 6.20 Mean points per question above the mean for the ABNAS factors. . * indicates significance at the 0.05 level but did not reach the alpha statistic of 0.01; ** significance at this level; *** significance at the 0.001 level.

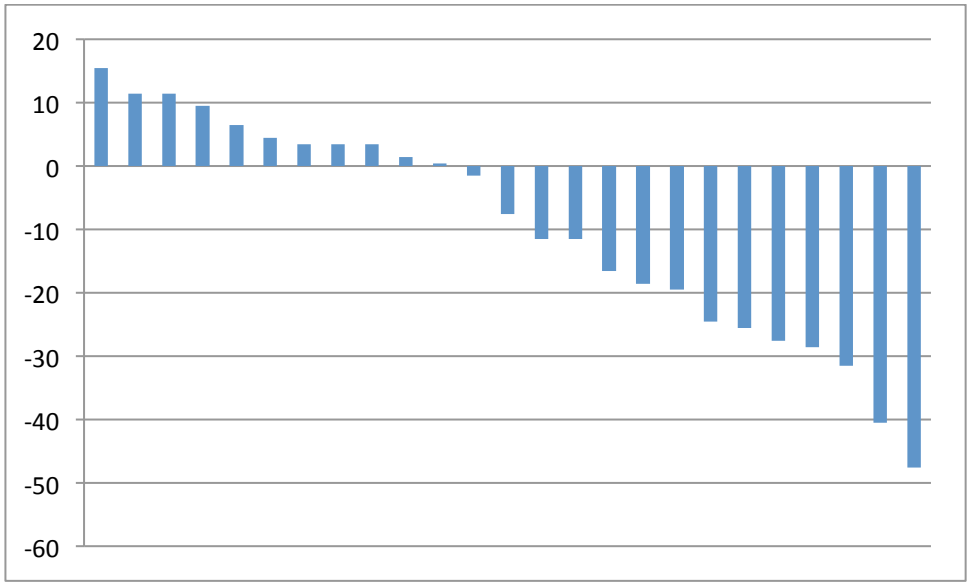


Figure 6.21 Variation from the total mean ABNAS score (19.46). Individuals on the left scored less than this on the ABNAS (fewer symptoms of neurotoxicity) whereupon the majority (right) scored above the control mean on this test.

The range of ABNAS scores was very wide (figure 6.21) with the majority however scoring above the mean (more symptoms, right most and negative bars on 6.21). Again not everyone who scored highly on one factor would also report high levels on symptoms on every factor; figure 6.22 shows this well. The ABNAS scores are shown by factor and ranked by total ABNAS score – language particularly seems to behave differently to the other factors whereupon slowing seems to mirror total ABNAS score well.

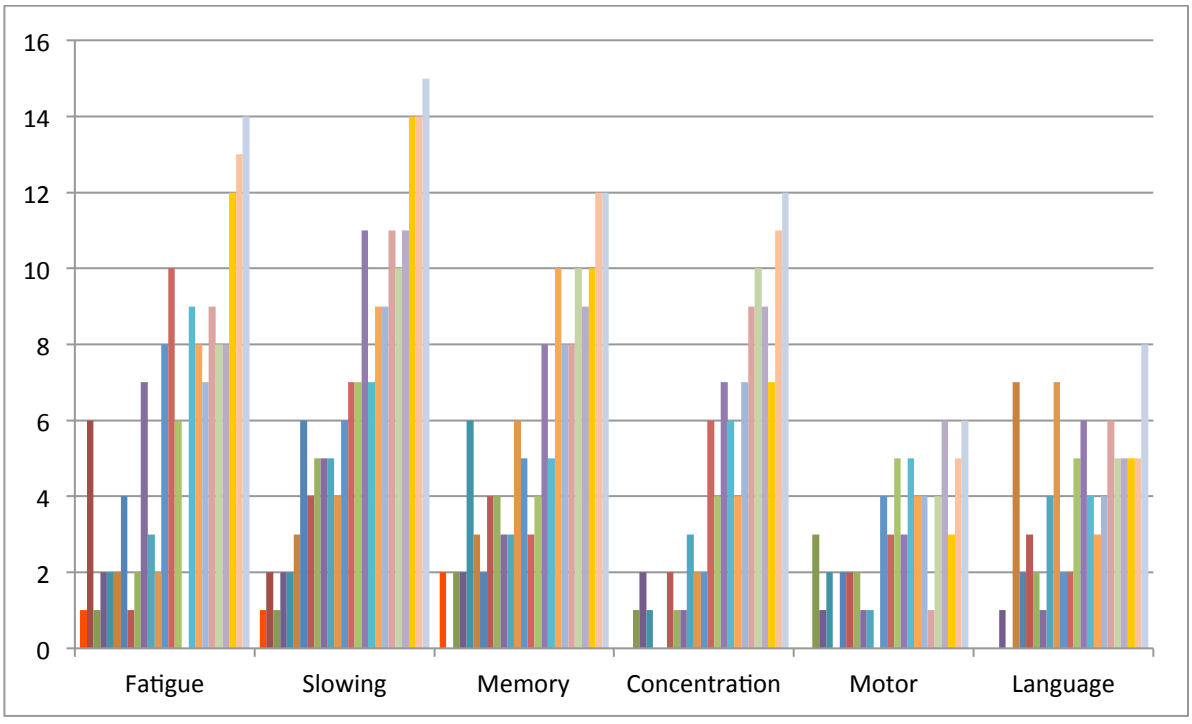


Figure 6.21 (previous page). All scores on the ABNAS factors demonstrating the variation between individuals. Care should be taken in interpreting this graph. Fatigue and slowing are comprised of five factors, memory and concentration of four and motor and language of three each. The graph is ranked by total ABNAS score.

6.3.6.4 HADS

The mean score for the HADS-A (table 6.15) was in a range that would be in keeping with a pathological level of anxiety symptoms (8 or above) whereupon the mean HADS-D score stopped just short of this threshold for depression (7.22). Both were significantly raised compared to control scores however. Figure 6.23 demonstrates the range and spread of HADS scores. The pattern of mixed affective symptoms with anxiety dominant is seen in figure 6.23, but what figure 6.24 makes clear is that for eight individuals their depressive symptoms outstrip those of anxiety.

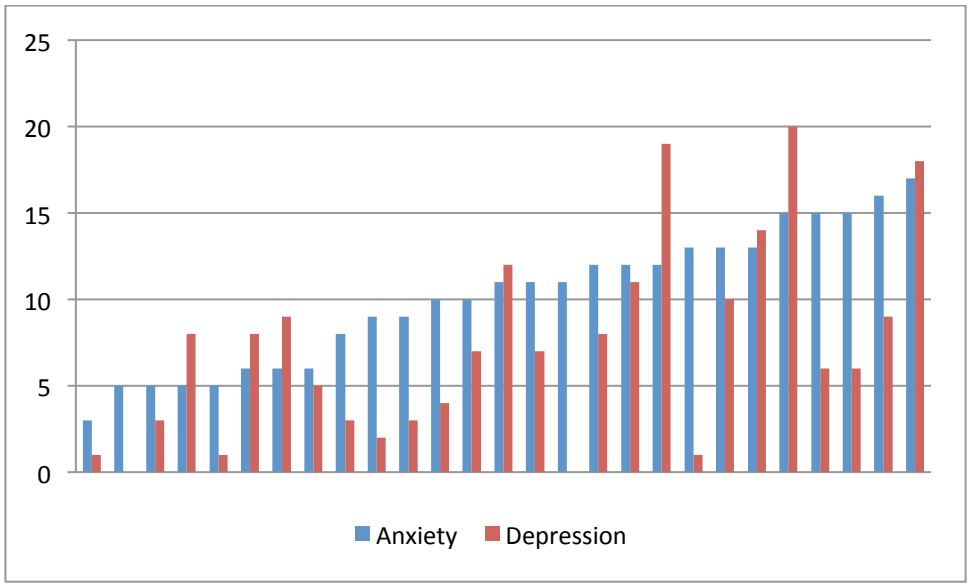


Figure 6.23 Paired anxiety and depression scores for the HADS demonstrating a clear discrepancy between the two scores for some individuals.

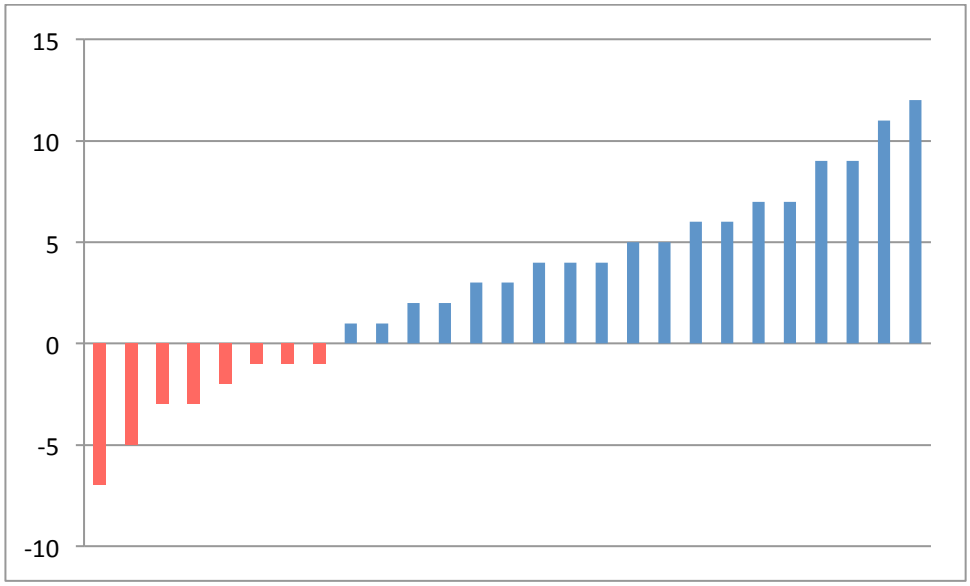


Figure 6.24. Difference between HADS anxiety and HADS depression scores. Negative scores (red) show higher depression scores and positive scores (blue) show higher anxiety scores. This was a statistically different difference (p>0.03).

6.4 Identification of subgroups

6.4.1 JME with low IQ

Having collected wide ranging data on IQ and with low IQ often used as an exclusion criterion for JME cohort studies, it was clear that JME with low IQ would be the first subgroup I would investigate. Using a cut off of one SD below the mean full scale IQ provided eight cases with an IQ of 85 or less. The table below (6.16) shows the differences in the mean scores and the significance between the groups. The low IQ group consisted of 7 females and had a mean age of 38; the normal IQ group had 23/31 females and a mean age of 30.9 (ns difference).

As you would expect selecting on the basis of the WAIS FSIQ, verbal and performance IQs (which feed into the FSIQ metastatistic) are 23 and 30 points higher in the main group than the low IQ group. Working memory (both WIAS and WMS) and general memory are also 18 to 28 points higher in the main group. People with JME and low IQ also performed less well on executive function tests. Performance was in the normal range for the normal IQ group (mean scores on the D-KEFS Verbal Fluency test ranged between 9.5 and 11.2) in stark contrast to the impaired scores in the low IQ group (5.0 to 6.0). This is borne out by statistical significant differences between the groups – seen most clearly on letter fluency

(FAS test), and category switching / category accuracy. People with low IQ scored poorly on the CWIT - with dramatically low means of 3 and 3.6 for verbal inhibition and inhibition switching (the purer tests of executive function); the differences were statistically different on colour naming and verbal inhibition. The poor scores of those with normal IQ on inhibition switching prevented this difference from being statistically demonstrable. Colour naming is baldly a test of learning a new test rule and 'reading out' the colour of blocks. The poor performance on this is mirrored by low IQ's poor scoring on trails task two (linking numbers up in order) rather than trails three (letters in order). That is perhaps they were less confident or able when given this new task but improved their score on the similar following task. Trails four is the purest executive function test of the trails and a clear discriminator between the groups.

People with low IQ and JME scored 17.8 and 18.4 points higher on the DEX self and other questionnaires respectively. These were statistically significant differences that identify that both individuals and significant others recognise their poor everyday function at executive tasks. Total BADS scores were also lower in the low IQ group (by over 29 points – almost two SD). Of the elements that comprise the BADS it was key search that was scored the most differently (mean score 1.3 in low IQ vs. 3.2 in the normal group, $p<0.0006$). The rule shift cards were also a discriminatory test with low IQ JME scoring 2.4 and the normal group 3.6, $p <0.006$). Those with low IQ were also more likely to have higher anxiety scores on the HADS and much higher neurotoxicity scores on the ABNAS. Although both groups' mean HADS anxiety scores were in the pathological range those with low IQ were over five points higher at a significance of greater than $p=0.003$. The ABNAS factors demonstrating the most striking differences were 'Slowing' (mean score 12 in low IQ, 5.5 in normal IQ group; $p< 0.0008$). Memory and concentration ABNAS factors were also strikingly different (low IQ 9.4 and 8.4 mean score vs. 4.7, 3.3; $p<0.005$ for both). Low IQ did not predict performance on the first and last trails tests nor extrovertism traits.

	Low IQ	Normal IQ	p	
VIQ	72.38	102.35	0.00001	***
PIQ	78.75	102.06	0.00002	***
WM –WAIS	73.38	101.77	0.00001	***
PS	79.25	98.17	0.00419	**
GM	85.50	104.20	0.00003	***
WM –WMS	78.88	100.03	0.00005	***
Letter Fluency	5.00	9.45	0.00018	***
Category fluency	6.00	10.68	0.01113	**
Category switching	5.13	10.74	0.00008	***
Category accuracy	5.88	11.19	0.00008	***
Colour naming	5.63	9.10	0.00079	***
Word reading	6.75	9.20	0.06014	
Verbal inhibition	3.00	9.47	0.00004	***
Inhibition switching	3.63	7.80	0.01450	
T1	7.29	7.76	0.73520	
T2	3.71	8.41	0.00072	***
T3	5.43	8.72	0.01684	
T4	3.57	9.10	0.00005	***
T5	7.86	10.00	0.05204	
BNT	47.75	53.59	0.01859	
DEX-Self	39.00	21.19	0.00224	**
DEX-Other	40.00	21.64	0.00791	**
TYM	40.86	46.63	0.00067	**
HADS Anxiety	14.17	8.95	0.00239	**
HADS Depression	11.67	5.95	0.02642	

ABNAS	50.00	23.90	0.00131	**
Impact Epilepsy Scale	30.00	22.35	0.03415	
EPQ Neurotic	46.00	33.78	0.08440	
EPQ Extrovert	24.67	32.78	0.20474	
BADS	64.50	94.17	0.00032	***

Table 6.16. JME with low IQ (* is significant at 0.01, ** between 0.01 and Bonferroni corrected value, *** significant at Bonferroni correction level – 0.00125).

6.4.2 Low BADS scores

Having demonstrated that people with drug resistant JME score less well at the BADS it was the next important stage to look at levels of BADS performance. I divided them into three groups: one SD below the mean (i.e. 84 and below, n=12/39), mean score or over (i.e. 100 or more, n=13) and a third group scoring within one SD of the mean (i.e. 99 to 85, n=13). The mean scores for these three groups the correlation statistic and the corresponding p value re given in the table below. Of the thirty comparisons made – twenty one are stronger than the alpha statistic and twelve of these survive a Bonferroni correction (p=0.001667). Of these twelve they include all five of the indices from the WAIS as well as three of the subtests of the CWIT and three of the trails tests. BADS performance was therefore a much greater predicator on these last two mentioned D-KEFS tests than verbal fluency and the DEX questionnaires. Despite this both DEX self and DEX other as well as letter fluency and category switching were particularly poorly performed by people with low BADS scores.

BADS performance did not predict performance on IES, HADS depression, or BNT.

	Lowest	Mean to low	Mean or more	r	p	
VIQ	83.2	99.3	112.5	0.517	0.0009	***
PIQ	83.1	102.8	112.4	0.533	0.0006	***
FSIQ	81.5	101.2	112.2	0.560	0.0002	***
WM - WAIS	77.9	100.8	115.2	0.694	0.0001	***
PS	80.6	100.3	109.2	0.500	0.0016	**
GM	91.2	104.8	112.2	0.453	0.0042	**
WM -WMS	82.2	100.1	111.2	0.604	0.0001	***
Letter Fluency	6.4	9.2	10.5	0.476	0.0025	**
Category fluency	8.7	10.5	10.7	0.148	0.3763	
Category switching	7.0	10.7	11.6	0.486	0.0019	**
Category accuracy	7.7	11.4	11.8	0.455	0.0040	**
Colour naming	6.9	8.4	10.3	0.512	0.0012	**
Word reading	6.8	9.1	10.7	0.521	0.0009	***
Verbal inhibition	4.3	9.3	11.1	0.556	0.0003	***
Inhibition switching	4.5	7.0	9.6	0.438	0.0066	**
T1	6.8	7.3	8.2	0.438	0.0084	**
T2	4.4	8.5	10.5	0.677	0.0001	***
T3	5.7	8.3	11.2	0.550	0.0006	***
T4	5.0	10.3	10.5	0.633	0.0001	***
T5	8.3	8.8	11.0	0.324	0.0572	
BNT	49.6	53.3	58.9	0.171	0.3192	
DEX-Self	22.5	15.9	22.4	-0.493	0.0055	**
DEX-Other	22.7	12.8	21.1	-0.514	0.0059	**
TYM	42.4	46.0	51.1	0.407	0.0207	*
HADS Anxiety	13.9	8.7	9.8	-0.507	0.0081	**
HADS Depression	9.9	6.7	6.6	-0.256	0.2064	
ABNAS	8.8	5.6	5.7	-0.367	0.0773	
Impact Epilepsy Scale	8.6	6.6	5.8	-0.235	0.2682	
EPQ Neurotic	13.5	4.1	7.7	-0.436	0.0612	
EPQ Extrovert	9.5	3.2	6.2	0.242	0.3170	

Table 6.17. JME with low BADS scores. (* is significant at 0.01, ** between 0.01 and Bonferroni corrected value, *** significant at Bonferroni correction level – 0.001667).

6.4.3 Verbal versus performance IQ

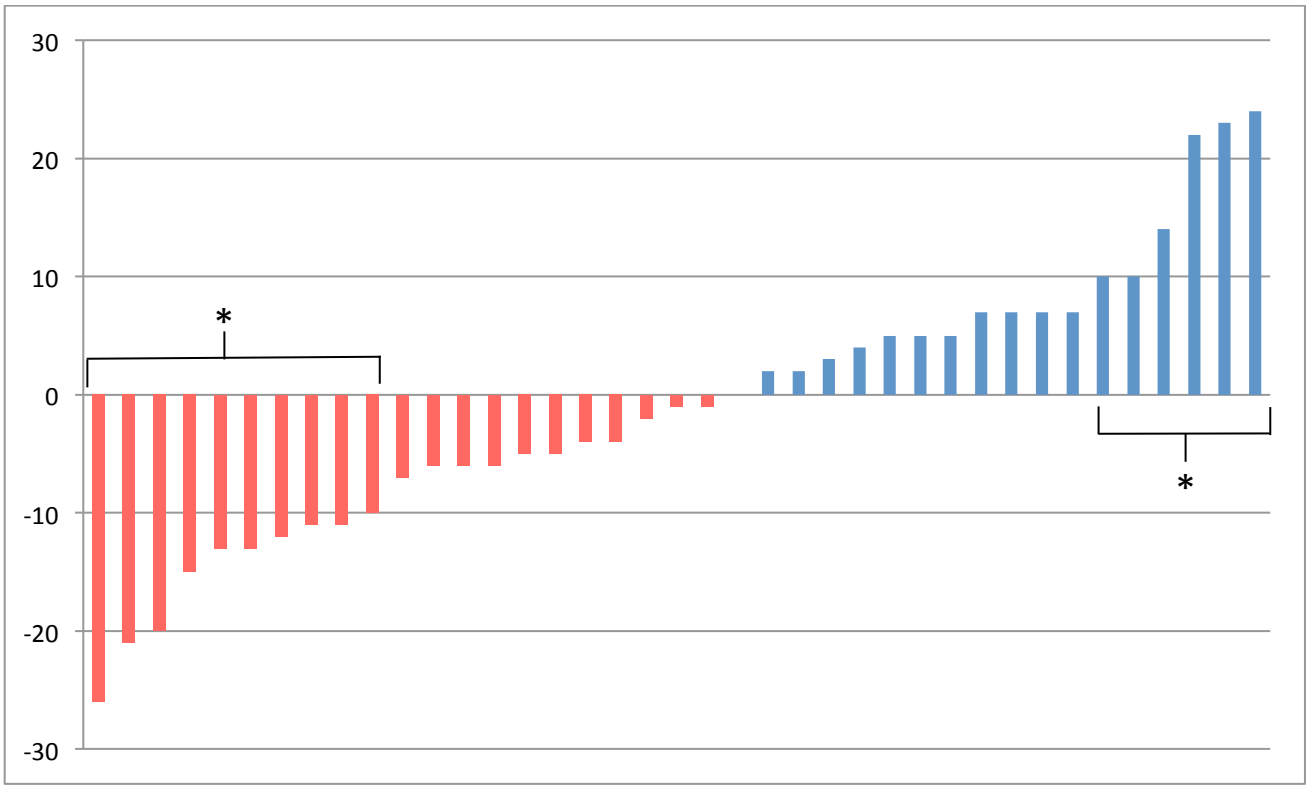


Figure 6.25 Histogram demonstrating difference between Verbal IQ and Performance IQ. Red bars illustrate a higher PIQ and blue a higher VIQ. On a group basis there is no significant difference between the performances (PIQ is a point higher) – but sixteen individuals have a significant difference ($p<0.05$) between their performance of the two subsets of scales (* on the above graph). Ten of these have a higher PIQ than VIQ.

The dominance of verbal or picture IQ could give an indication of lateralisation versus generalisation in the JME cohort. 16/39 had a significant difference between their VIQ and PIQ scores (10 exhibiting a higher PIQ than VIQ; 6 higher VIQ than PIQ), (figure 6.25). There was no significant difference between the age and sex of the groups. There are only three left hand dominant cases and they were all in the equal PIQ and VIQ category. The table below provides mean scores and the results of an unpaired t-test between the groups. As there are an extreme number of comparisons here I used a more extreme alpha statistic then normal; * equates to a p statistic between 0.01 and 0.001 and ** to a p-value below 0.001.

What the table clearly shows is that consistently the group without a VIQ or PIQ dominance (equal group) performs least well on the WAIS and WMS tests. The working memory of this

group was particularly poor. The PIQ dominant group being larger than VIQ can demonstrate significance in some comparisons (such as FSIQ) despite the difference being less than the equal group and VIQ. PIQ had the best processing speed results. The VIQ group had the highest FSIQ, the least anxiety symptoms and a much stronger auditory than visual memory for both immediate and delayed information.

The VC-PO comparison is an extreme version of VIQ versus PIQ; VC being a purer verbal composite and PO a pure index of perceptual abilities. However the comparisons between VC and processing speed show just how skewed the three groups can be in terms of ability. The superior PO in the PIQ group accounts for their vastly superior PO versus WM results. The comparison between immediate and delayed visual memory corroborate the original categorisation.

	Picture IQ	Equal	Verbal IQ	PIQ v E	E v VIQ	PIQ v VIQ
Sum of VIQ scales	56	52	76			
VIQ	95	92	116		*	*
Sum of PIQ scales	58	43	50			
PIQ	110	91	99	*		
FSIQ	101	91	108	*		
WM WAIS	110	90	111			
PS	107	87	96	*		
AI	100	97	109			
VI	104	92	92			
HADS A	12	11	6			*
HADS D	4	9	5			
VC-PO	-21	-2	11	**	*	**
VC-PS	-14	7	19	**		**
PO-WM	12	6	-7			**
AI-VI	-4	5	16			*
AD-VD	3	9	19			

Table 6.18. PIQ v VIQ. * equates to a p statistic between 0.01 and 0.001 and ** to a p-value below 0.001.

Within the metastatistics a number of subscales showed very biased performances. For example matrix reasoning was one of the subtests on which the PIQ group excelled (mean score 13 versus 9 for equals and 10.8 in VIQ; p=0.0008 for PIQ versus equals); the other being digit symbol coding (PIQ versus equals 11 v 7, p=0.007). For VIQ dominant comprehension of the WAIS was the most discriminating test where they scored a mean of 13.7 versus 9 for both of the other groups.

6.4.4 EPQ-BV Subgroups

The use of EPQ-BV to create subgroups is limited by the fact that there are only twenty-one responses; with that caveat I split the group into three groups. I looked at the difference between EPQ-BV neuroticism and extrovertism scores and split the group into 15 points (or more) difference favouring extrovertism (n=5), 15 points or more difference favouring neuroticism (n=7) and not 15 points difference (n=9). Figure 6.26 below demonstrates the range of differences.

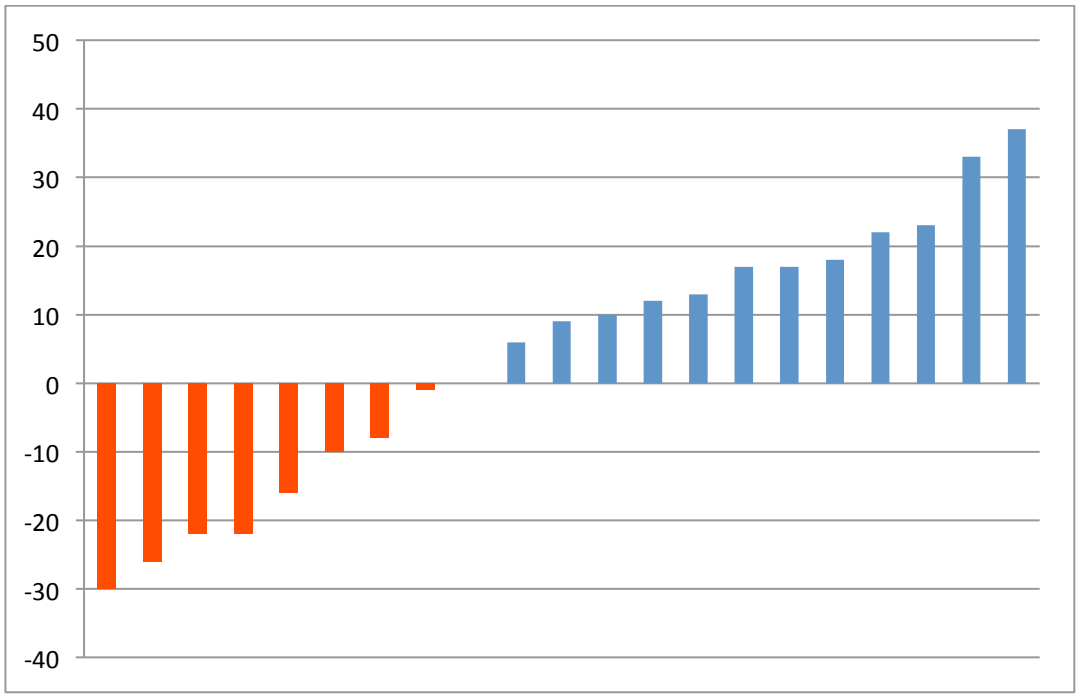


Figure 6.26 Histogram demonstrating the variation in EPQ-BV scores between participants. Red scores (left most) have the highest difference between high extrovertism and low neuroticism scores; blue bars show higher neuroticism than extrovertism scores.

Table 6.19 below clearly demonstrates that those with higher extrovertism than neuroticism scores performed better across a range of psychological tests including the WAIS and BADS. Across the WMS (not shown) extroverts scored equally with the equal group and both better than the neurotic subgroup – e.g. GM 108, 108 and 94. This pattern was seen across the D-KEFS tests (again not shown) – the sum of trails scaled score was 10.0 for extroverts, 9.0 for the equal group and 6.7 for the neurotic group. The small sample sizes undoubtedly contribute to the inability to determine statistical significance here. Nonetheless some sub tests did reach significance levels (as shown in table 6.19).

People with drug resistant JME and higher neuroticism than extrovertism scores had lower VIQ and PIQ subtest scores; comprehension (VIQ) and digit symbol coding (PIQ) were particularly discriminatory. They also had lower scores on working memory, processing speed and a lower BADS standardised score (indicating higher levels of executive dysfunction). They also had higher levels of both anxiety (unsurprisingly) and depression and were much more affected by neurotoxicity symptoms. Particularly people with drug resistant JME and higher neuroticism scores self-reported higher fatigue scores, greater slowing, poorer concentration and greater motor difficulties.

	Extrovert	Equal	Neurotic	Extro v Equal	Equal v Neurot	Neurot v Extro
Sum of VIQ scales	66	62	50		***	***
VIQ	105	102	89			*
Sum of PIQ scales	55	49	43			*
PIQ	107	99	91			
FSIQ	105	101	89			
WM WAIS	106	100	92			
PS	110	96	87			*
BADS	103	90	81			
HADS A	6	10	14		*	***
HADS D	2	9	12	*		**
ABNAS –fatigue	2	7	11		*	***
ABNAS –slowing	3	7	11			**
ABNAS –memory	3	6	9			*
ABNAS – concentration	2	4	8		*	**
ABNAS –motor	1	3	4			**
ABNAS –language	3	4	5			

Table 6.19. EPQ-BV subtypes. Extrovert ('extro', people scoring 15 points or more on EPQ-BV E, than EPQ-BV N), Neurotic ('neurot', people scoring 15 points or more on EPQ-BV N, than EPQ-BV E), equal (not a 15 point difference between tests scores). * significance at 0.05 level, ** significance at 0.01 level, *** significance at 0.005 level.

6.5 Conclusion

It is clear that within the limited number of studies available that people with JME exhibit considerable heterogeneity in executive dysfunction (De Toffol *et al.* 1997, Wandschneider

et al. 2012). This is further complicated by the fact that there are many forms of executive dysfunction. People all of whom could reasonably be described as dysexecutive, may exhibit markedly different profiles on tests – this has been comprehensively shown by the breadth of abnormalities demonstrated here. Also, neuropsychological tests are not sensitive to many forms of executive dysfunction. There is also a dislocation between test performance and real world executive ability, so a person who performs normally on a range of 'frontal' tests may appear quite abnormal in real life settings (Wood, Liossi 2007). This is where test batteries such as the BADS are helpful as their tests have greater ecological validity.

Psychology is at its most limited when used as a purely descriptive tool – but when coupled to other research methods such as advanced imaging (Wandschneider *et al.* 2012) or genetics it can help interpret patterns and produce mechanistic explanations. Therefore those participants who had comprehensive psychology in this chapter also had DNA extracted for advanced genetic projects. The analysis of copy number variation is explored in chapter seven.

Chapter Seven

Copy number variation

Copy number variation in JME and hyperekplexia

7.1 Introduction

It is recognised that certain phenotypes are more prone to large and frequent variations in copy number. Large (over 1000 kbp) deletions or duplications are seen more frequently in generalised genetic epilepsies than in focal genetic epilepsies and are over represented in autism and schizophrenia but less so in bipolar disorder. Some CNVs cluster in genomic regions producing so called 'CNV syndromes' – but the genotype/phenotype correlations of these are loose as many can be inherited from unaffected parents. They are without doubt of significance – but at most likely convey susceptibility - rather than are pathogenic in the way a point mutation can be in a gene of interest. As the CNVs delete or duplicate genomic regions which may not be gene rich it is tempting to speculate whether the susceptibility in many cases may be conferred by mechanisms controlled within the intron. In this chapter I will look at CNVs in people with JME – predominantly using SNP genotyping – but a small number also had CGH array. I will see whether people with drug resistant JME may have CNVs associated with CNV syndromes and whether rare CNVs may disrupt genes of interest.

As previously described in chapter four – between forty and fifty percent of cases with infantile onset-hyperekplexia remain unexplained depending on the case definition. It is also well established that indels and exomic deletions are common causes of hyperekplexia in non-Caucasian cases. Could larger deletions and duplications of known glycinergic genes be important in 'gene negative' hyperekplexia cases? Using CGH array techniques I will look to see whether I can identify CNVs which could be causative at a monogenic level – and also explore whether they may help explain some of the extended phenotype seen in certain genetically explained cases.

7.1.1 Chapter highlights

1. The identification of a novel *DMD* deletion identified in a woman with JME and low IQ; implicating her as a Duchenne's carrier.
2. The identification of a *H19* indel which is a plausible cause of Beckwith-Wiedermann syndrome in a child with co-existing hyperekplexia.
3. A novel pattern of 15q13.3 deletion producing a 15q13.3 CNV syndrome (with *CHRFAM7A* homozygosity) in a person with a severe BECCTs phenotype.
4. The deletion of transcription factor *SP1* in a person with familial genetic generalised epilepsy – this is predicted to cause reduced activation of neuronal potassium channels.
5. The identification of the possible cause of a severe hyperekplexia like phenotype in a child with premature death: creatine transporter deficiency syndrome.
6. The identification of Pitt-Hopkins syndrome as the likely diagnosis of an adult with a neurodevelopment syndrome thought to be hyperekplexia like.
7. Duplication in a gene strongly linked to malignant tachyarrhythmia (*NKX2-5*) in a young adult who died a SUDEP death.
8. The discovery of the 16p12.2 deletion CNV syndrome in someone with drug resistant JME. This CNV syndrome is associated with a neuropsychological phenotype in people with autism which - in the most part – she also shares.
9. Clustering of some rare CNVs in people with drug resistant JME – such as
    a. 12p13.31, 12p13.33 – disrupting *CACNA1C*

7.2 Results

In this chapter I will present first the results for CGH array and then the SNP genotyping. It is not feasible to present all the data on each potential CNV although each was studied in detail. Appendix H contains the analysis of CNVs which were scrutinised because they were near regions previously reported as 'pathogenic' in people with epilepsy. Appendix I contains the CNVs which are likely to be benign – but cannot be discounted and may need re-appraisal as we learn more about the role of the intronic regions. Appendix J contains the

results of the CNVs which clustered in a way which appeared to suggest an artefact of the technique and not a true CNV at all.

7.2.1 CGH Array

I will start by first describing the pattern of CNVs identified, then by looking at those which the *BlueFuse* software called as 'pathogenic', then at CNVs near previous CNVs called as pathogenic by previous authors, then by looking at CNVs which may involve genes of interest.

24 cases were selected for CNV analysis using competitive CGH array yielding 24 datasets. 12 CNVs were deemed to be pathogenic by the automated *BlueFuse Multi* software, but a further 176 were called as 'unknown' – 27 of which were recurrent. Of the unknowns 22 were on chromosome 1, 18 on Ch 2, 3 on Ch 3, 6 on Ch 4, 5 on Ch 5, 12 on Ch 6, 8 on Ch 7, 2 on Ch 8, 1 on Ch 9, 5 on Ch 10, 8 on Ch 11, 3 on Ch 12, none on thirteen, 6 on Ch 14, 4 on Ch 15, 8 on Ch 16, 5 on Ch 17, 2 on Ch 18, one on Ch 19, 6 on Ch20, none on twenty-one, 3 on Ch 22 but 46 on X and 2 on Y. This demonstrates the difficulty of analysing X chromosome copy number variations; 26% of all potential CNVs in this study. Table 7.1 below gives the briefest of phenotypic description with which cases had automated calls of 'pathogenic' CNVs. These CNVs will be discussed in detail below in the order they appear in the table.

Case	Sex	Phenotype	Pathogenic
S1	M	Noonan's and JME	
S2	F	JME and LD	Xp21.1
S3	M	*GLRA1* Hyperekplexia and LD	11p15.5
S4	M	*GLRA1* del plus LD	8p22
S5	M	Possible seizures, early death	
S6	M	IGE and LD, SUDEP	
S7	M	Jeavon's syndrome and Di George's	22q11.21
S8	F	JME and LD	
S9	M	IGE and LD	

S10	M	Atypical BECCTS and LD			
S11	F	GEFS+ and LD	9q12		
S12	M	LD and myoclonic epilepsy	2q11.1	Xp22.33	Xp22.33
F1	F	AiW			
F2	M	AiW, Epilepsy, LD			
F3	F	AiW			
F4	F	IGE			
F5	F	IGE			
F6	F	IGE			
F7	F	Unaffected	5p13.2	22q11.22	
F8	F	TLE			
F9	F	TLE	22q11.22		
F10	F	Unclassified epilepsy	9q12		
F11	M	Unclassified epilepsy			
F12	F	TLE	15q26.2		

Table 7.1 The brief phenotypes of the cases with the 'automated pathogenic' calls. BECCTS (benign epilepsy of childhood with centro-temporal spikes), AiW (Alice in Wonderland phenomena), TLE (temporal lobe epilepsy).

7.2.1.1 'Pathogenic' CNVs

7.2.1.1.1 Case S2 is a carrier for muscular dystrophy

A deletion of exons 54-56 within a single allele of the dystrophin gene was identified in a female with JME. Although this CNV appears to be novel (not seen in either of the online dystrophin CNV repositories) it is likely that it would be pathogenic; there are similar CNVs in exons 50 to 60 that are of a similar size that are causative and at 141kbp it is not an insignificant loss – removing two exons (figure 7.1). The Leiden Muscular Dystrophy pages (www.dmd.nl/nmdb2/home.php?select_db=DMD_d) identify 32 CNVs which include exon 54, 55 which include exon 55 and 11 that include exon 56; clearly some CNVs include all three. It is possible therefore to speculate that the loss of exons 54 and 55 are either biologically more plausibly lost due to an inherent instability – or (as is more likely) more prone to producing a DMD phenotype.

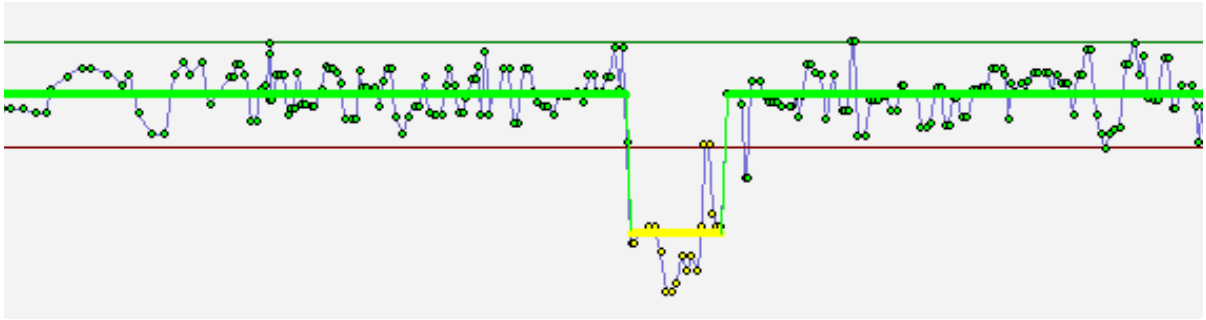


Figure 7.1 Log2 Ratio chart of CGH probes demonstrating DMD gene deletion in case S2

Her physical examination was remarkable for her short stature (four foot eleven), bilateral hearing aids and a webbed appearance of her toes (syndactyly). However it was not my practice to ask specifically for symptoms of myopathy or exercise tolerance during the semi-structured interview – and I remain open to the suggestion that she may have mild symptoms as a manifest carrier. Similarly she was not previously diagnosed with any learning disability – however her full scale IQ was calculated at 64. This result was deemed to be clinically significant and in keeping with our ethics and consent procedure, we felt this result needed to be verified by a diagnostic laboratory and the returned to the patient and her children. It would be predicted that any male offspring would have a 50% chance of developing a Duchenne or Becker muscular dystrophy phenotype. Follow-up since this result has lead me to understand that she has a son who is symptomatic. I am very grateful for the assistance of the clinical genetics department at the Institute of Medical Genetics, University Hospital of Wales in this regard.

7.2.1.1.2 Case S3 was identified as having a small deletion at 11p15.5

This microdeletion (428bp) is within *H19* gene; technically this is too small to be called a CNV and would be called an indel. *H19* is a maternally imprinted expressed transcript (which is a non-protein coding RNA). It functions as a tumour suppressor and is located in an imprinted region of chromosome 11 near the insulin-like growth factor 2 (*IGF2*) gene. Expression of this gene and *IGF2* are imprinted so that this gene is only expressed from the maternally-inherited chromosome, and *IGF2* is only expressed from the paternally-inherited chromosome. A region of paternal-specific methylation upstream of this gene is required for

the imprinting of these genes. Mutations in this gene are associated with Beckwith-Wiedermann Syndrome and Wilms tumorigenesis.

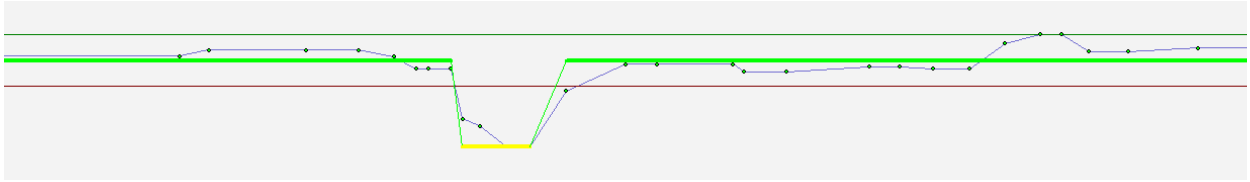


Figure 7.2 Log2 ratio zoomed to whole of H19 gene for sample S3.

A male with hyperekplexia secondary to a *GLRA1* mutation was included for CNV analysis because of a number of atypical features that could not be explained by the *GLRA1* mutation alone. As described in chapter four *GLRA1* hyperekplexia has quite a limited spectrum of comorbidity and learning difficulty is rare. In addition this child was noted to be unusually large - over the 99th centile for both height and weight at the ages 1, 3 and 6. DGV only has a single deletion here (a much larger CNV) in all its databases.

Chromosome 11p15 imprinting is well recognised as important for human growth with both Silver-Russell and Beckwith-Wiedermann syndromes (SRS, BWS) linked to mutations here. The region is particularly complex with imprinting making interpretation of copy number variations here particularly difficult. Begemann (2012) and colleagues correctly suggest that the size of the CNV, the positions of the breakpoints, parental inheritance and the imprinting status will all influence pathogenesis.

H19 is maternally imprinted and acts as a tumour suppressor on the *IGF2* gene. There are two different imprinting control regions (ICR) called ICR1 and ICR2 at 11p15.5. Interestingly both BWS – an overgrowth syndrome and SRS one of severe growth restriction are linked to errors in these areas. In addition to large stature, cases also exhibit exomphalos and macroglossia; less frequently cardiomyopathy and embryonal tumours such as Wilms' tumour are seen. It can be difficult to identify by phenotype alone. It is most commonly (50% of cases) caused by mutations that produce ICR2 hypomethylation but uniparental disomy at 11p15 is also an important cause. It is most commonly sporadic in its occurrence.

The CNV I present is a 428bp deletion at 11: 1973286-1973713– which is predicted to delete part of exon 5 (34bp), the intron 5-6 and 314bp of exon 6 including the 3' end of the gene. The *H19* gene is very small (729bp) and so this is a significant CNV in the context of this gene.

A recent review (Begemann *et al*. 2012) brought together CNVs at ICR1 and 2 causing both SRS and BWS phenotypes. They report two SRS case with a duplication affecting *H19*: one of 562 kb, the other of 596kb. They argue that increased *H19* transcription would bring about a large reduction in *IGF2* – producing the SRS phenotype. By extension a pathogenic deletion within *H19* could be expected to produce a BWS phenotype. Both of their cases inherited this duplication maternally, a paternally inherited duplication of 562kb was reported in Demars *et al.* 2011 as not producing either phenotype. Gronskov *et al*. 2011 reported a single case of a paternally inherited deletion removing all of H19 – but also the H19 differently methylated region and other bases in ICR1 – curiously associated with a SRS phenotype.

Mice with a loss of *H19* function express an overgrowth phenotype similar to babies with BWS (Leighton *et al.* 1995a). This has led researchers to suggest that perhaps the only function of *H19* RNA expression is to regulate the expression of *IGF2* (Insulin Growth Factor 2 (Leighton *et al.* 1995a). Overexpression of *IGF2* can be responsible for overgrowth, and generally, *IGF2* is expressed in the absence of *H19*.

Identifying whether this CNV may be pathogenic will involve methylation specific studies and ideally analysis of parental samples. I am actively attempting to recruit the parents through local services in Australia. Currently available tests can identify methylation errors at the *H19* differently methylated region. The hypermethylation of the *H19* promoter on the paternal allele plays a vital role in allowing the expression of the paternal allele of *IGF2*. A reason for the close coupling of *H19* and *IGF2* expression may be that they share the same 3' gene enhancer. It has been suggested that *H19* is preferentially activated by the 3' enhancer instead of *IGF2* because *H19* has a stronger promoter than *IGF2* and that the *H19* gene is physically closer to the 3' enhancers than the *IGF2* gene (Leighton *et al.* 1995b).

7.2.1.1.3 Case S4 was identified as having a deletion in *MSR1*

Case S4 has a 69,674bp deletion at 8p22. A single gene is affected in this microdeletion – *MSR1* (macrophage scavenger receptor 1). OMIM links this gene to both Barret's oesophagus / oesophageal adenocarcinoma and hereditary prostate cancer. The macrophage scavenger receptors include three different types (1, 2, 3) generated by alternative splicing of this gene. These receptors are integral membrane glycoproteins and have been implicated in many macrophage-associated physiological and pathological processes including atherosclerosis, Alzheimer's disease, and host defence.

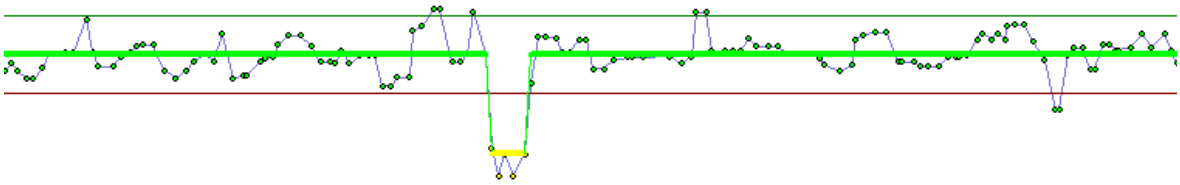


Figure 7.3 Log2 Ratio showing *MSR1* gene deletion in case S4

The deletion (8: 15996412-16066086) would nearly delete the whole gene (8 : 15965387-16050300) which has a low haplotype index (15.2%) which suggests that it may be pathogenic when only one allele is present. Six similar deletions are seen in DGV. S4 is male and so we should consider prostate cancer risk here. The converging evidence comes from deletions in prostate cancer cells (Latil and Lidereau, 1998), linkage studies of hereditary prostate cancer families (Xu *et al*., 2001) and from sporadic cases too. *MSR1* mutations were seen five times more frequently in men with sporadic prostate cancer than those who were unaffected.

7.2.1.1.4 Case S7 had his chromosome 22q11 deletion confirmed

Case S7 was known to have DiGeorge's syndrome and was included as a positive control. CGH array identified a large (2,560,388bp) deletion at 22q11.21 which would reduce 59 genes to heterozygosity: *DGCR6, PRODH, DGCR2, TSSK1A, DGCR14, TSSK2, GSC2, SLC25A1, CLTCL1, HIRA, MRPL40, C22orf39, UFD1L, CDC45L, CLDN5, SEPT5, GP1BB, TBX1, GNB1L, C22orf29, TXNRD2, COMT, ARVCF, C22orf25, MIRN185, DGCR8, MIRN1306, TRMT2A,*

RANBP1, ZDHHC8, RTN4R, MIRN1286, DGCR6L, GGTLC3, TMEM191C, PI4KAP1, RIMBP3, SUSD2P2, SUSD2P1, USP41, ZNF74, SCARF2, KLHL22, MED15, FAM108A5, POM121L4P, PI4KA, SERPIND1, SNAP29, CRKL, AIFM3, LZTR1, THAP7, P2RX6, SLC7A4, MIRN649, P2RX6P, POM121L7 and *POM121L3P*. This CNV is one of the largest identified in this project. The *TBX1* gene is considered to be the major candidate gene for the main features in 22q11.2 deletion syndrome, including congenital heart malformations and craniofacial abnormalities. This CNV is in keeping with the size of reported CNVs causing DiGeorge's (around 3 megabases) and very much falls across the classic CNV region as describes on the DECIPHER database.

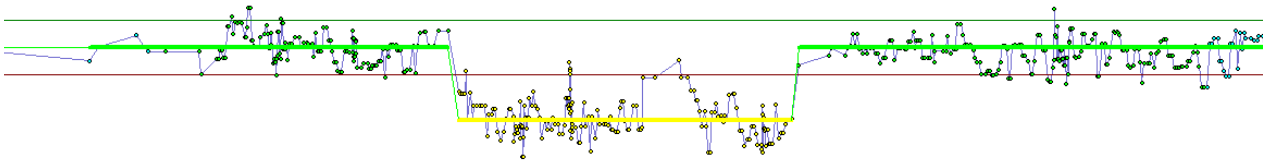


Figure 7.4 Log2 ratio demonstrating the large 22q11 deletion in case S7

Unlike psychosis and schizophrenia which have been strongly linked to CNVs here, epilepsy has not had its relationship well characterised. Of the 348 people with 22q11.2 deletion identified by Kao and colleagues (2004) in Philadelphia, 27 (7%) had unprovoked seizures and these were thought to be generalised seizures. Lemke and colleagues (2009) reported a child with JME and a terminal 22q11 deletion. She had a 2.5mb deletion confirmed with MLPA of thirty genes between *CDC45L* to *LZTR1*; her CNV fits entirely with this one presented in S7. The genes which may cause the neurological phenotypes in DiGeorge are not fully understood. It is possible that they are not within the 22q11 region but nearby; Berkovic *et al.* (2004) described families with focal epilepsy with linkage evidence to 22q12.

Of the 59 genes lost in this CNV– a number are of interest. *SLC25A18* is a candidate gene for epileptic encephalopathy and its neighbour *ATP6V1E1* was identified by next generation sequencing as potentially an epilepsy pathway gene (appendix F). *SLC25A18* encodes for a glutamate carrying, solute carrier family 25 (member 18), and *ATP6V1E1* for the H^+ transporting ATPase, lysosomal V1 subunit E1. *SLC25A18* (in keeping with many solute carriers tolerates haploinsufficiey well and has a haplotype index of 65%. *ATP6V1E1* (with an

indeterminate haplotype index of 49%) is an enzyme that appears to be important for synaptic vesicle proton gradient generation. Su *et al.* (2004) have found that it is heavily expressed in nervous tissues. A further number of genes had a very low haplotype index suggesting they are more likely to be pathogenic when heterozygous – these include *HIRA* (HIR histone cell cycle regulation defective homolog A, 2.3%), *CDC45* (cell division cycle 45 homolog, 0.8%) and *CECR6* (cat eye syndrome chromosome region, candidate 6 ,3.6%). The role of *HIRA* is not fully elucidated however it is expected to play a role in transcriptional regulation and chromatin and histone metabolism. Some authors have considered it central to the 22q11 deletion phenotypes. A little more is speculated about *CDC45* function. It is a member of the highly conserved mini-chromosome maintenance proteins. The protein has been shown to interact with *MCM7* and DNA polymerase alpha. Studies of the similar gene in *Xenopus* suggested that it may play a pivotal role in the loading of DNA polymerase alpha onto chromatin. With regards to *CECR6*, cat eye syndrome is characterised by coloboma and other dysmorphic features. It is a candidate gene for this syndrome but very little is known about its function or proteome.

A large deletion at 9q12 in case S11 **-** Please see appendix I - likely artefact

7.2.1.1.5 A large pericentromeric deletion at 2p11.1-2q11.1 in S12

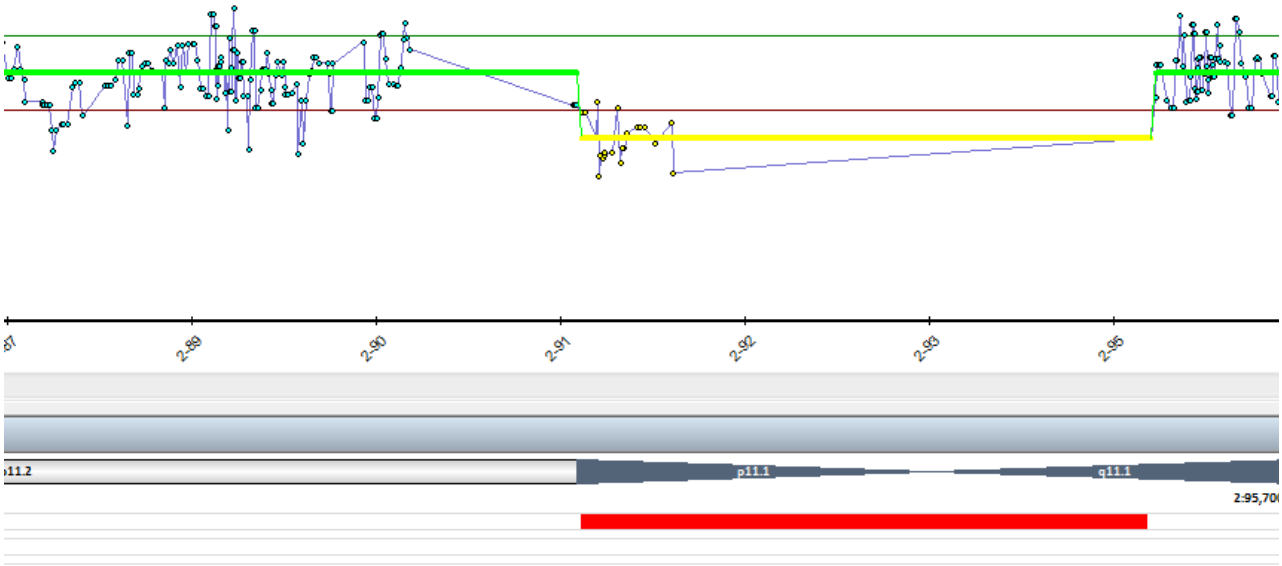


Figure 7.5 (previous page) The Log2Ratio (top) shows the probes and the estimated copy number, the cytobands and centromere are shown below that and the CNV is shown in red.

It is difficult to know how much of this deletion is artefact and how much may be genuine. The loss of the centromere would be deleterious. The size and the co-ordinates of this CNV may not be accurate due to a lack of CGH probes at the centromere of chromosome two. The weighted log2 ratio graph (figure 7.5) strongly suggests a deletion at p11.1 – but then there are no probes until q11.1 to suggest when this CNV ends; it is unlikely that the CNV is in fact 3,696,822bp – and indeed the centromere may not be fully deleted here. S12's 2p11.1 deletion would directly remove four proposed pseudogenes *ACTR3BP2, GGT8P, LOC654342* and *DRD5P1.* The nearest protein genes to the deleted 2p area (2: 91,034,048-94,730,870) are *RPIA* (ribose 5-phosphate isomerase A at 88991176-89050452) – essential in carbohydrate metabolism and *EIF2AK3* (eukaryotic translation initiation factor 2-alpha kinase 3). These two genes are linked to two OMIM phenotypes, the former to Ribose 5-phosphate isomerase deficiency and the latter to Wolcott-Rallison syndrome. The first is a progressive leucoencephalopathy and the second epiphyseal dysplasia with early onset diabetes.

The 2p11.1-2q12.2 locus has been of interest to groups looking for the cause of familial cortical myoclonic tremor with epilepsy – an autosomal dominant condition. The second locus (FAME2) is in this region (Saint-Martin *et al.* 2008) and yet no mutation in the region's genes has proven to be causative. It is very plausible that there is an intronic microRNA that may be pathogenic to both phenotypes. Our case (S12) may well have autosomal dominant epilepsy, his pedigree is shown in figure 7.6.

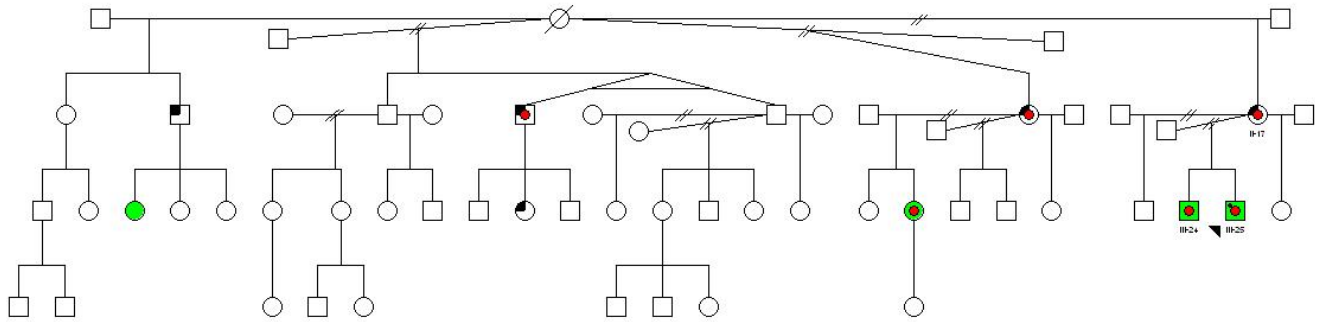


Figure 7.6 (previous page) Pedigree of case S12. Green figures (epilepsy), red dots (migraine), black quadrants (febrile seizures). If this was autosomal dominantly inherited then we have failed to ascertain the seizure history from the deceased matriarch in the first line of the pedigree. The proband (S12) is highlighted with an arrow.

What is the importance of the Xp22.33 duplications for S12?

The duplication of *SHOX* appears to be well tolerated and not related to either the phenotypes published, nor likely to cause his epilepsy. The CNV is discussed in appendix I.

A 5p13.2 deletion in case F7 is intronic

This is described in appendix I - likely benign CNV

What are the implications of 22q11.22 duplication?

22q11.22 duplication (as opposed to the terminal deletion) appears to be much better tolerated and is described in appendix I.

7.2.1.1.6 The 15q26.2 deletion affects *NR2F2* in F12 – could it cause hippocampal sclerosis?

15: 94674054- 94676153 a 2,099bp loss in gene *NR2F2.* DGV shows four duplications and two deletions in this region. The gene is a member of the nuclear receptor subfamily 2, group F, which encodes a member of the steroid thyroid hormone superfamily of nuclear receptors. The encoded protein is a ligand inducible transcription factor that is involved in the regulation of many different genes.

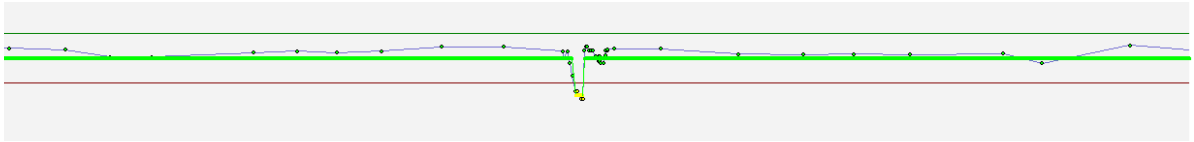


Figure 7.7 Log2 ratio around *NR2F2* gene in case F12

This deletion would result in the loss of the 5' region and the majority first exon of *NR2F2*. Exon one is 1,830bp long and the CNV stops 627bp before the end of exon one. It has a remarkably low haplotype index of 4.9% and there are very few overlapping CNVs described (3 gains in DGV). There are 13 overlapping CNVs in DECIPHER of the 7 which had limited phenotype data five mentioned learning disabilities, most dysmorphic features including microcephaly and none epilepsy. Case F12 is subtly dysmorphic with microcephaly and temporal lobe epilepsy with hippocampal sclerosis.

NR2F2 is not expressed widely in brain tissues but is preferentially expressed in the caudal ganglionic eminence. Recently Tang *et al.* (2012) generated mouse mutants to study the role of *NR2F2* in telencephalon development. The mutant mice had severe defects in the formation of the amygdala complex, including the lateral, basolateral and basomedial amygdala nuclei. Further work demonstrated that caudal ganglionic eminence derived cells failed to settle in the basomedial amygdala nuclei owing to reduced expression of neuropilin 1 and 2 (two semaphorin receptors that regulate neuronal cell migration and axon guidance) coded for by Nrp1 and Nrp2. These two genes are the direct targets of *NR2F2*.

If *NR2F2* affects neuronal cell migration it becomes a very plausible candidate gene for learning difficulties and microcephaly. If it also affects amygdala assembly and the creation of the forebrain – it could plausibly be indicated in faulty neuronal development at the hippocampus and liability to hippocampal sclerosis and temporal lobe epilepsy. Furthermore Fuentealba *et al.* (2010) identified that in the dorsal hippocampal CA1 area in rats, *NR2F2* was restricted to GABAnergic interneurons and expressed in several, largely non-overlapping neuronal populations. They concluded by saying that the characterisation of *NR2F2* expressing neurons "suggests that this developmentally important transcription factor plays cell type-specific role(s) in the adult hippocampus."

7.2.1.2 Epilepsy Associated CNVs?

After investigating the 'pathogenic' CNVs – I then looked to see which CNVs mapped to cytobands where epilepsy associated CNVs had been previously reported. 107 matching CNVs were identified (table 7.2 below) an average of 4.5 per case. Clearly they could not all

be pathogenic – but each deserved to be investigated in detail with particular attention as to i) whether the CNV overlaps the epilepsy associated CNV, ii) whether the CGH array call was a convincing one and iii) genes of interest in the regions. If the CNVs were unlikely to be pathogenic the fuller descriptions are in appendices H, I and J.

CNV	Cases
1p21.1 duplication	F4
1q21.1 deletion	S1, S2, S3, S5, S7, S12, F9
2p11.2 deletion	F7
3q26.1 duplication	S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, F4, F9, F10
4p16.3 duplication	F3
5p15.33 deletion	S1, S4, S5, S7, S10, S12, F12
5p15.33 duplication	F8, F10, F11
5q13.2 deletion	S2, S8
6p21.32 deletion	S6, S7, F1, F9, F6, F10
6q22.31 duplication	S8, F11
7q35 deletion	S1
7q36.3 duplication	F3
8p23.1 deletion	F8
8p23.1 duplication	S4
12p13.31 deletion	S8, F5, F8
12p13.31 duplication	S1, S7, S9, F2, F10, F12
12q24.33 deletion	F12
14q11.1-q11.2 deletion	S9, S10, S12, F2, F4, F9, F11
14q11.1-q11.2 duplication	S5, S8, F12
14q32.33 deletion	S3, S11, S12, F4, F5, F6, F7, F9
15q11.2 deletion	S2, S3, S7, S8, S12, F4, F5, F8, F9
15q11.2 duplication	S11, F3, F6, F7, F8, F10, F11, F12
15q13.2, 15q13.3 deletion	S10
16p11.2 deletion	S2, S5
16p11.2-p11.1 duplication	S7, S8, S9, S11, F8, F9, F10, F12
16p13.11	F7
17p11.2 deletion	S4
19q13.31 deletion	S5

Table 7.2 CNVs identified by CGH array that correspond to cytobands where previously reported CNVs were published as associated with epilepsy.

1q21.1 deletion, duplication

Likely to be a benign and recurrent CNV – appendix I.

2p11.2 deletion

Likely to be a benign and recurrent CNV – appendix I.

3q26.1 duplication

This may well represent a recurrent CNV of uncertain significance – appendix H.

4p16.3 duplication

This may well represent a recurrent CNV of uncertain significance – appendix H.

5p15.33 deletion and duplication

These may well represent a recurrent CNV of uncertain significance – appendix H.

5q13.2 deletion

These deletions may well represent artefact due to the peri-centromeric position of the 'CNV' – appendix H.

Six deletions at 6p21.32

These may well represent a recurrent CNV of uncertain significance, likely to be benign– appendix I.

7.2.1.2.1 Does the intronic 6q22.31 duplication affect splicing in *NKAIN2?*

Two cases have CNVs at 6q22.31. The epilepsy associated duplication is at 6:123581123-124208500, however the CNV in case S8 is a deletion at 119729388-119791615 of 62,227bp (no genes) as opposed to F11 which is a duplication much nearer the reported CNV at 124 478246-124505043 (26,797bp). This CNV includes *NKAIN2* which encodes the transmembrane Na^+/K^+ transporting ATPase interacting 2 protein which interacts with *ATP1B1* (the beta subunit of Na^+/K^+ ATPase) which is highly expressed in nervous tissues and has been linked previously to epileptogenesis. *NKAIN2* has a low haplotype index of 30%. The other gene directly duplicated by the previously reported CNV is *TRDN* (triadin) thought to have a role in skeletal muscle; but that CNV also impairs *NKAIN2.* Although this CNV is intronic (within intron 1-2) – there is no overlap in DGV (8 partially overlap) and only five overlapping cases in DECIPHER.

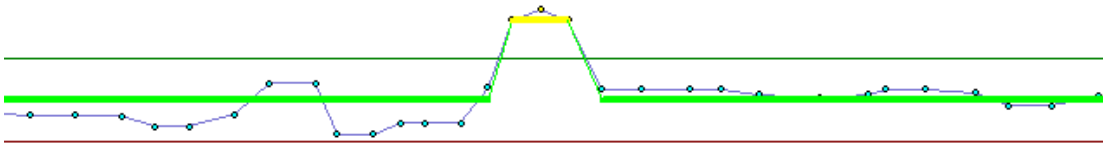


Figure 7.8 Weighted log2 ratio demonstrating duplication in the *NKAIN2* gene seen in F11

The Na+ K+ ATPases have often been quoted as perhaps explaining some of the shared heritability between epilepsy and migraine. They are integral membrane proteins that actively exchange ions across cell membranes; the energy required for this process is derived from hydrolysis of one molecule of ATP. Mutations affecting the $\alpha 2$ subunit of the Na^+, K^+-ATPase, which is expressed in astrocytes, is linked to familial hemiplegic migraine type 2 (FHM2); mutations affecting the $\alpha 3$ subunit, which is expressed in neurons, are linked to rapid onset dystonia and parkinsonism. The β subunit is heavily glycosylated and there are three b isoforms; of which $\beta 1$ is expressed by neurones. The β subunit targets the α subunit to the plasma membrane and modulates its transport functions. (Benarroch 2011). Of interest in F11's family there is autosomal dominant epilepsy and migraine (figure 7.9).

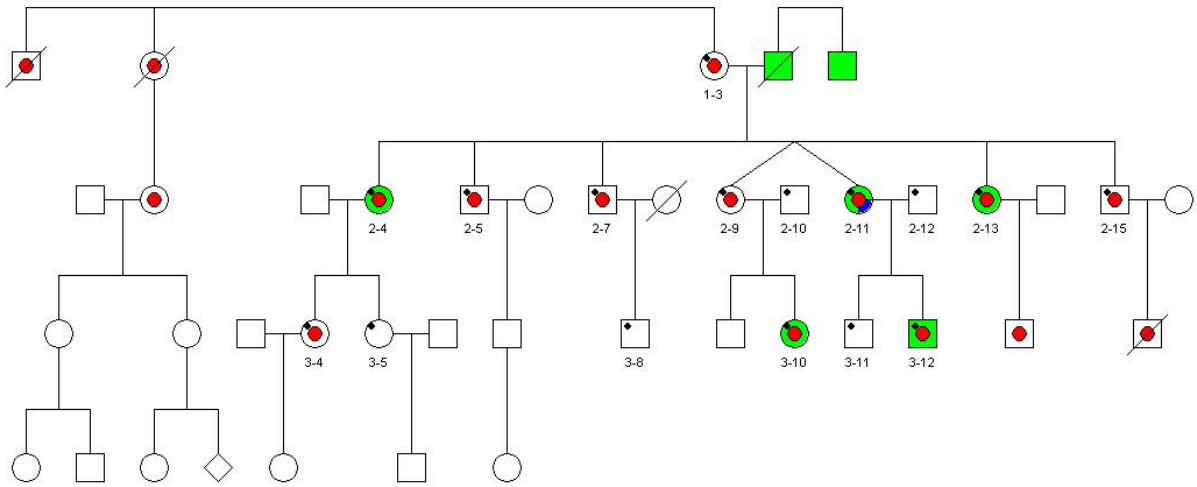


Figure 7.9 Pedigree above – epilepsy (green), migraine (red circles) – proband (arrow).

7q35 deletion

This CNV is likely benign and is in appendix I.

7q36.3 duplication

These may well represent recurrent CNVs of uncertain significance – appendix H.

8p23.1 deletion

These may well represent recurrent CNVs of uncertain significance – appendix H.

12p13.31 deletion / duplication

These may well represent recurrent benign CNVs– appendix I.

12q24.33 deletion

These may well represent recurrent benign CNVs– appendix I.

14q11.1-q11.2

These may well represent recurrent benign CNVs– appendix I.

14q32.33 deletion

These may well represent recurrent benign CNVs– appendix I.

15q11.2 deletion / duplication

This is discussed in appendix H – recurrent CNVs of unknown significance.

7.2.1.2.2 S10 harbours the recognised 15q13.2, 15q13.3 deletion

S10 has two deletions nearby at 15: 28294142-28606779 (312,638bp) and 15:30298126-30661360 (363,234bp). The first would reduce *CHRFAM7A* to heterozygosity, the second would delete *FAM7A1* and *FAM7A3;* both of the latter two are thought to be pseudogenes. The recognised 15q13.3 microdeletion syndrome is between 15: 30910306 -32445407 so just adjacent to these two CNVs. However the genes that they disrupt are likely to be relevant to epilepsy pathogenesis. *CHRFAM7A* codes for CHRNA7 (cholinergic receptor, nicotinic, alpha 7, exons 5-10). 15q13.3 is discussed further under JME samples below when the SNP genotyping results are described.

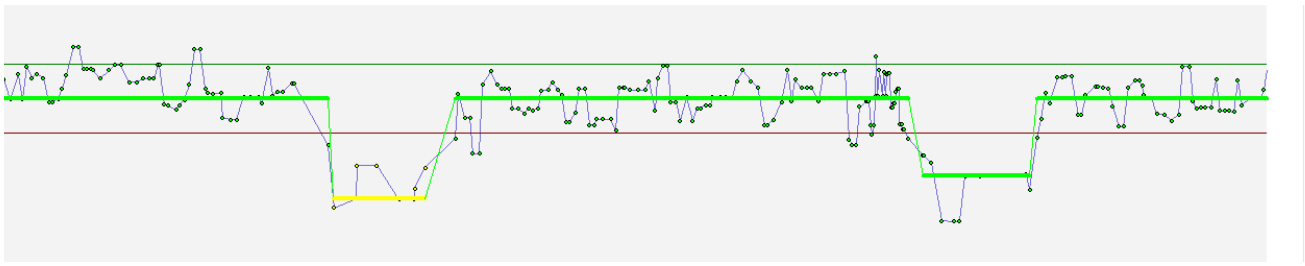


Figure 7.10 (previous page) Weighted log2 ratio of the two deletions at 15q13.2, 13.3 in case S10 at the area of the known microdeletion syndrome.

16p11.2 deletion/ duplication

These may well represent a recurrent benign CNV– appendix I.

A rare CNV is at 16p13.11

This may well represent a benign CNV and is distinct from the 16p13.11 CNV syndrome– appendix I.

17p11.2 deletion

This may well represent a benign CNV– appendix I.

19q13.31 deletion

This may well represent a benign CNV– appendix I.

In summary the published epilepsy CNVs did not occur in any great frequency in this sample. I will now discuss other CNVs which may harbour genes of interest to the sample.

7.2.1.3 Other CNVs of interest identified by CGH array

7.2.1.3.1 Three cases with a 11q13.1 deletion

There are three deletion CNVs at 11q31.1; the largest of which is seen in case S11 at 11: 64769762-65648716 (878,954bp). Of note is that two other individuals (both related to each other) also have 11q13.11 CNVs which differ (below). F7 has a shorter CNV that starts in the

same place whereas F8 starts later and then there is a second separate deletion following it (below).

As F7 is an unaffected relative one could conclude one of three things: i) the 11q13.1 deletion is a benign CNV or one not associated with epilepsy; ii) the CNV confers an increased epilepsy risk or is a modifier but other mutations are needed to create the phenotype; or iii) variations in the genes involved may help explain which are crucial for the epilepsy phenotype. The gene list below is extensive – but only twelve genes are seen in S11 and F8's CNV but not in F7: *FOSL1, C11orf68, DRAP1, TSGA10IP, SART1, EIF1AD, BANF1, CST6, CATSPER1, GAL3ST3, SF3B2* and *PACS1.*

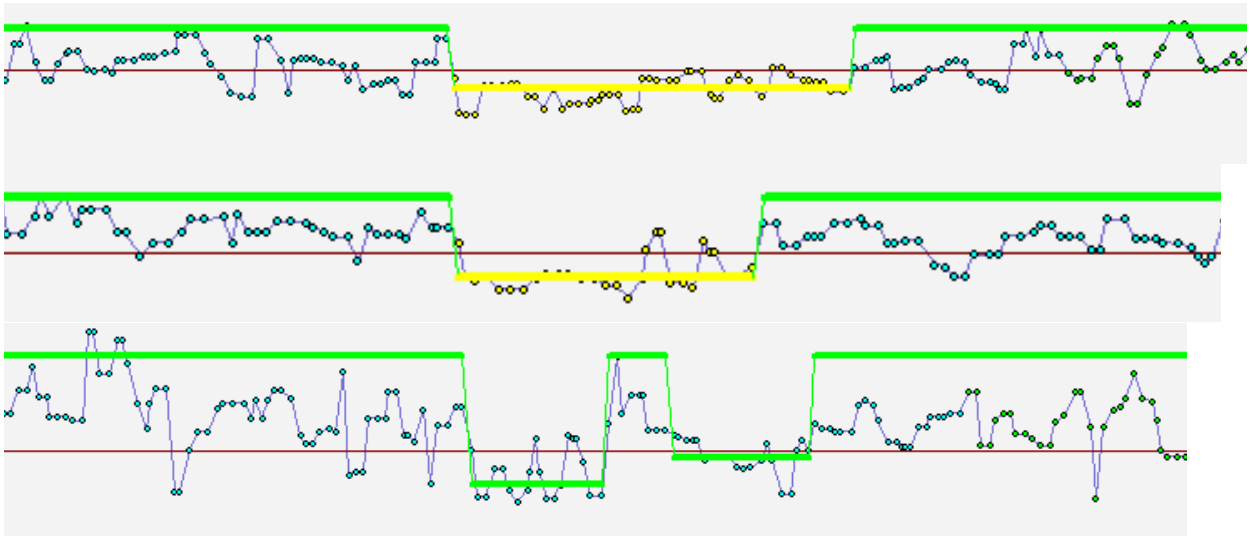


Figure 7.11 Three weighted log2 ratios showing the deletions at 11q13.1 in S11, F7 and F8 in that order.

S11	F7	F8	Name
POLA2	*POLA2*		Polymerase (DNA directed), alpha 2, accessory subunit
CDC42EP2	*CDC42EP2*		CDC42 effector protein (Rho GTPase binding) 2
DPF2	*DPF2*		D4, zinc and double PHD fingers family 2
TIGD3	*TIGD3*		Tigger transposable element derived 3
SLC25A45	*SLC25A45*		Solute carrier family 25, member 45;
FRMD8	*FRMD8*	*FRMD8*	FERM domain containing 8
MIRN612	*MIRN612*	*MIRN612*	microRNA 612
SCYL1	*SCYL1*	*SCYL1*	SCY1-like 1
LTBP3	*LTBP3*	*LTBP3*	latent transforming growth factor beta binding protein 3
SSSCA1	*SSSCA1*	*SSSCA1*	Sjogren's syndrome/scleroderma autoantigen 1

FAM89B	*FAM89B*	*FAM89B*	family with sequence similarity 89, member B
EHBP1L1	*EHBP1L1*	*EHBP1L1*	EH domain binding protein 1-like 1
SIPA1	*SIPA1*	*SIPA1*	signal-induced proliferation-associated 1
KCNK7	*KCNK7*	*KCNK7*	Potassium channel, subfamily K, member 7
MAP3K11	*MAP3K11*	*MAP3K11*	Mitogen-activated protein kinase kinase 11
PCNXL3	*PCNXL3*	*PCNXL3*	Pecanex-like 3
RELA	*RELA*	*RELA*	V-rel reticuloendotheliosis viral oncogene homolog A
KAT5	*KAT5*	*KAT5*	K(lysine) acetyltransferase 5
RNASEH2C	*RNASEH2C*	*RNASEH2C*	Ribonuclease H2, subunit C
OVOL1			Ovo-like 1
SNX32			Sorting nexin 32
CFL1			Cofilin 1 (non-muscle)
MUS81			MUS81 endonuclease homolog
EFEMP2			EGF containing fibulin-like extracellular matrix protein 2
CTSW			Cathepsin W
FIBP			Fibroblast growth factor (acidic) intracellular binding protein
CCDC85B			Coiled-coil domain containing 85B
FOSL1		*FOSL1*	FOS-like antigen 1; cell proliferation
C11orf68		*C11orf68*	Chromosome 11 open reading frame 68
DRAP1		*DRAP1*	DR1-associated protein 1
TSGA10IP		*TSGA10IP*	Testis specific, 10 interacting protein
SART1		*SART1*	Squamous cell carcinoma antigen recognized by T cells
EIF1AD		*EIF1AD*	Eukaryotic translation initiation factor 1A domain containing
BANF1		*BANF1*	Barrier to autointegration factor 1
CST6		*CST6*	Cystatin E/M
CATSPER1		*CATSPER1*	Cation channel, sperm associated 1
GAL3ST3		*GAL3ST3*	Galactose-3-O-sulfotransferase 3
SF3B2		*SF3B2*	Splicing factor 3b, subunit 2
PACS1		*PACS1*	Phosphofurin acidic cluster sorting protein 1

Table 7.3 Comparison of the three CNVs at 11q13.1 for cases S11 (affected), F7 (unaffected) and F8 (affected).

Of the genes that are seen in S11 – three could be thought to contribute an epilepsy phenotype. *SLC25A45* (solute carrier family 25, member 45) is a mitochondrial carrier protein and but it may not be neuronally expressed. The product of *KCNK7* (potassium

channel, subfamily K, member 7) has not been shown to be a functional channel. However *RNASEH2C* (ribonuclease H2, subunit C) has a recognised role in epileptogenesis - mutations in this gene cause Aicardi-Goutieres syndrome-3 an encephalopathy syndrome. This gene encodes a ribonuclease H subunit that can cleave ribonucleotides from RNA: DNA duplexes. These three individuals would be carriers for Aicardi-Goutieres syndrome – where bi allelic mutations are needed to produce the phenotype. Examples of genes which may cause a phenotype (from the above list) are those with a low haplotype index such as *POLA2* (13.7%), *DPF2* (25.1%), *PCNXL3* (13%), *RELA* (0.4%), *OVOL1* (22.8%) and *SART1* (6.2%).

POLA2 encodes the enzyme –beta subunit of DNA polymerase alpha and therefore has a role in DNA repair and replication; little is known about mutations in *POLA2*. *DPF2* encodes a member of the d4 domain family, characterized by a zinc finger-like structural motif. This protein functions as a transcription factor and possibly serves a regulatory role in rapid hematopoietic cell growth and turnover. This gene is considered a candidate gene for multiple endocrine neoplasia type I. *PCNXL3* has no known function at present. *RELA* encodes the transcription factor p65 but beyond exploring its proteome little more is known. *OVOL1* encodes a zinc finger containing transcription factor that is likely involved in hair formation and spermatogenesis. *SART1* encodes two proteins; one is expressed in the nucleus of proliferating cells – the other in skin cancers.

The 2q21.1 deletion in F6 is unlikely to be pathogenic

This is described in appendix I.

7.2.1.3.2 Does the CNV at 12q13.13 cause a potassium channelopathy?

A single CNV was seen in F6 at 12: 52083350-52139497, deleting 56,147bp which contains four genes. *SP1* (Sp1 transcription factor), *AMHR2* (anti-Mullerian hormone receptor, type II), *PRR13* (proline rich 13) and *PCBP2* (poly(rC) binding protein 2). There is a great deal of interest in the function of *SP1* including investigations about its role in epilepsy. Feng *et al.* (1999) identified that it increased in activity in rat hippocampi following induced seizures.

More recently (Mucha *et al.* 2010) demonstrated that *SP1* activates expression of both *KCNQ2* and *KCNQ3*. Mutations in the potassium channels *KCNQ2* and 3 have been implicated in idiopathic generalised epilepsies since 1999. Steinlein *et al.* 1999, Neubauer *et al.* 2008). If *SP1* activates expression - and *REST* (repressor element 1-silencing transcription factor) represses expression of both of these genes – then a loss of SP1 function would effectively reduce the expression of these channels.

This CNV is not seen in DGV and there was only one overlapping case in DECIPHER who was reported to have developmental delay – but this was a much larger CNV (12:48876965-53157968 – 4.28megabases). *SP1* has the lowest possible haplotype index (0.1%) suggesting that reduction to heterozygosity would certainly be pathogenic.

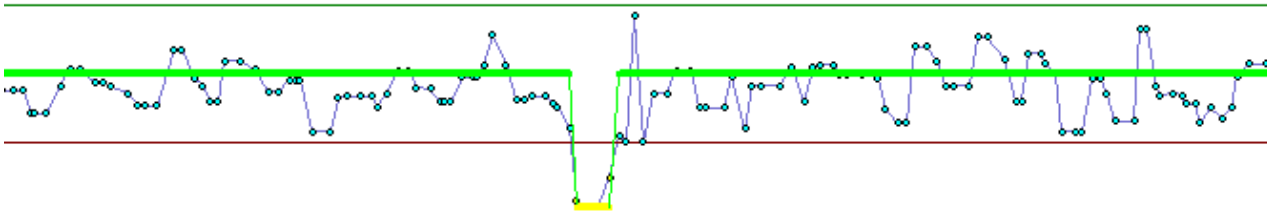


Figure 7.12 Weighted log2 ratio of case F6 at the 12q13 cytoband

F6 has an idiopathic generalised epilepsy. However F6 was included because of her relationship to F4 and F5 (both have a similar phenotype to F6); and they do not share this CNV. Their pedigree is shown below (figure 7.13).

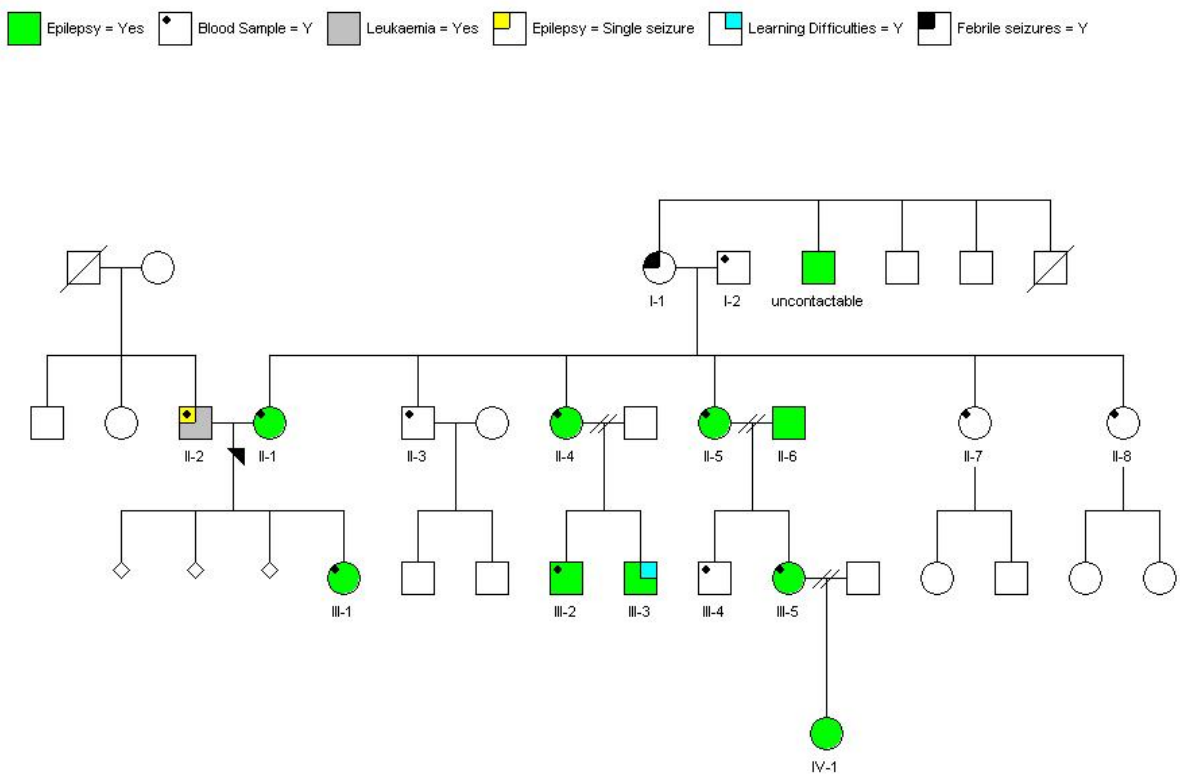


Figure 7.13 Pedigree of F6

7.2.1.3.3 Does S5 have creatine transporter deficiency syndrome?

Two unrelated cases both had X chromosome CNVs at q28 that involved *SLC6A8* (solute carrier family 6 (neurotransmitter transporter, creatine), member 8). S5's CNV is a deletion at X: 152610206-152611927 (1,721bp) and F8's is a nearby but not overlapping duplication at X: 152608145-152609984 of 1,839bp. It is important to state that S5 is male and F8 female. *SLC6A8* is unusual in that it has a low haplotype index (20.2%) and three of the JME cases have duplications CNVs overlapping it (J4, J23 and J27 – all female). It is the gene responsible for creatine transporter deficiency syndrome Valayannopoulos *et al*. 2012. This disorder is characterised by learning disability and epilepsy.

The deletion in S5 may be pathogenic: it would delete intron 3-4, exon 4, intron 4-5, exon 5 and 15bp of exon 6. Urinary creatine: creatinine ratio is increased in males with *SLC6A8* deficiency and creatine uptake in cultured fibroblasts is impaired. In the vast majority of males affected with *SLC6A8* deficiency, direct genomic DNA sequencing analysis reveals a

pathogenic mutation in *SLC6A8.* The definitive diagnosis is often based on at least two of the above-mentioned analyses. Since the creatine transporter defect is an X-linked disorder, in males a hemizygous mutation is expected, whereas in females heterozygous mutations are expected.

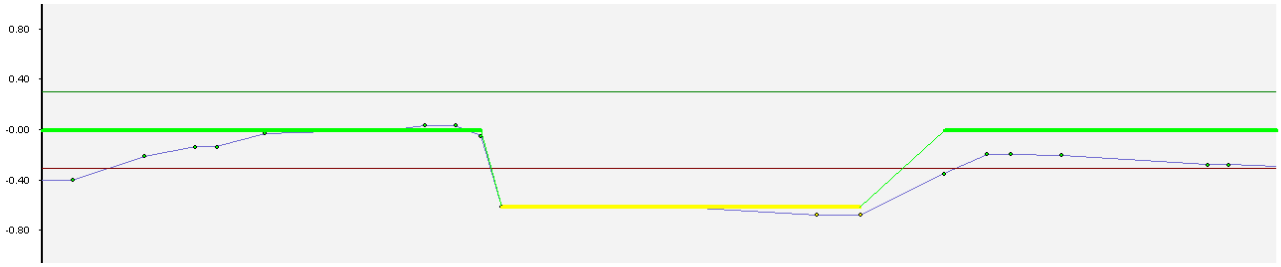


Figure 7.14 Weighted Log2 ratio for the *SLC6A8* gene deletion in case S5

S5 is a child who had recurrent neonatal onset seizures that proved difficult to control and the paediatric neurologists considered hyperekplexia amongst the differential diagnoses. The child unfortunately did not develop normally and died in early infancy. *SLC6A8* deficiency syndrome was first described in 2001 by Solomons *et al*.; since then a staggering 45 families (94 cases in total) have been published (Betsalel *et al.* 2011). The phenotype in affected males is incomplete (as many of these reports did not include sufficient phenotypic data) but it includes a spectrum of manifestations from mild intellectual disability and speech delay to severe intellectual disability, seizures, and behavioural symptoms that may become more marked during the course of the disease. Diagnosis normally is in the first years of life (now that it is better recognised) and for some life expectancy can be near normal.

Epilepsy is common in affected males but it is usually drug responsive, but cases of drug refractory epilepsy have been published (Almeida *et al.* 2006, Fons *et al.* 2009, Mancardi *et al.* 2007). A neuropsychological profile in four affected boys from two unrelated families from the Netherlands revealed hyperactive impulsive attention deficit and a semantic-pragmatic language disorder with oral dyspraxia (Mancini *et al.* 2005). Individuals with *SLC6A8* deficiency may also exhibit growth retardation, a degree of generalised muscular hypotrophy, dysmorphic facial features (such as broad forehead and flat mid-face), microcephaly, and brain atrophy on MRI (Mancini *et al.* 2005, Poo-Arguelles *et al.* 2006). This is in stark contrast to the lack of dysmorphism seen in true hyperekplexia cases.

Could this be *SLC6A8* deficiency disorder? The majority of cases are a *deficiency* caused by an intragenic mutation; this is a gene truncation and may cause a complete failure of this transporter – producing the more severe phenotype. Sudden death is likely at any age with poorly controlled epilepsy – although SUDEP is not a particularly common cause of death in infancy outside of status epilepticus. There have been reported cardiac arrhythmias in affected males and so perhaps this child died a sudden cardiac death related to his underlying and undiagnosed metabolic disorder (Anselm *et al.* 2008). This finding requires parental testing (his mother may be a hemizygous carrier) and further clinical correlation.

S4 - 10q11.22

Despite the involvement of *GPRIN2* (G protein regulated inducer of neurite outgrowth 2) - this is likely a recurrent CNV. It is also seen in the SNP genotyping data – appendix I.

17q25.1 duplication – a rare and large CNV of uncertain significance

A CNV of this size could be considered to be pathogenic and it certainly is both real and not frequently occurring; however the biological context of this CNV in relation to the case's phenotype in lacking. It possibly is conferring susceptibility to an (as yet) unknown phenotype *in vivo* (appendix J).

7.2.1.3.4 Could variation at 7q11.23 be responsible for the LD in four cases?

S2, S9, S10 and S11 all have epilepsy and LD and variation at 7q11.23; which is associated with a CNV syndrome (table 7.4). There are three CNVs in DECIPHER spanning the region of S2's CNV; they are all linked with LD or behavioural problems. Of the CNVs I present– the three duplications are so short they should really be called indels, however they are all within the Williams Beuren syndrome area (figure 7.15). This neurodevelopment syndrome is characterised by a distinctive, 'elfin' facial appearance, along with a low nasal bridge, an unusually cheerful demeanour and ease with strangers; developmental delay coupled with strong language skills; and cardiovascular problems, such as supravalvular aortic stenosis

and transient hypercalcaemia. There is a strong genotype-phenotype relationship here and so if not all of the twenty-six genes are affected it is plausible that the attenuated phenotype we see here is related to the smaller genomic disruption. Recent studies suggest that besides the role of the genes in the deleted/duplicated region, factors such as regulatory sequences, epigenetic mechanisms and parental origin of the CNV may be important in determining the variation in 7q11.23 CNV phenotypes.

7q11.23	Case	Min	Max	Size kbp
Deletion	S2	76421871	76507311	85.440
Duplication	S9	74127568	74127763	0.195
Duplication	S10	74127568	74127712	0.193
Duplication	S11	74127568	74127763	0.195

Table 7.4 The size of the CNVs at 7q11.23

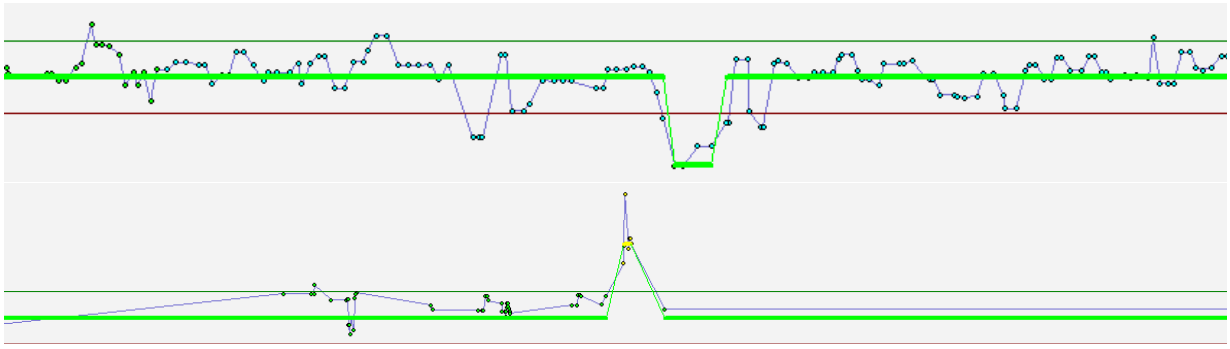


Figure 7.15 Weighted log2 ratios for cases S2 (top) and S9 (bottom) not scaled to each other.

For example Sanders *et al.* (2011) identified four people with 7q11.23 duplications which partially span our indels (7:71970679–74254837). Their study was of 1124 people with autistic spectrum disorder; the mean IQ of those four was 84 – comparable with the cases I present. Their replication study found further 7q11.23 duplications at 7:72411506–73782113. They concluded by saying that CAP-GLY domain containing linker protein 2 (*CLIP2*), LIM domain kinase 1 (*LIMK1*), General transcription factor II, i (*GTF2i*), and Syntaxin

1A (*STX1A*) are the most promising candidates among the 22 genes within their CNV region for involvement in cognitive and social phenotypes.

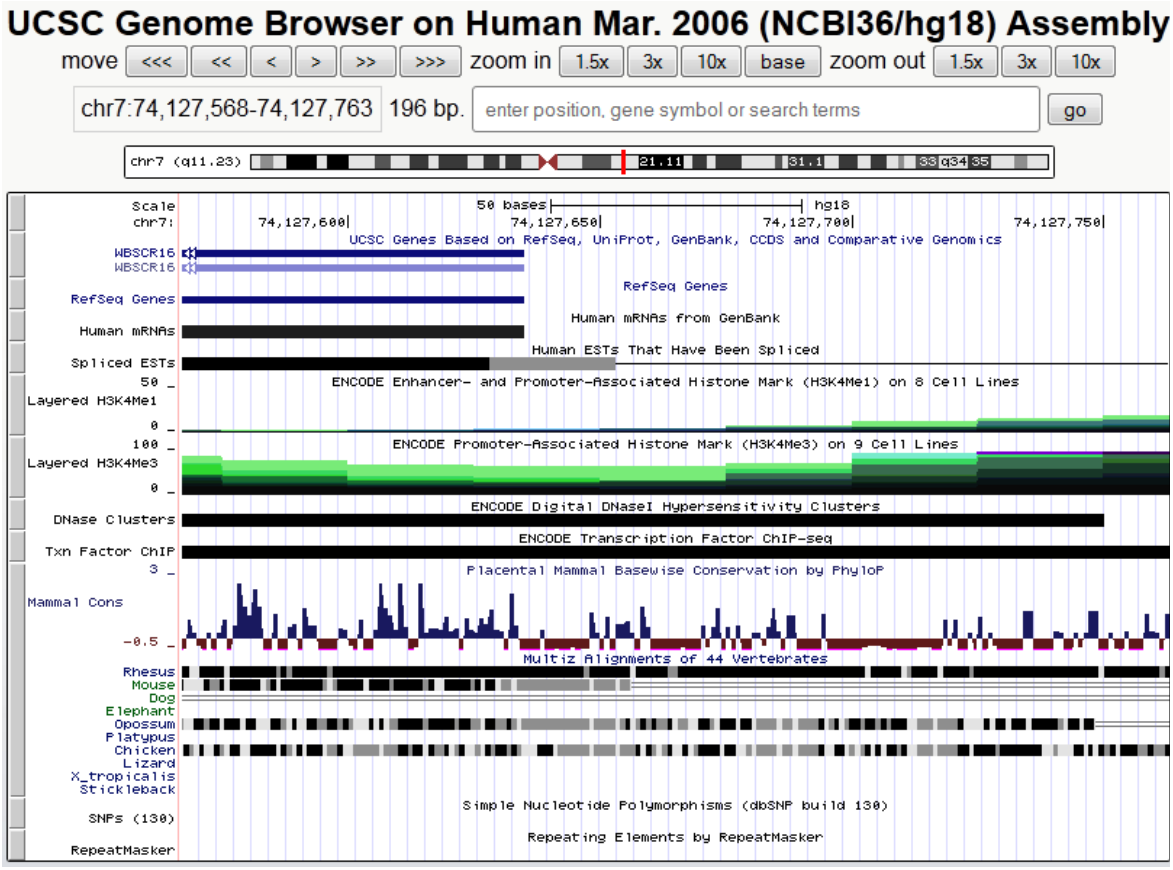


Figure 7.16 Screen capture of the UCSC genome browser - http://genome.ucsc.edu demonstrating only the 195bp of the S9, S11 7q11.23 duplication

The ENCODE project has taught us to be a lot more suspicious of intronic disruption. The duplicated region includes the gene *WBSCR16* (Williams-Beuren syndrome chromosome region 16) –shown in blue as the top line on figure 7.16. The duplication would be of the first exon and the 5' upstream sequence. The next line of note is the promoter-associated histone mark (H3K4Me3). Chemical modifications (such as methylation and acylation) to the histone proteins present in chromatin influence gene expression by changing how accessible the chromatin is to transcription. A specific modification of a specific histone protein is called a histone mark. This track shows the levels of enrichment of the H3K4Me3 histone mark across the genome as determined by a ChIP-seq assay. The H3K4Me3 histone mark is

associated with promoters that are active or poised to be activated. This area shows heavy layering. Regulatory regions in general, and promoters in particular, tend to be DNase sensitive A grey box indicates the extent of the hypersensitive region. The darkness is proportional to the maximum signal strength observed in any cell line. It is plausible that the 7q11.23 indels are pathogenic in this context.

7.2.1.3.5 Is *NKX2-5* a cardiac cause of SUDEP in S6?

Case S6 had unclassified epilepsy, learning difficulties and unfortunately died a probable SUDEP death subsequently. He was identified as having a 5q35.2 duplication (172,592,209-172,592,473) of just 264bp. This indel would be within *NKX2-5*. This gene is just two exons long and the duplication would wholly be within exon two (figure 7.17) Nkx2.5 is one of the most widely studied cardiac-specific transcription factors. Genome-wide association studies implicate the NKX2.5 locus as causative for lethal cardiac arrhythmias (Pfeufer *et al.* 2010). They studied 28,517 people looking for associations with PR interval duration. *NKX2-5* was one of nine loci associated with PR interval duration and one of five associated with atrial fibrillation.

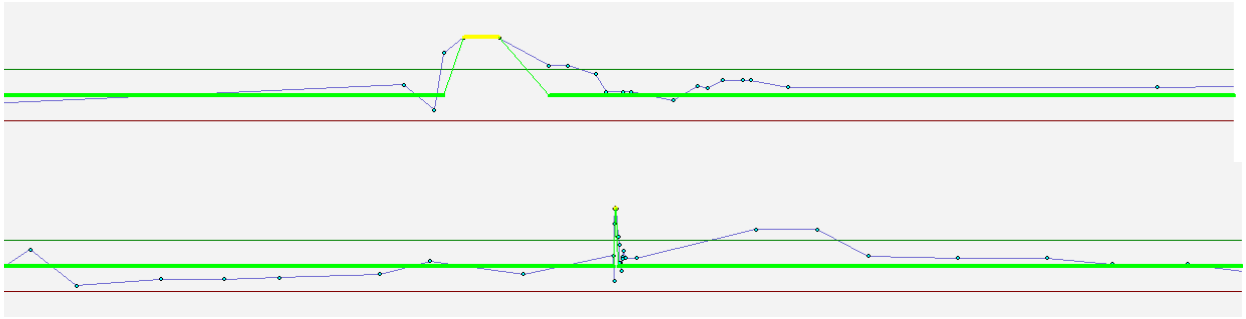


Figure 7.17 Weighted log2 ratios for the duplication in case S6 at 5q35.2. The top illustration is of the whole gene NKX2-5 and below the 5q325.2 cytoband.

Briggs *et al.* (2008) studied Nkx2-5 knockout mice that demonstrated conduction defects – accompanied by a reduction in the expression of cardiac voltage-gated Na+ channel pore-forming alpha-subunit. It is thought that Nkx2.5 works alongside genes such as Tbx5 (T-box 5) to regulate the expression of important genes such as Shox2 (Short stature homeobox 2)

and Bmp4 (Bone morphogenetic protein 4). Together they help create the pace making region in the developing embryo (Puskaric *et al.* 2010). As well as being a risk for cardiac arrhythmia it may also produce structural heart defects. Beffagna *et al.* (2012) identified missense mutations at a low frequency (2/100).

If the activity of this transcription factor can be proven to be affected by this duplication then there is a plausible hypothesis that S6 had either cryptic congenital heart disease or more likely an undiagnosed cardiac arrhythmia. His SUDEP death may in fact have been a sudden cardiac death and it is important that we screen the rest of his family (both with and without epilepsy) for this indel and then refer them to both clinical genetics and cardiology as this is a possible risk factor for sudden death.

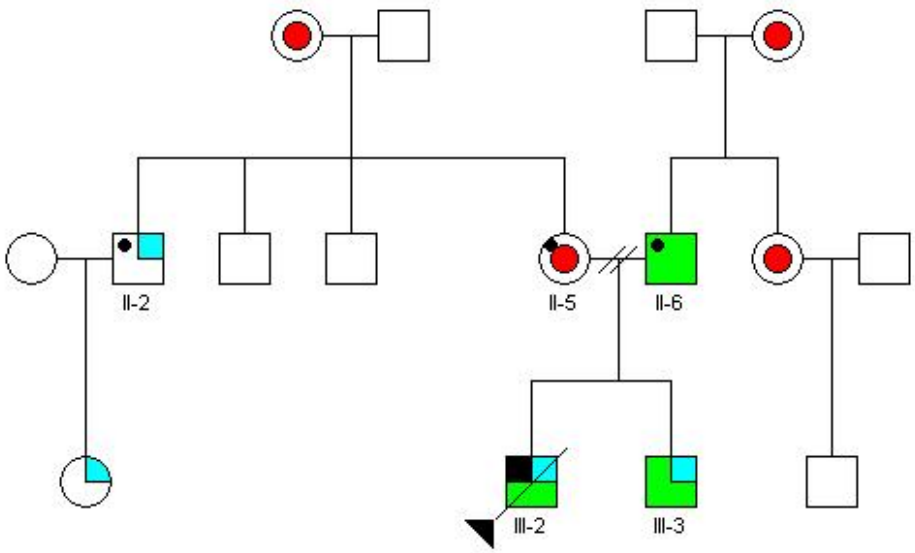


Figure 7.18 Pedigree of the family of S6. Green figures (epilepsy), red dots (migraine), black quadrants (febrile seizures), light blue quadrants (learning disability); S6 (proband) identified by the arrow.

7.2.1.4 CNVs in five patients with hyperekplexia

These patients were part of a cohort submitted for CNV analysis because they did not harbour a pathogenic variant in any hyperekplexia gene, nor were they classical cases that one would take on for next generation sequencing. None of these five presented cases have

CNVs near the known hyperekplexia genes: *GLRA1* (5q32), *GLRB* (4q31.1), *SLC6A5* (11p14.1). Interrogating DECIPHER with respect to these CNVs brought variable results: Case 2 (known CNV syndrome) case 4 (25 overlapping CNVs), case 5 (4 overlapping), case 8 (103 overlapping) and case 9 (8 overlapping). The CNVs are shown in table 7.5 and the genes are described in table 7.6.

Case	Location		Min	Max	Size kbp	Genes
H2	22q11.21	Loss	17274835	17895356	620.521	*DGCR6, PRODH, DGCR2, DGCR14, GSC2, SLC25A1, CLTCL1, HIRA, MRPL40, C22orf39, UFD1L, CDC45L, CLDN5*
H4	18q21.2-q21.31	Loss	49306327	54492889	5186.56	*DCC, MBD2, POLI, STARD6, C18orf54, C18orf26, RAB27B,CCDC68, TCF4, TXNL1, WDR7, ST8SIA3, ONECUT2, FECH, NARS, ATP8B1, NEDD4L, ALPK2*
H5	19q13.42	Loss	59492650	59497108	4.458	*LILRA3*
H8	Xp22.31	Gain	7920059	8420846	500.787	*VCX2, VCX3B*
H9	7q11.22	Gain Gain	71121333 71454833	71217585 71554996	96.252 100.163	*CALN1* *CALN1*

Table 7.5 The six largest CNVs in the cases submitted for CGH array

Gene	Official Full Name	NCBI description
DGCR6	DiGeorge syndrome critical region gene 6	This gene is a candidate for involvement in DiGeorge syndrome pathology and in schizophrenia
PRODH	Proline dehydrogenase (oxidase) 1	Mutations in this gene are associated with hyperprolinemia type 1 and susceptibility to schizophrenia 4
DGCR2	DiGeorge syndrome critical region gene 2	The DGCR2 gene encodes a novel putative adhesion receptor protein, which could play a role in neural crest cells migration, a process which has been proposed to be altered in DiGeorge syndrome.
DGCR14	DiGeorge syndrome critical region gene 14	The encoded protein may be a component of C complex spliceosomes

GSC2	Goosecoid homeobox 2	Because many of the tissues and structures affected in VCFS/DGS derive from the pharyngeal arches of the developing embryo, it is believed that haploinsufficiency of a gene involved in embryonic development may be responsible for its aetiology. The gene is expressed in a limited number of adult tissues, as well as in early human development.
SLC25A1	Solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1	The mitochondrial tricarboxylate transporter (also called citrate transport protein, or CTP) is responsible for the movement of citrate across the mitochondrial inner membrane
CLTCL1	Clathrin, heavy chain-like 1	This gene is a member of the clathrin heavy chain family and encodes a major protein of the polyhedral coat of coated pits and vesicles.
HIRA	HIR histone cell cycle regulation defective homolog A	This gene encodes a histone chaperone that preferentially places the variant histone H3.3 in nucleosomes. Orthologs of this gene in yeast, flies, and plants are necessary for the formation of transcriptionally silent heterochomatin. This gene plays an important role in the formation of the senescence-associated heterochromatin foci. These foci likely mediate the irreversible cell cycle changes that occur in senescent cells. It is considered the primary candidate gene in some haploinsufficiency syndromes such as DiGeorge syndrome, and insufficient production of the gene may disrupt normal embryonic development.
MRPL40	Mitochondrial ribosomal protein L40	
C22orf39	Chromosome 22 open reading frame 39	
UFD1L	Ubiquitin fusion degradation 1 like	The protein encoded by this gene forms a complex with two other proteins, nuclear protein localization-4 and valosin-containing protein, and this complex is

		necessary for the degradation of ubiquitinated proteins. In addition, this complex controls the disassembly of the mitotic spindle and the formation of a closed nuclear envelope after mitosis.
CDC45L	Cell division cycle 45 homolog	Cdc45 is a member of the highly conserved multiprotein complex including Cdc6/Cdc18, the minichromosome maintenance proteins (MCMs) and DNA polymerase, which is important for early steps of DNA replication in eukaryotes.
CLDN5	Claudin 5	Claudins are integral membrane proteins and components of tight junction strands. Tight junction strands serve as a physical barrier to prevent solutes and water from passing freely through the paracellular space between epithelial or endothelial cell sheets.
DCC	Deleted in colorectal carcinoma	This gene encodes a netrin 1 receptor. The transmembrane protein is a member of the immunoglobulin superfamily of cell adhesion molecules, and mediates axon guidance of neuronal growth cones towards sources of netrin 1 ligand.
MBD2	Methyl-cpg binding domain protein 2	The protein encoded by this gene may function as a mediator of the biological consequences of the methylation signal. It is also reported that the this protein functions as a demethylase to activate transcription, as DNA methylation causes gene silencing.
POLI	Polymerase (DNA directed) iota	
STARD6	Star-related lipid transfer (START) domain containing 6	Cholesterol homeostasis
C18orf54	Chromosome 18 open reading frame 54	
C18orf26	Chromosome 18 open	

	reading frame 26	
RAB27B	RAB27B, member RAS oncogene family	Members of the Rab protein family, including RAB27B, are prenylated, membrane-bound proteins involved in vesicular fusion and trafficking.
CCDC68	Coiled-coil domain containing 68	
TCF4	Transcription factor 4	This gene encodes transcription factor 4, a basic helix-loop-helix transcription factor. The encoded protein recognizes an Ephrussi-box ('E-box') binding site ('CANNTG') - a motif first identified in immunoglobulin enhancers. This gene is broadly expressed, and may play an important role in nervous system development. Defects in this gene are a cause of Pitt-Hopkins syndrome.
TXNL1	Thioredoxin-like 1	
WDR7	WD repeat domain 7	WD repeats are minimally conserved regions of approximately 40 amino acids typically bracketed by gly-his and trp-asp (GH-WD) that may facilitate formation of heterotrimeric or multiprotein complexes. Members of this family are involved in a variety of cellular processes, including cell cycle progression, signal transduction, apoptosis, and gene regulation. The encoded protein forms the beta subunit of rabconnectin-3 and binds directly with Rab3A GDP/GTP exchange protein and indirectly with Rab3A GDP/GTP activating protein; these proteins are regulators of Rab3 small G protein family members involved in control of the calcium-dependant exocytosis of neurotransmitters.
ST8SIA3	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 3	ST8SIA3 belongs to a family of sialyltransferases that form sialyl-alpha-2,8-sialyl-R linkages at the nonreducing termini of glycoconjugates
ONECUT2	One cut homeobox 2	This gene encodes a member of the onecut family of transcription factors, which are characterized by a

		cut domain and an atypical homeodomain. The protein binds to specific DNA sequences and stimulates expression of target genes, including genes involved in melanocyte and hepatocyte differentiation.
FECH	Ferrochelatase	The protein encoded by this gene is localized to the mitochondrion, where it catalyses the insertion of the ferrous form of iron into protoporphyrin IX in the heme synthesis pathway. Mutations in this gene are associated with erythropoietic protoporphyria.
NARS	Asparaginyl-trna synthetase	Aminoacyl-tRNA synthetases are a class of enzymes that charge tRNAs with their cognate amino acids.
ATP8B1	Atpase, aminophospholipid transporter, class I, type 8B, member 1	Mutations in this gene may result in progressive familial intrahepatic cholestasis type 1 and in benign recurrent intrahepatic cholestasis
NEDD4L	Neural precursor cell expressed, developmentally down-regulated 4-like, E3 ubiquitin protein ligase	This gene encodes a member of the Nedd4 family of HECT domain E3 ubiquitin ligases. HECT domain E3 ubiquitin ligases transfer ubiquitin from E2 ubiquitin-conjugating enzymes to protein substrates, thus targeting specific proteins for lysosomal degradation. The encoded protein mediates the ubiquitination of multiple target substrates and plays a critical role in epithelial sodium transport by regulating the cell surface expression of the epithelial sodium channel, ENaC.
ALPK2	Alpha-kinase 2	
LILRA3	Leukocyte immunoglobulin-like receptor, subfamily A (without TM domain), member 3	Leukocyte Ig-like receptors (LIRs) are a family of immunoreceptors expressed predominantly on monocytes and B cells and at lower levels on dendritic cells and natural killer (NK) cells.
VCX2	Variable charge, X-linked 2	The VCX gene cluster is polymorphic in terms of copy number; different individuals may have a different number of VCX genes. VCX/Y genes encode small and

		highly charged proteins of unknown function.
VCX3B	Variable charge, X-linked 3B	Although the exact function of this family member has yet to be determined, a role in mRNA stability regulation can be inferred from the ability of the highly similar family member, VCX-A, to inhibit mRNA decapping. A possible role in the regulation of ribosome assembly during spermatogenesis has also been suggested
CALN1	Calneuron 1	This gene encodes a protein with high similarity to the calcium-binding proteins of the calmodulin family. The encoded protein contains two EF-hand domains and potential calcium-binding sites.

Table 7.6 The genes and their descriptions disrupted by CNVs in these hyperekplexia cases.

7.2.1.4.1 H2 has terminal 22q deletion syndrome

The 22q11.21 deletion syndrome has been described above. Case H2 has a 620kbp deletion of twelve genes in this region including previously discussed genes such as *HIRA*. It is most likely that this is happenstance. This CNV would not be expected to cause a severe neurological phenotype at birth, but undoubtedly is clinically relevant.

7.2.1.4.2 Does H4 have Pitt-Hopkins syndrome?

Case H4 has a large (5186kbp) deletion at 18q21.2-q21.31 which could be expected to reduce 18 genes to heterozygosity – one of which is *TCF4*. Transcription factor 4 (TCF4) gene mutations are recognised as a cause of Pitt-Hopkins syndrome. The *TCF4* gene has a particularly low haplotype index of 1.9% - which strongly suggests that haploinsufficiency would produce the phenotype. The majority of Pitt-Hopkins cases are caused by de novo haploinsufficiency; 40% point mutations, 30% small deletions or insertions and 30% deletions. A de novo deletion in this area of 797kb was described in conjunction with a child with speech delay, mental retardation, sleeping problems, facial dysmorphism, and feet anomalies (van Diepen *et al.* 2011).

Pitt-Hopkins's is a disorder recognised by neurodevelopmental delay, learning disability, epilepsy and distinctive facial features. Additionally they have a respiratory rate disorder with alternating periods of hyperventilation and apnoeas. These apnoea attacks (unlike the ones seen in hyperekplexia) can lead to transient loss of consciousness. This case was referred by a geneticist in Melbourne who suspected an atypical hyperekplexia phenotype. Curiously Pitt-Hopkins was first described in that city. The phenotype here may not be typical for many reasons, not least the fact that the individual is now in his fifties, but perhaps that this is a contiguous gene disorder: a 'Pitt-Hopkins plus'. The deletion of *TCF4* probably has to be taken in the context of the neighbouring losses too.

Case H5 - the *LILRA3* loss is probably well tolerated. It has a haplotype index of over 85%

Duplication of both *VCX3B, VCX2* in case H8

The sex of the case in not recorded and so knowing how to interpret this X linked duplication is difficult. Furthermore there are a great number of overlapping DECIPHER cases (103) – without sufficient evidence from the literature to support either of these genes as pathogenic.

7.2.1.4.3 *CALN1* duplication at 7q11.22 in case H9

The mouse orthologous gene (Caln1) shows little prenatal expression, with highest expression at postnatal day 21. If deletion of *CALN1* in humans is pathogenic – it may mimic a hyperekplexia-like presentation which classically presents in the first month of life. The murine gene Caln1 is highly expressed exclusively in neural tissue particularly the hippocampus and cortex. It has a high homology with calmodulin which indicates a potential role in signal transduction, and the cellular localization of the mRNA suggest that *CALN1* has a significant role in the physiology of neurons and is potentially important in memory and learning. Although eight CNVs overlap in DECIPHER there are no similar ones and neurological phenotypes abound in their descriptions. The role of this CNV is unclear and it

cannot be claimed to be pathogenic without further confirmatory work. Correlating with the phenotype is unhelpful as she is of normal IQ.

7.2.2 SNP Genotyping

34 individuals with a clinical diagnosis of JME were submitted for SNP genotyping. The number of CNVs per case is given in the table below - mean 18.4. There were 624 CNVs identified using the methods previously described. 83 of these (13.3%) were at cytobands that corresponded to previously described regions of interest for GGE and these are shown in the table below.

A number of these epilepsy associated CNVs will be discussed in full. The data are both here and in the appendices. Analysis identified that some were near known CNVs – but did not overlap the area; some were duplications whereas the CNV syndrome was normally associated with a deletion (or vice versa); some were associated with a number of CNVs seen in healthy individuals; and finally some apparent CNVs when analysed manually appeared to be unconvincing false positives. I will start by analysing the cytobands where CNVs have been previously reported as associated with similar epilepsies.

Patient	Total number of CNVs	Location	Gain / Loss	Start	Stop	Size in kbp
1	14	12p13.33	Gain	2252689	2266120	13.431
2	20	17q12 17q21.31	Gain Gain	34437481 44187491	34477480 44292676	39.999 105.185
3	28	8p23.3 12p13.33 17q12	Gain Gain Gain	1701168 2252689 34440082	1820583 2266120 34477480	119.415 13.431 37.398
4	42	5p15.33 8q24.3 8q24.3	Gain Gain Gain	2723344 142463825 140596802	2851756 142604262 140644429	128.412 140.437 47.627

		8q24.3 15q13.2 16p23.1 Xq28	Gain Loss Gain Gain	143145546 31073735 78344586 152770650	143429623 32446830 78401357 152868453	284.077 1373.095 56.771 97.803
5	12	16p11.2	Gain	34449594	34755816	306.222
6	24					
7	25	5q35.1 17q12 17p11.2 Xp22.31	Loss Gain Gain Gain	170373942 34440082 21311748 6699291	170407987 34477480 21565102 6703035	34.045 37.398 253.354 3.744
9	13	17q12	Gain	34440082	34475680	35.598
10	20	14q11.2	Loss	22835777	22878421	42.644
11	17	1p31.1 11q21.31	Loss Gain	73012022 44187491	73098114 44784970	86.092 597.479
12	5					
13	15	16p11.2 17q21.31	Gain Gain	32564621 44187491	33789190 44254379	1224.569 66.888
14	19	14q11.2 17q21.31 17q21.31	Loss Gain Loss	22746791 44212823 43937108	22944507 44276618 43978535	197.716 63.795 41.427
15	19	7q11.22 17q21.31	Loss Gain	69755346 44212823	69811718 44292742	56.372 79.919
16	15	14q11.2 17q21.31	Loss Gain	22747746 44187491	22941375 44292742	193.629 105.251
17	24					
18	13	16p11.2 17q21.31	Gain Gain	34466474 44187491	34755816 44254379	289.342 66.888
19	24	14q11.2 17q21.31 17q12	Loss Gain Gain	22749288 44187491 34426244	22854720 44254413 34477480	105.432 66.922 51.236
20	8	16p11.2 17q21.31	Gain Gain	34449594 44187491	34755816 44784639	306.222 597.148
21	15	1p31.1 14q11.2	Loss Loss	73012022 22772662	73098114 22959362	86.092 186.7

22	13	14q11.2	Loss	22676928	22959362	282.434
		14q11.2	Loss	22594506	22620607	26.101
		17p11.2	Gain	21334350	21707933	373.583
23	64	14q11.2	Loss	22641679	22940386	298.707
		15q11.2	Gain	25303130	25327709	24.579
		16p12.2	Loss	21946522	22431357	484.835
		17q21.31	Gain	44187491	44244930	57.439
		Xp22.31	Gain	6671973	6673662	1.689
		Xp22.31	Gain	6607440	6611469	4.029
		Xp22.31	Gain	6696213	6698697	2.484
		Xp22.31	Gain	6484793	6500902	16.109
24	12	9p24	Loss	2598943	2761174	162.231
		14q11.2	Loss	22562098	22612060	49.962
		14q11.2	Loss	22730865	22943573	212.708
		17q21.31	Gain	44212823	44292742	79.919
25	8	17q12	Gain	34440082	34477480	37.398
		17q21.31	Gain	44212823	44254379	41.556
26	13	6p12.3	Gain	49432184	49459866	27.682
		16p13.11	Loss	15054174	15182587	128.413
		17q21.31	Gain	44187491	44254379	66.888
27	22	2q11.2	Gain	99841949	99924411	82.462
		Xp22.31	Gain	6607440	6611469	4.029
		Xp22.31	Gain	6696273	6698697	2.424
28	15	14q11.2	Loss	22827208	22941375	114.167
		16p11.2	Loss	32538258	32906257	367.999
		17q21.31	Gain	44212823	44276618	63.795
		17q12	Gain	34440082	34849704	409.622
29	17	17q21.31	Gain	44187491	44288442	100.951
30	12	16p11.2	Gain	34449594	34755816	306.222
		17q12	Gain	34437481	34477480	39.999
31	14	14q11.2	Loss	22599355	22960819	361.464
		17q12	Gain	34437481	34477480	39.999
32	17	14q11.2	Loss	22496582	22943573	446.991
33	19	10q21.3	Loss	68077578	68122519	44.941

		14q11.2	Loss	22560402	22943215	382.813
		17q12	Gain	34440082	34477480	37.398
		17q21.31	Gain	44187491	44288442	100.951
34	16	14q11.2	Loss	22747482	22941375	193.893
		16p11.2	Gain	34466474	34755816	289.342
		17q21.31	Gain	44187491	44288442	100.951
35	10	12p13.33	Loss	2235940	2257908	21.968
		17q21.31	Gain	44212414	44785669	573.255

Table 7.7 Table showing apparent CNVs per person (total number), then the details of those previously reported to be associated with an IGE phenotype

7.2.2.1 Epilepsy Associated CNVs

The full list of CNVs which have been linked to epilepsy are in appendix F, the majority of CNVs in table 7.7 in the cytobands of those linked to epilepsy do not overlap and they are published in appendix H.

1p31.1 deletions

Thought to be a rare benign CNV – appendix I.

2q11.2 duplication

Thought to be a recurrent CNV of unknown significance - appendix J.

7.2.2.1.1 Over representation of a rare CNV (14q11.12 deletion) in the JME sample

The cytoband 14q11.12 has been associated with epilepsy and deletions of between 42,644 bp and 446,991bp were reported in 14 cases. This particular deletion was seen frequently - 9 more times in the WMRGL file of cases and 27 times in EU file of controls. DECIPHER does not recognise it as a known microdeletion syndrome however – and only 7 cases in that

database overlap with these cases. 13 similar deletions are recorded in DGV, and many more microduplications – 164. As the EU control set has approximately 450 cases this CNV (seen in 14/35 – 40%) is over expressed compared to the EU dataset of controls (27/450 – 6%) - $p<0.0001$, Chi statistic - 48.5.

Case	Min	Max	Size	Dist	Mark
10	22835777	22878421	42.644	775	56
14	22746791	22944507	197.716	708	280
16	22747746	22941375	193.629	730	266
19	22749288	22854720	105.432	717	148
21	22772662	22959362	186.7	781	240
22	22594506 22676928	22620607 22959362	26.101 282.434	511 643	52 440
23	22641679	22940386	298.707	650	460
24	22562098	22612060	49.962	594	85
24	22730865	22943573	212.708	679	314
28	22827208	22941375	114.167	771	149
31	22599355	22960819	361.464	605	598
32	22496582	22943573	446.991	646	692
33	22560402	22943215	382.813	600	639
34	22747482	22941375	193.893	726	268

Table 7.8 The 14q11.12 deletions seen in people with JME

Each CNV (table 7.8 below) is covered by a good number of probes (up to 692) with a relatively small mean marker distance (511 to 781bp); it appears to be a convincing CNV by eye too. 14q11.12 could represent a benign recurrent CNV that has a higher prevalence in the South Wales population. This microdeletion is in a gene poor region. The nearest genes being many kilobases away and do not include any genes directly implicated in epilepsy phenotypes such as *DAD1* (defender against cell death 1) and *SLC7A7* (solute carrier family 7 (amino acid transporter light chain, y+L system), member 7) – found in skin. Or however it could represent an intronic area of interest for JME – harbouring regulatory or enhancing

regions (figure 7.19). Furthermore this area is linked to immune mediated disorders such as IgA nephropathy and inflammatory bowel disease through GWAS studies.

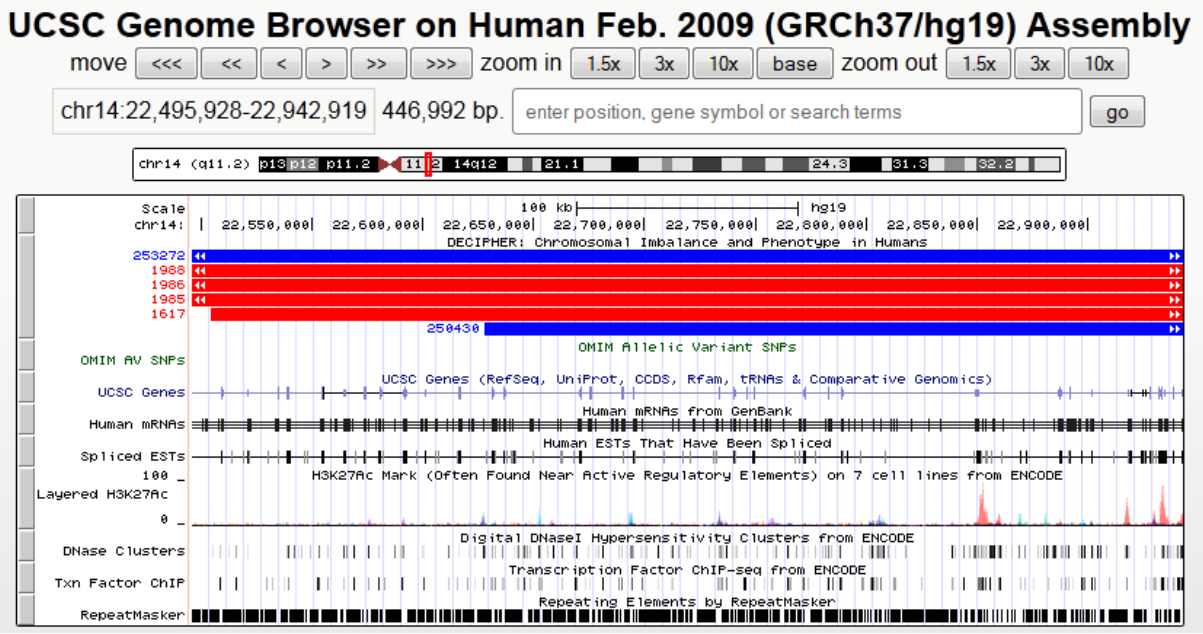


Figure 7.19 Screen capture of the UCSC genome browser - http://genome.ucsc.edu demonstrating the 446,991bp of the J32, the 14q11.12 deletion. The top bars (red – deletion, blue duplication) are CNVs in DECIPHER. The layered H3K27AC line demonstrates areas of histone mark density – which could be important here.

7.2.2.1.2 Identification of a 15q13.2-13.3 deletion

Case 4 was identified as having a 15q12.3-13.3 deletion (15: 31073735-32446830) – a site of a known epilepsy associated CNV syndrome (15: 30910306 – 32445407). The overlap of this deletion (size 1,373,095bp) is nearly exactly that described by DECIPHER as typical for the CNV syndrome (1.5megabase stretch). At time of writing there were 169 patients listed on that database with a 15q13.3 syndrome, 149 directly overlapping our case. Manual confirmation of the deletion is shown in figure 7.20. It is convincing by eye from both the weighted log2 ration (top scatter plot) and the smooth signal (below single line) suggesting that the copy number drops down to one. The mean marker distance is 941bases, there are 1460 probes spanning the CNV and the ChAS system gives an automated confidence of this CNV of 91.6%.

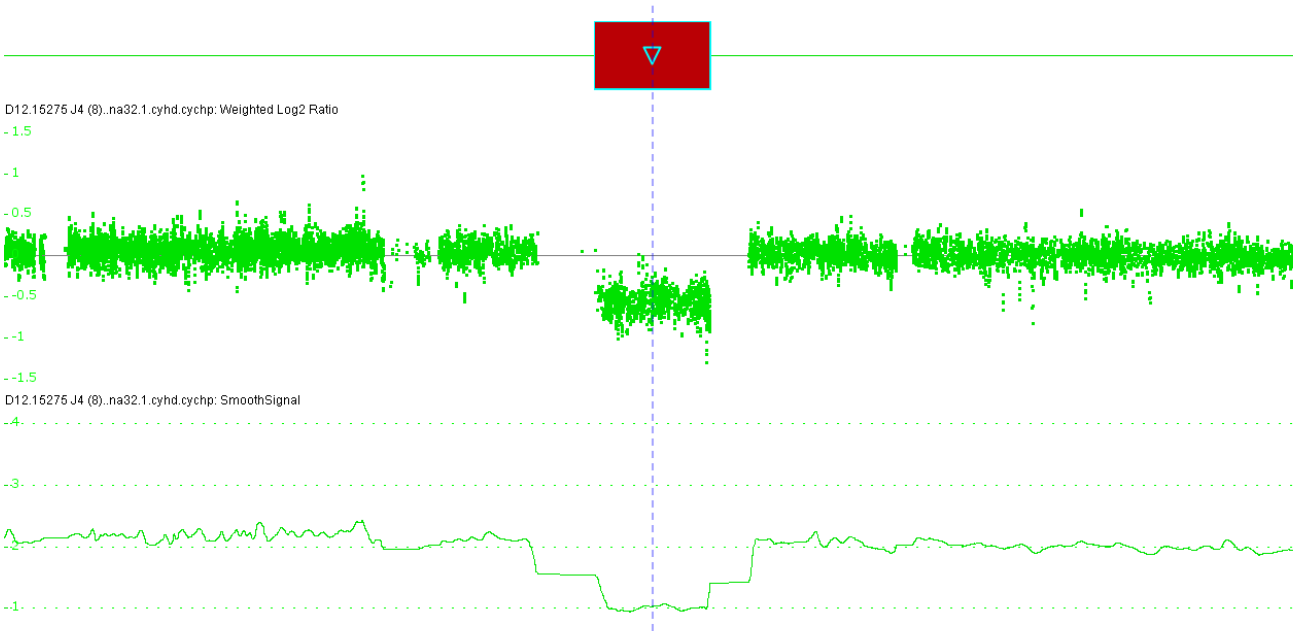


Figure 7.20 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 15q13.2-13.3 deletion

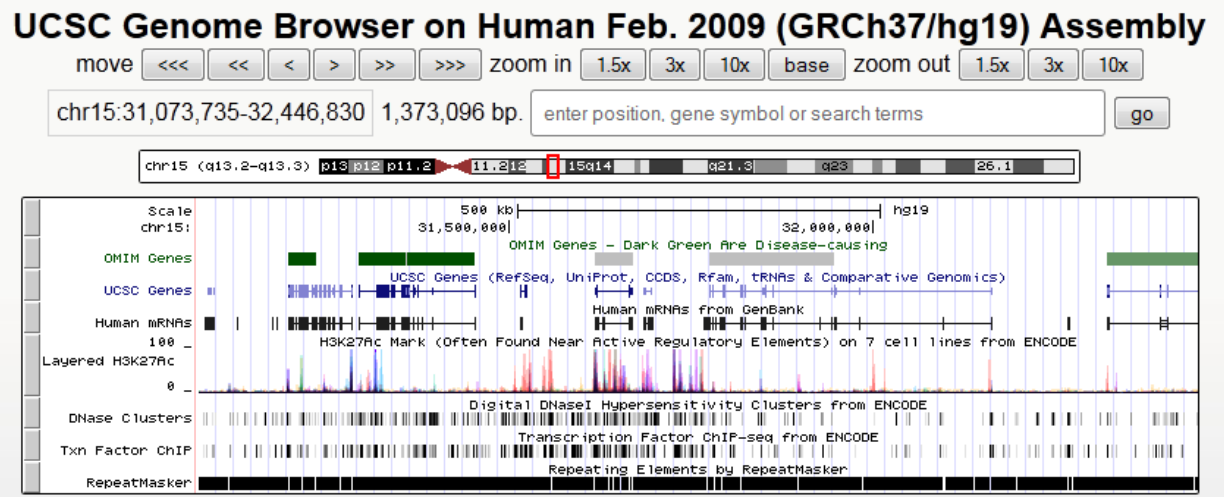


Figure 7.21 Screen capture of the UCSC genome browser - http://genome.ucsc.edu demonstrating the 15q13.2-q13.3 deletion in case 4. OMIM genes and UCSC genes are overlaid. The high activity in certain regions of the layered H3K27AC line demonstrates areas of histone mark density -suggesting regulatory roles for large parts of the CNV.

Case four is heterozygous for seven genes: *FAN1, MTMR10, TRPM1, MIR211, KLF13, OTUD7A and CHRNA7* (table 7.9). The gene which has been of most interest as a candidate in this region is *CHRNA7* – which codes for the nicotinic acetylcholine receptor subunit alpha-7. It has a predicted pathogenicity in a heterozygous state of 20% and four exons are lost in this microdeletion syndrome presented. The nicotinic acetylcholine receptors

(nAChRs) are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. The family member *CHRNA7*, which is located on chromosome 15 in a region associated with several neuropsychiatric disorders, is partially duplicated and forms a hybrid with a novel gene from the family with sequence similarity 7 (*FAM7A*). Alternative splicing has been observed, and two variants exist, for this hybrid gene. The N-terminally truncated products predicted by the largest open reading frames for each variant would lack the majority of the neurotransmitter-gated ion-channel ligand binding domain but retain the transmembrane region that forms the ion channel.

Gene	Official full name	NCBI description
FAN1	FANCD2/FANCI-associated nuclease 1	This gene encodes a member of the myotubularin-related class 1 cysteine-based protein tyrosine phosphatases.
MTMR10	Myotubularin related protein 10	None
TRPM1	Transient receptor potential cation channel, subfamily M, member 1	The encoded protein is a calcium permeable cation channel that is expressed in melanocytes and may play a role in melanin synthesis. Specific mutations in this gene are the cause autosomal recessive complete congenital stationary night blindness-1C.
MIR211	microRNA 211	microRNAs (miRNAs) are short (20-24 nt) non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs
KLF13	Kruppel-like factor 13	Transcription factor that contains 3 classical zinc finger DNA-binding domains
OTUD7A	OUT (ovarian tumour) domain containing 7A	Has deubiquitinating activity that is directed towards 'Lys-48' or 'Lys-63'-linked polyubiquitin chains
CHRNA7	Nicotinic acetylcholine	nACRs are ligand-gated ion channels that mediate

	receptor subunit alpha-7 (neuronal)	fast signal transmission at synapses

Table 7.9 Genes contained within J4's 15q13.2-q13.3 deletion

Six duplications and one loss at 16p11.2-p11.1

Thought to be a recurrent CNV of unknown significance – and is distant from the microdeletion syndrome - appendix I.

7.2.2.1.3 J23 has the 16p11.2 deletion CNV

In a single case (23) the 16p12.2 deletion was identified. This has previously been associated with epilepsy and is part of the 16p11.2-p12.12 microdeletion syndrome (16: 21512062-30199854). This deletion (484,835bp) is at 16:21946522-22431357 – so partially within the 8.69megabase region which is said to comprise the CNV syndrome. Very few large CNVs have been reported and there are two clusters of CNVs within the 16p11.2 region; one actually at 16p11.2 (the microdeletion syndrome) and the other at the boundary between the p12.3 and p12.2 cytobands which better corresponds to this microdeletion. Eight genes are reduced to heterozygosity: *UQCRC2, PDZD9, C16orf52, VWA3A, EEF2K, POLR3E, CDR2* and *RRN3P3* (table 7.10). 46 cases overlap in DECIPHER – as you would expect with a known CNV syndrome.

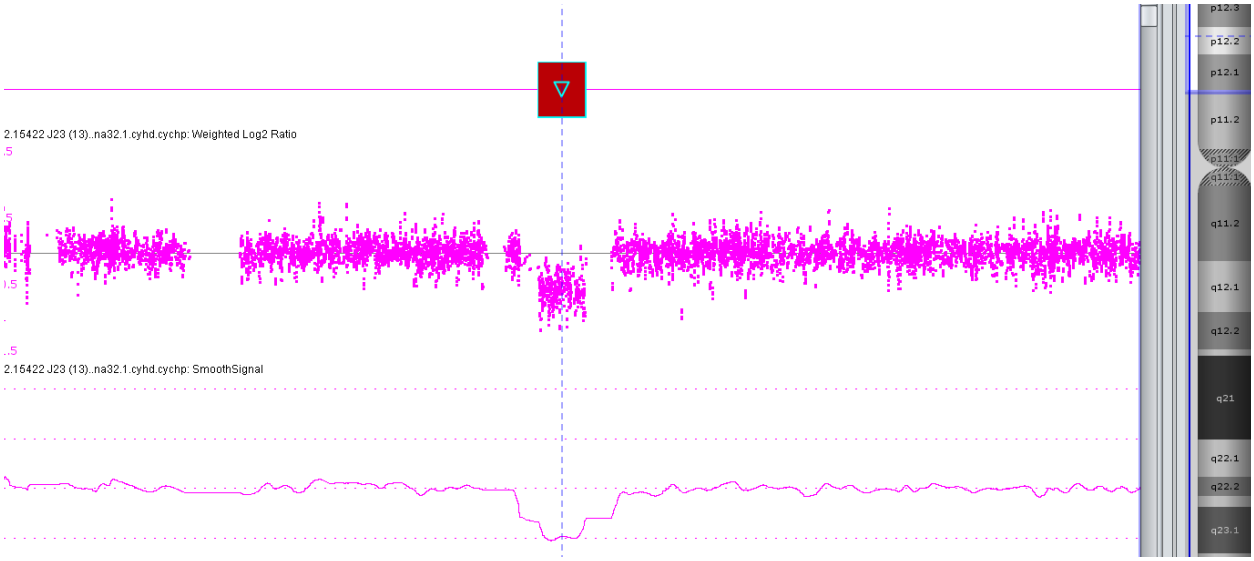


Figure 7.22 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 16p11.2 deletion in J23.

Gene	Official Full Name	NCBI description
UQCRC2	Ubiquinol-cytochrome c reductase core protein II	
PDZD9	PDZ domain containing 9	
C16orf52	Chromosome 16 open reading frame 52	
VWA3A	Von Willebrand factor A domain containing 3A	
EEF2K	Eukaryotic elongation factor-2 kinase	This gene encodes a highly conserved protein kinase in the calmodulin-mediated signaling pathway that links activation of cell surface receptors to cell division. This kinase is involved in the regulation of protein synthesis
POLR3E	Polymerase (RNA) III (DNA directed) polypeptide E	
CDR2	Cerebellar degeneration-related protein 2	
RRN3P3	RNA polymerase I transcription factor homolog	Pseudogene

Table 7.10 Genes contained within J23's 16p12.2 deletion

7.2.2.1.4 The 15q11.2 duplication in J23 may be needed to complete the phenotype

Case J23 in addition to having the 16p12.2 microdeletion harbours a microduplication at 15q11.2 (15: 25303130-25327709) of 24,579bp. This 15q11.2 duplication falls within the Angelman's / Prader-Willi area on 15q at 22749354 to 28438266. There are 51 cases that overlap with this one in the DECIPHER database and although the genes directly within this CNV represent fourteen micro-RNAs (such as SNORD116-9 – encoding a small nucleolar

RNA, C/D box 116-9). This SNORD116 cluster are thought by some to be important in producing the Prader-Willi phenotype when deleted. There are also genes of interest nearby- such as *UBE3A* (15: 25582396-25684175) which is only 254,687 bp away. Ubiquitin protein ligase E3A is a maternally imprinted gene and caused the Angelman's phenotype. Of note – but further away are *GABRB3, GABRA5* and *GABRG3* which encode the GABA A receptor beta 3 subunit (26788693-27018935), GABA A receptor alpha 5 subunit (27111866-27194357) and GABA A receptor, gamma 3 subunit (27216429-27778373) respectively. OMIM recognises the nearest of these three *(GABRB3*) as a susceptibility gene for childhood absence epilepsy.

Overlapping CNVs are seen 36 times in DGV, once in the EU dataset of normal controls (one gain, not a perfect match) and in one case in the WMRGL dataset (again partial match duplication).

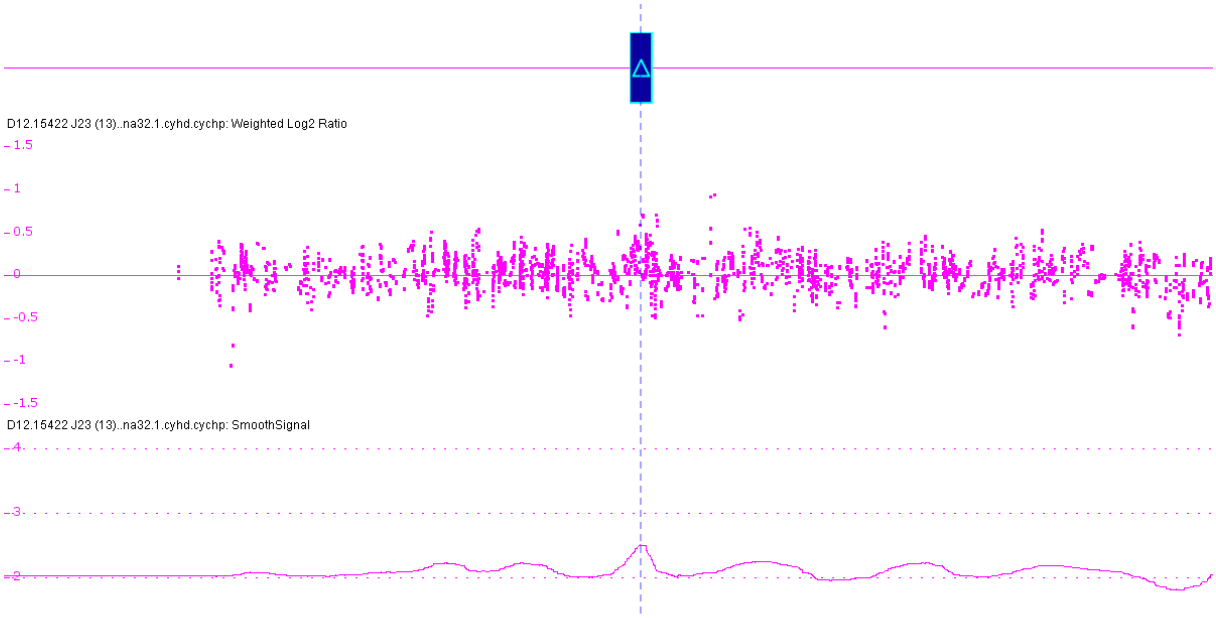


Figure 7.23 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 15q11.2 duplication in J23.

7.2.2.1.5 Case J26 has the 16p13.11 deletion

The microdeletion at 16:150541740-15182587 is seen in case 26 and is 128,413bp in size. The weighted log2 ratio and smooth signal trace are shown below (figure 7.24). There is a known microdeletion / microduplication syndrome at 16p13.11 between 14986684 and

16486684 (1.5 megabases) involving fourteen genes. The *CytoScan HD* method has allowed us to identify a much more modest microdeletion here reducing just three genes to heterozygosity: *PDXDC1, NTAN1* and *RRN3.*

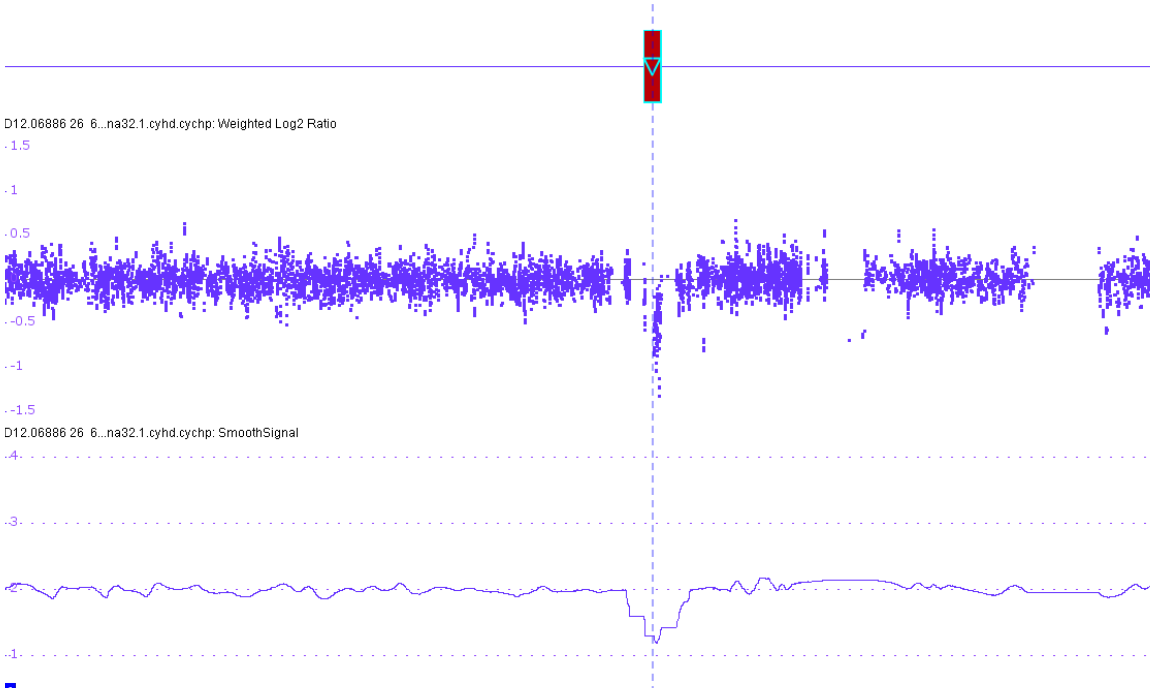


Figure 7.24 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 16p13.11 deletion in J26.

The figure below (7.25) demonstrates just how little of the 16p13.11 microdeletion region is lost with this specific CNV. Girirajan *et al.* (2012) state that in children with LD this CNV is de novo in no more than 25% of cases and is associated with another large CNV in almost 10% of cases. J26 has a large CNV of uncertain significance at 2q12.3 (839kbp); this is a deletion in a gene poor area. Similarly he has a 489kbp duplication at 14q32.33 – which is thought to be recurrent and benign. Heinzen *et al*. (2010) reported 23 patients with 16p13.11 deletions and epilepsy larger than 100kbp. The size varied in their cohort – and all but one covered *NDE1* (which this CNV does not). There were no genotype / phenotype correlations to be made and indeed heterogeneity was very prominent. Psychiatric comorbidities did not exceed those in the community. Our case (J26) has a HADS anxiety score of 16 and a depression score of 9 (a score above 8 is thought to be significant and the mean score in controls were 6.14 and 3.68 respectively).

Of Heinzen *et al*. (2010)'s 23 16p13.11 deletions only three spanned the region covered by the CNV in J26 - the remainder began just after it terminated with relatively uniform breakpoints. Could this help identify the pathogenic loci of this CNV? Or does it represent the populations that are studied as it can be inherited from unaffected individuals. Their epilepsy syndromes were heterogeneous with only three having a classical GGE electroclinical syndrome – three with JAE and one with CAE – and none with JME.

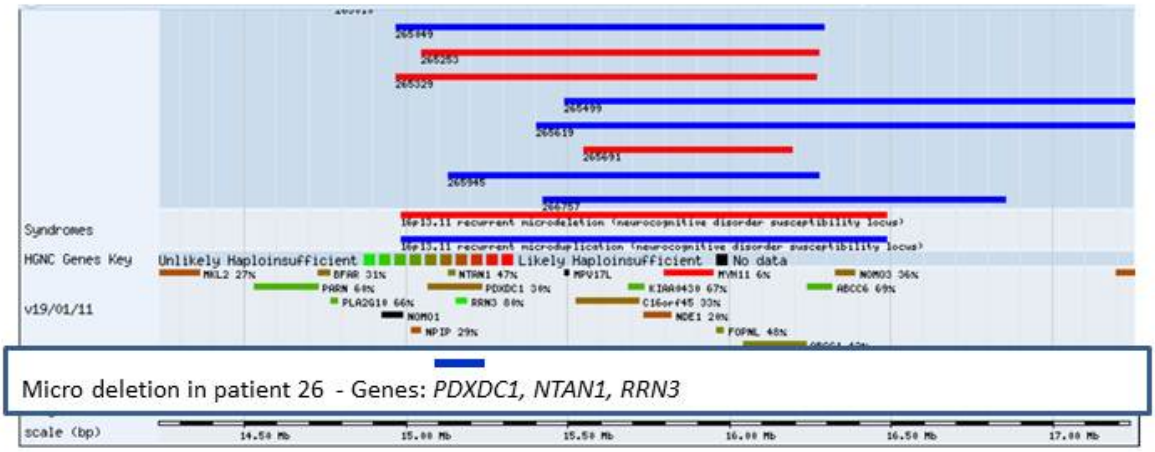


Figure 7.25 DECIPHER lists the CNVs in the area associated with the 16p13.11 deletion syndrome; blue are duplication and red are deletions. This is a screen capture demonstrating how relatively modest this CNV is in size compared to other reported CNVs.

Gene	Official Full Name	NCBI description
PDXDC1	Pyridoxal-dependent decarboxylase domain containing 1	
NTAN1	N-terminal asparagine amidase	The protein encoded by this gene functions in a step-wise process of protein degradation through the N-end rule pathway. This protein acts as a tertiary destabilizing enzyme that deamidates N-terminal L-Asn residues on proteins to produce N-terminal L-Asp. L-Asp substrates are subsequently conjugated to L-Arg, which is recognized by specific E3 ubiquitin ligases and targeted to the proteasome.
RRN3	RRN3 RNA polymerase I	

	transcription factor homolog	

Table 7.11 Genes contained within the 16p13.11 deletion in case J26.

17q21.31 represents a benign recurrent CNV that does not overlap the epilepsy CNV there – appendix H.

7.2.2.2 Recurrent CNVs with genes of interest

7.2.2.2.1 Is the intronic *CTNNA3* deletion relevant?

There were two 10q21.3 deletion CNVs identified – in cases 11 and 33. Both the losses were identical – at 10: 68077578-68122519 and being of 44,941bp. This CNV was infrequently seen in cases (four overlaps in DECIPHER, one in WMRGL) but often in control patients (9 times deleted in EU database, 20 deletions in DGV). A frequency of 9/450 (2%) in controls versus 2/35 (5.7%) is not statistically significant. However it would create an intronic deletion within the *CTNNA3* gene which has been strongly linked with neurodevelopmental phenotypes such as autism, late-onset Alzheimer's in females and idiopathic continuous spike and waves during slow-wave sleep syndrome. There is striking similarity with that last report – as their CNV was also intronic (10:68550481-68668009); Lesca *et al.* (2012) were able to ascertain that this CNV was maternally inherited. *CTNNA3* encodes the catenin (cadherin-associated protein), alpha 3 protein.

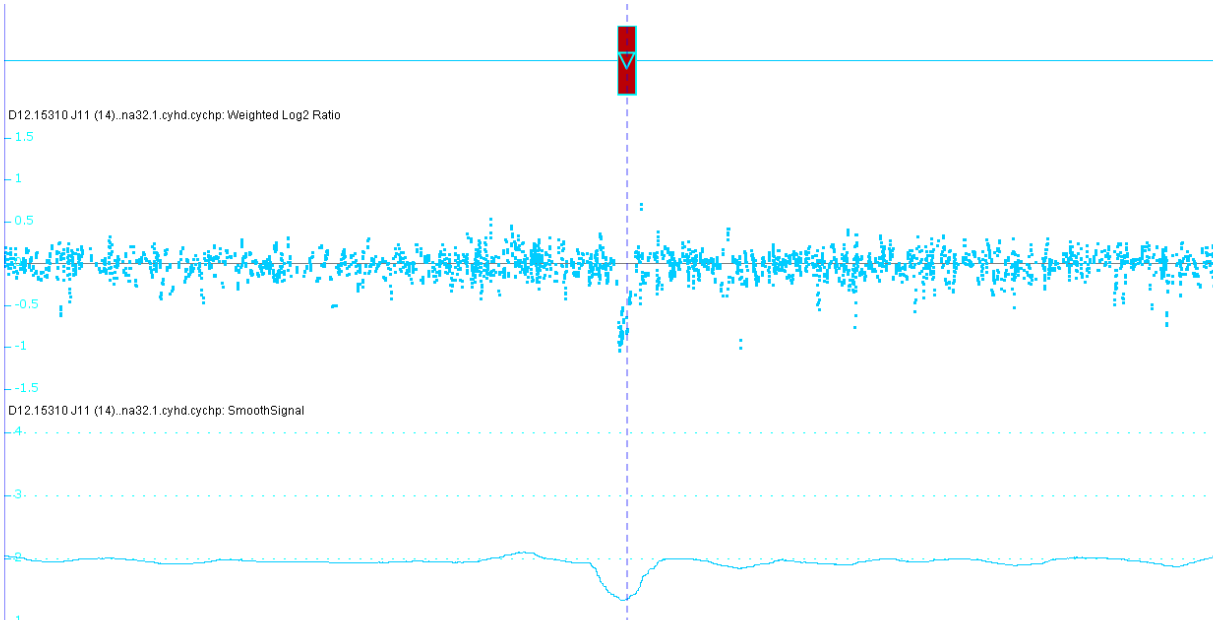


Figure 7.26 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 10q21.3 deletion in case J11.

7.2.2.2.2 Clustering of CNVs at 12p13.31, 12p13.33

Although separated by many megabases – I will consider these two CNVs together as both J6 and J35 had both CNVs.

Analysis identified a cluster of ten CNVs at 12p13 in eight individuals (cases 6 and 35 had both). Five involved *CACNA1C* (two deletions) and five *SLC2A14* and *SLC2A3* (two deletions). It is debatable as whether to classify this region as 'epilepsy associated'; a single case with both a de novo 8p23.2p23.3 deletion and a 12p13.31-p13.33 duplication was described in 2012. (Margari *et al.*) - epilepsy was one of the phenotypes. Of our six cases with 12p13 duplications – none had 8p23.2 – 8p.23.3 deletions. Case 3 has a 119.415kbp duplication at 8p23.3 alongside a 12p13.33 duplication.

Case	CNV	Start	Stop	Size kbp	Dist	Mrk	Genes
35	Loss	2235940	2257908	21.968	252	88	*CACNA1C*
15	Loss	2246103	2257908	11.805	149	80	*CACNA1C*
1	Gain	2252689	2266120	13.431	213	64	*CACNA1C*

3	Gain	2252689	2266120	13.431	213	64	*CACNA1C*
6	Gain	2252689	2272567	19.878	228	88	*CACNA1C*
32	Gain	8004356	8124048	119.692	1246	97	*SLC2A14, SLC2A3*
9	Gain	8004356	8124048	119.692	1246	97	*SLC2A14, SLC2A3*
35	Gain	8004411	8142583	138.172	1341	104	*SLC2A14, SLC2A3*
2	Loss	8004411	8124048	119.637	1259	96	*SLC2A14, SLC2A3*
6	Loss	8004411	8135534	131.123	1324	100	*SLC2A14, SLC2A3*

Table 7.12 The CNVs at 12p13.31, 12p13.33, Distance is mean marker distance and Mrk is the number of markers that cover the CNV.

Mutations in the *CACNA1C* gene are associated with a variant of long QT syndrome called Timothy's syndrome (Pagon *et al.* 1993). and also with Brugada syndrome (Hedley *et al.* 2009) None of these cases were known to have cardiac arrhythmias, however it has been implicated with other cryptogenic neuroscience disorders. A large-scale genetic analysis conducted in 2008 shows the possibility that *CACNA1C* is associated with bipolar disorder (Ferreira *et al.* 2008) and subsequently also with schizophrenia (Green *et al.* 2010, Curtis *et al.* 2011). Also, A *CACNA1C* risk allele has been associated with disruption in brain connectivity in patients with bipolar disorder, and in a much attenuated way their unaffected relatives (Radua *et al.* 2012). *CACNA1C* has a haplotype index of 12% - strongly suggesting pathogenicity when haploinsufficient. The haplotype index for *SLC2A3* is 31% and for *SLC2A14* is 73%.

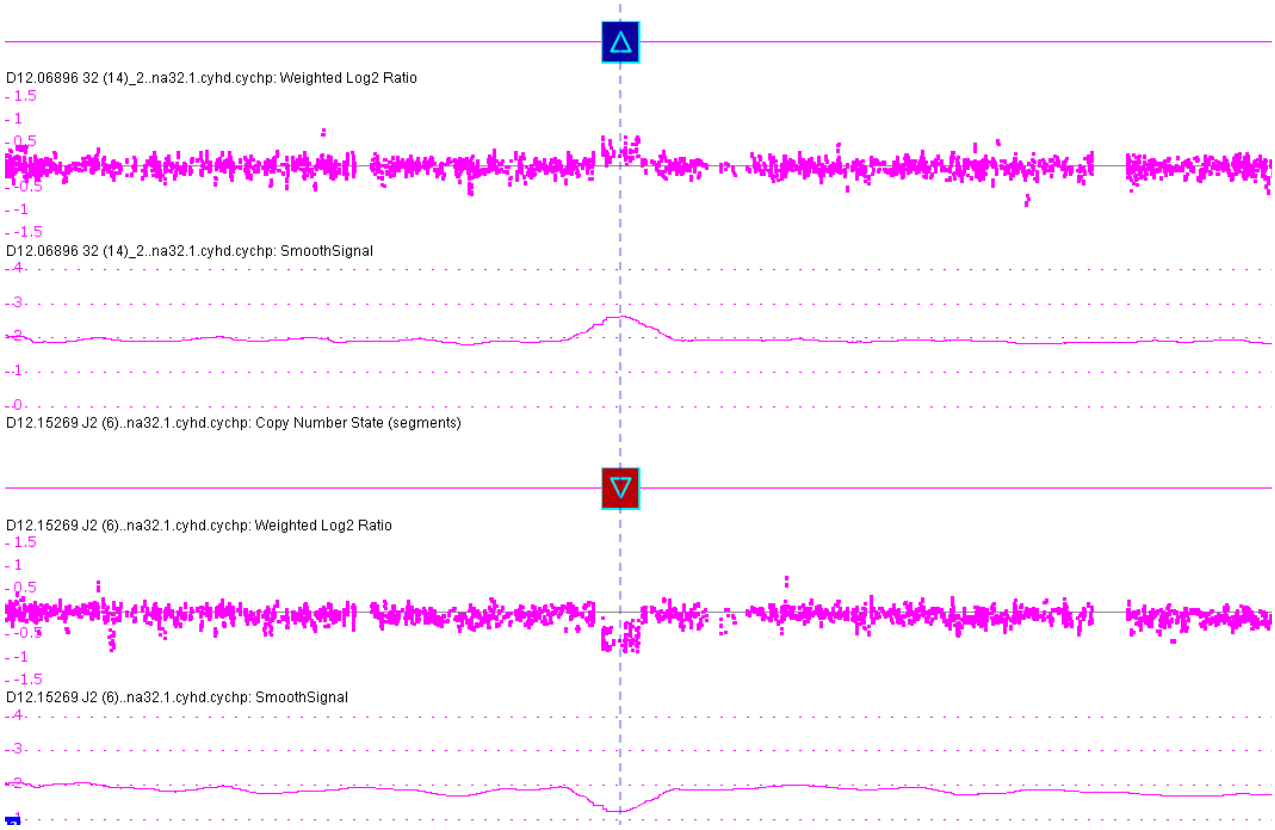


Figure 7.27 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating 12p13.31 *CACNA1C* deletions and duplications (J15, J1).

Gene	Official full name	NCBI description
CACNA1C	Calcium channel, voltage-dependent, L type, alpha 1C subunit	Calcium channels mediate the influx of calcium ions into the cell upon membrane polarization. The alpha-1 subunit consists of 24 transmembrane segments and forms the pore through which ions pass into the cell. The calcium channel consists of a complex of alpha-1, alpha-2/delta, beta, and gamma subunits in a 1:1:1:1 ratio.
SLC2A14	Solute carrier family 2 (facilitated glucose transporter), member 14	Members of the glucose transporter (GLUT) family, including *SLC2A14*, are highly conserved integral membrane proteins that transport hexoses such as glucose and fructose into all mammalian cells. Known as GLUT14
SLC2A3	Solute carrier family 2	As above, known as GLUT3

	(facilitated glucose transporter), member 3	

Table 7.13 Genes within the 12p13.31, 12p13.33 cluster

These are infrequently occurring CNVs. *CACNA1C* has 47 exons and spans 2,162,464 to 2,802,108. All cases call within intron 3-4 which is 328,545bp in length. In these cases it is of note that the three microduplications affecting *CACNA1C* are identical (and overlap with the microdeletions). It is possible that these CNVs have a direct splicing effect on *CACNA1C*- or perhaps a direct effect on the intragenic region confers risk to the phenotype. The figure below (7.28) shows the histone mark layer of exon 3-4.

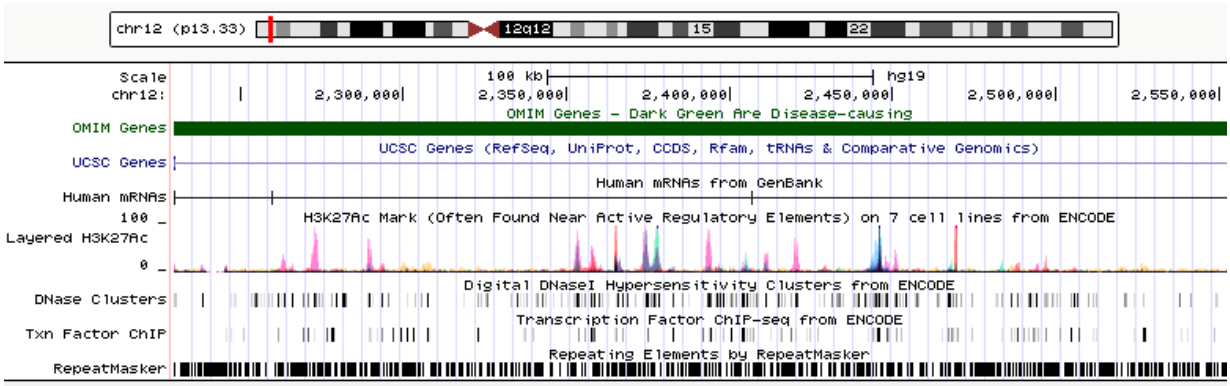


Figure 7.28 Screen capture of the UCSC genome browser - http://genome.ucsc.edu demonstrating intron 3-4 of *CACNA1C*. The high activity in certain regions of the layered H3K27AC line demonstrates areas of histone mark density -suggesting regulatory roles for this intron.

Regarding the second nearby CNV affecting the solute carriers there are CNVs recorded for the EU cases (15 similar gains, 2 losses) and in DGV 37 overlapping gains, 34 losses. The CNV would affect the whole of *SLC2A3* and 3 exons of *SLC2A14*. The haplotype index makes *SLC2A3* (GLUT3) a more promising candidate. GLUT3 facilitates the transport of glucose across the plasma membranes of mammalian cells. GLUT3 is most known for its specific expression in neurons and has originally been designated as the neuronal GLUT. GLUT3 also has a higher affinity for glucose than GLUT1 (*SLC2A1*); whereupon it is GLUT1 that has an established relationship with epilepsy and in particular early onset absence epilepsy (Suls *et*

al. 2009, Brockmann *et al.* 2001, Mullen *et al*. 2010). However mice heterozygous for GLUT3 demonstrate abnormal spatial learning and working memory, abnormal cognitive flexibility with intact gross motor ability, electroencephalographic seizures and perturbed social behaviour with reduced vocalization (Zhao *et al.* 2010).

7.2.2.3 Rare CNV with genes of interest

7.2.2.3.1 Intronic *AUTS2* gene deletion at 7q11.22

A second rare intronc microdeletion was seen at 7q11.22 in case 15 (intron 4-5). This CNV (7: 69755346-69811718) was 56,372bp in size and occurs within the *AUTS2* gene. This CNV appears to be very unusual: it is not in the WMRGL or EU datasets, nor in DGV – occurring four times in DECIPHER. The *AUTS2* gene encodes a protein called the autism susceptibility candidate 2. It has a very low haplotype index (7.3%). Small CNVs have been seen within *AUTS2* previously (133-319 kb). When they have occurred they have been more clearly deleterious: two patients had duplications involving single exons, whereas two had deletions that removed multiple exons. All patients had developmental delay, whereas two patients had autistic features (Nagamani *et al.* 2012). *AUTS2* has also been suggested as a risk gene for a number of behavioural traits including alcohol misuse (Schumann *et al.* 2011). Deletions of *AUTS2* have been described by Mefford *et al.* (2011) in one individual with JME and another with an unclassified epilepsy phenotype.

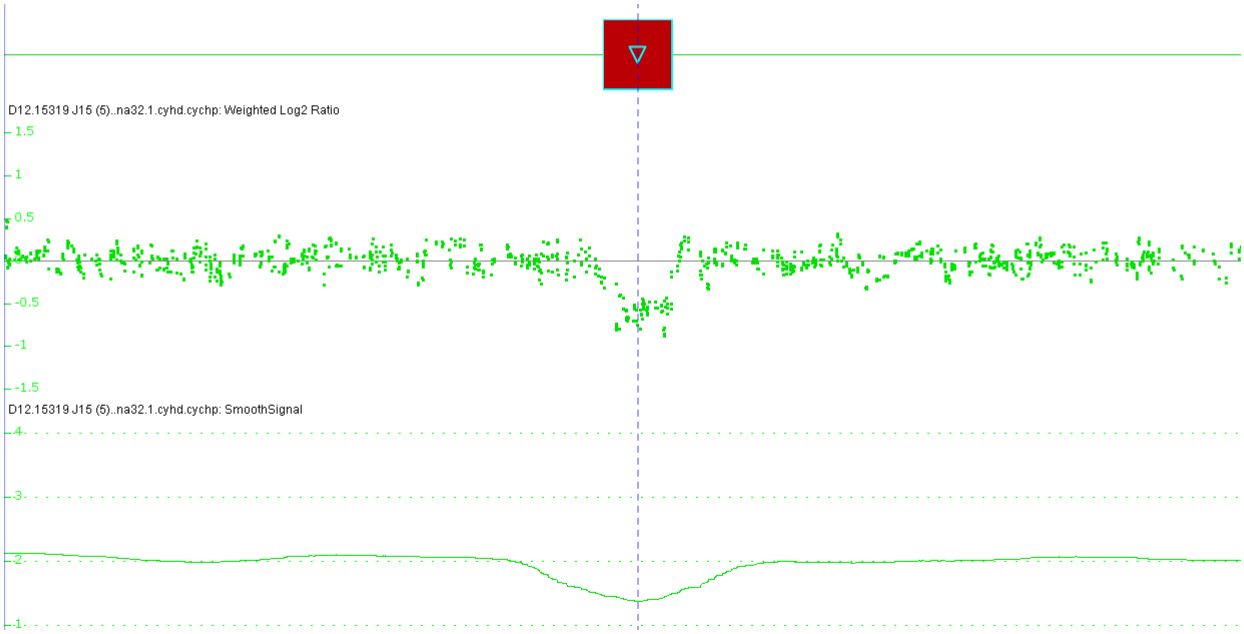


Figure 7.29 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the intronic *AUTS2* gene deletion at 7q11.22 in J15.

7.2.2.3.2 The 9p24.2 deletion and modifier *KCNV2*

The 9p24.2 deletion was seen once in the cohort – in patient 24. It was seen twice in cases in the WMRGL dataset but has not been reported in controls in either the EU dataset or the DGV database. It has been reported 28 times in the DECIPHER database and therefore should be considered a rare CNV, potentially pathogenic. In case 24 three genes are reduced to heterozygosity: *KIAA0020, VLDLR* and *KCNV2.* Their haplotype indices 12% are 31.4% and 19.8% respectively.

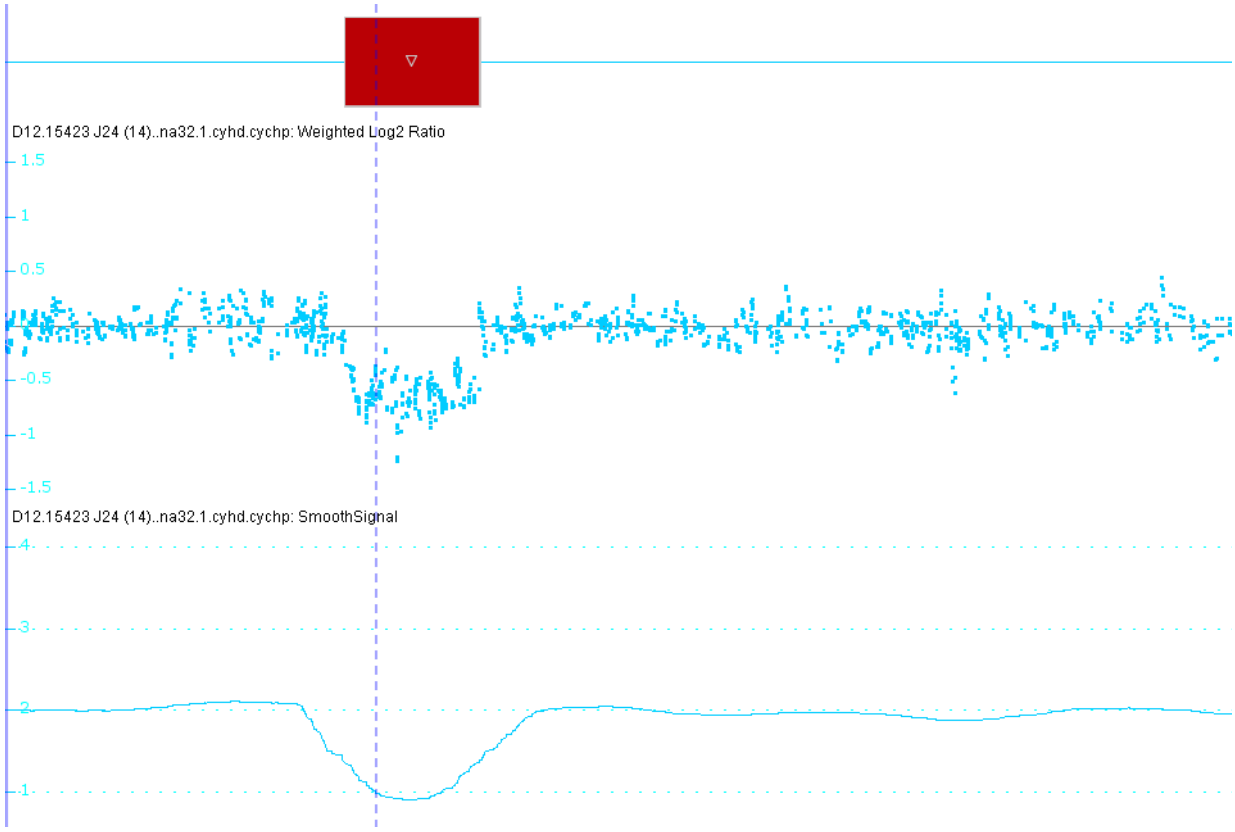


Figure 7.30 SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 9p24.2 deletion in J24.

Gene	Official Full Name	NCBI description
FLJ35024	Uncharacterized LOC401491	Misc RNA
VLDLR	Very low density lipoprotein receptor	This gene encodes a lipoprotein receptor that is a member of the LDLR family and plays important roles in VLDL-triglyceride metabolism and the reelin signaling pathway. Mutations in this gene cause VLDLR-associated cerebellar hypoplasia.
KCNV2	Potassium channel, subfamily V, member 2	Voltage-gated potassium (Kv) channels represent the most complex class of voltage-gated ion channels from both functional and structural standpoints. Their diverse functions include regulating

		neurotransmitter release, heart rate, insulin secretion, neuronal excitability, epithelial electrolyte transport, smooth muscle contraction, and cell volume. This gene encodes a member of the potassium voltage-gated channel subfamily V. This member is identified as a 'silent subunit', and it does not form homomultimers, but forms heteromultimers with several other subfamily members. Through obligatory heteromerization, it exerts a function-altering effect on other potassium channel subunits. This protein is strongly expressed in pancreas and has a weaker expression in several other tissues.
KIAA0020	Pumilio domain-containing protein KIAA0020	A minor histocompatibility antigen

Table 7.14 Genes within the 9p24.2 deletion

KCNV2 deletion is expected to produce cone dystrophy and supernormal rod response – a rare retinal disorder (Wissinger *et al.* 2011). However Jorge *et al.* (2011) also described it as an epilepsy susceptibility gene. It was identified as a modifier of Scn2a in transgenic mice. A threefold increase in hippocampal Kcnv2 expression was associated with a more severe epilepsy. They went on to screen 209 children with epilepsy and found nonsynonymous variants in 2/209 (not present in 368 controls). These were inherited from unaffected relatives and again they concluded that these were phenotype modifiers rather than directly pathogenic.

7.2.2.4 Rare CNV – unknown significance

6p21.2 duplication

This is a rare CNV of uncertain significance– appendix J.

10q11.22 duplication

This is a recurrent CNV – also seen in the CGH array data – appendix I.

11q13.4 duplication

This is a rare CNV of uncertain significance– appendix J.

7.2.2.5 *EFHC1*

EFHC1 (as described in full in the introduction) is the single most studied gene in relation to JME and some authors believe has a causative relationship with JME. Not a single sample had a deletion in *EFHC1*– however sample J7 (and J7 alone) had a region of LOH (loss of heterozygosity over the whole gene).

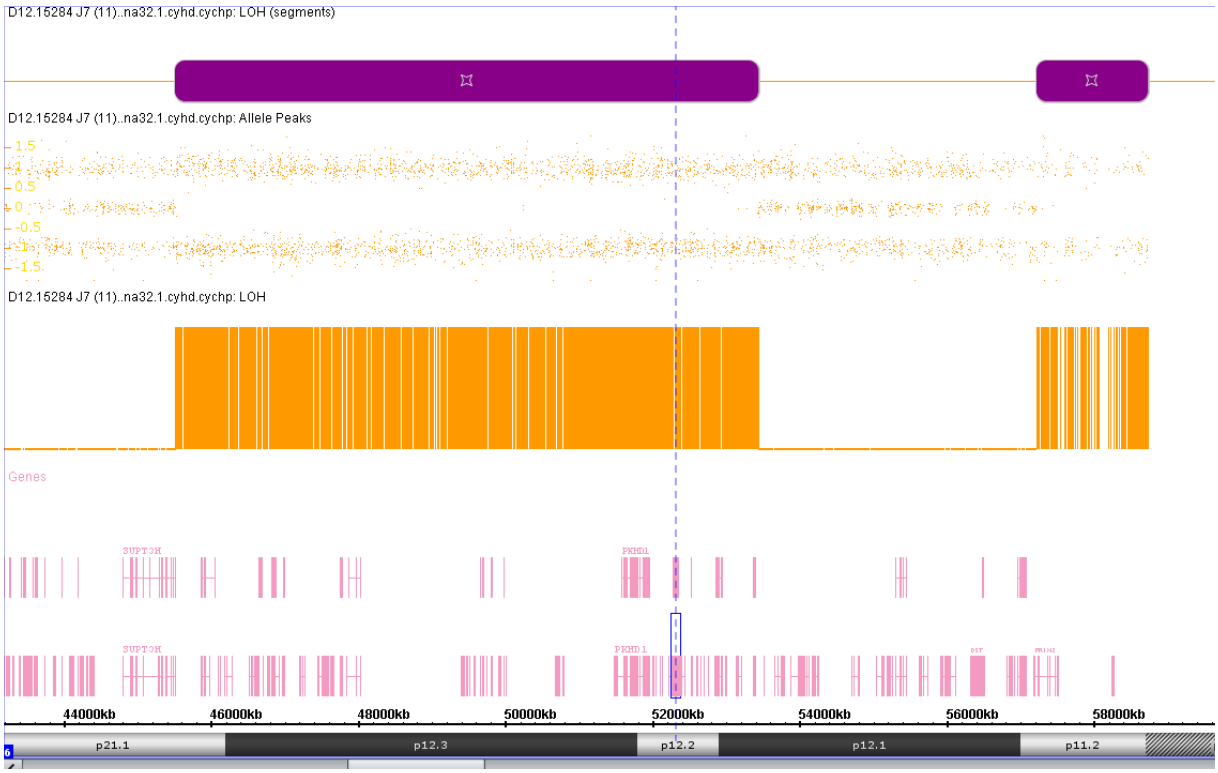


Figure 7.31 The dotted vertical line denotes the position of *EFHC1.* The LOH (shown in purple) is very large indeed - 6: 45521010-53447628 encompassing 7,926.618kbp. This is the single largest LOH in J7 by a large margin.

Comparison

In appendix K is a comparison between CGH array and SNP genotyping. I look at an example where CGH array failed to provide an answer and then focus on the two cases which were studied with both techniques.

7.3 Summary

The data presented here from both SNP genotyping and CGH array demonstrates the complexities of analysing CNV data. Firstly all genetic results need to be analysed in the context of the individual (their manifest phenotype) and of their family (have they inherited this CNV?). Despite the sophisticated phenotypic analysis of people with drug resistant JME seen in the previous chapter – I have in some cases not been able to marry up the CNV data and phenotype. Furthermore the method of ascertainment and the ethical framework that I worked within made collection and analysis of asymptomatic relatives impossible for the main body of my cohort. Furthermore there is a pipeline of confirmatory laboratory work and functional analysis necessitated by these discoveries that are outside of the scope of this thesis.

Despite these caveats this is the largest study of CNVs in both people with JME and people with hyperekplexia. I found no evidence for CNVs to be common in 'gene negative' hyperekplexia – but intriguingly hyperekplexia can be mimicked by other single gene disorders such as Pitt-Hopkins syndrome and creatine deficiency syndrome which *can* be identified by CNV analysis. Understanding the scope of the *GLRA1* phenotype (chapter four) permitted the identification of an atypical case – and perhaps the unearthing of a novel indel producing Beckwith-Wiedermann syndrome.

No single CNV or group of CNVs can account for the pathogenesis of drug resistant JME. Rarely (such as the clustering of *CACNA1*) did CNVs include ion channels or genes involved in the proteome of ionic transmission. The role of rare clustering of CNVs in JME is discussed as was the potential of intronic regions. CNV syndromes were identified: one 16p12.2 deletion in a female with drug resistant JME and a 15q13.3 deletion in a male with atypical BECCTS. The most important single findings perhaps were not directly due to the pathogenesis of

epilepsy. The discovery that a female with JME carries a novel deletion in *DMD* is clinically significant and even more so – perhaps – was the identification of a duplication in *NKX2-5* in a young adult who died a SUDEP death. Not only may this be the cause of his sudden death but screening his family may identify others who are similarly affected and help prevent a premature cardiac arrest.

Chapter Eight

Conclusions

8.1 Introduction

Chapters three to seven have taken us from the individual to the cohort; from their psychological performance to their genes. This aim of this journey is to better describe people with paroxysmal disorders to facilitate second generation genetic testing. Although there is a revolution underway in bioinformatics helping biologists sift through huge data files, clinical correlation will remain crucial to this process.

1. We should not fear patient participation even in complex neuro-biology projects. A peer-to-peer relationship may assist with recruitment and retention.
2. Collection of rarer cohorts is possible with patience and collaboration. This provides the opportunity to identify homogeneous groups such as in hyperekplexia.
3. Analysing cohorts drawn from a variety of sources may help us challenge expected norms, such as in JME. Phenotypic definitions cannot always be accepted as other authors may draw on groups biased for understandable reasons – such as ethnicity.
4. Non seizure features can be used to sub-categorise cohorts. The use of scales designed for other indications (such as the BADS and traumatic brain injury) can be used to identify significant deficits in people with JME.
5. Copy number variation analysis – although complex and in many ways still in its infancy – can identify large deletions and duplications. However the implications of these can only be understood within the clinical context of the individual, as many large CNVs appear to convey little additional risk of disease.

The conclusions drawn from the results chapters will be discussed in the order that the chapters were presented and the themes that span this thesis will be expanded.

8.2 Involvement

8.2.1 Negative impact of involvement

Over promoting the importance of involvement can risk sounding pious. It is fair to say that the level of patient involvement such as the patient RDG sits uncomfortably with some researchers and can evoke cynicism. Is the patient RDG really patient centred and patient run? I admit that the patient RDG exists within certain parameters (cost, size, focus, influence) and that these may not be within the control of the patient members of the RDG. I admit that although the RDG is not chaired by a clinician, the health professional members - although a minority - may have a disproportionately sized say in certain matters. The 'doctor / patient relationship' may prevent the meetings from ever taking place on an equal footing.

One of our continued concerns is about 'professional distance' as the boundaries between a social and a 'doctor / patient' relationship get blurred. How do you cope with requests for general epilepsy advice? For opinions about drug treatment? Complaints about colleagues? Does this decision change if the questions are posed in clinic, after a meeting, or perhaps when giving someone a lift to the station? A greater challenge is the inequality in the relationship: if I know someone from clinic, I may be aware (for example) of their recent divorce, failed IVF treatment and brother's mental health problems. The patient may or may not choose to disclose these during discussions in the meeting – but what would it be appropriate for me to share? And as my disclosures are carefully measured (rather than the open and indiscriminate nature of medical notes) the relationship is always unequal.

8.2.2 Social media - Twitter

In contrast to the open 'broadcasting' of *facebook* (chapter three) *Twitter* is currently the rival social medium. *Twitter* is an application which allows people to write brief statements and link to interesting articles and webpages. To read someone's tweets you select them (follow them) – or alternatively you can search through all updates as all the content is in the public domain. Using a hashtag (#epilepsycongress for example) makes this kind of searching more straightforward.

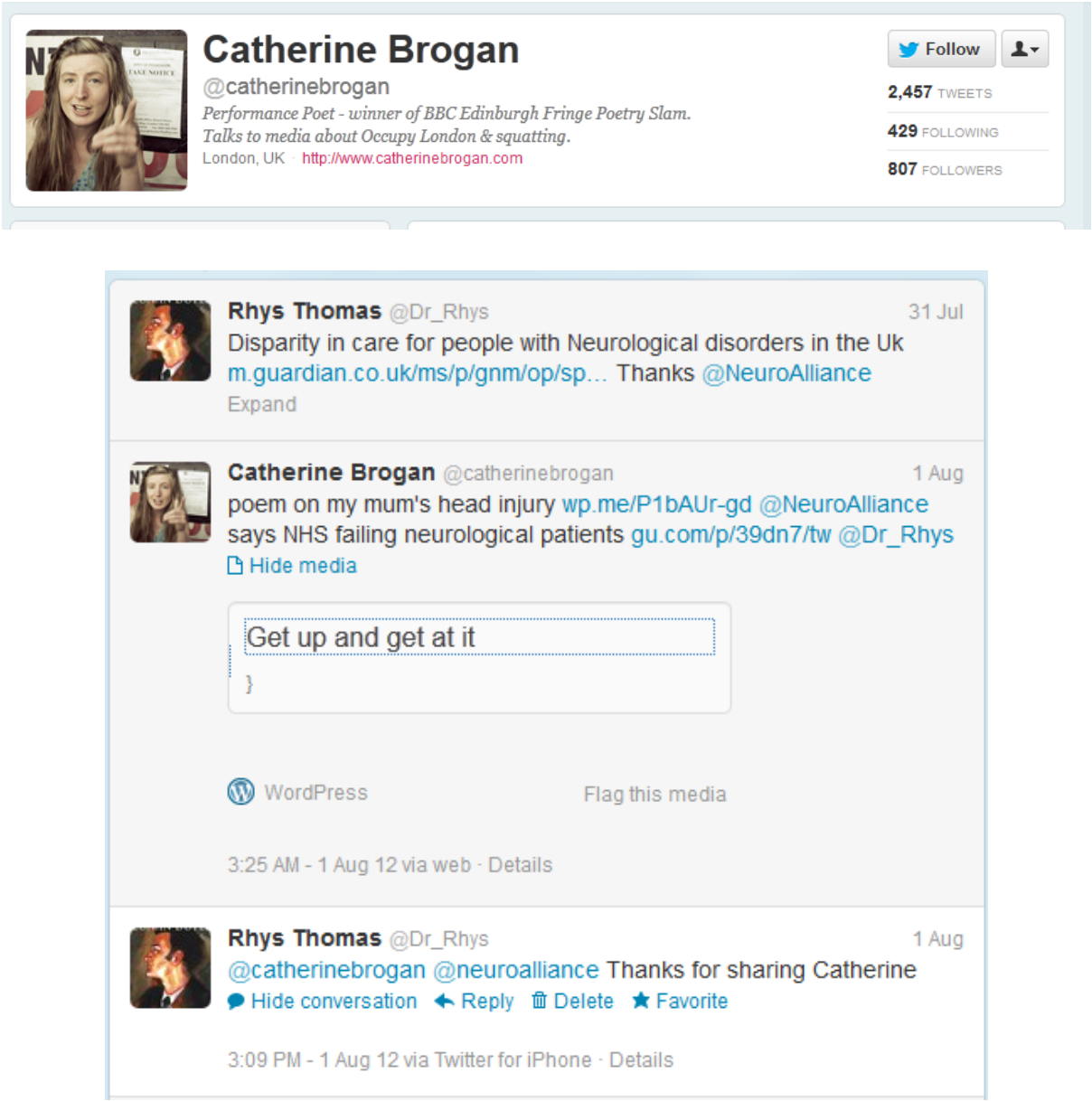


Figure 8.1 A typical exchange on Twitter. I give by advertising the existence of a *Neurological Alliance* report on services for people with neurological disorders (reported in the Guardian) and receive a poem from an award winning poet – about her mother's traumatic brain injury.

There's an immediacy about tweets: a whole stream of contemporaneous views in your pocket (via your smart phone). Early adopters of *Twitter* included people in IT, hipsters and media agencies. People in IT liked the fact that although they were programming at home they could 'get the wisdom of the crowd' and get advice about bugs and quirks of their programming language back quickly. I'm aware of a number of emergency department doctors who do the same thing. There are a growing number of epilepsy charities, organisations and private individuals who have active Twitter accounts; furthermore the

BMJ or Lancet stable journal, the University or health board, even chief executives have accounts. There's also a thriving Sunday night journal club (@TwitJournalClub). Beyond this there are plenty of doctors on Twitter who either tweet under their real names (figure 8.1) – or use a pseudonym (such as @grumpyGP).

Twitter is, furthermore, a great place for people with epilepsy to meet likeminded friends. Your confidence is low... and your driving licence suspended... and your nearest meeting a couple of buses away… and you need to speak to someone now…. tweet it. If you want to know whether other people had this side effect, or how they chose between two different meds.. tweet it. If you want to tap into the experiences of other mothers who had children with the same rare but devastating childhood epilepsy... tweet them. The American Epilepsy Society featured a poster last year about attitudes to epilepsy on Twitter (generally positive and supportive; but 40% negative - McNeil, and colleagues from Dalhousie University) compared this to the braying, bullying and misinformation often discovered on *YouTube* (Cossburn, Smith 2007). There is a wealth of micro-conversations going on about epilepsy (20 tweets used the word 'epilepsy' in the last half an hour for example). How soon will you be able to tweet your epilepsy specialist nurse for advice? You can already do this for *Epilepsy Action* (figure 8.2).

New technologies complement the traditional ways of meeting (such as the RDG, DUETs focus groups below). For a condition such as epilepsy where there is no 'typical journey' and where there are limitations to mobility and confidence, there is a prime role for social media. For conditions as rare as the epileptic encephalopathies or hyperekplexia the need for an internet based virtual society is even greater. Whenever sufficient motivated people can gather and are facilitated to share their experience there are always opportunities for new discoveries; this is particularly true of the DUETs project discussed next.

Figure 8.2 An exchange on Twitter; A patient challenging me to learn more about exclusions for people with epilepsy. *Epilepsy Action* joins the conversation and provides advice to a boxer from Australia.

8.2.3 DUETs Conclusions

The DUETs study demonstrates that patients and clinicians have different agendas when asked independently to produce and prioritise lists of treatment uncertainties. I recognise some limitations including that the presence of a doctor at the three patient meetings may have introduced an observer bias and that there were no newly diagnosed patients in those groups. The uncertainties generated by our participants are certainly not exhaustive, in part because we concentrated on treatment rather than natural history, causation and investigation. Our list of epilepsy therapeutic uncertainties needs to be supplemented by 'fresh' prospectively gleaned uncertainties by scouring recent Cochrane and other systematic reviews, and clinical guidelines. Furthermore, the database, no matter how dynamic, is redundant if we do not attempt to confirm whether the questions posed (by both clinicians and patients) are genuine uncertainties, that is, 'known unknowns.' The questions have been scrutinised and the outcomes published at NHS Evidence (www.library.nhs.uk/DUETs). This project provides the framework and content which can be used by professionals in targeting genuinely patient-focussed research.

8.2.3.1 Practical considerations

I expected patients to focus more on the needs of the individual regarding their epilepsy treatment; however the qualitative and statistical analysis in the main does not support this. Not only did epilepsy professionals produce an equal number of questions under the theme of *"considering the patient as an individual"* (n=14) but they ranked these uncertainties as more important than patients did. Both groups prioritised practical considerations that were specific to them.

Fifteen questions raised exclusively by patients and carers concerned how to take prescribed medication, (figure 3.12). Four questions were raised about the general practitioner's role in the treatment of epilepsy: all came from patients and carers. There were two questions about access to repeat prescription amongst the 7 of the 8 uncertainties raised about services. Strikingly, not only did patients and carers generate 8 of the 9 questions regarding improving public awareness of the treatment of epilepsy and

seizures, but three of these questions were ranked among the 20 most important by patients and carers.

Professionals also prioritised practical considerations from *their* point of view all of which clustered around 'the challenging consultation'. All of the questions about treatment of non-epileptic attack disorder (NEAD) were posed by professionals (n=6, 2 were highly rated). Side effects *in utero* and prescribing issues in pregnancy, although ranked as important by people with epilepsy, were also rated as more important by clinicians than by patients and carers. Patients did not volunteer questions about prevention of sudden unexpected death in epilepsy (SUDEP) (n=3); all ten questions that focused on the needs of older people were by professionals (6 regarding prescribing AEDs, 4 focussing more general therapeutic needs); most of questions on the considerations before starting treatment (8/9), medication compliance (10/11) and nutritional support (11/12) were asked by professionals.

8.2.3.2 Priorities

When discussing uncertainties about epilepsy treatment, I expected to receive questions about 'curing epilepsy' and achieving life-long seizure freedom: these questions were conspicuous by their absence. Patients asked two questions and epilepsy professionals eleven, regarding how best to *manage* seizure freedom – most of these questions were about safe drug withdrawal. Does this indicate that pragmatism (as opposed to idealism) is paramount in the important priorities? Epilepsy is not necessarily a life-long condition – a number of participants had achieved seizure freedom, both with surgery and medication.

Promoting an environment which engenders genuine patient involvement in posing questions and developing outcomes that matter, is still at an early stage (Firkins 2008). Mapping mismatches between researchers' and patients' priorities, for example in rheumatoid and osteoarthritis, demonstrated that the priority treatment outcome for patients with rheumatoid arthritis is not pain reduction, but fatigue and sleep quality (Tallon *et al.* 2000; Hewlett *et al*. 2006; Kirwan *et al*. 2005; Carr *et al.* 2003). The identification of uncertainties has also been undertaken for a number of conditions including schizophrenia

(Lloyd *et al.* 2006; Lloyd and White, 2011) and urinary incontinence (Buckley *et al.* 2007). One of the key themes from the James Lind Alliance priority setting partnership for incontinence mirrors the practical considerations of people with epilepsy: people with continence difficulties want more public toilets.

8.2.3.3 Shared agendas

I encourage researchers in other disciplines to engage with patients and carers to help people with a personal experience of a condition to express their research priorities and condition-specific uncertainties. I would welcome any group keen to address an epilepsy uncertainty or undertake a systematic review to consult DUETs. We will need the help of the epilepsy community in both continuously updating the uncertainty database and answering the questions that matter. 'Uncertainties' is an occasional BMJ feature (Chadwick *et al.* 2008; Chalmers 2008). I would like to encourage authors and editors to explicitly consider the *uncertainties* apparent in a body of work, in addition to the areas that they directly address.

8.2.4 Involvement - Summary

I started chapter three by stating that communicating with patients in a full and engaged way is the only possible way to attempt to collect the rich and nuanced data of everyday living with a chronic condition. The limitations of coding someone from a clinic letter or a scale or two is made absurd when one considers the bounty of information that is yielded by copy number analysis (chapter seven). To communicate fully it is no longer sufficient to simply be willing to call a witness to their seizure. So much of what makes epilepsy a fascinating topic for research happens in the life in between the seizures. It is therefore important for me to consider non-seizure features such as executive function, personality, impulsivity, mood and memory when attempting to identify novel phenotyping tools for juvenile myoclonic epilepsy (chapter six). Not only can these attempt to collect data on what it is like to live amongst the seizures, but we know from the DUETs study that patients identify and prioritise research into cognition and mood in epilepsy.

DUETs identified that patients' approach to research questions focuses upon practical solutions to problems. The patient RDG provides a forum where research of these practical problems can be developed into RDG research projects and project-specific grant applications. Future projects currently under investigation include methods to reduce dental and tongue injury during a seizure, how to give advice about medication (missing doses and medication mistakes) and how to minimise the cognitive side-effects of antiepileptic drugs. Genuine consideration of participants' experiences in research is rare and via our patient RDG high priority is given to service–user opinions. It was therefore important for me to choose research strategies which could produce data that could be returned to the participants. The JME volunteers could opt in to receive a report following the analysis of their interview data; and those who gave us blood or saliva for DNA either consented to be told about important findings – or not, after all facets were explained.

It can sometimes be difficult to explain to colleagues why I feel passionately about the patient RDG meetings. David Oglivy (thought of by some as the father of modern advertising) is thought to have coined the aphorism "search all city parks and you won't find a statue of a committee." Furthermore the saying "a camel is a horse designed by committee" (attributed to Sir Alec Issigonis) again atones to the perils of group working producing less than the sum of the talents involved. Despite this, I think working with patients and patient groups is essential both for common and rare disorders. The participants in both the RDG and the DUETs project have often challenged me and provided me with a fresh viewpoint or a novel explanation. It is against this prejudice (which I fully understand) that I must assert the value of the RDG as a 'talking-shop'. I would counter Mr Oglivy by saying that you wouldn't choose to read the biography of a critic, nor the poems of a pessimist. And of course he is wrong about the statue of a committee: Rodin's 'Burghers of Calais' is displayed in twelve locations worldwide, including outside Parliament.

Figure 8.3 The Burghers of Calais by Auguste Rodin1886, cast 1908 "Les Bourgeois de Calais" Victoria Tower Gardens, Westminster -London, October 2009

Chapter three describes the involvement of volunteers and rediscovering the individual at the heart of genetic research. It demonstrates the benefits of mixed methods; both qualitative and quantitative; both case reports and patient lead digital stories; art and print; digital new media and traditional press reporting. All of these forms have a part to play – and the next few years will see the rise of sophisticated online communities as conditions that are more common than hyperekplexia gather together with social, educational and research aims. I think electronic resources will outstrip and replace the traditional 'patient group' for pastoral support for all but the oldest of generations within ten years in the United Kingdom. Researchers who fail to adopt social media and adapt to the expectations of their community will fail to involve their volunteers fully.

8.3 Hyperekplexia

8.3.1 Genotype-phenotype

This is the first description of how the hyperekplexia genotypes affect the associated clinical phenotypes outside of reports of large kindreds. I report definitive indications for gene-specific phenotypic differences and an association of recessive inheritance with increased risk of learning difficulties and developmental delay – particularly speech acquisition. I also highlight the risk of severe recurrent neonatal apnoeas with hyperekplexia (particularly associated with *SLC6A5* and *GLRB*) which has hitherto been under recognised. I also acknowledge some limitations to the study: it has proved impossible for me to examine each patient individually and so I am dependent on the information provided by the referring clinicians. This is a cross-sectional cohort study; patients of differing ages are being compared, some are too young for certain features (learning difficulties or falls for example) to exhibit themselves. Similarly, there is a recall bias from older patients as their neonatal apnoeas may be forgotten, under-reported or ignored. It is very probable that a lack of familiarity with this condition leads to under-reporting and misdiagnosis (or an over diagnosis of seizures). Despite this being the largest description of hyperekplexia families and cases by a significant margin, it is quite possible that I have been unable to recognise or prove the existence of rarer but relevant co-morbidities and characteristics.

8.3.1.1 Recessive versus Dominant Inheritance

Hyperekplexia was initially misreported as demonstrating solely autosomal dominant inheritance, because these families were easier to identify, with greater numbers of affected family members; ultimately leading to an ascertainment bias (Kok and Bruyn 1962, Kirstein and Silfverskiold 1958). Autosomal dominant inheritance does occur, but it only accounts for 16% of the gene-positive cases in this study. This may be an under-estimate; we may be more likely to receive requests for a genetic diagnosis when the clinical pattern is ambiguous - such as a lack of family history. However, it is important to recognise the importance of recessive inheritance in the context of genetic counselling. It is likely that in consanguineous families, other undetected recessive mutations may be responsible for the

extended phenotype. In addition to the autosomal dominant bias, the over-reliance of interpreting the physical characteristics from the proband (often the most clearly affected individual in a family) has left us unable to identify 'major' and 'minor' forms of hyperekplexia. There is heterogeneity, however, derived from the variation in the physical characteristics over an individual's life (worst in the first year) and over the course of a day (improved in sleep, with alcohol and benzodiazepines - provoked by external stimuli). This means that this delineation (major versus minor) may not represent a distinct pathology, not least because of other considerations such as *in vivo* pathophysiological mechanisms, genetic incomplete penetrance or the possible occurrence of digenic/multigenic cases.

Parental sampling has identified hyperekplexia as a highly-penetrant condition. Only rare examples of incomplete penetrance are reported (Zoons *et al.* 2012). There is a small degree of variability within families, for example, a family from the UK with autosomal dominant inheritance due to the common *GLRA1* R271Q mutation exhibited mild learning difficulties, until the present generation, where an affected individual completed mainstream schooling. However, this may represent the success of early diagnosis and prompt treatment with clonazepam (rather than chlordiazepoxide which her ancestors used; Lindahl 2005). Of the twelve pairs of siblings who share the same genotype, there appears to be an extensive degree of homology. When the core features of hypertonia, startle, falls, LD, response to drugs and apnoea are compared there is 4% discordance, driven mainly by the ambiguity due to variable questionnaire entries.

8.3.1.2 Hyperekplexia and Apnoeas

Neonatal apnoeas have been recognised in hyperekplexia following a case report in 1983 (Kurczynski *et al*., 1983) and were estimated to occur in 10% of patients (Giacoia *et al*., 1994); this under-estimates the risk. Apnoeas occurred in 56% of all patients, but over 80% with *SLC6A5* / *GLRB* mutations. These events are often associated with the triggered tonic, abdominal splinting attacks and can be life-threatening. A patient who was compound heterozygous for *SLC6A5* mutations (Y491C and Q630X) suffered 47 respiratory arrests in an eight-week period (Rees *et al*., 2006). It was previously unclear why apnoeas were regarded as an ‘exclusive’ feature of sporadic hyperekplexia (Bakker *et al*., 2006; Bakker *et al.,* 2009).

However, the explanation lies within the inheritance patterns; we have shown that patients with *SLC6A5*/*GLRB* mutations are significantly more likely to describe serious neonatal cyanotic attacks and since the vast majority of known *SLC6A5*/*GLRB* mutations are recessive the cases will appear sporadic. The same trend applies for *GLRA1* mutations where sporadic index cases have been linked to recessive mutations (Rees *et al*. 2002; Chung *et al*. 2010). Cases that appear to reverse this trend can be explained through functional studies. For example, a proband with a clear family history has a dominant *GLRA1* mutation (P250T). From a molecular perspective, this mutation exhibits a dominant negative effect, which mimics the recessive scenario by creating a total knockdown of α1β GlyRs.

8.3.1.3 Co-morbidity with learning difficulties and developmental delay

Forty percent of people with *GLRA1* mutations had neither neonatal apnoeas nor delayed development, in contrast to *SLC6A5*/*GLRB* mutations where just one case had neither co-morbidity. It has been incorrectly stated that the cognitive profile of people with hereditary hyperekplexia is unaffected or mildly impaired – although recognising that 'sporadic' cases were more complex (Zhou *et al*., 2002); and later attainment of motor milestones has been previously described (Tsai *et al.,* 2004) in a family with recessive *GLRA1* hyperekplexia. It is likely that the apnoea attacks do not directly cause cognitive impairment: forty per cent of the cases with LD or developmental delay had no prior apnoea attacks (all had *GLRA1* mutations).

Magnetic resonance imaging is ubiquitously normal in genetically explained hereditary hyperekplexia, which may be unusual if repeated serious hypoxic / ischaemic brain injury was occurring in the prenatal or neonatal period, where basal ganglia ischaemia or gliosis may occur after prolonged apnoea. It is likely that in consanguineous families, other undetected recessive mutations may be responsible for the extended phenotype or bias the reporting of rare co-morbidities. Although a dysexecutive syndrome has been described alongside hyperekplexia symptoms (Gaitatzis *et al.,* 2004), the late age of symptom onset and abnormal brain imaging suggest a symptomatic hyperekplexia. It is perhaps no surprise that the patient concerned had no detectable *GLRA1* mutation.

The developmental delay demonstrated in some patients may represent defective neuronal migration and targeting within a context of an environment where glycine is depleted from presynaptic terminals, or incomplete neural networks due to the lack of GlyR activation during brain development. In favour of this theory is a description of a family with an atypically severe hyperekplexia syndrome (Seidahmed *et al.* 2012). This Saudi family with consanguinity had six infants that had a severe and lethal form of hyperekplexia. Although they were gene negative, the microcephaly, optic atrophy and neuronal underdevelopment with prominent CSF spaces suggest a failure of neuronal migration. In particular frontal and temporal structures appear most affected.

8.3.1.4 Synaptogenesis

The phenomenon of last trimester intrauterine startle first described by Leventer *et al.* (1995) and confirmed in chapter four suggests that the glycinergic synaptopathy is first apparent months before birth – during the time of greatest neurocortical development. Synaptogenesis is particularly important during early life where there is neuronal pruning due to competition for neural growth factors by neurons and synapses. Glycinergic neurons appear during development from embryonic day 12.5. (Chalphin and Saha 2010). In the neonatal brain many inhibitory synapses initially are mixed GABAergic and glycinergic, which changes during CNS maturation. *GLRA1* and *GLRB* in rats are expressed at very low levels in spinal neurones until postnatal day 15, with the $\alpha 2$ subunit expression declining prenatally (Malosio *et al.* 1991).

Castaldo *et al.,* suggested (2004) that the glycinergic synapse and GlyRs may well participate in cognitive development. GlyRs have been described in septal cholinergic neurons, which play a major role in learning and memory by means of their connections with the hippocampus and the cerebral cortex and their function can be modulated by psychotropic medication. Our finding that speech acquisition is preferentially affected implicates glycinergic pathways in expressive language – however, many patients reported later developmental 'catch-up' suggesting a sophisticated compensatory mechanism which plausibly may involve up-regulation of inhibitory GABA, or down-regulation of antagonistic glutamate/ NMDA systems. Whatever the mechanism, it appears that compensation for

GLRA1 mutations seem to be more efficient in humans than in animal models, as *GLRA1* abolition produces a milder human phenotype than seen in animals (Harvey *et al.* 2008, Schaefer *et al*. 2012). In particular, rats do not seem to receive the symptomatic benefits that humans do from clonazepam. Homozygous mutant mice for human *GLRA1* and *GLRB* mutations have a progressive disease that is lethal at post natal days 15 to 21 (Findlay *et al*. 2003).

There are two further GlyR alpha-subunits (α2, α3) that have a much lower expression pattern in adults that may provide some contingency for the lack of functional GlyR α1β by forming functional α2β and α3β heteropentamers. There seems to be much less innate contingency for transporter or *GLRB* pathology; possibly because GlyT2 is a specialised presynaptic monomer, and loss of GlyR β also causes the disruption of gephyrin-mediated clustering and anchoring of GlyRs reducing postsynaptic channel density.

It has been suggested that mutations that lead to a complete lack of function may be more likely to exhibit a severe phenotype with learning difficulties, such as R218Q, or the nonsense mutation Y202X in *GLRA1* (Brune *et al.* 1996; Castaldo *et al.* 2004). The R65W mutation (recessive) results in drastically reduced functional channels and surface receptors (Chung *et al.* 2010). The phenotype displayed included delayed milestones at a year as well as speech and language delay and recurrent neonatal apnoeas. R252C (recessive), R392H (recessive), E103K (compound heterozygote) all have demonstrably reduced current and functional channels and developmental delay/LD but without apnoeas (Vergouwe *et al.* 1999; Villmann *et al.* 2009; Chung *et al.* 2010). The pattern of learning difficulties and developmental delay is yet to be described using standardised tests.

8.3.1.5 Compensation

The recovery seen in patients with hyperekplexia suggests an adaptation to living with a faulty glycinergic system. Either there is adaptation at the glycineric synapse (other glycine receptor subunits are preferentially expressed, or glycine reuptake is preferentially by the GlyT1 astrocyte system), or perhaps the GABAnergic system compensates. The pathophysiologly of startle and tone suggests a dual role for both inhibitor systems and the

symptom relief from alcohol and the benefits seen by benzodiazepines are suggestive of a GABAnergic influence on the condition. Therefore the effects of disruption to the glycinergic system would be maximal at an early stage, prior to compensation – any early life neuronal process such as or neuronal development, the formation of higher networks, or myelination could be disrupted in the glycine poor environment. Interestingly there is no compensation seen in knock in mice (universally lethal as mentioned above) but there is for spontaneously occurring murine hyperekplexia (Schaefer *et al.* 2012). The mechanism is unknown but it is neither from GABAnergic up regulation nor compensation from other GlyRs in mice.

8.3.1.6 Response to clonazepam

A prompt diagnosis is of importance, as the vast majority of people respond well to clonazepam, which may offer more relief from startling than hypertonia (Tijssen *et al.* 1997). Physical activity (for hypertonia) and patient education should form part of a treatment regime for anyone with hyperekplexia (The Hyperekplexia Society- *facebook*- chapter three). The major non-pharmacological treatment for infants with hyperekplexia is the Vigevano manoeuvre (Vigevano *et al.* 1989). The description was *"during a long seizure, one of us intervened in an attempt to counteract the hypertonia: the patient was held by the head and legs and forcibly flexed towards the trunk. The seizure ceased immediately. The procedure was repeated several times and always proved effective."* It has also had varying success in abating tonic attacks. I recommend cardio-respiratory monitoring for infants who have frequent apnoea attacks or tonic non-epileptic attacks with bradycardia.

Understanding that there appears to be a difference in clinical presentation (between *SLC6A5/GLRB* and *GLRA1* hyperekplexia) means that I must recommend both early genetic testing for suspected neonates and pre-conceptual counselling for couples who have a family history of hyperekplexia.

8.3.1.7 Imaging

Our gene positive cases had ubiquitously normal conventional imaging (the majority were MR brain scans, occasionally CT). This finding is supported by the literature. There have been attempts to utilise advanced imaging such as MR spectroscopy (Bernasconi *et al.* 1998). The Saudi case discussed above had abnormal conventional imaging but was gene negative (Seidahmed *et al.* 2012). Four unrelated individuals with 'major' hyperekplexia were studied. They demonstrated a reduction of N-acetylaspartate / creatine and choline contacting compounds in frontal and central regions. It was taken to represent frontal neuronal dysfunction. What is striking is how atypical the cases presented are from the gene positive individuals that I have presented (Bernasconi *et al.* 1998, table 1 p1508). Not one of the four had startle from birth – and the hypertonia described is transitory in two, persistent in a third and absent in the fourth. However all four have normal MR imaging and respond (to some extent) to clonazepam.

8.3.1.8 Gene negative cases

Hindsight allows us to reappraise previously published gene-negative patients. Shiang *et al.* (1995) described seventeen patients: 11 had definite autosomal dominant inheritance and uncomplicated hyperekplexia without LD, whilst five of the six gene-negative patients had developmental delay. All had an equivocal family history with no clear autosomal dominant inheritance. We now know that one had compound *SLC6A5* mutations (delCT 1459-1467 (fs+40X), G657A appendix G, Carta *et al.* 2012) and another has a recessive mutation in *GLRB* (Chung *et al.* 2012). The thirty-five gene-negative cases form a looser collection of cases with exaggerated startle co-presented with occasional hypertonia, falls and apnoea. It leads us to speculate that the gene-negatives fall into the following categories: 1) classical hyperekplexia where intragenic DNA variations in *GLRA1*, *GLRB* and *SCL6A5* remain untested (ENCODE consortium *et al.* 2012); 2) classical hyperekplexia with genes yet undiscovered; 3) neonatal / early-infancy acquired hyperekplexia that is a phenocopy of genetic hyperekplexia (e.g. autoantibody driven hypertonia and startle) (Hutchinson *et al.* 2008); 4) other undiagnosed or unrecognised neuromotor disorders presenting as mimics, sharing

common features such excessive startle or hypertonia (table 2.2). These conclusions are supported by the identification of a rogue Pitt-Hopkins' syndrome and potentially a child with creatine transporter deficiency syndrome amongst the referred cases (chapter seven).

It is possible that the largest gene of effect is yet to be identified in hyperekplexia or that multiple minor genes are still elusive. Eight typical cases and their parents are currently submitted for CNV analysis (CGH array) and whole exome sequencing as part of a programme grant awarded by *Action Medical Research* to Professor Rob Harvey, Professor Mark Rees and myself in 2011. As part of the preparation for this project we reanalysed our typical 'gene negative' cases and identified a number of new mutations in *GLRB* - the third gene of effect in hyperekplexia (Chung *et al*. 2012).

8.3.2 Phenotype of Hyperekplexia Associated with *GLRB*

In keeping with hyperekplexia associated with *GLRA1* and *SLC6A5* mutations, every child demonstrated pathogenic startle and symptoms were diagnosed within the first week of life. On occasion (cases 3, 5 and 6 – p114-116) these triggered startle episodes were recognised prenatally as 'recurrent hiccups' *in utero* during the last trimester. These startle episodes are identical in clinical presentation to those previously described in genetic hyperekplexia. Initially these movements mimicked myoclonus in their speed of onset. Some cases (such as the sisters 2 and 2*) had such prominent startle that it appeared to occur spontaneously. I can also confirm that clonazepam appears to be as efficacious in *GLRB* hyperekplexia as in *GLRA1* and *SLC6A5*: 10/11 had taken it and all had a satisfactory or dramatic response from it.

For most cases generalised hypertonia was most prominent in the first year of life but did persist beyond this point; the hypertonia would be exacerbated by tactile stimuli, strong emotions or occasionally on attempting to reach or grasp an object. Reflecting the age of the children at last clinical contact mostly, 6/11 reported prominent falls. These are probably a representation of the startle response once the child is ambulant; the falls are commonly triggered by auditory stimuli and are frequently injurious as the child is unable to protect itself fully when falling stiff to the floor.

The inheritance pattern is - thus far - autosomal recessive (in keeping with *SLC6A5* and the majority of *GLRA1* cases). The ethnicities represented are of interest – with 5/10 cases of Indian origin and only two Caucasian – originating from North West Europe and the first kindred with Chinese ethnicity. It is predicted that hyperekplexia is recognised clinically in India and only a tiny minority get referred for genetic testing, however the disproportionate number of Indian cases is of interest.

8.3.2.1 Development

Five of the eleven children presented had delayed motor milestones (not walking at two years) and a further three more were recognised as having delayed speech (for example case 1 had no words at 22 months and was only making simple phrases at 4 years). Six were thought to have learning difficulties (mild to severe) and two were thought to have generalised tonic clonic seizures *in addition* to the non-epileptic startle attacks.

8.3.2.2 Comparison with other *GLRB* cases

Al-Owain *et al.*'s report (2011) of a Saudi family with a homozygous missense mutation in *GLRB* identified eye misalignment as a clinical feature. In our series this has recurred at least once with cases 8 and her sister (not tested); they are reported as having a convergent squint with left sided amblyopia. Rare features occurring at this anecdotal level however may not truly be more prevalent than the background frequency. Furthermore in James *et al.* (2012) we identified one case with constitutional short stature (3rd centile) – this is also seen in case 8 (Chung *et al.* 2012). The *GLRB* case identified by Leet *et al*. (2012) was an eight year old female whose symptoms started at 2 hours of life. She had a very good response to clobazam and unremarkable tests and physical examination.

8.3.3 Ethnicity

8.3.3.1 Ethnicities in Hyperekplexia

This is the first review of hyperekplexia ethnicities and describes the novel finding that ethnicity can predict *GLRA1* genotype and therefore predict the mode of inheritance. There are some limitations inherent in estimating ethnicity and I recognise these. Self-declared ethnicity may not be fully accurate for a legion of reasons including a subjective feeling of national identity, non-paternity and failing to recognise or appreciate mixed heritage in previous generations. These limitations however, should only make it more difficult to find the associations I present.

Hyperekplexia has been genetically identified in many populations, but has not yet been described in anyone of Slavic, North-African or Polynesian heritage (although one clinical case from Papua New Guinea has been described clinically; McMaster *et al.,* 1999). A single family of African-American heritage has been recently diagnosed - the first with any identifiable links with Africa (Gregory *et al.* 2008); in addition I can now report two siblings of North African Jewish heritage.

There appears to be three clusters of cases: firstly, Caucasian cases predominate (Forsyth *et al.* 2007) and indeed the initial genes were discovered in these Northern Europe populations. It is hard, however, not to expect a referral bias due to the proximity of local tertiary testing centres, and a greater number of paediatric neurologists and clinical geneticists per head of population leading to diagnosis and referral for testing. The second cluster is from Turkey: of the 23 Turkish cases, eight are specifically described as Kurds, there is also one Iraqi Kurdish family identified. Kurds appear to be of greater risk and it is in part due to a common deletion allele (of six exons) creating a founder effect within the Turkish Kurdish population (Becker *et al.* 2006; Siren *et al.* 2006). Seven families have been identified from Pakistan, two of which are specifically of Kashmiri origin. The Asian cases show the same varied causes as white Caucasian cases; in contrast to Arabic and Israeli/Jewish families where homozygosity brought about by consanguinity was the sole cause. Transplanted populations (migrants) appear at increased risk – driven by culturally determined patterns of marriage.

No clear founder effect was seen in R271 cases (in contrast to previous descriptions of exon deletions in Turkish communities). Hyperekplexia due to R271 mutations shows dominant inheritance and the deletions of exons one to six are recessive; meaning that the inheritance pattern can be in part predicted by someone's ethnicity. Hyperekplexia does show regional and ethnic variation but is it likely to also be present but under-recognised in populations where it is less prevalent. There are also regionally specific startle syndromes which have not yet been genetically characterised such as "Jumping Frenchmen of Maine" and "Latah"; these too may be ethnically specific hyperekplexia syndromes.

8.3.4 Proposed classification of the hyperekplexias

Despite the classical features being present from birth (and in some reports exaggerated startle responses are felt *in utero*) the majority of definitive diagnoses are made during infancy. A positive test in an infant can result in older related adults receiving an answer to their life-long 'jitters'. Alternatively, a proportion of early neonatal referrals with primary hypertonia can sometimes develop into more sinister degenerative disorders (after testing) which is not classical inherited hyperekplexia and always negative for glycinergic mutations. However, I am also eager to differentiate between these congenital cases and adult onset cases of hyperekplexia; I would triage the latter acquired cases for testing for anti-glycine receptor antibodies (Hutchinson *et al.* 2008). These cases with PERM – progressive rigidity with encephalomyelitis are dominated by hypertonia as much as startle. Hypertonia (predominantly lower limbs) is the presenting feature of stiff-person syndrome (which is associated with anti-GAD antibodies). The association between acquired hypertonia and startle response (elegantly shown to abate after intravenous immunoglobulin / plasma-exchange) and anti-glycine receptor antibodies remains a rare finding (McKeon *et al.* 2012). In eighty 'stiff-persons' they discovered ten with GlyR antibodies (six in serum and six in CSF; two in both).

Furthermore, attempts to definitively describe the hyperekplexia phenotype(s) are challenged by potential referral bias – limiting the ethnicities we can study. This may be of importance because there are some culturally-bound neuropsychiatric conditions (e.g. the 'Jumping Frenchmen of Maine') which share some characteristics with hereditary

hyperekplexia (Kurczynski, 1993). Whether this represents the exaggerated startle seen in some anxiety conditions is unknown, since some investigators describe these cultural startle conditions as predominantly psychosomatic in nature (Bartholomew 1994). Exaggerated startle may be symptomatic, secondary to predominately pontine pathology: brainstem infarction, infection, haemorrhage or hypoxia may all produce hyperekplexia-like symptoms. In addition paraneoplastic syndromes, multisystem atrophy and multiple sclerosis can all exhibit startle responses (Bakker 2006).

Type	Name	Test	Clinical
I	**Classic**	*GLRA1* > *SCL6A5* Up to 60% may have no identifiable mutation Dominant and recessive cases	Neonatal or inter uterine provoked startle episodes (100%) Generalised hypertonia (over 75%) worse in trunk and lower limbs – improves with clonazepam Hypertonia and startle improves with time but might not completely remit Apnoeas (under 40%) Learning difficulty and developmental delay (~35% - more marked if recessive) No dysmorphism MRI normal
II	**Extended**	*SCL6A5* > *GLRA1* *GLRB*>*GLRA1* Predominantly autosomal recessive	Neonatal or inter uterine provoked startle episodes (100%) Generalised hypertonia (over 60%) worse in trunk and lower limbs – improves with clonazepam Hypertonia and startle improves with time but might not completely remit Apnoeas (~90%) Learning difficulty and developmental delay (~65%) No dysmorphism MRI normal Seizures possible
III	**Progressive**	*GLRA1* G342S recognised as risk allele	Presents as typical *GLRA1* hyperekplexia but symptoms do not resolve with age – but may respond to clonazepam and alcohol

			Dysmorphism common Dystonia common Myopathic muscle biopsy CK mildly elevated
VI	**Plus**	Unknown cause, likely heterogeneous	Hyperekplexia symptoms from neonatal period Premature death within first two years of life Occasional dysmorphism / seizures
V	**Autoimmune**	Anti-glycine receptor antibodies	Later onset (adult) evoked startle episodes Generalised hypertonia worse in trunk and lower limbs – improves with clonazepam Lymphocytic CSF Cranial nerve palsies seen Improves with plasma exchange
VI	**Symptomatic**	Heterogeneous	MRI may show cause Trauma Demyelination Sub arachnoid haemorrhage Neurodegenerative
VII	**Startle Mimics**	'Hyperekplexia' as a symptom of another disorder	Post-traumatic stress disorder Generalised anxiety disorder Coffin-Lowry Syndrome Tourette's syndrome Crisponi syndrome Paroxysmal extreme pain disorder

Table 8.1 Proposed classification of the hyperekplexias

8.3.5 Unanswered questions

Despite the efforts of cohort studies such as I present in chapter four I recognise that there is much that is yet unanswered about hyperekplexia. Although the molecular genetics have provided a wealth of information, there are a large proportion of clinical cases that are negative for known genes of effect. In the most part these 'phenocopies' are indistinguishable from those who have an identified gene mutation: is there still a major gene of effect to be discovered?

From a clinical point of view the rarity of hyperekplexia means that the characteristics have – in the main – been described as case reports and a concerted effort would need to be made to collect a cohort for phenotypic evaluation. Is there a characteristic EMG pattern during startle, or during the hypertonic attacks? (King and Stephenson 2009). If we could capture EEG during these episodes and eradicate the background trace – could there be an EEG signature? What could MEG or next generation neuroimaging tell us about neuronal function during this attacks?

It is also not clear what the best second agent would be for hyperekplexia therapy. Indeed although clonazepam is the best therapy, a fifth of patients do not take it – no doubt concerned about the global sedating effects and cautious about initiating medium to long term drug therapy in very young children. Drug trials in rare conditions are notoriously conspicuous by their absence; however one was attempted in hyperekplexia demonstrating that vigabatrin was inferior to clonazepam therapy (Tijssen *et al.* 1997). In general spasticity medications and anti-epileptic drugs have been used with varying success: the numbers being too limited to draw any statistical conclusions.

8.3.6 Conclusions

Although it was first described just over fifty years ago, we are closer to understanding the molecular genetics that underpins this condition than we are for many other, more common conditions – despite the fact that the causes of hyperekplexia are heterogeneous. The variety of synaptic structures which can be affected to produce a very similar phenotype may well offer insight into more common disorders of inhibitory synaptic transmission, such as epilepsy. Although hyperekplexia can be controlled with clonazepam and the symptoms improve with age, it can no longer be described as a benign condition due to the high prevalence of apnoea attacks, developmental delay and learning difficulties.

This study represents, by far, the largest cohort of hyperekplexia cases worldwide, supported by twenty years of genetic hierarchical analysis. I firmly establish that the constellation of symptoms caused by failure of glycinergic synaptic transmission can be generated by mutations affecting presynaptic and postsynaptic proteins. Mutations in

SLC6A5 and *GLRB* cause repeated apnoea attacks in the majority of cases and most either report developmental delay (with speech delay prominent) or learning difficulties. Sporadic cases of this type are explained by recessive mutations, with consanguinity a risk factor and treating early may help alleviate the morbidity due to recurrent apnoeas. Hyperekplexia remains a clinical diagnosis, but I argue that prompt genetic analysis will help estimate prognosis, help in pre-conception counselling and plan safe neonatal care. For those patients that remain gene-negative we can now begin a process of phenotypic re-assessment and further stratification of hyperekplexia in preparation for next-generation genetic analysis.

8.4 JME clinical phenotype

The DUETs project suggested that opportunism can be as much of a driver of research direction as scientific intrigue; too often projects are designed to meet the time scale and resources available. Similarly when collecting patient cohorts it is tempting to collect the patients you see frequently or those who are most accessible. This of course introduces inherent biases.

As clinical and genetic heterogeneity are two of the main themes of this thesis it was important before analysing the neuropsychological and copy number data to identify the potential biases within the cohorts I have collected. I elected to do this by comparing large numbers of people with JME; people with familial JME; people with familial genetic epilepsies; and people with drug resistant JME. Wherever it was appropriate I also drew reference to other authors' similar cohorts with JME.

8.4.1 St George's cohort of JME families

Absence seizures are only described by a third of people with JME and familial epilepsy and in those who report absences they are less frequent than monthly in half of cases. The histogram (figure 5.1) does suggest that CAE evolving to JME – or JME with a young onset of absence seizures is a robust sub category – despite the reduced frequency in this collection when compared to the Latin-American families (Martínez-Juárez *et al*. 2006). Similarly I can confirm that this category is seen more frequently in females – but I did identify a strong relationship with photosensitivity. I applaud the scope given by this team to collect atypical JME cases and identified a number within this cohort also. This included JME with: onset before ten years; febrile seizures; learning difficulty; normal EEGs; and focal sounding seizures. One particular oddity was the variety of EEG patterns (table 5.1) and I expect that future work – particularly looking at the EEGs with 2 and 2.5hz spike and wave may help identify mild Lennox-Gastaut type epilepsies.

The importance of negative myoclonus (almost always alongside the positive myoclonus) is brought out of the familial JME cohort. This feature also occurs in sporadic JME. In 2009 I

reported a correlation between traumatic dental injury and juvenile myoclonic epilepsy (Thomas *et al.* 2009). In this study I scrutinised a clinical epilepsy database of 1,673 persons and identified that of the 14 people with dental injuries eight had juvenile myoclonic epilepsy. This equated to injury to almost 10% of people with JME in the database. The injuries were to the front teeth, often incisors and often it was repeated. Although at the time at a loss to explain the findings, thinking it was a consequence of early morning seizures occurring in cramped environments such as the bathroom, I now understand that it is probably the result of astatic seizures. Negative myoclonus if felt in the lower limbs can result in sudden loss of tone and 'drop attacks'. These are unheralded and dramatic and so are frequently injurious. Negative myoclonus was a feature of 'pubertal JME' in this cohort peaking between 11-15 and not seen in outliers.

8.4.2 New sub groups and the Cardiff cohort

I found that using data collected for one intention (supporting a genetics project) did not necessarily lend itself to another (identifying novel subgroups). This was predominantly because only known factors associated with JME were collected routinely by the researchers. Interesting patterns were identified however, such as the reduction in PPR seen with increasing age of onset with familial JME. In addition to the high levels of PPR that we saw throughout these studies, 60% of the 1990s Cardiff cohort were photosensitive and 48% of those with familial JME (ns difference). There was also a sex bias seen in both cohorts favouring females. It is impossible to know whether this is a genetic clue for familial JME, a feature of catamenial disturbance provoking JME seizures, or a bias of recruitment. The Cardiff cohort was sequential patients and so the ratio of 1.6 females to males is least likely to be biased for the latter reason.

Like the 1990s Cardiff cohort Guaranha *et al.* (2011) collected consecutive JME cases from clinic and performed EEG and looked for PPR (76 enrolled, 65 followed up). They had an equal sex incidence, 95% had GTCS and 48% experienced absence seizures. There was a family history in 70% and they also subdivided their cases as per Martínez-Juárez *et al*. (2006), see table 5.2. Their proportions were: classic JME (74%); CAE evolving to JME (11%); JME with adolescent absences (15%); and JME with astatic seizures (0%). They had less

classic JME than the St George's cohort (ns); more CAE to JME (ns); more JME with adolescent absences (ns, p=0.051); and no astatic seizures (ns).

Of interest when considering the psychology cohort below, Guaranha *et al.* (2011) report that those with all three seizure types are most likely to have sub-optimal seizure control and 11/65 (17%) had normal EEGs at baseline. Praxis and language induced myoclonus was associated with poor seizure control but a PPR was only equivocally linked. Those with easier seizure control had a later age of onset (15.4 years vs. 12.6years). Also higher trait anxiety scores were associated with poorer seizure control.

8.4.3 The psychology cohort

In this collection of people with drug refractory JME 18% reported having a history of depression or post natal depression (12% in the Guaranha *et al*. 2011 study). The HADS depression screening test however rated 45% as having significant levels of depressive symptoms (chapter six). The mean age of onset (14.1 years) was not as young as the poor responders reported in Guaranha and colleagues' study. I saw a very high number with absence seizures (70%) which is in keeping with the finding that those with all three seizure types are least likely to achieve full seizure remission. Oddly none had CAE evolving to JME – is this a JME subcategory with a good chance of seizure freedom? Praxis and language activated myoclonus was not actively asked about – however it was never spontaneously reported. Furthermore not once in this cohort – nor the complimentary ReJUMeC collection – was myoclonus observed during the battery of psychometric testing (in excess of seventy interviews).

8.4.3.1 The effect of anti-epilepsy drugs

It is currently impossible to describe the comorbidities and psychometric profile of JME without actually describing 'drug treated JME'. This is because JME is considered to respond very well to treatment – but drug treatment may be for the long-term. JME is thought to be a life-long condition and seizure relapse is common if people discontinue medication

(Panayiotopoulos *et al.* 1994). Therefore by the time that the clinical diagnosis is secure – it is almost certain that everyone will be on an anti-epilepsy medication - although there are variable degrees of poor concordance, and mild cognitive problems make people more likely to forget to take their medication.

Delgado-Escueta and Enrile-Bacsal (1984) studied 43 patients, 12 out of 13 of whom had a relapse when their medication was withdrawn. A study of 66 Arabic patients for five years again suggested that early relapse (9/11 who discontinued valproate) is to be expected. (Panayiotopoulos *et al.* 1994). In contrast to our findings Martínez-Juárez *et al*. (2006) suggested that childhood absence epilepsy prior to JME provided the least favourable conditions for drug withdrawal; however even in the 'classic JME group' 8 out of 161 managed seizure freedom without medication. A small study (Camfield and Camfield 2009) of longer-term outcomes in consecutively identified JME (25 years after diagnosis) was revealing. Although all had been prescribed drugs, half (48%) were no longer taking them – and that's because half of those now drug free, were also seizure free. All patients in this study had seizure onset before the age of sixteen and maybe this is a predictor of a better outcome. Studying people with JME is synonymous with studying people with JME taking anti-epilepsy drugs. However the drugs themselves (by definition psychoactive compounds) have been associated with mood, cognition and behaviour change – not least of which is suicidality.

In contrast to the risk in overdose and dose related sedation associated with the traditional anti-epilepsy drugs, many of the new generation of AEDs are associated with specific psychiatric problems. Topiramate has been associated in several studies with cognitive problems (Shorvon 1996) particularly at higher doses. Additional psychiatric adverse events such as irritability, aggressive behaviour, agitation, anxiety and poorer performance on cognitive speed, memory, and language tasks have been associated particularly with rapid dose titration presumably through impaired attention and concentration (Mula *et al.* 2003). Zonisamide can occasionally cause irritability, emotional liability, and, rarely, mania or psychosis.

How do newer and older agents compare in JME? Independent studies are scarce but one comparison has been made of valproate and topiramate in Brazilian patients with JME (Filho

et al. 2006). Topiramate's mode of action is unique, including sodium channel blocking, effects on the GABA system and carbonic-anhydrase inhibition. However it is thought not to have any effect on serotonergic function. The patients had similar seizure control and similar intellectual functioning (WAIS-III). However on tests of concentration, psycho-motor speed, short-term memory (digit span - forward and symbol search) and verbal fluency (FAS test), patients taking topiramate performed at a significantly poorer level. Patients taking topiramate performed poorly on tests of frontal lobe functioning (Stroop and trail tasks) but this was not significant. The sample (26 on valproate, 16 on topiramate) may have been too small to overcome the heterogeneity inherent in JME and identify any genuine associations here.

8.4.4 Borderline GEFS+

From analysing individual data collected on epilepsy families I report a novel epilepsy familial sub-phenotype – borderline GEFS+. Not long after the paper reporting this as an entity was published, Professor Ingrid Scheffer (Melbourne University) and team embarked on a similar reanalysis of their smaller GEFS+ families without Dravet syndrome. The data presented at the London ECE meeting in October 2012 (but not yet published) can draw upon a larger dataset. Broadly their data confirm my findings – and found many more mildly affected individuals than they had previously published.

Borderline GEFS+ is likely to be a polygenic epilepsy syndrome sharing many – but not all - points of variation with classical GEFS+. The Wales Epilepsy Research Network (WERN) classification (Table 5.4) has features which may help focus gene screening projects. I recognise some limitations to this type of work. Not every individual had an electroencephalogram (EEG), and although, where possible, we corroborated the stories given with medical records, it is likely that there is a degree of non-disclosure in all the families studied. However, I believe that identifying the concept of borderline GEFS+ and its relationship with migraine (also believed to be a channelopathy) may help to explain their shared pathogenesis (Haan *et al.* 2007). Recently, a dominant negative mutation in the TRESK potassium channel has been identified in a family with migraine with aura (Lafrenière *et al*. 2010). The frameshift mutation in the *KCNK18* gene renders the channel non-

functional and may offer a therapeutic target. Anti-epileptic drugs such as topiramate and sodium valproate are commonly used as second-line agents for migraine prophylaxis; indeed the novel anti-epilepsy agent retigabine is thought to act at the KCNQ/Kv7 potassium channel (Brodie *et al.* 2010). The concept of a genetically homogenous GEFS+ syndrome is difficult to support by intra-familial heterogeneity – even within the subcategories proposed; borderline GEFS+ families had twice the number of adults with a symptomatic focal epilepsy (40% vs. 20%) than seen in classical GEFS+.

8.4.4.1 Febrile seizures

The most common seizure phenotype in UK GEFS+ families was the febrile seizure, occurring in over half of the individuals among classical or borderline GEFS+ families. Most did not progress to either further generalised seizures or to an adult epilepsy pattern. Febrile seizures are notoriously under-reported by older relatives, (Wallace *et al.* 2003) potentially artificially reducing their prevalence. 50% of the people who had seizures from classical or borderline GEFS+ families, experienced generalised seizures (compared with 33-67% in the original Australian families) (Scheffer and Berkovic 1997). Febrile seizures plus (FS+) were rare, and were specific (97.5%) but not sensitive for identifying GEFS+ or GEFS+ borderline families, accounting for 24% to 19% of febrile seizures identified in these families, respectively. In the families with borderline GEFS+ the median age of onset of febrile seizures was 12 months. This is similar to reported median age of onset ranging between 11-12 months in individuals with *SCN1A* mutations (Sijben *et al.* 2009) but earlier than the general population average of 18 months.

8.4.4.2 Future directions for borderline GEFS+

I have identified a novel familial sub-phenotype within this UK based cohort – borderline GEFS+ - and suggest criteria for identifying these families. Borderline GEFS+ families have prominent FS+, but have twice the migraine prevalence of other familial epilepsy and a greater preponderance of adults with symptomatic epilepsies. Identifying families with

either a classic or borderline phenotype will be important for efficient direction of resources to inform gene discovery.

Whole genome sequencing for medical benefit is already accessible (quick and cheap enough) for exceptional cases. It is only the informatic challenges that need to be overcome before it can permit us to be more certain about how individuals may respond to drugs, dosing and side effects. If routine analysis at birth is both far-fetched and unpalatable to those who fear that the information could be leaked to nefarious organisations capable of understanding the complexity of the genome – for example insurance companies - then perhaps an individual may consent to having a whole genome scan following a clinical diagnosis.

The individual genome will be scoured for known pathological patterns by bio-informatic software. Certain combinations of gene mutations will be coalesced into new genetically defined syndromes 'drug resistant CAE developing into JME' or 'JME like epilepsy with high likelihood of remittance in mid age' or more likely named after their gene combinations and pathways. The hope is that this could offer prognosis and provide some stability and security to the individual. Living with a paroxysmal condition is terribly anxiety provoking – so any attempt to accurately predict the future has to be welcomed. Improving the quality of information that we give about prognosis will permit accurate genetic counselling for individuals and families. It may also help us predict the likelihood of comorbidities such as depression or post-ictal psychosis.

It is tantalising to imagine how a fuller understanding of individual differences could inform personalised treatments. When selecting a medication to be used the likelihood of certain side effects could be predicted, or a possible toxic level for the individual could be estimated. Certain combinations of drugs may be avoided after understanding how they may be individually metabolised. Very early surgery could be offered with confidence to some people. Of course the fuller the understanding of epilepsy causality – the more sophisticated the drug treatments. I look forward to the smarter drugs will be developed following a better understanding of the condition.

8.4.5 JME Clinical Phenotype Summary

Chapter five captures both the variation within the electroclinical syndrome of JME and between different cohorts of supposedly the same condition. The example of identifying a borderline GEFS+ demonstrates that subgroups can be identified and their robustness challenged by further genetic analysis. The difficulties of identifying homology is in direct contrast to hyperekplexia (chapter four) and explained by the recognition that hyperekplexia is predominantly monogenetic and JME presumed to be of polygenetic inheritance. Chapter six explores whether non-seizure phenotypes identified through neuropsychological testing may be employed to identify novel subgroups.

8.5 JME neuropsychometry

8.5.1 Recruitment

The pattern of slow recruitment was mirrored by the national MRC cohort collection, ReJuMEC, which had the unprecedented indignity of having it's funded withdrawn because of sluggish case accrual. This problem was not predicted – as shown in chapter five there were sufficient cases from South Wales alone to ensure we reached the 200 person threshold. It appeared that the target population were not as 'motivated' to participate as other patient population groups. Anecdotally colleagues involved in enrolling people with movement disorders have found that this cohort is strongly motivated and fastidious about details and timing. This certainly was not the case in the JME collection.

Modifying the strategy for reminding participants about their appointment did not significantly improve the chances of them attending an appointed session. It is my impression that this is a real world demonstration of the deficits identified by neuropsychological testing. Problems with attention, concentration, memory and planning will all lead to people to fail to reply to letters and fail to attend appointments. This pattern is also replicated in clinical practice; epilepsy clinics have one of the largest 'did not attend' rate of medical outpatient clinics – only mental health clinics are more disrupted by non-attenders.

The JME cohort are predominantly young (mean age 32), female (31/39) and therefore of working age and potentially with a young family. Life can be chaotic, it can be difficult to find the time to reply to research and prioritise an altruistic project such as this. Additionally constraints put upon me by our ethical approval may have contributed to the difficulties in recruiting. The framework prevented consent in clinic and the three page standard study information sheet is particularly unfriendly and off-putting. Cosmetic changes have been made when re-applying for ethical approval. However these differences between the idiopathic epilepsies and movement disorders could be biologically driven. The psychopathology (described in the introduction) in JME is predominantly of bipolar symptoms and dysexecutive behaviour, whereas people with Parkinson's disease have obsessive and compulsive traits; these are co-morbid features of a basal ganglia pathology.

As demonstrated clearly in chapter six, people with drug resistant JME have higher anxiety and depression scores, are more likely to be introverted, neurotic and have planning and sequencing difficulties. It is no wonder then that they were a difficult cohort to recruit and retain.

8.5.2 WAIS

The WAIS tests produced IQ values and can be used to challenge the maxim that JME is only seen in people with normal IQ. The relevance of having four participants with FSIQs two SDs below the mean is that this is an IQ of 70 or below is a working definition for learning difficulties (LD). One of these participants was clearly identified as having LD (JM14); he had his mother as a carer, worked for Remploy and had special schooling. Another (JM 23) however had never been identified as functioning at this level yet had had two of her children taken from her care due to concerns about her ability to function as a lone parent for them.

	This study	Standardised means	Pascalicchio's controls	Pascalicchio's JME
WAIS-III				
Picture completion	11.1	10	12.4	12
Vocabulary	9.7	10	9.6	8.6
Digit symbol	8.4	10	11.8	10.7
Similarities	9.3	10	10.7	10.5
Block design	9.5	10	12.1	11.5
Arithmetic	8.5	10	10.7	9.6
Matrix reasoning	10.3	10	11.7	11.1
Digit span	9.8	10	11	9.9
Information	9.2	10	10.7	10.8
Comprehension	9.8	10	11.6	11
Picture arrangement	10.3	10	8.5	7.8
L-N Sequencing	9.8	10	10.9	10.2
Symbol Search	8.8	10	13	11.5
BNT	52.32	Raw scores used	55.9	53.7

Table 8.2. (previous page) **Comparison of JME psychology cohorts** Demonstrating the difference between my results, the standardised means and the results of Pascalicchio and colleagues (2007).

Pascalicchio *et al.* (2007)'s study of fifty Brazilian's with JME and fifty control subjects is the largest published cohort. Instead of relying on the standardisation of tests they matched people with JME to controls. They also made no attempt at correcting for multiple comparisons and accepted a flat alpha statistic of 0.05. Many of their people with JME scored well with seven of the 13 WAIS domains administered being *above* the standardised mean. Pascalicchio and colleagues' work must be interpreted with caution as their control subjects appear to be particularly able and have outscored the standardised score for every test except picture arrangement. This is a common fault with psychology research – control subjects tend to be drawn from the acquaintances of the researchers (commonly undergraduate University students) or suffer from a healthy volunteer bias if drawn from a clinical population. The authors only state that controls were 'consecutively selected from the community' which is insufficiently accurate to understand where they were sourced. Supranormal controls question the validity of all the results. Were the psychologists over generous or inaccurate with their scoring?

	This study	**Pascalicchio's JME**		**p=**	
WAIS-III		**Mean**	**SD**		
Picture completion	11.1	12	2.34	0.0184	*
Vocabulary	9.7	8.6	1.9	0.0006	***
Digit symbol	8.4	10.7	2.84	0.0001	***
Similarities	9.3	10.5	1.58	0.0001	***
Block design	9.5	11.5	3.16	0.0003	***
Arithmetic	8.5	9.6	2.84	0.0167	*
Matrix reasoning	10.3	11.1	2.61	0.0656	
Digit span	9.8	9.9	2.63	0.8513	
Information	9.2	10.8	2.91	0.0011	**
Comprehension	9.8	11	2.39	0.0038	**
Picture arrangement	10.3	7.8	1.74	0.0001	***
L-N Sequencing	9.8	10.2	2.86	0.4445	
Symbol Search	8.8	11.5	2.92	0.0001	***
BNT	52.32	53.7	4.48	0.0619	

Table 8.3. Comparison of drug resistant JME with published values for drug sensitive JME. * denotes significance at 0.05; ** significance at 0.01; *** significance at 0.001 level.

What is striking is how poorly my cohort scored compared to the drug responsive JME cases they have presented (table 8.2). As Pascalicchio *et al*. presented their data as means with SDs it is possible to compare my drug resistant cohort with the results of their drug sensitive one. This comparison (a one sample t-test as described in methods and materials) is shown in table 8.3 above. Without resorting to using Pascalicchio's inflated values for controls as a comparison there are significant differences between people with drug sensitive and drug resistant JME across the WAIS. These are seen in verbal subtests (vocabulary, similarities, information and comprehension) as well as picture subtests (digit symbol coding, block design, picture arrangement, and symbol search). Of note is that the drug resistant cohort I present performed better in picture arrangement than those with drug sensitive JME (all others scored less well). And furthermore one of the few tests to not demonstrate any difference is digit span. Digit span is a test of concentration and attention and is well performed (and equally well performed) by people with drug sensitive or drug resistant JME. This argues against the hypothesis that attentional deficits may underlie the executive function results. There is a much greater range in mean subtest scores in the Pascalicchio paper: range 4.2 – compared to my range of 2.7 mean scaled points. The mean values differ significantly. The difference in the sum of scaled scores is 10.7 points higher in Pascalicchio's drug sensitive group, ($p<0.05$).

Full scale IQ has rarely been published in studies – although it is often calculated to compare JME and control groups. The dogma is that IQ is not affected in JME, however as IQ is a culturally affected compound function of aptitude, attention (negatively impacted by many anti-epilepsy medications) and schooling – it would not be surprising to discover that people with JME fared less well when tested. Absence seizures have frequently been described as a cause of suboptimal schooling, even when identified and diagnosed correctly. In fact some people with JME clearly identify concentrating on certain cognitive tasks as a trigger for myoclonic jerks. Vollmar and colleagues (2010) have investigated this phenomenon using fMRI and showed functional coupling between the frontal lobes and the pre-motor cortex as individuals performed tasks during the scan. Compared to controls there was increased co-activation, particularly in the motor cortex.

8.5.2.1 Subtest performance on the WAIS

Although comparison of index scores did not identify that people with drug resistant JME performed differently on the WAIS, they did score less well on a number of sub-tests. Care must be taken not to over interpret the discovery of results where the p statistic was around the level of the alpha statistic. A Bonferroni correction for all the t-tests used would have required the ultra-conservative significance level of $p< 0.00076$. It is therefore with relevance to this that I dismiss the variation in performance on picture arrangement ($p<0.011$) and picture completion ($p<0.031$). This does leave digit-symbol coding (an element of PIQ) and arithmetic (an element of VIQ) that were answered particularly poorly.

Table 8.4 below identifies all WAIS and WMS subtests published on people with JME. Care must be taken in interpreting the results, as demonstrated by the analysis of the Pascalicchio paper above (2007). There is variation in the performance of people with JME on the digit span tests (as discussed above); both the cohort I present and Pascalicchio did not identify a significant difference when compared to standardised controls. Three studies with a moderate sample size produced varying results: Sonmez *et al.* (2004), n=35 – no difference; Moschetta *et al.* (2009), n=40 – JME scored less well than matched controls; Kim *et al.* (2007) n=27 – JME scored less well than matched controls.

Cognitive function

WAIS-III / WMS

Full scale IQ	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
	JME results not reported (Piazzini *et al.* 2008)
	JME (n=35) not different from matched controls (Sonmez *et al.* 2004)
	(Short form) JME (n=27) not different from matched controls. [26]
	No difference between VAL (n=26) and TOP (n=16) in JME (de Araujo Filho et al. 2006)
Verbal IQ	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
Performance IQ	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
Picture Completion	JME (n=50) no different to matched controls (Pascalicchio *et al.* 2007)

Vocabulary	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
	Not reported (Karachristianou *et al.* 2004)
	JME (n=9) better than controls. (Iqbal *et al.* 2009)
	JME (n=28) no different from matched controls (O'Muircheartaigh *et al.* 2011)
Digit Symbol	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
Similarities	JME (n=50) no different to matched controls (Pascalicchio *et al.* 2007)
	Not reported (Karachristianou *et al.* 2004)
	JME (n=28) worse than matched controls (O'Muircheartaigh *et al.* 2011)
Block Design	JME (n=50) no different to matched controls (Pascalicchio *et al.* 2007)
	Not reported (Karachristianou *et al.* 2004)
Arithmetic	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
	Not reported (Karachristianou *et al.* 2004)
	JME (n=28) no different from matched controls (O'Muircheartaigh *et al.* 2011)
Matrix Reasoning	JME (n=50) no different to matched controls (Pascalicchio *et al.* 2007)
Digit Span	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
	JME (n=35) not different from matched controls (Sonmez *et al.* 2004)
	JME (n=40) worse than matched controls (Moschetta *et al.* 2009)
	JME (n=27) worse than matched controls. (*Kim et al.* 2007)
	No difference between JME (n=20) and matched controls (Roebling *et al.* 2009)
	JME (n=28) no different from matched controls (O'Muircheartaigh *et al.* 2011)
	JME (n=8) difference from matched controls (n=16) or siblings (n=8) not reported. (Iqbal *et al.* 2009)
	JME (n=9) result not reported (Swartz *et al.* 1994)
Information	JME (n=50) no different to matched controls (Pascalicchio *et al.* 2007)
Comprehension	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
Picture Arrangement	No difference between JME (n=20) and matched controls (Roebling *et al.* 2009)
	JME (n=50) no different to matched controls (Pascalicchio *et al.* 2007)
Letter–Number (L-N) Sequence	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)

Symbol Search	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)

Other tests

Vocabulary Test (Schmidt & Metzler)	No difference between JME (n=20) and matched controls (Roebling *et al.* 2009)
WASI	IQ - not reported (Moschetta *et al.* 2009)
Block Design	JME (n=8) difference from matched controls (n=16) or siblings (n=8) not reported. (Iqbal *et al.* 2009)
Matrix Reasoning	JME (n=8) difference from matched controls (n=16) or siblings (n=8) not reported. (Iqbal *et al.* 2009)
Vocabulary	JME (n=8) difference from matched controls (n=16) or siblings (n=8) not reported. (Iqbal *et al.* 2009)
Mattis dementia rating scale	JME (n=9) no difference from matched controls (De Toffol *et al.* 1997)
Clock Drawing Test	JME (n=35) not different from matched controls (Sonmez *et al.* 2004)
Brooks letter-outline task	Not reported (Karachristianou *et al.* 2004)
Copying a cube	JME (n=35) not different from matched controls (Sonmez *et al.* 2004)
Benton Continuous Word Association Test	JME (n=9) worse than controls. (Swartz *et al.* 1994)

Table 8.4 Summary of cognitive and memory test results from studies investigating people with JME.

From the data I presented in chapter six, processing speed was diminished in people with drug resistant JME and correlated well the with ABNAS self-reported score; this is not unexpected as ABNAS factors are designed to test slowing, fatigue and motor problems.

8.5.3 WMS

Memory complaints are common in epilepsy (are described in the DUETs project, chapter three) and the WMS is a test of immediate and delayed memory. The poorest scored tests were spatial span followed by family pictures I and II. Both of these are visual processing tests and of course performance on family pictures II is dependent on the score of family pictures I. There are six individuals from the cohort with a significantly lower PIQ than VIQ (figure 6.25) however across the board there is a great deal of variation and indeed ten had higher PIQ than VIQ. Both of these tests need a great deal of attention and concentration, this is clearly so for spatial span. Spatial span is analogous to digit span and letter-number

sequencing and requires the participant hold a visual pattern in the head and then repeat it by pointing. Any disturbance in attention here and the test is failed. The fact that family pictures I and II are the last of the immediate and delayed WMS tests I think contributes to their poor scores. I suggest that people with JME will fatigue more quickly and suffer from cognitive overloading more quickly which may explain the stepwise deterioration in WMS performance (figure 6.9). There was no difference between the performances of people with drug resistant JME on tests of immediate memory compared with delayed memory.

Sonmez *et al.* (2004) (table 8.5) did identify differences in the WMS. Their sample size (35) is similar to mine but again their reliance on collected controls that have outperformed the standardised scores makes it very difficult to interpret their results as reliable. For example people with JME scored 11.89 on WMS total recall (above the standardised mean) but were 'statistically significantly different' – with reference to an uncorrected 0.05 – because their controls were supra-normal scoring 12.94. Added to these concerns are that they create their own scoring system by unnecessarily abbreviating certain tests (such as the BNT and the WMS) and do not seem to have scaled scores correctly as they are still looking at age related differences - which are corrected for by scaling the scores.

Memory	
WMS-R	JME (n=35) worse than matched controls (Sonmez *et al.* 2004)
Logical Memory I and II immediate	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
	Difference not reported VAL (n=26) and TOP (n=16) in JME (de Araujo Filho *et al*. 2006)
Logical Memory I and II delayed	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)
Visual Reproduction I and II immediate	JME (n=50) no different from matched controls (Pascalicchio *et al.* 2007)
	Difference not reported VAL (n=26) and TOP (n=16) in JME (de Araujo Filho *et al*. 2006)
Visual Reproduction I and II delayed	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007)

Rey Osterrieth Figure Test	JME (n=20) no different from matched controls (Roebling *et al.* 2009)

	JME (n=8) result not reported comparing with (n=16) or siblings (n=8) (Iqbal et al. 2009)
California Verbal Learning Test	JME (n=8) result not reported comparing with (n=16) or siblings (n=8) (Iqbal et al. 2009)
MMSE	JME (n=35) no different from matched controls (Sonmez *et al.* 2004)
Benton's Facial Recognition Test	JME (n=35) no different from matched controls (Sonmez *et al.* 2004)

Table 8.5 Review of memory tests administered in JME

8.5.4 TYM

TYM was designed for use as a self-administered (clinically supervised) screening tool for people with dementia; the aim was to identify those with Alzheimer's dementia and a cut off of 42/50 was identified as the best compromise between sensitivity and specificity. Six of the 34 who completed the TYM had a score of 42 or below in this cohort. As table 6.9 shows there is a much stronger correlation between TYM score and WAIS and WMS indices than for EPQ-BV, HADS or BADS scales and therefore TYM appears to be a clinically useful screening test for identifying who will score poorly on more formal standardised testing. The fact that they lost single marks across a number of tests perhaps suggests that it is identifying a failure to fully attend and concentrate rather than a significant failure across a specific cognitive domain.

I have started to use TYM in the epilepsy unit to quickly screen for cognitive difficulties when people with GGE complain about memory problems. It appears to be well tolerated by those who answer it; despite the seemingly facile questions. I use it to either briefly monitor memory performance and to record a level of performance or to ascertain who should be referred to neuropsychology for a more formal description of their strengths and weaknesses.

8.5.5 D-KEFS

Subtests included within the D-KEFS have been often used to test executive function in JME. Interestingly the straight FAS test (verbal fluency) from the D-KEFs rather than the category

fluency or category switching was the least well performed compared to control means. This test has been administered to people with JME before and a poorer performance than controls or other people with epilepsy is seen in all but the smallest of studies (table 8.6). The FAS test is associated with left prefrontal function and appears to be very sensitive to the effects of JME. Pulsipher and colleagues (using a smaller sample size of twenty children) could not identify a difference in category switching accuracy either. However Sonmez *et al.* (2004) did identify a difference in category fluency using an abbreviated test (animals only). My concerns with this study are mentioned above.

Phonemic fluency

FAS test	JME worse than matched controls (Pascalicchio *et al.* 2007) JME worse than TLE or control; same as FLE (Piazzini *et al.* 2008) JME worse than matched controls (De Toffol *et al.* 1997) JME (n=40) worse than matched controls (Moschetta *et al.* 2009) JME (n=27) worse than matched controls. (*Kim et al.* 2007) Worse when JME treated with TPM than VAL (de Araujo Filho *et al*. 2006) JME (n=9) result not reported (Swartz *et al.* 1994) JME (n=25) worse than matched controls (Kim *et al*. 2012)
Greek language FAS (Μ, Π, Α)	Not reported (Karachristianou *et al.* 2004)

Semantic fluency

Supermarket test	JME worse than matched controls (Roebling *et al.* 2009) JME worse than matched controls (Pascalicchio *et al.* 2007)
Animal naming	JME worse than matched controls (Sonmez *et al.* 2004) No difference when JME is being treated with TPM than VAL (de Araujo Filho et al. 2006) Worse when JME treated with TPM than VAL (de Araujo Filho et al. 2006)
Category Switching Accuracy from DKEFS	JME not significantly different from controls or BECETS (Pulsipher *et al.* 2009)
Category fluency	JME (n=19) worse than matched controls but not siblings (Wandschneider et al. 2010)

Key: TLE – temporal lobe epilepsy, FLE – frontal lobe epilepsy, TPM – topiramate, VAL – valproate, BECTS – benign partial epilepsy of childhood with centrotemporal spikes.

Table 8.6 Review of verbal executive tests administered in JME

8.5.5.1 CWIT

Colour word interference sub tests were all significantly poorly answered by the study cohort. Each of the four sub components were scored in the impaired range by the mean scaled score. Colour naming and word reading are more surprising a result than verbal inhibition and inhibition switching because they are seen to be more a test of processing speed. The participant reads the words of one of three colours for the first test and in the second names the colour. Care must be taken not to 'trip over your tongue' and say compound words 'bleen' 'gred' for example – but they are not strong tests of executive function. Verbal inhibition is however an established executive function test and inhibition switching a potent test of function. Table 8.7 below shows that in experiments where the sample size exceeds 35 all have identified poorer performance on Stroop-like tests, although Pulsipher *et al.* (2009) could not differentiate between the performance of people with JME and those with BECTS.

This surprising pattern was mirrored in TMT. The first trail (visual scanning) is as much a test of visual processing as it is an executive function test and (like all of the five subtests) also tests processing speed. The final trail (motor speed) involves the 'least executive function' as such it is mostly a pure processing speed task and the difference is not significant between the groups. In contrast the fourth trail (number- letter sequencing) which is much more a test of executive function is significantly less well performed by people with drug-resistant JME. It can be approximated that as the subtests of the CWIT and TMT involve greater need for executive function the difference between people with drug resistant JME and controls gets wider. The proverbs subtest was not as poorly answered across the cohort as one may have expected.

8.5.5.2 Comparison with previous tests of executive function in JME

Eleven papers were identified as likely to be informative and the data was extracted. Very few papers used similar tests, had a similar design or gave individual level data and so a formal meta-analysis was impossible. Instead the conclusions have been grouped together (table 8.7) by the test used. I have included information as to the sample size and the control group (if any).

Executive Function tests	Result
Stroop test	JME (n=19) worse than matched controls but not siblings (Wandschneider et al. 2010) JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007) JME (n=40) worse than matched controls (Moschetta *et al.* 2009) JME (n=35) worse than matched controls (Sonmez *et al.* 2004) JME (n=20) no difference from matched controls (Roebling *et al*. 2009) JME (n=8) no difference from matched controls (n=16) or siblings (n=8) (Iqbal *et al.* 2009) JME (n=9) no difference from matched controls (De Toffol *et al.* 1997) No difference between VAL (n=26) and TOP (n=16) in JME (de Araujo Filho *et al.* 2006) JME (n=9) result not reported (Swartz *et al.* 1994) JME (n=25) worse than matched controls (Kim *et al*. 2012)
Trail Making Task	JME (n=50) worse than matched controls (Pascalicchio *et al.* 2007) JME (n=40) worse than matched controls (Moschetta *et al.* 2009) JME (n=9) worse than controls. (Swart *et al.* 1994) JME (n=27) worse than matched controls. (Kim *et al.* 2007) No difference between JME (n=20) and matched controls (Roebling *et al.* 2009)

	No difference between VAL (n=26) and TOP (n=16) in JME (de Araujo Filho *et al*. 2006) JME (n=28) worse than matched controls (O'Muircheartaigh *et al.* 2011) JME (n=25) worse than matched controls (Kim *et al*. 2012)
Wisconsin Card Sorting Test	JME (n=9) worse than matched controls (De Toffol *et al.* 1997) JME (n=40) worse than matched controls (Moschetta *et al.* 2009) JME (n=50) worse than controls or TLE but same as FLE (Piazzini *et al.* 2008) JME (n=9) result not reported (Swart *et al.* 1994)
D-KEFS inhibition D-KEFS - Category switch, Correct card sort Five-point test (design fluency)	JME (n=20) worse than matched controls; no different from BECTS (Pulsipher *et al*. 2009) JME no different from matched controls nor BECTS (Pulsipher *et al*. 2009) JME (n=20) no difference from matched controls (Roebling *et al.* 2009) JME (n=9) worse than controls. (Swart *et al.* 1994)
DEX-Q (self) DEX-Q (other)	JME worse than siblings and matched controls (Iqbal *et al.* 2009) JME no different from siblings or matched controls (Iqbal *et al*. 2009)
Brixton Spatial Anticipation Test	JME no different from matched controls nor siblings (Iqbal *et al.* 2009)
WAIS-III - Block Design	JME (n=50) no difference to matched controls (Pascalicchio *et al.* 2007) JME no different from siblings or matched controls (Iqbal *et al.* 2009) Not reported (Karachristianou *et al*. 2004) No difference between VAL (n=26) and TOP (n=16) in JME (de Araujo Filho *et al.* 2006)
WAIS-III Picture Arrangement	JME (n=50) no difference to matched controls (Pascalicchio *et al.* 2007) JME (n=20) no difference from matched controls (Roebling

	et al. 2009) No difference between VAL (n=26) and TOP (n=16) in JME (de Araujo Filho *et al.* 2006)
Matching Familiar Faces Test	JME (n=40) worse than matched controls (Moschetta *et al*. 2009)
Key: TLE – temporal lobe epilepsy, FLE – frontal lobe epilepsy, TPM – topiramate, VAL – valproate, BECTS – benign partial epilepsy of childhood with centrotemporal spikes.	

Table 8.7 Review of executive function tests administered in JME

8.5.5.3 Stroop

The Stroop test has been performed in many fashions since John Ridley Stroop published it 1935; I used the CWIT (above) in the ReJUMeC cohort. Ten papers reported outcomes for people with JME using the Stroop and one using the modified Stroop (D-KEFS inhibition). In total 233 people with JME had data presented regarding their outcome. Five papers (n=169, sample size 19 to 50, mean 34) found that people with JME performed less well on the Stroop and four papers (n=37, sample size 8 to 20, mean 12) failed to find a difference. The larger sample sizes suggest that the papers that failed to find a difference were not adequately powered to identify a significant difference. This is in keeping with the strongly positive results in the data I present. Three papers also reported results compared to other groups: valproate versus topiramate control (no difference), siblings (no difference), children with BECTS (no difference).

8.5.5.4 Trail Making task

Eight papers used the trail making task to examine sequencing – a total of 225 people with JME were studied. Six papers (n=179, sample size 9 to 50, mean 30) found that people with JME performed less well on the TMT than matched controls did. Two papers failed to find a difference - one of which was compared to controls (n=20) the other aiming to compare the effect of two AEDs. This favours an outcome suggesting that larger sample sizes can identify that people with JME score less well at the Trail Making Task. This is in keeping with the data I present. Not every paper has presented all TMTs.

8.5.5.5 Wisconsin Card Sorting Test

The WCST is a test of mental flexibility and set-shifting. I did not use a similar test in my battery. 108 people in four papers were reported as having used the WCST, but the outcome was only reported for 99 people. All of these were said to have scored less well on the WCST than matched controls. One paper also added that people with JME scored less well on the WCST than people with TLE – but no better or worse than people with frontal lobe epilepsy. It would have been interesting to add the WCST to the battery administered; perhaps substituting for the proverbs test. There was no consensus for the five point test – with two studies of 20 and 9 people disagreeing.

8.5.6 BADS

The BADS executive function tests were not too time consuming to perform and were well tolerated by the participants. This is the first time that I am aware that the BADs has been used to describe the executive function of people with epilepsy. The study was not designed to fully evaluate the function of the BADS against other executive function tasks however table 6.17 demonstrates how well BADS score correlates with i) executive function tests such as verbal fluency, CWIT, TMT, DEX self and DEX other as well as ii) performances indices from the WAIS and WMS, but not iii) BNT, HADS depression, ABNAS nor the Impact of Epilepsy Scale. The BADS elements are much shorter to administer than the D-KEFS, the materials needed are less cumbersome and the rule shift test is designed to be less of a test of memory than the Stroop / CWIS involves. As such I would recommend BADS as a test that can differentiate between people with drug resistant JME and controls and may have a role in the assessment of people with JME because the BADS subtests are designed to have an ecological validity. Tests such as key search and zoo map particularly test planning and sequencing with a ‘real world’ feel.

8.5.6.1 DEX

The self-completed dysexecutive questionnaires are underutilised in studies of JME. One study (table 8.7) looked at DEX-Q and found the self-rated questionnaire identified more dysexecutive symptoms in those with JME than in their siblings or controls; the user completed questionnaire found no differences. This is in stark contrast to the data I present regarding the DEX where DEX-other was markedly more able to identify dysexecutive differences. Iqbal and colleagues (2009) compared eight sibling pairs to 16 controls. This was a UK study and used many of the same tests as I've administered. They reported DEX-self scores of 29.4 and DEX-other scores of 16.1 for people with JME (the cohort I present were 24.1 and 24.7). This is very interesting – it appears that people with drug resistant JME under-report their executive dysfunction compared to those with drug sensitive JME – if the relatives' score is seen as accurate.

The identification that DEX-other produced a higher score for people with drug resistant JME and the DEX-self does not (although their two scores are less than a point away from each other) probably reflects a fault of the study in not identifying appropriate control means. As explained in the methods chapter I elected to choose an elevated mean for DEX-S to prevent the identification of false positive results. The failure to identify a significant difference with the DEX-self prompted a post-hoc analysis of DEX-self results (table 6.14) to identify just what the questionnaire was correlated with and TMTs, proverbs and DEX-other were the strongest results. This is particularly interesting as proverbs had not previously been identified in this study as a discriminatory test of executive function. Concrete proverb interpretations have been found to be related to set shifting, planning, problem solving and working memory impairment in schizophrenia (Thoma *et al.* 2009). However the exact anatomical localisation of the ability to form novel verbal abstractions has not been yet studied in sufficient detail.

8.5.7 Eysenck Personality Questionnaires

The score for EPQ-BV neuroticism is at the cusp of significance at the alpha level chosen to mitigate for multiple comparisons elsewhere in the dataset. There were also only twenty-one returned EPQ-BV questionnaires. If the rest of the sample – or a further sample – could

be encouraged to return their forms it would soon become clear whether this result was of genuine statistical significance. Does this description of the drug resistant JME cohort of 'neurotic introverts' explain why so few were enthusiastic about replying to my invitations to interview?

Gelisse and colleagues (2001) looked at personality in JME and found that a borderline personality disorder (6.5%) was most prevalent; which is unsurprising considering Janz and Christian's first description (1957). Other studies with similar methods suggested that personality disorders may be more prevalent but also more widely distributed: histrionic (7%), passive–aggressive (6%), borderline (4%), dependent (2%) and obsessive–compulsive personalities (2%) (Devinsky *et al.* 1997). A well-constructed study of people with JME from a tertiary centre estimated that 23% had one or more personality disorders, a figure roughly double the prevalence found in the community (Mula *at al.* 2003). These estimates were attained via semi-structured interview rather than a custom tool for personality disorders such as Hare's psychopathy checklist. It is likely that a questionnaire for psychopathy, or antisocial personality disorder, would show a high frequency of cases in a JME sample.

These differences are to be expected due to the challenges of study design; for example tertiary referral centres will have more atypical, intractable and complex cases. In addition there are a variety of methods used to determine the psychopathy in a population: prospective screening using tools that may not have been necessarily validated in an epilepsy population (e.g. HADS, Becks); retrospective case note review looking at clinical diagnoses; or semi-structured psychiatric interviews (e.g. Schedule Clinical Interview for DSM-IV – which does not test for attention deficit disorder). The population studied will also have an effect as cultural factors will impact on the stigmatisation of epilepsy.

A cohort of patients reassessed 25 years after diagnosis (Camfield and Camfield, 2009) noted that three quarters had one or more indicator of social dysfunction; these included failure to complete high school, unplanned pregnancy, depression, unemployment, living alone, never in a romantic relationship longer than 3 months. A particularly novel finding was that of a high rate of unplanned pregnancy outside of a stable relationship (80%). The study also confirmed a high rate of depression and social isolation in patients with JME.

8.5.8 BNT

The Boston naming test specifically examines nominative and semantic language. Language was traditionally thought to be a lateralised function; however fMRI studies have challenged the theory of hemispheric dominance of language even in strong right handers (Cuzzocreo *et al.* 2009). Only Sonmez and colleagues (2004) exclusively studied people who were one hand dominant (right in their case). In the data I present three people self-report as left hand dominant. The data presented in chapter six is one of the few studies to demonstrate a very clear difference between BNT performance for people with JME compared to controls. The laterality implied with a specific language difference is tantalising as JME is taught to be a GGE without focal seizures and yet this suggests an asymmetry of function; or perhaps a lateralised dysfunction.

8.5.9 Impact of Epilepsy Scale

People with JME do have higher rates of psychosocial distress than controls measured using the Global Assessment of Functioning scale. This is part of the multiaxial evaluation of DSMIV (Axis V) and measures global functioning on a hypothetical mental health–illness continuum; from 0–100)(Filho *et al.* 2007). A great deal of this discrepancy can be explained by lower employment rates. Unemployment (in the face of preserved IQ) could itself be a consequence of the number of people who fail to keep a driving licence as in many countries even myoclonic jerks result in twelve months of ineligibility (Thomas et al. 2010).

8.5.10 ABNAS

The ABNAS identified that people with drug-resistant JME self-report problems with i) memory, ii) slowing and iii) fatigue, and, to a lesser extent –iv) language. These problems are tested with i) WMS and TYM, ii) PS and TMT5, iii) not formally tested and iv) VIQ and BNT.

As shown above the WMS scores were not significantly different from controls and this may be because it tests only certain types of memory. Delayed memory to a psychologist is recall after half an hour; however it in increasingly apparent that people with epilepsy are prone

to accelerated forgetting. Forgetting is a normal and important process. Pruning of neuronal connections prevents us from having neural networks that are overly rigid and inflexible. Consequences of the 'unforgetting brain' would be a lack of adaptability and ironically seizures (as hypersynchronous activity would spread too easily across supra-connected brain regions). However it is clear that people with epilepsy have an accelerated form of this cognitive pruning which can be demonstrated to result in poorer recall as early as twenty-four hours following a stimulus and is more prominent at a week and three weeks. (Butler and Zeman, 2008). This can erode both semantic and anecdotal memory and best reflects the day-to-day memory complaints that are reported in clinic. Accelerated forgetting is not tested by the WMS. Information on the WAIS does test semantic memory (mean score 9.2, p=0.086), but it is so determined by educational level that it can be difficult to interpret out of context. TYM, despite not directly asking about long-term memory, was less well scored by people with drug resistant JME.

Processing speed and trail making test five (motor speed) test slowing. As previously shown PS is poorer in people with drug resistant JME (94, $p<0.03$) but not TMT5 (9.6, p=0.4). There are many timed and non-timed tests however that PS influences, such as the other TMT tests, digit symbol coding. Language is tested across a number of domains including VIQ (no significant difference) and BNT (clear significant difference).

8.5.11 HADS

Identifying that people with drug resistant JME have higher levels of depressive and anxiety symptoms is neither surprising nor new. 45% of individuals here score in the pathological range for depressive symptoms and 75% for anxiety symptoms. Studies have suggested that up to half of people with JME may have a concurrent psychiatric disorder (Filho *et al.* 2008) Although the most frequently seen co-morbidity is a mood disorder (26%) anxiety disorders are more prevalent when people with JME are compared to people with mesial temporal lobe sclerosis and temporal lobe epilepsy (19% versus 14%). The high prevalence may be related in part to the high numbers of people with poor seizure control in that study (42%). In marked contrast a series showed that less than 2% of people with JME met DSM IV

criteria for a depressive disorder and only 3.5% for generalised anxiety: a level comparable with population averages (Gelisse *et al.* 2001).

8.5.12 Subgroups

8.5.12.1 JME with low IQ

The ability of the full WAIS to identify 8 cases with an IQ of 85 or below (one SD below the mean) is an important finding in its own right. This equates to 20.5% of those studied with drug refractory JME. The bottom performing quintile also had significantly poorer processing speed, working memory and scored less well on a host of executive function tests too. These tests included all four of the 'FAS' test components; verbal inhibition (the 'most executive' part of the CWIT); the most difficult trails from the TMT - trails two and four (but oddly not trail three – p=0.01684); the DEX questionnaires and the BADS. Interestingly they also had more drug side effect symptoms as measured by the ABNAS neurotoxicity scale. Looking back at their medication history (chapter five), three were on polytherapy (two on two drugs and one on three) – the rest on monotherapy. This is in proportion with the cohort.

8.5.12.2 Low BADS scores

I present the first use of the BADS that I am aware of to identify dysexecutive symptoms in people with epilepsy. It appears to be sensitive to identification of symptoms and does not exactly duplicate the D-KEFS scales. The cohort was separated into three near equally sized groups: mean BADS and above; mean to one SD below the mean; and below one SD below the mean. The lowest group performs particularly poorly across a range of tests including IQs (WAIS), memory and processing speed. BADS also correctly identifies those that score least well on some DKEFs subtests including the FAS tests (except category fluency), all CWIT components, and all TMT trails (except the fifth which is a test of processing speed alone). It also identifies poor scores on the DEX self and other questionnaires. Higher anxiety scores

(mean of 13.9 – in the pathological range) were seen in the lowest BADS group – but no personality type (EPQ) or impact of epilepsy was related to this subgroup.

8.5.12.3 Verbal versus performance IQ

The groups were split into those who scored statistically better on VIQ than PIQ, PIQ than VIQ and those that scored equally well: 16/39 had a significant difference between their VIQ and PIQ scores (10 exhibiting a higher PIQ than VIQ; 6 higher VIQ than PIQ). Those who had neither a dominant VIQ, nor a dominant PIQ fared less well across a range of tests (table 6.18). Despite having the highest FSIQ – those who were VIQ dominant had a lower processing speed than those with PIQ dominant. Those who were PIQ dominant had pathological levels of anxiety (mean 12) – double that of VIQ dominant group.

8.5.12.4 EPQ-BV Subgroups

An unexpected finding – giving that the return of EPQ questionnaires was incomplete – was how discriminatory these were when creating subgroups. Like the IQs above the groups were split into i) extrovert dominant, ii) no dominance and iii) neuroticism dominance (after sex adjustments were made). Unlike the IQs however – this time the 'balanced' group were not the poorest performers. High neuroticism scores were not compatible with a strong performance across a range of tests including WAIS and WMS. Particularly this group scores in the pathological range for anxiety (14) and depression (12) compared to those who were extrovertism dominant (6 and 2 respectively). Curiously the neurotic group also had a greater burden of neurotoxicity side effects as measured by the ABNAS – in particular concerns about memory, fatigue and slowing.

8.5.13 Why executive dysfunction?

Controversial histopathological studies by Meencke and Janz (1985) reported microdysgenesis in JME – reports which although they could not be replicated – have come back in to fashion as they are in keeping with some genetic and psychological findings in

JME. Quantitative analyses of high resolution MR images implicate the prefrontal cortex as a region of greatest abnormality in JME (O'Muircheartaigh *et al*. 2011). Structural imaging does not reveal underlying pathology on an individual level but microstructural abnormalities have been described on a group level (Vollmar *et al.,* 2012). The supplementary motor area is often implicated and makes an attractive locus as it is a crucial hub in the thalamo-fronto-cortical network. Wandschneider *et al.* (2012) suggest that frontal lobe tests need to be sufficiently challenging in order to identify deficits. Some have suggested that there is also a subconscious failure to fully commit to testing that may produce reflex myoclonus. I do not feel that the poor performances across the range of BADS tests support their hypothesis; rather there is innate heterogeneity in the population of people with JME and this is reflected in their executive function.

8.5.13.1 Heterogeneity in JME

As chapter six clearly demonstrates people with drug resistant JME perform poorly across a range of executive function tests. Although many people in each study (as in the one presented in chapter six) have no dysfunction whatsoever (e.g. figure 6.15, table 6.17). A study of forty patients with JME (and twenty-two controls) using the temperament dimension of the 'temperament and character inventory', demonstrated much greater levels of novelty seeking behaviour in people with JME. In addition those with poorer seizure control had greater novelty seeking and impulsive behaviour (Valente *et al.* 2009). The distribution of epileptiform activity seen over frontocentral regions in JME corroborates with poorer executive function: however test results are heterogenous within each study - some patients have marked deficits, others none. In particular, mental flexibility and concept formation-abstract reasoning are abnormal, even when compared to people with temporal lobe epilepsy (Devinsky *et al.* 1997).

Valente (2009) and colleagues, employed six tests of executive function in forty patients: five percent had no deficits (taken as a result lower than one standard deviation in one or no tests), ten percent had two low scores, 17.5% had three or four and 67.5% were scored lower than one standard deviation on five or six of the tests. The IQs of those scoring in the affected ranges were not published, but it clearly shows both heterogeneity but also that

two thirds are severely affected. This sample included ten patients with poor control (despite high doses of sodium valproate) and it is not clear to what extent these individuals may have influenced the results.

8.5.14 Summary

There are some limits to this study which I want to acknowledge. This test battery is probably as complex as can be administered in a single sitting and yet some interesting areas such as decision making could not be tested (Zamarian *et al.* 2012). Indeed Moschetta and Valente in November 2012 described ' the most comprehensive' neuropsychological battery for JME that used just a fraction of the tests described here (digit span, Stroop, TMT, WCST, Matching Familiar Figures Test, verbal fluency, matrix reasoning and vocabulary from the WAIS). The argument about the confounding effect of drugs – or even worse - pseudo-resistance due to non-concordance with prescribed drugs is discussed above but yet needs reiterating. Finally not every author would support the broad definition of JME that I have used to create the cohort – however I believe the descriptions in chapter five support the definitions used. Despite these caveats I present one of the largest descriptions of psychological function in JME (Pascalicchio *et al.* 2007 and Piazzini *et al*. 2008 excluded); it is as far as I am aware the most in depth test battery administered. It is the largest clinical description of drug resistant JME and utilised a number of tests (BADS, TYM, DEX-Q, and EPQ-BV) that have not previously been employed in the study of JME before. Specifically these novel methods have been crucial in the identification of a number of novel subgroups. The next challenge is to test how robust these groupings are in the face of next generation and advanced genetic testing.

8.6 Copy number variation

8.6.1 Polygenetic pathogenesis of JME

The phenotype of JME even within the context of consensus guidelines is heterogeneous and this is supported by the fact that although up to a third of people may have a family history of epilepsy, JME is 'inherited' in a complex polygenic fashion. Some major contributing genes have been identified in certain situations – such as *EFHC1* in familial JME and specifically in people of Latin American / Southern European heritage. This gene however is found in no more than 10% of families from any population and it is not yet known whether familial and sporadic JME are phenotypically indistinguishable (chapter five). The implicated genes are broadly in two categories – genes potentially involved in neuronal development (such as *EFHC1, BRD2, RING3 connexin 36*) and ion channel genes (*GABRA1, CACNB4, GABRD*). As polygenic inheritance of a number of risk factor genes that each play an unknown role in producing the heterogeneous JME phenotype is a currently well supported hypothesis. I wanted to look at the role of rare copy number variants (CNVs) in JME compared to a control population.

We each have a number of copy number variations (deletions or duplications) of large areas of DNA which on their own appear to convey no association with ill-health. However it has been established that certain large CNVs cluster at genomic regions and are associated with variable phenotypes. In particular the large CNVs at 15q13.3, 16p12.2 and 16p13.11 are both commonly seen in idiopathic and focal genetic epilepsies but have also been associated with schizophrenia and autism. Genomic medicine is facilitated by rapid technological advancements and high-density SNP genotyping allows us to identify smaller CNVs with greater confidence than some CGH array techniques.

Using two techniques, but primarily SNP genotyping I looked at copy number variation in 63 individuals; 34 with drug resistant JME. This structure of this discussion will mirror the format of the results in the previous chapter. I will start by discussing selected findings identified by CGH array in 29 people with mixed phenotypes (hyperekplexia and epilepsy); then I will discuss the SNP genotyping – starting with CNVs in areas of interest; then areas

with CNV clustering in this sample; then individual CNVs of importance. I will finish by linking the psychological subgroups (above) with identified CNVs.

8.6.2 CGH Array findings

8.6.2.1 *DMD* Deletion

CGH identified a novel *DMD* deletion in a female with JME and low IQ; implicating her as a Duchenne's carrier. This was confirmed via SNP genotyping. Novelty was confirmed by scrutinising two online *DMD* specific databases (www.dmd.nl/DMD_deldup.html) and (www.umd.be/DMD/W_DMD/index.html). Unfortunately follow-up has indicated that her son is being investigated for a muscle problem and therefore I probably have *in vivo* confirmation that this deletion is pathogenic. Between 2.5 and 20% female DMD carriers develop weakness – although a JME phenotype and low IQ are not part of the expected phenotype.

Her full scale IQ is 64 (VIQ 65, PIQ 69) and although her working memory was low (74) she scored better on the WMS than the WAIS (scores in the mid 80s). Her verbal fluency was one of the worst series of scores (3, 3, 3 and 4) and she scored 'at floor' on verbal inhibition (the most taxing part of CWIT (1) and well on the rest (7, 9, 7) demonstrating great effort. Her BADS score was 70 – confirming she had wide ranging executive difficulties. She demonstrated unequivocally pathological levels of depression and anxiety on the HADS (18 and 17). There is no clear alternative CNV which may lead to her learning difficulties, short stature, bilateral hearing loss and syndactyly. Some of these features do sound mitochondrial – however results of sequencing her exome are awaited.

8.6.2.2 15q13.3 CNV syndrome

The 15q13.3 CNV is one of (if not *the*) most important CNV in neuroscience. It has been repeatedly shown to be over-represented in studies of schizophrenia, autism, ADHD and epilepsy (Williams *et al.* 2012, Galizia *et al*. 2012, Stewart *et al*. 2011). It is not pathogenic for any one of these conditions *per se* but gives a tantalising glimpse into the shared

heritability of these disorders. If at times the biological causation of epilepsy appears obscure – ADHD and schizophrenia are much less well understood.

The 15q13.3 CNV was identified twice in this programme of work: once via CGH array in a boy with atypical BECCTS and LD and then again in the drug refractory JME cohort via SNP genotyping. The first had a novel pattern of 15q13.3 deletion producing a 15q13.3 CNV syndrome (with *CHRFAM7A* homozygosity) and the second identified a large (1.4 megabase) deletion. Although *CHRNA7* is not lost in the first case, on the break point edges are *CHRFAM7A, FAM7A1* and *FAM7A3* on the other. Until recently the FAM7As were seen as pseudogenes – but there is growing evidence that they form a complex between *CHRFAM7A* and *FAM7A*.

CHRNA7 has attracted a lot of attention as the primary pathogenic locus within the CNV (Hoppman-Chaney *et al*. 2012). However not every 15q13.3 CNV includes this gene (estimates are that 75% do) and parents can pass this CNV on without any neurological deficit themselves. Against this, de novo deletions of just *CHRNA7* can produce a phenotype in keeping with 15q13.3 CNV syndrome (Hoppman-Chaney *et al.* 2012). I believe the importance of 15q13.3 is likely to be seen by considering the region (exomic and intragenic) as a whole – rather than seeing it as a region of common variation that happens to harbour *CHRNA7.* That said the nicotinic acetylcholine receptors have long been linked to epilepsy (predominantly autosomal dominant frontal lobe epilepsy, (Steinlein *et al.* 1995)) but also to JME (Taske *et al.* 2002). Taske and colleagues predicted the current understanding of this gene and CNVs by stating “however, none of the variants alone appeared either necessary or sufficient to cause JME”- but they no doubt identified a cluster of importance and therefore it is unsurprising to find a 15q13.3 deletion in JME. In the 8 known CNVs that Mefford *et al*. (2010) identified in people with JME – three were 15q13.3 deletions (no gains). Overall the team identified 15q13.3 CNVs in just under 1% of the 517 people with epilepsy they screened. It is unclear how many people they screened had JME and so a frequency for 15q13.3 in JME is unknown. Certainly this rate of 2/52 in people with any epilepsy (3.8%) appears to be an over representation which may prove to be important.

8.6.2.3 Pitt-Hopkins Syndrome

CGH array identified Pitt-Hopkins syndrome as the likely diagnosis of an adult with a neurodevelopment syndrome previously thought to have a hyperekplexia like syndrome. Case 4 (of the hyperekplexia series) had a large (5,186kbp) deletion at 18q21.2-q21.31 which could be expected to reduce 18 genes to heterozygosity – one of which is *TCF4*. Transcription factor 4 (*TCF4*) gene mutations are recognised as the cause of Pitt-Hopkins syndrome. The *TCF4* gene has a particularly low haplotype index of 1.9% - which strongly suggests that haploinsufficiency would produce the phenotype.

Pitt-Hopkins is a disorder recognised by neurodevelopmental delay, learning disability, epilepsy and distinctive facial features. Additionally they have a respiratory rate disorder with alternating periods of hyperventilation and apnoeas. These apnoea attacks (unlike the ones seen in hyperekplexia) can lead to transient loss of consciousness. This case was referred by a geneticist in Melbourne who suspected an atypical hyperekplexia phenotype. Curiously both paediatrician David Pitt and paediatric neurologist Ian Hopkins practiced at the Royal Children's Hospital in Melbourne. The phenotype here may not be typical for many reasons, not least the fact that the individual is now in his fifties, but perhaps that this is a contiguous gene disorder: a 'Pitt-Hopkins plus'.

Within the 18 deleted genes of this CNV are a number which could be expected to be pathogenic – or perhaps alter the epilepsy to some degree. One of these genes is *CALN1* (calneuron 1). The mouse orthologous gene (Caln1) shows little prenatal expression, with highest expression at postnatal day 21. If deletion of *CALN1* in humans is pathogenic, it is clear how it may complicate or mimic a hyperekplexia-like presentation which classically presents in the first month of life. The murine gene Caln1 is highly expressed exclusively in neural tissue, particularly the hippocampus and cortex. It has a high homology with calmodulin which indicates a potential role in signal transduction, and the cellular localization of the mRNA suggest that *CALN1* has a significant role in the physiology of neurons and is potentially important in memory and learning. As stated in the results chapter however there are a further five genes with a low haplotype index within the region (*PRODH* (11.7%), *HIRA* (2.3%), *CDC 45*(0.8%), *RANBP1(*6.4%), *DCC* (1.4%)) – which should

have a high chance of being pathogenic in a hemizygous state. The deletion of *TCF4* probably has to be taken in the context of these losses too.

8.6.2.4 Frequently occurring CNVs – artefacts – benign or important?

The duplication at 3q26.1 is a good example of an inconclusive result which cannot be ignored. As a general rule deletions are easier CNVs to interpret the results of – and ones that clearly delete exons or whole genes may be more likely to have an effect than intronic variation (Appendix J). However there are clusters of CNVs, such as the 14/24 at 3q26.1. Clustering of CNVs may represent 1) artefact with the procedure; 2) an artefact of the software or bioinformatic approach; 3) a frequently occurring CNV that is benign; 4) a frequently occurring CNV producing a not pathological phenotype – such as blood group or HLA status; 5) a frequently occurring CNV reflecting the location from which the sample was taken; 6) a CNV conferring an increased risk of the condition which the cases share.

In the specific case of 3q26.1 the duplication appears genuine but occurs in a gene poor area and the sample is geographically diverse. All these cases have epilepsy and it could be that 3q26.1 represents a location for an important enhancing or promoting region (ENCODE et al. 2012).

Similarly ten cases had CNVs at 5p15.33 – which (depending on the size) either involved one or two genes (Appendix J). Of these however one (S4) did not have an epilepsy clinical picture (hyperekplexia) and another (S5) may have a *SLC6A8* transmitter disorder to explain the clinical presentation. This collection of seven deletions and three duplications did not present a convincing argument for a CNV-phenotype difference between the cases with a deletion – or those with a duplication. Again it could be that it is neither the zinc finger gene (*ZDHHC11*) nor the neighbouring *TPPP* (the tubulin polymerization promoting protein) that are of importance here – but perhaps enhancing regions hidden within the intronic DNA. However both the above genes are of interest – as is the fact that the region is the common site for abnormalities in Cri du Chat.

Of course the genes directly deleted or duplicated in a CNV may not be those which cause the phenotype; position of effect and the juxtaposition of bringing distant genes together

can create the phenotype. For example in the six cases with 6p21.32 deletion – it may neither be the loss of HLA genes, nor the inscrutable intron, but perhaps neighbouring channel genes which increase the risk of seizures (Appendix J). *GABBR1* (gamma-aminobutyric acid (GABA) B receptor 1) is at 6: 29570005- 29600962; *GRM4* (glutamate receptor, metabotropic 4) is at 6: 33989623- 34123399; and intriguingly *BRD2* (bromodomain containing 2) is nearer still at 6: 32936437 -32949282. *BRD2* has been reported to be implicated with JME. S6, S7 and F6 have a GGE, F1 is not thought to have epilepsy, F9 has a cryptogenic focal epilepsy and F10 unclassified.

The recurring CNVs at 12p13.31 are very similar – with very similar break points and 8/9 being identical. This makes an artefact of the process much more likely – or, as a second explanation, a degree of interrelatedness. Comparison with the SNP array datasets (if this area is well covered) may help decide which the better explanation is. The area lost or gained is gene poor and therefore a clinical correlation cannot be made. A very similar argument can be made for the eight deletions at 14q32.33 – however their location (distal chromosome) makes a sampling artefact all the more likely. A more organic looking CNV cluster is shown by the ten cases at 14q11.1. These have differing size and breakpoints but all overlap and mostly just affect olfactory receptors – although other genes are also involved. This is likely to represent a recurrent benign CNV cluster (all in appendix J).

8.6.3 SNP genotyping

The method of focussing on cytobands which had previously been linked with epilepsy associated CNVs was not a successful one (table 7.7). The epilepsy associated CNVs were rare and did not recur at any frequency in this project. Similarly attempting to identify CNV clusters with only 34 samples has proven to be difficult – but confirming known pathogenic CNVs and identifying rare CNVs have been more successful.

8.6.3.1 The 16p11.12 CNV syndrome in JME

The full 16p11.2 microdeletion syndrome is associated with learning disability, developmental delay and autistic spectrum behaviour. Language and cognitive problems are more pronounced than motor delay; in particular expressive language appears to be more affected than receptive language. Obesity and epilepsy are recognised comorbidities. Case J23 is overweight and does clearly have JME. Her verbal IQ is 72, yet her performance IQ is 98 (FSIQ is 82); the differences between VIQ and PIQ are statistically significant at ($p<0.05$). On the six verbal IQ tests (mean score 10) she scores 4 on vocabulary, 4 for similarities, 6 on arithmetic, 9 for digit span, 5 for information and 4 for comprehension. On the WMS her auditory immediate memory is poorer than visual immediate (77 vs. 84) and auditory delayed poorer than visual delayed (89 v 109). She had some of the poorest tests scores on the CWIT (modified Stroop) – scaled scores of 2, 7, 1 and 1 across the four tests – despite normal function on the nonverbal trials tests (10, 10, 11, 7, 11). Her proverb analysis was poor (mean score 5) even when answering the straight-forward multiple choice questions (20/32 – ranked in the bottom percentile). Her Boston Naming Test score was 39/60 (joint lowest of all the tested individuals). She has a HADS anxiety score of 15 and a depression score of 6; both are elevated compared to controls and the anxiety score would suggest clinical significance.

Girirajan *et al.* (2012) studied more than 30,000 samples genotyped with array CGH, and then selected 2,312 children with LD who carried deletions or duplications associated with known CNV syndromes. In total, they included 39 genomic regions with 39 possible deletions and 33 corresponding duplications. They went on to analyse these patients for the burden of second or third additional large deletions or duplications. While they found an average frequency of 10% for second hits, this frequency was highly variable between CNV syndromes. The 16p11.2 locus is thought to be inherited from unaffected relatives (95%) and then a second CNV confers additional risk of the phenotype (Girirajan *et al.* 2012). They reported 16p12.1 deletions as the second mostly likely CNV to be seen with a second large variant (20% of occasions), the largest being the 16p11.2 duplication. Table 7.7 demonstrates that case J23 has the highest CNV burden of any individual and also harbours a 14q11.22 deletion (of uncertain significance – as described above) and the 15q11.2

duplication (described in below). It may be that her overall CNV burden produces her cognitive and epilepsy phenotype – for example she has a 793kbp duplication at 14q32.33 of unknown significance.

8.6.3.2 Rare CNVs

Until a larger collection of people with JME are studied it is impossible to identify whether specific genes disrupted in this cohort are of greater importance. The identification of a rare *AUTS2* intronic variant and the *KCNV2* modifier may well be important on an individual level in JME. The AUTS2 variant occurs in not one of the EU control dataset and never in DGV. I await functional work to ascertain whether this deletion may disrupt the gene. Similarly the deletion of *KCNV2* is once seen in the control data set (a partially overlapping deletion) although three of the WMRGL set of children with developmental problems have a CNV affecting this gene. I think it this rare CNV should be considered pathogenic and I await further confirmatory tests.

8.6.3.3 Apparent Clusters

Once known pathogenic CNVs are identified (3/34 – 15q13.3, 16p11.2 and 16p13.11, 8.8%) it is important to look at clusters of CNVs and estimate whether they may confer additional risk. Five people had a similarly located CNV within intron 3-4 of *CACNA1C* (alpha 1C subunit of the voltage-dependent L type calcium channel) on chromosome twelve (table 7.12, figure 8.4); 14.7% of the sample. It is also seen in healthy controls – as are all rare CNVs - and was reported 73 times in 2,026 cases in CHOP - the Copy Number Variation project at the Children's Hospital of Philadelphia (3.6%) ($p<0.006$). Fourteen cases in DECIPHER have CNVs that involve *CACNA1C*, these are predominantly very large (1MB or more). Nine had phenotypic information listed including: mental retardation/developmental delay (3); speech delay (3); microcephaly (2); and autistic behaviour (1).

However it was also seen in the EU control dataset; it occurs 74 times – 16.4%. It is likely that this intragenic cluster is better identified by SNP genotyping than CGH array and this

best explains why it is seen five times more often in my cohort and the EU control dataset than in other repositories. Each CNV had only 11-17 CNVs overlap it in DGV for example.

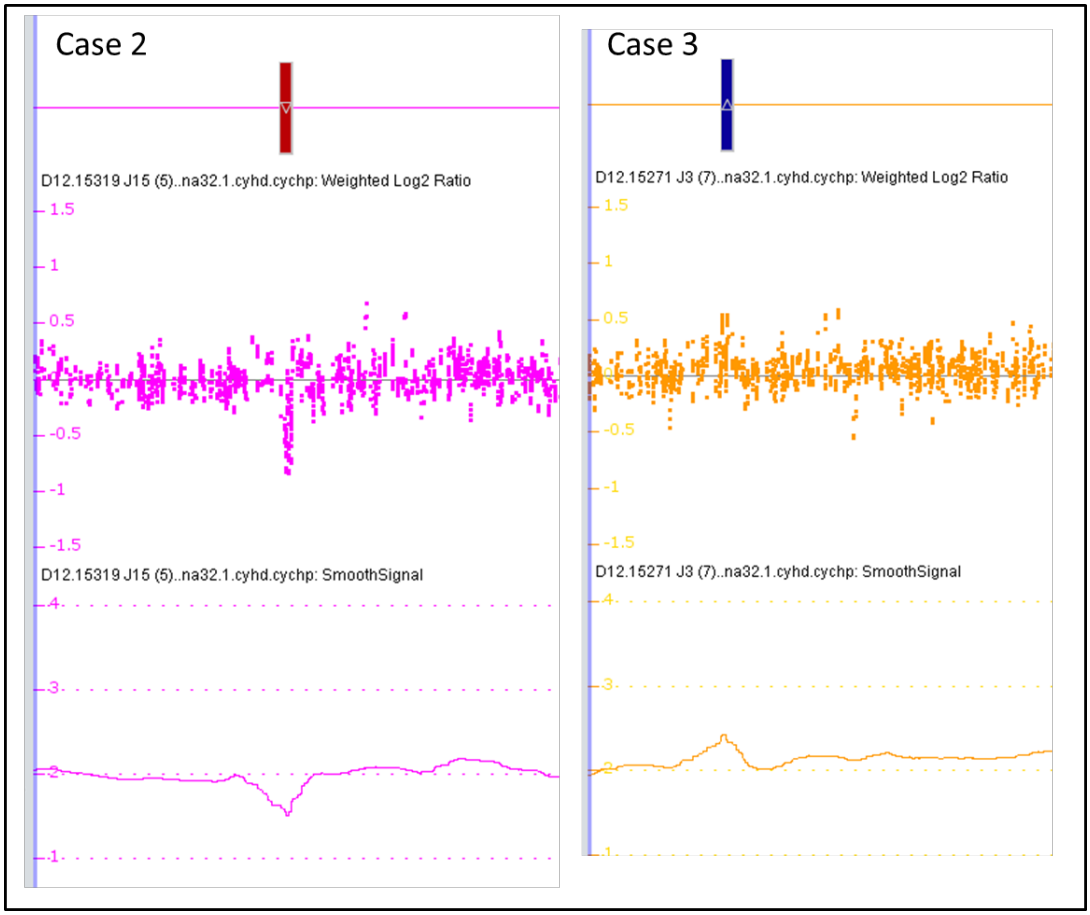


Figure 8.4. SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating *CACNA1C* CNV deletion (case 2) and duplication (case 3).

Calcium channels such as that (in part) coded for by *CACNA1C* mediate the influx of calcium ions into the cell upon membrane polarization. The alpha-1 subunit consists of 24 transmembrane segments and forms the pore through which ions pass into the cell. The hypothesis neutral approach of CNV analysis or WES can be biased by private theories – such as focussing on ion channels in the epilepsies.

8.6.4 Future directions – Exomic sequencing

I have been able to secure funding to take the gene negative hyperekplexia cohort and the drug refractory JME cohort on for exomic sequencing thanks to project grants from *Action Medical Research* and *Epilepsy Research UK* respectively. Therefore to discuss the future of this project – describing paroxysmal disorders to enable genetic analysis – I will discuss next generation sequencing.

There has been an academic 'gold rush' with researchers mining the deep seams of whole exome and whole genome sequencing since 2008. Although undoubtedly initially a major advance for identifying new disease associated genes for rare monogenetic disorders, more recently common and complex conditions have been successfully studied using these techniques. With great power comes great responsibility however and we must not forget that next generation sequencing produces unique ethical conundrums.

The traditional attempts at genetic studies have concentrated on candidate gene analyses by either screening previously identified disease associated genes or identifying a novel gene based on linkage or other hypothesis-led instincts. *Sanger* sequencing to find mutations in candidate genes is a powerful tool, however, it is time-consuming and can be a very costly method for genetically heterogeneous disorders such as adults with epilepsy. Also ascertaining pathogenicity of novel variants can be notoriously difficult – even with the ion-channel genes that have predominated the early discoveries in epilepsy genetics (Chung *et al.* 2010). More recently, it has been noted that the human genome contains a number of missense and deleterious nonsense mutations that do not appear to have any phenotypic effect (MacArthur *et al.* 2012; Klassen *et al.* 2011). Although linkage analysis has provided invaluable insights into the underlying genetic causes of many disorders, there are inherent difficulties including the ascertainment of large multiply-affected families, the ambiguity of large linkage interval regions and the amount of work trawling through interval candidate genes or further fine mapping.

Protein coding genes constitute approximately 1% of the human genome but harbour 85% of the mutations with large effects on disease-related traits. Recently, rapid advances in next generation sequencing technologies have made the prospect of being able to sequence individual genomes an increasingly realistic prospect. Where initially sequencing of the

human genome was a 13 year long endeavour costing approximately $2.7 billion (Lander *et al*. 2001; McPherson *et al*. 2001; Sachidanandam *et al*. 2001), just a decade later that financial and temporal burden has been decimated, with comparative sequencing of a whole human genome costing around the $4,000 mark which can be completed in a matter of days (Metzker 2010). This makes the utilisation of such techniques to search for novel disease causing variants, where currently there is no known disease aetiology, an attractive prospect. However, at a cost of $4,000 - 10,000, whole genome sequencing (once salaries, patient acquisition and bioinformatics analysis is fully costed) remains an unfeasible technique to employ for routine cases in clinic. One way of circumventing this substantial issue of affordability, is to search only the coding regions, which together account for approximately 1% of the whole genome sequence but hold more than 85% of known disease causing mutations (Botstein and Risch 2003). Whole exome sequencing has become a popular tool in the search for novel pathogenic variations in a number of conditions – as shown in appendix A. The massively parallel sequencing process allows the investigation of megabases of DNA at one time.

Historically, finding causal mutations for Mendelian-inherited traits has relied upon the use of techniques such as linkage mapping and candidate gene sequencing for identification. This has been very successful in uncovering pathogenic mutations in over a third off all Mendelian disorders (www.OMIM.org). However, there are still a large number of rarer disorders where application of this method cannot find such mutations due to issues such as incomplete penetrance and poor locus heterogeneity compounded by the relatively small samples sizes seen. The importance of rare disorders should not be discounted clinically; taken as a group they represent a large area of clinical neurology and genetics - often with the most devastating outcomes and the greatest need for services. WES can be performed effectively in cases where these rare disorders are clearly present and result in the identification of new pathogenic gene variants (appendix A) with the additional bonus of being more time and cost effective.

8.6.4.1 Limitations

Although WES encapsulates an exciting new era in genetic technology, its widespread use is presently subject to a number of caveats. One of the major challenges posed by exome sequencing is how to define the regions of capture. Currently, the exomic regions covered by commercially available technologies stem from sequence data held within public databases such as *RefSeq* and the Consensus Coding Sequence Project (www.ncbi.nlm.nih.gov/projects/CCDS). However, it is accepted that our knowledge of what exactly constitutes the protein coding sections of the human genome is not yet complete and so vital regions may be overlooked. Further, exome coverage does not extend to other important non-coding regions of the human genome such as microRNAs and promoter regions, which may have a significant impact on the pathogenicity of disease.

Secondly, WES produces a large amount of data which need to be carefully considered before making diagnostic inferences. Although WES produces less data than whole genome sequencing, you may still be faced with more than 10,000 variants within a whole exome. This requires careful and precise filtering by comparison to reference genomes using such tools as dbSNP (Schossig *et al.* 2012) to sort SNPs from pathogenic variants. These results can then be classified further on the basis of their predicted physiological impact using tools such as Scale-Invariant Feature Transform (SIFT) (Ng and Henikoff 2003) and Multivariate Analysis of Protein Polymorphism (MAPP) (Stone and Sidow 2005).

Lastly it is also important to remember that this technology still has the capacity to produce false-positive results whether that is as a result of ineffective coverage, poor capture efficiency or errors in base calling. This can be mediated by ensuring that criteria for a true variant are stringent and as a cover-all any putative mutation can always be validated though traditional Sanger sequencing methods.

One of the ways to overcome the complex heterogeneity of epilepsy genetics is the simultaneous screening of multiple epilepsy genes by using epilepsy-specific panels (targeted NGS). A recent study presented approximately 50% mutation detection rate (16 out of 33 patients) by utilising a sequencing panel containing 256 genes relevant to epilepsy phenotypes (Klassen *et al.* 2011). However, it is highly debatable whether it makes sense to use direct exomic sequencing in place of hypothesis-based gene panels. The beauty of

whole exome sequencing is that by remaining agnostic one can identify genes in families and systems that either have no current known function, or have been previously (and erroneously) discounted or suggest a pathogenicity which had not previously been mooted. NGS studies identify causative genes that would not have been selected using traditional methods (Corbett *et al*. 2010, Chen *et al*. 2011). Although the gene panels currently present a marginally better quality of coverage over exomic sequencing (for the selected genes), it would be inevitable, given progress in the field, to adopt exome sequencing or even the whole-genome sequencing as the diagnostic tool of choice in the near future (Dixon-Salazar *et al*. 2012).

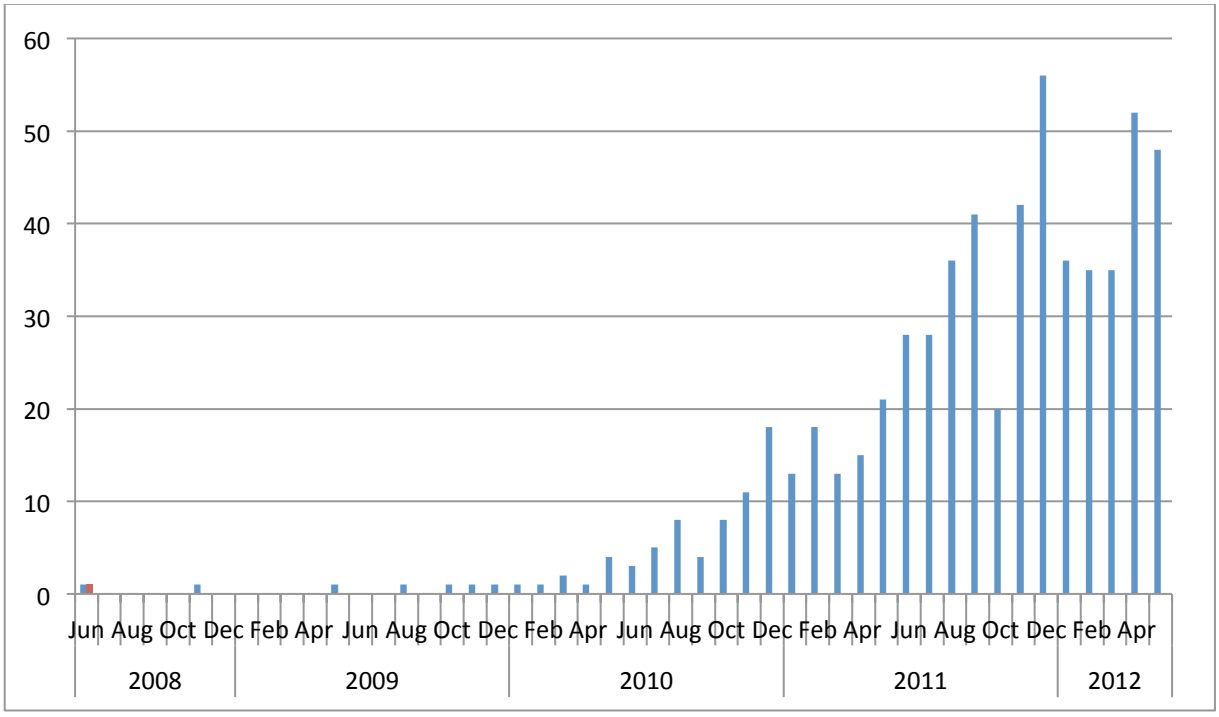


Figure 8.5 Illustration demonstrating the inexorable rise of exome sequencing in the medical literature. The y axis is the number of papers published per month.

The graph above (figure 8.5) is an illustration of how the technique has been embraced by both clinical and research communities. Using the search terms 'exome' and 'sequencing only' and selecting papers published prior to June 2012 using the PubMed index yielded 611 papers. This includes reviews and original papers and uses the earliest publication date (printed article or electronic publication). I do not begin to suggest that this search strategy

is exhaustive – or indeed even specific – but is does capture the 'gold rush' and the pioneer spirit seen in a boom industry.

The graph demonstrates how quickly this field is moving and just how difficult it has been to keep abreast of the developments. The adoption of this technique has been fuelled partially by the accessibility of the technique by a dramatic reduction in price per exome – itself secondary to better automatisation and 'mass production' to meet this increased demand. Figure 8.5 clearly shows that close to sixty papers can be published monthly using this technique – both demonstrating new genes associated with rare and common disorders – and also improved post exomic strategy; bioinformatics and modelling. Appendix A provides a time line - describing the journey of exomic sequencing in literature (partially published as Thompson *et al.* 2012).

8.6.4.2 Arguments for and against WES in clinical practice

8.6.4.2.1 In favour of WES in clinical practice

1. Single gene tests can cost in excess of £800 per gene

2. Some genetic analysis is relatively incomplete (no MLPA (multiplex ligation-dependent probe amplification) for example)

3. It may prevent you from having to perform as many muscle biopsies; a biopsy may still be needed for diagnostic/confirmatory reasons – but a repeat biopsy would be rare indeed

4. You can give diagnoses earlier – preventing repeat clinic visits and ancillary tests such as muscle MRI

5. There may be less need for second opinions outside of the area to confirm unusual phenotypes

6. You will more swiftly identify atypical presentations of common disorders

7. There are clear reasons why knowing the gene involved is important in muscle disorders for cardiac and respiratory / genetic counselling reasons

8. There will be a permanent dataset that can be re-scrutinised if further genetic 'tests' are warranted later

8.6.4.2.2 However it is important to consider

1. Will this change how you return 'important but unrelated' information back to patients, and specific consent may be needed?

2. Some genetic results will take time to validate and so may be slow to return to the patient

3. Dystrophin (for example) is such a big component of paediatric muscle disease that it still may be cheaper to sequence this directly and then to take on the gene negative children

4. WES may not be ethically appropriate for new-born screening (e.g. Huntingdon's disease risk being known at birth – the data would need to be handled very sensitively)

5. We would need to collect DNA from parents more routinely

6. Some important pathological variants will be intronic

7. Some disorders will depend on gene dosing

8. Copy number variation and translocations may add additional challenges

9. Coverage of the exome will be variable and so some regions will be difficult to call with full certainty

8.6.4.2 Translation back into the clinic

We are clearly not yet in a position where we can confirm a case of JME with genetic testing, use NGS for predictive testing when someone presents with CAE, or exclude epilepsy through exome analysis. However perhaps traits and characteristics associated with JME could have an associated gene test soon. This is already the case when one considers the chances of someone having a hypersensitivity reaction after taking carbamazepine. It has been established that having Han Chinese ethnicity increases the chances of developing anything from a rash necessitating discontinuation – up to Stevens-Johnsons syndrome. In Taiwan, 7.7% of people with epilepsy have the HLA-B*1502 allele, conferring risk of skin reactions to carbamazepine. A study advised those with the allele to avoid carbamazepine – all others were prescribed the drug. Among the 4,877 people studied, none developed either Stevens–Johnson syndrome or toxic epidermal necrolysis; 4% had mild transient rash, clearly demonstrating the power of pharmacogenomics on a population level (Chen *et al.*

2011). Furthermore an international consortium performed a genome-wide association study of 22 subjects with carbamazepine-induced hypersensitivity syndrome and 43 with carbamazepine induced maculopapular exanthema. The HLA-A*3101 allele was significantly associated with carbamazepine hypersensitivity: its presence increased the risk in Caucasians from 5% to 26%, whereas its absence reduced the risk from 5% to 3.8% McCormack *et al.* 2011).

8.6.4.3 Considerations for the use of NGS in clinic

There are a number of logistical and theoretical considerations that will need consideration when utilising the power of NGS in clinical practice. These include (but are not limited to) the identification of pathogenic genes not associated with the phenotype of interest (such as *BRCA1* for breast cancer) and the safe storage of such data. NGS produces enormous data files that can endlessly be re-examined by researchers but unscrupulous use of such data could leave the individual vulnerable to exploitation. It is also very difficult to return a finding when it is of unknown significance such as conferring an indeterminate degree of risk of an incurable condition, or when it is very difficult to fully estimate pathogenicity *in vitro* or indeed *in vivo*. These challenges are only increased when returning a genetic finding is considered in the context of familial epilepsy (Hammond *et al*. 2010). As bio-informatic pipelines fully develop and harness the power of protein modelling and other *in silico* techniques we will be able to meet these challenges. NGS is going to be applied to individuals and families with JME in the near future regardless as to whether we face up to the challenges of improved phenotyping; but to facilitate the assimilation of the data bounty of NGS brought about by technological advances we need to adopt a similarly sophisticated clinical approach.

8.7 Final Conclusions

8.7.1 Conclusions

This thesis - *'Phenotyping paroxysmal disorders to empower genetic research'* uses many strategies to describe how clinical skills can complement traditional and second generation genetics.

This thesis has addressed the stated aims and objectives – even where it has not been able to fully answer them. Indeed many of the more interesting outcomes have been either when a question could not be fully resolved, or when the analysis identified unexpected results. There are a number of themes that run across the chapters.

8.7.1.1 The individual

The importance of the individual clearly runs through chapter three and in particular the digital storytelling project. The widespread difficulties identified by the neuropsychology project, in terms of mood, neurotoxicity and memory, has made me employ these tests more frequently in my routine clinical practice. Individualised differences brought out from 'epilepsy is different' in the introduction are most important when considering the copy number variation data; there are very few variants of note that are frequently recurring. Copy number variation is no doubt important, and in some cases pathogenic, in JME, however they must be working in concert with variation elsewhere.

8.7.1.2 Heterogeneity

Once the individuals are analysed in detail – they can be considered as a group. The opinions of scientists, patients and clinicians were collected and compared in the DUETs project. Variation within groups and between groups is a key conclusion of the hyperekplexia work; in chapter four these variations are (for the most part) explicable by the genotype. In contrast, in chapter five, when JME is considered, the variation is more difficult to sub-classify. This is another line of evidence supporting the polygenetic nature of

JME. However *familial* variation enabled the identification of a borderline GEFS+ pattern. The cognition and neuropsychological tests – for the most part – support deficits across specific domains – and yet many people function well (some very well) in these areas.

8.7.1.3 Translation

This work was reliant on my ability and opportunity to collect information from the clinical domain and use it to plan the genetic testing strategy (chapters four, seven). What was more unexpected for me was to identify findings that could be returned to the clinic. These included some group differences such as the genotype-phenotype correlations in hyperekplexia, the ethnicity differences in hyperekplexia; there were also results of potential importance to individuals such as the *DMD* carrier status and the potential cause of SUDEP. Additionally, being able to correlate the large CNVs in someone with JME to their psychology results allowed me to confirm a specific verbal language deficit that has only previously been identified in autism.

8.7.2 The future

There are conclusions from this work that may further the work of others. The 'research agenda' is freely available for the use of the research community through the philosophy of the James Lind Alliance. Identifying developmental delay and in particular speech difficulties in the hyperekplexias strongly implicates glycinergic synaptopathy in learning difficulties. I am certain that a prospective and sophisticated analysis of children with hyperekplexia will identify further deficits. The identification of a suspicious CNV in a 'SUDEP' case produces a hypothesis for screening people who have sudden young cardiac deaths.

What I find most exciting about this work is how well it has laid the foundation for our future projects. The JME and hyperekplexia samples have been submitted for second generation sequencing. The neuropsychology data is currently being used to inform a study on connectivity using diffusion tensor imaging. I have ambitions that either through these strategies – or through technological advances yet to have been devised – the people who

have supported this work by participating in interviews or donating DNA will help us identify the fourth major gene for hyperekplexia and help identify the matrix of genetic variation needed to produce JME. It is only with a fuller understanding of the pathogenesis of paroxysmal disorders that we can hope to design more efficacious and less toxic therapy. I hope that the work I present here can form part of the platform that realises this, for direct patient benefit.

Appendix A

Published papers from this thesis

Appendix B

Involvement: patient voices

Appendix C

ReJUMeC protocol

Refractory Juvenile Myoclonic Epilepsy Cohort (ReJuMEC)

Version 2.0 18/06/2009

Funder reference: MRC – G0800637

Authorised on behalf of sponsor(s) (University of Liverpool / Walton Centre for Neurology and Neurosurgery NHS Trust)

Signed _____ Date 23 July 2009

Name JAMES FOX

Title MANAGER, CONTRACT SERVICES

Authorised by Chief Investigator

Signed _____ Date 22.7.2009

Name A MARSON

Title PROFESSOR OF NEUROLOGY

UNIVERSITY OF LIVERPOOL

The Walton Centre NHS for Neurology and Neurosurgery NHS Trust

Clinical Trials Research Centre

MRC Medical Research Council

mcrn Medicines for Children Research Network

General Information

This document describes the Refractory Juvenile Myoclonic Epilepsy Cohort (ReJuMEC) study and provides information about procedures for entering participants into it. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to the registered investigators in the study, but centres entering participants for the first time are advised to contact the coordinating centre (Clinical Trials Research Centre (CTRC), University of Liverpool) to confirm they have the most up to date version. Clinical problems relating to this study should be referred to the Chief Investigator via the CTRC.

Statement of Compliance

This study will be carried out in accordance with the World Medical Association Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989) and South Africa (1996) amendments and will be conducted in compliance with the protocol, CTRC Standard Operating Procedures, The Research Governance Framework for Health and Social Care (2nd edition, 2005) and ICH GCP guidelines.

Contact Details: Institutions

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Contact Details: Study Steering Committee

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Table of Contents

Glossary

AE	Adverse Event
ci	Chief Investigator
CRF	Case Report Form
CTRC	Clinical Trials Unit
gp	General Practitioner
GCP	Good Clinical Practice
iec	Independent Ethical Committee
JME	Juvenile Myoclonic Epilepsy
lrec	Local Research Ethics Committee
NCR	No Carbon Required
CTRC	Clinical Trials Research Centre
mrec	Multi-centre Research Ethics Committee
PI	Principal Investigator
PISC	Patient Information Sheet and Consent Form
r&d	Research & Development
SAE	Serious Adverse Event
SMG	Study Management Group
SDV	Source Data Verification
Ssc	Study Steering Committee
VPA	Valproic Acid and/or Sodium Valproate

1 PROTOCOL SUMMARY

Title: Refractory Juvenile Myoclonic Epilepsy Cohort Study - ReJuMEC

Phase: N/A – Cohort Assembly

Population: The target population is 200 participants aged between 14 and 65 years with a diagnosis of Juvenile Myoclonic Epilepsy (JME) who have failed to achieve seizure control with sodium valproate (VPA), and have experienced an average of 4 days with myoclonic seizures per month over the past 3 months or an average of 4 days with absence seizures per month over the past 3 months.

Number of Sites: 14 sites in the United Kingdom

Study Duration: Participants will be enrolled on to the study for the duration of 3 months, in which time they will undertake neurophysiology and neuropsychology testing, as well as providing an optional DNA sample for storage and use in future research projects. Participants may be approached after the ReJuMEC study to take part in studies assessing the aetiology, management and sequelae of JME.

Objectives:

Primary:

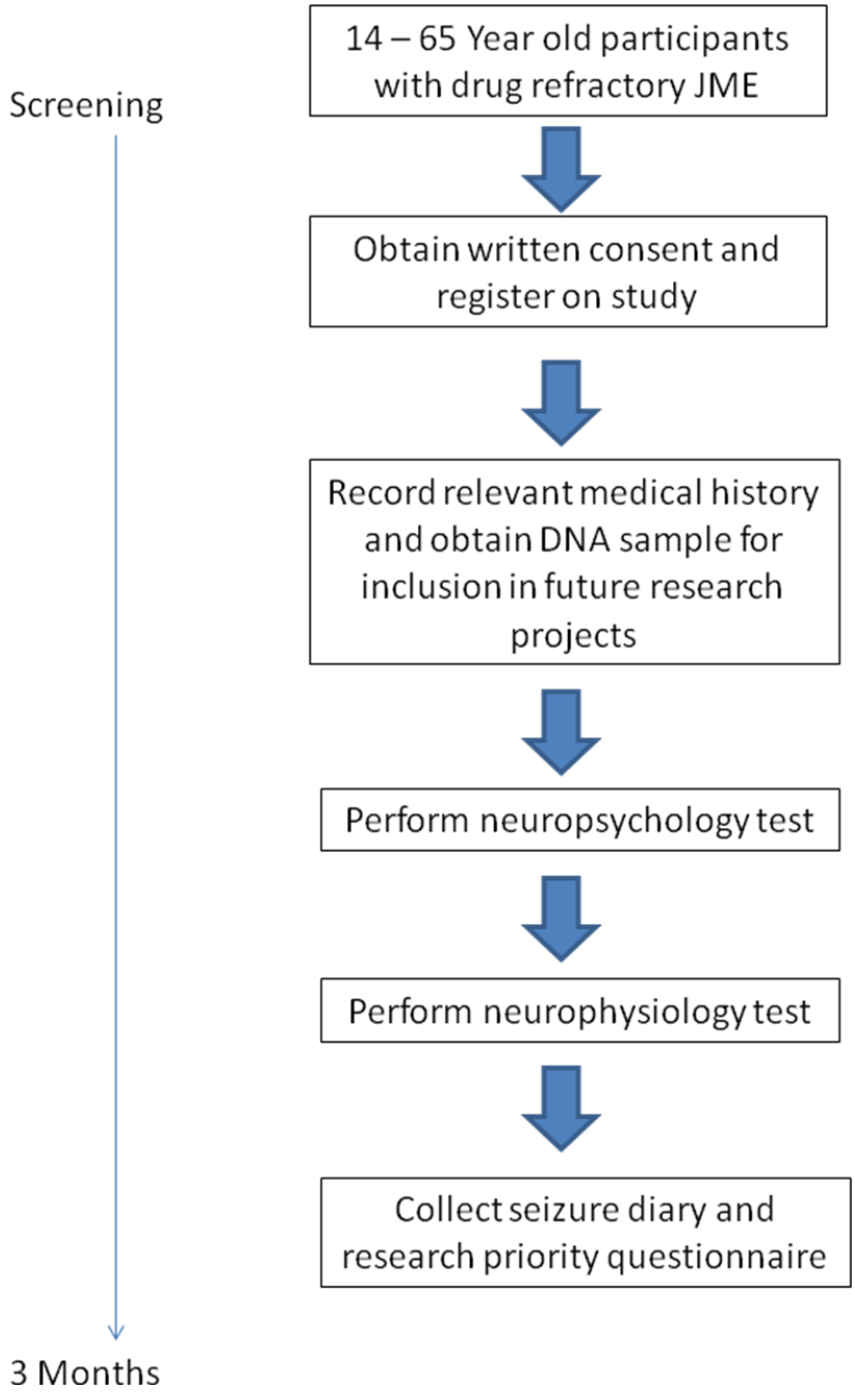
To establish a cohort of participants with JME that is resistant (or "refractory") to treatment with conventional antiepileptic drug medication.

Secondary:

- To permit the development of EEG characteristics as surrogate markers of efficacy in idiopathic generalised epilepsy
- To provide a uniformly phenotyped resource for investigation of the genetic aetiology of JME, genetic markers of JME endophenotypes, and for inclusion in pharmacogenetic studies
- To support functional imaging studies addressing basic mechanisms in epilepsy and the investigation of previously unrecognised frontal lobe involvement in JME
- To encourage observational studies of neuropsychological functioning in participants undergoing lifelong drug treatment for idiopathic generalised epilepsy
- To enable more rapid development of treatments for idiopathic generalised epilepsies by employing JME as the primary screen for novel therapies.

Protocol Summary - continued

Schematic of Study Design:



2 BACKGROUND INFORMATION

2.1 Introduction

Epilepsy is a common serious disorder with a prevalence of 0.5 – 1% and a life time cumulative incidence of around 3% [1]. Approximately 40% of patients have idiopathic generalised epilepsy, a group of syndromes thought to have a genetic aetiology. The common idiopathic generalised epilepsy syndromes include JME, childhood and juvenile absence epilepsy, and epilepsy with tonic clonic seizures on awakening. JME typically presents in the teenage years and is characterised by the presence of myoclonic and tonic clonic seizures, with around one third of participants also experiencing absence seizures [2,3]. An electroencephalograph (EEG) typically shows generalised polyspike and wave abnormalities and approximately 30% of JME participants are photosensitive. Diagnostic magnetic resonance imaging (MRI) is usually normal. JME accounts for around 10% of all epilepsies presenting in adolescence and early adulthood, and is an ideal surrogate for the investigation of outcomes and drug treatment in the wider group of idiopathic generalised epilepsies, as participants have multiple seizure types and well characterised EEG abnormalities.

JME requires lifelong treatment with antiepileptic drugs. Around 30% of people with JME fail to respond to medication and continue to suffer debilitating seizures despite otherwise optimal therapy. Drug resistant epilepsy, irrespective of seizure type or syndrome, is associated with significant physical and psychosocial morbidity, dependent behaviour, poor quality of life, and an increased risk of sudden unexpected death [4]. People with frequent, uncontrolled seizures are among the most disadvantaged in society and represent a considerable burden to their families, carers and healthcare resources alike. VPA is the current first line drug for JME [5] but is associated with significant dose-related adverse effects, including somnolence, dizziness, tremor and weight gain, and can occasionally cause hepatotoxicity and thrombocytopenia. Of more significant concern is the association with polycystic ovarian syndrome in young women [6] and propensity for teratogenicity and neurodevelopmental delay. VPA can increase the risk of major congenital malformations, whilst up to one third of children exposed to sodium valproate *in utero* may have a significant decline in their verbal IQ [7,8]. In terms of seizure control, sodium valproate remains first-line treatment for JME but, given the risks, there are clear trade-offs for women of childbearing potential, who represent one third of people with epilepsy.

New, effective, and safe treatments for people with idiopathic generalised epilepsy are urgently required. The past 20 years has witnessed an unprecedented development of new antiepileptic drugs, but the primary focus of this effort has been focal epilepsies. A number of new agents have been introduced as add-on treatment in drug resistant focal epilepsy, with some subsequently being licensed as monotherapy [9]. Regulatory trials are typically performed in this population because focal epilepsies are more common in adults. Where the use of new antiepileptic drugs has emerged in idiopathic generalised epilepsies, it has often been the result of off-label prescribing and observational studies, with inadequate assessment of either efficacy or safety. Lamotrigine and topiramate may be considered as broad spectrum antiepileptic agents with utility in idiopathic generalised epilepsy but both were shown to be inferior to sodium valproate in terms of seizure control in a recent pragmatic study reported by our group [5]. Vigabatrin and tiagabine can exacerbate

seizures in several idiopathic syndromes, gabapentin and pregabalin are at best ineffective, and there is only anecdotal evidence of efficacy for levetiracetam [10]. Accordingly, the development of new drugs for idiopathic generalised epilepsies is required for those participants who do not respond to sodium valproate, those who do not tolerate it, or those in whom its use might be inadvisable.

Although the idiopathic generalised epilepsies account for around 40% of all human epilepsies, new treatments have rarely been formally studied in this participant group. This is, at least in part, due to the ability to distinguish between individual syndromes on the basis of clinical presentation and EEG data and a disease classification and drug licensing system which favours clinical features over aetiology. Idiopathic generalised epilepsies accordingly appear more heterogeneous than focal epilepsies, even though they may possess fewer underlying causes. The consequence is a requirement for licensing of new products in individual syndromes rather than in idiopathic generalised epilepsy as a whole, in stark contrast to focal epilepsy where universal approval is granted on the basis of seizure type alone [11]. There is no reason why, with adequate characterisation, people with idiopathic epilepsies cannot be included in regulatory studies. A cohort of participants with a single, well-defined idiopathic syndrome is likely to be far more homogeneous than any group of randomly recruited focal epilepsy participants. The availability of a clearly-defined cohort of participants with refractory idiopathic generalised epilepsy would relieve a significant bottleneck for drug development in this population.

2.2 Rationale

JME is ideal for proof-of-concept and Phase II/III studies of interventions that may have efficacy in the wider group of idiopathic generalised epilepsies as it is the most common idiopathic syndrome and participants have multiple seizure types and well characterised EEG abnormalities. Spontaneous and evoked epileptiform discharges on the EEG of JME participants can be used as surrogate endpoints, particularly in proof of concept studies [12]. Assembling a large cohort of well-phenotyped JME participants will offer a unique resource, unrivalled internationally, for the future research of this important and often under-appreciated syndrome, facilitating the investigation of novel seizure- and syndrome-specific therapies, a detailed examination of its aetiology, and the opportunity to dissect the long-term consequences of epilepsy and its treatment in a relatively homogeneous participant group.

We will collect a cohort of 200 participants with drug refractory JME which will enable the bottleneck in drug development for idiopathic generalised epilepsy to be bypassed. The cohort will be characterised by clinical history, 48-hour EEG findings and neuropsychological testing. Where possible a DNA sample will be obtained for use in future genetic and pharmacogenetic research projects.

2.3 Potential Benefits and Risks

2.3.1 Known Potential Benefits

Participating in the study will not directly benefit the participant, but will facilitate future research in JME.

2.3.2 Known Potential Risks/Inconvenience

There are no *major* risks/inconveniences involved by taking part in the study. The known potential risks/inconveniences are:

- Confidentiality – Participants' confidential information may be breached if not stored properly at site or at the CTRC.
- Blood Sample – If participants agree to give a blood sample, they may experience some bruising around the injection site.
- Genetic Information – Participants' genetic information will be collected and studied, all samples will be pseudonymised and it will not be possible for researchers to link results to participants.
- EEG Testing – Participants will have to wear EEG equipment (electrodes and an EEG recorder) for a period of 48 hours, in that time they will have to attend hospital three times, once for fitting, the next day for checking and the last day for removal.
- Neuropsychology Testing – Participants will be asked to undergo a battery of neuropsychology tests which may last up to four hours.

3 SELECTION OF CENTRES/CLINICIANS

Study centres will be initiated once all the requirements from section 8.2 of ICH-GCP guidelines have been met and they are in compliance with CTRC Standard Operating Procedures (SOPs).

All centres will have a consultant (paediatrician, paediatric neurologist or adult neurologist) with a special interest in epilepsy. All centres must be equipped with the ability to perform a 48 hour EEG. Centres must also be able to perform DNA sampling. Checks will be performed during initiation from a CTRC representative that the centres are suitably equipped and qualified to undertake neuropsychology and neurophysiology testing.

3.1 Centre/Clinician Inclusion Criteria

a. Positive SSA by LREC
b. Local R&D approval
c. Receipt of evidence of completion of (a) & (b) by CTRC
d. Completion and return of 'Signature and Delegation Log' to CTRC
e. CV to accompany all research personnel recorded on the 'Signature and Delegation' log
f. Up-to-date GCP training certificate.
g. Contractual agreements signed between site and study sponsor, including 'Material Transfer Agreement'.
h. Written confirmation that centres are equipped and qualified to perform a 48 hour EEG.
i. Sites can undertake neuropsychological testing, or there is agreement that this can be provided by another participating centre.

3.2 Centre/Clinician Exclusion Criteria

a. Not meeting the above inclusion criteria

4 STUDY POPULATION

4.1 Inclusion Criteria

- Adults and young people aged more than or equal to 14 and less than or equal to 65 years of age
- Positive diagnosis of JME made by an epilepsy specialist
- The participant has experienced (and/or):
    - An average of 4 days with myoclonic seizures per month over the past 3 months
- An average of 4 days with absence seizures per month over the past 3 months The participant has previously failed to achieve seizure control with VPA (at least 1,000mg daily) over a period of at least 3 months exposure.

4.2 Exclusion Criteria

Inability to provide informed consent

4.3 Participant Transfer and Withdrawal

In consenting to the study, participants are consenting to study assessments and data collection.

4.3.1 Participant Transfers

Should a participant transfer to another hospital, a copy of the participant's CRFs should be provided to the new site. The participant (or their parent/legal representative) will have to sign a new consent form at the new site, and until this occurs the participant remains the responsibility of the original centre. The CTRC should be notified in writing of any participant transfers. The receiving site should have gained ethics approval prior to the participant being transferred.

4.3.2 Withdrawal from Study

Participants are free to withdraw consent at any time without providing a reason. The participant will not contribute further data to the study; the CTRC should be informed in writing by the responsible consultant and a withdrawal of consent form should be completed.

Participants may be withdrawn from the study for any of the following reasons:

The participant (or parent/legal guardian as appropriate) withdraws consent.
The participant is re-classified during the phenotyping/characterisation phase and accordingly may no longer be eligible to be included in the cohort.

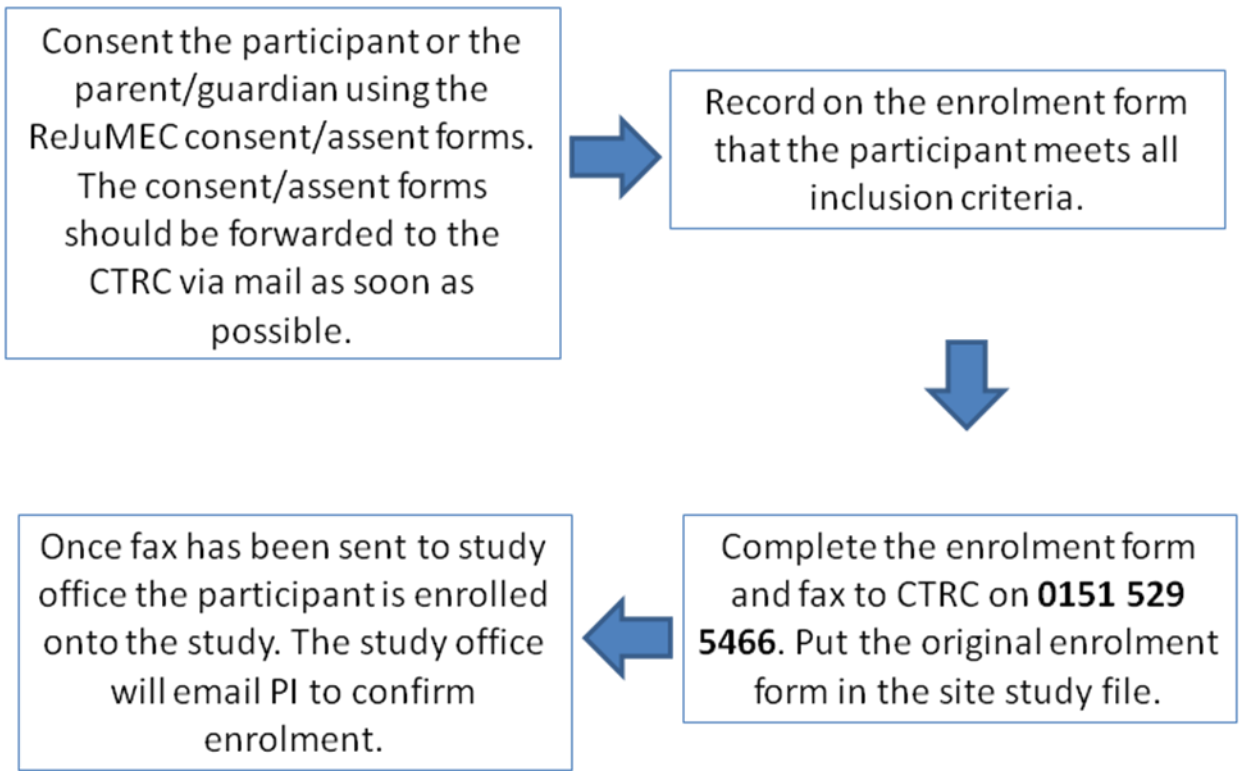
5 ENROLMENT AND REGISTRATION

5.1 Screening

Participants will be identified from clinical records by a hospital site clinician. During an outpatient appointment the clinician will approach the participant with details of the study and ask for their consent (from the participant, or if applicable, the participant's parent/guardian) Recruitment will be undertaken by an experienced epilepsy specialist. who will check that the participant is eligible by following the eligibility criteria in section 5 of the ReJuMEC protocol, and record their eligibility in the ReJuMEC Case Report Form. If the participant has consented to give a DNA sample then this should be taken at the time of consent, or at the participant's earliest convenience. For further information on informed consent please consult section 9.3 of this protocol.

5.2 Enrolment

Participants will be enrolled into the study via fax. The enrolment procedure will follow the diagram below:



The CTRC will collect each participant's contact details for future contact. If the participant is a minor then contact will be made through the minor's parent or legal guardian. Contact details will be stored separately from all other study documents. The contact details will be mailed to the study office using the contact details form. On all study documents throughout the study, a participant will only be identified by their unique study number and initials. The unique study number will be made up of

the site number and the number of the participant at the site for example site number 003 and participant 14 would make 00314. We anticipate that a single site will not recruit more than 99 participants as it is a 200 participant cohort with a planned 14 hospital sites.

5.3 Co-enrolment Guidelines

Participants are free to enter other studies; we do ask that the participant can still commit fully to the ReJuMEC study whilst on other studies.

6 ASSESSMENTS AND PROCEDURES

6.1 Schedule

A participant will be involved in the study for a period of three months. The table below shows the schedule for each participant.

Procedures	Screening	Baseline*	Post Baseline to 3 Months
Review of medical history	X		
Assessment of eligibility	X		
Written informed consent	X		
Enrolment to study		X	
Contact details completed		X	
DNA sampling		X	
Ethnicity form completed		X	
Seizure diary given		X	
Seizure diary completed			X
48 hr EEG*			X
Neuropsychology test**			X
Research priorities questionnaire			X

* See section 7.2.3
** See section 7.2.4

6.2 Procedures/ Assessments

6.2.1 Research Priorities From a Participant's Perspective questionnaire

This questionnaire has been developed in partnership with 'Epilepsy Action' and its volunteers. The volunteers are people with epilepsy or who have family members with epilepsy. Copies of the questionnaire were sent out to the volunteers for their comments and opinions. The questionnaire aims to get responses from participants regarding research priorities into epilepsy.

The questionnaire will be sent to participants after three months of participation by the coordinating centre (CTRC), and should be completed and returned to the CTRC using a pre addressed envelope provided to the participant.

6.2.2 DNA Sample

All participants will be invited to provide a sample of whole blood or saliva for DNA extraction and storage in a central repository at the University of Liverpool. Samples will be frozen and stored at the University of Liverpool for future analysis. DNA sampling will be optional and refusal will not preclude participation in other aspects of the cohort assembly. Samples and associated clinical information will be anonymised prior to further utilisation.

If a blood sample is given then the participant should give 10ml of blood. If a saliva sample is given then the participant should give 5ml of saliva.

The DNA sample should be taken at the participant's earliest convenience between consent and 3 months.

6.2.3 48 Hour EEG

All participants will be referred for a 48-hour ambulatory EEG which will be used to confirm the diagnosis, assess the extent of any generalised spike and wave discharges, and quantify the severity of the seizure disorder. The number, duration and frequency (Hz) of discharges in each 48-hour period will be recorded.

The EEG is attached to the participant's scalp via electrodes and is connected to a small unit. The EEG unit will record all brainwave activities for further study. Participants can still attend work and go about their normal daily activities; this will not negatively affect the outcome.

The participant will attend hospital for the attachment of the EEG equipment. The participant will need to return to hospital the next day to check the EEG is still working. A final visit will be made after 48 hours so that the EEG equipment can be removed.

6.2.4 Neuropsychology Assessments

A single battery of standard neuropsychometric tests will be performed on all adult participants (an adult is classified as being aged 16 years or above), comprised of the Wechsler Adult Intelligence Scale (WAIS III), Wechsler Memory Scale (WMS III), Boston Naming Test (BNT), Delis-Kaplan Executive Function System (D-KEFS), Hospital Anxiety & Depression Scale (HAD), Aldenkamp-Baker Neuropsychological Assessment Schedule (ABNAS) and Impact of Epilepsy Scale (IES). This battery will permit evaluation of intellectual ability, verbal and non-verbal memory, frontal lobe-mediated executive functions, language functioning, depression and generalised anxiety, participant-perceived cognitive impairment, and the psychosocial impact of a diagnosis of JME. It will provide a platform for future longitudinal studies of neuropsychological functioning, a baseline for assessing the neuropsychological effects of novel therapies, and a measure of frontal lobe involvement in JME.

For participants aged 14 -15 years (when the participant reaches 16 years they are no longer a minor) a different battery of tests will be administered, comprised of the Wechsler Intelligence Scale for Children IV (WISC IV), Children's Memory Scale, Expressive One Word Naming Test, Delis-Kaplan Executive Function System (D-KEFS), Hospital Anxiety & Depression Scale (HAD), Aldenkamp-Baker

Neuropsychological Assessment Schedule (ABNAS) and Impact of Epilepsy Scale (IES).

A detailed summary and justification for use of all of the above tests is available from Professor Gus Baker (clinical psychologist); contact can be made via the study office.

The neuropsychology assessments should be completed at the participant's local hospital site. If this is not possible a member of staff from University of Liverpool will travel to the participant's local hospital site and administer the tests. The study office should be made aware as soon as possible if this outreach programme is required.

6.2.5 Seizure Diaries

Seizure diaries will be issued to participants at baseline together with instructions on how to complete them. These diaries will keep a record of the participant's seizures over a 3 month period. After 3 months the diaries should be sent back to the CTRC using a pre addressed envelope which will provided to the participant.

6.2.1 Ethnicity Form

The ethnicity form will be given to participants if they agree to be part of the DNA study. The participant should complete the form at the time of consent. They are free to state that they do not wish to give ethnicity details - a box is available for this option.

6.3 Loss to Follow-up

Seizure diaries and questionnaires should be sent back to the CTRC after 3 months. If the CTRC notes that they have not been returned, CTRC will notify the relevant site. Sites will then attempt to contact the participant and ask them to return the forms. Participants will be asked to inform the site of any change of address using the change of address card. The site will then need to notify CTRC.

7 STATISTICAL CONSIDERATIONS

7.1 Introduction

The cohort size has been estimated on the basis of expected future utilisation and the requirements of power calculations therein. This is most applicable to trials of novel interventions (i.e. antiepileptic drugs) which are, by design, randomised and powered to demonstrate a specific treatment effect. Most previous interventions in JME have been small; 16 participants in a double-blind, dose-comparison, crossover trial of sodium valproate monotherapy [13], 28 participants in a randomised, open-label comparison of topiramate and sodium valproate [14], and 33 participants in a randomised, double-blind, placebo-controlled trial of adjunctive lamotrigine [15]. As a result, they have often been inadequately powered, most likely due to difficulties in large-scale recruitment from a single centre or standardisation of phenotyping across multiple sites. Recruiting 200 individuals to this cohort will enable unique Phase II/III regulatory trials in the JME population and will also support multiple, concurrent smaller-scale proof-of-principle studies. Other expected future uses, such as neuroimaging and neuropsychological examination, are expected to be more observational in nature.

7.2 Analysis Plan

A separate and full statistical analysis plan (SAP) will be developed prior to the analyses of any study data. The SAP will be agreed by the study steering committee before being implemented. Studies recruiting participants from this cohort will be expected to develop study-specific statistical analysis plans.

7.3 Utilisation of the Cohort

1. Drug studies: this cohort will be suitable for investigator-initiated and regulatory studies of novel therapeutic interventions. We expect this to be the principal use of the cohort and it has been sized accordingly. *If we reasonably anticipate a 75% participation rate in Phase II trials amongst cohort members (these individuals are motivated to participate in trials due to the frequency and debilitating nature of their seizures), then 150 individuals randomised in a double-blind manner to adjunctive active treatment or placebo in a parallel-group design would have 80% power to demonstrate an achievable 20% increase in responder rates at a conservative placebo response of 15% and a significance level of $p<0.05$.* This cohort will aid in the development of novel treatments specifically aimed at those participants with idiopathic generalised epilepsies. We have already identified several pharmaceutical industry partners and will to continue to promote interest in this cohort amongst other pharmaceutical companies who are active in the development of broad spectrum antiepileptic drugs (AEDs).

2. Neuroimaging: JME is currently classified as idiopathic generalised epilepsy, with no known pathology on MRI or computed tomography (CT) scanning. However, some of the behavioural and neuropsychological characteristics of the disorder suggest selective involvement of the frontal lobes. Preliminary neuroimaging studies have identified neurotransmitter changes in the cerebral cortex using

positron emission tomography, abnormalities of cortical grey matter in medial frontal areas using quantitative MRI, and evidence of progressive thalamic dysfunction using ^{1}H-magnetic resonance spectroscopy [16]. The availability of this well-phenotyped cohort, together with recent advances in high-resolution neuroimaging, will allow further investigation of the neurophysiology and neuroanatomy of JME and might ultimately lead to an improved understanding of the disorder. Such work has already commenced at the Institute of Neurology, University College London under the direction of Dr Matthias Koepp, who has provided unreserved support for the assembly of this cohort and is our academic neuroimaging partner.

3. Genetics: JME is presumed to have a genetic aetiology. There is a positive family history in 50-60% of cases and inheritance is complex. Genetic mapping studies have identified provisional susceptibility loci on chromosomes 6p and 15q but none has unequivocally identified a candidate gene or genes [17]. This failure may be explained by genetic heterogeneity within JME itself but might equally reflect inadequate or inconsistent phenotyping in previous genetic association studies. The availability of a well-phenotyped cohort of known JMEs, with additional endophenotype data derived from neurophysiological and neuropsychological assessments, is likely to improve the accuracy and power of genetic analysis and may help to pinpoint susceptibility genes more definitively. DNA samples from this cohort will be made available to researchers with an interest in the genetic aetiology of JME. These may be investigated in isolation or used to augment existing collections. Due to the co-existence of a detailed drug history, the samples will also be made available for both internal (University of Liverpool) and external pharmacogenetic research projects. Professor Mark Rees (University of Swansea) has provided unreserved support for the assembly of this cohort and collection of their DNA and is our academic genetics partner.

4. Neuropsychological functioning: Memory difficulties are the most frequently reported cognitive problem in people with epilepsy [18]. The sensitivity of individual memory sub-types to epilepsy-related deficits is believed to be associated with aetiology. People with seizures arising in the temporal lobe are likely to have difficulties with recent memory due to the association of this structure with new learning, whereas those with left-sided seizure initiation typically have word-finding difficulties as a result of language localisation [18]. Participants with idiopathic generalised epilepsies have more diffuse seizure onset, and are likely to have less specific memory deficits, but little is known about their nature or extent. The battery of neuropsychological tests employed in the phenotyping/characterisation phase of cohort assembly will provide a baseline for the longitudinal assessment of memory in idiopathic generalised epilepsy. This cohort will also facilitate investigation of anecdotal reports suggesting specific defects in prospective memory in JME which might again indicate unexpected frontal lobe involvement in seizure generation.

5. Participant-based research recommendations: As part of the characterisation phase of the study, cohort participants will be given the unique opportunity to rank specific pre-defined areas of epilepsy research of most immediate impact or interest to them. The highest ranked areas and any research questions raised therein will be discussed by the steering committee, with appropriate UK researchers identified and encouraged to utilise the cohort in order to address those specific concerns. This is an entirely novel approach in the epilepsy research field, allowing study participants to engage in the research process and empowering participants to play a role in their own disease management.

8 ETHICAL CONSIDERATIONS

8.1 Ethical Considerations

The study will abide by the principles of the World Medical Association Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989) and South Africa (1996).

Methods used in this study are in common usage, such as DNA sampling, neurophysiology and neuropsychology testing.
The following list of items highlights the ethical considerations of the study:

The study initially will focus on the collection of a cohort of participants between the age of 14 and 65 years. In recruiting minors, the study will be dealing with a vulnerable population.
The DNA sample should be taken at the participant's earliest convenience between consent and 3 months.
The participant will need to return to hospital for neurophysiology and neuropsychology testing. Reimbursement for participant travel will be arranged.

8.2 Ethical Approval

The study protocol and all substantial amendments will be submitted for review by the Cheshire Research Ethics Committee and each centre must undergo Site Specific Assessment (SSA) by the relevant Local Ethical Research Committee (LREC). The CTRC should receive notification of positive SSA for each new centre: usually this will be through the CI. A copy of local Research & Development (R&D) approval should be forwarded to CTRC before participants are entered.

8.3 Informed Consent Process

Informed consent is a process initiated prior to an individual agreeing to participate in a study and continues throughout the individual's participation. In obtaining and documenting informed consent, the investigator should comply with applicable regulatory requirements and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki.

Where the potential participant is a minor, proxy consent from the parent or legally acceptable representative should be obtained prior to each participant being registered in the study, after a full explanation has been given of the study regime. Age and stage-of-development specific Patient Information and Consent Leaflets will also be implemented and patient assent obtained where appropriate. The right of the parent/ legal representative to refuse consent for the minor to participate in the study without giving reasons must be respected.

8.3.1 Informed Consent (Competent Adults)

The consent process must be carried out by a medically qualified member of the research team. All participants will receive written and verbal information concerning the nature of the study. This information will emphasise that participation in the study is voluntary and that the participant may withdraw from the study at any time and for any reason. All participants will be given opportunity to ask questions and will be given sufficient time to consider before consenting.

Both the person taking consent and the participant must personally sign and date the form. One copy of the signed consent form will be retained by the Investigator in the Study Site File and must be made available for inspection by relevant CTRC personnel. The original copy will be filed in the participant's medical notes and a further copy of the signed consent form will be given to the participant. One final copy of the consent form should be sent to the CTRC.

The consent forms will contain full explicit details regarding the consent procedure; all information will be explained to the participant via the patient information sheet.
The participant will be asked to sign the following consent forms:

- Consent form for ReJuMEC study
- Consent form for DNA Sampling

The participant may withdraw consent at any time without reason and without effect on any further treatment.

In accordance with GCP, the person taking consent should ensure that a reasonable time has lapsed between the participant/parent being given the study information sheet and the time of consent. This time interval will depend on clinical circumstances but the participant's consultant and the person taking consent should ensure the participant and family feel under no time pressure in giving consent. The person taking consent should check that the participant and/or parent has fully understood the information sheet and has been given the opportunity to ask questions. We would ideally like the participant to consent on the day of the outpatient appointment so that a DNA sample can be taken on the same day. If consent cannot be taken on the day of outpatient appointment then another visit will need to be made with the clinician.

8.3.2 Informed Consent (Minors)

Discussion of objectives, risks and inconveniences of the study and the conditions under which it is to be conducted are to be provided to participants by staff with experience with minors. Age and stage-of-development appropriate Patient Information and Consent forms and assent, describing in detail the study, study procedures and risks, will be approved by an independent ethical committee (IEC) and the participant and their parent/legal representative will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the participant and their parent/legal representative and answer any questions that may arise. A contact point where further information about the study may be obtained will be provided.

The parent or legal representative of the minor will sign the informed consent document. If capable, the participant should assent and sign and personally date a separate IEC-approved assent form, describing (in simplified terms) the details of the

study, study procedures and risks. Assent forms do not substitute for the consent form signed by the participant's legally acceptable representative.
The participant and/or their parent/legal representative should have the opportunity to discuss the study with their surrogates and think about it prior to agreeing to participate. The participant may withdraw informed consent at any time which will lead to them being removed from the study.

The parent or legal representative may, without the minor being subject to any resulting detriment, withdraw the minor from the study at any time by revoking the informed consent. The rights and welfare of the participants will be protected by emphasising to them that the quality of medical care will not be adversely affected if they decline to participate in this study. One copy of the signed consent form will be retained by the Investigator in the Study Site File and must be made available for inspection by a representative of the CTRC. The original copy will be filed in the participant's medical notes and a further copy of the signed consent form will be given to the participant. One final copy of the consent form should be sent to the CTRC.

The consent forms will contain full explicit details regarding the consent procedure; all information will be explained to the participant via the patient information sheet.
When the participant reaches the age of 16 they will need to consent to the study using the adult consent forms. The consent forms should be completed as in section 9.3.1 and forwarded to the CTRC as soon as possible.

The following documents will need to be signed and dated:

- Parental consent form for ReJuMEC study (parent/guardian to sign)
- Parental consent form for DNA Sampling (parent/guardian to sign)
- Minor assent form (minor to sign)

The participant may withdraw consent at any time without reason and without effect on any further treatment

In accordance with GCP, the person taking consent should ensure that a reasonable time has lapsed between the participant/parent being given the study information sheet and the time of consent. This time interval will depend on clinical circumstances but the participant's consultant and the person taking consent should ensure the participant and family feel under no time pressure in giving consent. The person taking consent should check that the participant and/or parent has fully understood the information sheet and has been given the opportunity to ask questions. We would ideally like the participant to consent on the day of the outpatient appointment so that a DNA sample can be taken on the same day. If consent cannot be taken on the day of outpatient appointment then another visit will need to be made with the clinician.

According to the Children's Act (1989) the following when dealing with minor consent applies:
"A mother always has legal responsibility for her child, however a father only has legal responsibility if he is married to the mother or has acquired legal responsibility for his child in the following ways:
For children born before 1 December 2003, unmarried fathers can get parental responsibility by:

- marrying the mother of their child or by obtaining a parental responsibility order from the court

- registering a parental responsibility agreement with the court or by an application to court

For children born after 1 December 2003, the situation is different. Unmarried fathers can get parental responsibility by:

- registering the child's birth jointly with the mother at the time of birth - this is now quite common and many parents choose to do this
- re-registering the birth if you are the natural father
- marrying the mother of their child or by obtaining a parental responsibility order from the court
- registering with the court for parental responsibility”

9 STUDY MONITORING

Site monitoring is conducted to ensure protection of participants taking part in the study, study procedures, laboratory, and data collection processes are of high quality and meet sponsor and, when appropriate, regulatory requirements. A monitoring plan document will be developed to describe who will conduct the monitoring, at what frequency monitoring, and what level of detail monitoring will be conducted.

Initially the study manager will undertake a single site visit to each collaborative centre in the early stages of enrolment for compliance purposes and will ensure data veracity for the duration of recruitment and characterisation.

9.1 Risk Assessment

In accordance with the CTRC Standard Operating Procedure this trial has undergone a risk assessment, completed in partnership between the CTRC, study sponsor (University of Liverpool), co sponsor (Walton Centre for Neurology and Neurosurgery NHS Trust) and co-lead investigators. In conducting this risk assessment, the contributors considered potential patient, organisational and study hazards, the likelihood of their occurrence and resulting impact should they occur.

9.2 Source Documents

Source data: All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies). (ICH E6, 1.51).

Source documents: Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial). (ICH E6, 1.52).

In order to resolve possible discrepancies between information appearing in the Case Report Form (CRF) and any other patient related documents, it is important to know what constitutes the source document and therefore the source data for all information in the CRF.

The following parameters that will be recorded in the CRF are not source data:

- Relevant medical history and diagnosis, including drug history (medical notes are source documents).

Data where no prior record exists and which is recorded directly in the CRF, e.g. inclusion/exclusion criteria the CRF will be considered the **source document**, unless otherwise indicated by the investigator. For remaining data, where no prior record exists and which is recorded directly in the paper CRF e.g., seizure diary and

questionnaires, the paper CRF will be considered the **source document**, unless otherwise indicated by the investigator.

The following list identifies whether the information we are collecting is considered as source data or source document.

- Clinical History – date of epilepsy diagnosis, seizure type, date of onset, current seizure frequencies, first degree family member with epilepsy, febrile seizure, other medical conditions and current non-epilepsy medications. (Medical notes – source data)
- Drug History – current anti-epileptic drugs, previous anti-epileptic drugs. (Medical notes – source data)
- Investigations – EEG, date of EEG, provocation, result, photo convulsive response, MRI, date of MRI, MRI result. (Medical notes – source data)
- Seizure Diaries – date of data collection start, date of data collection stop, number of days with myoclonic seizures in 3 months, number of days with absence seizures in 3 months, total number of tonic-clonic seizures in 3 months, time of day of seizures. Also recorded the number of seizures in one day. (Medical notes – source document)
- Research priorities of epilepsy questionnaire. (Medical notes – source document)
- 48 Hour EEG Results – Date and time of recording start, date and time of recording stop, problems with recording / recording equipment, clinical seizures reported during recording, discharge on EEG, type of discharge, frequency of discharges, duration of individual discharges, total duration of abnormal discharges in 48hr period, time of day of discharge. (Medical notes – source data)
- Neuropsychology testing results (see section 7.2.4). A result of these tests will be entered into the CRF. Only the end score of each test will be recorded after the neuropsychologist has calculated and checked the results. (Medical notes – source data)

9.3 Data Capture Methods

9.3.1 Case Report Forms

The CRF is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write “N/D”. If the item is not applicable to the individual case, write “N/A”. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialled and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

The CRF will be in paper form and should be photocopied at site. Other documents such as the characterisation of refractory JME and seizure diaries will be in paper form on a single sheet. The PISC and consent forms will again be on paper format and will be presented on a single sheet with no NCR format.

The top copy of the CRF should be sent to the CTRC for data check and data inputting within seven days of completion.

9.4 Monitoring at CTRC

Data stored at CTRC will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. If any such problems are identified, a query sheet detailing the problematic CRF(s) will be returned to the local site by post or fax for checking and confirmation or correction, as appropriate. The answered query sheet will be attached to the original CRF as proof of query resolution. The completed CRFs with any answered query sheets will be stored at the CTRC. CTRC will send reminders for any overdue and missing data.

9.4.1 Central Monitoring

The CTRC is to receive a copy of the signed consent form within a week of registration of a patient. If consent forms are not forwarded regularly by a participating centre, the study coordinator will conduct a site visit to check the presence of a signed PISC in the case notes of all enrolled participants.

Data submitted to the database will be centrally monitored by the CTRC to ensure as far as possible that CRF data collected are consistent with adherence to the study protocol. Data will be checked for missing or unusual values (range checks) and checked for consistency within participants over time. Discrepancies that have been raised will be queried. The MACRO data management system will automatically keep a log of what data has been changed, the time of each change, and the person who changed it. The study coordinator will review rates of recruitment, missing data, study withdrawals and losses to follow-up across sites, and remedial action will be taken as necessary. If heterogeneity in reporting is noted across centres then the study co-ordinator will arrange site visits to undertake source data verification. Standardised paper CRFs should be sent to the CTRC within seven days of completion. The study coordinator will conduct data entry checks and use automated validation checks at data entry. A site visit will be conducted if inconsistencies, unresolved queries or missing data are noted at a given site.

Weekly recruitment reports will be provided by the study coordinator, monitoring reasons cited for consent refusal and querying reasons for slow recruitment. The Study Management Group (SMG) is charged with providing solutions to problems where possible. In order to perform their role effectively, monitors and persons involved in Quality Assurance will need direct access to primary subject data, e.g. participant records, test results, appointment books, etc. Because this affects the participant's confidentiality, this fact is included on the Patient Information Sheet and Informed Consent Form. The study coordinator will keep a central protocol deviation log which will be updated with all deviations reported from study sites. If the study coordinator identifies significant and/or persistent noncompliance on the part of the PI, this will be documented in the monitoring report and the SMG will discuss any further action required. A site visit will be conducted if data are consistently missing from a given site. The study coordinator will be in regular contact with the PIs in order to monitor the impact that the study may have on the running of the service.

9.4.2 Confidentiality

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited with the exceptions noted below.

Paper CRFs will collect the participant's name and address for future involvement in JME trials as discussed on PISC.

Saliva or blood samples will be transferred to external laboratories and will be identified by unique identifiers only; these unique identifiers will be used throughout the trial on various documents such as seizure diaries etc., and will be given to the participant at the time of enrolment.

Medical information may be given to the participant's medical team and all appropriate medical personnel responsible for the participant's welfare. Verification of appropriate informed consent will be enabled by the provision of copies of participants' signed informed consent/assent forms being supplied to the CTRC by recruiting centres. This requires that name data will be transferred to the CTRC, which is disclosed in the PISC. The CTRC will preserve the confidentiality of participants taking part in the study and The University of Liverpool is registered as a data controller with the information commissioner's office.

9.4.3 Quality Assurance and Quality Control of Data

QA includes all the planned and systematic actions established to ensure the study is performed and data generated, documented/recorded and reported in compliance with applicable regulatory requirements. QC includes the operational techniques and activities done within the QA system to verify that the requirements for quality of the trial-related activities are fulfilled. This trial has undergone a risk assessment, the outcome of which indicates it to be a low risk trial. As such, site visits will be conducted and source data verification performed if indicated as a result of central monitoring processes. To this end:

- The Principal Investigator, and designated staff from each centre will attend the study launch meeting, coordinated by CTRC in conjunction with co-lead investigators,
- The study coordinator is to verify appropriate approvals are in place prior to the initiation of a site, and that the relevant personnel have attended study specific training
- The study coordinator is to monitor screening, recruitment and drop-out rates between centres
- The study coordinator is to conduct data entry consistency checks and follow up data queries
- Independent oversight of the study will be provided by the Study Steering Committee and the Study Management Group.

9.5 Records Retention

The investigator at each investigational site must make arrangements to store the essential study documents, (as defined in Essential Documents for the Conduct of a

Clinical Trial (ICH E6, Guideline for Good Clinical Practice)) including the Investigator Study Site File, until the CTRC informs the investigator that the documents are no longer to be retained. This study will provide baseline data for future clinical trials, and therefore this study will adhere to GCP standards for clinical trials.

In addition, the investigator is responsible for archiving of all relevant source documents so that the study data can be compared against source data after completion of the study.

The investigator is required to ensure the continued storage of the documents, even if the investigator, for example, leaves the clinic/practice or retires before the end of required storage period. Delegation must be documented in writing.

The CTRC undertakes to store originally completed CRFs and separate copies of the above documents for the same period, except for source documents pertaining to the individual investigational site, which are kept by the investigator only.

Data from the study will be retained for use in further research for a period of over three years. The CTRC policy is to store data for a maximum of 15 years. When data is no longer need in the trials office it will be archived at the University of Liverpool, following the CTRC Archiving SOP TM021 and the University of Liverpool archiving procedures.

Before or at 15 years the data will be destroyed following the University of Liverpool procedures.

10 INDEMNITY

The University's insurance, professional indemnity and clinical trials insurances will apply as appropriate and this will extend to cover for non-negligent harm.

11 FINANCIAL ARRANGEMENTS

The study is funded by the Medical Research Council. Contractual agreements will be in place between the sponsor and collaborating sites that will incorporate financial arrangements.

12 STUDY COMMITTEES

12.1 Study Management Group (SMG)

A Study Management Group (SMG) will be formed comprising the Chief Investigator Professor Tony Marson, other lead investigators Dr John Paul Leach, Dr Michael Johnson, Dr Graeme Sills, Professor Paula Williamson, Mr Ben Hardwick, Dr Pete Dixon and Miss Laura Bonnett. The SMG will be responsible for the day-to-day running and management of the study and will meet approximately 6 times a year for year one and then quarterly after that.

12.2 Study Steering Committee (SSC)

The Study Steering Committee (SSC) will comprise of Professor Tony Marson (Chief Investigator, expert in neurology and epilepsy), Dr John Paul Leach (clinical neurophysiology and neurology specialist, expert in epilepsy and clinical neuro-physiology), Dr Paul Cooper (consultant neurologist, expert in epilepsy and neurological problems, Dr Michael Johnson (expert in general neurology and epilepsy), Dr Markus Reuber (consultant neurologist and expert in epilepsy) and Mr Ben Hardwick (ReJuMEC study co-ordinator).

Requests for access will be made by formal application, prioritised on the basis of greatest clinical or scientific impact, and not unreasonably withheld. The SSC will monitor cohort involvement in utilisation studies on a six-monthly basis to ensure a balanced portfolio of research and to limit potential overuse.

13 PUBLICATION

Individual clinicians must undertake not to submit any part of their individual data for publication without the prior consent of the SMG.

The SMG will form the basis of the Writing Committee and advise on the nature of publications. The Uniform Requirements for Manuscripts Submitted to Biomedical Journals (http://www.icmje.org/) will be respected. All publications shall include a list of participants, and if there are named authors, these should include the study's Chief Investigator(s), statistician(s) and study coordinator involved at least. If there are no named authors (i.e. group authorship) then a writing committee will be identified that would usually include these people, at least.

14 PROTOCOL AMENDMENTS

Version 1 (03/04/2009)

Original Approved version.

Version 2 (18/06/2009)

Amendments made to the:
Enrolment process
48hr EEG process
Inclusion criteria
Participant schedule
Neuropsychology process
Participating sites
General typological errors throughout

15 REFERENCES

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16 APPENDICES

Appendix A – List of participating sites

16.1 Appendix A – List of Participating Sites

Site Name	Principal Investigator	Address	Contact
Walton Centre for Neurology and Neurosurgery	Professor Tony Marson	Department of Neurological Science Clinical Sciences Centre Lower Lane Fazakerley Liverpool L9 7LJ	Tel: 0151 529 5770 Fax: 0151 529 5465 Email: a.g.marson:liv.ac.uk
Southern General Hospital	Dr John Paul Leach	Institute of Neurological Sciences Southern General Hospital 1345 Govan Road Glasgow G51 4TF	Tel: 0141 232 7359 Fax: 0141 201 2999 Email: johnpaul.leach@ggc.scot.nhs.uk
Charing Cross Hospital	Dr Michael Johnson	Division of Neurosciences and Psychology Imperial College London Charing Cross Hospital Fulham Palace Road London W6 8RF	Tel: 0208 846 1194 Fax: 0208 846 5482 Email: m.johnson@imperial.ac.uk
The Royal Hallamshire Hospital	Dr Stephen Howell	Neurology Department The Royal Hallamshire Hospital Glossop Road Sheffield South Yorkshire S10 2JF	Tel: 0114 271 2942 Fax: 0114 271 1901 Email: stephen.howell@sth.nhs.uk
Royal Victoria Infirmary	Dr Margaret Jackson	Neurology Department Newcastle General Hospital Westgate Road Newcastle Upon Tyne NE4 6BE	Tel: 0191 233 6161 Fax: 0191 201 0155 Email: margaret.jackson@newcastle.ac.uk
Kings College Hospital	Dr Lina Nashef	Neurology Department Kings College Hospital Denmark Hill London SE5 9RS	Tel: 0203 299 8343 Fax: 0203 299 3445 Email: lina.nashef@kch.nhs.uk
University Hospital of Wales	Dr Phil Smith	Department of Neurology UHW Heath Park Cardiff CF14 4XW	Tel: 02920 742834 Fax: 0290 744 577 Email: smithpe@cf.ac.uk
Hope Hospital	Dr Paul Cooper	Department of Neurology Hope Hospital Salford Royal NHS Foundation Trust Stott Lane Salford M6 8HD	Tel: 0161 206 5482 Fax: 0161 206 4809 Email: paul.cooper@manchester.ac.uk

Wrexham Maelor Hospital	Dr Dave Smith	Department of Neurology Wrexham Maelor Hospital Croesnewydd Road Wrexham LL137TD	Tel: 01978 725082 Fax: 0197 829 1397 Email: dave.smith@doctors.org.uk
City Hospital	Dr Peter Cleland	Department of Neurology City Hospitals Sunderland NHS Foundation Trust Kayll Road Sunderland SR4 7TP	Tel: 0191 565 6256 Fax: 0191 569 9643 Email: Peter.cleland@chs.northy.nhs.uk
James Cook University Hospital	Dr Paul McKee	Department of Neurology James Cook University Hospital Marton Road Middlesbrough TS4 3BW	Tel: 01642 854208 Fax: 0164 228 2770 Email: paul.mckee@stees.nhs.uk
Royal Victoria Hospital	Dr John Craig	Department of Neurology Royal Victoria Hospital Grosvenor Road Belfast BT12 6BA	Tel: 028 9024 0503 Fax: 028 9024 0899 Email: john.craig@belfasttrust.hscni.net
Selly Oak Hospital	Dr Doug McCorry	Department of Neurology Selly Oak Hospital Raddlebarn Road Selly Oak Birmingham B29 6JD	Tel: 0121 627 1627 Fax: 0121 627 2834 Email: dougallmccorry@yahoo.com
Alder Hey Hospital	Dr Rachel Kneen	Department of Neurology Littlewoods Neurosciences Unit Alder Hey Children's NHS Foundation Trust Eaton Road Liverpool L12 2AP	Tel: 0151 2525163 Fax: 0151 228 0328 Email: rachel.kneen@alderhey.nhs.uk
Western Infirmary	Prof Martin Brodie	Epilepsy Unit Western Infirmary Glasgow G11 6NT	Tel: 0141 211 2572 Fax: 0141 334 9329 Email: martin.j.brodie@clinmed.gla.ac.uk

Appendix D

Consent forms

What Do I Do Now?

Please take time to consider whether you are willing to participate in this research study. Discuss it with others if you wish and please feel free to contact us for additional information, or clarification of this information document. Dr Rhys Thomas will be happy to hear from you, his contact details are as follows:

Phone Number: 01792 295134

E-mail: Rhys.Thomas@Swansea.ac.uk

Address; Room 330
Institute of Life Sciences
College of Medicine
Swansea University
Singleton Park
SA2 8PP

Once you have made a decision then please let us know. You can do this by contacting Dr Thomas directly or by returning the enclosed decision form in

If we do not receive a response within 3 months then you may be contacted again about the study. This is simply to ensure that individuals who wish to participate in the research are not missed for any reason.

Thank you for taking the time to read this; we look forward to your response

WALES EPILEPSY RESEARCH NETWORK

WERN

The Wales Epilepsy and Transient Loss of Consciousness BioBank

Patient Information Sheet

(Neuropsychological Testing)

NISCHR

Sefydliad Cenedlaethol ar gyfer Ymchwil Gofal Cymdeithasol ac Iechyd | National Institute for Social Care and Health Research

Why Neuropscyhological Testing?

In order for us to learn the most from the BioBank it is important for us to understand as much as we can about the epilepsy of each individual who donates a sample for the BioBank. Some kinds of epilepsy are associated with characteristic patterns of memory, planning or sleeping difficulties. Because of the specific kind of epilepsy that you have, we would find it helpful to do some testing to learn more about you and your epilepsy. This testing is in addition to the BioBank research process which is described in the Participant Information Sheet.

What are the possible benefits of taking part in the additional testing?

We can provide a written copy of your test results and a brief report. It is possible that greater awareness of your sleep-wake cycle may help the epilepsy team make suggestions as to ways to better improve your seizure control. However, there are unlikely to be any immediate or direct benefits for your epilepsy: the findings will benefit individuals with epilepsy in the future.

What are the possible disadvantages and risks of taking part in the additional testing?

Volunteering will involve giving up the best part of a day to be with us in the Epilepsy Unit.

What will happen to me if I take part?

What will happen to me if I decide to take part?

- If you decide to take part in these additional tests you will be contacted by Dr Rhys Thomas (an epilepsy Registrar working at the Heath Hospital) or Jordanna Walsh (a research assistant working on the study). They will guide you through the information provided and ensure that you are clear about what the research and this additional testing will involve.

- If you are happy to proceed, a time will be arranged for the testing in the Epilepsy Unit, Heath Hospital, Cardiff or at Swansea University. They will send out a questionnaire for you to fill in before the visit.

- At the meeting you will be asked to sign a consent form which states that you have understood all of the information received and you are willing to participate in the research and this additional testing.

- The testing will take a couple of hours to complete. You will have plenty of opportunities to take breaks and there are no right or wrong answers to the tests.

What will happen to me if I take part?

Will my taking part in this testing be kept confidential?

All the information about your participation in the BioBank will be kept confidential and you are free to withdraw from the study at any time. Complete testing records will be maintained for a minimum of 5 years after the last contact with the chief investigator, and a summary will then be maintained for an additional 12 years before disposal. The procedures for handling, processing, storage and destruction of their data are compliant with the American Psychological Association Ethical Principles of Psychologists and Code of Conduct (APA, 1992).

What if there is a problem?

Any complaint about the way you have been dealt with during the study will be addressed. In the first instance you can contact Dr Rhys Thomas on 01792 295134 or Cheney Drew on 01792 602310. Thereafter, you can contact Professor Rodger Wood on 01792 295778.

r

Participant Reference Number:

CONSENT FORM

Consent for Neuropsychology

Title of Project:

The Wales Epilepsy and Transient Loss of Consciousness BioBank

Name of Researchers: Dr Rhys Thomas, Professor Phil Smith, Professor Mark Rees, Professor Rodger Wood & Clinical Consultants of the Wales Epilepsy Research Network (WERN)

Please initial box

1. I consent to answering questions and performing tasks to test my memory, intelligence, impulsivity and the impact of epilepsy on my life. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that the data collected during the study may be looked at by responsible individuals from the Neuropsychology Unit at the Psychology Department of Swansea University (Supervisor, Professor Rodger Wood). I give permission for these individuals to have access to my records. ☐

4. Information gathered from the tests remains confidential and will be anonymised before use in research. Results cannot be passed on to anyone else without my express written permission. ☐

5. I would like to receive a copy of the results and a brief report after the study has finished. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Participant Reference Number:

CONSENT FORM

Consent to Give Blood

Title of Project:

The Wales Epilepsy and Transient Loss of Consciousness BioBank

Name of Researchers: Professor Mark Rees, Dr Cheney Drew & Clinical Consultants of the Wales Epilepsy Research Network.

Please initial box

1. I agree to give a blood sample for research and long-term blood product archiving. ☐
2. I agree that my sample can be used in collaborative UK and overseas epilepsy research. ☐
3. I agree that my sample can be used in the development of new anti-epileptic drugs, and in studies on established anti-epileptic drugs which may mean submitting small amounts of my sample to reputable Pharmaceutical Companies. ☐
4. I understand how the blood sample will be collected and I have had an opportunity to discuss any concerns that I may have about the procedure. ☐
5. I understand that giving this sample is voluntary and that I am free to withdraw my approval for use of the sample at any time, without giving a reason, and without medical treatment or legal rights being affected. ☐
6. I understand that this research project aims to better understand epilepsy. On rare occasions this may lead to research findings which have clear implications for me and my family - such as how the epilepsy is treated:
   I *would / *would not like to be informed of such findings"
7. I understand that analysis of my blood sample may very rarely identify important information about me and my family which is not related to epilepsy. I have been told about the potential implications of this and have had the opportunity to ask questions:
   I *would / *would not like to be informed of such findings"

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Participant Reference Number:

CONSENT FORM

Consent to Store Data

Title of Project:

The Wales Epilepsy and Transient Loss of Consciousness BioBank

Name of Researchers: Professor Mark Rees, Dr Cheney Drew & Clinical Consultants of the Wales Epilepsy Research Network.

Please initial box

1. I understand that sections of any of my medical notes may be looked at by responsible individuals and I give permission for these individuals to have access. ☐

2. I agree that my clinical details can be inserted into a secure and dedicated epilepsy research database. ☐

3. I understand that access to this data is strictly limited to Clinicians and Health workers within the Wales Epilepsy Research Network. ☐

4. I agree that an anonymous version of my clinical data can be used in collaborative UK and overseas research. ☐

5. I agree that an anonymous version of my clinical data can be used to cross-reference with other anonymous data sources from NHS and similar records for the purposes of healthcare analysis and clinical trials support. ☐

6. I have had an opportunity to discuss any concerns that I may have about the database. ☐

7. I understand that this consent is voluntary and that I am free to withdraw my approval for my clinical details to be in the database at any time, without giving a reason, and without medical treatment or legal rights being affected. ☐

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix E

Neuropsychological tests in detail

WAIS - IQ

The Wechsler Adult Intelligence Scale (WAIS III) is a structured and standardised test of cognitive abilities frequently used in neuropsychological research. It is time consuming with patients (can take over an hour to complete) and as such many authors extract a number of subtests or used abbreviated versions of this test. I elected (in keeping with the ReJuMEC protocol) to perform a full WAIS examination. The WAIS III comprises 14 subdomains; two of these are optional and parts two and three of subdomain three (digital symbol coding) are also option. The subdomains used are described below.

1. **Picture Completion**

This test helps comprise the performance IQ (PIQ) meta statistic. It requires participants to communicate to the tester what is missing from a series of standardised illustrations. These hand drawn coloured cartoons include examples such as a pair of glasses without the nasal bridge to link the two sides of the frame and a picture of someone walking on wet sand but not leaving footprints. It is a test of processing speed (20 seconds to answer each question) but not one of naming (an illusion to what is missing, where it is on the picture suffices). The twenty-five questions get progressively more difficult and a failure to answer the first questions correctly (starting at number six: a door missing its handle) required that you then go backwards rather than forwards. This concept (reverse rule) is used in many of the WAIS III tests as it permits the test to start a quarter of the way through the questions – so able candidates need not get bored answering facile questions.

2. **Vocabulary**

This requires people to define a word and is scored as incorrect (0 marks), partially correct (1) or correct (2). There are thirty-three questions and so the maximal score attainable is sixty-six. The scales score is part of the verbal IQ (VIQ) statistic. Scoring can be complex and so answers are written verbatim and scored from the manual following the interview. This has a reverse rule (test starts at question four – ‘winter’). There is also a ‘discontinue rule’ (as there is for picture completion above). In this case six consecutive scores of zero require that you abandon the test. The most complex words to define are ‘ominous’, ‘encumber’ and ‘tirade’.

3. **Digital Symbol Coding**

The digital symbol coding test is part of the PIQ tests and requires good concentration, attention and processing speed. As such the score also goes towards the Processing Speed statistic. The page has the figures one to nine written in their numeric form with a crude but non-intuitive symbol underneath the. For number one the symbol resembles a long hyphen, two two an inverted capital letter T, for six it is a circle. Participants are given seven examples before being asked to complete as many as they can correctly in a minute. If the number – symbol relationship enters working memory (so that they do not need to continue to refer to the top key) ensures that they manage to complete more of the test. The numbers occur at random and one must not complete all the ones, then all the twos etc – rather each question in turn. An incorrect response counts double (i.e. it is negatively marked).

4. Similarities

This is a component part of VIQ. Starting at question six – participants are asked to describe the similarities between words, terms and concepts. They begin in the physical world 'why is a piano like a drum?' and 'what do an orange and a banana have in common?' but end with 'why is an enemy like a friend?' These are scored like vocabulary was for questions six to nineteen, but the easiest ones are either correct (one mark) or not; the maximum score is thirty-three. The discontinue rule necessitates that four incorrect answers ends the test.

5. Block Design

Block design involves the use of plastic cubes. The six faces have two which are red, two white and two which are red and white separated with a diagonal line. The participant is asked first to replicate the pattern which the interviewer has made using the blocks – then in more difficult questions – to replicate a pattern from a book. Initially the participant needs four blocks (making a square), then later nine (initially for a square and then later for a diamond). The task starts at question five, has a reserve rule and discontinues after three consecutive scores of zero. Scoring is based on time to completion. The first six questions are scored out of two (these are trial attempts) and the following eight questions have a maximum score of 7. The total for the task is 68 and this informs the PIQ statistic. Although it appears complex it was understood well by all participants.

6. Arithmetic

This is a task of mental mathematics. It is a timed test – although only the last and penultimate questions have any score differential based on time to answer. There is a time limit for each question – but this rarely caused an individual to lose marks; if they had no answered by sixty seconds it was likely that they had forgotten an element of the question. All questions are based on 'real world conundrums' and as such the answers are often in units, e.g. pounds, kilos. All can be answered with addition, subtraction, multiplication and division and the penultimate question requires that one has an understanding of 'chance'. There is a reverse rule (questions start at five), a discontinue rule (four null answer questions in succession) and the maximum score is twenty-two. This task's score goes towards the VIQ statistic. This is very much a test of processing auditory information and a significant test of concentration.

7. Matrix Reasoning

This task requires the participant to work through examples in a companion book looking at sequences. Each sequence is a full colour series of geometrical designs and underneath them is a choice of five items which could be the missing item. The rules for which is the correct answer for each series changes item by item. Participants start at question four after having seen three examples (unscored). The test is discontinued after four consecutive scores of zero or four wrong answers out of five questions. This leniency – and the fact that guessing is not penalised here (no advantage of saying 'don't know') means that the task is frequently completed. The maximum score is 26 (one point per question) and goes towards PIQ.

8. Digit Span

The digit span task is both an element of the VIQ and working memory statistics. It requires a great deal of concentration. Starting at the first question participants are asked to repeat back strings of digits spoken to them in a standardised and controlled manner; to prevent them being broken down into three digit chunks (the way one recites a phone number) or in an overly musical way (which aids recall). Initially the request is for two digit strings "1-7" and "6-3" but the test ends on nine digit strings. There are two tests at each length before the task continues to a longer example. Each completely correct string recalled scores a point. The participant must score a point at least on one of the pairs to continue to a more

difficult string. The maximum score is sixteen. Once this is complete the same task is performed – but this time participants must reply reversing the order of the digits – so "6-2-7" becomes "7-2-6". This time there are seven questions and the maximum score is fourteen. There are two optional subdomains to this task which I have chosen not to score.

9. Information

This is also a VIQ test and asks general knowledge questions of the participant. The questions include those on history (both twentieth century such as 'Who was Martin Luther King?' and 'Name me a British Prime Minister during the Second World War' and ancient such as 'Who was Cleopatra?'); geography (such as 'on which continent would you find Brazil?' and 'What is the capital of Rome?'); literature, science and religion are also covered in twenty-eight questions. The discontinue rule requires that six consecutive questions halts the test and one starts at question five. The maximum score is 28 and the test is untimed.

10. Picture arrangement

This is a timed test and the last task to contribute towards PIQ. The participant is given a number of cards – upon which are drawn a series of pen and ink cartoons which tell a story. They are rearranged into a standardised order in front of the participant and the time it takes them to correct the order is taken. The first question (trial) is scored 0, 1 or 2 depending on whether the participant took over two attempts, two attempts or one respectively. Each subsequent series of cards is administered just once. A correct series (within time) scores two. Questions 5-9 have an opportunity for frequently used partially correct series to score one mark. The maximum score is twenty-two.

11. Comprehension

This is the final component of VIQ and is a test of understanding of real world problems. This can be complex to score and so replies are transcribed verbatim and can be scored later with reference to the scoring manual. Participants are asked about concepts such as 'Why do we have child employment laws?' and 'Why might someone prefer to be tried in front of a jury of their peers?' Some queries are about problem solving 'How would you find your way out of the forest if you were lost during the daytime?' There are eighteen questions (starting at number four – reverse rule in place) and five questions require two answers to

score fully. Each question is scored out of two – part from items one to three so that the maximum score is thirty-three.

12. Symbol Search

The symbol search task score goes towards the Processing Speed index. A separate answer book is used and the participant enters their answers directly into it. They are shown three examples, then watched performing three more before they are given two minutes to complete the task. They are shown two geometric designs to the left – then five more towards the right – then the words yes / no on a row. They are asked whether either of the first two symbols on the left are seen amongst the five on the right – if they see them they circle 'yes', if not – they circle 'no'. The maximum score is sixty and incorrect answers are negatively marked.

13. Letter-Number sequencing

This task informs the Working Memory statistic. It has the same format as the digit span in so much as the participant must get a correct score at a string length to progress onwards and that each tie gets progressively more difficult as string length increases by a digit. The aim of this task is to rearrange the alphanumeric characters spoken to them in order; first numbers in numeric order, then letters in alphabetical order. This is a task of both auditory processing and concentration. It is more difficult than the digit span and so three attempts at each string length are offered –with a maximum score of twenty-one.

WAIS Index Scores

VIQ - Verbal IQ is comprised of six subdomains: vocabulary, similarities, arithmetic, digit span, information and comprehension. That is all the questions are presented verbally to the participant.

VC – Verbal comprehension is seen as a purer test of verbal acquired knowledge and reasoning. Arithmetic and digit span are not included in this index score.

PIQ – Picture IQ is a composite of five subdomains: picture completion, digit symbol-coding, block design, matrix reasoning, and picture arrangement.

PO – Perceptual organisation is a measure of nonverbal, fluid reasoning, attentiveness to detail and visual-motor integration. The inclusion of untimed tests such as matrix design and the exclusion of digit-symbol coding and picture arrangement make this less dependent on processing speed.

FSIQ – The full scale IQ is an aggregate score of PIQ an VIQ. It is seen as a representative score of global function.

WM – Working memory is highly correlated with WM calculated from the WMS. It includes subtests that require the participant to hold information in the mind, concentrate and formulate an answer. The WAIS WM includes arithmetic and digit span (verbal tasks) whereupon the WM from the WMS includes spatial span (non-verbal) amongst the subtests.

PS – Processing speed is a measure of the ability to handle visual information quickly. It includes the coding and symbol search subtests.

WMS - Memory

Wechsler Memory Scale (WMS III) contains seventeen stems of which seven are option. Two are reproduced in the WAIS – the digit span (optional) and letter number sequencing. Nine stems were asked of all participants and the details of these tasks are given below.

1. **Logical Memory I**

For this task the participant is read a brief story which is three lines long with a number of sub-clauses. They are asked to concentrate as they will be asked to recall as much of the story immediately afterwards. The recall is scored as a 'story unit' – which is a key piece of

detail of the story and as a 'thematic unit' which is a broader conceptual understating of the narrative. Story A has seven thematic units and twenty five story units (2 -8 per thematic unit).

The second story (story B) is presented just as story A was – this time there are twenty five story units and eight thematic units (1-6 per theme). Story B differs however in that it is immediately repeated and the participant is asked to recall all the information again; providing an opportunity to sample the effects of repeated verbal information on immediate memory. This test contributes towards the Auditory Immediate index and the meta-statistic of immediate memory. The maximum score is out of seventy-five for story units and twenty-three for thematic units.

2. **Faces I**

This task contributes towards visual immediate memory (and therefore in turn immediate memory). Participants are asked to observe and learn a series of colour photographs of faces which are presented to them for two seconds per face. After learning these faces they are presented with a series of forty-eight similar faces and asked whether they have seen the face before. These yes / no answers score one each and the maximum score is forty-eight from the same number of questions.

3. **Verbal Paired Associates I**

This task also contributes towards auditory immediate memory. Participants are presented with a series of eight words which are paired with an unrelated partner word. They must remember these as linked in such a way that when presented with the first word they can recall the partner word. The order that they are presented in is altered so as to remove the primacy and recency biases. The first words are presented four times and the score out of eight is tallied. To aid recall if an incorrect answer is provided (or no answer at all) then the correct partner is provided. Participants have five seconds to reply with the partner word and the maximum score is thirty-two.

4. **Family Pictures I**

This task is the second which contributes towards visual immediate memory. Participants are shown a cartoon of a family that consists of seven members: Mother, father, grandmother, grandfather, son, daughter and their dog. Once they are introduced to these

characters they are they shown four cartoons of these people in a scene. The first is of a picnic, the second in a shop, the third in the garden and the fourth at the dinner table. Each picture is shown for ten seconds and contains four of the case. Once the forty seconds is over they are asked to remember the first scene and provide: which people where in the picture; which quadrant they were in; what tasks or actions they were performing. This provides each member with a score of one for identification, one for location and one or two for the action – totalling four per person. They must have identified the character correctly to attain the mark – a correct action and location misremembered as a different person scores no marks. The maximum score is sixty-four. Commonly the garden and the picnic are confused; mother and daughter are confused; and the detail needed to answer the meal questions is not recalled.

9. Spatial span

Spatial span alongside letter-number sequencing is taken to produce a working memory index for WMS (this is analogous to the working memory statistic produced by the WAIS). Using a peg board participants are asked to perform a physical version of the digit span. Pre-determined patterns of tapping pegs in order need to be repeated immediately after being demonstrated. There are two attempts per trial and once no score is produced in a pair of digit strings the task is repeated but asking the participant to reverse the order of the pattern shown. The maximum score forwards is sixteen and backwards it is the same producing a total score of thirty-two at best.

12. Logical Memory II

This task should be administered thirty minutes after the first logical memory task. It requires delayed recall of the stories – first A then B. It is scored just as logical memory I is scored with the same number of story and thematic units. If they cannot remember the stories at all a reminder can be given of the general gist of the story which gives no details away. The maximum story unit recall score is fifty and thematic unit score is fifteen. Following this a series of thirty yes/no choice questions are administered regarding story A and B (fifteen each). The maximum score for this is thirty. This score contributes towards the auditory delayed memory statistic.

13. Faces II

The second administration of forty-eight faces uses different faces from the first trial – but tests upon the originally learned faces (not the newly exposed). Again yes/no answers are required and a perfect score is forty-eight. This goes towards producing the visual delayed memory index.

14. Verbal Paired Associates II

Returning to verbal paired associates after half an hour participants are again provided with the first word and asked for the partner. This time if incorrect the correct response is not provided. Full marks scores eight points. The participant is then asked to consider twenty-four pairs identify the previously learned pairs from within them. This task is particularly facile and therefore a good measure of effort. No incorrect answers scores twenty-four here.

15. Family Pictures II

The participant is finally asked to consider the pictures of the family that they saw for just ten seconds each half an hour ago. The same questions are asked of them and scored in the same way to produce a maximum possible attainment of sixty-four.

WMS Index scores

Auditory Immediate – This comprises Logical Memory I and Verbal Paired Associates I scaled scores.

Auditory Delayed – This is put together by Logical Memory II and Verbal Paired Associates II scores.

Low scores on these domains (relative to an individual's FSIQ and attentional functioning) may suggest a verbal memory impairment. Clearly delayed recall is contingent on the immediate recall.

Visual Immediate - This includes Faces I and Family Pictures I subdomains.

Visual Delayed – This is the aggregate of Faces II and Family Pictures II.

Differences between the two subtests (faces and family pictures) can be lost by summation as the two paradigms differ and prosopagnosia would be lost in the index score.

Immediate memory – This is the summation of all part one tests: logical memory I, verbal paired associates I, faces I and family pictures I.

ARD - Auditory Recognition Delayed – This is created by adding the recognition raw scores of logical memory II and verbal paired associates II and then converting that sum to a scaled score and then an index score. i.e. it only includes the multiple choice components (the recognised parts) and is a test of 'easier' recall.

General Memory – This is a score of delayed memory and includes all part II tests plus the ARD scaled score. It is considered the best overall measure of memory critical for day to day activities.

As well as the WAIS domains described above participants were asked to complete a test your memory (TYM) questionnaire. This is designed as a user-completed tool for screening people with dementia in the out-patient setting. I asked participants to complete it at home and return the questionnaires to me. There are ten questions and a maximum score of fifty. The first tests orientation (name, year, date of birth etc.); the second immediate visual recall (write down the above sentence); then semantic memory (Prime Minister's name, date of World War One); then four arthimetic questions and then five animals that begin with S to test executive function; then two conceptual questions 'why is a carrot like a potato'; then nominative memory – naming items on a shirt and jacket including low frequency words; then a visual test; then a clock drawing tests (circle provided); then a test of delayed memory – recalling that first sentence. The final five marks are scored dependent on how much support the participant needed to complete the test. TYM is copyright free and a

detailed scoring sheet is provided at www.tymtest.com TYM is a British designed test and there are no data on how well people with epilepsy may score on this.

Language

Language is formally and informally tested throughout the interview. However the Boston Naming Test (BNT) is a specific test of nominative memory. Participants are shown a series of pen and ink cartoons which depict a word. They must name what is shown directly – circumlocutions and descriptions will not suffice. Although there are more complicated ways to score the BNT including provided verbal cues and timing the speed of the answer I am reporting a straight yes/no per question. There are sixty pictures and I used a reverse rule starting at number thirty-six and discontinued if six consecutive scores of zero were produced.

Executive function

To test executive function I used elements from the Delis-Kaplan Executive Function System (D-KEFS) and the BADS (Behavioural Assessment of Dysexecutive Syndrome). The D-KEFS is a widely used North American system and there are nine elements; eight in the test booklet and the ninth a user completed questionnaire. ReJuMEC required three elements (described in full below): the Trail Making task, Verbal Fluency and the Colour-Word Interference task. I augmented my protocol with the dysexecutive questionnaires (DEX) and the proverbs task.

1. **Trail Making Task**

The trail making task (TMT) is a standard test which had been adopted into the D-KEFS and has been used before with people with epilepsy. Participants are given five simple answer booklets – each has a front cover with a panel containing an example, and the booklet opens to the centre paegs to reveal the trails. The cover example is worked with the participant and then the page is turned and they are timed to complete a set task accurately. The first requires that the put a line through all the threes on the page hidden amongst other numbers. This ensures that the participant has the visual scanning needed to

complete tasks 2 to 5 and no significant inattention or hemianopia. The second trail involves linking numbers one to sixteen together in order using a solid unbroken line. The numbers are hidden amongst letters. Task three is the opposite – linking the alphabet up (A to P) in order. Task four is the most challenging and involves all the alphanumeric characters on the page. The participant must link the numbers and letters up in series in an alternating ordered manner. The sequence begins at one, then A, then two, then B, then three etc. The fifth trail is a measure of processing speed as much as anything as unlabelled circles with a dotted line to connect them must be linked in the correct order. The five tasks are marked for time to completion and the number of errors identified. This produces a scaled score, which is summated and can also be expressed by a composite score.

2. Verbal Fluency Test

The D-KEFS provides a comprehensive version of the commonly used verbal fluency or 'FAS' test. The first portion is the standard FAS – where the participant is asked to produce as many words as he or she can in a minute that begin with a specific letter. The names of people and places are forbidden such are multiple uses of the same word. The test is scored in 15 second intervals and a total score is calculated. In addition incorrect answers (set-loss errors) and repeated answers are recorded. The second component of the test looks at category fluency and the participant is given a minute to name as many items within a set as they can (no restriction of the first letter of the word). The first category is animals and the second is boys' names. Category switching is tested next; the participant is given two categories to name items from. They requirement is that they alternate the set members. The example is 'fruit' and 'furniture' – and so might proceed 'banana… chair…. melon… table..' The D-KEFS provides for multiple interpretations of the scores however I will tabulate the raw and scaled scores for language fluency, category fluency, category switching and category accuracy. The last of these indices is calculated from the category switching task.

3. Colour-Word Interference Test

This is a modification of the Stroop test used by D-KEFS. There are four stages to this test. This first requires the participant to name the colours (effectively read the words 'red, 'blue' and 'green') across five lines with ten words in each row. The test is timed and errors are differentiated between acknowledged and changed by the participant (self-corrected) and

those which go unnoticed (uncorrected). In the second part instead of words there are blocks of colour and similarly the participant is asked to 'name' these. Although these are unchallenging these are important component parts to the participant learning the rules of the test, as the tasks build on previously learned elements. The third part (inhibition) has the words blue, green and red written in ink that is not the same colour as the word would suggest, e.g. blue would be written in either green or red ink. The participant is asked to name the colour of the ink and not the word. The fourth stage (inhibition/ switching) begins with the rules of stage three but adds that if there is a box around the word you say the word and ignore its colour.

Each test has fifty replies within it and the score is both the time taken to complete the task and the summation of errors. If any task is not completed within 180 seconds then the unanswered questions are counted as uncorrected errors.

4. Proverbs test

The proverbs test asks the participant to interpret eight phrases. The first five saying are well known 'e.g. Rome wasn't built in a day' and the last three are uncommon 'no bread is without a crust'. Each answer is marked in two ways – for accuracy and abstraction. The first can score zero, one or two depending of accuracy and the second is absolute – with an abstract answer scoring two marks and a literal interpretation no marks. This produces a total score of thirty-two. This score is augmented by a secondary series of questions where the participant is provided with four multiple choices as to the meaning of the original sayings. Choosing an abstract answer scores four marks, a concrete one two marks and picking either a phonemically similar or unrelated answer scores nothing. This adds a further thirty two marks to the proverbs test.

5. Dysexecutive questionnaires

The DEX questionnaires are a pair of user-completed surveys consisting of twenty questions each. One is completed by the participant (DEX-self) and the other by a close friend or relative (DEX-other). Each question has a Likert scale response (never, occasionally, sometimes, fairly often and very often) which score 0 to 5 respectively. The maximum score is therefore eighty and the minimum is zero. Questions include 'I find it difficult to stop

myself from doing something even though I shouldn't' and 'I really want to do something one minute, but couldn't care less about it the next').

BADS

The BADS is a UK designed series of tasks initially designed for people with an acquired brain injury. It is designed to have a greater degree of ecological validity than other tests. There are six tests of which I administered four. These are described in detail below.

1. **Rule Shift Cards**

Rule shift cards are not dissimilar to the Colour-Word Intereference (CWIT) or Stroop test in that it requires the passive learning of rules and then quick 'unlearning' of these rules. It is a much less challenging test that the Colour-Word Interference Test and was included to help score those who had scored poorly on the CWIT. The participants are given a flip book containing the faces of twenty-one playing cards. They also are given a rule – with a written reminder of this palced next to them (to help those with poor recall). The rule is say 'yes' to red and 'no' to black. The tests starts witht the second card and the maximum score is twenty. The time taken to complete the task is also noted.

The second part of the test is changing the rule so that it is now - say 'yes' if the card is the same colour as the last one, otherwise say 'no'. This rule card is again placed next to the participant. The test is started at the first card and there are twenty responses. A profile score of four is maximum on the BADS. If the time taken to complete the second part of the task is greater than 67 seconds than a profile point is lost. The total errors also contribute to reducing the profile score: 1-3 errors is 3 profile points, 4-6 is two and 7-9 errors is a score of one.

2. **Key Search**

This task requires the participant to draw their search strategy for finding lost keys within a fixed area. They are provided with a sheet of paper which has a dot towards the bottom centrally placed and above this an empty square. They are told that they have walking everywhere in the field and they keys are lost somewhere within it. The instruction is 'Starting from this point I want you to draw a line with the pen to show me where you

would walk to search the field.' It is scored according to the manual with a top score of sixteen possible. This is broken into three marks for entering within 10mm of a corner (two for the base of the square); 3 marks for finishing within 10mm of a corner (2 for at the base of the square); 1 each for making a continuous line, for using parallel lines, and for making all lines horizontal / vertical; the search pattern is scored out of five; there is one mark for making an effort to cover the ground and another for whether their pattern would have found the keys. A profile point is lost if the time taken to draw the strategy is longer than 95 seconds.

3. Temporal Judgement

This task is the briefest consisting of four simple questions on estimating the time things take. The accepted intervals are narrow and are scored one profile point for each answer. The four questions are –

1. How long does it take to do a routine dental check-up? (5 to 15 minutes)
2. How long does it take a window cleaner to clean the windows of an average sized house? (15 to 25 minutes)
3. How long do most dogs live for? (9 to 15 years)
4. How long does it take to blow up a party balloon? (50 to 70seconds)

4. Zoo Map

This task requires a degree of planning and sequencing. They are provided with a map of a zoo with certain locations on – e.g. lions, bears, picnic area. Each path can only be used once unless it is shaded and another path is demarcated as a 'camel ride'. They are told that they can only take one camel ride which in effect does not change this path's rule- you may still only use it once.

They clearly must not walk outside the paths nor jump from one location to another. They are asked to start in a specific location (entrance) and end at the picnic area visiting six locations along the way (and electing not to visit locations that are not part of the route). They are not given the order in which to visit them and as such there are three different, but similar, solutions to the puzzle. The task is timed but they are told that accuracy is more

important than time. Eight points (for eight locations) is the maximum score; marks can be deducted for breaking any of the above rules.

The second stage provides the participants with the same map – but this time an order to visit the same locations which if followed would provide the solution to the puzzle. On this stage the time to completion is crucial and if the planning time is greater than fifteen seconds or the total time is greater than 123 seconds then a profile point is removed.

Mood

Hospital Anxiety & Depression scale (HAD) is used under licence granted 20/7/2009. It is a commonly used screening test for affective symptoms in hospital practice. Alone it is insufficient to diagnose anxiety or depression but as severe symptoms can cause poor concentration and mimic a dementia like presentation it is important to try to describe contemporaneous affective symptoms. Fourteen questions are posed with Likert style response underneath such as ’definitely as much’, ‘not quite as much’, ‘only a little’ and ‘hardly at all’. Seven questions ask about anxiety symptoms, seven about depressive symptoms and are scored from zero to three. This produces a maximum depressive score of twenty-one and the same for anxiety symptoms.

Impact of epilepsy

Two scales developed by Professor Gus Baker, Liverpool University are used to estimate the impact of epilepsy and I am grateful to him for giving me permission to use them and a copy of the scoring systems. The scales used were the ABNAS (Alderkamp-Baker Neuropsychological Assessment Schedule) and the Impact of Epilepsy Scale (IES). The first of these is a series of twenty-four questions answered on a Likert scale (no problem, a mild problem, a moderate problem and a serious problem). Questions ask about yourself and your interactions with the world inquiring into issues such as self-esteem. The scoring system links questions 1, 7, 13, 18 and 24 under the heading ‘Fatigue’, 2, 8, 14, 19 and 23 under ‘Slowing’, 3, 9, 15, 20 under ‘Memory’, 4, 10,16,21 under ‘Concentration’, 5,11,17

under ‘Motor’ and 6,12,22 under ‘Language’. They can also be taken together as a metastatistic which would be scored out of ninety-six. The IES is ten questions long and asks about how epilepsy changes your opportunities in life – such as employment, relationships and standard of living. It is again a Likert scale scoring 1 to 4 for ‘not at all’ to ‘a lot’. The maximums core therefore is 40 – and because two answers can score at zero for ‘not applicable’ the minimum score is eight.

Personality

The Eysenck Personality Questionnaire brief version (EPQ-BV) requires participants to complete twenty four Likert scaled questions with one of five answers (A to E). A (not at all) scores one and E (extremely) scores five. Questions 13 and 19 however are reverse scored. Odd questions contribute towards the extroversion element of the EPQ-BV and even numbers towards neuroticism. The minimum score for each domain is therefore twelve and sixty is the maximal score attainable. This test was chosen because it is quick to administer and can act as a screen for personality disorders (PD). Taken alone it cannot diagnose PD and neither can it inform on other forms of PD.

The EPQ-BV (Sato 2005) is a 24-item short version of the EPQ-R, which was developed from Eysneck and Eysneck (1975) EPQ. It was chosen for it brevity and likert scale format. Alternative personality scales (such as the Eysenck Personality Questionnaire (Eysenck and Eysenck 1975)) take longer to administer, are time consuming, giving only the option to answer yes or no. In addition to these advantages it was also chosen for its re-test reliability and validity (Sato 2005). The coefficient alpha scores for the EPQ-BV have been reported to be .92 and .90 for E and N, respectively (Sato 2005; Sato 2007). An example extrovertion question is ‘are you a talkative person?’ and for neuroticism ‘does your mood often go up and down?’

Appendix F

Custom data files for SNP genotyping

Ion channels

Accessory Genes/Other		*Dopamine Receptor Genes*		*Serotonin s*
ANK2				DRD1
BSND		DRD2		HTR1A
KCNIP1		DRD3		HTR1B
MINK1		DRD4		HTR1D
PSMD1		DRD5		HTR1E
SLC12A5		*GABA Receptor Genes*		HTR1F
TNRC15		GABBR1		HTR2A
Voltage-gated Calcium Channel Genes	GABBR2			HTR2C
CACNA1A		hEP Gene	**GABRA1**	HTR3A
CACNA1B		GABRA2	hEP Gene	HTR3B
CACNA1C		GABRA3		HTR3C
CACNA1D		GABRA4		HTR3D
CACNA1E		GABRA5		HTR3E
CACNA1F		GABRA6		HTR4
CACNA1G		GABRB1		HTR5A
CACNA1H		GABRB2		HTR6
CACNA1I		**GABRB3**		HTR7
CACNA1S		GABRD	hEP Gene	*Voltage-gated*
CACNA2D1		GABRE		**KCNA1**
CACNA2D2		GABRG1		KCNA10
CACNA2D3		**GABRG2**		KCNA2
CACNA2D4		GABRG3	hEP Gene	KCNA3
CACNB1		GABRP		KCNA4
CACNB2		GABRQ		KCNA5
CACNB3		GABRR1		KCNA6
CACNB4		GABRR2		KCNA7

CACNG1		*Glycine Receptor Genes*		KCNAB1
CACNG2		Gcom1		KCNAB2
CACNG3		GLRA1		KCNAB3
CACNG4		GLRA2		KCNB1
CACNG5		GLRA3		KCNB2
CACNG6		GLRB		KCNC1
CACNG7		GRIA1		KCNC2
CACNG8		GRIA2		KCNC3
Cholinergic Receptor Genes	GRIA3			KCNC4
CHRNA1			GRIA4	KCND1
CHRNA10		*Ionotropic Glutamate Receptor Genes*		KCND2
CHRNA2	hEP Gene	GRID1		KCND3
CHRNA3		GRID2		KCNE1
CHRNA4	hEP Gene	GRIK1		KCNE1L
CHRNA5		GRIK2		KCNE2
CHRNA6		GRIK3		KCNE3
CHRNA7		GRIK4		KCNE4
CHRNA9		GRIK5		KCNF1
CHRNB1		GRIN1		KCNG1
CHRNB2	hEP Gene	GRIN2A		KCNG2
CHRNB3		GRIN2B		KCNG3
CHRNB4		GRIN2C		KCNG4
CHRND		GRIN2D		KCNH1
CHRNE		GRIN3A		KCNH2
CHRNG		GRINA		KCNH3
Chloride Channel Genes	*Metabotropic Glutamate Receptor Genes*	KCNH5		KCNH4
CLCN1				GRM1
CLCN2	hEP Gene	GRM2		KCNH6
CLCN3		GRM3		KCNH7

CLCN4
CLCN5
CLCN6
CLCN7
CLCNKA
CLCNKB

GRM4
GRM5
GRM6
GRM7
GRM8

Cyclic Nucleotide-gated Channel Genes

HCN1
HCN2
HCN3
HCN4

KCNH8
KCNQ1
KCNQ2
KCNQ3
KCNQ4
KCNQ5
KCNRG
KCNS1
KCNS2
KCNS3
KCNT1
KCNV1

Genes identified by NGS of IGEs

MYO15A
USH2A
NUP205
HECTD1
HEXA
LAMC2
UTRN
MXRA5
UNC45B
ABCC11
ANXA1
CCDC77
CYP2C9
SCN7A
SLC27A5

TDRD6
UROS
ATP10D
C12orf63
C14orf155
CNDP2
DEPDC1B
DNAH8
DZIP3
KIAA0100
KLF3
LAMA3
MDN1
PIK3R3
PIWIL3

PLCE1
PROX1
SPECC1L
SPEN
VWF
FAAH2
UBQLN2
ATXN7
KIAA0556
ARID4A
C1orf164
CEACAM5
CSMD2
DNAH7
DSP

EMILIN2	ABCB5	HSPA4L
FICD	ABCC3	KIAA1109
HIVEP1	AKAP8	MAP2
KIAA0922	ALPK3	MARVELD2
KIAA1632	ARL6	MX1
LRBA	ATBF1	MYOM2
MYH6	ATP10A	NR1I2
MYOM1	ATXN1	OR4B1
OIT3	BSN	PLCB3
PCDH9	CILP	PLEKHA6
PHF3	CNTNAP2	PLXNA2
PKHD1	DNAH3	PTPRD
PLXNA3	DNAH5	SALL4
PPL	DOCK8	SH3PXD2B
PPP1R13L	EPHB4	SILV
RNF123	FAM83H	SPG11
RPE65	FAT2	STOX1
RPUSD2	FCGBP	TRAK2
SLC16A4	FGD6	TXNDC3
SLC18A1	GCN1L1	XKR8
SPHKAP	GPNMB	ZBED4
STON1-GTF2A1L	GPR177	ZNF592
TULP4	GSS	
VPS13D	HMCN1	

Linkage areas of interest in the IGEs

10p11.2 Kinirons P et al. Am J Med Genet A. 2008 Mar 1;146A(5):578-84. PMID:18241056

10q25.2-26.11 Puranam RS et al. Ann Neurol. 2005 Sep;58(3):449-58. PMID:16130088

9q32-33 Baykan B et al. Epilepsia. 2004 May;45(5):479-87. PMID:15101829

8q24 Plaster NM et al. Neurology. 1999 Oct 12;53(6):1180-3. PMID:10522869

8q24 Fong GC et al. Am J Hum Genet. 1998 Oct;63(4):1117-29. PMID:9758624

8p23-p21 Baulac S et al. Arch Neurol. 2008 Jul;65(7):943-51. PMID:18625863

8p12 Durner M et al. Am J Hum Genet. 1999 May;64(5):1411-9. PMID:10205274

6p21.32 Pal DK et al. Am J Hum Genet. 2003 Aug;73(2):261-70. Epub 2003 Jun 25. PMID:12830434

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EFHC1 Suzuki T et al. Biochem Biophys Res Commun. 2001 Nov 2;288(3):626-36. PMID:11676489

GABRA1 Cossette P et al. Nat Genet. 2002 Jun;31(2):184-9. Epub 2002 May 6. PMID: 11992121

5q13.3-5q23.1 Durner M et al. Ann Neurol. 2001 Mar;49(3):328-35. PMID:11261507

3q26 Sander T et al. Hum Mol Genet. 2000 Jun 12;9(10):1465-72. PMID:10888596

3q13.2-21.2 Kapoor A et al. Ann Neurol. 2008 Aug;64(2):158-67. PMID:18756473

3p23-p14 Chioza BA et al. Epilepsy Res. 2009 Dec;87(2-3):247-55. Epub 2009 Oct 17. PMID:19837565

3p22.3-22.2 Blair MA et al. Epilepsia. 2011 May;52(5):993-9. doi: 10. 1111/j. 1528-1167. 2011. 03059. x. Epub 2011 Apr 11. PMID:21480884

3p14.2-p12.1 Zara F et al. Neurology. 1998 Aug;51(2):493-8. Erratum in: Neurology 1998 Nov;51(5):1520. PMID:9710024

2q36.3-37.1 Klein KM et al. Epileptic Disord. 2008 Mar;10(1):13-8. PMID:18367426

2q36 Sander T et al. Hum Mol Genet. 2000 Jun 12;9(10):1465-72. PMID:10888596

2q33-q36 Ratnapriya R et al. Hum Genet. 2010 Aug;128(2):123-30. Epub 2010 May 14. PMID:20467754

2q24.1 Layouni S et al. Epilepsy Res. 2010 Jun;90(1-2):33-8. Epub 2010 Apr 7. PMID:20378313

2q23.3 Layouni S et al. Epilepsy Res. 2010 Jun;90(1-2):33-8. Epub 2010 Apr 7. PMID:20378313

23-q31 Lopes-Cendes I et al. Am J Hum Genet. 2000 Feb;66(2):698-701. PMID:10677328

19q13.1 Wallace RH et al. Nat Genet. 1998 Aug;19(4):366-70. PMID:9697698

18q12.2-18q21.32 Durner M et al. Ann Neurol. 2001 Mar;49(3):328-35. PMID:11261507

15q13.3 Elmslie FV et al. Am J Hum Genet. 1996 Sep;59(3):653-63. PMID:8751867

14q23 Sander T et al. Hum Mol Genet. 2000 Jun 12;9(10):1465-72. PMID:10888596

11q22.1–23.3 Yang MS et al. Neurosci Lett. 2003 Aug 7;346(3):133-6. PMID:12853102

5p14.1 Durner M et al. Ann Neurol. 2001 Mar;49(3):328-35. PMID:11261507

Genes associated with infantile epileptic encephalopathy

ARX	MEF2C	SCN8A
CDKL5	CNTNAP2	GRIN2A
SLC25A22	NRXN1	GRIN2B
STXBP1	FOXG1	ZEB2
SPTAN1	UBE3A	POLG
SCN1A	SLC9A6	MAGI1
ARHGEF9	GABRG2	SLC2A1
PCDH19	KCNQ2	HNRNPU
PNKP	PLCB1	EFCAB2
SCN2A	MAPK10	CACNA2D1
TCF4	SLC2A1	PCLO
MECP2	SCN9A	CACNA2D1

SLC26A1

SYT2

LRRK2

SCLT1

GPR1

ZDBF2

ADAM23

GDNF

NHEG

TMEM185A

DOK5

EPHA6

GABRR3

SLC1A3

SLC25A18

ALDH7A1

BRD2

CACNA1A

CACNA1H

CACNB4

CASR

CHRNA2

CHRNA4

CHRNB2

CLCN2

CSTB

EFHC1

EPM2A

GABRA1

GABRB3

GABRD

GABRG2

GPR98

GRIN2A

GRIN2B

KCNMA1

KCNQ2

KCNQ3

KCTD7

MBD5

ME2

NHLRC1

PCDH19

PRICKLE1

PRICKLE2

SCARB2

SCN1A

SCN1B

SCN2A

SCN9A

SLC2A1

TBC1D24

ARHGEF9 [Early Infantile Epileptic Encephalopathy]

ARX [Early Infantile Epileptic Encephalopathy]

CDKL5 [Early Infantile Epileptic Encephalopathy]

CNTNAP2 [Pitt Hopkins Syndrome]

FOXG1 [Rett Syndrome]

GABRG2 [Early Infantile Epileptic Encephalopathy]

GRIN2A [Early Infantile Epileptic Encephalopathy]

GRIN2B [Early Infantile Epileptic Encephalopathy]

MAPK10 [Lennox Gastaut Syndrome]

MECP2 [Rett Syndrome]

NRXN1 [Pitt Hopkins Syndrome]

PCDH19 [Early Infantile Epileptic Encephalopathy]

PNKP [Early Infantile Epileptic Encephalopathy]

RNASEH2A [Aicardi-Goutieres Syndrome]

RNASEH2B [Aicardi-Goutieres Syndrome]

RNASEH2C [Aicardi-Goutieres Syndrome]

SAMHD1 [Aicardi-Goutieres Syndrome]

SCN1A [Early Infantile Epileptic Encephalopathy]

SCN1B [Early Infantile Epileptic Encephalopathy]

SCN2A [Early Infantile Epileptic Encephalopathy]

SCN9A [Early Infantile Epileptic Encephalopathy]

SLC2A1 [GLUT1 Deficiency Syndrome]

SLC25A22 [Early Infantile Epileptic Encephalopathy]

SLC9A6 [Angelman Syndrome]

SPTAN1 [Early Infantile Epileptic Encephalopathy]

STXBP1 [Early Infantile Epileptic Encephalopathy]

TCF4 [Pitt Hopkins Syndrome]

TREX1 [Aicardi-Goutieres Syndrome]

UBE3A [Angelman Syndrome]

ZEB2 [Mowat-Wilson Syndrome]

ARHGEF9 [Early Infantile Epileptic Encephalopathy]

ARX [Early Infantile Epileptic Encephalopathy]

ATP6AP2 [Epilepsy with XLMR]

ATRX [Epilepsy with XLMR]

CASK [Mental Retardation and Microcephaly]

CDKL5 [Early Infantile Epileptic Encephalopathy]

CUL4B [Epilepsy with XLMR]

CXORF5 [Simpson-Golabi-Behmel Syndrome]

DCX [Lissencephaly]

FGD1 [Aarskog Scott Syndrome]

GPC3 [Simpson-Golabi-Behmel Syndrome]

GRIA3 [Epilepsy with XLMR]

HSD17B10 [Epilepsy with XLMR]

JARID1C [Epilepsy with XLMR]

OPHN1 [Epilepsy with XLMR]

PAK3 [Epilepsy with XLMR]

PHF6 [Boerjeson Forsmann Lehmann Syndrome]

PLP1 [Pelizaeus-Merzbacher Disease]

PQBP1 [Epilepsy with XLMR]

RAB39B [Epilepsy with XLMR]

SLC9A6 [Angelman-Like Syndrome]

SMC1A [Cornelia De Lange Syndrome]

SMS [Epilepsy with XLMR]

SRPX2 [Rolandic Epilepsy]

SYP [Epilepsy with XLMR]

Genes identified from Epilepsy pathways and proteomes

CFTR, ABCA8, ABCG4, ABCA4, ABCC3, ABCC2, ABCB5, ABCC11, ABCC9

SLCO2A1, SLC1A7, TF, SLC18A1, CFTR, SLC5A2, SLC14A2, NUP133, GABRB2, ATP10D, ABCB5, ATP4B, SLC3A1, SLC2A10, SLC6A19, SLC5A9, ABCA8, ABCG4, SLC35C1, NUP188, SLC47A1, NUP205, SLC30A6, ATP6V1E1, SLC7A6, SLC22A1, SLC7A3, ATP10A, SLC20A2, ADCY7, ABCC2, ATP1A1, SLC17A5, ATP8B1, NUP153, NUP160, SLC27A6, ABCA4, ABCC3, SLC39A6, SLCO1A2, ABCC11, ABCC9, HMOX2

ABCC3, CFTR, ABCC2, ABCC9, ABCC11

ENO3, GYS2, PHKG2, PYGB, GALE, PRPS1, NUP133, GAPDHS, TREH, SI, LCT, NUP153, NUP160, ENO2, NUP188, PGAM2, NUP205

GRM7, GABBR2, TAS1R2, CASR, GRM3

TREH, SI, LCT

ERBB3, EGF, ERBB2, NRG1

GAD1, GAD2

GPR77, C5

HEXB, HEXA

CNVs of relevance

14q11.2	is an area of interest IGE
17q21.3	is the most promissing area of interest for IGE
15q13.3,	IGE
16p13.11	IGE
15q11.2	IGE

IGE

1q21.1 Y Chr1: 145.0–145.9 900 kb Del

15q11.2 Y Chr15: 20.2–20.8 600 kb Del - IAE 4 CYFIP1

15q11.2 Y Chr15: 20.2–20.8 600 kb Del - CAE 4 CYFIP1

15q11.2 Y Chr15: 20.2–20.8 600 kb Del Inh (P) CAE 4 CYFIP1

15q11.2 Y Chr15: 20.2–20.8 600 kb Del Inh (P) IGE 4 CYFIP1

15q13.3 Y Chr15: 28.7–30.1 1.4 Mb Del - IAE 6 CHRNA7

15q13.3 Y Chr15: 28.7–30.1 1.4 Mb Del - JME 6 CHRNA7

15q13.3 Y Chr15: 28.7–30.1 1.4 Mb Del - JME 6 CHRNA7

15q13.3 Y Chr15: 28.7–30.1 1.4 Mb Del - JME 6 CHRNA7

15q13.3 Y Chr15: 28.7–30.1 1.4 Mb Del - IGE + ID 6 CHRNA7

16p11.2 Y Chr16: 29.5–30.2 700 kb Dup - JME 30 SEZ6L2

16p13.11 Y Chr16:15.4–16.3 900 kb Del Inh (M) CAE 6 NDE1

16p13.11 Y Chr16:15.4–16.3 900 kb Del - JME 6 NDE1

16p13.11 Y Chr16:15.4–16.3 900 kb Del - JME 6 NDE1

16p13.11 Y Chr16: 15.4–18.5 3.1 Mb Del - JME 7 NDE1

1p31.1 Chr1: 72.04–72.15 111.3 kb Del Inh (P) CAE 1 NEGR1

4q22.2 Chr4: 94.18–94.83 646.6 kb Del Inh (M) CAE 1 GRID2

5p15.33 Chr5: 0.72–1.43 713.0 kb Dup Inh (M‘

EP007.1 5q33.2 Chr5: 153.2–160.3 7.1 Mb Del Not in M IGE + ID 44 CYFIP2

6q12 Chr6: 65.03–66.09 1.06 Mb Dup - JME 1 EYS

7q11.22 Chr7: 69.38–69.46 78.7 kb Del - JME 1 AUTS2

q36.1 Chr7: 151.35–151.43 85.8 kb Del Inh (P) MAE 1 GALNT11

8q21-q22 Chr8: 83.97–97.20 15.9 Mb Dup Inh (P) JME+ID 50 many

9p21.3 Chr9: 21.21–21.63 427.5 kb Del - JME 9 KLHL9

9q21.32 Chr9: 83.9–85.2 1.30 Mb Del Inh (M) IGE 2 RASEF

9q31.3 Chr9: 113.33–114.33 1.01 Mb Dup Inh (M) CAE 10

13q31.1 Chr13: 84.69–85.36 671.8 kb Del - JME 1 SLITRK6

14q24.2 Chr14: 70.96–71.23 268.6 kb Del - JME 1 SIPA1L1

15q25.2 Chr15: 83.00–83.12 117.4 kb Dup - IAE 3 NBM

16q23.1 Chr16: 74.49–75.27 785.8 kb Dup - GTCS only 1 CNTNAP4

17p11.2 Chr17: 19.92–19.94 13.3 kb Del - GTCS only 1 CYTSB

17p11.2 Chr17: 19.92–19.94 17.5 kb Del - JME 1 CYTSB

17q12 Chr17: 30.53–30.87 338.5 kb Del - IAE 7 UNC45

18q11.2 Chr18: 19.66–20.50 840.4 kb Dup - JME 6

18q11.2 Chr18: 19.66–20.50 840.4 kb Dup - JME 6

21q21.1 Chr21: 16.21–18.81 2.59 Mb Dup Inh (M) IGE + ID 7

Xp22.31 ChrX: 7.78–8.39 605.5 kb Dup - IGE 4 PNPLA4

Idiopathic Focal Epilepsies (n = 63)

1q21.1 Y Chr1: 145.0–145.9 900 kb Del - BECTS 8

16p12.1 Y Chr16: 21.8–22.3 500 kb Del - BECTS 7

21 16p13.11 Y Chr16: 15.4–16.3 900 kb Del - BECTS 6 NDE1

4q35.1 Chr4: 186.30–186.61 302.4 kb Dup - BECTS 8 SLC25A, SNX25

8p23.1 Chr8: 10.19–10.37 173.1 kb Del - BECTS 1 MSRA

Other (n = 55)

K 047 15q11.2 Y Chr15: 20.2–20.8 600 kb Del brother‘ IC 4 CYFIP1

2q35 Chr2: 218.36–218.94 571.9 kb Dup - SIGEI 11

5q35.1 Chr5: 167.62–167.89 268.7 kb Dup - West 4 WWC1

5q35.1 Chr5: 169.43–169.64 230.0 kb Dup - West 4 DOCK2, FOXI1

7q11.22 Chr7: 69.38–69.42 38.3 kb Del - Unclassified 1 AUTS2

7q35 Chr7:146.06–146.36 304.4 kb Del Inh (P‘) NC 1 CNTNAP2

15q13.3-q14 Chr15: 30.66–32.44 1.78 Mb Dup Inh (M) Unclassified 15

17p13.1

UCL/King’s paper

15q11.2q13.1 Deletion 5356.7kb

1p36.33p36.32 Deletion 2333.6kb

9p24.3p24.2 Deletion 3644.9kb

15q11.1q13.1 Duplication 8369.1kb

16p13.11 Deletion 946.8kb

6q22.31q22.33 Deletion 4060kb

4p16.3p12 Duplication 48570kb

15q13.2q13.3 x1 1.981 Mb

Xp22.33q28

16p11.2

15q11.2q13.2(19,109,124e28,153,416)

15q13.2q13.3(28,910,478e30,226,235)

16p13.11 x1 1.144 Mb

7q35 x0 908 kb

8p23.2p23.3 deletion

12p13.31p13.33

Appendix G

Hyperekplexia cases

GLRA1 and SLC6A5

Sex	Self declared ethnicity	Gene mutation(s)		Inheretance	Affected family memebrs	Hypertonia	Startle	Falls	Learning difficulties or developmental delay	Response to Clonazepam	Neonatal apnoeas	Delayed motor milestones at one year	Speech delay	Epilepsy
M	Irish / Dutch / French	P250T		D	+	+	+		-		+			
M	White Australian	R271Q		D	-	+	+	+	-	+/-	-			
M	Caucasian	R271Q		D	-	-	+	+	-	+	+			
F	White Australian	R271Q		D	+	+	+	-	-	x				
M	Caucasian	R271Q		D	+	+	+	++	+	x				
F	Caucasian	R271Q		D	+	+	+	+	-	+				
F	Caucasian	R271Q		D	+	+	+		+	x				
M	French Canadian	R271Q		D	-	-	+	-	-	+				
M	White British	R271Q		D	+	+	+	+	-		+			-
M	Caucasian	Q226E		?D	+	-	+	na	-	not tried	-	-	-	
F	Japanese / Caucasian	Y279C		D	-	+	+	+	+	+	+	-	-	
F	Pakistani - Kashmirir	C986A		D	-	+	++	++	-	+				
F	Turkish	D165G	D165G	R	-	-	+	+	+	+	+		+	-
M	Caucasian	del ex 7		?R		+	+	+					+	+
F	Turkish	del ex1-ex6		R	Sib	+	+	-		+				
M	Turkish	del ex1-ex6	del ex1-ex6	R	Sib	-	+			++				
M	Turkish	del ex1-ex6	del ex1-ex6	R	Sib	+	+	-	-	++				
M	Turkish	del ex1-ex6	del ex1-ex6	R	Sib	+	+	-	-	+				
M	Caucasian	E103K	del634-635CT	R	-	+	+	-	+	+		+	+	?
M	Turkish	ex5B del CT	ex5B del CT	R	-	+	+	+	+	+				

M	Jordanian	G254D	G254D	R	Sib		+	+	?	+				
M	Jordanian	G254D	G254D	R	Sib	+	+	+		+	+			
M	Jordanian	G254D	G254D	R	-	+	+	+	-	+	+			
M	Jordainian	G254D	G254D	R	-	+	+	-	-	++	+			-
F	Turkish	L291P	D388A	R	-	+	+		+	+			+	
M	French	R93L	del ex4-7	R	-	+/-	+	+	-	++	-	-	-	+/-
M	Turkish	R252C	R252C	R	+	-	+	-	+	+				
M	Italian Canadian	R392H	R392H	R	?Sib	+	+	+	+	+				
M	UAE / Arab	R65W	R65W	R	-	+/-	+	-	+	+	+	+	+	?
M	Tukish	R65W	R65W	R			+	+		+				+
M	White Australian	S231N	S296X	R	Sib		+		++	+		+	++	
M	White Australian	S296X	S296X	R	Sib		+		+			+	+	
M	Jordanian	Y202X	Y202X	R	++	+	+	+	+	not tried	+	+		
M	Asian	E383X	E383X	R	-	+	+	-	-	+	-	-		
M	Pakistani	R218W	R218W	R	-	+/-	+	na	-	++	+	-		
M	Turkish	delEX1-6	delEX1-6	R	+	normal	+	-		+	+			
F	Turkish	delEX1-6	delEX1-6	R	+	normal	+			+	+			
M	Moroccan / Lybian Jews	E103K	E103K	R	-	+	+		+	+	+	+		+
M	Jordainian	Y197X	Y202X	R	+	+	+	-	-	+	+			
F	Jordanian	Y197X	Y197X	R	-	+	+	-	-	++				
M	Caucasian	A830G	DelC	R	-	+	+	++	-		+			
F	Welsh Romany Gypsy	AT1112A	AT1112A	R	-	+	+	++	-	+/-				+/?
M	Pakistani	Y202X	Y202X	R	-	++	+	-	+					
F	Pakistani	Y202X	Y202X	R	Sib	+	+	+	-	+				
F	Pakistani	Y202X	Y202X	R	Sib	+	+	+		++				
M	Jordianian	Y202X	Y202X	R	Sib	-	+	+	+	x		+		
M	Jordanian	Y202X	Y202X	R	Sib	-	+	+	+	x				
		R218Q	S296X	R		+	+	-						
		T162K	T162K	R		+/-	+	-	-		+	+		
		E280K	E280K	R		+	+	+			+			

	South Africa	R271Q		D	+	+	+	+						
	Lebanon	Y197X	Y197X	R	-	+	+	+	+	+	+	+		
	Australia	R414H	R414H	R	-	+	+				+	-	-	
	Iraeli	R72C	R72C	R	+	+	+	+			+			
M	Iraeli	R72C	R72C	R	+	+	+	++	+		+			
	Iraeli	R72C	R72C	R	+	+	+	++	+		+			
		R65W	P230S	R	-	+	+	-	+					
		R27C	R27C	R	Sib	+	+	+	+		+			
		R27C	R27C	R	Sib	Normal	+	+			+			
		R218Q	R218Q	R	-	+/-	+	na	-	++	+	-		
		R271Q		D	Sib	normal as an adult	+	+	-	+	-	-		
M	Spanish	W151X		?D	-	+	+		+/-	+	+	+	+	
F	Pakistani	R439X	R439X	R	Sib	+	+	-	+	-	+			
	Unknown	delCT 1459-1467 (fs+40X)	G657A	R	?+		+	-	+		?			
M	Caucasiain	delTG 593-594 (fs+121X)		?R	+	+	+		+	+	+	+	+	+/-
F	Caucasiain	delTG 593-594 (fs+121X)		?R	+	+	+		+	+	+	+	+	+/-
F	Caucasian	E248K	IVS8+1 G> A	R	-	+	+	+	-	+	+			
F	Asian	P243T	P243T	R	Sib	+	+	+	+	+	+	+	+	
F	Asian	P243T	P243T	R	Sib	+	+	-	+	+	+	+	+	
F	French	R439X		?R	-	+	+		+	++	+	+		?
F	Caucasian British	R439X	R439X	R	-	+	+	+	?	+	+			
M	Pakistani / Persian	R439X	R439X	R	-	-	+	+	+	+	+	+	++	+
M	Caucasian	IVS 13+1 G>T	R439X	R	-	+	+	+	+	++	++			
M	Jordanian	Y297X	Y297X	R	Sib	-	+	-	+	+	+			
M	Jordanian	Y297X	Y297X	R	Sib				+	+				
M	Caucasian British	F547S	Y656H	R	-		+	+	+	++	+	+		?
F	Caucasian British	Y705C		?R	-	+	+	na	na	na	+	na	+	+
M	Caucasian British	S256R	Y656H	R		+	+	na	na	na	+	na	+	+/-
		G225R	G225R	R										
		G449E	?											

F	Caucasian	Y377X	F547S	R	-	+	+	-	-	++	+			
M	Turkish	Y287X	Y287X	R	+	+	+	+	-	+	-			
F	Turkish	Y287X	Y287X	R	+	-	+	+	-	+	-			
M	Indian	G449E	G449E	R	-	+	+	-	+	+	-			
F	Indian	V134V	fs 53 X	R	-	+	+	-	-	+	-			

GLRB

Case	Code	Origin	Origin	Age (in 2012)	Ethnicity	Mode of Inheritance	Genotype	Class of Mutation	Protein Mutation†
1#	**H119/03**	Belgium	Belgium	15	Caucasian	Compound Heterozygote	c. G920A (P)	Missense	p. G229D
							IVS5+5 G→A (M)	Splice Site	Loss of Exon 5
2	**H249/3**	Germany	Germany	4	Caucasian	Compound Heterozygote	c. C634T (DN)	Nonsense	p. R190X
							c. 849-852 delCCT (M)	in-frame deletion	p. ΔS262
3	**H155/03**	Japan	Japan	25	Chinese	Recessive	c. G136T (H)	Nonsense	p. E24X
3*	**H155/04**	Japan	Japan	24	Chinese	Recessive	c. G136T (H)	Nonsense	p. E24X
4	**H250/03**	New Delhi	India	6	Indian	Recessive	Δex1-8 (H)	Large Deletion	Null
5	**H261/03**	Germany	Germany	3	Turkish	Recessive	c. C572T (H)	Missense	p. P169L
6	**H289/03**	India	India	4	Indian	Recessive	c. 8_9 dup(TT), 8_9 ins(A) (H)	Frameshift	p. F-19I (fs3X)

7	**H138/03**	UK	UK	10		Recessive	IVS5+5 (H)	Splice Site	Loss of Exon 5
8	**H291/03**	India	India	3	Indian	Recessive	IVS5+5 (H)	Splice Site	Loss of Exon 5
9	**H313/03**	India	India	15	Indian	Recessive	IVS5+5 (H)	Splice Site	Loss of Exon 5
10	**H286/03**	India	India	7	Indian	Recessive	IVS5+5 (H)	Splice Site	Loss of Exon 5
11	**H272/03**	Jordan	Jordan	11	Arabic	Recessive	c. G1415A (H)	Nonsense	p. R450X
12	**H324/03**	UK	UK	9 months	Caucasian	Heterozygote Dominant??	c. A1475C (D?)	Missense	p. Y470C

Case	Onset	Stiff	Startle	Triggered	Falls	Neonatal apnoeas	Response to Clonazepam	Epilepsy	Squint	MR Brain	Improvement over time	Learning Difficulties	Motor milestones	Speech
1#	3 hours after birth	+	+	+	+	No	+	No	NR	NR	Yes - startles to tactile stimuli at age six	No	No delay	No speech at 22mo
2	In utero	+	+	+	-	Yes	++	No	No	Normal	Yes	Severe learning difficulties - secondary to language delay. Bayley scale 42	Walked at 19 months	Mild speech delay

3	18 hours after birth	+	+	+	-	Yes	+*	No	No, but ocular apraxia	NR	Yes - startles to tactile stimuli at age seven	Severe learning difficulties	Walked at 2 years	One word at a year, severe speech delay
3*	Just after birth	+	+	+	-	Yes	++	No	No, but ocular apraxia	NR	Yes - startles to tactile stimuli at age six	Severe learning difficulties	Walked at 18 months	Severe speech delay
4	First week of life	+	+	+	+	Yes	+	No	Yes	Normal	Yes. Startles to tactile stimuli; falls prominent.	Mild learning difficulties	Walked at 2 years	Delayed with subsequent catch up: Sentences only at 4 years
5	In utero	+	+	+	NR	Yes	+	No	No	Normal	Yes - discontinuation of clonazepm at 1 year lead to tonic attack and apnoea recurrence	Severe learning difficulties, Bayley Scales 52	Walked at 18 months	Speech delay, 2 word sentence at 3 years
6	In utero	+	+	+	+	Yes	+	No	No	CT normal	Yes. Reduction in startle, spastic gait	Mild learning difficulties	Mild motor delay	Speech delay
7	NR	+	NR	NR	+	NR	+	NR	Yes	NR	NR	Yes	NR	Speech delay
8	First week of life	+	+	+	-	NR	+	Yes	No	Normal	Yes. Tactile stimuli still trigger startles and falls	No	Delayed	No problems
9	First week of life	+	+	+	+	NR	+	Yes	No	Normal	Yes. Startle still present	Mild learning difficulties	Delayed	Speech delay

10	First week of life	+	+	+	+	NR	+	No	No	Normal	Yes. Startle still present, mild stiffness	Mild learning difficulties	Delayed	Speech delay
11	At birth	+	+	+	+	Yes	+	No	No	Normal	At 2.5 years still needs clonazepam and phenobarbital; discontinuation lead to tonic attacks and apnoea recurrence	Yes	Walked at 2 years	Speech delay
12	In utero	+	+	+	+	Yes	+	No	NR	Normal	NR	NR	NR	Maternal concerns

Gene Negative

Case number	Normal EEG	Normal MRI	Stiff	Startle	Nosetap	Falls	Apnoeas	Developmental Delay	Dystonia	Dysmorphism	Seizures	Progression
1	Yes	Yes	+	+	+	NR						Onset at 20 days
2	Yes	NR	+	+	+	NR		+				Onset as an infant. Mild - did not need clonazepam
3	NR	Yes	Normal	+	+	NR					+	Onset at 2 days
4	Yes	Yes	+	+	+	NR					+/-	
5	Yes	Yes	+	+	+	+		+				Onset at birth. Clonazepam helpful
6	Yes	Yes	+	+	NR	NR		+				Onset at birth. Clonazepam helpful

7	Yes	Yes	+	+	+	NR	+					Onset at birth, normalised at 2 months
8	Yes	Yes	NR	+	+	NR	+				+	
9	Yes	NR	Normal	+	NR	+						Falls since childhood
10	Yes	Yes	+	+	+	NR	+		+		+	
11	Burst suppression - no clear seizures	Yes	+	+	+	NR						Continuous coarse tremor
12	Yes	Yes	NR	+	NR	NR	+			+		
13	GTCS	Yes	+	+	+	NR		+			+	
14	Yes	Large cisterna magna	NR	+	+	NR	+	+	+			Onset at birth
15	Yes	NR	+	NR	NR	NR		+		+		
16	NR	Yes	+	+	+	NR	+					Onset at birth
17	Yes	Yes	+	+	NR	+		+				
18	Yes	Yes	+	+	NR	NR						Stiffness noticed at four months
19	Two foci in left hemisphere, no paroxysomal activity	Yes	+	+	+	NR		+	+		+	Startle attacks from 8 months
20	Yes	Yes	+	NR	NR	NR						
21	Yes	Yes	+	+	NR	NR			+			
22	NR	NR	NR	+	NR	+						
23	Yes	NR	NR	NR	+	+					+	
24	Yes	NR	NR	+	NR	+		+				Onset from early childhood

25	NR	NR	+	+	+	NR						Onset at birth
26	NR	NR	+	+	+	NR						Onset at birth
27	4-5Hz bitemporal	Yes	NR	+	NR	+		+	+		+	Onset at four years
28	Yes	Yes	NR	+	+	NR						
29	NR	NR	+	NR	NR	NR		+				
30	NR	NR	+	+	+	NR		+				Onset as a baby
31	Yes	Yes	+	NR	NR	NR						Onset as a neonate
32	Generalised slowing during apnoea attack	Yes	+	+	+	NR	+		+	+		
33	NR	NR	+	+	+	+					+	
34	Eeg (day 10)- mild excess of sharp waves	Yes	+	+	NR	NR	+					
35	GTCS	Yes	NR	+	NR	NR					+	Adult onset

Appendix H

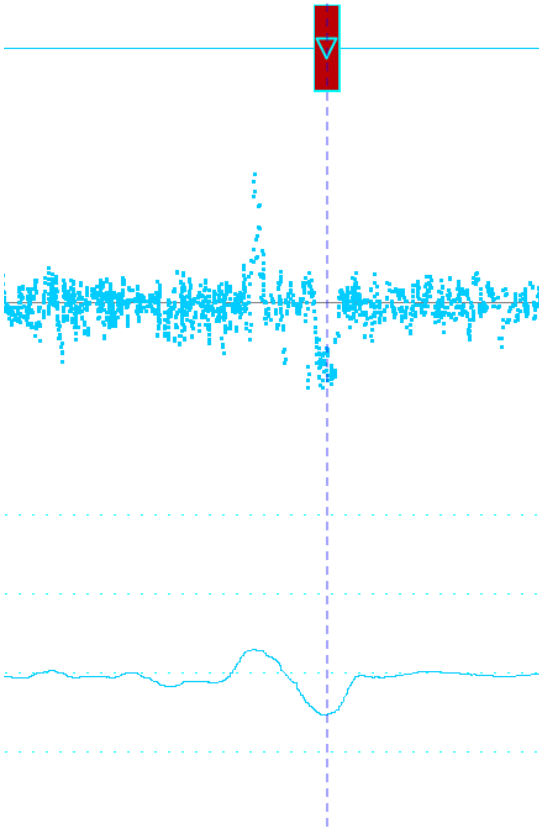
Copy number variants – near epilepsy regions

CNVs which are near published epilepsy CNVs but not overlapping

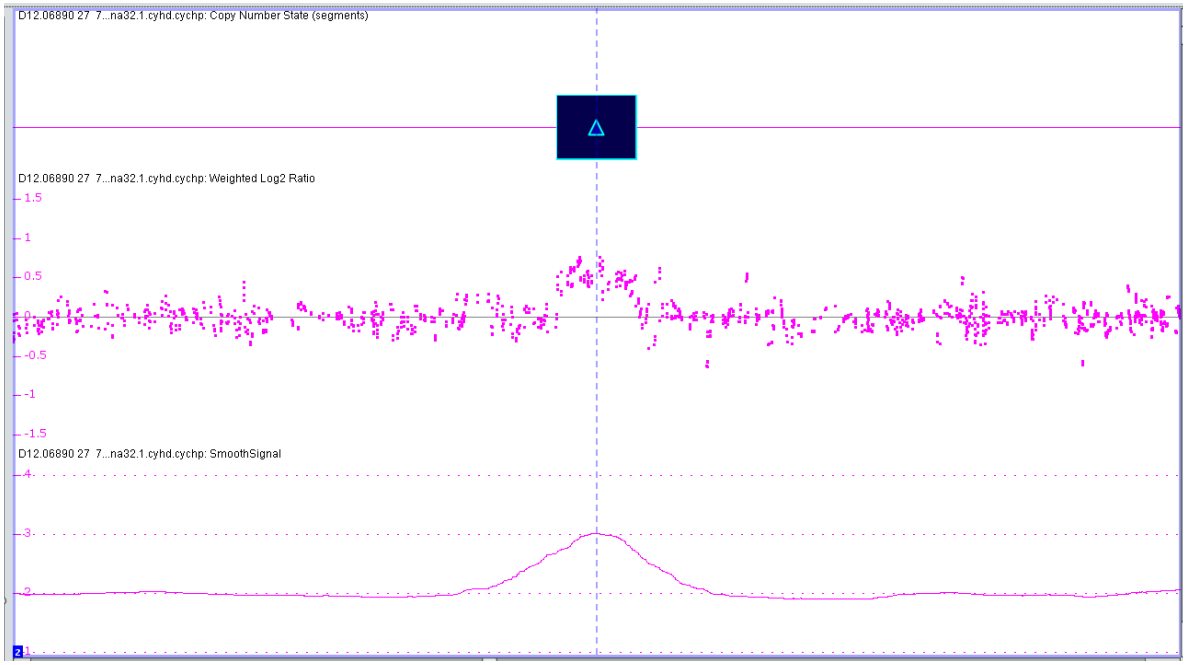
1p31.1 deletions

Although 1p31.1 deletions have been reported in association with epilepsy – these deletions (1: 73012022-73098114) do not overlap the area 111.3kbp between 72.04 and 72.15. Cases 11 and 22 have the 1p31.1 deletion – but it appears in a gene poor area. One of the nearest genes however is –*NEGR1* (the neuronal growth regulator 1). Deletions of *NEGR1* have previously been reported in association with CAE (Mefford et al. 2010).

This CNV (86,092bp) has eight overlaps in DECIPHER, none in the WMRGL, four deletions in the EU control dataset and seven deletions in DGV. It appears to represent a rare benign CNV.



The cytoband 2q11.2 has an epilepsy associated CNV. The epilepsy CNV was at 2: 96143158 -97387872. Case 27 has a duplication at 2: 99841949-99924411 of 82,462bp; which does not overlap the previously described CNV. Case 27 has a frequently occurring CNV – but when it goes occur it increases the copy number to three. It is seen once in WMRGL dataset, once in the EU dataset and seven times in DGV: all are duplications. In this case there would be duplication of the whole of both - *LYG2* and *LYG1.* These genes encode for lysozyme G-like proteins 2 and 1.



16p11.2-p11.1

There is a known epilepsy associated CNV syndrome –at 16p11.2. However this microduplication syndrome (16: 29606852-30199855) does not directly overlap the seven cases presented. Of our cases one is a deletion (case 28) and there are six duplications that overlap. In total there are 4 entries in the DECIPHER corresponding to this area.

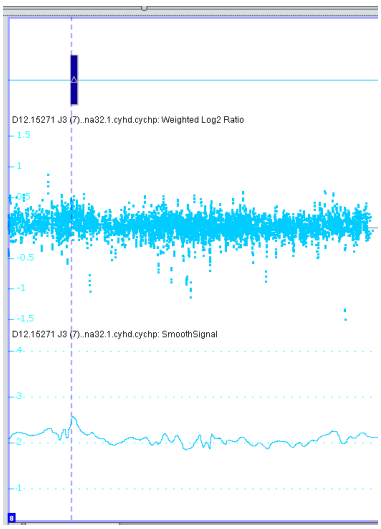
This 16p11.2-p11.2 region is relatively poorly covered with probes (marker distances of over 1672 and up to 5935 bases, but with a good number of markers within the CNV (63 to 176).

The largest CNV (case 13) has overlap with ten cases from the WMRGL, and ten duplications and nine deletions in the EU control database. There are a further 11 deletions in DGV and 25 duplications. This makes this CNV at 16p11.2-11.1 likely to be recurrent and probably benign. The genes within the CNV include protein coding genes with no known function (such as *TP52TG3B*) and pseudogenes such as *SLC6A10P*.

Case		Min	Max	Size	Dist	Mrk	Genes
28	L	32538258	32906257	367.999	5935	63	TP53TG3B, TP53TG3, SLC6A10P
13	G	32564621	33789190	1224.569	7559	163	TP53TG3B, TP53TG3, SLC6A10P, LOC390705
30	G	34449594	34755816	306.222	1749	176	LOC283914, LOC146481, LOC100130700
5	G	34449594	34755816	306.222	1749	176	LOC283914, LOC146481, LOC100130700
20	G	34449594	34755816	306.222	1749	176	LOC283914, LOC146481, LOC100130700
34	G	34466474	34755816	289.342	1672	174	LOC283914, LOC146481, LOC100130700
18	G	34466474	34755816	289.342	1672	174	LOC283914, LOC146481, LOC100130700

8p23.3 – duplication

The 8p23.3 duplication is seen is case 3 at 8: 1701168-1820583. This 119,415bp CNV was reported five times in WMRGL database of cases but never at this size in EU control dataset. The DGV database of controls reports one gain and two losses (both partially overlapping) and the gain avoids *CLN8* entirely. The weighted log2 ratio is not particularly convincing, the smoothsignal perhaps more so. This potential CNV is covered by 140 markers at a mean distance of 859bases. It covers three genes directly *CLN8, MIR596* and *ARHGEF10*. CLN8 has a haplotype index of 68% and *ARHGEF10* of 44%.



Gene	Official Full Name	NCBI description
CLN8	Ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)	Mutations in this gene are associated with progressive epilepsy with mental retardation (EMPR), which is a subtype of neuronal ceroid lipofuscinoses (NCL). Patients with mutations in this gene have altered levels of sphingolipid and phospholipids in the brain.
MIR596	microRNA 596	
ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	Rho GTPases play a fundamental role in numerous cellular processes that are initiated by extracellular stimuli that work through G protein coupled receptors. The encoded protein may form complex with G proteins and stimulate Rho-dependent signals.

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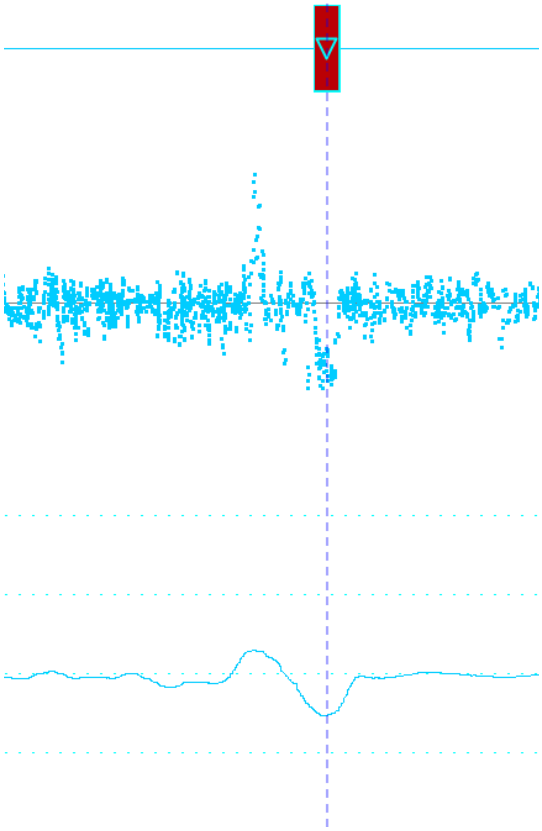

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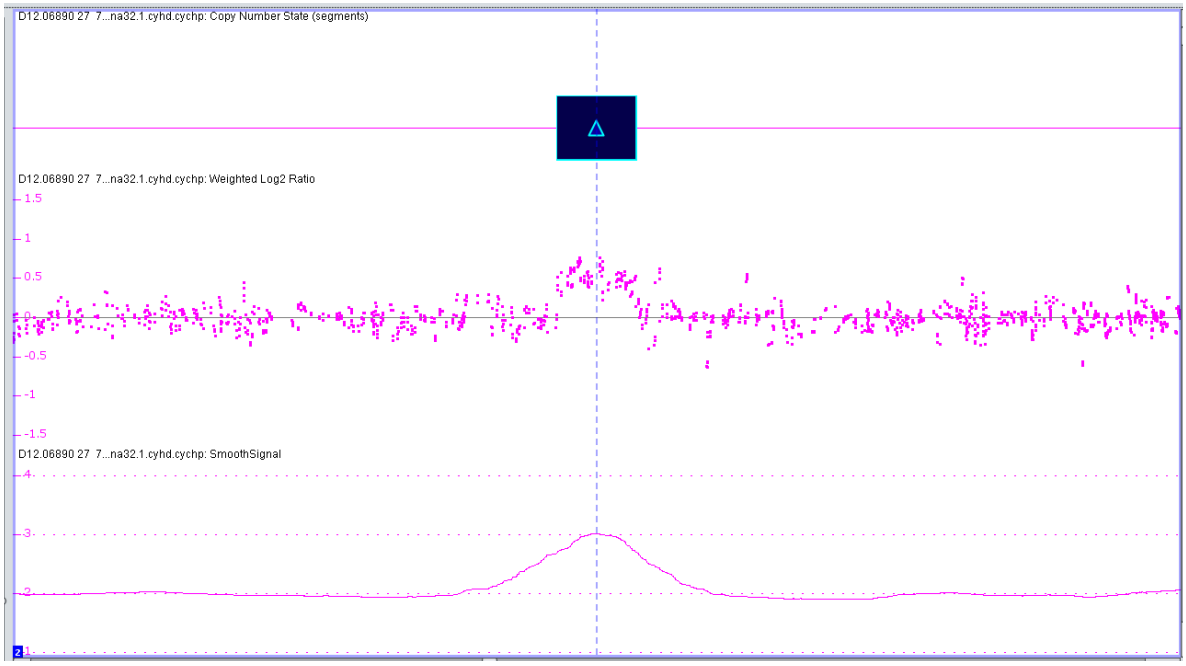


16p11.2-p11.1

There is a known epilepsy associated CNV syndrome –at 16p11.2. However this microduplication syndrome (16: 29606852-30199855) does not directly overlap the seven cases presented. Of our cases one is a deletion (case 28) and there are six duplications that overlap. In total there are 4 entries in the DECIPHER corresponding to this area.

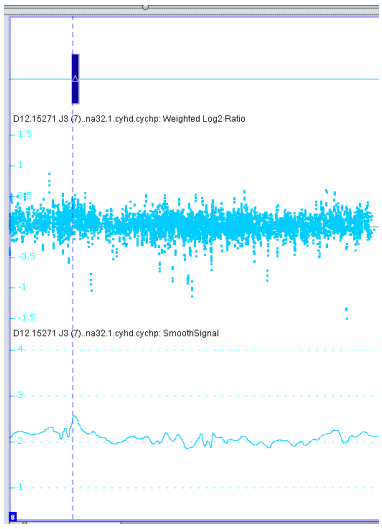
This 16p11.2-p11.2 region is relatively poorly covered with probes (marker distances of over 1672 and up to 5935 bases, but with a good number of markers within the CNV (63 to 176). The largest CNV (case 13) has overlap with ten cases from the WMRGL, and ten duplications and nine deletions in the EU control database. There are a further 11 deletions in DGV and 25 duplications. This makes

this CNV at 16p11.2-11.1 likely to be recurrent and probably benign. The genes within the CNV include protein coding genes with no known function (such as *TP52TG3B*) and pseudogenes such as *SLC6A10P*.

Case		Min	Max	Size	Dist	Mrk	Genes
28	L	32538258	32906257	367.999	5935	63	TP53TG3B, TP53TG3, SLC6A10P
13	G	32564621	33789190	1224.569	7559	163	TP53TG3B, TP53TG3, SLC6A10P, LOC390705
30	G	34449594	34755816	306.222	1749	176	LOC283914, LOC146481, LOC100130700
5	G	34449594	34755816	306.222	1749	176	LOC283914, LOC146481, LOC100130700
20	G	34449594	34755816	306.222	1749	176	LOC283914, LOC146481, LOC100130700
34	G	34466474	34755816	289.342	1672	174	LOC283914, LOC146481, LOC100130700
18	G	34466474	34755816	289.342	1672	174	LOC283914, LOC146481, LOC100130700

8p23.3 – duplication

The 8p23.3 duplication is seen is case 3 at 8: 1701168-1820583. This 119,415bp CNV was reported five times in WMRGL database of cases but never at this size in EU control dataset. The DGV database of controls reports one gain and two losses (both partially overlapping) and the gain avoids *CLN8* entirely. The weighted log2 ratio is not particularly convincing, the smoothsignal perhaps more so. This potential CNV is covered by 140 markers at a mean distance of 859bases. It covers three genes directly *CLN8, MIR596* and *ARHGEF10*. CLN8 has a haplotype index of 68% and *ARHGEF10* of 44%.



Gene	Official Full Name	NCBI description
CLN8	Ceroid-lipofuscinosis, neuronal 8 (epilepsy, progressive with mental retardation)	Mutations in this gene are associated with progressive epilepsy with mental retardation (EMPR), which is a subtype of neuronal ceroid lipofuscinoses (NCL). Patients with mutations in this gene have altered levels of sphingolipid and phospholipids in the brain.
MIR596	microRNA 596	
ARHGEF10	Rho guanine nucleotide exchange factor (GEF) 10	Rho GTPases play a fundamental role in numerous cellular processes that are initiated by extracellular stimuli that work through G protein coupled receptors. The encoded protein may form complex with G proteins and stimulate Rho-dependent signals.

Appendix I

Copy number variants – benign and recurrent

CNVs which appear genuine and are recurrent

What is the importance of the Xp22.33 duplications for S12?

Two separate duplications were called as pathogenic on Xp22.33 in S12; the first taking the copy number to three for 315,189bp (X: 206554-521743), the second doing the same between X:534705-553662 for 18,957kb. However by eye – you would suggest they were the same CNV. Of note is the possibility of a microdeletion in a marker sparse area nearby X:17607-32362 (14,755bp). However this potential microdeletion was seen in 9 of the 24 samples making it more likely to be artefactual.

DECIPHER lists an Xp22.33 deletion syndrome between X: 460558 and 753877 (Leri-Weill dyschondrostosis (LWD) - *SHOX* deletion). At its mildest this causes short stature – at its most severe limb deformities. These duplications involve both *SHOX* (short stature homeobox) and *PPP2R3B* (protein phosphatase 2, regulatory subunit B'', beta) genes. This duplication is seen 17 times in the DECIPHER database, although no similar CNVs were seen in DGV. *SHOX* duplications have been implicated in cases of Müllerian aplasia (irrelevant here as the case is male) (Sandbacka *at el.* 2011), Leri-Weill dyschondrosteosis (Benito-Sanz *et al.* 2011) and type I Mayer-Rokitansky-Kuster-Hauser syndrome (Gervasini *et al.* 2010). Our case had none of these features and it is tempting to suggest that it is possible to make false correlations between rare disorders and CNV pathogenicity without utilising an adequate control sample.

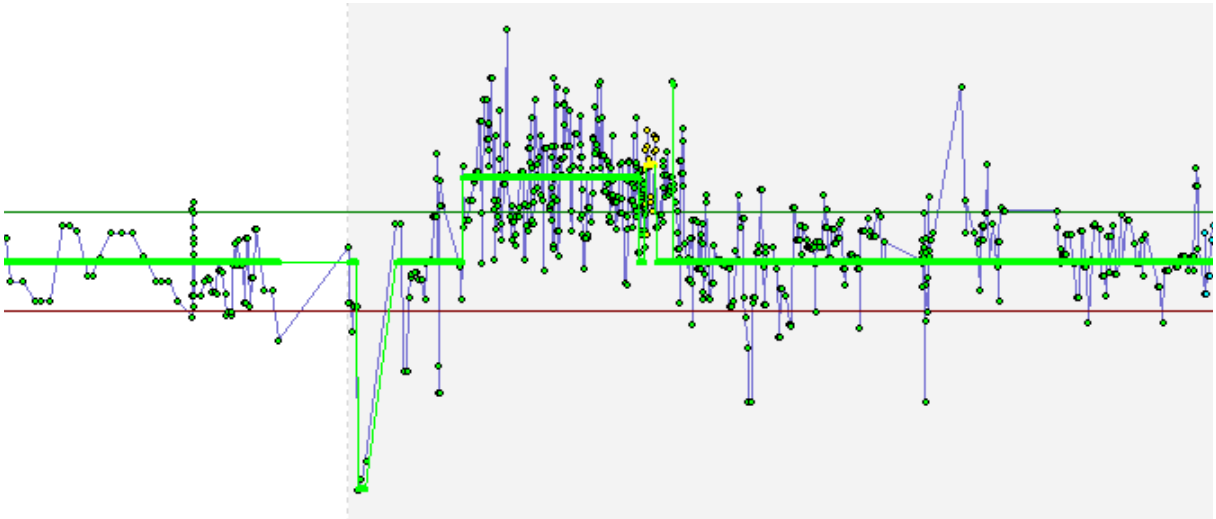


Figure Weighted log2 ratio for CNVs at Xp22

What are the implications of 22q11.22 duplication?

As opposed to 22q11.22 deletion (described above) – 22q11.22 duplication is less clearly pathogenic. Two unrelated individuals had 22q11.22 duplications of 313kbp and 196kbp (F9 and F7) which may be asymptomatic; particularly because case F7 does not have epilepsy. 22q11 duplication syndrome is not a clinically recognisable disorder; it is diagnosed following molecular genetic analysis. The most frequent reported symptoms in probands with duplication of 22q11.2 duplication syndrome (according to the DECIPHER database) are mental retardation/learning disability (97%), delayed psychomotor development (67%), growth retardation (63%) and muscular hypotonia (43%). However, these are common and relatively non-specific indications for cytogenetic analysis, and the extent to which duplication of 22q11.2 is causal for these features is currently unknown. The majority of 22q11 duplications are inherited often from an unaffected parent. This is in sharp contrast to the 22q11 deletion syndrome where approximately 90% of cases are *de novo*.

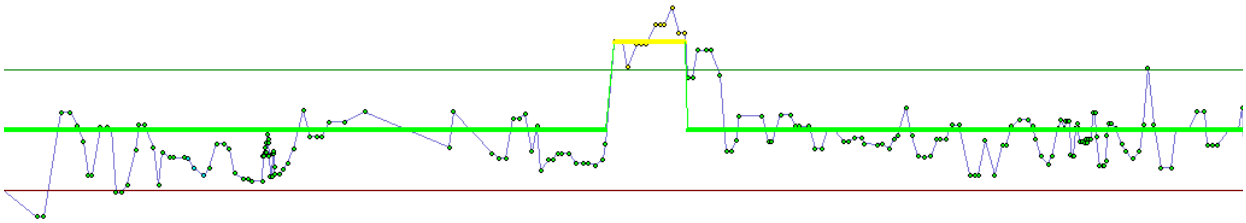


Figure Weighted log2 ratio demonstrating 22q11.22 duplication in F7

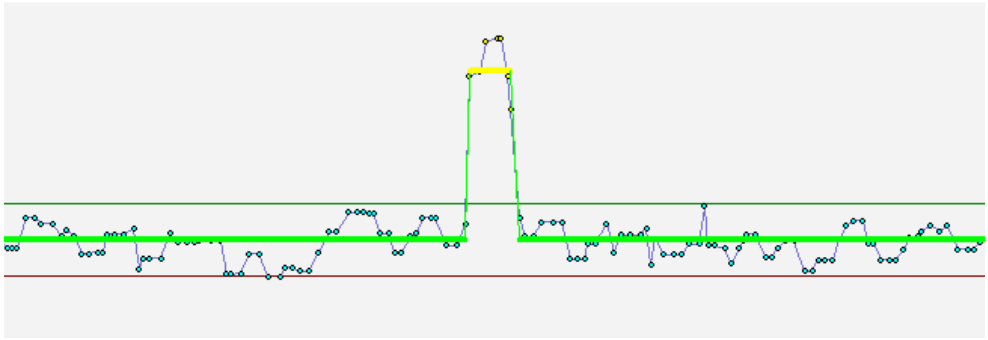
In F7's 22q11.22 duplication at (22:20591480-20788440) there are four genes in this area (table below). The controvertial 22q11 duplication syndrome – is described as CNV between 19009792 and 21452445; so this CNV sits within it. In contrast the 22q11.22 duplication seen in F9 is at 22: 20591480-20904791and is 313,311 bp long, containing two more genes than the above duplication. This is a common area of variation – there are 97 overlapping CNVs in DGV. The area is close by but separate from the velocardiofacial / DiGeorge syndrome locus.

Gene	Official Full Name	NCBI description
ZNF74	Zinc finger protein 74	
SCARF4	Scavenger receptor class F, member 2	Mediates the binding and degradation of acetylated low density lipoprotein
KLHL22	Kelch-like 22	
MED15	Mediator complex subunit 15	Subunit of the multiprotein complexes PC2 and ARC/DRIP and may function as a transcriptional coactivator in RNA polymerase II transcription.

Table Genes in the 22q11.22 duplications

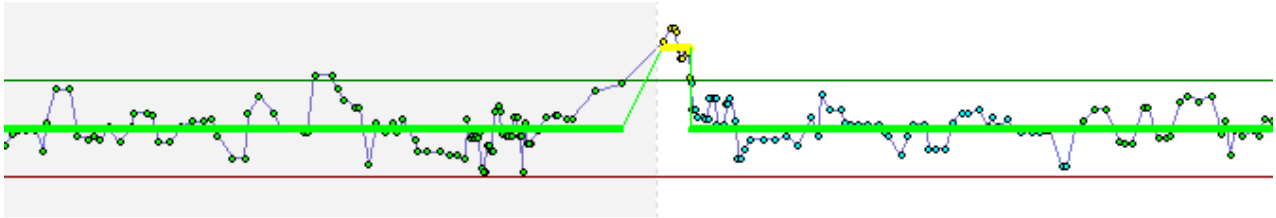
3q26.1 duplication (cases S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, F4, F9, F10)

The 3q26.1 variant was autocalled as a duplication in 14 of 24 cases analysed (including two cases S3 and 4 who were not thought to have epilepsy. Manual inspection suggests it is convincing (below). It is an area of little variation – it does not appear in DGV, nor the WMRGL nor EU datasets and only four CNVs overlap it in DECIPHER. This is in striking contrast to the frequency that it appears in this cohort. These cases are ethnically and geographically diverse: S1, S2, S7, S8, S10, S11, F4, F9 (white Caucasian, South Wales) S3 – (white Caucasian – Melbourne, Australasia) S4 (Pakistani) S5 (white Caucasian, North of England) S6, S9 (white Caucasian, midlands), F10 (white Caucasian, South West of England). It could represent a rare CNV seen most frequently in South Wales. The area has no protein coding genes within it and no genes associated with epilepsy in its proximity.



4p16.3 duplication (case F3)

This 52kbp duplication – seen in a single individual but not in her sister (F2) nor nephew (F1) involves two zinc finger genes: *ZNF595, ZNF718.* Nine DECIPHER CNVs overlap and seven similar CNVs are seen in DGV. This is a very distal CNV and could not be mapped by CytoScan HD (no probes for the area).



5p15.33 deletion and duplication

As the ten CNVs show below the duplications were a little longer and included an additional gene. *TPPP* is interesting as it's a tubulin polymerization promoting protein. *ZDHHC11* is a DHHC-type containing 11 zinc finger gene. There is not a great deal known about the DHHC type zinc finger family. There are 20 overlapping entries in DGV, and 13 in DECIPHER. Of those 13, four had phenotype information on DECIPHER and one of these cases included seizures. This CNV was a duplication at 5: 204849- 6753953 - 6,549,104bp in length and so therefore a much greater CNV, containing 34 genes.

Intriguingly the important area for Cri du Chat syndrome (the very start of Chromosome 5 up until 10001bp) overlaps these CNV partially. Outside the CNV described but nearby are genes such as *SLC6A19* which is associated with Hartnup disorder – where seizures are a recognised feature alongside dermatological, nephrological and gastrointestinal symptoms. The epilepsy associated duplication was described as 5: 0.72–1.43, so all of these CNVs sit within the reported CNV. ZDHHC11 has a haplotype index of 88% and TPP of 31% and so they are very unlikely to be directly pathogenic due to haploinsufficiency.

5p15.33	Case	Min	Max	Size kbp	Genes
Deletion	S1	786482	848774	62.292	ZDHHC11
	S4	786482	874909	88.427	ZDHHC11
	S5	786482	848774	62.292	ZDHHC11
	S7	786482	848774	62.292	ZDHHC11
	S10	763524	848774	85.250	ZDHHC11

	S12	786482	874909	88.427	ZDHHC11
	F12	812199	874909	62.710	ZDHHC11
Duplication	F8	739315	859205	119.890	TPPP, ZDHHC11
	F10	739315	848774	109.459	TPPP, ZDHHC11
	F11	745881	848774	102.893	TPPP, ZDHHC11

Table xx Clustering of CNVs in our sample spanning phenotypes and ethnicities

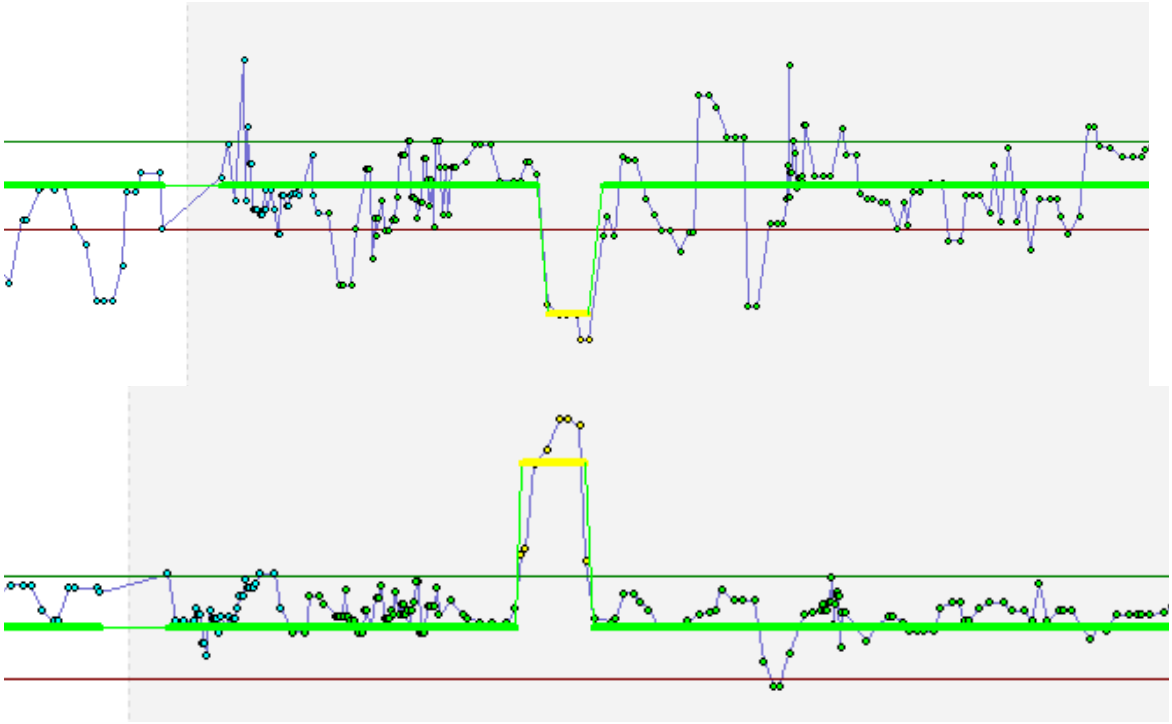
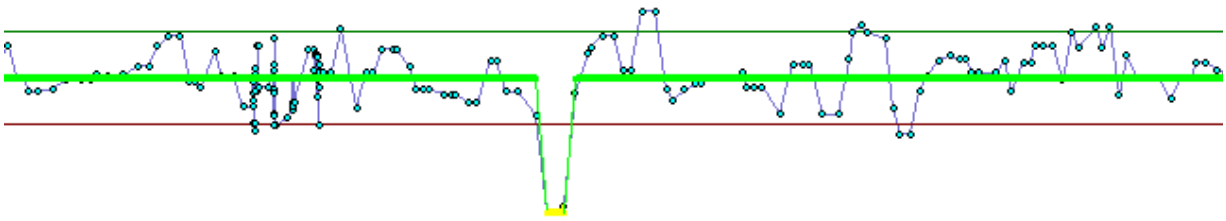


Figure Weighted Log2 ratios of F12 (top) and F8 (below) demonstrating the 5p15.33 CNVS

Six deletions at 6p21.32



Case	Min	Max	Size kbp

S6	32558700	32586161	27.461
S7	32565191	32605309	40.119
F1	32558700	32629878	71.178
F6	32558700	32629878	71.178
F9	32565191	32703025	137.834
F10	32558700	32672786	1140.86

This reduces two major histocompatibility complex genes to heterozygosity – DQ beta 1 (*HLA-DQB1*) and DQ alpha 1 (*HLA-DQA1*). The former of these has been reported to convey susceptibility to multiple sclerosis and coeliac disease and resistance from variant Creutzfeldt-Jakob disease; the latter again increases the likelihood to coeliac disease. Both of these two genes have robust haplotype indices (above 70%) and it is well recognised that there is considerable variation tolerated within the HLA genes.

However nearby there are some epilepsy associated genes: *GABBR1* (gamma-aminobutyric acid (GABA) B receptor 1) is at 6: 29570005- 29600962; *GRM4* (glutamate receptor, metabotropic 4) is at 6: 33989623- 34123399; and intriguingly *BRD2* (bromodomain containing 2) is nearer still at 6: 32936437 -32949282. *BRD2* has been reported to be implicated with JME. S6, S7 and F6 have a GGE, F1 is not thought to have epilepsy, F9 has a cryptogenic focal epilepsy and F10 unclassified.

Perhaps the suggestion that this 6p21 deletion may be having an effect at BRD2 is tenuous- it is over 250,000bp away. And as the figure below shows – it is in an area of frequently reported variation

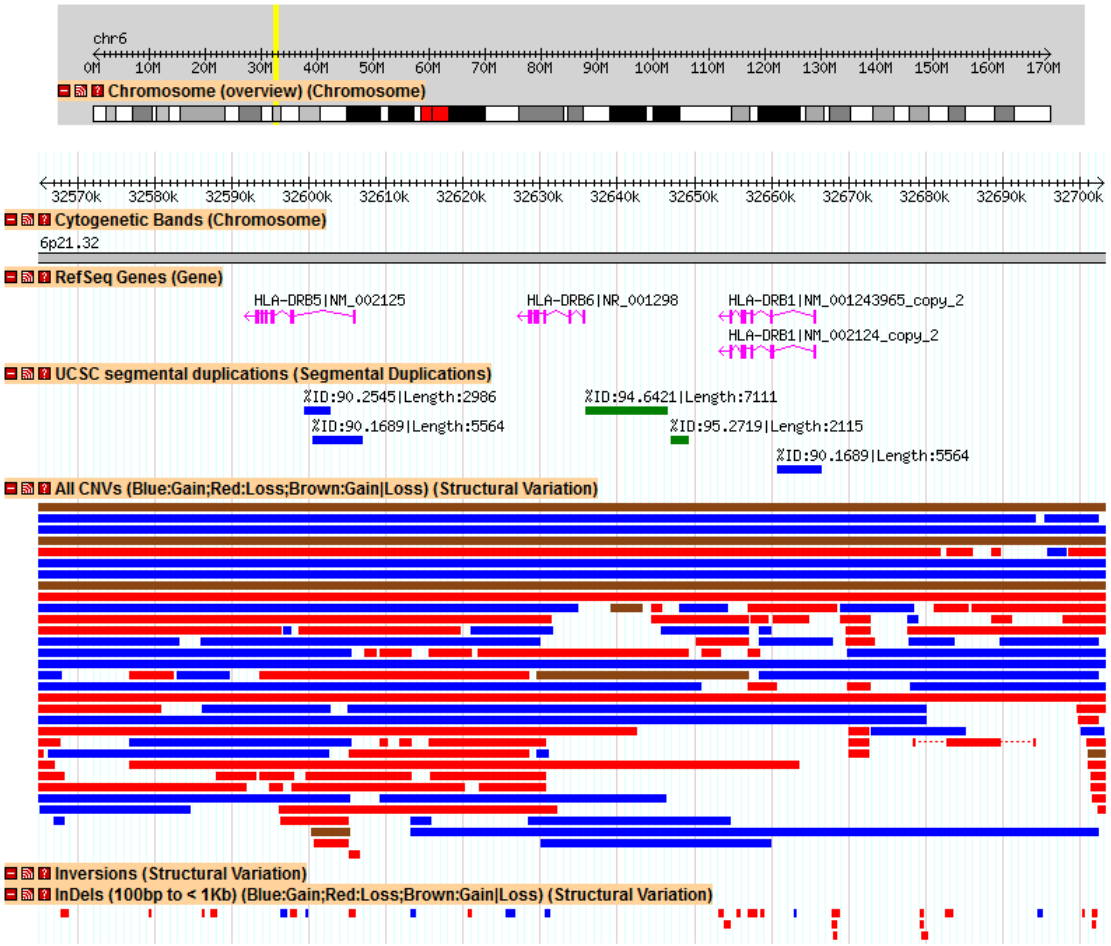
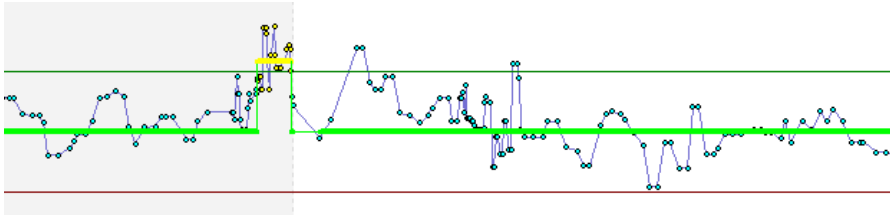


Figure Screen capture from DGV demonstrating the frequently reporte variation in the area (red and blue bars represent reported experiments with CNVs here).

7q36.3 duplication

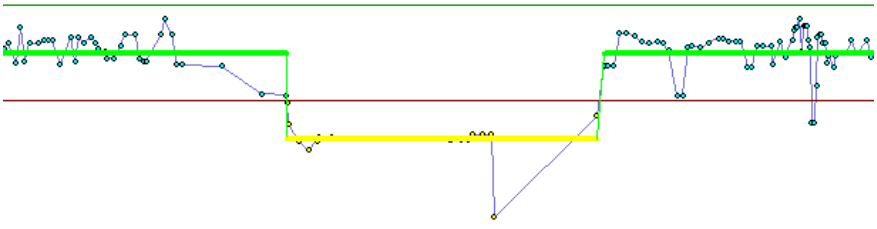
This duplication is in a gene poor area – 7:158736443-158816863 and of a modest size - 80,420bp. The epilepsy associated CNV does not overlap but is nearby at 7: 156123654-158388155. This CNV at the distal end of chromosome 7. Only two CNVs overlap in DGV - but eighteen do in DECIPHER.



This CNV again was seen as a duplication in a single case (S4) and as a deletion in another (F8). The location is 8:7230389-8117301 produces a large deletion - 886,912 bp containing many genes, not all of them protein coding*: DEFB103A, SPAG11B, DEFB104B, DEFB106B, DEFB105B, DEFB107B, FAM90A8, FAM90A17, FAM90A16, FAM90A18, FAM90A19, FAM90A7, FAM90A10, FAM90A22, FAM90A8, FAM90A17, FAM90A9, FAM90A16, FAM90A18, FAM90A19, FAM90A23, FAM90A2P, OR7E154P, FAM90A15, FAM90A14, FAM90A13, DEFB107A, DEFB105A, DEFB106A, DEFB104A, SPAG11A, DEFB103B, DEFB4, DEFB109P1, FAM90A11, FAM90A24P, FAM90A12* and *MIRN548I3.* There are no OMIM diseases associated with these and a great deal of recognised variation in DGV: 31 overlapping areas; and DECIPHER - a further 30 areas.

The duplication is at 8: 7,156,930-8,117,301 and is a little larger at 960,371bp; however the epilepsy associated CNV does not overlap (8: 10.19–10.37) and was 173.1kbp. A further three pseudogenes are involved with this CNV: *DEFB109P1, DEFB108P1* and *DEFB108P2.* DGV recognises a great degree of variation here with 95 overlapping CNVs described.

There is a 8p23.1 deletion syndrome (8: 8100055-11764629) which only just fails to overlap with this CNV. The genes starting DEFB (numbering 12 in total) are defensin genes –coding for host defence peptides. The genes starting FAM90A are a primate-specific gene family, orginating from multiple duplications and rearrangements; 21 members are listed above some of which are actually considered to be pseudogenes. The genes not covered by these families are in the box below. There are no genes of interest to epilepsy in flanking regions.



Gene	Official Full Name	NCBI description
SPAG11B	Sperm associated antigen 11B	This gene encodes several androgen-dependent, epididymis-specific secretory proteins

SPAG11A	Sperm associated antigen 11A	
MIRN548I3	microRNA 548i-3	Non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.
OR7E154P	Olfactory receptor, family 7, subfamily E, member 154 pseudogene	Pseudogene

14q11.12 deletion

14q11.1-q11.2

There were ten CNVs at the 14q11.1 cytoband, seven of which were deletions (table below). The CNVs ranged from a 59kbp deletion to 1029kbp. The majority of CNVs overlapped the same area – with just F11 (14: 22589160-22648367) being distinct, reflected in the genes involved too. All genes listed below that start 'OR' are olfactory receptor family members; there are only four that do not belong to one of these families (table below).

The area covered by most of the CNVs here is pericentromeric on chromosome 14 and very little variation is called in DGV that spans this area. DECIPHER does not have a single record for 14q11.1; although eighteen for neighbouring 14q11.2 (where F11 creeps in to and 7 records overlap). Indeed samples J31, J32, J33 all have deletions spanning this area and J3 and J23 have microdeletions within it and there are 14 deletions in DGV. The relevance to this 14q11.2 CNV is discussed below when the JME samples are described.

14q11.1	Case	Min	Max	Size kbp	Genes
Deletion	S9	18798671	19490660	691.989	*OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	S10	19268806	19490660	221.854	*OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*

	S12	18187184	19216393	1029.210	*OR11H13P, OR11H12, POTEG*
	F2	18139146	19490660	1351.514	*OR11H13P, OR11H12, POTEG, OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	F4	19268806	19490660	221.854	*OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	F9	18446792	19490660	1043.868	*OR11H13P, OR11H12, POTEG, OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	F11	22589160	22648367	59.207	*CDH24, ACIN1, C14orf119*
Duplication	S5	18798671	19515811	717.140	*OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1, OR4K15*
	S8	19273319	19467542	194.223	*OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5*
	F12	18544802	19515811	971.009	*POTEG, OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1, OR4K15*

Gene	Official Full Name	NCBI description
POTEG	POTE ankyrin domain family, member G	
CDH24	Cadherin 24, type 2	

ACIN1	Apoptotic chromatin condensation inducer 1	Apoptosis is defined by several morphologic nuclear changes, including chromatin condensation and nuclear fragmentation. This gene encodes a nuclear protein that induces apoptotic chromatin condensation after activation by caspase-3, without inducing DNA fragmentation. It has also been shown to be a component of a splicing-dependent multiprotein exon junction complex that is deposited at splice junctions on mRNAs, as a consequence of pre-mRNA splicing. It may thus be involved in mRNA metabolism associated with splicing.
C14orf119	Chromosome 14 open reading frame 119	

The cytoband 14q11.12 has been associated with epilepsy and deletions of between 42,644 bp and 446,991bp were reported in 14 cases. This particular deletion was seen frequently - 9 more times in the WMRGL file of cases and 27 times in EU file of controls. DECIPHER does not recognise it as a known microdeletion syndrome however – and only 7 cases in that database overlap with these cases. 13 similar deletions are recorded in DGV, and many more microduplications – 164. Each CNV (table below) is covered by a good number of probes (up to 692) with a relatively small mean marker distance (511 to 781bp); it appears to be a convincing CNV by eye too. 14q11.12 probably represents a benign recurrent CNV that has a higher prevalence in the South Wales population. This microdeletion is in a gene poor region. The nearest genes being many kilobases away and do not include any genes directly implicated in epilepsy phenotypes such as *DAD1* (defender against cell death 1) and *SLC7A7* (solute carrier family 7 (amino acid transporter light chain, y+L system), member 7) – found in skin.

Case	Min	Max	Size	Dist	Mark
10	22835777	22878421	42.644	775	56
14	22746791	22944507	197.716	708	280

16	22747746	22941375	193.629	730	266
19	22749288	22854720	105.432	717	148
21	22772662	22959362	186.7	781	240
22	22594506 22676928	22620607 22959362	26.101 282.434	511 643	52 440
23	22641679	22940386	298.707	650	460
24	22562098	22612060	49.962	594	85
24	22730865	22943573	212.708	679	314
28	22827208	22941375	114.167	771	149
31	22599355	22960819	361.464	605	598
32	22496582	22943573	446.991	646	692
33	22560402	22943215	382.813	600	639
34	22747482	22941375	193.893	726	268

14q32.33 deletion

There were eight near identical CNV deletions at 14q32.33 (below). The CNV reported to be associated with epilepsy was at 14: 103375996- 106312055 and so these CNVs fall within this area, however it is a 2936kbp deletion – larger by a factor than in this series. The genes involved with F5 (the largest CNV) here are *IGHVIII-26-1, IGHVII-26-2, IGHV7-27, IGHVII-28-1, IGHV3-29, IGHVII-30-1, IGHV3-30-2, IGHVII-31-1, IGHV3-32, IGHVII-33-1, IGHV3-33-2, IGHV7-34-1, IGHV3-36, IGHV3-37, IGHVIII-38-1, IGHV7-40, IGHVII-40-1, IGHV3-41, IGHV3-42, IGHVII-43-1*. All of these genes are of the immunoglobulin heavy variable family – many of them pseudogenes. Illustrations of 14q32.33 are given in the discussion of comparing cases in chapter seven.

14q32.33	Case	Min	Max	Size kbp
Deletion	S3	105610764	105630119	19.355
	S11	105610764	105630119	19.355
	S12	105977754	106002209	24.455
	F4	105857223	105881301	24.079
	F5	105833402	106002209	168.807
	F6	105857223	106002209	144.986

	F7	105857223	105881301	24.079
	F9	105857223	105881301	24.079

15q11.2 deletion / duplication

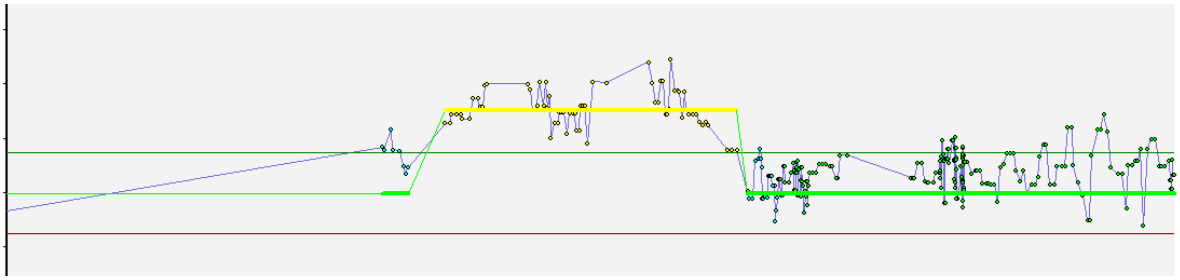
There are nine cases with deletions and eight with duplications from the 24 cases studied. F3 was called as having two duplications adjacent to each other and F8 as having a duplication followed by a deletion in the area. This is a pericentromeric region on chromosome 15 and of note is the fact that the start of 15q up until 23619912bp is the region known to cause Angleman and Prader-Wili Syndromes.

The published epilepsy associated CNVs were deletions at 15:20.2-20.8 of around 600kb, a large 5,356kb deletion between 15q11.2 and 15q13.1 and a larger one still between 15: 19109124-28153416. Some of the CNVs presented here are large – six are over a megabase – and all bar the two smallest directly involve genes. 15q11.2 is discussed again later with reference to the JME cases under 'Recurrent CNVs with genes of interest'.

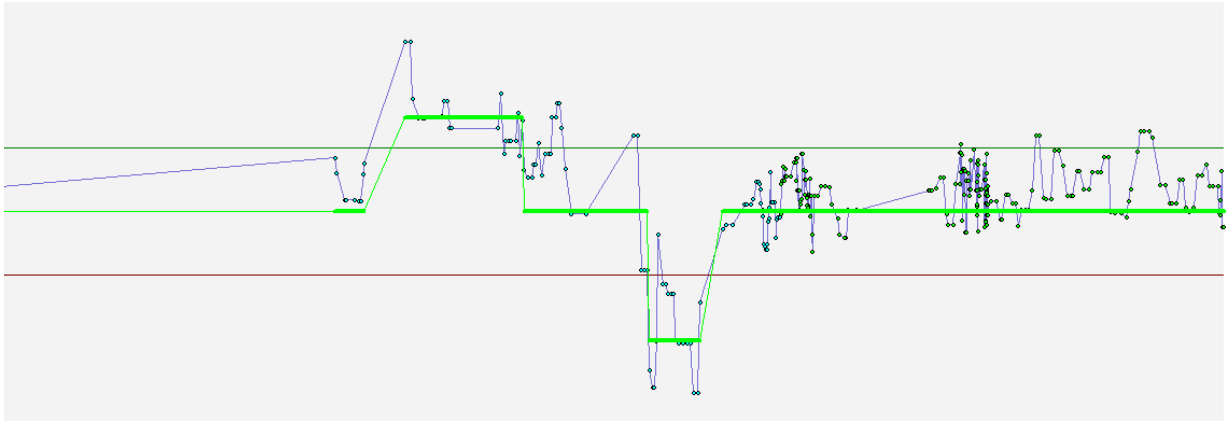
15q11.1	Case	Min	Max	Size kbp	Genes
Deletion	S2	18884466	19786685	902.219	*HERC2P2, HERC2P3, POTEB, POTEC*
	S3	18692895	19833572	1,140.677	*HERC2P2, HERC2P3, POTEB, RPS8P10, OR4N4*
	S7	18722617	20091146	1,368.529	*HERC2P2, HERC2P3, POTEB, , RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	S8	18790619	19833572	1,042.953	*HERC2P2, HERC2P3, POTEB, RPS8P10, OR4N4*
	S12	19171413	19765882	594.469	*POTEB,*
	F4	19418630	19537065	118.435	
	F5	18810028	19786685	976.657	*HERC2P2, HERC2P3, POTEB,*
	F8	19845972	20091146	245.174	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F9	19465389	20091146	625.757	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*

Duplication	S11	18657418	20249916	1,592.498	*HERC2P2, HERC2P3, POTEB, RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F3	18657418	19121787	464.369	*HERC2P2, HERC2P3*
		19765882	20091146	325.264	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F6	18810028	19206809	396.781	*HERC2P2, HERC2P3*
		19338213	20091146	752.933	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F7	18458177	19195328	737.151	*HERC2P2, HERC2P3*
	F8	18657418	19227572	570.154	*HERC2P2, HERC2P3*
	F10	18315236	20091146	1,775.910	*VSIG7, HERC2P2, HERC2P3, POTEB, POTEC, RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F11	21901595	22055583	153.988	
	F12	18657418	20091146	1,433.728	*HERC2P2, HERC2P3, POTEBRPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*

S11



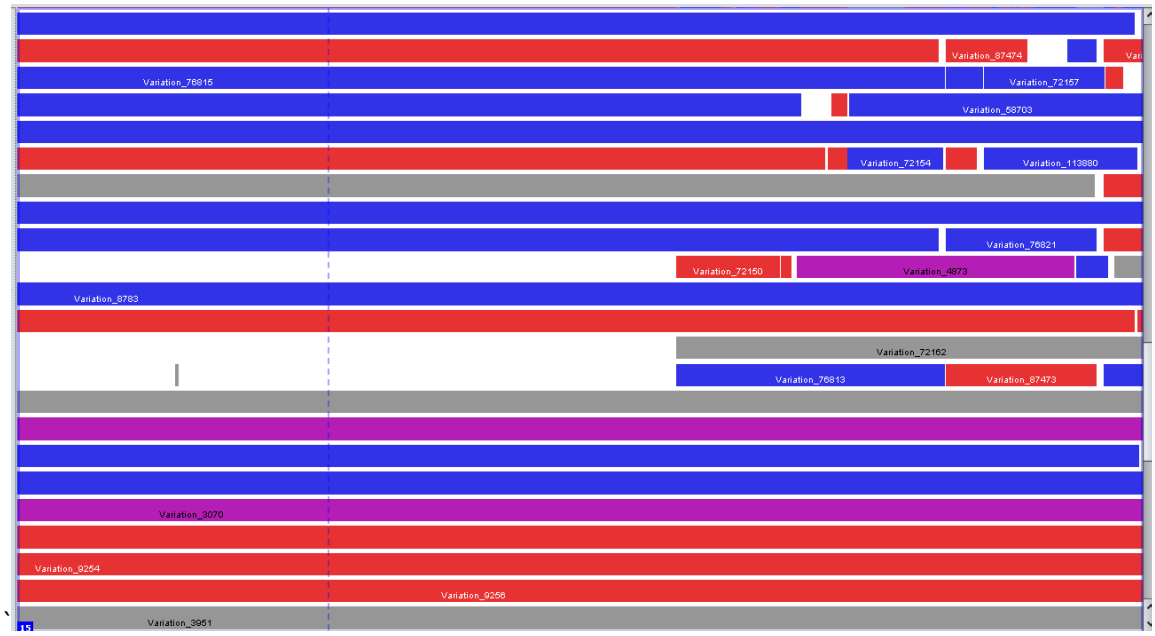
F8



It appears that this CNV coming out the centromere (gene poor and hard to map) is an area of great variation and with few genuine genes of interest (below). The illustration of the number of DGV variants (below) confirms that this is an area with a great number of probably benign CNVs, despite having been linked to epilepsy previously.

Gene	Official Full Name	NCBI description
VSIG7	immunoglobulin heavy variable 1/OR15-9	Pseudogene
HERC2P2	Hect domain and RLD 2 pseudogene 2	Pseudogene
HERC2P3	Hect domain and RLD 2 pseudogene 3	Pseudogene
POTEB	POTE ankyrin domain family, member B	
RPS8P10	Ribosomal protein S8 pseudogene 10	Pseudogene
OR4N4	Olfactory receptor, family 4, subfamily N, member 4	
OR4M2	Olfactory receptor, family 4, subfamily M, member 2	
VSIG6	V-set and immunoglobulin domain containing 6	
MIRN1268	microRNA 1268a	Non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability

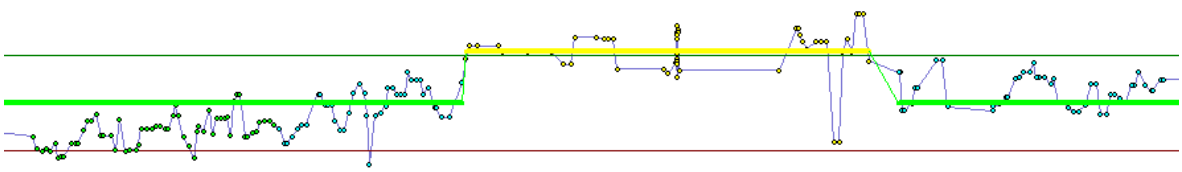
		and translation of mRNAs



16p11.2 deletion/ duplication

The cytoband 16p11.2 is an apparent hot spot for CNVs in this study: there are three deletions reported and eight cases with ten duplications. Some of the CNVs are over a megabase – and most involve *TP53TG3B* (TP53 target 3B). *IGHV2OR16-5* is non-functional and *SLC6A10P* is a pseudogene.

None of these CNVs are within the recognised area for the 16p11.2 microduplication/microdeletion syndrome (16:16-29606852). The area covered by the largest of the CNVs here (F8) has very few overlapping areas reported on DECIPHER (4), but 35 overlapping regions in DGV. Even though the area is marker poor (below) the CNV appears genuine. F8 below.



16p11.2	Case	Min	Max	Size kbp	Genes
Deletion	S2	32809724	33734826	925.102	TP53TG3, TP53TG3
	S5	32378156	32545380	167.224	
	S5	32378156	32545380	167.224	
Duplication	S7	34339573	34584822	245.249	
	S8	34375339	34605060	229.721	
	S9	34375339	34524469	149.130	
	S11	31893658	33734826	1,841.168	TP53TG3, TP53TG3, IGHV2OR16-5, SLC6A10P, TP53TG3, TP53TG3
		34339573	34605060	265.487	
	F8	31958995	33561829	1,602.834	TP53TG3, TP53TG3, IGHV2OR16-5, SLC6A10P, TP53TG3, TP53TG3
	F9	34339573	34605060	265.487	
	F10	31862607	32799600	936.994	TP53TG3, TP53TG3, IGHV2OR16-5, SLC6A10P
	F12	32801349	33680606	879.257	SLC6A10P, TP53TG3, TP53TG3
		34339573	34559455	219.882	

17q25.1 duplication – a rare and large CNV of uncertain significance

S3 is only case to have a large (861,201bp) duplication at 17: 69344027-70205228. This stretch contains 16 genes: *RPL38, TTYH2, DNAI2, KIF19, BTBD17, GPR142, GPRC5C, CD300A, CD300LB, CD300C, CD300LD, C17orf77, CD300E, RAB37* and *CD300LF*.

Gene	Official Full Name	NCBI description
RPL38	Ribosomal protein L38	This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs

		to the L38E family of ribosomal proteins. It is located in the cytoplasm.
TTYH2	Tweety homolog 2	Members of this family function as chloride anion channels. The encoded protein functions as a calcium(2+)-activated large conductance chloride(-) channel, and may play a role in kidney tumorigenesis.
DNAI2	Dynein, axonemal, intermediate chain 2	Mutations in this gene are associated with primary ciliary dyskinesia type 9
KIF19	Kinesin family member 19	
BTBD17	BTB (POZ) domain containing 17	
GPR142	G protein-coupled receptor 142	
GPRC5C	G protein-coupled receptor, family C, group 5, member C	Members of this superfamily are characterized by a signature 7-transmembrane domain motif. The specific function of this protein is unknown; however, this protein may mediate the cellular effects of retinoic acid on the G protein signal transduction cascade.
CD300A	CD300a molecule	This gene encodes a member of the CD300 glycoprotein family of cell surface proteins found on leukocytes involved in immune response signaling pathways.
CD300LB	CD300 molecule-like family member b	CD300LB is a nonclassical activating receptor of the immunoglobulin superfamily expressed on myeloid cells.
CD300C	CD300c molecule	The CMRF35 antigen, which was identified by reactivity with a monoclonal antibody, is present on monocytes, neutrophils, and some T and B lymphocytes.
CD300LD	CD300 molecule-like family member d	
C17orf77	Chromosome 17 open reading frame 77	

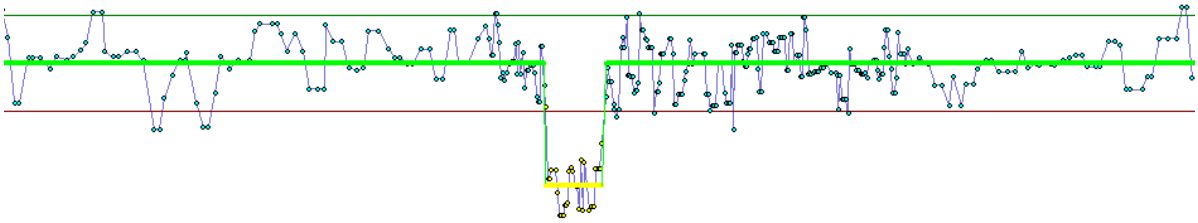
CD300E	CD300e molecule	CD300LE is an activating receptor of the immunoglobulin (Ig) superfamily expressed on myeloid cells.
RAB37	RAB37, member RAS oncogene family	Rab proteins are low molecular mass GTPases that are critical regulators of vesicle trafficking.
CD300LF	CD300 molecule-like family member f	CD300LF is an inhibitory receptor of the Ig superfamily expressed on myeloid cells. It mediates negative regulatory signals by recruiting SHP1 or SHIP.

In DGV there are seven similar duplications reported and only one in DECIPHER. A CNV of this size could be considered to be pathogenic and it certainly is both real and not frequently occurring; however the biological context of this CNV in relation to the case's phenotype in lacking.

Case S5 has a NRG3 deletion at 10q23.1

The 10: 83776117-83924137 CNV was seen just once, in sample S5. At 148,020bp it is modest in size and directly disrupts just one gene *NRG3*. *NRG3* codes for neuregulin 3 and has been shown to activate the tyrosine phosphorylation of its cognate receptor, ERBB4, and is thought to influence neuroblast proliferation, migration and differentiation by signalling through ERBB4. Linkage studies have implicated this gene as a susceptibility locus for schizophrenia and schizoaffective disorder. However *NRG3* has a haplotype index of over 60% and so won't be pathogenic due to haploinsufficieny alone.

This deletion would be entirely intronic; intron 1-2 spans 482.574kbp between 10: 83625900 -84108474. Neuroregulins are thought to act as repellents for migrating GABAergic interneurons. Li et al. 2012 Neuregulin repellent signaling via ErbB4 restricts GABAergic interneurons to migratory paths from ganglionic eminence to cortical destinations.



17q21.31 represents a benign recurrent CNV – appendix x.

Nineteen of our cases appear to have variation at 17q21.31 (table page x). 17q21.31 is a cytoband with previously reported association to epilepsy. However 17q21.31 microdeletion syndrome is described as between 17: 43705166 and 44294406. It classically involves *C17orf69, MAPT, KANSL- 1* and the deletion seen in case 14 sits within this known CNV. The gene *MAPT* encodes the microtubule-associated protein tau (MAPT). MAPT transcripts are differentially expressed in the nervous system, depending on stage of neuronal maturation and neuron type. MAPT gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.

Cases		Min	Max	Size	Dist	Mark	Genes
14	Loss	43937108	43978535	41.427	702	60	*LOC100128977, MAPT, LOC100130148*
2	Gain	44187491	44292676	105.185	1001	106	*KIAA1267, LOC644246*
11	Gain	44187491	44784970	597.479	5481	110	*KIAA1267, LOC644246, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, NSF*
13	Gain	44187491	44254379	66.888	735	92	*KIAA1267*
14	Gain	44212823	44276618	63.795	1012	64	*KIAA1267, LOC644246*

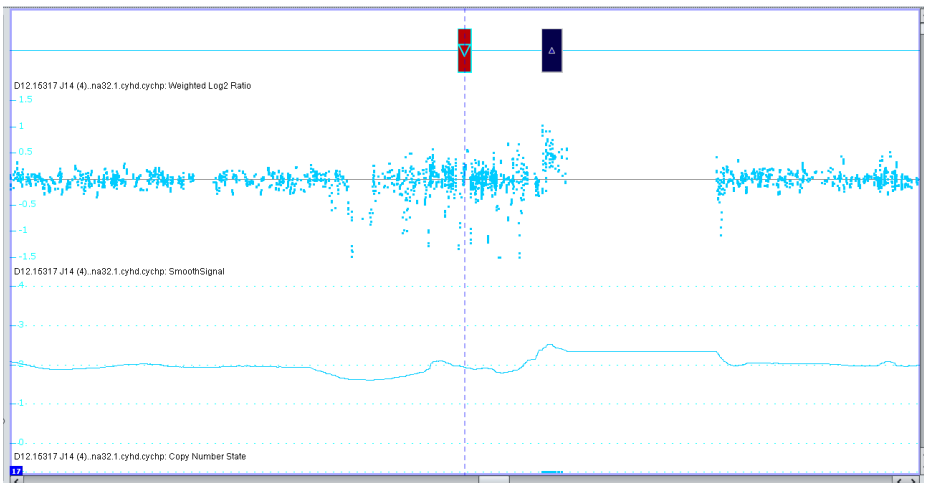
15	Gain	44212823	44292742	79.919	1141	71	*KIAA1267, LOC644246*
16	Gain	44187491	44292742	105.251	992	107	*KIAA1267, LOC644246*
18	Gain	44187491	44254379	66.888	735	92	*KIAA1267*
19	Gain	44187491	44254413	66.922	727	93	*KIAA1267*
20	Gain	44187491	44784639	597.148	5580	108	*KIAA1267, LOC644246, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, NSF*
23	Gain	44187491	44244930	57.439	765	76	*KIAA1267*
24	Gain	44212823	44292742	79.919	1141	71	*KIAA1267, LOC644246*
25	Gain	44212823	44254379	41.556	755	56	*KIAA1267*
26	Gain	44187491	44254379	66.888	735	92	*KIAA1267*
28	Gain	44212823	44276618	63.795	1012	64	*KIAA1267, LOC644246*
29	Gain	44187491	44288442	100.951	980	104	*KIAA1267, LOC644246*
33	Gain	44187491	44288442	100.951	980	104	*KIAA1267, LOC644246*
34	Gain	44187491	44288442	100.951	980	104	*KIAA1267, LOC644246*
35	Gain	44212414	44785669	573.255	7444	78	*KIAA1267, LOC644246, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, NSF*

Gene	Official Full Name	NCBI description
KIAA1267	Withdrawn	

LOC644246	KANSL1 antisense RNA 1	RNA protein
LRRC37A	Leucine rich repeat containing 37A	
ARL17A	ADP-ribosylation factor-like 17A	
ARL17B	ADP-ribosylation factor-like 17B	
NSFP1	N-ethylmaleimide-sensitive factor pseudogene 1	Thought to be a pseudogene
LRRC37A2	Leucine rich repeat containing 37, member A2	
NSF	N-ethylmaleimide-sensitive factor	

The eighteen duplications here probably represent benign common CNVS – perhaps more frequently seen in a South Wales population. They are also distinct in location from the 17q21.31 deletion syndrome. They were seen to completely overlap 5 times in the WMRGL dataset, 20 times in the EU control population. They are also seen frequently in control subjects: at least 200 times in DGV of this size or larger – all were duplications.

It can also be argued that the deletion seen in case 14 is not convincing (shown below) with a poor smooth signal trace – despite a good marker distance (under 800 bases) and 60 markers covering the CNV.



Appendix

CNVs which appear genuine and are recurrent

What is the importance of the Xp22.33 duplications for S12?

Two separate duplications were called as pathogenic on Xp22.33 in S12; the first taking the copy number to three for 315,189bp (X: 206554-521743), the second doing the same between X:534705-553662 for 18,957kb. However by eye – you would suggest they were the same CNV. Of note is the possibility of a microdeletion in a marker sparse area nearby X:17607-32362 (14,755bp). However this potential microdeletion was seen in 9 of the 24 samples making it more likely to be artefactual.

DECIPHER lists an Xp22.33 deletion syndrome between X: 460558 and 753877 (Leri-Weill dyschondrostosis (LWD) - *SHOX* deletion). At its mildest this causes short stature – at its most severe limb deformities. These duplications involve both *SHOX* (short stature homeobox) and *PPP2R3B* (protein phosphatase 2, regulatory subunit B'', beta) genes. This duplication is seen 17 times in the DECIPHER database, although no similar CNVs were seen in DGV. *SHOX* duplications have been implicated in cases of Müllerian aplasia (irrelevant here as the case is male) (Sandbacka *at el.* 2011), Leri-Weill dyschondrosteosis (Benito-Sanz *et al.* 2011) and type I Mayer-Rokitansky-Kuster-Hauser syndrome (Gervasini *et al.* 2010). Our case had none of these features and it is tempting to suggest that it is possible to make false correlations between rare disorders and CNV pathogenicity without utilising an adequate control sample.

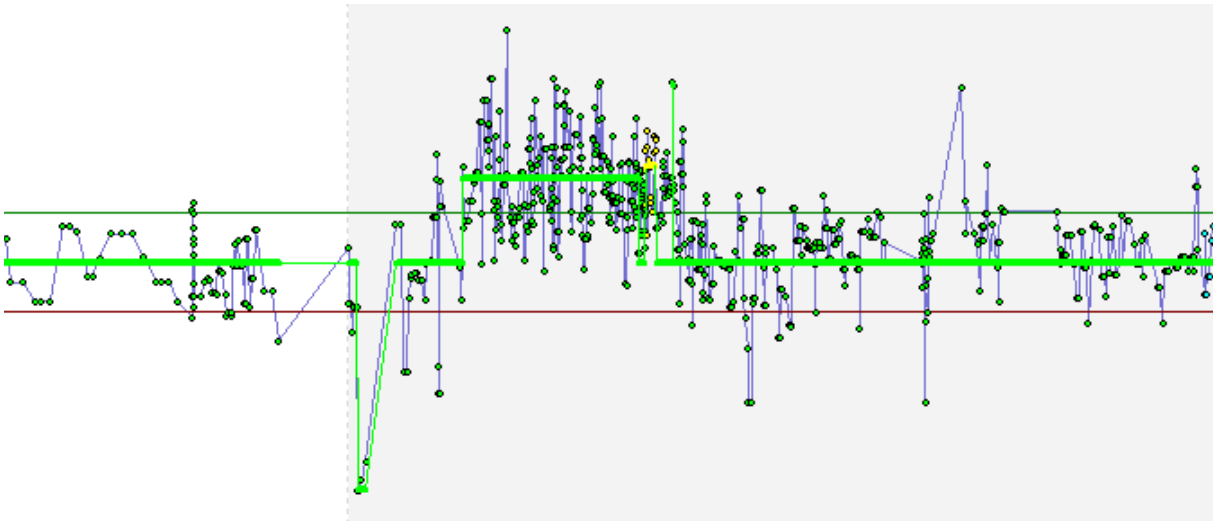


Figure xx Weighted log2 ratio for CNVs at Xp22

What are the implications of 22q11.22 duplication?

As opposed to 22q11.22 deletion (described above) – 22q11.22 duplication is less clearly pathogenic. Two unrelated individuals had 22q11.22 duplications of 313kbp and 196kbp (F9 and F7) which may be asymptomatic; particularly because case F7 does not have epilepsy. 22q11 duplication syndrome is not a clinically recognisable disorder; it is diagnosed following molecular genetic analysis. The most frequent reported symptoms in probands with duplication of 22q11.2 duplication syndrome (according to the DECIPHER database) are mental retardation/learning disability (97%), delayed psychomotor development (67%), growth retardation (63%) and muscular hypotonia (43%). However, these are common and relatively non-specific indications for cytogenetic analysis, and the extent to which duplication of 22q11.2 is causal for these features is currently unknown. The majority of 22q11 duplications are inherited often from an unaffected parent. This is in sharp contrast to the 22q11 deletion syndrome where approximately 90% of cases are *de novo*.

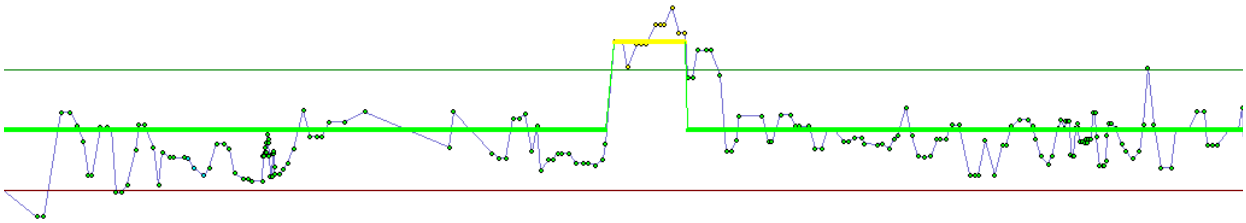


Figure xx Weighted log2 ratio demonstrating 22q11.22 duplication in F7

In F7's 22q11.22 duplication at (22:20591480-20788440) there are four genes in this area (table xx below). The controvertial 22q11 duplication syndrome – is described as CNV between 19009792 and 21452445; so this CNV sits within it. In contrast the 22q11.22 duplication seen in F9 is at 22: 20591480-20904791and is 313,311 bp long, containing two more genes than the above duplication. This is a common area of variation – there are 97 overlapping CNVs in DGV. The area is close by but separate from the velocardiofacial / DiGeorge syndrome locus.

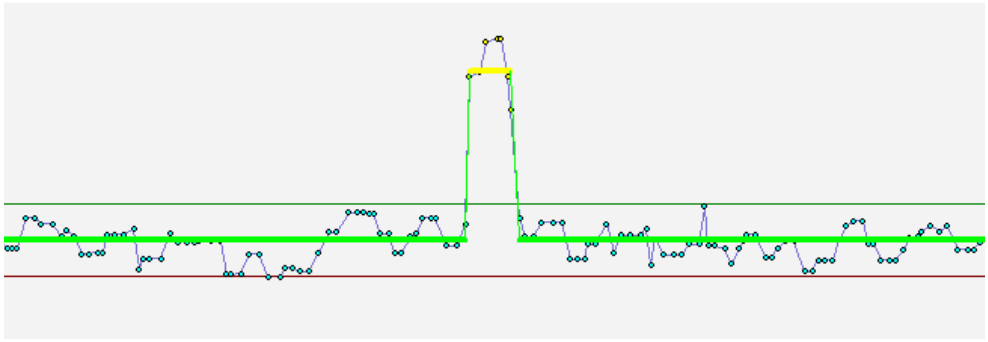
Gene	Official Full Name	NCBI description
ZNF74	Zinc finger protein 74	
SCARF4	Scavenger receptor class F, member 2	Mediates the binding and degradation of acetylated low density lipoprotein
KLHL22	Kelch-like 22	

MED15	Mediator complex subunit 15	Subunit of the multiprotein complexes PC2 and ARC/DRIP and may function as a transcriptional coactivator in RNA polymerase II transcription.

Table xx Genes in the 22q11.22 duplications

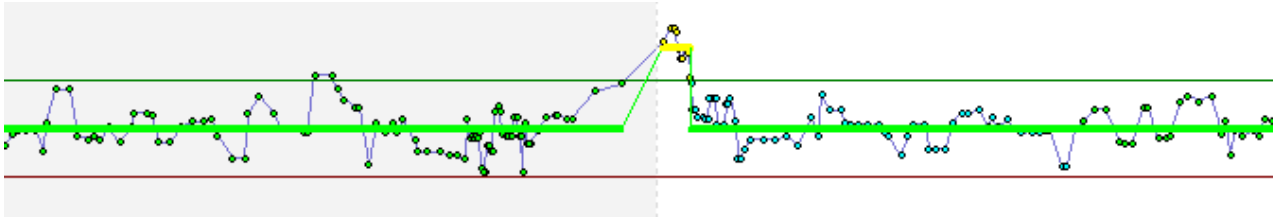
3q26.1 duplication (cases S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11, F4, F9, F10)

The 3q26.1 variant was autocalled as a duplication in 14 of 24 cases analysed (including two cases S3 and 4 who were not thought to have epilepsy. Manual inspection suggests it is convincing (below). It is an area of little variation – it does not appear in DGV, nor the WMRGL nor EU datasets and only four CNVs overlap it in DECIPHER. This is in striking contrast to the frequency that it appears in this cohort. These cases are ethnically and geographically diverse: S1, S2, S7, S8, S10, S11, F4, F9 (white Caucasian, South Wales) S3 – (white Caucasian – Melbourne, Australasia) S4 (Pakistani) S5 (white Caucasian, North of England) S6, S9 (white Caucasian, midlands), F10 (white Caucasian, South West of England). It could represent a rare CNV seen most frequently in South Wales. The area has no protein coding genes within it and no genes associated with epilepsy in its proximity.



4p16.3 duplication (case F3)

This 52kbp duplication – seen in a single individual but not in her sister (F2) nor nephew (F1) involves two zinc finger genes: *ZNF595, ZNF718.* Nine DECIPHER CNVs overlap and seven similar CNVs are seen in DGV. This is a very distal CNV and could not be mapped by CytoScan HD (no probes for the area).



5p15.33 deletion and duplication

As the ten CNVs show below the duplications were a little longer and included an additional gene. *TPPP* is interesting as it's a tubulin polymerization promoting protein. *ZDHHC11* is a DHHC-type containing 11 zinc finger gene. There is not a great deal known about the DHHC type zinc finger family. There are 20 overlapping entries in DGV, and 13 in DECIPHER. Of those 13, four ahd phenotype nformation on DECIPHER and one of these cases included seizures. This CNV was a duplication at 5: 204849- 6753953 - 6,549,104bp in length and so therefore a much greater CNV, containing 34 genes.

Intriguingly the important area for Cri du Chat syndrome (the very start of Chromosome 5 up until 10001bp) overlaps these CNV partially. Outside the CNV described but nearby are genes such as *SLC6A19* which is associated with Hartnup disorder – where seizures are a recognised feature alongside dermatological, nephrological and gastrointestinal symptoms. The epilepsy associated duplication was described as 5: 0.72–1.43, so all of these CNVs sit within the reported CNV. ZDHHC11 has a haplotype index of 88% and TPP of 31% and so they are very unlikely to be directly pathogenic due to haploinsufficiency.

5p15.33	Case	Min	Max	Size kbp	Genes
Deletion	S1	786482	848774	62.292	ZDHHC11
	S4	786482	874909	88.427	ZDHHC11
	S5	786482	848774	62.292	ZDHHC11
	S7	786482	848774	62.292	ZDHHC11
	S10	763524	848774	85.250	ZDHHC11
	S12	786482	874909	88.427	ZDHHC11
	F12	812199	874909	62.710	ZDHHC11
Duplication	F8	739315	859205	119.890	TPPP, ZDHHC11
	F10	739315	848774	109.459	TPPP, ZDHHC11
	F11	745881	848774	102.893	TPPP, ZDHHC11

Table xx Clustering of CNVs in our sample spanning phenotypes and ethnicities

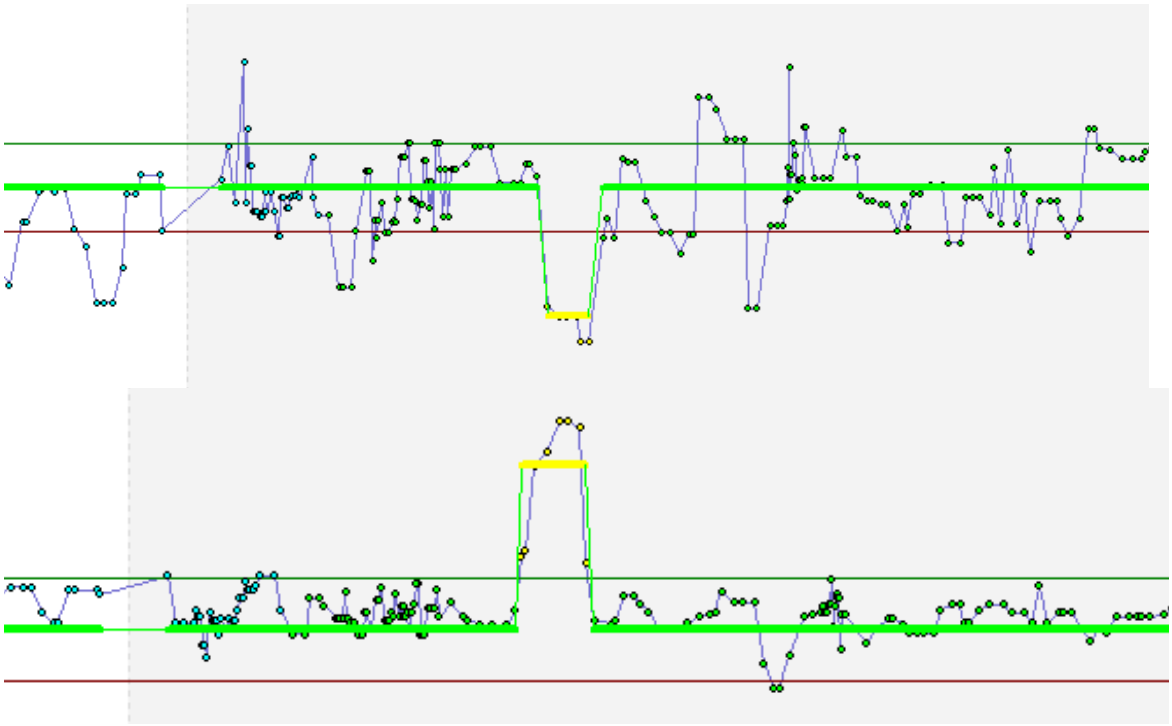
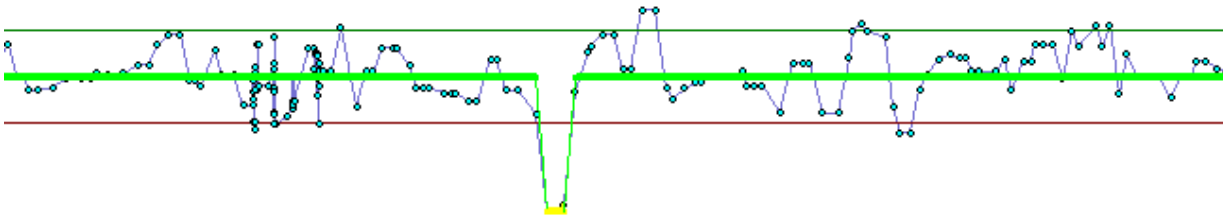


Figure xx Weighted Log2 ratios of F12 (top) and F8 (below) demonstrating the 5p15.33 CNVS

Six deletions at 6p21.32



Case	Min	Max	Size kbp
S6	32558700	32586161	27.461
S7	32565191	32605309	40.119
F1	32558700	32629878	71.178
F6	32558700	32629878	71.178
F9	32565191	32703025	137.834
F10	32558700	32672786	1140.86

This reduces two major histocompatibility complex genes to heterozygosity – DQ beta 1 (*HLA-DQB1*) and DQ alpha 1 (*HLA-DQA1*). The former of these has been reported to convey susceptibility to multiple sclerosis and coeliac disease and resistance from variant Creutzfeldt-Jakob disease; the latter again increases the likelihood to coeliac disease. Both of these two genes have robust haplotype indices (above 70%) and it is well recognised that there is considerable variation tolerated within the HLA genes.

However nearby there are some epilepsy associated genes: *GABBR1* (gamma-aminobutyric acid (GABA) B receptor 1) is at 6: 29570005- 29600962; *GRM4* (glutamate receptor, metabotropic 4) is at 6: 33989623- 34123399; and intriguingly *BRD2* (bromodomain containing 2) is nearer still at 6: 32936437 -32949282. *BRD2* has been reported to be implicated with JME. S6, S7 and F6 have a GGE, F1 is not thought to have epilepsy, F9 has a cryptogenic focal epilepsy and F10 unclassified.

Perhaps the suggestion that this 6p21 deletion may be having an effect at BRD2 is tenuous- it is over 250,000bp away. And as the figure below shows – it is in an area of frequently reported variation

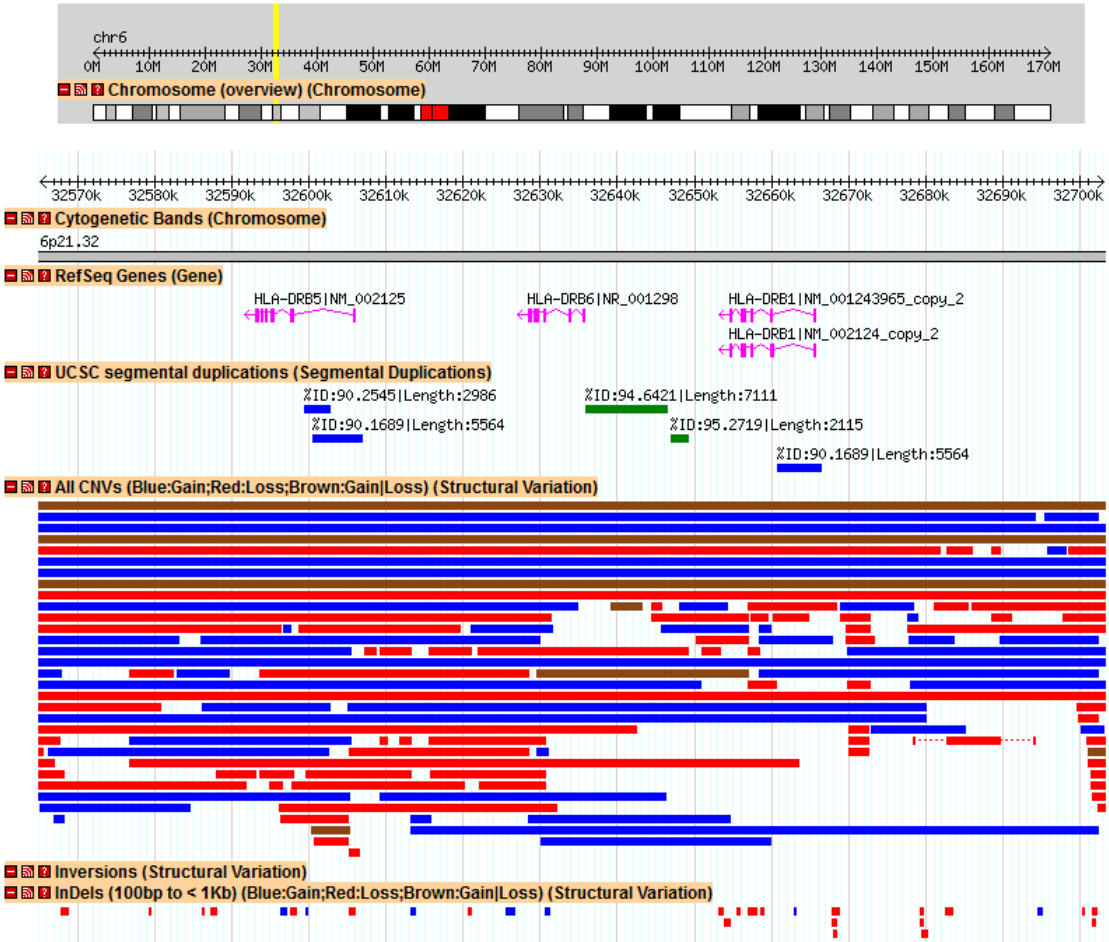
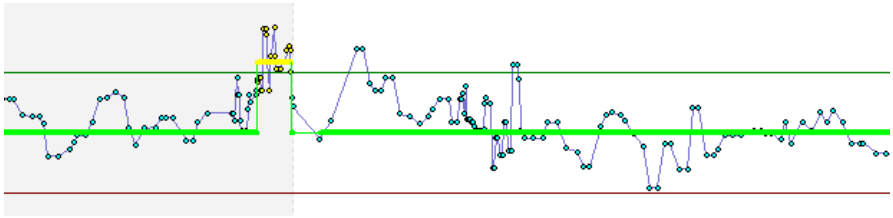


Figure xx Screen capture from DGV demonstrating the frequently reporte variation in the area (red and blue bars represent reported experiments with CNVs here).

7q36.3 duplication

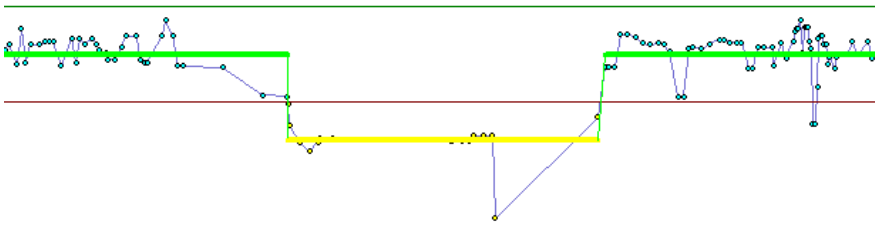
This duplication is in a gene poor area – 7:158736443-158816863 and of a modest size - 80,420bp. The epilepsy associated CNV does not overlap but is nearby at 7: 156123654- 158388155. This CNV at the distal end of chromosome 7. Only two CNVs overlap in DGV - but eighteen do in DECIPHER.



This CNV again was seen as a duplication in a single case (S4) and as a deletion in another (F8). The location is 8:7230389-8117301 produces a large deletion - 886,912 bp containing many genes, not all of them protein coding*: DEFB103A, SPAG11B, DEFB104B, DEFB106B, DEFB105B, DEFB107B, FAM90A8, FAM90A17, FAM90A16, FAM90A18, FAM90A19, FAM90A7, FAM90A10, FAM90A22, FAM90A8, FAM90A17, FAM90A9, FAM90A16, FAM90A18, FAM90A19, FAM90A23, FAM90A2P, OR7E154P, FAM90A15, FAM90A14, FAM90A13, DEFB107A, DEFB105A, DEFB106A, DEFB104A, SPAG11A, DEFB103B, DEFB4, DEFB109P1, FAM90A11, FAM90A24P, FAM90A12* and *MIRN548I3.* There are no OMIM diseases associated with these and a great deal of recognised variation in DGV: 31 overlapping areas; and DECIPHER - a further 30 areas.

The duplication is at 8: 7,156,930-8,117,301 and is a little larger at 960,371bp; however the epilepsy associated CNV does not overlap (8: 10.19–10.37) and was 173.1kbp. A further three pseudogenes are involved with this CNV: *DEFB109P1, DEFB108P1* and *DEFB108P2.* DGV recognises a great degree of variation here with 95 overlapping CNVs described.

There is a 8p23.1 deletion syndrome (8: 8100055-11764629) which only just fails to overlap with this CNV. The genes starting DEFB (numbering 12 in total) are defensin genes –coding for host defence peptides. The genes starting FAM90A are a primate-specific gene family, orginating from multiple duplications and rearrangements; 21 members are listed above some of which are actually considered to be pseudogenes. The genes not covered by these families are in the box below. There are no genes of interest to epilepsy in flanking regions.



Gene	Official Full Name	NCBI description
SPAG11B	Sperm associated antigen 11B	This gene encodes several androgen-dependent, epididymis-specific secretory proteins
SPAG11A	Sperm associated antigen 11A	
MIRN548I3	microRNA 548i-3	Non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs.
OR7E154P	Olfactory receptor, family 7, subfamily E, member 154 pseudogene	Pseudogene

14q11.12 deletion

14q11.1-q11.2

There were ten CNVs at the 14q11.1 cytoband, seven of which were deletions (table below). The CNVs ranged from a 59kbp deletion to 1029kbp. The majority of CNVs overlapped the same area – with just F11 (14: 22589160-22648367) being distinct, reflected in the genes involved too. All genes listed below that start 'OR' are olfactory receptor family members; there are only four that do not belong to one of these families (table below).

The area covered by most of the CNVs here is pericentromeric on chromosome 14 and very little variation is called in DGV that spans this area. DECIPHER does not have a single record for 14q11.1; although eighteen for neighbouring 14q11.2 (where F11 creeps in to and 7 records overlap). Indeed samples J31, J32, J33 all have deletions spanning this area and J3 and J23 have microdeletions within

it and there are 14 deletions in DGV. The relevance to this 14q11.2 CNV is discussed below when the JME samples are described.

14q11.1	Case	Min	Max	Size kbp	Genes
Deletion	S9	18798671	19490660	691.989	*OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	S10	19268806	19490660	221.854	*OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	S12	18187184	19216393	1029.210	*OR11H13P, OR11H12, POTEG*
	F2	18139146	19490660	1351.514	*OR11H13P, OR11H12, POTEG, OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	F4	19268806	19490660	221.854	*OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	F9	18446792	19490660	1043.868	*OR11H13P, OR11H12, POTEG, OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1*
	F11	22589160	22648367	59.207	*CDH24, ACIN1, C14orf119*
Duplication	S5	18798671	19515811	717.140	*OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1, OR4K15*
	S8	19273319	19467542	194.223	*OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5*

F12	18544802	19515811	971.009	*POTEG, OR11H13P, OR11H2, OR4Q3, OR4H12P, OR4M1, OR4N2, OR4K2, OR4K5, OR4K1, OR4K15*

Gene	Official Full Name	NCBI description
POTEG	POTE ankyrin domain family, member G	
CDH24	Cadherin 24, type 2	
ACIN1	Apoptotic chromatin condensation inducer 1	Apoptosis is defined by several morphologic nuclear changes, including chromatin condensation and nuclear fragmentation. This gene encodes a nuclear protein that induces apoptotic chromatin condensation after activation by caspase-3, without inducing DNA fragmentation. It has also been shown to be a component of a splicing-dependent multiprotein exon junction complex that is deposited at splice junctions on mRNAs, as a consequence of pre-mRNA splicing. It may thus be involved in mRNA metabolism associated with splicing.
C14orf119	Chromosome 14 open reading frame 119	

The cytoband 14q11.12 has been associated with epilepsy and deletions of between 42,644 bp and 446,991bp were reported in 14 cases. This particular deletion was seen frequently - 9 more times in the WMRGL file of cases and 27 times in EU file of controls. DECIPHER does not recognise it as a known microdeletion syndrome however – and only 7 cases in that database overlap with these cases. 13 similar deletions are recorded in DGV, and many more microduplications – 164. Each CNV

(table below) is covered by a good number of probes (up to 692) with a relatively small mean marker distance (511 to 781bp); it appears to be a convincing CNV by eye too. 14q11.12 probably represents a benign recurrent CNV that has a higher prevalence in the South Wales population. This microdeletion is in a gene poor region. The nearest genes being many kilobases away and do not include any genes directly implicated in epilepsy phenotypes such as *DAD1* (defender against cell death 1) and *SLC7A7* (solute carrier family 7 (amino acid transporter light chain, y+L system), member 7) – found in skin.

Case	Min	Max	Size	Dist	Mark
10	22835777	22878421	42.644	775	56
14	22746791	22944507	197.716	708	280
16	22747746	22941375	193.629	730	266
19	22749288	22854720	105.432	717	148
21	22772662	22959362	186.7	781	240
22	22594506 22676928	22620607 22959362	26.101 282.434	511 643	52 440
23	22641679	22940386	298.707	650	460
24	22562098	22612060	49.962	594	85
24	22730865	22943573	212.708	679	314
28	22827208	22941375	114.167	771	149
31	22599355	22960819	361.464	605	598
32	22496582	22943573	446.991	646	692
33	22560402	22943215	382.813	600	639
34	22747482	22941375	193.893	726	268

14q32.33 deletion

There were eight near identical CNV deletions at 14q32.33 (below). The CNV reported to be associated with epilepsy was at 14: 103375996- 106312055 and so these CNVs fall within this area, however it is a 2936kbp deletion – larger by a factor than in this series. The genes involved with F5 (the largest CNV) here are *IGHVIII-26-1, IGHVII-26-2, IGHV7-27, IGHVII-28-1, IGHV3-29, IGHVII-30-1, IGHV3-30-2, IGHVII-31-1, IGHV3-32, IGHVII-33-1, IGHV3-33-2, IGHV7-34-1, IGHV3-36, IGHV3-37,*

IGHVIII-38-1, IGHV7-40, IGHVII-40-1, IGHV3-41, IGHV3-42, IGHVII-43-1. All of these genes are of the immunoglobulin heavy variable family – many of them pseudogenes. Illustrations of 14q32.33 are given in the discussion of comparing cases later in this chapter.

14q32.33	Case	Min	Max	Size kbp
Deletion	S3	105610764	105630119	19.355
	S11	105610764	105630119	19.355
	S12	105977754	106002209	24.455
	F4	105857223	105881301	24.079
	F5	105833402	106002209	168.807
	F6	105857223	106002209	144.986
	F7	105857223	105881301	24.079
	F9	105857223	105881301	24.079

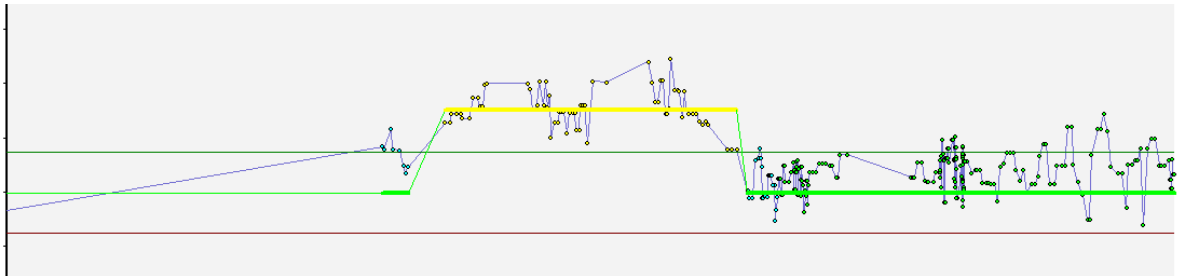
15q11.2 deletion / duplication

There are nine cases with deletions and eight with duplications from the 24 cases studied. F3 was called as having two duplications adjacent to each other and F8 as having a duplication followed by a deletion in the area. This is a pericentromeric region on chromosome 15 and of note is the fact that the start of 15q up until 23619912bp is the region known to cause Angleman and Prader-Wili Syndromes.

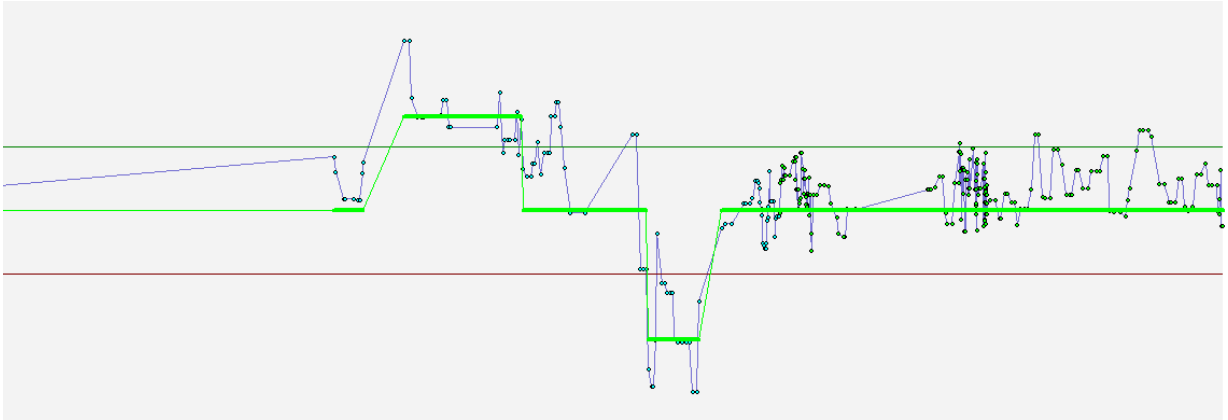
The published epilepsy associated CNVs were deletions at 15:20.2-20.8 of around 600kb, a large 5,356kb deletion between 15q11.2 and 15q13.1 and a larger one still between 15: 19109124-28153416. Some of the CNVs presented here are large – six are over a megabase – and all bar the two smallest directly involve genes. 15q11.2 is discussed again later with reference to the JME cases under 'Recurrent CNVs with genes of interest'.

15q11.1	Case	Min	Max	Size kbp	Genes
Deletion	S2	18884466	19786685	902.219	*HERC2P2, HERC2P3, POTEB, POTEC*
	S3	18692895	19833572	1,140.677	*HERC2P2, HERC2P3, POTEB, RPS8P10, OR4N4*
	S7	18722617	20091146	1,368.529	*HERC2P2, HERC2P3, POTEB, , RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*

	S8	18790619	19833572	1,042.953	*HERC2P2, HERC2P3, POTEB, RPS8P10, OR4N4*
	S12	19171413	19765882	594.469	*POTEB,*
	F4	19418630	19537065	118.435	
	F5	18810028	19786685	976.657	*HERC2P2, HERC2P3, POTEB,*
	F8	19845972	20091146	245.174	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F9	19465389	20091146	625.757	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
Duplication	S11	18657418	20249916	1,592.498	*HERC2P2, HERC2P3, POTEB, RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F3	18657418	19121787	464.369	*HERC2P2, HERC2P3*
		19765882	20091146	325.264	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F6	18810028	19206809	396.781	*HERC2P2, HERC2P3*
		19338213	20091146	752.933	*RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F7	18458177	19195328	737.151	*HERC2P2, HERC2P3*
	F8	18657418	19227572	570.154	*HERC2P2, HERC2P3*
	F10	18315236	20091146	1,775.910	*VSIG7, HERC2P2, HERC2P3, POTEB, POTEC, RPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*
	F11	21901595	22055583	153.988	
	F12	18657418	20091146	1,433.728	*HERC2P2, HERC2P3, POTEBRPS8P10, OR4N4, OR4M2, VSIG6, MIRN1268*



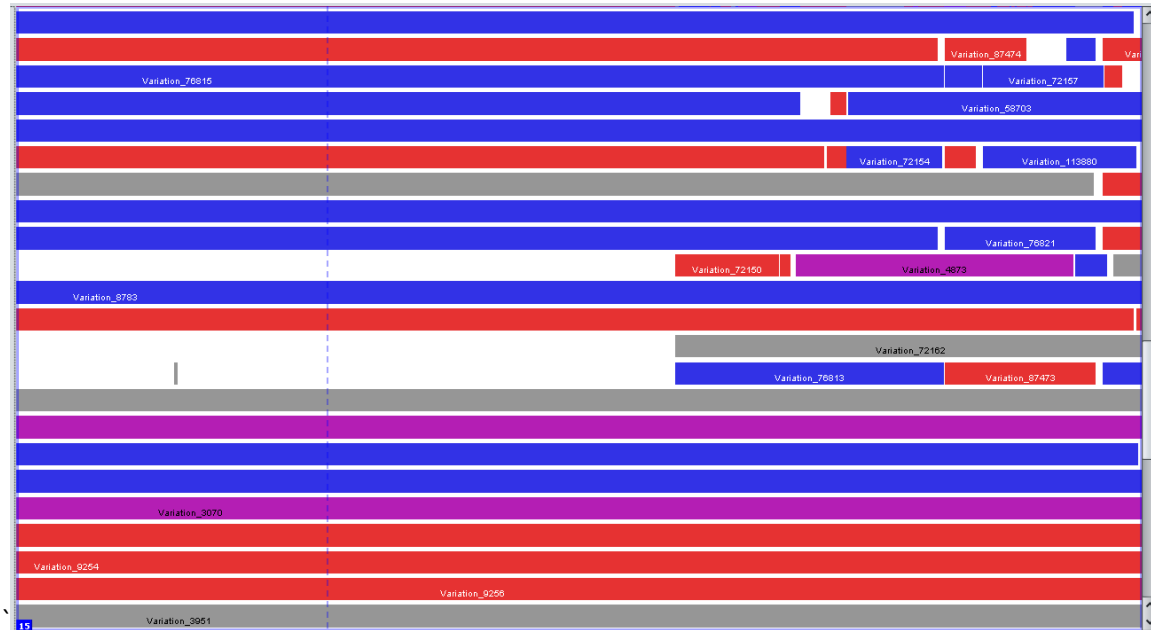
F8



It appears that this CNV coming out the centromere (gene poor and hard to map) is an area of great variation and with few genuine genes of interest (below). The illustration of the number of DGV variants (below) confirms that this is an area with a great number of probably benign CNVs, despite having been linked to epilepsy previously.

Gene	Official Full Name	NCBI description
VSIG7	immunoglobulin heavy variable 1/OR15-9	Pseudogene
HERC2P2	Hect domain and RLD 2 pseudogene 2	Pseudogene
HERC2P3	Hect domain and RLD 2 pseudogene 3	Pseudogene
POTEB	POTE ankyrin domain family, member B	
RPS8P10	Ribosomal protein S8 pseudogene 10	Pseudogene
OR4N4	Olfactory receptor, family 4, subfamily N, member 4	

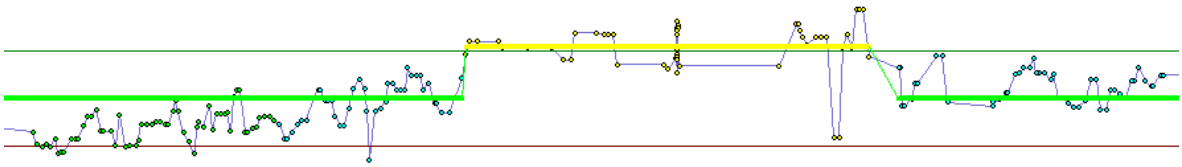
OR4M2	Olfactory receptor, family 4, subfamily M, member 2	
VSIG6	V-set and immunoglobulin domain containing 6	
MIRN1268	microRNA 1268a	Non-coding RNAs that are involved in post-transcriptional regulation of gene expression in multicellular organisms by affecting both the stability and translation of mRNAs



16p11.2 deletion/ duplication

The cytoband 16p11.2 is an apparent hot spot for CNVs in this study: there are three deletions reported and eight cases with ten duplications. Some of the CNVs are over a megabase – and most involve *TP53TG3B* (TP53 target 3B). *IGHV2OR16-5* is non-functional and *SLC6A10P* is a pseudogene.

None of these CNVs are within the recognised area for the 16p11.2 microduplication/microdeletion syndrome (16:16-29606852). The area covered by the largest of the CNVs here (F8) has very few overlapping areas reported on DECIPHER (4), but 35 overlapping regions in DGV. Even though the area is marker poor (below) the CNV appears genuine. F8 below.



16p11.2	Case	Min	Max	Size kbp	Genes
Deletion	S2	32809724	33734826	925.102	TP53TG3, TP53TG3
	S5	32378156	32545380	167.224	
	S5	32378156	32545380	167.224	
Duplication	S7	34339573	34584822	245.249	
	S8	34375339	34605060	229.721	
	S9	34375339	34524469	149.130	
	S11	31893658	33734826	1,841.168	TP53TG3, TP53TG3, IGHV2OR16-5, SLC6A10P, TP53TG3, TP53TG3
		34339573	34605060	265.487	
	F8	31958995	33561829	1,602.834	TP53TG3, TP53TG3, IGHV2OR16-5, SLC6A10P, TP53TG3, TP53TG3
	F9	34339573	34605060	265.487	
	F10	31862607	32799600	936.994	TP53TG3, TP53TG3, IGHV2OR16-5, SLC6A10P
	F12	32801349	33680606	879.257	SLC6A10P, TP53TG3, TP53TG3
		34339573	34559455	219.882	

17q25.1 duplication – a rare and large CNV of uncertain significance

S3 is only case to have a large (861,201bp) duplication at 17: 69344027-70205228. This stretch contains 16 genes: *RPL38, TTYH2, DNAI2, KIF19, BTBD17, GPR142, GPRC5C, CD300A, CD300LB, CD300C, CD300LD, C17orf77, CD300E, RAB37* and *CD300LF*.

Gene	Official Full Name	NCBI description
RPL38	Ribosomal protein L38	This gene encodes a ribosomal protein that is a component of the 60S subunit. The protein belongs to the L38E family of ribosomal proteins. It is located in the cytoplasm.
TTYH2	Tweety homolog 2	Members of this family function as chloride anion channels. The encoded protein functions as a calcium(2+)-activated large conductance chloride(-) channel, and may play a role in kidney tumorigenesis.
DNAI2	Dynein, axonemal, intermediate chain 2	Mutations in this gene are associated with primary ciliary dyskinesia type 9
KIF19	Kinesin family member 19	
BTBD17	BTB (POZ) domain containing 17	
GPR142	G protein-coupled receptor 142	
GPRC5C	G protein-coupled receptor, family C, group 5, member C	Members of this superfamily are characterized by a signature 7-transmembrane domain motif. The specific function of this protein is unknown; however, this protein may mediate the cellular effects of retinoic acid on the G protein signal transduction cascade.
CD300A	CD300a molecule	This gene encodes a member of the CD300 glycoprotein family of cell surface proteins found on leukocytes involved in immune response signaling pathways.
CD300LB	CD300 molecule-like family	CD300LB is a nonclassical activating receptor of the

	member b	immunoglobulin superfamily expressed on myeloid cells.
CD300C	CD300c molecule	The CMRF35 antigen, which was identified by reactivity with a monoclonal antibody, is present on monocytes, neutrophils, and some T and B lymphocytes.
CD300LD	CD300 molecule-like family member d	
C17orf77	Chromosome 17 open reading frame 77	
CD300E	CD300e molecule	CD300LE is an activating receptor of the immunoglobulin (Ig) superfamily expressed on myeloid cells.
RAB37	RAB37, member RAS oncogene family	Rab proteins are low molecular mass GTPases that are critical regulators of vesicle trafficking.
CD300LF	CD300 molecule-like family member f	CD300LF is an inhibitory receptor of the Ig superfamily expressed on myeloid cells. It mediates negative regulatory signals by recruiting SHP1 or SHIP.

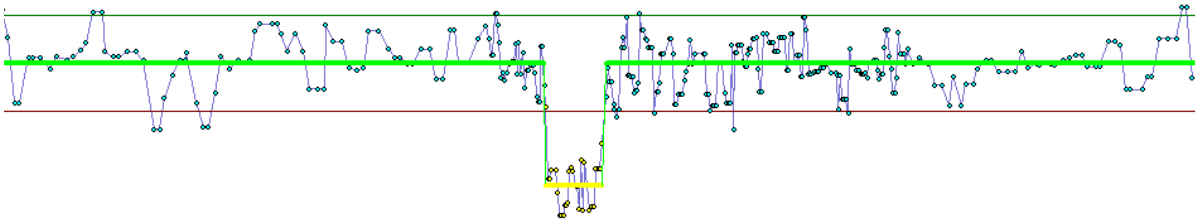
In DGV there are seven similar duplications reported and only one in DECIPHER. A CNV of this size could be considered to be pathogenic and it certainly is both real and not frequently occurring; however the biological context of this CNV in relation to the case's phenotype in lacking.

Case S5 has a NRG3 deletion at 10q23.1

The 10: 83776117-83924137 CNV was seen just once, in sample S5. At 148,020bp it is modest in size and directly disrupts just one gene *NRG3*. *NRG3* codes for neuregulin 3 and has been shown to activate the tyrosine phosphorylation of its cognate receptor, ERBB4, and is thought to influence neuroblast proliferation, migration and differentiation by signalling through ERBB4. Linkage studies have implicated this gene as a susceptibility locus for schizophrenia and schizoaffective disorder.

However *NRG3* has a haplotype index of over 60% and so won't be pathogenic due to haploinsufficieny alone.

This deletion would be entirely intronic; intron 1-2 spans 482.574kbp between 10: 83625900 - 84108474. Neuroregulins are thought to act as repellents for migrating GABAergic interneurons. Li et al. 2012 Neuregulin repellent signaling via ErbB4 restricts GABAergic interneurons to migratory paths from ganglionic eminence to cortical destinations.



17q21.31 represents a benign recurrent CNV – appendix x.

Nineteen of our cases appear to have variation at 17q21.31 (table page x). 17q21.31 is a cytoband with previously reported association to epilepsy. However 17q21.31 microdeletion syndrome is described as between 17: 43705166 and 44294406. It classically involves *C17orf69, MAPT, KANSL- 1* and the deletion seen in case 14 sits within this known CNV. The gene *MAPT* encodes the microtubule-associated protein tau (MAPT). MAPT transcripts are differentially expressed in the nervous system, depending on stage of neuronal maturation and neuron type. MAPT gene mutations have been associated with several neurodegenerative disorders such as Alzheimer's disease, Pick's disease, frontotemporal dementia, cortico-basal degeneration and progressive supranuclear palsy.

Cases		Min	Max	Size	Dist	Mark	Genes
14	Loss	43937108	43978535	41.427	702	60	*LOC100128977, MAPT, LOC100130148*
2	Gain	44187491	44292676	105.185	1001	106	*KIAA1267, LOC644246*

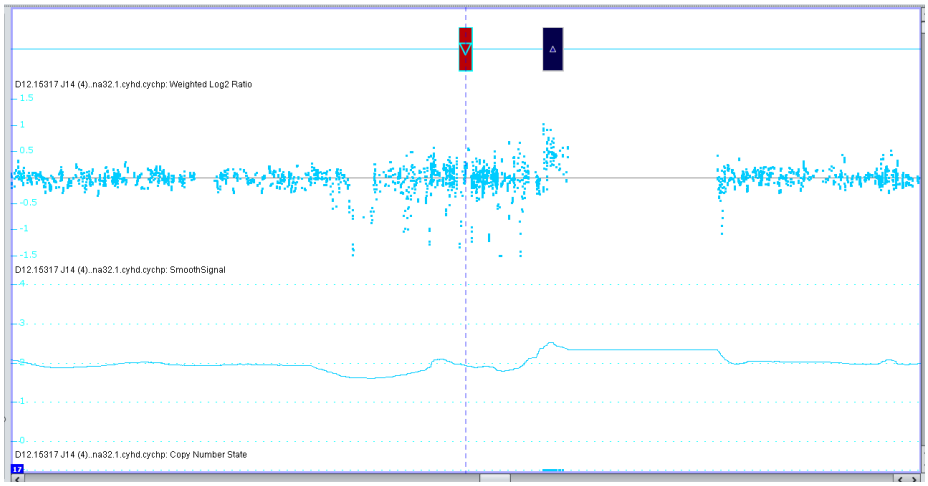
11	Gain	44187491	44784970	597.479	5481	110	*KIAA1267, LOC644246, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, NSF*
13	Gain	44187491	44254379	66.888	735	92	*KIAA1267*
14	Gain	44212823	44276618	63.795	1012	64	*KIAA1267, LOC644246*
15	Gain	44212823	44292742	79.919	1141	71	*KIAA1267, LOC644246*
16	Gain	44187491	44292742	105.251	992	107	*KIAA1267, LOC644246*
18	Gain	44187491	44254379	66.888	735	92	*KIAA1267*
19	Gain	44187491	44254413	66.922	727	93	*KIAA1267*
20	Gain	44187491	44784639	597.148	5580	108	*KIAA1267, LOC644246, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, NSF*
23	Gain	44187491	44244930	57.439	765	76	*KIAA1267*
24	Gain	44212823	44292742	79.919	1141	71	*KIAA1267, LOC644246*
25	Gain	44212823	44254379	41.556	755	56	*KIAA1267*
26	Gain	44187491	44254379	66.888	735	92	*KIAA1267*
28	Gain	44212823	44276618	63.795	1012	64	*KIAA1267, LOC644246*
29	Gain	44187491	44288442	100.951	980	104	*KIAA1267, LOC644246*
33	Gain	44187491	44288442	100.951	980	104	*KIAA1267, LOC644246*
34	Gain	44187491	44288442	100.951	980	104	*KIAA1267, LOC644246*

35	Gain	44212414	44785669	573.255	7444	78	*KIAA1267, LOC644246, LRRC37A, ARL17A, ARL17B, NSFP1, LRRC37A2, NSF*

Gene	Official Full Name	NCBI description
KIAA1267	Withdrawn	
LOC644246	KANSL1 antisense RNA 1	RNA protein
LRRC37A	Leucine rich repeat containing 37A	
ARL17A	ADP-ribosylation factor-like 17A	
ARL17B	ADP-ribosylation factor-like 17B	
NSFP1	N-ethylmaleimide-sensitive factor pseudogene 1	Thought to be a pseudogene
LRRC37A2	Leucine rich repeat containing 37, member A2	
NSF	N-ethylmaleimide-sensitive factor	

The eighteen duplications here probably represent benign common CNVS – perhaps more frequently seen in a South Wales population. They are also distinct in location from the 17q21.31 deletion syndrome. They were seen to completely overlap 5 times in the WMRGL dataset, 20 times in the EU control population. They are also seen frequently in control subjects: at least 200 times in DGV of this size or larger – all were duplications.

It can also be argued that the deletion seen in case 14 is not convincing (shown below) with a poor smooth signal trace – despite a good marker distance (under 800 bases) and 60 markers covering the CNV.


D12.15317 J14 (4)..na32.1.cyhd.cychp: Weighted Log2 Ratio
1.5
1
0.5
-0.5
-1
-1.5
D12.15317 J14 (4)..na32.1.cyhd.cychp: SmoothSignal
4
3
2
1
0
D12.15317 J14 (4)..na32.1.cyhd.cychp: Copy Number State

Appendix J

Copy number variation – clusters and artefact

Frequently occurring CNVs which may be artefactual

A large deletion at 9q12 in case S11

The software would be more likely to call large CNVs as pathogenic and more likely to call deletions than duplications. This CNV seen in case S11 is nearly two megabases in size (1,936,086bp) and contains seven potential genes: *AQP7P4, AQP7P1, FAM88B, FAM27E3, FAM27B, ANKRD20A3* and *RPL7AP46.* As you can see from the table below only *ANKRD20A3* is a protein coding gene, but the role of microRNAs is being increasingly investigated.

Gene	Official Full Name	NCBI description
AQP7P4	Aquaporin 7 pseudogene 4	Pseudogene
AQP7P1	Aquaporin 7 pseudogene 1	Pseudogene
FAM88B	No entry	
FAM27E3	Family with sequence similarity 27, member E3	Protein coding
FAM27B	Family with sequence similarity 27, member B	RNA
ANKRD20A3	Ankyrin repeat domain 20 family, member A3	Protein coding
RPL7AP46	Ribosomal protein L7a pseudogene 46	Pseudogene
PGM5P2	phosphoglucomutase 5 pseudogene 2	Pseudogene
MIRN1299	Micro RNA	RNA

Table Genes identified at the large 9q12 deletion

Ankyrin repeat domain 20 family, member A3 (*ANKRD20A3*) does not have a great body of literature describing its function however ankyrin repeats (which are a 33-residue motif in proteins – consisting of two alpha helices separated by loops) have associations with human diseases. The Notch protein – which is a key cell signalling pathway component – can cause CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and

leukoencephalopathy) when the repeat domain is disrupted by mutations. *ANKRD20A3* contains an ankyrin repeat.

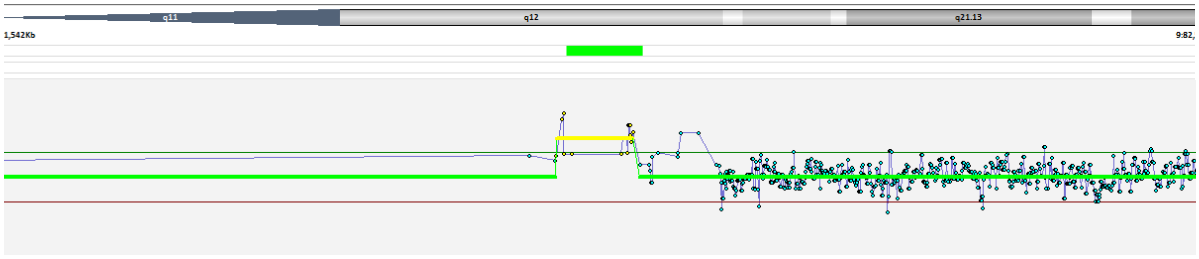


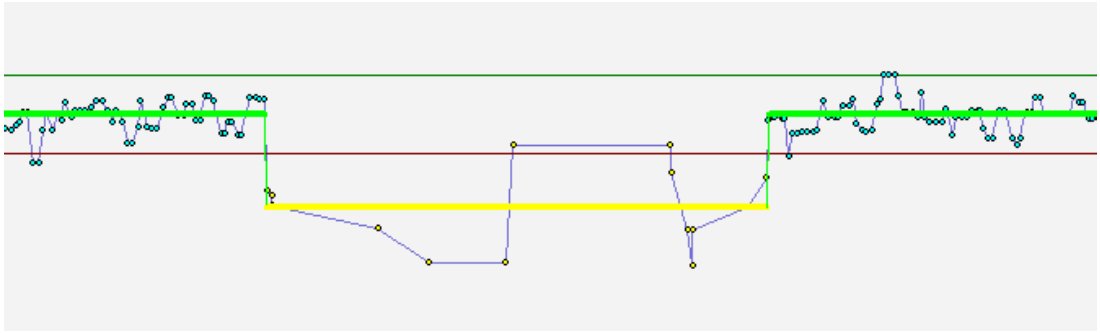
Figure Log2ration (bottom), CNV call size (green bar) and chromosomal position for case 11.

A second 9q12 was reported as of unknown significance in case F10. S11's is at 9: 66,062,668 67,998,754 and F10 overlaps it at 9: 66,256,494-68,397,902. F10's CNV also includes the genes - *PGM5P2* and *MIRN1299.* At best I must report that this is of uncertain significance, and it could quite possibly be either tolerated and benign or an artefact (above).

5q13.2 deletion

This deletion seen in two cases (S2, S8) is in a marker poor peri-centromeric region of chromosome five. As a result it is difficult to have confidence in this. The 1,824,731bp region 5: 68868743 -70693474 has eight overlapping entries in DECIPHER and 45 in DGV; so even if it is genuine it may represent a benign CNV. However there are a number of neurologically relevant genes within the region: *OCLN, GTF2H2D, GTF2H2C, GTF2H2B, SERF1B, SMN2, SERF1A, SMN1, NAIP* and *GTF2H2.*

5q13 is notoriously difficult to map. *SERF1B* to *GTF2HC* for example are part of a 500 kb inverted duplication on chromosome 5q13. This duplicated region contains repetitive elements which make it prone to rearrangements and deletions. The repetitiveness and complexity of the sequence have also caused difficulty in determining the organisation of this genomic region.



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GTF2H2D	General transcription factor IIH, polypeptide 2D	Part of SMA duplicated region on 5q13
GTF2H2C	General transcription factor IIH, polypeptide 2C	
GTF2H2B	General transcription factor IIH, polypeptide 2B	Pseudogene
SERF1B	Small EDRK-rich factor 1B	This gene is the centromeric copy which is identical to the telomeric copy.
SMN2	Survival of motor neuron 2, centromeric	Mutations in the telomeric copy are associated with spinal muscular atrophy, mutations in this gene, the centromeric copy, do not lead to disease. Thought to be a possible disease modifier.
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NAIP	NLR family, apoptosis inhibitory protein	It is thought that this gene is a modifier of spinal muscular atrophy
GTF2H2	General transcription	Often deleted along with SMN1; unknown function

	factor IIH, polypeptide 2	

Xq28 duplications

Three cases had similar CNVs at Xq28 – a duplication of between 25,226 and 97,803bp (table below). Within each duplication (cases 3, 4 and 14) lies the gene *ATP2B3* (ATPase, Ca2+ transporting, plasma membrane 3). This is a calcium pump which appears to be expressed in both skin and brain.

Case	CNV	Start	Stop	Size kbp	Dist	Mrk
3	Gain	152770650	152829492	58.842	478	124
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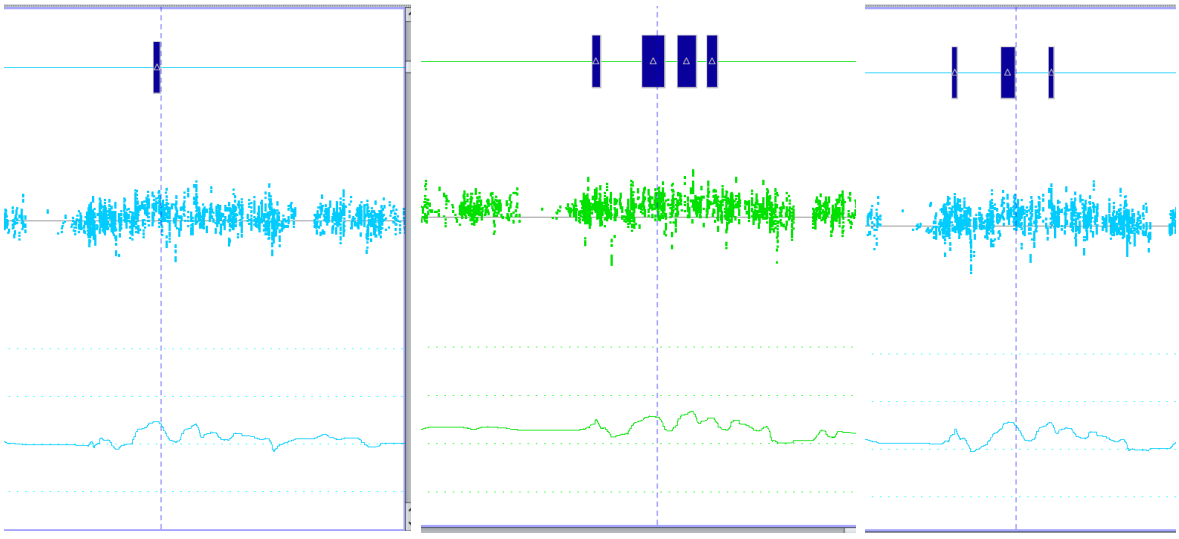
14	Gain	152804266	152829492	25.226	458	56

3, 4, 14 in order below



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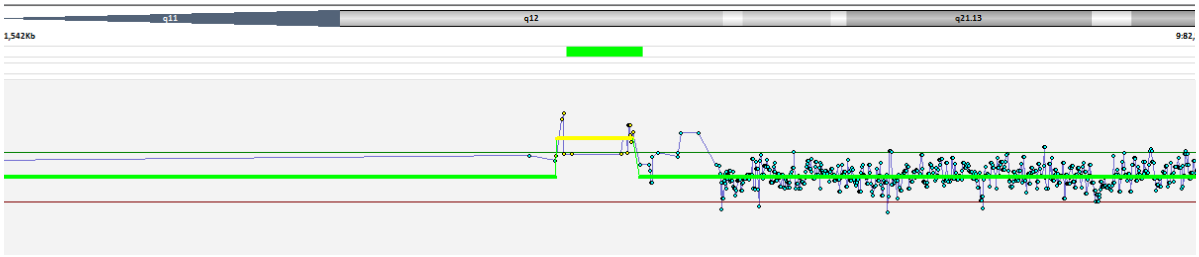

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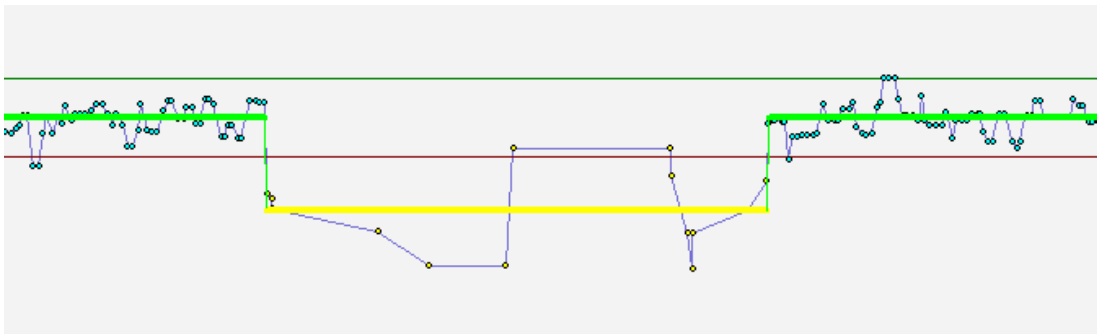

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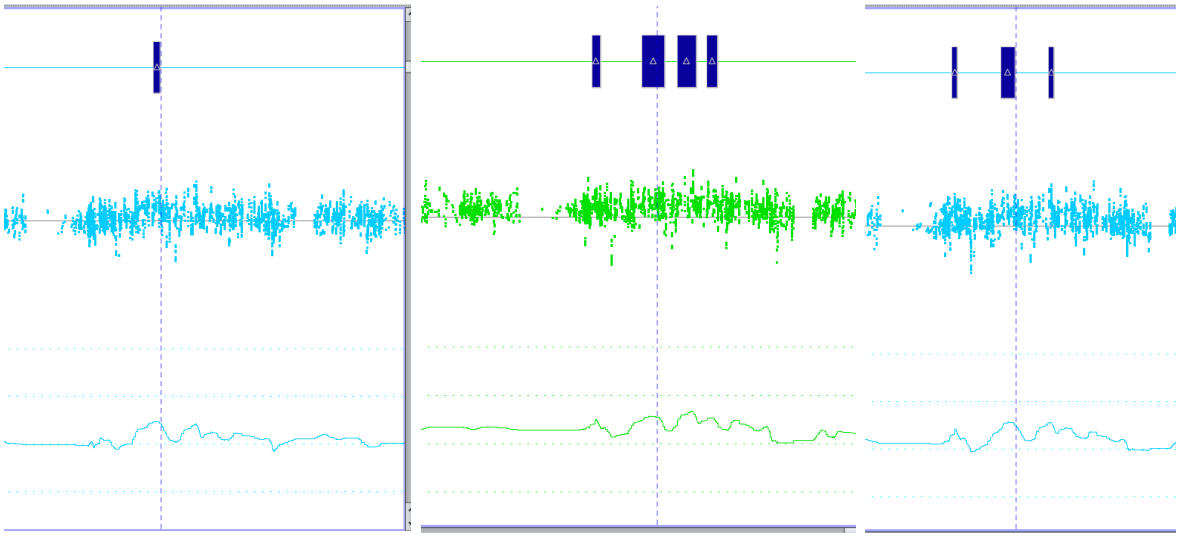

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Appendix K

Copy number variation - comparisons

Comparison

In appendix K is a comparison between CGH array and SNP genotyping. I will start by looking at an example where CGH array failed to provide an answer and then focus on the two cases which were studied with both techniques.

CGH array fails to identify a four exon deletion

Sample S4 – was known to have a homozygous partial deletion of *GLRA1* (5q32) – confirmed by *Sanger* sequencing. The weighted log2 trace from this gene is shown in figure 7.33 Exons six (151,234,738-151,234,601), seven (151,231,165-151,230,951), eight (151,208,628-151,208,482) and nine (151,202,524-151,202,074) Exons 4 to 9 were covered by just two array markers – making identification of this deletion impossible to confirm. There was no innuendo of a deletion in this region whatsoever.

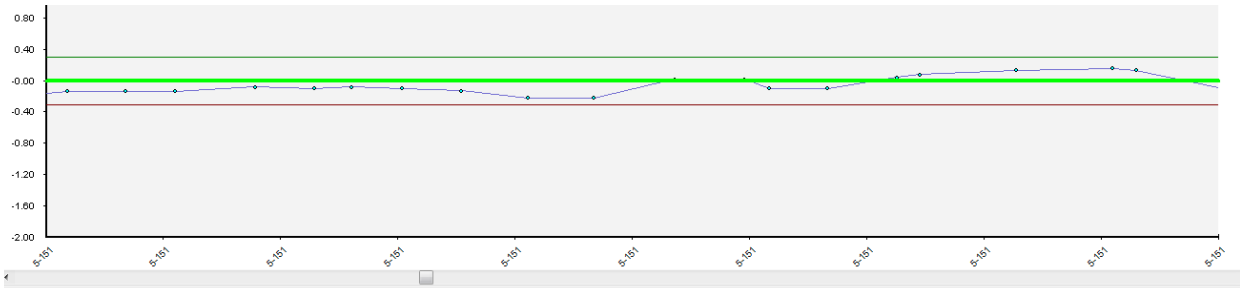


Figure Weighted log2 ratio for the CGH array of case S4 focussing on *GLRA1.*

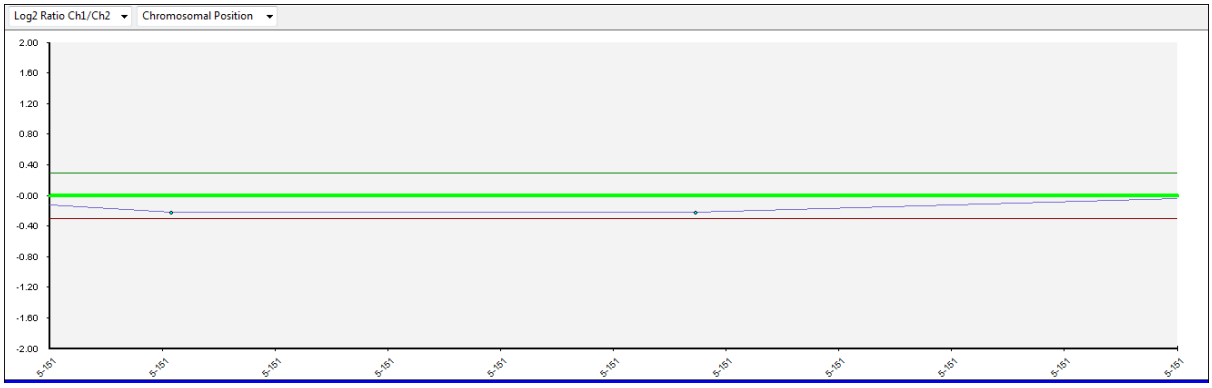


Figure Weighted log2 ratio for the CGH array of case S4 focussing on exons four to nine.

Two samples were both submitted for CGH array and SNP genotyping: samples S1 and S2 are also J26 and J34.

Samples S1 (CGH) versus J26 (SNP genotyping).

CGH array identified 14 potential CNVs. SNP genotyping identified 13 CNVs with a minimum standard of 50 markers per CNV; changing the analysis setting to require a minimum of ten markers would increase the number of potential CNVs to 83. However only three were concordant – 1p13.3 duplication, 2q12.3 deletion and 7q33 deletion (shown below). The largest CNV (2q12.3, 835 – 839 kbp) was identified with both platforms – but SNP genotyping called three of 489kbp, 359kbp, 273 kbp at 14q32.33, 22q11.22 and 1p13.3 that were not identified similarly via CGH array. The largest CGH CNVs not called by SNP genotyping were at 7q35 (203,614 bp), and 4q13.2 (78,088 bp) – proportionately much smaller.

Platform	Cytoband				Min	Max	Size	Genes
SNP	1	p13.3	p13.3	Gain	108769288	109042722	273.434	*NBPF4, NBPF6*
CGH					108590640	108824964	134.324	*NBPF5, NBPF6*
CGH	1	q21.1	q21.1	Loss	143720908	143738839	17.931	*PDE4DIP*
CGH	2	q21	p21	Gain	45025537	45025863	0.326	*SIX3*
SNP	2	q12.3	q12.3	Loss	107593216	108432565	839.349	
CGH					106956802	107792272	835.47	
SNP	3	q13.33	q13.33	Gain	121789285	121795895	6.61	*CD86*
CGH	3	q26.1	q26.1	Gain	163997258	164101806	104.658	
CGH	4	q13.2	q13.2	Gain	69057765	69116517	58.752	*UGT2B17*
CGH	4	q13.2	q13.2	Loss	70159720	70237808	78.088	*UGT2B28*
CGH	5	p15.33	p15.33	Loss	786482	848774	62.292	*ZDHHC11*
SNP	6	p12.3	p12.3	Gain	49432184	49459866	27.682	*CENPQ*
CGH	6	p21.33	p21.33	Gain	29981668	30010264	28.596	*HCG4P6*
SNP	7	q33	q33	Loss	133064191	133118605	54.414	*EXOC4*
CGH					132720780	132762671	41.891	*EXOC4*
CGH	7	q35	q35	Loss	143501841	143705455	203.614	*OR2A1, OR2A42, OR2A20P, OR2A9P, OR2A1, ARHGEF5*

SNP	8	p11.22	p11.22	Gain	39247097	39386952	139.855	*ADAM5P, ADAM3A*
SNP	11	q11	q11	Gain	55374175	55439978	65.803	*OR4P4, OR4S2, OR4C6*
CGH	12	p13.31	p13.31	Gain	9528620	9585186	56.566	
SNP	14	q32.33	q32.33	Gain	106246713	106736227	489.514	*KIAA0125, ADAM6*
SNP	16	p13.11	p13.11	Loss	15054174	15182587	128.413	*PDXDC1, NTAN1, RRN3*
SNP	17	q21.31	q21.31	Gain	44187491	44254379	66.888	*KIAA1267*
SNP	22	q11.22	q11.22	Gain	22899276	23258438	359.162	*PRAME, LOC648691, POM121L1P, GGTLC2, MIR650, IGLL5*
CGH	22	q11.23	q11.23	Loss	22677989	22715949	37.960	*GSTT1*
SNP	X	p22.33	p22.33	Gain	2368245	2393265	25.02	*DHRSX*
CGH	X	p22.33	p22.33	Loss	17607	32362	14.755	
SNP	X	p21.3	p21.3	Loss	29160655	29174711	14.056	*IL1RAPL1*

Table Comparison of CGH array and SNP CNVs. The cytobands are presented in chromosome order with CGH rows white and SNP in light blue.

14q32.33 duplication

This is a very distal segment of chromosome 14 and these are notoriously difficult to get right – however there do seem to be sufficient markers across this area (186) and manual inspection suggests that it is a convincing call. It is frequently seen using the CytoScan HD approach; 14 times in the WMRGL dataset, 26 times in EU controls and strikingly in 25 of the first 34 JME cases submitted. It is possibly an artefact – however it is seen very frequently in DGV and there are 25 DECIPHER entries (no CNV syndrome recognised). It is telling that there are no CGH markers beyond point 106358520 – making it impossible to identify this CNV. If it is genuine it appears to be benign however.

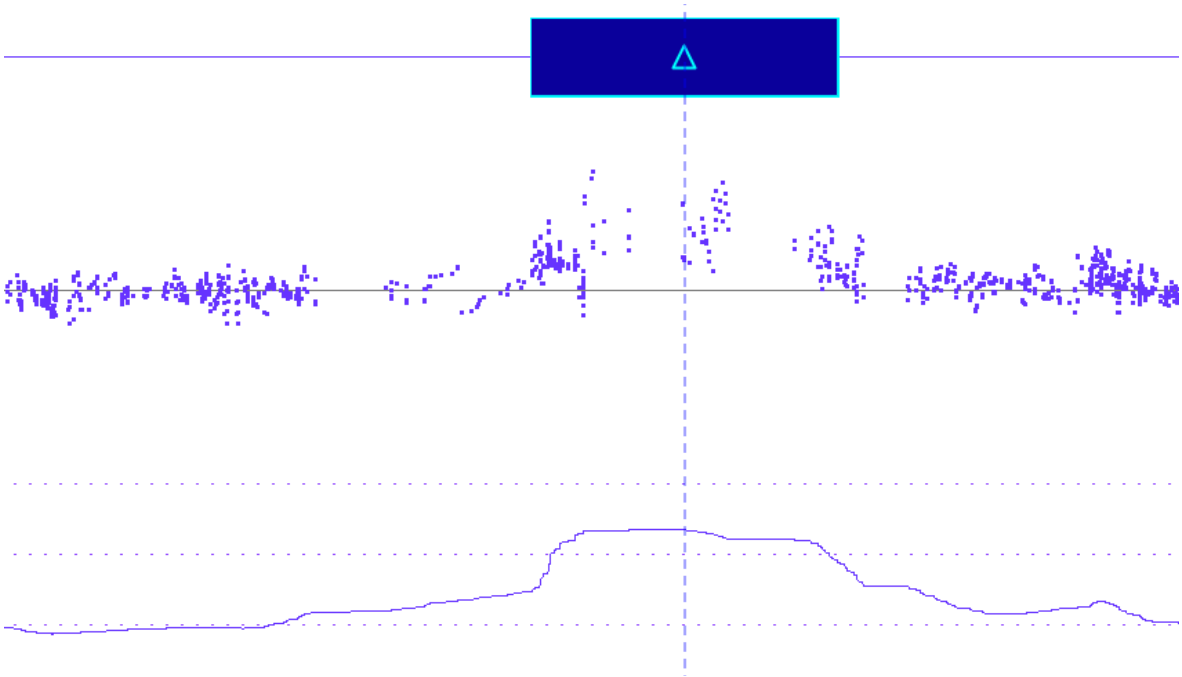


Figure SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 14q32.33 duplication in J26 (S1 - CGH).

22q11.22 duplication

Figure 7.36 shows the comparison between SNP genotyping and CGH array for the 22q11.22 duplication for J26 (S1 – CGH). Close inspection of the SNP genotyping data when zoomed in (left) is not as convincing as when zoomed out (right) and seen with the smooth signal line (bottom of two lines). The CGH array data (bottom two diagrams) are much less convincing by eye.

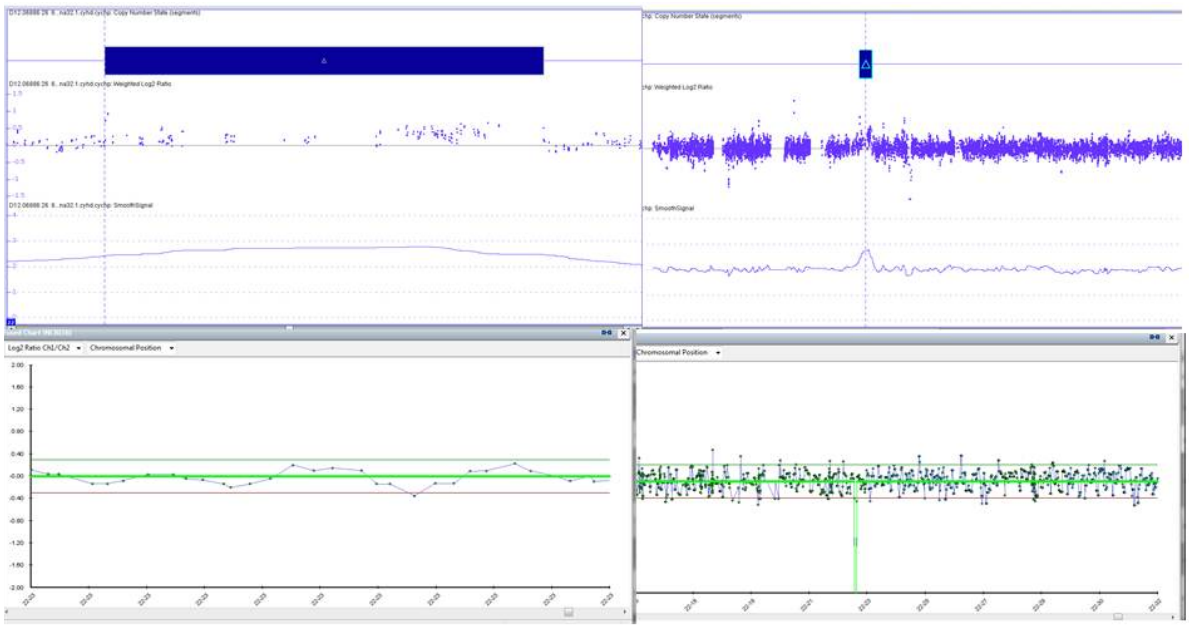


Figure (Top left) SNP probe chart and weighted log2 ratio and smooth signal line and CGH array (bottom left). The images on the right are the same areas viewed from a greater chromosomal distance.

1p13.3 duplication

The illustrations below demonstrate that the greater number of markers (103) used in SNP genotyping has been better able to map the right most end of this CNV. Here SNP genotyping probably better estimates the size of this duplication.

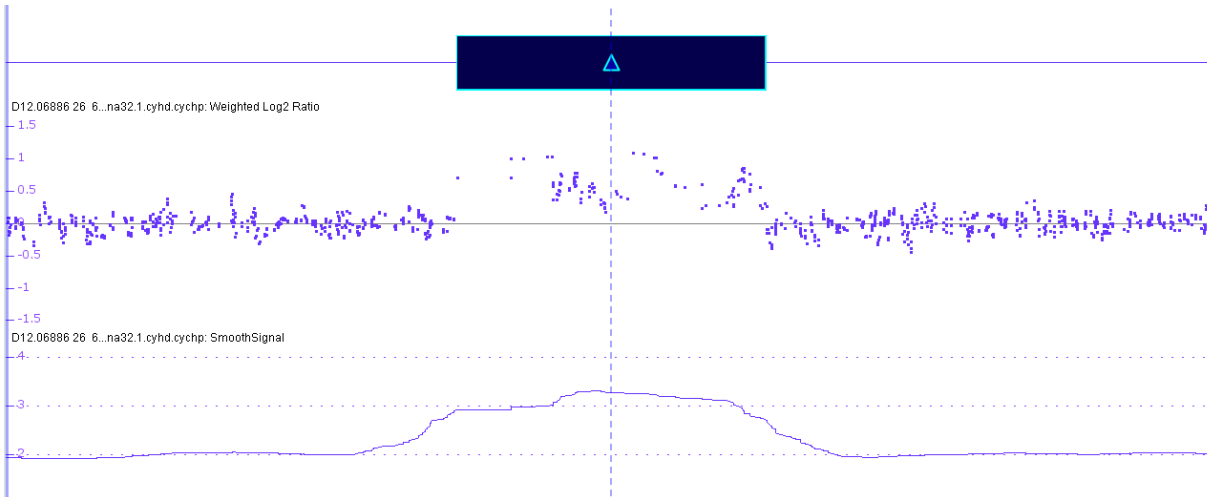


Figure SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 1p13.3 duplication in J26 (S1 - CGH).

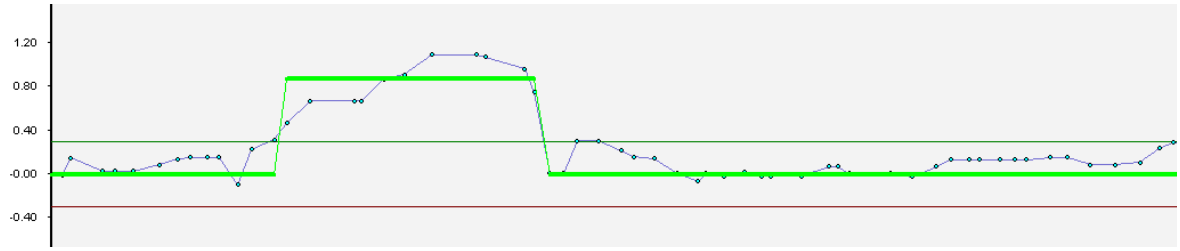


Figure Weighted log2 ratio for the CGH array of case J26 (S1 - CGH), 1p13.3 duplication.

7q35 deletion

This area on SNP genotyping is not well served by probes for a proportion (below). Although on the rightmost part of this location – the smooth signal is flat and the markers show very little scatter.

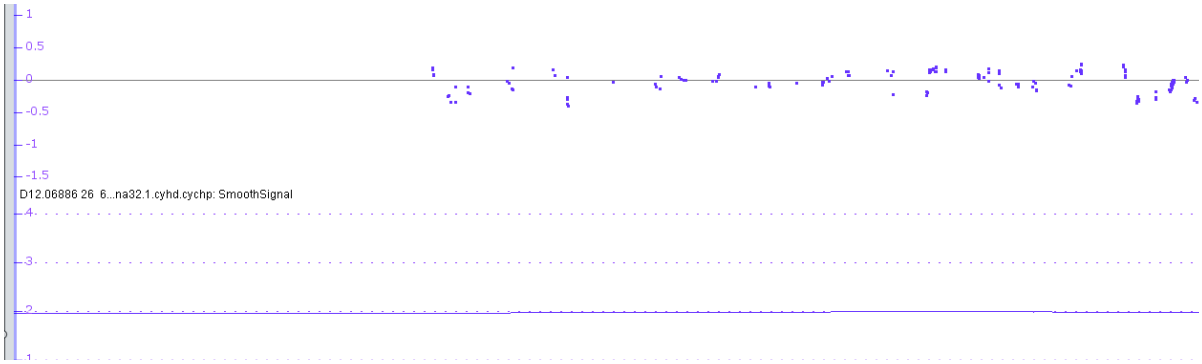


Figure SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 7q35 deletion in J26 (S1 - CGH).

However expanded views of the area clearly show that there are four markers in the CGH experiment demarcating the deletion – and good coverage either side; in comparison to no clear loss in SNP genotyping and scanty coverage in places. In this instance it is most likely a technical failure by SNP genotyping and the CNV is genuine. It is recognised as an area of variation in DGV and there are 11 entries in DECIPHER.

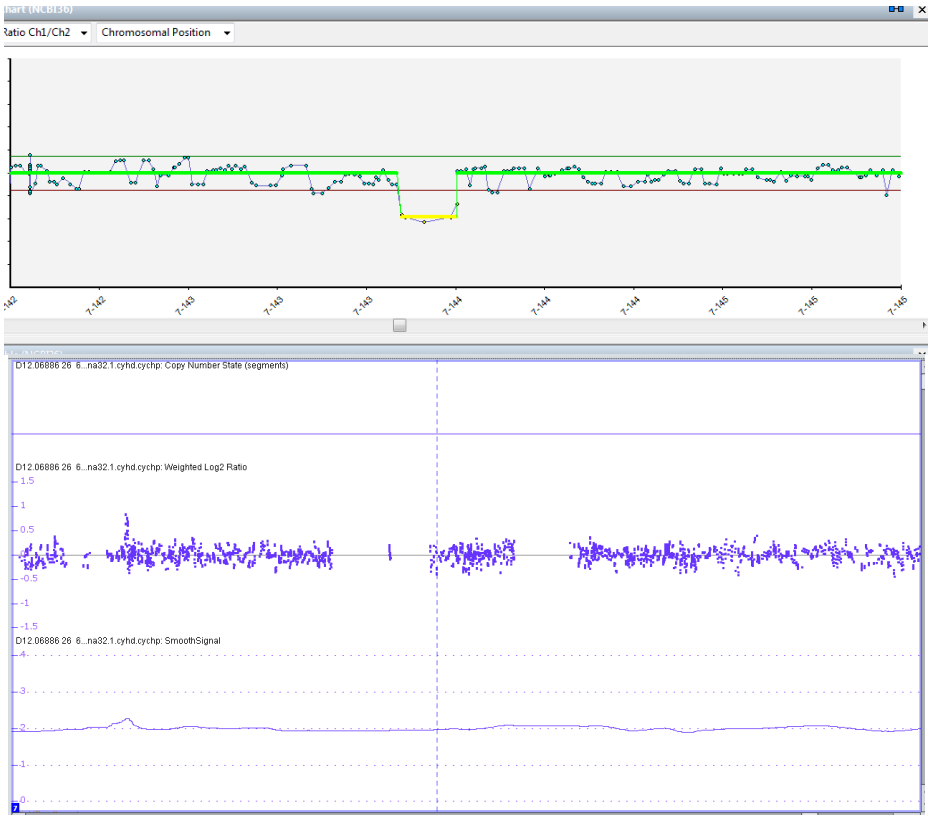
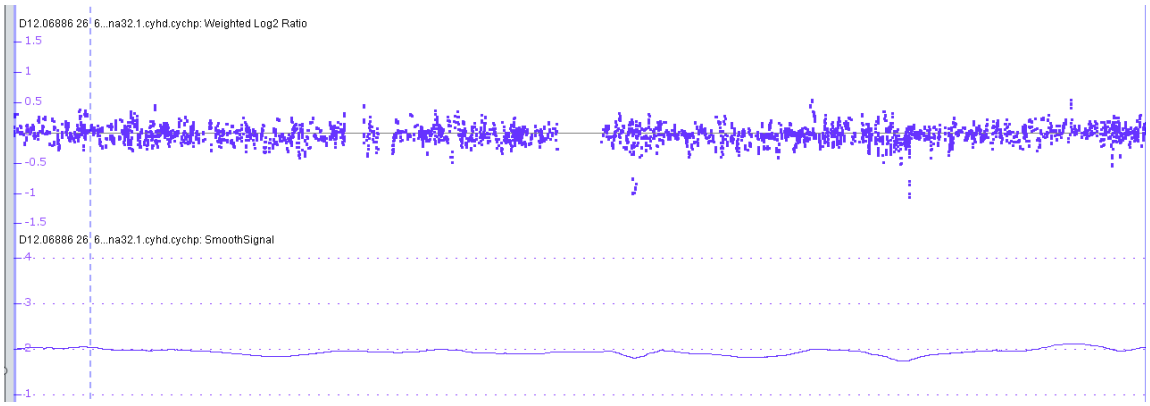


Figure Weighted log2 ratio for the CGH array of case J26 (S1 - CGH), 1p13.3 duplication (top)

4q13.3 deletion

The explanation for why SNP genotyping has failed to recognise this CNV is much easier to explain: there is not one single marker between 4: 70159720-70237808! There also appears to be a paucity of markers at the region that CGH calls a duplication nearby. However a degree of caution is needed: if an area is 'difficult to map' then it may be that CGH is not accurate and producing false calls.



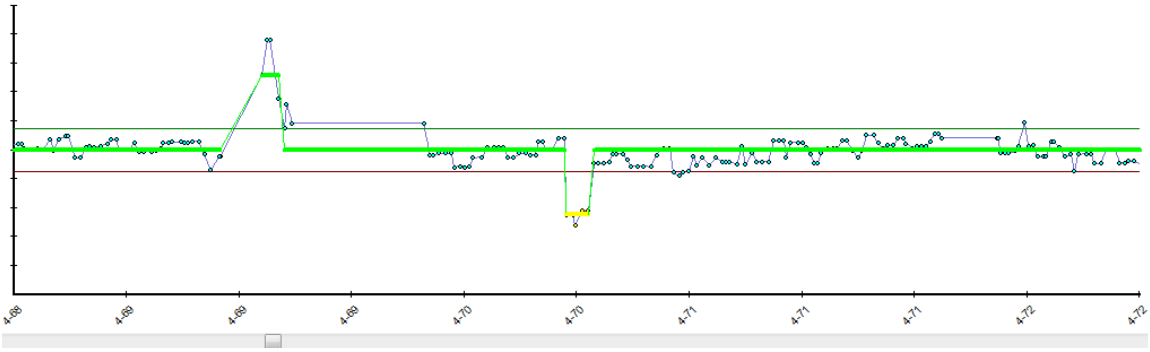


Figure Weighted log2 ratio for the CGH array of case J26 (S1 - CGH), 1p13.3 duplication (bottom) and SNP probe chart and weighted log2 ratio and smooth signal line (top) demonstrating the position of the 4q13.3 deletion called by CGH array.

Comparison (S2 with J34)

When comparing S2 with J34 – there appears to be a very similar level of discrepancy. CGH identifies 18 CNVs and SNP genotyping 16; of which only two overlap 16p11.2-p11.1 and a duplciation, Xp22.33 duplication. A large gain not seen by CGH (at distal chromosome 14) was explained above (figire 7.35). The largest CNV not identified by CGH is again at a distal chromosome – 22q11.22 (figure 7.36). The CGH array only CNVs are not necessarily smaller: there are three areas of discrepancy of around a megabase or more: 16p11.2 deletion; 15q11.2 deletion and a 5q13.2 deletion. The illustrations below suggest that the CGH array bio-informatics are a tfault for protentially 'over calling' these as CNVs.

16p11.2 deletion

The 16p11.2 deletion location is very poorly served by SNP probes – making CNV detection here impossible. Thankfully this area does not seem to be of pathogenic importance; it does not overlap the 16p11.2 CNV syndrome and there are only four overlapping cases in DECIPHER. As well as a number of pseudogenes it contains TP53TG3B (TP53 target 3B) a protein coding gene with very little known function. Four other JME cases have sufficient makers to call a deletion nearby and one a duplication.

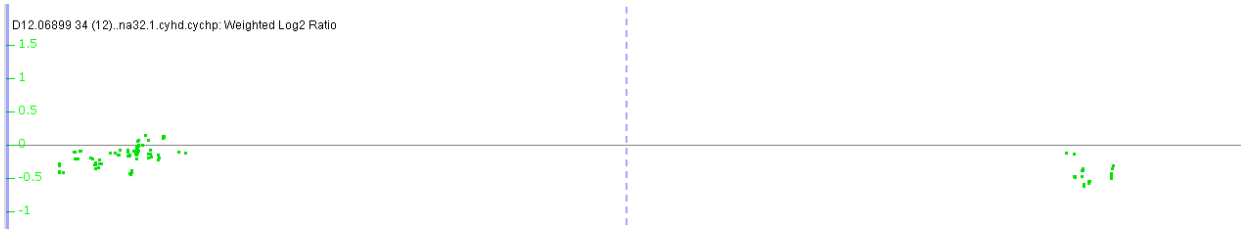


Figure SNP probe chart demonstrating the 16p11.2 deletion in J34 (S2 – CGH)

15q11.2 deletion

15: 18,884,466 - 19,786,685 is at a very gene poor region – the centromere of chromosome 15. There are virtually no SNP probes at all across this region. However the CGH trace is more convincing of a deletion here, although it has difficulties with markers for a portion of the trace (left most). The genes involved include *HERC2P2, HERC2P3, POTEB* and *POTEC*. The first two are pseudogenes; the third codes for POTE ankyrin domain family, member B and the fourth for the same family but member C.

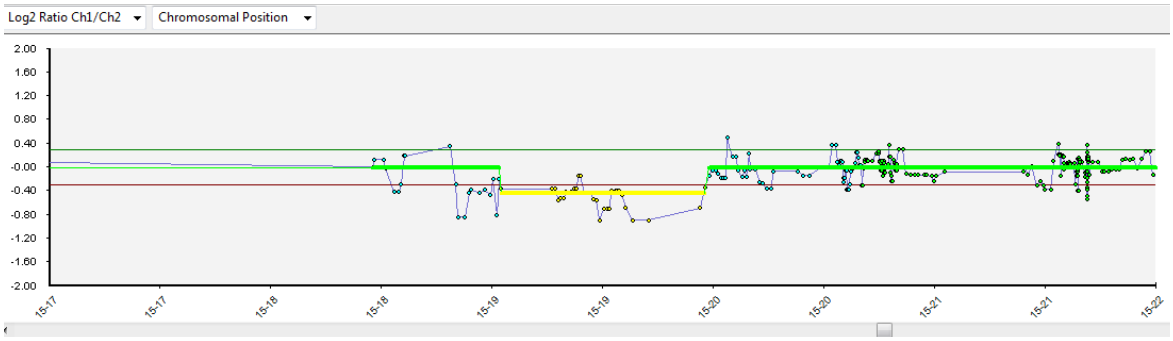


Figure SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 15q11.2 deletion in J34 (S2 – CGH).

5q13.2 deletion

This again is an area that is particularly poorly covered by CytoScan probes. This area contains a number of genes – *OCLN, GTF2H2D, GTF2H2C, GTF2H2B, SERF1B, SMN2, SERF1A, SMN1, NAIP* and *GTF2H2.* The 1.7megabase area however is also poorly covered by CGH array (below) and so this must be considered a dubious call.

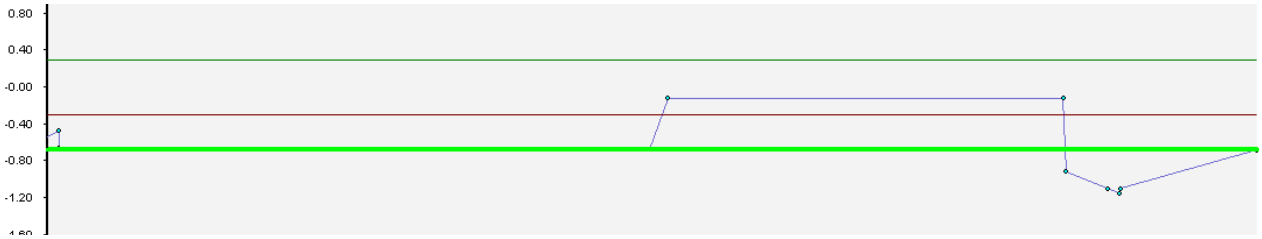


Figure SNP probe chart and weighted log2 ratio and smooth signal line (bottom) demonstrating the 5q13.2 deletion in J34 (S2 – CGH).

Appendix L

Exomic sequencing timeline

Time line

This is by no means a comprehensive or systematic review – but it gives an illustration as to how the field has advanced so rapidly over the last five years. We have chosen to focus on the theme of exome sequencing here.

Oct 2007 Selective genomic enrichment is possible - the challenges of the size and complexity of dealing with single mammalian genomes can be overcome by working with the protein coding regions. (Albert et al., 2007)

Nov 2007 Hodges and colleagues (Hodges et al., 2007) boldly state that "Increasingly powerful sequencing technologies are ushering in an era of personal genome sequences and raising the possibility of using such information to guide medical decisions." They describe their technique of focussed micro-arrays that capture the protein coding parts of the genome – approximately 200,000 exons – and identify 98% of these. The excitement is tangible within the paper as to what the future may hold.

June 2008 *Roche NimbleGen* publish a technical paper on genomic sequencing where they state they are "currently re-sequencing the complete human exome" (Droege and Hill, 2008). There is a feeling that some of the promises made following the first sequencing of the whole human genome may soon begin filtering down to multiple laboratories.

Aug 2009 Twelve human exomes are sequenced in parallel. As a proof of concept their paper is compelling; eight exomes were from people who had been well mapped using HapMap and the Human Genome Structural Variation projects – this would permit them to look at how well certain areas of the exome had been identified by the technique. They also looked at four new cases - they attempted to look at a rare presumably Mendelian disorder Freeman-Sheldon syndrome (FSS). (Ng et al., 2009) At this stage 8x coverage was achieved in 96.3% of the exome – which they claimed covered 99.7% of targeted bases. They concluded that there was both a low false positive and a

low false negative rate – and looked at how to manage copy number variation. FSS is an autosomal dominant disorder characterised by severe multiple congenital contractures; it is thought to be a monogenetic disorder and the myosin heavy chain (*MYH3*) gene was identified by the group above in 2006. (Toydemir et al., 2006) – The *MYH3* gene was correctly identified using the strategies described in the 2007 paper.

Nov 2009 The utility for identifying pathogenic genes in monogenetic disorders is established again with Miller syndrome. Using four affected – but unrelated individuals (two siblings and two unrelated kindreds) – and improving their techniques to guarantee a 40x coverage Ng and colleagues identify a single candidate gene. (Ng et al., 2010b) Miller syndrome is characterised by severe micrognathia, cleft lip/palate, hypoplasia or aplasia of the posterior elements of the limbs, coloboma of the eyelids, and supernumerary nipples. Their efficient filtering of variants lead to only *DHODH* being considered as the gene of effect – the gene encodes for the enzyme, dihydroorotate dehydrogenase. They were able to identify different pathogenic variants within the same gene – and then confirm its role as the only plausible candidate by sequencing the gene in further families with Miller syndrome and confirming that it is mutated there too. The paper correctly identified the strength of exome sequencing – you needn't have an *a priori* hypothesis about the gene(s) of effect or even about the mode of inheritance – modelling these options after the sequence data are available.

Dec 2009 Using 'serial subtraction' to identify candidate genes clearly worked – but could exome sequencing also be used for familial genetics? Hedges and colleagues studied a multigeneration family.(Hedges et al., 2009) Primarily looking for de novo variation seen in offspring but not parents the team were able to identify 47 changes suggestive of a new mutation; whereupon the suggested de novo mutation rate is thought to be nearer two per generation. Are these false positives? Their 8x coverage looks very light by current standards – which they identify as a weakness of their report

June 2010 Exome sequencing could be used when DNA is limited – such as cases of sudden death or early foetal death. The numbers needed to identify pathogenic mutations can be very small – particularly when the phenotype is exquisitely well demarcated. There were fewer than 40 reported cases of Fowler syndrome when Lalonde and colleagues published their work. (Lalonde et al., 2010b) Not only were they able to find causative variants in two foetuses – but they identified four separate variants (two per case in a compound heterozygote mechanism) is the feline leukaemia virus subgroup C cellular receptor family member 2 gene (*FLVCR2*). The Fowler syndrome in this series is the lethal disorder of prenatal children associated with hydrocephalus–hydrencephaly – not the syndrome of urinary retention as described by Professor Clare Fowler of Queen Square, London.

June 2010 Consanguinity may help researchers hone in on causative genes – but is it a barrier on exome sequencing? Walsh and colleagues studied a Palestinian family with nonsydromic hearing loss and identified a single candidate gene following extensive filtering of known SNPs from their dataset. (Walsh et al., 2010) They initially sequenced just one individual – confident that he had the same phenotype as the rest of the affected family members. *GPSM2* was identified which codes for the G-protein-signalling modulator 2 – it harboured a nonsense mutation. To demonstrate that it was a credible candidate they had to verify that it was clearly expressed in the ear. This demonstrates one of the challenges of tackling a problem without an *a priori* hypothesis – some genes that are identified do not have a large body of literature behind them, supporting their expression, roles and interacting proteins.

Aug 2010 A number of the above papers (Walsh et al., 2010), (Lalonde et al., 2010a) were assisted by previous work suggesting where to focus their work. How successful can exome sequencing be when truly blind to where to first look? Perrault syndrome is a sex-influenced disorder characterized by sensorineural deafness in both males and females and ovarian dysgenesis in females. Neurological features are seen in some individuals such as mild mental

retardation and cerebellar and peripheral neuropathy. A single family with two affected daughters was identified for study. Only one gene - HSD17B4 which encodes for 17β-hydroxysteroid dehydrogenase type 4 fitted their bioinformatics approach. (Pierce et al., 2010) After identifying that both transcripts were expressed protein modelling of the protein was undertaken. This identified that Perrault syndrome was a *forme fruste* of D-bifunctional protein deficiency – which is normally fatal in early life. Identifying that it is possible to survive with this metabolic disorder may open up further therapeutic avenues.

Aug 2010 How do exome sequencing results fit in when used as a suite of techniques and can they be used to identify genes of effect in polygenic disorders? Using a combination of family-based linkage, whole-exome sequencing, direct sequencing and association methods Bowden and colleagues identified rare variants of large effect. (Bowden et al., 2010) They were studying plasma adiponectin levels both in the population and in 240 Hispanic Americans with familial clustering of affected status. They identified that ADIPOQ (adiponectin protein coding gene) could explain 17% of the population variance of blood levels and 63% in families. The variant (G45R) that was studied was rare (1.1% population frequency) in the study population.

Aug 2010 An oft cited example of the strategy of serial subtraction of variance in unrelated individuals was in Kabuki syndrome. (Ng et al., 2010a) This is recognised as having a distinctive facial appearance, cardiac and skeletal abnormalities, immunological deficiencies and mild to moderate intellectual impairment. *MLL2*, a Trithorax-group histone methyltransferase was identified as the causative gene – however they had to use a secondary genotypic and phenotypic stratification analysis to produce this result. Initially there was a favoured gene (*MUC16*) which was shared by all ten individuals. *MUC16* is extremely large making it at risk of being identified as a false positive. Ranking the cases in terms of confidence of clinical diagnosis helped exlude phenocopies and identify *MLL2*.

Aug 2010 In addition to gene discovery – the reduction in the cost of whole exome sequencing and the analysis opens the door for personalised medicine.(Worthey et al., 2011) Rios and colleagues used WES to study an 11 month child who had very high plasma cholesterol levels – something potentially reversible if identified accurately enough and sufficiently early. (Rios et al., 2010) Their strategy was excluding common disease causing genes before attempting WES; prioritising nonsynonymous substitutions; then excluding those in dbSNP and other sources of well characterised sequenced data; then using modelling of presumed inheritance and biological plausibility to identify the candidate gene. Here they identified an atypical presentation of a known disorder – sitosterolemia caused by two nonsense mutations in *ABCG5*.

Aug 2010 The discovery of new genes of effect can help you better understand the pathogenicity of other known genes – and help widen the search for new genes. Bilguvar and colleagues used WES to look at children with cortical malformations and identified *WDR62* (WD repeat domain 62) as the compelling candidate gene. The discovery of an 'unexpected gene' necessitates a programme of functional biology to create a second or third line of evidence behind the identified gene. The discovery of the association with *WDR62* upsets some of the accepted norms believed to be central to cortical malformations. *WDR62* expression in the neocortex is transient, spanning the period of embryonic neurogenesis. Unlike other known microcephaly genes, *WDR62* does not apparently associate with centrosomes and is predominantly nuclear in localisation.

Aug 2010 Exome sequencing has a great role to play in 'genetic but not inherited disorders' – the de novo disorders such as in moderate and severe intelluectual disability. (Vissers et al., 2010) Krawitz and co-workers used biomarkers such as elevated serum alkaline phosphatase and characteristic facial features seen in three non-consanguineous siblings to identify the causative gene. (Krawitz et al., 2010) *PIGV,* the second mannosyltransferase in the GPI anchor biosynthesis pathway, is of particular interest because

alkaline phosphatase is a GPI-anchored protein. This discovery was later replicated in three unrelated kindreds.

Sep 2010 In a story reminiscent of the DBP-deficiency children and their *forme fruste* above (Pierce et al., 2010) – identification of the genetic cause of the disorder immediately puts the eponymous syndrome into its biological context: this was the story with Sensebrenner syndrome. (Gilissen et al., 2010) The disorder is synonymous with cranioectodermal dysplasia and compound hetereozygous mutations in *WDR35* were the cause in two cases (sequenced by two separate teams). *WDR35* is homologous to *TULP4* (from the Tubby superfamily) and has previously been characterized as an intraflagellar transport component, confirming that Sensenbrenner syndrome is a member of the ciliary disorder family.

Oct 2010 Moving away from a small number of sequenced exomes in rare disorders – Li and colleagues sequenced 200 individuals from Denmark (12x coverage) to look at the patterns of SNP frequency to inform future bioinformatics. (Li et al., 2010) Even if the mean read coverage was 12x – it is not equally covered across the exome (60% here had 10x or more) they felt that the sequencing depth was not adequate to call genotypes of each individual accurately. Instead they developed a SNP calling and frequency estimation method based on population data that simultaneously used genotype likelihood information from all individuals to detected SNPS with a low (2%) false positive rate. They later explored themes of natural selection on protein coding genes.

Nov 2010 Some disorders – such as Charcot-Marie-Tooth and the spinocerebellar ataxias (SCA) already have a large number of known genes associated with them; currently – to identify a gene to screen one would need to use subcategorisation of phenotype or inheritance – or know the frequency in a certain ethnic group – to create a strategy for testing.(Erlich et al., 2011) However as many disorders present atypically it may be more cost effective to use WES initially – it also enables new gene discover such as in the paper

by Wang and colleagues. (Wang et al., 2010) Here *TGM6* was identified initially in a four-generation Chinese family and replicated in an unrelated kindred. Transglutaminase 6 produces an autosomal dominant SCA – and this was its first link to a pathology in man.

Dec 2010 The animal models of motor neurone disease may not represent fully the pathogenesis in humans – a better understanding of the family of genes associated with this devastating disorder in man will help develop more complete models. Johnson and team identified the *VCP* gene co-segregated with phenotype in an Italian family with autosomal dominant motor neurone disease. (Johnson et al., 2010) Valosin-containing protein variants were then screened for in a further cohort of 2880 individuals with motor neurone disease. Four more cases were identified – confirming a rare but important cause of MND.

Jan 2011 Many teams had identified WES' potential for identifying susceptibility variants or pathways for tumourgenesis. A *Nature* paper from Varela et al. looked at renal cancer specifically. (Varela et al., 2011) Their conundrum was that the known clear cell carcinoma related genes were only identified in less than 15% of cases. They identified variants in the SWI/SNF chromatin remodelling complex gene *PBRM* in a remarkable 41% of cases. This discovery came from sequencing just seven cases initially – however they went on to screen 227 in a validation cohort.

Jan 2011 Can exome sequencing and subsequent analysis keep up with the demands of a genetics service? How quickly can results be returned to the clinical domain? As discussed above there are over 35 known genes associated with Charcot-Marie-Tooth and serial (or parallel / batch) testing of these even using pragmatic or clinical algorithms would take many months. WES was used to identify variants in a known gene (*GJB1*) in a CMT family by sequencing the exomes of someone with a clear phenotype and one with an ambiguous clinical pattern. (Montenegro et al., 2011) The whole project took eight weeks in total.

Feb 2011 Sometimes the pattern of inheritance identified can add to the clinical phenotype – such as uncovering a potential founder effect. Whilst studying a family with retinitis pigmentosa, Zuchner and colleagues named *DHDDS* (dehydrodolichol diphosphate synthase) as the gene of interest – but furthermore the variant most likely arose from an ancestral founder, because eight of the nine identified alleles in 27,174 control chromosomes were of confirmed Ashkenazi Jewish ethnicity. (Zuchner et al., 2011)

March 2011 Animal models are still important when novel genes are postulated through WES; however in this paper copy number variation analysis identified the gene when exome sequencing could not. The zebrafish model of dilated cardiomyopathy helped link the BCL2-associated athanogene 3 (*BAG3*) with the phenotype after exome sequencing and genome wide copy number analysis in a large family produced a candidate gene. (Norton et al., 2011)

Mar 2011 Further illustration of exome sequencing's ability to identify atypical presentations of known (but rare) disorders – and rapidly enough for clinical practice- were given by Tsurusaki and colleagues. (Tsurusaki et al., 2011) In a child with X linked leucoencephalopathy common genes were sequenced traditionally and no cause was identified. M*CT8* associated leucoencephalopathy was discounted as the boy had normal thyroid function and *MCT8* is normally associated with a dysthyroid state. Rapidly identifying the nonsense mutation in *MCT8* helped widen the phenotype and was diagnostic in this family.

Apr 2011 The main reasons to identify genes associated with cancers are to i) understand pathogenesis, ii) look for markers of prognosis and iii) identify novel mechanisms to target treatment. The discovery therefore of mutation at a single point in *TRRAP* in 6 out of 67 people with melanoma and *GRIN2A* (mutated in 33% of samples) represents a great leap forward. (Wei et al., 2011) *TRRAP* encodes the transformation/transcription domain-associated protein, whereupon *GRIN2A* (glutamate (N-methyl-(D)-aspartic acid (NMDA)) receptor subunit ε-1) is part of the class of ionotropic glutamate receptors

and bears the agonist binding site for glutamate. This is a fascinating link between melanoma and the glutamate signally pathway and one that could perhaps be modulated to help improve prognosis from a highly lethal cancer.

May 2011 Confirming that 'psychogenic' or 'psychiatric' disorders have a firm genetic underpinning helps to reduce stigma. A study of a single family with Tourette's syndrome could narrow down the potential genes to three: *MRPL3, DNAJC13*, and *OFCC1.* They discovered variants in two patients from an unrelated kindred who harboured mutations in *OFCC1*, strengthening its biological case. (Sundaram et al., 2011)

May 2011 Some disorders (see CMT again above) have many disease associated genes and therefore the underlying biology of the disorder is well known; others have either scanty or absent genetic support and the discovery of the 'first genes' can kick-start the search for new therapeutics. Saarinen and colleagues studied nodular lymphocyte predominant Hodgkin lymphoma with familial clustering discovering that *NPAT* (nuclear protein, ataxia-telangiectasia) segregated with phenotype. In unrelated individuals a point mutation in *NPAT* increased the risk of developing the disorder four fold.(Saarinen et al., 2011)

June 2011 How many people with a sporadic complex genetic disorder do you need to sequence to discovering meaningful results? O'Roak and colleagues sequenced 20 individuals with autistic spectrum disorder. (O'Roak et al., 2011) They identified 21 de novo mutations, of which 11 were protein-altering and 4 of these 11 were deemed to be causative. The genes were *FOXP1, GRIN2B, SCN1A*, and *LAMC3*; interestingly they were identified in the phenotypically more severely affected individuals. Furthermore one of the individuals (with a *FOXP1* mutation) had a second gene that looked likely to be relevant – with a rare inherited *CNTNAP2* mutation. It would be expected in candidate gene screening that you would stop sequencing once a likely gene had been identified; exome sequencing permits you to look beyond this at multigenic disease, disease modifying variants and polygenic disorders.

June 2011 What's the best 'control' for an exome? One of the published online datasets? Internal controls? How about the same individual. Sequencing leukemic and normal cells from the same individual identified five potential genes – one of which (*BRAF*) was a known oncogene. Astonishingly 47 further patients with hairy cell leukaemia were evaluated and the V600E mutation was present in all of them (but 0/195 of other types of leaukaemia). (Tiacci et al., 2011)

June 2011 The era of personal genomics (or exomics) is most exciting when there are a smorgasbord of therapeutics – drugs, devices and surgery – which ones would best suit the individual? Using a targeted strategy focusing on 237 channels in idiopathic epilepsy Klassen and colleagues described an attractive model of how one might give a personalised multi-gene diagnosis to an individual using massively parallel sequencing. (Klassen et al., 2011) Someone's susceptibility to seizures may be part of a continuum of normal and therefore 'risk' SNPs may be seen in the normal population. For example looking at GABA receptor subunits specifically 24% of cases had two or more non synonymous SNPs in one of the six GABA receptor alpha subunits (6.5% of controls) and in the three beta subunits 14.5% of cases had two or more nsSNPs compared with 5% of controls.

July 2011 In terms of the clinical context exome sequencing is evidently suited to catastrophic disorders of infancy – but can it also identify variants in later onset conditions? If so this would clearly enable pre-symptomatic testing. Zimprich and colleagues investigated a single Austrian family with sixteen individuals who had late onset Parkinson's disease (PD). (Zimprich et al., 2011) A single missense mutation in the *VPS35* gene was identified in the seven surviving affected family members. Two second cousins were sequenced (genetically distant – but phenotypically similar). One variant also cosegregated with late-onset PD in three unrelated families. *VPS35* is a component of the retromer complex and is involved in retrograde transport from the endosomes back to the trans-Golgi network.

July 2011 Could exome sequencing help explain historical cases and previous oddities? Proteus syndrome is one of the conditions that 'The Elephant man' was thought to have had. 26 of 29 people with a clinical diagnosis of Proteus syndrome had a single mutation in a known oncogene *AKT1*. The syndrome of organomelgaly and tissue overgrowth is particularly non-uniform adding to the 'lopsided' and unsightly clinical picture. Lindhurst and colleagues identified that the mixture of mutant alleles varied in the tissues studied from 1% mutants to 50% - perhaps explaining the asymmetry. (Lindhurst et al., 2011)

July 2011 What do to with unexpected findings? The ethics of whole genome research had been well rehearsed – but this paper describing a group's experiences of unrelated findings is a good example of the new challenges that teams found themselves exposed to. (Lyon et al., 2011) They discovered when they were looking for rare variants that may predispose towards ADHD one case of idiopathic haemolytic anaemia. Many other possible genetic diagnoses may be preventable, or have implications for the wider family. We discuss this in full below.

July 2011 Not every gene discovery is deleterious or in a rare disorder. The study of head and neck squamous cell carcinomas identified *NOTCH1* as a potential tumour suppressor gene. (Agrawal et al., 2011) Specifically studying those without environmental risk factors (tobacco smokers, alcohol drinkers, negative for the human papilloma virus) they found 28 *NOTCH1* mutations in 32 individuals. The work confirmed previously identified associations with genes and reported a novel link with *FBXW7* (a member of the F-box protein family and constitutes a component of the ubiquitin protein ligase complex). *FBXW7* has itself been recognised as a tumour suppressor gene previously.

Aug 2011 Schizophrenia has been a notoriously difficult 'nut to crack' using traditional genetic methods – but Xu and colleagues reported some success using WES. (Xu et al., 2011) 53 people without a known family history of schizophrenia were studied yielding 40 *de novo* variants in 27 individuals (but 7 out of 22

controls also harboured *de novo* mutations). Crucially all mutations occurred in different genes – demonstrating just how genetically heterogeneous what we call schizophrenia really is.

Aug 2011 Alongside all the excellent clinical and translational work are leaps forward in the bioinformatics approaches. Although these are outside the scope of this work it would be churlish not to recognise them. For example the ability for WES to detect copy number variation is a welcome one. The accuracy depends on depth-o-coverage and the design of the capture probes. (Sathirapongsasuti et al., 2011)

Sep 2011 Does exome sequencing have a role in mitochondrial disorders? Galmiche and team studied a family with four siblings with a mitochondrial cardiomyopthay. (Galmiche et al., 2011) They identified that the siblings were compound heterozygotes for a missense mutation and a large deletion affecting *MRPL3*. A single case was whole exome sequenced, all four had SNP genotyping. *MRPL3* codes for a mitochondrial ribosomal protein and this was the first report of human pathology associated with this gene.

Sep 2011 Even if multiple genes are identified within the same disorder – it can provide an opportunity. The discovery that multiple genes within the RNA splicing machinery were mutated in 29 people with myelodysplastic syndromes was a novel one. These findings were frequent (up to 85% of the sample) and only found in MDS. The 3'-splice site appeared to be particularly vulnerable. (Yoshida et al., 2011) Here WES helped identify an unusual mode of action – specifically affecting major splicing components.

Oct 2011 Dystonia can be a challenge to treat and often of unknown cause. The identification that *GCDH* (glutaryl-CoA dehydrogenase) deficiency is the cause in a nuclear family after WES of just one sibling is very promising. (Marti-Masso et al., 2012) This produces a nice biomarker (GCDH activity) to enable quasi-genetic screening of generalised dystonia.

Nov 2011 Another movement disorder had its origins unravelled via exome sequencing. Paroxysmal kinesigenic dystonia is now known to be caused by mutations in the PRRT2 gene (coding for proline-rich transmembrane protein 2). (Chen et al., 2011) Features that reassure teams trying to demonstrate pathogenicity: it co-segregated exactly with affected status; mutations were not seen in 1,000 healthy controls; it was replicated in other unrelated kindreds. It was also a finding discovered independently by others. (Wang et al., 2011)

Dec 2011 How often are variants missed by WES but identified by other techniques? No approach to a complex problem will be flawless and often the best results occur when techniques can be combined and cross referenced. Bloch-Zupna and colleagues report the value of homozygosity mapping and candidate prioritisation alongside exome sequencing. (Bloch-Zupan et al., 2011) The family were highly consanguineous and further understanding of the role of the candidate gene (*SMOC2*) in zebra fish helped add authority to the delineation of pathogenesis.

Dec 2011 Sometimes a disorder can be so linked to its causative gene that researchers overlook it as a possible cause of other pathologies; this was the case between *NOTCH3* and CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). However WES of an individual – part of a Turkish family with clinical Alzheimer's disease identified a mutation in *NOTCH3* as the likely causative variant. (Guerreiro et al., 2012) Although this finding was not replicated in other unrelated individuals – it is undoubtedly true that hypothesis neutral WES is more likely to identify atypical presentations of known disorders and widen the phenotype associated with established genes.

Dec 2011 Chronic lymphocytic leukaemia (CLL) is the most prevalent leukaemia seen in developed health care systems. An ambitious study of 105 people with CLL identified 1246 mutations that could be pathogenic (excluding those in immunoglobulin loci) – 78 of these were recurrent (seen in more than one case). Quesada and co-workers sifted through this detailed and valuable

dataset to identify *SF3B1* as a gene that demands further attention. (Quesada et al., 2012) Of note there was a median of 45 somatic mutations per case (1100 different genes in the study population). *SF3B1* encodes a subunit of the spliceosomal U2 snRNP. *SF3B1* does not seem to be specific for CLL however – being mutated in rare cases with breast, pancreatic cancers and malignant melanoma.

Dec 2011 The true 'agnostic approach' for identifying variants using exome sequence – with *no a priori* hypothesis – can be skewed by the way one chooses to exclude or prioritise genes due to their biological plausibility. Ramagopalan and colleagues chose to focus on *CYP27B1* because some – but not all – people who study multiple sclerosis are intrigued by the association between vitamin D levels and myelination. (Ramagopalan et al., 2011) *CYP27B1* was one of over 58,000 variants seen per individual and was seen in just one of the 43 individuals who were sequenced with familial MS. However to strengthen their story they then looked for validation in a larger (over 12,500 people) cohorts. Although gene variants were not seen in controls – they were present only at a very low level in people with MS (less than 1% of those with the condition). Association has been demonstrated – the evidence for causation is not yet there.

Jan 2012 Some kindreds have been published and described – too small to have their causative genes identified with linkage analysis; too rare to be recruited into genome wide association studies – just waiting for the right technique. Therefore when Velinov and team started to analyse the eight generations of the 'Parry family' they must have hoped that exomic sequencing could explain what was causing Kufs disease, (an adult-onset form of NCL –one of the neuronal ceroid lipofuscinoses). (Velinov et al., 2012) Their discovery was that mutations in *DNAJC5* are associated with autosomal dominant Kufs. They WES sequenced three people from the family and reduced 14,683 potential variants to 111 by filtering through dbSNP and then focusing on those with the most promising GERP conservation scores; this yielded 8 first rank targets. GERP (Genomic Evolutionary Rate Profiling) is a statistically

rigorous and biologically transparent framework for identifying constrained elements. Furthermore they looked at copy number variation and 109 of the 5,464 variants that segregated between the three exomes were in coding regions or areas that may affect splicing. Only four were not in dbSNP and in one of these four was the gene *DNAJC5.* This gene had previously been linked to the phenotype of interest. The Parry family had additional features – and the authors postulated that there may be a mixing of phenotypes as the gene *PRPF6* (associated with retinitis pigmentosa) was mutated in many affected family members.

Jan 2012 Rare disorders are not just a fringe novelty as the elucidation of what underlies these disorders can either help us better understand biological systems; or the disorder may have an increased frequency in certain genetically isolated groups. The Amish and Mennonite people are descended from groups of German/Swiss immigrants who have a cultural and religious practice of marrying from within their communities for over 12 generations. They are currently located in specific locations such as Pennsylavnia, USA or Belize. This population should therefore be at risk of recessive, homozygous disorders – but the bioinformatics pipelines need to be efficient as individuals may be homozygous for many allelic variants. Puffenberger and colleagues identified associated genes for seven disorders, six of them novel. (Puffenberger et al., 2012) This approach – and the mapping of allelic frequency within specific communities can help pre-conceptual counselling, parental testing and help provide targeted advice. The accuracy of diagnosis to aid targeted advice is all the more important when dealing with communities who have specific cultural practices.

Jan 2012 The genetics underpinning hypertension will undoubtedly be complex – but cracking this will have important morbidity and mortality implications and help usher in an era of rational therapeutics. Boyden and colleagues identified 52 families who had pseudohypoaldosteronism type II (PHAII). (Boyden et al., 2012) After exluding known associated genes (*WNK1* and *WNK4)* they performed WES on eleven unrelated individuals; 23 genes

harbouring variants were shared by five or more individuals. *KLHL3* was identified as the strongest candidate and this lead the team to then look at *CUL3* – as it's a presumed functional partner of *KLHL3*. It is reassuring to know that all individuals with PHAII associated with *WNK1*, *WNK4*, *KLHL3* and *CUL3* mutations are helped by thiazide diuretics.

Feb 2012 Can exome sequencing as a first line investigation help identify a range of causes for heterogeneous disorders? Is it cost effective enough yet and swift enough to be a front line diagnostic test? Dias and colleagues looked at 88 genes in 125 people presenting with myopathies or stiff-man syndrome (hereditary spastic paraplegia). (Dias et al., 2012) They used only 20x coverage and therefore the mean coverage of bases dropped off somewhat (perhaps as much as 5% of the exome was not covered at all); they conclude that additional *Sanger* sequencing may be necessary for some genes that are not easily covered by WES. This technique has a number of major advantages over traditional sequencing: it was cheaper; it has the potential to identify new genes; the dataset can be reinterpreted later if needed.

Feb 2012 What is the best strategy for new gene discovery in patients with rare disorders? Many causes of intellectual disability and brain malformation are presumed to be *de novo* – and a trio design has been used with great success by authors such as Rivière and colleagues. (Riviere et al., 2012) They used three proband-parent trios to identify *ACTB* and *ACTG1* as causes of Baraitser-Winter syndrome (BWS). This was validated in 15 further probands – identifying a cause in all affected children studied. This study was made possible by initial high quality dysmorphology -using the characteristic features of congenital ptosis, high-arched eyebrows, hypertelorism, ocular colobomata and anterior predominant lissencephaly to make a syndromal diagnosis of BWS.

Feb 2012 Bioinformatics and software predications can only take you so far when assessing the potential pathogenic consequences of a variant. You have to also ask yourself – is this biologically plausible; are there too many variants

for them all to be pathogenic? The population prevalence of long QT syndrome (LQTS) is known to be between 1 in 2000 and 1 in 5000. However when looking at a meta-analyses of 5,400 sequenced exomes 33 variants were identified in 173 alleles – this would project to a population estimate for LQTS of 1 in 31. As the group who had been sequenced were not selected for arrhythmia, sudden death or LQTS trait – care must be taken not to over-interpret results say Refsgaard and colleagues. (Refsgaard et al., 2012) This more than any previous paper we've discussed explains the need for segregation, clinical interpretation of results and detailed functional analysis. Specifically protein modelling and *in silico* chemistry will have to help researchers interpret variation when it occurs in critical areas – such as these polymorphous channel genes.

Mar 2012 False positives are the demons that stalk this type of association research – more so when the size of the exome is taken into account. Can you be certain that the variant identified really is pathogenic and causal for the phenotype of interest? Fajardo and team drew our attention to a number of situations whereby variants may be falsely called. (Fuentes Fajardo et al., 2012) Some genomic regions are highly polymorphic; some have characteristics which suggest assembly misalignment; some are labelled as variants based in misleading reference genome information. Of great interest to researchers trying to weed out unlikely results are the 23,389 positions with excess heterozygosity suggestive of alignment errors and 1,009 positions in which the hg18 human genome reference sequence appeared to contain a minor allele identified in this paper.

Mar 2012 Linkage analysis had previously permitted researchers to narrow down genes responsible for phenotypes to small loci – such as 14q32 for an autosomal dominant spinal muscular atrophy. (Harms et al., 2012) Targeted exome sequencing was therefore focussed on the 73 genes in this region to identify *DYNC1H1* as the gene of effect. This gene has been recently associated with Charcot-Marie-Tooth and learning difficulty – demonstrating the breadth of

the clinical phenotype associated with mutations and the tail domain of the heavy chain of cytoplasmic dynein.

Apr 2012 *De novo* mutations are often explained to parents as a genetic cause for their child's problems – but not one that their child had inherited. It appears that *de novo* mutations are an important cause of autism. (Sanders et al., 2012) A huge undertaking – performing WES on 928 individuals identified 278 de novo coding mutations. Some (such as *SCN2A* – coding for the alpha subunit of the sodium channel, voltage-gated, type II) were recurrent. Perhaps more surprising is that recurrence was so rare: 125 non-synonymous variants were identified in 200 quartets; only 15 of which were nonsense (5 of these were in siblings rather than probands). All five occasions where a canonical splice site affected were seen in probands. Interestingly the rate of de novo variants associated with autistic spectrum disorder increased with paternal and maternal age. Alongside *SCN2A* the authors reinforce the importance of *KATNAL2* (katanin p60 subunit A-like 2) and *CHD8* (chromodomain helicase DNA binding protein 8).

Apr 2012 Some findings are difficult to report because so little is known about the gene associated with the phenotype; an exciting place for 21st century genetics to be. A recent paper by Hovarth and co-workers not only described a novel phenotype (iron accumulation with dystonia, optic atrophy, and peripheral neuropathy) but a gene not yet associated with human disease (chromosome 19 open-reading frame 12 gene). (Horvath et al., 2012) After excluding the main causes of iron accumulation by sequencing *PANK2* and *PLA2G6*, and exon 4 of the FTL1 gene the team whole exome sequenced two brothers. The team conclude by stating that further functional validation and sequencing of *C19orf12* is needed in an unrelated cohort of individuals with a similar phenotype.

Apr 2012 How to keep on top of this explosion of studies? Xia and team developed a database of next generation sequencing studies which they call the Next Generation Sequencing Catalog;

http://bioinfo.mc.vanderbilt.edu/NGS/index.html (Xia et al., 2012) This is not just a dynamic repository to help teams who are searching through large datasets – but it provides access to software. This project includes both whole exome and whole genome data and is an example of the kind of collaborative project which will help diagnostic and research focused projects over the next five years.

Apr 2012 The break-neck speed of WES papers being published means that relatively contradictory papers can be published in the same month with little in the way of cross commentary. One such case is a paper by Iossifov and team compared with Sanders et al above. (Iossifov et al., 2012) (Sanders et al., 2012) Iossifov's paper also looked at hundreds of children with autistic spectrum disorder (343 families) – but they found fewer *de novo* variants and in their study paternal age specifically was associated with *de novo* variants. That said they identified upwards of 350 potential susceptibility genes; in and of itself a life time of work using current techniques.

May 2012 So WES has demonstrated its ability to identify rare coding variants in complex phenotypes; how does it cope with complex traits? Large scale genome wide association studies have demonstrated that common variants have a modest effect at best and a sample size exceeding 10,000 is often needed. Kiezun and colleagues estimate that 10,000 exomes will be required to achieve sufficient power to robustly detect associations of rare variation with complex traits. (Kiezun et al., 2012) They also suggest that focussing solely on the exome is a methodological mistake.

May 2012 We end this time line with a hugely ambitious project: sequencing 15,585 genes to 111x in 2,440 people. (Tennessen et al., 2012) The mean individual harboured 15,595 single neucleotide variants (SNV); the majority (86%) were rare (frequency of <0.5%) and novel (82%). Of importance 2.3% of these SNVs were predicted to alter protein function – which equates to 313 genes per genome. From an evolutionary point of view 'explosive' recent population growth was thought to be the cause of this excess of rare functional variants.

The successes of genome sequencing

It is beyond the scope of this chapter to describe all the whole genome sequencing sequencing (WGS) papers in depth. However it would be churlish not to illustrate a few examples of the increased power of the whole genome. One such example is in examining balanced translocations as a cause of disease. Talkowski and colleagues sequenced 38 individuals with autism or related developmental delay using WGS and identified 33 loci. (Talkowski et al., 2012) They divided their discoveries in to four main categories: known genes; single genes associated with microdeletion syndromes; novel loci; genes associated with later onset psychiatric disorders. In order these included *AUTS2, FOXP1, CDKL5; MBD5, SATB2, EHMT1 AND SNURF-SNRPN; CHD8, KIRREL3, ZNF507;* and *TCF4, ZNFF804A, PDE10A, GRIN2B, ANK3.* As well as conclusively demonstrating the polygenic nature of autism (the genes sit in differnet gene systems and gene families), the paper suggests a genetic link between autism and schizophrenia. The other clear advantage of WGS is the ability to detect intronic variants. One such example is the identification common intronic deletions in the gene encoding BCL2-like 11 (*BIM*). *BIM* is a pro-apoptotic member of the B-cell CLL/lymphoma 2 (*BCL2*) family of proteins, and its upregulation is required to induce apoptosis in kinase-driven cancers. (Ng et al., 2012) This deletion is clinically relevant in people with chronic myeloid leukaemia (CML) – in that those with the variant respond less well to kinase drugs.

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