A CLINICAL AND GENETIC STUDY OF
ION CHANNEL DISORDERS IN CHILD NEUROLOGY

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DEDICATION

To all the families who helped with this work
- particularly my own

Dawn, Karim, Aliyah and Mariha
Abstract

Ion channels are macromolecular proteins in cell membranes that control the passage of charged particles including sodium, potassium and calcium ions in and out of cells. Rapid electrical signalling in the nervous system is mediated through the passage of ions through these channels. It is therefore not surprising that genetic mutations in the genes coding for these channels can result in neurological disease. Ion channel disorders or channelopathies have emerged in the last ten to fifteen years as an important new way of understanding neurological disease. Many of these conditions are paroxysmal in nature and include generalised and focal epilepsies, movement disorders and neuromuscular disorders. Some of these conditions follow simple Mendelian inheritance and are rare forms of common disorders such as epilepsy but they provide a useful model for more common neurological diseases with complex inheritance. Some conditions such as Dravet syndrome, a severe infantile onset epilepsy and sodium channelopathy produce devastating consequences for the affected child.

In this thesis I will describe the clinical work I have undertaken defining phenotypes of this emerging group of disorders. Detailed phenotyping is the first essential step in characterising new aspects of these genetic disorders. I have collaborated closely with molecular geneticists and cell physiologists in units around the world exchanging ideas in order to better understand the mechanisms of disease and hopefully translate this into better care for patients. The main themes covered in the thesis are episodic ataxias type 1 and 2 (EA1 & 2), benign familial neonatal convulsions, autosomal dominant nocturnal frontal lobe epilepsy, and Dravet syndrome and other SCN1A related epileptic encephalopathies. In the course of this work I have described novel relationships between EA1 and EA2 and epilepsy, described a novel gene and phenotypes associated with frontal lobe epilepsy, a novel
presentation of a potassium channelopathy, a family with a new genetic mechanism for their neonatal convulsions and epilepsy, and children with a novel mechanism for Startle disease (hyperekplexia). I have demonstrated the clinical utility of this translational research by establishing a molecular genetic diagnostic service for sodium channel (SCN1A) related infantile epilepsies. A study of the results from this national UK service shows that genetic diagnosis allows early diagnosis of these epilepsies. This can result in earlier focused treatment, and the hope for better epilepsy control and developmental outcome. I discuss the implications of this work and ongoing and future research projects.
DECLARATION OF ORIGINALITY

This thesis comprises only my original work. To the best of my knowledge and belief, the thesis contains no material previously published or written by any person, except where due acknowledgement is given in the text. The thesis contains no material which has been accepted for any other degree in any university. The thesis is less than 50,000 words in length, exclusive of tables, bibliographies and appendices.

Signed ...  

Sameer Mustafa Zuberi
There are many individuals who have provided invaluable advice and support throughout the long gestation of this thesis. The families and individuals who are at the core of this work, my patients and the patients of my close colleagues, deserve most thanks. They have shared with me their family histories, and many of the difficult experiences they have been through or they have witnessed affect their children and loved ones. They have been the most enthusiastic participants in this work and I hope that some of the findings will benefit them and future generations.

I came to Glasgow as a senior registrar in paediatrics with an interest in child neurology, Professor John Stephenson my teacher, mentor, friend, and supervisor, unlocked a passion for the subject. I have learned from his unbounded enthusiasm coupled with intellectual vigour and his ability to see the scientific importance in detailed clinical observation. To never stop thinking about your patients, to always discuss cases with colleagues, and to develop new hypotheses to find solutions are his philosophies of clinical practice and research.

I must thank my colleagues Dr Robert McWilliam, Dr Mary O'Regan, Dr Iain Horrocks, Sister Margaret Wilson and Dr Stewart Macleod for their support and help. Within our full time NHS positions they have seen the importance of clinical research in our discipline and supported my academic interests and at times, I am sure, tolerated my academic enthusiasm. Robert has accompanied me on field trips to the Outer Hebrides and proved a great companion as well as electromyographer. Dr Andreas Brunklaus, Specialist Registrar in Paediatric Neurology has guided me through databases and been a great help solving many technical problems. Dr John Tolmie, Clinical Geneticist in Glasgow, has provided valuable advice and I have benefited from his incisive mind in our joint
neurogenetic clinics. When the genetics becomes too complex for a paediatric neurologist he always has a straightforward and understandable explanation.

The EEG technologists, Hilary Reidpath, Angela Robertson and Susan McCusker never fail provide anything less than an outstanding service to the children and families attending Yorkhill. Whatever request, whether it be undertaking a recording an EEG at short notice or finding an old recording and videotape taken 20 years earlier was never too much for them.

The staff of the DNA lab at the Duncan Guthrie Institute for Medical Genetics in Yorkhill have been incredibly helpful in extracting DNA and forwarding it to my various research collaborators around the world. More recently we have developed a joint clinical and molecular genetic service for SCN1A gene analysis in Yorkhill. This wouldn’t have been possible without the support of Su Stenhouse head of molecular genetics. The most important member of this team is Rachael Birch, Clinical Molecular Geneticist, who has developed the service with me over the last few years. Her enthusiasm and hard work is responsible for any success this project has achieved.

My clinical collaborators in Scotland include Dr Ailsa McLellan, now Consultant in Paediatric Neurology, Royal Hospital for Sick Children, Edinburgh and formerly specialist registrar in Dundee, as well as Dr Martin Kirkpatrick, Consultant Paediatric Neurologist, Ninewells Hospital, Dundee. Ailsa and Martin worked closely with me in the study of Scottish families with autosomal dominant nocturnal frontal lobe epilepsy.

Professor Mike Hanna, Professor Nick Wood and Professor Dimitri Kullmann at the Institute of Neurology, Queen Square have collaborated on potassium and calcium channelopathies. They have appreciated the value of our clinical observations and have always listened to any ideas we have had. Their postgraduate students, Louise Eunson, Tracey Graves, Alex Spauschus and Ann Jouvenceau have worked on these projects.
Professor Sam Berkovic and Professor Ingrid Scheffer from the University of Melbourne and their molecular genetic colleague Professor John Mulley in Adelaide have collaborated with me on several projects including autosomal dominant nocturnal frontal lobe epilepsy, benign familial neonatal convulsions and SCN1A related epilepsies. The Melbourne/Adelaide group have been at the leading edge of discoveries in epilepsy genetics for more than ten years. Sam, Ingrid and John have had outstanding success in their field because of the meticulous nature of their clinical data collection and analysis and the value they place on close working relationships between clinicians and molecular geneticists. I hope that some of their philosophy has rubbed off on me.

Financial support through the course of this work has been received from the Muir Maxwell Trust and the Paediatric Neurology Research & Information Fund. The Muir Maxwell Trust funded the purchase of the SCN1A gene sequencer at Yorkhill and its running costs for the first three years. Without them this service would not have been established.

Most importantly I would like to thank my family. My parents Mohammad & Parveen have shown me the value and joy of lifelong learning. My wife Dawn and my children Karim, Aliyah and Mariha have been incredibly patient, loving, supportive and understanding as I have spent many evenings, weekends and holidays writing and revising the thesis at an inevitable cost to family time. My wife and children have helped me understand and appreciate the devastation that families feel when their child is affected by a serious or unexplained neurological condition. I hope that work included in this thesis will contribute some of the many small incremental advances in knowledge that are needed for better understanding and management of neurological disorders in childhood.
Published Papers Arising From This Work


BOOK CHAPTERS ARISING FROM THIS WORK

Zuberi SM Channelopathies. In: Co-morbidities. Edited by Bax MC & Gilberg C.
Mac Keith Press. In press 2009/10

Edited book

I am editing a multi-author textbook with the title Channelopathies for Mac Keith Press, Cambridge in the Clinics in Developmental Medicine Series. I am writing 3 chapters as well as editing the book.

Benign neonatal and infantile epilepsy syndromes
Dravet Syndrome and other SCN1A related epileptic encephalopathies
Autosomal Dominant Nocturnal Frontal Lobe Epilepsy
HONOURS RECEIVED IN THE COURSE OF THIS WORK

Millenium Young Physician Gowers Prize, 1999
- awarded by British Branch of International League Against Epilepsy for an essay “Ion Channels & Epilepsy: an Exciting Future”

Mac Keith Prize, 2000
- awarded by British Paediatric Neurology Association for my clinical research work.

Highly Commended
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ABBREVIATIONS AND GLOSSARY

ADNFLE  Autosomal Dominant Nocturnal Frontal Lobe Epilepsy
BFIC   Benign Familial Infantile Convulsions
BFNS   Benign Familial Neonatal Seizures
BFNIS  Benign Familial Neonatal and Infantile Seizures
BRE    Benign rolandic epilepsy
CHRNA2  Gene for neuronal nicotinic acetylcholine receptor α2 subunit
CHRNA4  Gene for neuronal nicotinic acetylcholine receptor α4 subunit
CHRNB2  Gene for neuronal nicotinic acetylcholine receptor β2 subunit
EA1    Episodic Ataxia Type 1
EA2    Episodic Ataxia Type 2
EEG    Electroencephalogram
DNA    Deoxyribose Nucleic Acid
DTP    Diphtheria, tetanus, pertussis (immunisation)
EMG    Electromyography
FANU   Fraser of Allander Neurosciences Unit
GABA   Gamma-Amino Butyric Acid
GEFS+  Genetic (Generalised) Epilepsy with Febrile Seizures Plus
Hz     Hertz
HEK    Human embryonic kidney
ICEGTCS  Intractable childhood epilepsy with generalised tonic clonic seizures
IGE    Idiopathic generalised epilepsy
ILAE   International League Against Epilepsy
KCNA1  Potassium channel gene
KCNQ2  Potassium channel subunit gene
KCNQ3  Potassium channel subunit gene
Kv1.1  Potassium channel protein (coded for by KCNA1 gene)
MLPA   Multiplex ligation probe amplification
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Chapter 1

Introduction

1.1 Ion Channels in neurological disease

In the last 15 years disorders of ion channel function have emerged as an important new class of neurological disorder. Ion channels are specialised trans-membrane proteins that are essential for controlling electrical signalling, the resting membrane potential of cells and neurotransmitter release throughout the nervous system. The term channelopathies was coined in 1995 to describe diseases caused by ion channel dysfunction (Griggs and Nutt 1995).

More than 40 genetic neurological channelopathies have been described (Zuberi 2003). The majority of these disorders are single gene syndromes within common classes of neurological disorders such as epilepsy, movement disorders and migraine. The study of monogenic channelopathies has resulted in important insights into these common disorders with complex inheritance, which are the bread and butter of the neurologist’s practice. One of the most exciting aspects of working in this field is seeing how close collaboration between clinicians, molecular geneticists, cell biologists and physiologists has led to major advances in knowledge which are now translating back into patient care.

Many of the genetic channelopathies have autoimmune counterparts some of which are well characterised such as autoimmune myasthenia gravis (acetylcholine receptor antibodies) and autoimmune neuromyotonia (potassium channel antibodies).
Recently new autoimmune channelopathies such as potassium (K+) channel antibody mediated limbic encephalitis have been recognised (Vincent et al 2004).

Many of the advances in the study of the neurological channelopathies have begun with detailed phenotypic studies of large kindreds which follow monogenic inheritance (Scheffer & Berkovic, 1997). These families are important as they allow the study of phenotypic variation among individuals with a shared genetic background. They also provide the best and most successful approach to molecular genetic analysis. Large families are more likely to have a single major gene expressing a neurological disorder than small families where polygenic inheritance is more likely to occur. By determining the mode of inheritance in a family, assessing the penetrance of a genetic trait and clearly characterising affected and non-affected individuals linkage studies can be performed. If there are problems in characterisation, such as diagnosing just one unaffected member of a family as affected because they have a similar but unrelated disorder (a phenocopy), then as detailed in Chapter 8 spurious results can emerge.

Linkage involves defining the regions of chromosomes which are shared among affected cases and differ from those in the unaffected cases (Pulst 1999). These studies provide a likelihood, logarithm of the odds score (LOD score), that a known chromosomal marker is associated with a particular trait. A LOD score of 3 is generally regarded as significant as it represents 1000:1 odds in favour of linkage. When a chromosomal region is identified then candidate genes in that part of the genome can be studied by a variety of techniques including direct sequencing to search for mutations. If no candidate genes are present or fail to yield positive results then alternative techniques are used to isolate new genes with mutations.

Many of the channelopathies have had successful genetic characterisation through this technique. The focus of this thesis is the phenotypic description of channelopathies that present to the child neurologist. The vast majority of the genetic channelopathies present in childhood and adolescence. The knowledge of their genetic basis will allow description of the phenotypic variability within the disorders.
and the boundaries of the disorders. As this is a new field in adult and child neurology there is great scope for characterisation of the phenotypes which in turn may aid diagnosis and management of individuals with channelopathies.

It is over 50 years since Hodgkin and Huxley first described the generation of the nerve action potential in the squid giant axon (Hodgkin & Huxley 1952). They determined that the electrical impulses were generated by the rapid passage of ions across the cell membrane through what they termed "active patches". We now call these patches ion channels and know that they are large macromolecular proteins which span the cell membrane.

Hodgkin and Huxley developed the patch clamp technique to measure ion movement as electrical currents (Armstrong & Hille 1998). They were able to mathematically deduce three of the key properties of ion channels: that they have a central aqueous pore through which the ions pass, that the pore can be rapidly opened and closed (gated) and that the channels are selective for different ion species. The advances in molecular biology of the 1980s which allowed the amino acid sequence of channel proteins to be described, and the beautiful 3-D pictures of bacterial potassium channels from X-ray crystallography in the 1990s have all confirmed these properties (Tempel et al 1987; Doyle et al 1999).

Many of the channelopathies are paroxysmal disorders. The ion channel mutation may impair channel function to a degree that only becomes significant when there are additional factors which affect channel function. These factors include temperature, the pH surrounding the channel, intracellular messengers and many other aspects of the channel micro-environment (Hille 2001). In some disorders chronic channel dysfunction can result in progressive impairment of function and interictal signs and symptoms.

The emergence of the channelopathies as a class of neurological disorder has benefited from the recent rapid advances in molecular biology. Mutations identified in humans can be incorporated into ion channel gene cDNA, the mutant channel
expressed in a cell membrane and the current across the mutant channel measured. These functional studies not only provide insight into the basic physiology of disease but may suggest novel therapeutic pathways. Functional studies on different channel types are discussed in the relevant chapters. Natural and genetically modified animal models of several channelopathies exist. The channelopathies are therefore an important source for translational research.

1.1.1 Structure and molecular biology of ion channels

There are two major classes or superfamilies of ion channels, voltage gated and ligand gated. Molecular genetic studies have shown that these two classes are structurally dissimilar. Amino acid sequencing suggests that the voltage-gated K⁺, Na⁺ and Ca²⁺ channels are all members of a family of proteins that are closely related to each other in evolutionary terms. It is likely that K⁺ and Ca²⁺ evolved from a single cation gene and that Na⁺ channels developed from Ca²⁺ channels (Hille 2001). The ion channel families are large with many closely associated channel species. Because of their critical role in electrical signalling in the nervous system the structure and amino acid sequence of large parts of the channel proteins are conserved through several species from drosophila to man. Most ion channels are assembled from distinct subunits each coded for by different genes (heteromultimers) though homomultimers do exist. The voltage gated channels are made up of large α subunits which make up the channel and may interact with smaller β subunits which may play a role in regulating channel function. The ligand gated channels comprise three major families; nicotinic receptors, glutamate receptors and the ionotropic ATP receptors.

Ion channels can be regarded as excitable molecules which allow passage of ions through changes in conformation in response to a variety of stimuli. The action potential along a nerve, as described by Hodgkin & Huxley, is transmitted by the sequential depolarisation and repolarisation of the cell membrane by the passage of sodium and potassium ions across the membrane (Figure 1.1). Changes in membrane voltage cause voltage gated sodium channels to open allowing a rapid influx of ions across the membrane with resultant depolarisation. This change in membrane voltage
leads to the opening of potassium channels and the rapid passage of potassium ions out of the cell resulting in repolarisation of the membrane (Figure 1.2).

When the action potential reaches the neuromuscular junction a sequence of events leads to release of acetylcholine (the ligand) which binds to the acetylcholine receptor, a ligand gated ion channel, on the postsynaptic membrane. The binding of the ligand leads to a change in conformation of the protein and opening of the channel allowing sodium ions to pass across the membrane and set in motion the train of events leading to muscle contraction (Figure 1.3).
The generation of the action potential.
The action potential $V$ is generated by the sudden influx of Na$^+$ ions across the membrane through voltage-gated sodium channels (a) with resultant depolarisation of the cell membrane. The opening of voltage-gated potassium channels allows passage of K$^+$ ions across the cell membrane (b) out of the cell and re-polarisation of the membrane (Hodgkin & Huxley 1952).
Figure 1.2
Cartoon of voltage gated ion channel. As the membrane voltage changes this is detected by a voltage sensor within the channel which changes conformation of the channel to allow passage of ions.

Figure 1.3
Cartoon of a ligand gated ion channel. A ligand such as a neurotransmitter binds to a receptor portion of the channel causing a change in channel conformation and passage of ions through the channel.
1.1.2 Potassium channels

There are over 50 genes which encode mammalian $K^+$ channels and many of these have multiple isoforms. The group most strongly associated with neurological disease is the 6 transmembrane domain family of $K^+$ channels. These include the voltage-gated and calcium gated $K^+$ channels. Potassium channels set the resting membrane potential and repolarise neurons following action potentials (Kullmann 2002). The voltage gated channels of the Kv family (Chapters 4 & 6) are made up of four homologous $\alpha$ subunits which come together to make up the channel. The six transmembrane segments (S1-6) linked by extracellular loops are illustrated in figure 1.4. The S4 region contains positively charged amino acids which cause the channel to change conformation and open as the transmembrane voltage changes. This region is called the voltage sensor. The S5-6 linker lines the pore and acts as the selectivity filter. The N and C terminals have several functions including interactions with other proteins and in targeting the channel to the cell membrane. Cytoplasmic $\beta$ regulatory subunits may also modulate channel function.

1.1.3 Sodium channels

The sodium channel $\alpha$ subunit is associated with two regulatory $\beta$ subunits as illustrated in figure 1.5. Sodium channels have four domains each of which is similar in structure to a potassium channel alpha subunit. Each domain has 6 transmembrane segments. It is likely that sodium channels evolved (possibly via calcium channels) from two duplications of the potassium channel (Kullmann 2002). Only one $\alpha$ subunit is required to form a channel. The S4 segment acts as the voltage sensor with S5-6 and the S5-6 linker acting as the selectivity filter and the pore forming region (Catterall 2000a).

1.1.4 Chloride channels

Chloride channels have 8-12 transmembrane segments and may exist as homodimers with two pores.
Figure 1.4

Kv1.1 voltage-gated potassium channel α subunit showing six transmembrane segments and intracytoplasmic N and C terminals. The loop between S5 & S6 known as the S5-6 linker is the selectivity filter. The arrow indicates the position of a point mutation discussed in chapter 4.
**Figure 1.5**

Cartoon of sodium channel showing 4 homologous domains in the α subunit each similar to a potassium channel α subunit, and two regulatory β subunits.
1.1.5 Calcium channels

Calcium channels have very similar structures to sodium channels with one major pore forming \( \alpha_1 \) subunit made up of 4 homologous, six segment transmembrane domains (Catterall 2000b). The structures of sodium and calcium channels differ in various amino acid sequences and mechanisms of channel inactivation. Calcium channels have different intracytoplasmic regulatory subunits the \( \alpha_2\delta \) and \( \beta \) subunits. The \( \alpha_2\delta \) subunit consists of a single transmembrane segment and is thought to be important in controlling surface expression of the channel.

1.1.6 Nicotinic ligand-gated channels

The nicotinic receptor family comprises GABA\( _A \), nicotinic acetylcholine, glycine and serotonin receptors. Ligand gated channels are also called ionotropic receptors. Serotonin receptor mutations are not known to be associated with neurological disease.

Nicotinic receptors are pentameric, meaning they are made up of five subunits as illustrated in figure 1.6. Each of the subunits contains four transmembrane domains. The second domain (M2) lines the pore and controls ion selectivity. The acetylcholine receptors are not very selective between different cation species (Kullmann, 2002). The glycine and GABA\( _A \) receptors are selective for small anions such as bicarbonate and chloride. The N and C terminals are extra cellular in contrast to the voltage gated channels. The pentamers can be homomultimeric or heteromultimeric with a variety of subunit types coming together to form the channel. The GABA\( _A \) receptor has at least 15 subunit types meaning that there are a great variety of potential channel combinations. The nicotinic acetylcholine receptors have fewer subtypes. It is important to note that different subtypes may be expressed at different periods of development. For example there are specific fetal subtypes of muscle acetylcholine receptors. This is important when a mother develops antibodies against fetal subtypes. She may have no symptoms but the fetus may suffer from antibody mediated attack causing congenital arthrogryposis.
Figure 1.6
Cartoon of neuronal nicotinic acetylcholine receptor illustrating how α4 subunits combine with β2 subunits to make up the pentameric structure of the channel.
1.2 The Neurological Channelopathies

Table 1.1 provides an overview of the central nervous system and neuromuscular disorders associated with mutations in ion channels. It is remarkable to consider that the first skeletal muscle channelopathy was described in 1991 and the first central nervous system channelopathy was described in 1994. Since then more than 40 disorders have been described.

As can be seen from the table a single phenotype may be caused by mutations in different genes. This emphasises the importance of identifying single large families to identify genes rather than pooling together multiple small families. Some of these genes code for different subunits of the same ion channel whereas in other cases genes from a different class of ion channel, such as in Genetic (Generalised) Epilepsy with Febrile Seizures plus, may associate with the same disorder. This suggests different genes may be involved at different points in the same pathway or network that controls neuronal excitability.

This list will continue to grow. I have not included some reports of association of an ion channel mutation with a single case unless this has been replicated.
Table 1.1
Central nervous system and neuromuscular channelopathies. Adapted from Zuberi (2003)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Mode of inheritance</th>
<th>Gene location</th>
<th>Gene symbol</th>
<th>Gene product</th>
<th>Key refs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ligand gated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autosomal dominant nocturnal frontal lobe epilepsy</td>
<td>AD</td>
<td>20q13.2</td>
<td>CHRNA4</td>
<td>Neuronal nicotinic acetylcholine receptor α4 subunit</td>
<td>Steinlein et al (1995)</td>
</tr>
<tr>
<td>Autosomal dominant nocturnal frontal lobe epilepsy</td>
<td>AD</td>
<td>8p12.3-8q12.3</td>
<td>CHRNA2</td>
<td>Neuronal nicotinic acetylcholine receptor α2 subunit</td>
<td>Aridon et al (2006)</td>
</tr>
<tr>
<td>Autosomal dominant juvenile myoclonic epilepsy (rare subtype)</td>
<td>AD</td>
<td>5q34-35</td>
<td>GABRA1</td>
<td>GABA A receptor α1 subunit</td>
<td>Cossette et al (2002)</td>
</tr>
<tr>
<td>Febrile seizures &amp; childhood absence epilepsy</td>
<td>AD, complex</td>
<td>5q32-33</td>
<td>GABRG2</td>
<td>GABA A receptor γ2 subunit. Extracellular domain</td>
<td>Wallace et al (2001)</td>
</tr>
<tr>
<td>Hyperekplexia</td>
<td>AD, AR</td>
<td>5q32</td>
<td>GLRA-1</td>
<td>Glycine receptor α1 subunit</td>
<td>Shiang et al 1993</td>
</tr>
<tr>
<td><strong>Voltage gated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign familial neonatal seizures</td>
<td>AD</td>
<td>8q24</td>
<td>KCNQ3</td>
<td>Potassium channel subunit</td>
<td>Charlier et al (1998)</td>
</tr>
<tr>
<td>Disorder</td>
<td>Mode</td>
<td>Chromosome</td>
<td>Gene</td>
<td>Gene Function</td>
<td>References</td>
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</tr>
<tr>
<td>Epilepsy &amp; paroxysmal dyskinesia</td>
<td>AD</td>
<td>10q22.3</td>
<td>KCNMA1</td>
<td>Subunit of BK channel</td>
<td>Du et al (2005)</td>
</tr>
<tr>
<td>Sodium channel</td>
<td>AD</td>
<td>2q22-23</td>
<td>SCN2A</td>
<td>Sodium channel α2 subunit</td>
<td>Heron et al (2002)</td>
</tr>
<tr>
<td>Benign neonatal-infantile seizures</td>
<td>AD, complex</td>
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<td>SCN1B</td>
<td>Neuronal sodium channel β regulatory subunit</td>
<td>Wallace et al (1998)</td>
</tr>
<tr>
<td>Generalised (genetic) epilepsy with febrile seizures plus</td>
<td>AD, complex</td>
<td>2q24</td>
<td>SCN1A</td>
<td>Neuronal sodium channel α1 subunit</td>
<td>Escayg et al (2000)</td>
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<tr>
<td>Sporadic severe myoclonic epilepsy of infancy and late onset variants</td>
<td>De novo mutations</td>
<td>2q24</td>
<td>SCN1A</td>
<td>Neuronal sodium channel α1 subunit</td>
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<td>Familial hemiplegic migraine</td>
<td>AD</td>
<td>2q24</td>
<td>SCN1A</td>
<td>Neuronal sodium channel α1 subunit</td>
<td>Dichgans et al (2005)</td>
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<tr>
<td>Generalised (genetic) epilepsy with febrile seizures plus</td>
<td>AD, complex</td>
<td>2q22-23</td>
<td>SCN2A</td>
<td>Neuronal sodium channel α2 subunit</td>
<td>Sugawara et al (2001)</td>
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<td>CACNA1A</td>
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<td>Ophoff et al (1996)</td>
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<tr>
<td>Familial hemiplegic migraine</td>
<td>AD</td>
<td>19p13</td>
<td>CACNA1A</td>
<td>Calcium channel</td>
<td>Ophoff et al (1996)</td>
</tr>
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<td>Episodic ataxia type 2</td>
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<td>19p13</td>
<td>CACNA1A</td>
<td>Calcium channel</td>
<td>Zhuchenko et al (1997)</td>
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<td>Disorder</td>
<td>Mode of inheritance</td>
<td>Gene location</td>
<td>Gene symbol</td>
<td>Gene product</td>
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<tr>
<td>Sodium channel</td>
<td>AD</td>
<td>17q23</td>
<td>SCNA4</td>
<td>Sodium channel α subunit</td>
<td>Rojas et al (1991)</td>
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<td>Hyperkalaemic periodic paralysis</td>
<td>AD</td>
<td>17q23</td>
<td>SCNA4</td>
<td>Sodium channel α subunit</td>
<td>McClatchey et al (1992)</td>
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<td>Paramyotonia congenita</td>
<td>AD</td>
<td>17q23</td>
<td>SCNA4</td>
<td>Sodium channel α subunit</td>
<td>Lerehe et al. (1993)</td>
</tr>
<tr>
<td>Potassium aggravated myotonia</td>
<td>AD</td>
<td>17q23</td>
<td>SCNA4</td>
<td>Sodium channel α subunit</td>
<td>Sternberg et al (2001)</td>
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<tr>
<td>Hypokalaemic periodic paralysis, type 2</td>
<td>AD</td>
<td>17q23</td>
<td>SCNA4</td>
<td>Sodium channel α subunit</td>
<td>Fertleman et al (2006)</td>
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<td>Sodium channel α subunit</td>
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<td>Calcium channel</td>
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<td>1q31-q32</td>
<td>CACNL1A3</td>
<td>Calcium channel-dihydropyridine receptor</td>
<td>Jurkatt-Rott et al (1994)</td>
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<td>Hypokalaemic periodic paralysis, type 1</td>
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<td>1q31-q32</td>
<td>CACNL1A3</td>
<td>Calcium channel-dihydropyridine receptor</td>
<td>Monnier et al (1997)</td>
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<tr>
<td>Malignant hyperthermia</td>
<td>AD</td>
<td>19q13.1</td>
<td>RYR1</td>
<td>Calcium channel-ryanodine receptor</td>
<td>Quane et al (1993)</td>
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<tr>
<td>Central core disease</td>
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<td>RYR1</td>
<td>Calcium channel-ryanodine receptor</td>
<td>Quane et al (1993)</td>
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<td>Stationary night blindness</td>
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<td>Xp11.23</td>
<td>CACNA1F</td>
<td>Calcium channel</td>
<td>Strom et al (1998)</td>
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<td>Chloride channel</td>
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<td>Myotonia congenita-Thomsens disease</td>
<td>AD</td>
<td>7q35</td>
<td>CLC-1</td>
<td>Chloride channel</td>
<td>Koch et al (1992)</td>
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<tr>
<td>Myotonia congenita-Becker’s myotonia</td>
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<td>7q35</td>
<td>CLC-1</td>
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<td>Koch et al (1992)</td>
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<td>Potassium channel</td>
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<td>11q13</td>
<td>KCNE3</td>
<td>Potassium channel</td>
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<td>12p13</td>
<td>KCNA1</td>
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<td>Eunson et al (2000)</td>
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<tr>
<td>Familial generalised myokymia</td>
<td>AD</td>
<td>17q23</td>
<td>KCNJ2</td>
<td>Potassium channel</td>
<td>Plaster et al (2001)</td>
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<tr>
<td>Condition</td>
<td>Inheritance</td>
<td>Chromosome</td>
<td>Gene(s)</td>
<td>Channel Type</td>
<td>Reference</td>
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<tr>
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<td>-----------------------------</td>
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<tr>
<td></td>
<td>AR</td>
<td>21q22</td>
<td>KCNE1</td>
<td>Potassium channel</td>
<td>Schulze-Bahr et al (1997)</td>
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<td>Autosomal dominant deafness type 2</td>
<td>AD</td>
<td>1p34</td>
<td>KCNQ4</td>
<td>Potassium channel</td>
<td>Kubisch et al (1999)</td>
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</table>

**Congenital myasthenic syndromes**

<table>
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<tr>
<th>Type</th>
<th>Inheritance</th>
<th>Chromosome</th>
<th>Gene(s)</th>
<th>Channel Type</th>
<th>Reference</th>
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<tr>
<td>Slow channel syndromes</td>
<td>AD</td>
<td>2q24-q32</td>
<td>CHRNA</td>
<td>Acetylcholine receptor (AChR) α subunit</td>
<td>Sine et al (1995)</td>
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<td></td>
<td>AD</td>
<td>17p11-p12</td>
<td>CHRNB1</td>
<td>AChR β subunit</td>
<td>Engel et al (1996a)</td>
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<tr>
<td>Fast channel syndrome</td>
<td>AR</td>
<td>17p13</td>
<td>CHRNE</td>
<td>AChR α subunit</td>
<td>Ohno et al (1996)</td>
</tr>
<tr>
<td>Acetylcholine receptor deficit</td>
<td>AR</td>
<td>17p13</td>
<td>CHRNE</td>
<td>AChR α subunit</td>
<td>Engel et al (1996b)</td>
</tr>
</tbody>
</table>

AR: autosomal recessive; AD: autosomal dominant; X: X-linked
1.3 Mutations in ion channel genes

Human DNA is constantly subject to mutation. Mutations drive the evolutionary process but they can also be pathogenic, either directly causing a disease phenotype or increasing the susceptibility to develop a disease. Large scale mutations involve loss or gain of chromosomal material. Smaller scale mutations can be grouped into three classes (Strachan & Reid, 2004). These are base substitutions (usually single bases), deletions of one or more nucleotides or insertion of one or more nucleotides. Each time a human cell divides a sequence of 6 billion nucleotides needs to be replicated. Despite the presence of proofreading exonucleases in DNA polymerases to prevent insertion of incorrect bases, this can occur in DNA replication.

Novel mutations can arise in the somatic cells or in the germline. Germline mutations are more common in the male germ line. We have recently shown in collaboration with colleagues in Australia that this is the case with mutations affecting the gene encoding the $\alpha_1$ subunit of the neuronal sodium channel (SCN1A) in infants with Dravet syndrome and related epileptic encephalopathies (Heron et al 2009).

There are several mutation types that can occur in ion channel genes including (Strachan & Read 2004):

- Deletions from one base pair to whole gene
- Insertions and duplications
- Frameshifts- small deletions, insertions or splicing errors adding or removing nucleotides that are not an exact multiple of three
- Dynamic mutations – tandem repeats that often change size on transmission to children
- Single base substitutions
  - Missense mutations replace one amino acid with another in the gene product
  - Nonsense mutations replace an amino acid codon with a stop codon
  - Splice site mutations create or destroy signals for exon-intron splicing
Missense mutations can be conservative or non-conservative substitutions depending on whether the amino acid is replaced by another similar to it. The change may result in a change in polarity of the amino acid coded for, a change in composition, or a change in volume. The degree of the physico-chemical change produced by a missense mutation can be scored in various ways (Grantham 1974). Deciding whether a particular mutation is pathogenic however is not straightforward and relies on a hierarchy of evidence (Strachan & Reid 2004):

- Functional expression studies show the change is pathogenic. At present this is only practical when performed in the research setting. Functional studies may give misleading results and results may differ in different biological systems.
- The change has been seen before in individuals with the same phenotype and not in controls
- Mutation is de novo in a de novo disease
- The sequence change is not seen in large number of controls
- Nature of the mutation
  - Whole gene deletions, nonsense mutations and frameshifts will almost certainly destroy gene function
  - Mutations affecting GT...AG nucleotides at exon-intron boundary usually abolish gene function.
  - Does the missense mutation occur in a functionally important part of the gene?
  - Is the amino acid conserved in other similar proteins and in other species?
  - Is the amino acid change polar to non-polar or acidic to basic?

Assessing the evidence as above is usually sufficient in clinical practice, without having to use functional studies, in order to judge whether a sequence change detected is significant or not.
CHAPTER 2

AIMS OF THIS WORK

The aims of this work are to advance the understanding of ion channel disorders (channelopathies) affecting the nervous system through studying the phenotypes of individuals and multi-generation families presenting to child neurology. By collaboration with molecular genetic colleagues in various centres around the world and latterly in Glasgow, I hope to advance the knowledge of phenotype genotype relationships in the channelopathies and the understanding of the underlying physiology of these conditions. I aim to assess whether close collaboration between clinicians and molecular geneticists can aid diagnosis and influence the management of patients.

2.1 Specific aims

1. To provide a phenotypic description of epilepsy in the syndromes of episodic ataxia type 1 and 2 and collaborate with colleagues to better understand the physiological relationships between epilepsy and movement disorders.

2. To describe the phenotype associated with a potassium channel mutation in a pure neuromuscular disorder.

3. To describe the genotype-phenotype relationships in benign familial neonatal seizures.

4. To compare the phenotypes of large families with frontal lobe epilepsy with mutations in two different but related ion channel gene subunits.
5. To describe the phenotypes of sodium ion channel α subunit (SCN1A) gene related infantile epileptic encephalopathies. To evaluate whether establishing a molecular genetic diagnostic service for these disorders aids diagnosis and influences management.
CHAPTER 3

GENERAL METHODS

Methods specific to individual disorders studied will be described in the relevant chapters.

3.1 Ascertainment of cases and families

The vast majority of individuals and families have been ascertained from the personal practice of the author and his supervisor, Professor John Stephenson. They comprise children and families presenting to the Fraser of Allander Neurosciences Unit (FANU), Royal Hospital for Sick Children, Glasgow. This work has continued over several years of my clinical practice, first as a Senior Registrar and then a Consultant in Paediatric Neurology in the FANU.

One family with Autosomal Dominant Nocturnal Frontal Lobe Epilepsy was ascertained through discussion with colleagues in Dundee. Cases have been referred to the Epilepsy Genetics Service in Glasgow for both molecular genetic analysis and a clinical opinion from throughout the United Kingdom and many overseas neurology centres.

3.2 Family studies

In many of the genetic channelopathies there are several affected individuals in a family. Indeed the diagnosis of a particular disorder may be dependant on identifying
a family history. When appropriate to the clinical care of the proband or for the study I reviewed the family history in detail. I found that identifying the oldest living female relative, or matriarch, in large families was often the most helpful initial step in ascertaining the presence and extent of a wider relevant family history. If other individuals were affected, with appropriate consent of the individual and managing clinician, I undertook telephone interviews, personal interviews and case record reviews. When individuals were referred from rural parts of Scotland and preferred local review I travelled to them.

3.3 Clinical evaluation

As most of the cases studied were seen in routine clinical practice the evaluation was that of a comprehensive history and neurological examination. Additional tests were performed as clinically indicated.

Many of the channelopathies present as paroxysmal disorders with a potentially wide differential diagnosis, and few if any interictal clinical signs or abnormalities on additional tests. A detailed history is therefore paramount. I took a structured general history including:

- Pregnancy, birth and developmental history
- General medical history
- Detailed family history including drawing a pedigree
- Social and educational history

The history of events, whether epileptic or non-epileptic, was taken from the affected individual relaying their own experience of the event and from witness accounts. The history included:

- When did the event occur?
- What was the individual doing at the beginning of the event?
- Were there any triggers to the event?
What did the individual feel at the beginning of the event?
A detailed description of the event from a witness
Did the individual have a warning of the event?
A detailed description of the event from the individual themselves
Duration of the event – including using techniques such as “replaying events in the
minds eye” to assess timing
Aftermath of the event
Frequency of events

Videotape recordings taken at home were reviewed. In some cases videotape
recorders (and tripods for nocturnal events) were lent to families to capture events at
home. Videotape was also used in the clinic if it was possible to provoke an episode
using special techniques.

I undertook a neurological and relevant general examination of individuals seen in
clinical practice and examined other family members if it contributed to the
diagnosis of that individual. Relevant clinical signs were videotaped.

Neurophysiological investigations were performed as clinically indicated. These
included EEG studies in channelopathies associated with epilepsy and EMG in
disorders affecting nerve and muscle. Video-EEG telemetry studies were required for
diagnostic purposes in selected cases.

I classified individuals with epilepsy, when possible, using the diagnostic scheme
and International League Against Epilepsy Classification of Seizures of Seizures and
Syndromes (Engel 2001, Commission of Classification and Terminology of the
International League Against Epilepsy1989). It is not always possible to make a
syndromic diagnosis using the ILAE classification but when possible I did so.
3.4 Collaboration with clinicians & molecular genetic labs in other institutions

The clinical work detailed in the thesis was performed in Glasgow. I have not undertaken any laboratory work myself though more recently I have supervised staff working in a molecular genetic service (see below). I have identified the patients and collaborated closely with genetic scientists to interpret and discuss the relevance of any molecular findings.

The channelopathies have been emerging as clinically important over the last decade. Many of these conditions are individually rare and therefore in the last ten years molecular genetic diagnostic analysis has been largely offered on a research basis by different laboratories around the world. A laboratory may have specific interests in certain conditions or genes.

Depending on the clinical condition studied I have collaborated closely with clinicians and scientists in different international centres. More recently as the importance of the channelopathies has been appreciated molecular genetic analysis for some conditions has been offered on a service basis.

In studies of Episodic Ataxias Type 1 and 2 I have collaborated with Professor Mike Hanna, Professor Nick Davis and Professor Dimitri Kullman at the Institute of Neurology, Queen Square, London. They have undertaken molecular genetic analysis of the potassium channel genes Kv1.1 and calcium channel gene CACNA1A. Their laboratory has also undertaken functional studies of the mutations detected in the individuals I have studied.

In studies of Autosomal Dominant Nocturnal Frontal Lobe Epilepsy, Benign Familial Neonatal Seizures, Benign Familial Infantile Convulsions and Paroxysmal Dyskinesia and Dravet Syndrome I have collaborated with Professor Ingrid Scheffer and Professor Samuel Berkovic at the Austin Medical Centre in Melbourne and Professor John Mulley at the Women’s and Children’s Hospital in Adelaide. Their
labs have undertaken genetic analysis of nicotinic acetylcholine receptor genes CHRNA4 and CHRN2, potassium channel subunit genes KCNQ2 and KCNQ3, and sodium channel gene SCN1A. Functional studies on mutations in CHRNA4 and CHRN2 were undertaken by Professor Daniel Bertrand's laboratory in Geneva.

PET studies of Scottish patients with ADNFLE were undertaken by Dr Fabienne Picard and colleagues in Paris.

In studies of children with hyperekplexia I have collaborated with Professor Mark Rees at the University of Swansea and Professor Robert Harvey at the School of Pharmacy in London. They have undertaken genetic analysis of the glycine transporter gene SLC6A5 and functional studies of mutations detected in cases I have identified.

### 3.5 Establishing an Epilepsy Genetics Service - molecular genetic diagnostic lab and clinical service - in Glasgow

Having worked with a research laboratory in Australia for several years, studying severe infantile onset SCN1A gene related epilepsies such as Dravet Syndrome I felt that this was likely to be the most clinically relevant gene and one which should be offered more widely on a service basis. I was successful in obtaining a grant from an epilepsy charity, The Muir Maxwell Trust, to purchase a DNA sequencer and support running costs of an SCN1A genetic diagnostic service. This is a joint clinical and molecular genetic service based at the Fraser of Allander Neurosciences Unit and Duncan Guthrie Institute of Medical Genetics both in the Royal Hospital for Sick Children, Glasgow. We have now secured National Services Division Scotland funding for the service as well as UK Genetic Testing Network (UKGTN) status. Australia is one of the countries which now uses our lab for diagnostic testing.

This remains the first and only SCN1A diagnostic service in the United Kingdom and one of few worldwide. It was important that it was established in such a way to facilitate audit and research. I developed a clinical form to accompany the DNA
sample. Referrers complete the form which I review prior to genetic testing. Given
the complex genotype phenotype relationships in SCN1A related epilepsy this allows
us to report the results of genetic testing in the context of the clinical referral
information. We have ethical approval to audit and publish results of the molecular
service and ethical approval for a study of genotype phenotype relationships using
data from the referral form.

3.6 Ethical aspects

All participating individuals, or carers in the case of minors, gave informed consent.
Confidentiality was preserved for all subjects. Many individuals and families were
seen in routine clinical practice. Written or recorded verbal consent (on video) was
obtained for the use of photographic or video recordings. Case record review was
approved by the Royal Hospital for Sick Children, Glasgow, Ethics Committee.
Ethical approval for genotype phenotype studies was obtained from the National
Research Ethics Service, Scotland A Research Ethics Committee. The review of
cases referred to the Epilepsy Genetics Service was approved by the Scotland A
Research Ethics Committee.
CHAPTER 4

EPISODIC ATAXIA TYPE 1 AND EPILEPSY

4.1 Introduction

Episodic ataxia type 1 (EA1) is a rare autosomal dominant movement disorder in which patients develop sudden brief episodes of paroxysmal ataxia (Van Dyke et al 1975). EA1 is associated with point mutations in the voltage-gated potassium channel gene (KCNA1) on chromosome 12p13. EA1 (Browne et al 1994, Litt et al 1994).

In this chapter I describe the clinical study of three Scottish families with this disorder and summarise collaborative work on these families undertaken by colleagues at the Institute of Neurology, Queen Square, London. The clinical and genetic study of family A has been published in Brain and has provided insights into the pathogenesis of the ataxia and epilepsy (Zuberi et al 1999). The links between epilepsy and movement disorders are discussed. The studies on families B and C support the original observations linking episodic ataxia type 1 and epilepsy with mutations in the KCNA1 gene. The study provides new insights into the phenotypic variability present within families. This relatively rare disorder can provide insights into pathophysiology of common neurological diseases such as epilepsy.

The KCNA1 gene codes for the Kv1.1 potassium ion channel (Browne et al 1994). The widespread expression of this channel in cerebellum, cerebral cortex and
peripheral nerve helps explain the symptoms associated with this disorder. Kv1.1 is a delayed rectifier potassium channel which has a key role in allowing the cell membrane to repolarise following an action potential and controlling neuronal excitability. It comes from the Shaker family of potassium channels which are highly conserved throughout evolution, with homologous channels in drosophila, mice and humans (Ramaswami et al 1990). Kv1.1 channels are tetrameric, consisting of four pore-forming subunits, each of which contain six transmembrane segments, linked by intracellular and extracellular loops (Ashcroft 2000).

EA1 usually has its onset in childhood and is characterised by brief episodes of cerebellar ataxia and dysarthria. The other key clinical feature is interictal myokymia also termed neuromyotonia. Some individuals describe a brief aura before the event such as a rising sensation up the body warning them that they will have to hold onto something for support. The ataxia may be associated with a coarse tremor and the movements may have features of chorea or dystonia. Some individuals describe weakness during an attack (Klein et al 2004).

In between attacks there is no interictal ataxia. Myokymia, the clinical sign most useful in making the diagnosis, may be subtle and missed if not looked for specifically. It is best observed as semi-rhythmic side to side movements of the fingers with the hands outstretched or as rippling of the muscles of the lower eyelid. Continuous motor unit activity can be demonstrated by surface electromyography without the necessity for needle insertion.

4.2 Methods

4.2.1 Clinical Studies

The probands from the three families were all referred for evaluation of paroxysmal events to the Fraser of Allander Neurosciences Unit (FANU). All families were of Scottish descent except for the mother of case II in family A, who was of German descent. The disease was documented over three generations in all 3 families. The
diagnosis of EA1 was based on the appropriate clinical history and the presence of myokymia on clinical examination and/or electromyography (EMG). The clinical evaluation of affected family members, children and adults, was undertaken by myself and followed the methods outlined in chapter 5. Some of the adults had imaging investigations undertaken at the Southern General Hospital in Glasgow. Case records were reviewed, including GP records and RHSC records from the 1960s held on microfiche. The diagnosis of epilepsy was based on the clinical history and witness account and was supported by ictal video-EEG in one case.

EMG was performed on four patients from family A and one member each from families B and C. Video recordings were made of all individuals who were examined. Ataxic episodes were induced and recorded in two patients from family A and home video of several ataxic episodes was available for one member of family B.

EEG studies were performed in the EEG department, Royal Hospital for Sick Children, Glasgow and interpreted by Professor John Stephenson and myself in family A and by myself in families B and C. EMG studies were performed by Dr Robert McWilliam and Dr Iain Horrocks, Consultant Paediatric Neurologists, Royal Hospital for Sick Children, Glasgow.

4.2.2 Molecular Genetic & Functional Studies (Work undertaken in London)

Informed consent was obtained for DNA analysis from all nine individuals in family A, 2 individuals in family B and 5 in family C. DNA was extracted using standard methods in Glasgow and forwarded to colleagues at the Muscle and Neurogenetics Sections, Institute of Neurology, Queen Square, London.

Louise Eunson under the supervision of Professor Mike Hanna and Professor Nick Wood undertook the sequencing of the KCNA1 gene in family A. Alex Spauschus worked on functional studies of family A under the supervision of Professor Dimitri
Kullmann. Human KCNA1 wild-type and mutant genes were amplified using PCR on genomic DNA extracted from a blood sample from one of the affected family members. Using cDNA techniques wild type and mutant clones were sequenced and transcribed in vitro. The mutant and wild type channels were expressed on Xenopus oocytes and patch clamp techniques used to measure currents across the channels. The mutant and wild type channel gene subunits were also expressed together in varying proportions to make up heterotetrameric channels.

DNA sequencing and haplotype studies from families B and C were performed by Tracey Graves under the supervision of Professor Mike Hanna. Tracey Graves and Stephanie Schorge undertook functional studies of the mutant channels in human embryonic kidney (HEK) cells in Professor Kullmann’s laboratory.

4.3 Results

4.3.1 Clinical study on family A

The pedigree is detailed in figure 4.1.

Case III2

The proband is a 3—year old boy who presented aged 7 weeks with recurrent apnoeic episodes associated with cyanosis. Pregnancy and delivery were unremarkable and the only abnormality noted prior to these episodes was a tendency to keep his fists clenched. Neurological examination was normal. Interictal EEG was normal. A 24-h video EEG study was performed during which several episodes were captured. These were shown to be focal epileptic seizures with impairment of awareness (complex partial seizures). Recordings showed the child staring forward or with his head turning to the left, the eyelids flickering, lip-smacking and the development of cyanosis. The episodes lasted up to 2 minutes and terminated with the infant fixing on his mother and the onset of regular respiration. EEG changes during the episode
Fig. 4.1 Pedigree of family A. Taken from Zuberi et al (1999).

![Pedigree Diagram](image)

**Fig. 1** Upper panel. Pertinent part of the pedigree of the EA1/epilepsy family. Filled symbols indicate individuals with EA1. The two individuals with epilepsy are indicated by ‘Ep’. The lower panel shows an agarose gel containing the DNA fragments following digestion with the restriction endonuclease *Ddel*. Normal individuals have the upper band only (159 bp undigested mismatch PCR product as described under Subjects and methods) as shown for cases III1 and III3 (cases I2 and III5 did not harbour the mutation; data not shown). The presence of the C677G mutation introduces a restriction site in the affected individuals (cases I1, II2, II4, III1, III2), and two bands are visible in these cases. These are the upper band, which is the undigested 159-bp product, and the lower band, which is the larger 131-bp product of digestion. The smaller 28-bp product of digestion is not shown. Lane 1, case III1; lane 2, case III1; lane 3, case II2; lane 4, case III2; lane 5, case II3; lane 6, case I1; lane 7, case II4. The proband (case III2) is indicated by an arrow.
consisted of rhythmical slow wave activity over the right hemisphere (possible right temporal lobe onset), becoming spike and slow wave complexes (Figure 4.2). This activity then spread to the left hemisphere, and asynchronous flattening of rhythms occurred periodically over both hemispheres. A CT scan with angled cuts through the temporal lobe was normal. Plasma electrolytes, calcium and magnesium were normal. The patient was treated with carbamazepine and had no further epileptic seizures. Subsequent developmental progress was normal and anti-epileptic medication was stopped at the age of 2 years.

At 20 months he developed irritability and swelling of his hands and feet. He had clenched fists and flexion of the toes. The dorsum of his hands and feet appeared swollen and oedematous and he refused to walk. Investigations for renal and joint disorders were negative and the problem resolved within a few days.

At the age of 2 years episodes of unsteadiness when walking developed. His legs would appear to buckle under him. There were several months between attacks and interictal general and neurological examination were normal. From the age of 3 years he has had episodes of ataxia at least once a week lasting seconds to minutes, precipitated by startle, exercise and sudden movement. Consciousness is preserved in the attacks. Examination shows periorbital and finger myokymia. Surface EMG shows continuous motor unit activity (Figure 4.3). The repetitive rhythmic discharges were heard clearly and recorded on videotape during the EMG. EEG at 3 years is normal.

Case II
The 50 year old paternal grandmother of the proband has had symptoms of ataxia since early childhood. They are precipitated by sudden movements, exercise, anxiety or loud noises, or occur spontaneously and last seconds to a few minutes. During episodes she becomes ataxic, has dysarthria but retains full consciousness. Phenobarbitone therapy as a child was not helpful. She attended a school for children with mild to moderate learning difficulties.
Figure 4.2. Ictal EEG of case III2, family A showing a focal sharp and slow wave activity with onset in right temporal region spreading to the left hemisphere. These changes were associated with an epileptic apnoea.

Figure 4.3. Surface EMG in case III2 showing rhythmic continuous motor unit activity
Clinical examination is normal apart from perorbital and finger myokymia. Acetazolamide was unhelpful.

Case II2
The 31 year old father of the proband was noted to have postural abnormalities of his upper and lower limbs at the age of 3 months when admitted with bronchiolitis. His wrists were flexed, the thumbs were adducted across his palms and his feet were held in equinovarus. There was no spasticity on examination. He was treated with splints. During this acute illness he developed transient oedema of his extremities. He was noted to have twitching of his eyelids but an EEG was normal. At 5 months he had surgery for a strangulated inguinal hernia. He walked at 14 months and his foot deformity had resolved, but he had a continued tendency to adduct his thumbs across his palms.

Episodes of ataxia began at the age of 4 years. They occur spontaneously, after a sudden movement or are precipitated by exercise. The attacks last seconds to minutes. Clinical examination showed myokymia, which was most prominent in the fingers and peri-orbital area. A typical ataxic episode, which lasted ~ 2 minutes was recorded on video after he was asked to walk briskly up a flight of stairs. He was markedly ataxic with dysarthria but eye movements were normal. EMG showed continuous motor unit activity consistent with myokymia. An interictal EEG was normal apart from showing prominent muscle activity with EMG at a frequency of ~10/s, which was more prominent with hyperventilation (Figure 4.4). A 1.5T MRI of brain and cerebral perfusion SPECT scan were normal. Acetazolamide produced resolution of attacks for 3 months but they returned at a reduced frequency. Attacks tend to cluster.

Case II4
This 30 year old woman has EA1 and epilepsy. She presented in early infancy with more prominent postural deformities of her limbs than in her brother. Her wrists were flexed to 50°, her thumbs were adducted and her knees were flexed to 20° and her feet were held in an equinovarus posture. Serum creatine phosphokinase was
normal. An EEG in infancy was normal. A diagnosis of atypical familial arthrogryposis was made. Like her brother she required surgery for an inguinal hernia. She walked late at 2 years of age and by 3 years the postural abnormalities had almost completely resolved. At the age of 9 years she was diagnosed as having stage IV Hodgkin’s disease. She responded to chemotherapy and localised radiotherapy and since then she has been disease free.

At the ages of 9 and 10 years she began to have epileptic seizures and episodic ataxia respectively. The clinical features of the seizures suggest they are focal seizures with impairment of awareness (complex partial) followed by secondary generalisation. There is no warning and the onset involves turning her head to the right accompanied by impaired awareness lasting up to 30 seconds followed by a generalised tonic clonic seizure. EEG and CT brain were normal at the age of 9 years. The ataxic episodes are clearly distinguished from the seizures and are similar to those described in her mother and brother. Consciousness is fully preserved. Neither the epileptic seizures nor the ataxic episodes have responded to phenytoin or sodium valproate. On examination she has periorbital and finger myokymia. EEG aged 29 was normal apart from prominent muscle artefact.

Case III1
This ten year old boy was also born with the postural abnormalities as described in other family members. At 8 months his fingers were held flexed and this would interfere with attempts to grasp and transfer objects. His knees were held slightly flexed (Figure 4.5). At 10 months, during an inter-current illness, he developed peripheral pitting oedema of his hands and feet, which resolved. By 14 months the postural abnormalities had resolved and he could cruise around furniture. At the age of 4 years he began to have typical ataxic episodes lasting from seconds to a few minutes. At age 10 an ataxic episode was videotaped in the hospital. It was induced by asking him to repeatedly step off a library stool. After 3 or 4 steps off the stool he could not speak, became very ataxic, lurching forward and having to be held by his father. The event lasted just a few seconds. EEG shows regular muscle activity more
Figure 4.4 Normal EEG in case II2, family A showing rhythmic EMG artefact

Figure 4.5. Case III1, family A in infancy. Flexed posture of knees, equinus posture at ankles and clenched fists are due to neuromyotonia.
prominent after hyperventilation but no other abnormality. EMG shows myokymia. Carbamazepine produced an initial decrease in ataxic episodes but this was not sustained.

4.3.2 Genetic and functional studies in family A

DNA sequence analysis of the KCNA1 gene revealed one significant heterozygous change, a C→G transition at position 677. This was not detected in 200 control chromosomes, it segregated with the disease and resulted in a radical amino acid substitution, threonine → arginine at position 226. This is a highly conserved position in the second transmembrane segment of the channel (Figure 4.6).

Expression studies showed that the mutant channel could be correctly translated and processed to the Xenopus oocyte cell membrane but it yielded currents with a significantly reduced amplitude, ~3%, compared with wild type. It was co-expressed with wild type channels to try to model the heterozygous state in vivo. The mutant allele seemed to dominate over the wild type with respect to current amplitude. This dominant negative effect is illustrated in figure 4.7.

4.3.3 Clinical study of family B

The pedigree is detailed in figure 4.8. There are 5 affected individuals of whom I have seen and examined 2 individuals.

Case IV1
The proband was referred to my paroxysmal disorders clinic for evaluation of possible epileptic seizures and dizzy turns aged 13y. His first events began around the age of 6 months. He would stop all activity, his lips would go blue, his face pale, his eyes would roll up and would he lose tone and slump to one side. The whole episode lasted one to two minutes. They occurred about once every two months. He
**Figure 4.6** Cartoon showing T226R mutation in S2 segment of the of Kv1.1 channel subunit in family A.

**Figure 4.7** Amplitude histogram showing whole cell currents in Xenopus laevis oocytes co-injected with wild type and mutant T226R channels. The mutant channels demonstrate a dominant negative effect.
was seen in the local hospital. An interictal EEG was normal. No diagnosis was made and the family stopped attending the hospital after about 2 years of age despite the events continuing until he went to school.

Around five years of age he began to have episodes he describes as “jelly legs”. These continue to occur most days with a frequency of up to 3 per day. The episodes are triggered by sudden movement such as tripping over feet, anxiety, sitting down and then trying to stand after exercise such as playing football. He feels a tingling sensation rising up his legs. Sometimes he tries to think of something to prevent the event progressing. This rarely helps. He feels unsteady as if he is going to fall, has to hold onto something for support and then his arms and legs start to shake. He staggers if he tries to walk. He remains fully aware. He has some dysarthria. The whole events lasts 30 seconds to 5 minutes. His mother managed to capture several events on home video. He is standing with a broad base but clearly ataxic with a coarse tremor of his upper limbs and titubation of his head. He is clearly aware and appropriately responsive in the events.

About once per month a typical unsteady episode progresses into a secondary generalised epileptic seizure. He collapses to the floor, is unconscious and has generalised clonic movements of all limbs. This lasts about 2 minutes. He has bitten his tongue in one event. Following the event he is confused. Neurological examination is normal apart from finger and eyelid myokymia. He has no educational difficulties. A standard EEG, sleep deprived EEG and 24h ambulatory EEG were normal. He had an episode of ataxia during the 24h EEG with no EEG change.

Lamotrigine and carbamazepine did not decrease frequency of either episodic ataxia or epileptic seizures. Acetazolamide has controlled the epileptic seizures but the frequency of episodic ataxia has reduced but still occurs about 5 times per week.
Figure 4.8 Pedigree of family B

Figure 4.9 Pedigree of family C
Case III2
The proband’s 44 year old father had brief episodes of ataxia from early childhood until about 15 years of age. He said that they were triggered by sudden movements and would last a few seconds to a minute. He retained consciousness throughout them. He did not seek medical advice. He has finger and eyelid myokymia evident on examination.

Other affected family members
I did not directly interview or examine the other family members. The history of their events came from case II2. The uncle of the proband, case III1, was reported to have had “dizzy turns” from childhood which continue as an adult. He is employed as a driver. He stops driving during an attack. He carries a supply of chocolates and sweets with him as he feels that taking something to eat stops an event. Case II2 only discovered this about his brother having similar episodes to himself during the evaluation of his son. The paternal grandmother, case II2, is also reported to have had “dizzy turns” since childhood and possibly epileptic seizures in her 20s. She lives in the USA and has apparently had extensive investigations with no diagnosis for her dizzy turns. The paternal great aunt of the proband, case III3, lives in Scotland and is also reported to have had brief “dizzy turns” since childhood. She has never been investigated and does not wish to be.

4.3.4 Clinical Study of family C

The pedigree is detailed in figure 4.9. There are 10 affected members of whom I have seen and examined 5 individuals over three generations. 2 cousins, cases III3 and III13, from this family were independently referred to my clinic for assessment of paroxysmal events.

Case III3
This 19 year old girl began having events age 6 or 7. They are triggered by sudden movement and emotion. They can occur spontaneously and in an intercurrent illness she can have up to 30 per day. She has events most days. They last seconds to up
to 20 minutes. She feels unsteady and has to hold on to someone or to a fence so she does not fall. She has dysarthria during episodes and has been stopped by police and accused of being drunk during an episode. Age 15 she had three episodes of collapse. One of these sounded clinically like a syncope. In two events she had a brief warning and then lost consciousness for a period followed by post ictal confusion and lethargy for about twenty minutes. One witnessed episode lasted 2-5 minutes. The history suggested a generalised tonic clonic epileptic seizure. An EEG was normal. On examination she has finger and eyelid myokymia. She has been treated with carbamazepine and acetazolamide these have not reduced the frequency of ataxic episodes.

Case III13
This 14 year old boy had neuromyotonia in early infancy. He tended to keep his arms flexed and fists clenched. This resolved by about a year of age. At about 1 year he had a generalised clonic febrile seizure. The clonic movements lasted between 5 and ten minutes. He began having brief episodes of ataxia lasting seconds to a few minutes aged 6 years. These are triggered by movement, startle or exercise but can occur spontaneously. They occur on most days and during intercurrent illnesses can almost be continuous throughout the day. A 24h EEG was normal. He has finger and eyelid myokymia. He has mild to moderate learning disability and attends a special needs school. His ataxic episodes have shown a partial response to carbamazepine.

Case II
This 64 year old lady has had episodes from about 12 years of age. They can be triggered by sudden movement such as getting up from a chair but also occur spontaneously when sitting or lying in bed. She feels unsteady and describes herself as “legless”. She has to hold onto something. Her voice becomes slurred. The episodes last from a few seconds to minutes. The longest was 10 minutes. At present they occur once a week but have occurred daily in the past. She has never seen a doctor about the events. There is no history of neuromyotonia when unwell. On examination she has finger and eyelid myokymia. None of her children had a clear
history of infantile neuromyotonia. She had 13 siblings and was not aware of any of them having similar episodes to herself.

Case II9
This 38 year old has had episodes from 7-8 years of age. The events are triggered by getting up too quickly or sudden movements. Her speech slurs and she feels dizzy. At the onset she feels a rising sensation coming up through her body. Attacks can last seconds to up to 10 minutes. During attacks she has to hold on to something. She had a bad attack when she was younger when dancing and wearing high heels causing her to fall and break a bone in her ankle. She has at least one episode a week but can have multiple episodes in a day particularly when she is unwell with a viral infection. Attacks may occur without any movement triggers when she is unwell. In the past her GP treated her for possible epilepsy and prescribed tegretol. It didn’t clearly help and she doesn’t take any regular medication. She has collapsed once and lost consciousness. I thought this was a syncope and not epileptic. On examination she has finger and eyelid myokymia.

Case II10
This 37 year old lady had events from 7 or 8 years of age. The episodes are triggered by getting up too quickly, any sudden movement and startle. The events are described as dizzy episodes. Her speech is slurred and she has to hold on to something or sit down. Sometimes they occur without any clear trigger when she is lying in bed. This is more common if she is unwell. She has at least 1 per week, but usually more, and can have a cluster of events in one day. She has never taken medication and has been told “it was all in her head”. On examination she has finger and eyelid myokymia.

Other family members
I did not interview or examine other family members. The following information is from their close relatives. Case II4 is the mother of the proband case III3 and daughter of case II. This 40 year old lady has had “dizzy spells” since early childhood. These are triggered by movement or stress. Since the diagnosis of her
daughter she has been on carbamazepine which has helped to a degree. Case II6 is the twin sister of case II4. She has had “dizzy spells” since childhood. She is on no medication. Case II12 had dizzy turns from childhood. She died in childhood from a respiratory illness. Case III5 has events less frequent than other family members. They only tend to happen when he is unwell. The started in early childhood and last a few seconds. He has mild learning disability. Case III8, the 21 year old daughter of II6, is said to have similar episodes to her mother. Case III16 the ten year old brother of III13 and son of II10 has had episodes of unsteadiness triggered by movement or startle since the age of 4 years. He has not received any treatment as yet.

4.3.5 Genetic and functional studies in families B and C

The KCNA1 gene was sequenced in 2 individuals from family B and 5 from family C. All affected individuals had a novel T to C substitution identified at position 1241 of KCNA1. This change was not seen in 128 control chromosomes. The mutation results in the substitution of phenylalanine, a large aromatic residue, for serine, a small polar residue, at amino acid 414 (F414S). This is at a highly conserved residue in the S6 transmembrane segment of the Kv1.1 channel. As the mutation was found in 2 apparently unrelated families haplotype analysis was performed. All except one marker was shared by all the affected individuals from both families. The results suggested a shared ancestral haplotype on which the mutation has arisen and that the two families are related. The families live approximately 20 miles apart and are not aware of any familial link with each others localities.

It was possible to express functional F414S mutant potassium channels in the HEK cell model. The channels were able to conduct potassium currents but these were approximately 40% of the wild type. The channels were slower to activate and inactivated more rapidly than wild type channels. The mutant channels therefore demonstrated a loss of function compared to wild type.
4.4 Discussion

4.4.1 Clinical Features and Investigations

In this study I have identified three families with the rare autosomal dominant disorder EA1 in which some individuals from each family also had epileptic seizures. Sequencing and haplotype studies suggest that families B and C share a common mutation in KCNA1 and are in fact related to a common ancestor. Therefore in the discussion I will refer to them collectively as family BC.

As in other paroxysmal conditions due to ion channel dysfunction, there was a long delay in making the correct diagnosis. The diagnosis of the three probands led to the correct diagnosis of an additional 17 individuals none of whom had a previous diagnosis of EA1. The lack of correct diagnosis is probably not a surprise given the rarity of the disorder and its paroxysmal nature. A factitious disorder was considered in at least two individuals. Some of the older affected individuals had events from childhood and although they continued to have episodic ataxia had become stoical and accepting of their symptoms and did not seek medical advice. It was also notable that close family members were sometimes not aware that a sibling or parent had exactly the same symptoms.

Neuromyotonia with postural abnormalities affected 4/5 individuals in family A but only 1 individual in family BC. This is presumed to be as a result of continuous muscle fibre activity. The inguinal hernias in family A were likely to be as a result of myokymia in the abdominal wall musculature. The postural abnormalities were so severe that they were termed an atypical arthrogryposis in family A but even more atypically for an arthrogryposis the postural abnormalities resolved with time. It is important that surgery is not considered for tendon release in infancy. Prior to the report of family A (Zuberi et al 1999) postural abnormalities had been described in one family previously (Hanson et al 1977). Another point of interest (and relevant to the next chapter) is that the postural abnormalities worsened during acute illnesses in
three cases in family A and 1 case in family BC. This may be due to acid-base or electrolyte changes exacerbating ion channel dysfunction.

All individuals had onset of ataxia before 12 years of age. The triggers including movement, startle, exercise and emotion were typical for EA1 but what has not been emphasised in previous literature is that many of the individuals can have spontaneous attacks with no clear trigger even when well. Attacks can occur in bed. When unwell, individuals in family BC described almost continuous, repeated attacks through the day. Attacks were usually brief but lasted up to 20 minutes in one case. During ataxic episodes two teenagers in family BC were questioned by police officers who thought they were drunk or taking drugs. The attacks captured on video had some significant differences between individuals. In family A, a father and son had episodes captured on video. Both appeared slightly distressed during episodes, they found it difficult to talk, they had a broad based gait and wanted to hold on to something to maintain standing. Case III1 lurched forward in the episode and had to be caught by his father. The home videos of case IV1 showed a prominent coarse tremor of the limbs and titubation of the head during events.

The most useful clinical sign was myokymia detected in all individuals by observation of small amplitude, semi-rhythmic, irregular, lateral finger movements with the hands held outstretched with forearms pronated. Eyelid myokymia was relatively subtle and seen more easily in the adults than the children. Surface EMG is a non-invasive way to demonstrate the myokymia (Figure 4.3). The observation of a continuous rhythmic muscle discharge artefact on EEG recordings in two individuals in family A was also a clue to the diagnosis. The muscle activity became more prominent during hyperventilation, perhaps reflecting the effect of changes in pH or CO₂ on channel function.

Overall the effect of medication on symptoms was disappointing. The effect of medication on epileptic seizures will be discussed in the section on epilepsy below. Acetazolamide, a carbonic anhydrase inhibitor, was partially effective in controlling ataxia in 1 member of family A and in two members of family B. Acetazolamide
may produce its effect by increasing the CO₂ in the channel vicinity, causing hyperpolarisation of the cell membrane and reducing neuronal excitability (Brunt & Van Weerden, 1990; Lubbers et al, 1995). Carbamazepine, an anti-epileptic medication and sodium channel blocker, was helpful in reducing ataxia for 3 individuals in family A, but this response was not sustained. It was also helpful in one individual in family BC. Other anti-epileptic medications including phenobarbitone and phenytoin have been reported as effective in some individuals in other families but when tried were not helpful in the families I have managed. These observations indicate that the variable response to medication may not only be influenced by the specific mutation in a family but also by the rest of an individual’s genetic and possibly environmental background.

Formal neuropsychological evaluations were not performed on any of the affected individuals with EA1. 2 individuals in family BC (one with and one without epilepsy) and one in family A (without epilepsy) attended schools for children with mild to moderate learning disability. My clinical impression was that several other family members may have specific learning difficulties but this was not formally assessed. This is a potential area for further study. The Kv1.1 channels are expressed throughout the nervous system and may have a role in cognitive processing. KCNA1 mRNA is known to be expressed in human amygdala, caudate nucleus, hippocampus, hypothalamus and thalamus (Albrecht et al 1995).

**4.4.2 Molecular genetic and functional studies**

There is good evidence that the mutations detected in these three families are pathogenic. The nature of the mutations as detailed in the results section and the expression studies support this. The mutant allele from family A could be translated and processed to the cell membrane of the Xenopus oocyte. The currents were significantly reduced compared to the wild type channel (Figure 4.7). When expressed together with the wild type channel the mutant allele seemed to dominate with respect to current amplitude. Therefore, heterotetrameric channels, as might be expressed in heterozygous patients, would be expected to have a reduced potassium
efflux during action potentials. Similar results have been achieved in functional studies of another EA1 associated mutation (Adelman et al 1995). Delayed repolarization is therefore likely to be the basis of the neuromyotonia and episodic ataxia. It is also possible that weakness may result from a depolarisation block.

The clinical, genetic and functional studies of families B and C have not been published as yet. The functional study of F414S in HEK cells performed by Tracey Graves and colleagues is the first we are aware of in which mutant Kv1.1 channels have been expressed in a human cell line. The mutant channel demonstrates a loss of function as detailed in the results section above. In many channelopathies expression studies give conflicting results in different models (Ragsdale 2008). EA1 mutants seem to be consistently associated with reduced channel function (Graves TD, personal communication).

4.4.3 The relationship between EA1 and epilepsy

Our 1999 Brain paper was the first to report the relationship between KCNA1 mutations and epilepsy as giving a wider insight into the pathogenesis of epileptic seizures (Zuberi et al 1999). Family A were the 11th family to be reported with EA1, comprising a total of 90 affected individuals (Van Dyke et al 1975; Hanson et al 1977; Gancher & Nutt 1986; Brunt & Van Weerden 1990; Vaamonde et al 1991; Browne et al 1994; Lubbers et al 1995; Comu et al 1996). 8 individuals from three families, including family A, were reported to have epilepsy. Eunson et al. reported a family who had a KCNA1 mutation associated with myokymia and epilepsy but not with episodic ataxia (Eunson et al 2000). The proband was a 40 year old woman who had tonic clonic seizures from the age of 9 and simple partial seizures from the age of 19. Her EEG showed sporadic sharp waves and slow waves over the left temporal region. Her 3 year old son had afebrile generalised tonic clonic seizures. Families B and C contain a further 2 individuals with recurrent epileptic seizures and one who has had a single generalised tonic clonic seizure. In all the families with EA1 and epilepsy none of the individuals who are unaffected by episodic ataxia or myokymia
have epilepsy. There is therefore a significant overrepresentation of epilepsy in individuals with KCNA1 mutations.

The phenotype of the epileptic seizures in infancy in the proband (III2) from family A, and the proband (IV1) from family B, are very similar. They appear to be brief focal epileptic seizures characterised by epileptic apnoea. They might best be described as benign partial seizures of infancy. Epileptic apnoeas are often associated with temporal lobe onset epileptic seizures in infancy. Kv1.1 is known to be expressed in human amygdala and hippocampus (Albrecht et al 1995). The seizure captured in case III2 from family A appeared to have temporal lobe onset. These seizures are not dissimilar to events seen in the syndrome of benign familial neonatal convulsions (see Chapter 7). These are known to be associated with mutations in the potassium channel subunits KCNQ2 and KCNQ3 (Biervert et al 1998; Charlier et al; Singh et al 1998).

Further evidence that dysfunction of Kv1.1 channels can cause epilepsy come from the mouse knockout for Kv1.1, which has a lethal epilepsy phenotype (Smart et al 1998). Drugs such as 4-aminopyridine that block potassium channels are proconvulsant in humans (Newsom-Davis 1993; Morales-Villagran et al 1996). Autosomal dominant lateral temporal lobe epilepsy also known as autosomal dominant partial epilepsy with auditory features is associated with mutations in the LGI1 gene. The protein product of this gene has recently been found to be part of a protein complex closely associated with the Kv1.1 protein. Mutations in LGI1 appear to alter fast inactivation of the channel once again implicating Kv1.1 in epileptogenesis (Schulte et al 2006).

Further evidence that Kv1.1 potassium channels are important in the pathogenesis of epilepsy and neuromyotonia comes from autoimmune limbic encephalitis. In this condition there is evidence of autoantibodies against the Kv1.1 channels (Vincent et al 2004). These channels are expressed in the limbic system and autoimmune attack can result in epileptic seizures, encephalopathy and long term cognitive impairment. Another feature seen in some individuals with limbic encephalitis is myokymia, indicating antibody mediated attack on channels in peripheral nerve.
Familial Generalised Myokymia

5.1 Introduction

In this chapter I describe a Scottish family from the Outer Hebrides with 3 affected individuals who have familial generalised myokymia. The syndrome of idiopathic familial generalised myokymia or neuromyotonia had been previously recognised however this is the first family reported with isolated neuromyotonia caused by a genetic defect. This work was published in Annals of Neurology in 2000 in a report containing three other families with related genetic abnormalities (Eunson et al 2000).

5.2 Methods

5.2.1 Clinical studies

The proband of this family presented to the neurology service, Royal Hospital for Sick Children, Glasgow as an emergency. Additional family members were studied on a field trip to the Outer Hebrides and cases were subsequently reviewed in clinic in Glasgow. I undertook the clinical evaluation of all family members. Dr Robert McWilliam accompanied me on the field trip. Electromyography studies were undertaken by my colleagues Dr McWilliam & Dr Iain Horrocks, Consultant Paediatric Neurologists. The examination of all affected members was videotaped as were the EMG studies.
5.2.2 Genetic and functional studies

Molecular genetic studies were undertaken by Dr Louise Eunson under the supervision of Professor Mike Hanna at the Muscle and Neurogenetics Sections, Institute of Neurology, Queen Square, London. Expression studies in a *Xenopus oocyte* system (using the methods described in chapter 6) were undertaken by Dr Alex Spauschus in Professor Dimitri Kullmann’s laboratory in the Institute of Neurology.

5.3 Results

5.3.1 Clinical study

The pedigree is detailed in figure 5.1. The family were first studied before the birth of case II3 and she was not included in the publication by Eunson et al (Eunson et al 2000).

Case II1

This 3 year old boy was airlifted to the Royal Hospital for sick children Glasgow from an island in the Outer Hebrides because of a 12 hour history of inability to walk associated with a 24h vomiting illness. His birth and development had been unremarkable up until that point apart from intermittent toe walking and running which his mother had noticed after he first walked aged 18 months. On the day of admission he had one recorded temperature of 38 degrees celcius but was otherwise afebrile. He was well perfused with a tachycardia of 160bpm and normal blood pressure. He was fully alert, answering questions appropriately. He had signs of a systemic viral infection with enlarged tonsils and injected tympanic membranes.

Cranial nerve examination was normal. There was increased tone in his upper limbs and his fists were held clenched with thumbs adducted. Small semi-rhythmic myokymic lateral movements of his fingers were visible. Upper limb tendon
Figure 5.1 Pedigree of family with generalised myokymia

Figure 5.2 Calf muscle hypertrophy in siblings with familial generalised myokymia
reflexes were present but diminished in amplitude. His knees were held in extension, and his ankles were plantar flexed. Rippling movements of his calf and hamstring muscles were palpable and these muscles were visibly hypertrophied. Tone was increased in his lower limbs. Deep tendon reflexes were diminished at the knees and not demonstrable at the ankles. There was no percussion myotonia. He did not seem to be weak. He could stand with minimal assistance but only on the tip of his toes. Plasma electrolytes, calcium, magnesium, bicarbonate, lactate, ammonia and urea were normal. Plasma alanine aminotransferase (80 IU/L; normal range, 10-40 IU/L) and creatine kinase (1,008 IU/L; normal range 15-90 IU/L) were elevated. There was no myoglobinuria. Muscle ultrasound was normal. Motor nerve conduction velocities were normal. Surface EMG over the right lateral gastrocnemius and right extensor digitorum showed continuous motor unit activity (myokymia) even at rest, with a regular periodicity of about 6 bursts per second.

His vomiting settled, and 24h after his admission, he was walking and running, but with a toe-to-toe gait. His legs felt less stiff, myokymia was still clinically evident but rippling movements of large muscles were less evident. Tendon reflexes were more easily demonstrated. His creatine kinase and other muscle enzyme levels normalized.

There was no family history of episodic ataxia, epilepsy or neuromuscular problems however the findings of myokymia / neuromyotonia on examination were reminiscent of that seen in family A with episodic ataxia that has been discussed in chapter 4. A DNA sample was therefore taken from the proband and forwarded to colleagues at the Institute of Neurology, Queen Square.

A year after this acute admission he was reviewed on a field trip to the Western Isles. He had remained well with no vomiting illnesses in the intervening period. Generalised upper and lower limb muscle hypertrophy was more prominent. There were no fixed deformities and the muscles were not stiff. A toe-to-toe gait was
evident on running. Surface EMG of the gastrocnemius, extensor digitorum and frontalis muscles showed continuous generalized myokymic activity.

This boy, who is now 15 years old, has continued under regular review in my clinic. He has not had another acute episode like the one aged 3 years. He has had no episodes of ataxia. His muscles are hypertrophied and rhythmic myokymia is clinically evident on observation of the calf muscles and fingers (Figure 5.2). He has a mild degree of tendo-achilles shortening bilaterally but he is able to bring his ankles to the neutral position. He complains of feeling tired after exercise but does not suffer from muscle cramps. He has been managed with physiotherapy, ankle foot orthoses at night and carbamazepine. He has no significant functional deficit due to his myokymia and I have prevented an orthopaedic surgeon from performing a tendo-achilles release.

Case II
The 39-year-old father of the proband was first seen on the field trip. He had no history of episodic ataxia, stiffness, or other muscle symptoms. There were no older living relatives to ask whether he toe walked as a child and review of general practice notes revealed no history of muscle or postural problems. The only abnormalities on clinical examination were calf muscle hypertrophy and subtle myokymia of his dorsal interossei. Surface EMG revealed myokymia with continuous motor unit activity within the right lateral gastrocnemius and right extensor digitorum.

Case I2 and II2
The 35-year-old mother of the proband had a normal neurological examination and surface EMG study. The 19 month old male sibling of the proband had a normal neurological examination and surface EMG study.

Case II3
The younger sister of the proband was born after the field trip. She is reviewed regularly with her brother in my clinic. She presented to her local district general
hospital aged 2 years unable to walk during a vomiting illness. She had increased tone in upper and lower limbs with her legs extended. Myokymia was clinically evident. Her increased tone settled within 24 hours. She had a further episode of neuromyotonia lasting 24 hours aged 6 years following a general anaesthetic for a tooth extraction. Her calf muscles are hypertrophied (Fig 5.2) and she has clinically evident myokymia. Surface EMG revealed continuous motor unit activity. She complained of occasional cramp like pain in her calf muscles which has responded to therapy with carbamazepine.

**5.3.2 Genetic and functional studies**

Sequence analysis of the KCNA1 gene was performed on DNA from all family members. A novel heterozygous mutation was identified in the three affected individuals. It was not present in the two unaffected cases. The point mutation C731A results in a radical amino acid substitution of praline to histidine at position 244 in the intracellular loop between transmembrane segments 2 and 3 (Figure 5.3).

Mutant cRNA was injected independently into Xenopus oocytes and current measured across the mutant channel and compared to the wild type channel. There was no difference in the current amplitude through mutant and wild type channels (Fig 5.4). When expressed together with wild type channels the current amplitude was relatively increased relative to wild type alone. Further studies looked at the activation and de-activation time course of the channel. When expressed on its own, the voltage activation of the P244H mutant channel was shifted to a more hyperpolarized potential and deactivation was slowed. These two effects were abolished by expressing the mutant channel with wild type channels.
Figure 5.3 Cartoon of Kv1.1 potassium channel subunit showing positions of various mutations including the P244H mutation in the family with generalised myokymia.

Figure 5.4. Amplitude histogram of currents through mutated and wild type channels. The current through the P244H channel is similar to the wild type
5.4 Discussion

This family is the first reported with a genetic basis for isolated neuromyotonia or as we term it familial generalised myokymia (Eunson et al. 2000). This clinical study expands the phenotypic spectrum associated with mutations in the KCNA1 gene. As is the case with many genetic channelopathies there is an autoimmune counterpart. Isaacs syndrome, or acquired neuromyotonia, is associated with autoantibodies directed against potassium channels in peripheral nerve and myokymia is sometimes seen in association with Kv1.1 antibody related limbic encephalitis (Shillito et al. 1995; Vincent et al. 2004).

I believe that the proband in our family had a viral infection associated with vomiting leading to subtle pH, CO2 or electrolyte changes in the area surrounding the cell membrane therefore aggravating the potassium channel dysfunction. The peripheral nerve hyperexcitability caused by the mutant channel caused continuous motor unit action potentials and clinical myokymia. This continuous involuntary muscle contraction over time leads to muscle hypertrophy. When the channel function was further compromised in the vomiting illness the continuous motor unit activity was so prominent that it resulted in neuromyotonia (stiffness), postural abnormalities and an inability to walk. Deep tendon reflexes were difficult to demonstrate because of the neuromyotonia. The creatine kinase levels were raised due to the excessive involuntary muscle activity.

Five families in three publications had been reported with features similar to this Scottish family (Ashizawa et al. 1983; Auger et al. 1984; Jamieson & Katirji 1995). Ashizawa et al. described a family with seven affected individuals. The proband, a three-year-old girl presented during an upper respiratory tract infection with diarrhoea in almost exactly the same way as the proband in our family. She was unable to walk, her legs were stiff and persistently extended, she had continuous twitching of muscles and her creatine kinase was raised. In one of the two families described by Auger et al in 1983 all three affected individuals (mother and two
daughters) also had neonatal convulsions. The authors commented that these may have been muscle contractions resulting from spontaneous muscle activity and misinterpreted as epileptic events. Dedek et al described a family with benign familial neonatal convulsions and myokymia associated with a KCNQ2 mutation (Dedek et al 2001). Therefore KCNQ2 as well as KCNA1 mutations can be associated with familial generalised myokymia. If benign neonatal convulsions occur, KCNQ2 would seem a more likely candidate.

The two affected children in this family have been treated with carbamazepine. It is difficult to know if this is producing a significant clinical benefit however they have not had any further episodes of severe neuromyotonia and do not suffer any muscle cramps.

The expression studies on the P244H mutant channel when co-expressed with the wild type showed it had the smallest effect on K+ currents. It would seem to have much less significant effects than the mutations reported with EA1 and myokymia or EA1, myokymia and epilepsy. This may go some way to explaining the milder phenotype with isolated myokymia. This family may not have presented to neurology if the proband had not had a significant vomiting illness. The only clinical sign had been intermittent toe walking. This is a relatively common presentation in infancy with a variety of causes but mostly unknown.

This study has produced novel insights into the variable phenotypes associated with KCNA1 mutations and through expression studies has suggested mechanisms at a cellular level that may influence the clinical phenotype.
Chapter 6

Episodic Ataxia Type 2 & Epilepsy

6.1 Introduction

In this chapter I describe a single case study of a child with episodic ataxia type 2 (EA2) which has provided early insights into the causes of absence epilepsy in humans. This individual’s clinical features, along with molecular genetic and expression studies, have been published in The Lancet (Jouvenceau et al 2001).

EA2 is the commonest of the paroxysmal ataxias and is associated with mutations in the CACNA1A gene which codes for the alpha subunit of a voltage gated P/Q type calcium channel (Ophoff et al 1996). The channel is made up of one major transmembrane alpha 1 (α1A) subunit and auxiliary β, α2δ, and γ subunits (Catterall WA 2000b). The alpha subunit is the pore forming part of the channel and is essential for voltage sensing and is the major determinant of the biophysical properties of the channel.

EA2 typically presents in autosomal dominant pedigrees. EA2 is allelic with familial hemiplegic migraine (a variant of migraine with aura) and spinocerebellar ataxia type 6 (a late onset slowly progressive ataxia). These are 3 distinct conditions however phenotypic overlap may occur (Jen et al 1999). In contrast to EA1 the episodes of ataxia in EA2 are much longer, lasting hours (Parker 1946; Hill & Shermann 1968; White 1969; Donat & Auger 1979; Baloh et al. 1997; Jen et al 2007).

Individuals with EA2 can have interictal cerebellar signs and a slowly progressive background ataxia (Yue et al 1997). Onset of symptoms is typically in childhood. Triggers for the ataxic episodes include physical and emotional stress. This includes
startle, movement and anxiety. Gait ataxia may be accompanied by dysarthria, nystagmus, vertigo, nausea and headache. Acetazolamide may be dramatically effective in EA2, so much so that it has been termed acetazolamide responsive ataxia (Griggs et al 1978). About 50% of EA2 patients report headache which fulfil the diagnostic criteria for migraine. The headache can have features of a hemiplegic migraine.

Gaze evoked nystagmus may be present between attacks but interictal cerebellar signs may be difficult to demonstrate. Failure to suppress the vestibular-ocular reflex with fixation may be the only early sign of cerebellar vermis dysfunction. With time spontaneous vertical nystagmus may appear. MRI may reveal subtle vermian atrophy and MR spectroscopy can show an alkaline cerebellar pH (Bain et al 1992; Sappey-Marinier et al. 1999). Channel dysfunction can therefore cause chronic and progressive symptoms as well as paroxysmal events.

6.2 Methods

6.2.1 Clinical study

The index case was referred to Professor JBP Stephenson in Glasgow for a second opinion by Dr Venkataswaran Ramesh, Paediatric Neurologist, Newcastle General Hospital. I undertook the clinical evaluation of the child along with Professor Stephenson. The clinical examination was videotaped. I arranged for DNA to be forwarded to colleagues in London and communicated with them regarding the child.

6.2.2 Molecular Genetic and expression studies

The molecular genetic and expression studies were undertaken by Ann Jouvenceau, Louise Eunson and Alex Spauschus and colleagues in Professor Mike Hanna and Professor Dimitri Kullman’s laboratories in the Institute of Neurology, Queen Square, London. DNA from the index case and both his parents was studied.
Following exon amplification by PCR the CACNA1A gene was sequenced. Genotyping to test for paternity was performed. Expression studies of the mutation identified were performed in a *Xenopus oocyte* system. The mutant and wild type α1A subunits were co-expressed together or independently with β and α2δ subunits.

**6.3 Results**

**6.3.1 Clinical details**

The index case was 11 years old when seen in Glasgow. He had an uneventful pregnancy and birth history. His mother was concerned that his development was slow at around 1 year of age. He walked at around 18 months but was said to fall frequently and was described as a clumsy child. His first words were at 18 months however his speech was unclear and he attended speech therapy pre-school. At the age of three years he developed nocturnal generalised tonic clonic epileptic seizures (GTCS) and daytime absences. The GTCS stopped at age 8 but the absences continued. Aged 6 years he had an EEG which showed interictal polyspike and wave discharges in the resting record and a further burst of generalised spike and wave during hyperventilation. He was commenced on lamotrigine and subsequently treated with gabapentin and clonazepam without significant effect on the absences.

From 8 years he developed attacks of unsteadiness of gait, dysarthria and diplopia. He retained a normal conscious level through these events. The episodes lasted up to 3 hours and had no clear precipitants. These attacks were clearly different from the absences. The episodes increased in frequency such that on most days he would feel unsteady for a period particularly in the mornings. He felt exercise made him less unsteady. He had been examined during an event and had marked gait and upper limb ataxia with slurred speech and prominent horizontal and vertical nystagmus. He was appropriately responsive. EEG during the events showed runs of slow wave activity and sharp waves over one or other hemisphere but no evidence of a non-convulsive status. Acetazolamide and flunarizine did not reduce the frequency of
events significantly. An MRI age 11 was reported as normal. He is in mainstream school with no evidence of a global learning difficulty.

There is no family history of episodic ataxia. He has three siblings who are all well. The only paroxysmal events in the family are night terrors in a sibling and syncopal events in his mother.

When he was reviewed in Glasgow he was said to be having a good day. He had a broad based stance and gait with minimal truncal ataxia. There was no tremor. There was no evidence of upper limb ataxia with no evidence of dysdiadochokinesia or dysmetria or on finger nose testing. Cranial nerve examination apart from eye movements was normal. He had a gaze evoked nystagmus with both right and left horizontal eye movements. He also had a gaze evoked vertical nystagmus. The other feature of note was failure to suppress his vestibular-ocular reflex with fixation. All aspects of the examination were captured on videotape.

Given the history of episodic ataxia and the findings on clinical examination we felt that a possible diagnosis, despite lack of response to acetazolamide, was episodic ataxia type 2 with absence epilepsy. As there was no family history this would most likely be associated with a de novo mutation in CACNA1A. In view of my collaborations with colleagues in the Institute of Neurology on cases with EA1 and epilepsy I communicated with them directly and they agreed to sequence the CACNA1A gene.

6.3.2 Molecular Genetic and expression studies

Colleagues at the Institute of Neurology identified a heterozygous point mutation (C5733T) in CACNA1A in the index patient. The mutation results in an arginine (CGA) to stop (TGA) change at amino acid position 1820 (Fig 8.1). The mutation was not present in either of his parents or in 200 control chromosomes. Genotyping suggested a less than one in a million chance of non-paternity. This confirmed the
clinical assessment that this was a de novo mutation. The R1820stop mutation is the most distal truncating mutation identified in the gene to date.

When the mutant channel was expressed in *Xenopus* oocytes the currents measured were indistinguishable from those across un-injected oocytes. This indicates that the mutant channel was non-functional. When the R1820stop mutant channels were injected along with wild type channels the current measured was significantly reduced compared to wild type alone. The mutant channels demonstrated a dominant negative effect on the wild type channels.

### 6.4 Discussion

This was the first report linking a calcium ion channel subunit to absence epilepsy in humans. There are several pieces of evidence that suggest the mutation identified in the index case was pathogenic. The phenotype of the index case with episodic ataxia, with interictal cerebellar signs was consistent with EA2. The mutation was heterozygous as seen in other cases of EA2. The mutation was not detected in control chromosomes or unaffected family members. The mutation was a premature stop codon at the beginning of the highly conserved C terminus. The expression studies indicated that the function of the channel was significantly impaired by the mutation. Epilepsy is not typically a feature of EA2 but EEG abnormalities including focal slow wave activity, sharp waves and spike and wave complexes have been reported in interictal EEG studies (Van Bogaert and Szliwowski 1996). The R1820stop mutation produces a more severe impairment in channel function than demonstrated in functional studies of other CACNA1A mutations associated with EA2. This may explain the absence epilepsy in this child.

Several spontaneous and mutagen induced mouse strains exist with mutations in the homologous murine CACNA1A gene (Fletcher & Frankel 1999). These include the *tottering*, *leaner and rolling*. The tottering mouse is remarkable in that it exhibits ataxia, and intermittent dystonia as well as absence epilepsy (Noebels & Sidman
Figure 6.1 Cartoon of voltage gated calcium channel α1 subunit showing positions of mutations associated with EA2. The star shows the position of the mutation detected in the child with EA2 and absence epilepsy (Taken from Jouvenceau et al 2001)
The leaner subtype of the homozygous tottering mouse has progressive ataxia, cerebellar degeneration and epileptic absences with spike wave discharges on EEG. These mice strains are regarded as very important models for studying the physiology of generalised epilepsies with generalised spike and wave discharges – the common idiopathic generalised epilepsies. Oscillations within the thalamocortical loop are thought to be important in generating spike wave discharges in absence epilepsy. There is evidence of abnormal control of thalamocortical activity in the tottering mouse (Caddick et al 1999). The index case in this report therefore supports the proposition that P/Q type calcium channels are important in the generation of generalised epileptic seizures in humans.

Since the index case and genetic study were published in the Lancet further evidence has emerged linking dysfunction of the CACNA1A channel and absence epilepsy. A spontaneously occurring rat strain called groggy, which has ataxia and absence epilepsy has been found to have mutations in the α1A subunit of the calcium channel (Tokuda et al 2007).

Further families with absence epilepsy, episodic ataxia type and CACNA1A mutations have been reported (Imbrici et al 2004; Jen et al 2004). In one family five individuals had generalised 3Hz spike and wave on EEG, absence epilepsy and ataxia (Imbrici et al 2004). One family member with absences developed marked cerebellar ataxia only when he was on anti-epileptic medication. This suggests that the mutation causing the epilepsy can also make an individual vulnerable to the side effects of medication and determine the nature of the side effect, in this case ataxia.
CHAPTER 7

BENIGN FAMILIAL NEONATAL SEIZURES

7.1 Introduction

The syndrome of benign familial neonatal seizures (BFNS), which is also termed benign familial neonatal convulsions, was the second idiopathic epilepsy syndrome (after autosomal dominant nocturnal frontal lobe epilepsy) with a defined genetic basis. In this chapter I describe the clinical evaluation of two large Scottish families with BFNS. These studies expand the genetic and clinical spectrum of this disorder. The molecular genetic study of family A has been published (Heron et al 2007).

BFNS is an uncommon disorder but probably unrecognised in many families. In the course of my clinical practice I have diagnosed two further families with BFNS who are discussed briefly. Several individuals from both families reported in this chapter have presented with neonatal seizures to the neuroscience service at RHSC, Glasgow over the last 30 years.

BFNS is an autosomal dominant disorder characterised by the onset, in the first week of life, of epileptic seizures, in otherwise well neonates (Rett & Teubel 1964; Ronen et al 1993). The seizures are typically tonic at onset, with cessation of movement, ocular deviation, sometimes apnoea and autonomic symptoms, with progression at times, to clonic features. The seizures usually cease spontaneously between a week and four months after onset. Some individuals may only have one or two days of
seizures. Previous studies have suggested a 5-15% risk of epileptic seizures later in life. The majority of affected individuals have no cognitive problems.

This was the first idiopathic genetic epilepsy on which successful linkage was reported, to chromosome 20q13.2 in 1989, and 8q in 1993 (Leppert et al 1989; Lewis et al 1993). BNFS is associated with mutations in two potassium channel subunit genes KCNQ2 (Chr 20) and KCNQ3 (Chr 8) (Biervet al 1998; Singh et al 1998; Charlier et al 1998). KCNQ2 and KCNQ3 code for the Kv7.2 and Kv7.3 channel subunits respectively. These two channel subunits are involved in the structure of the M-channel which allows flow of the M current (Wang et al. 1998). The M current is a slowly activating and deactivating potassium current that plays a critical role in determining the electrical excitability of neurons. It is reasonable to expect that its dysfunction may lead to epileptic seizures.

Infants born prematurely will have their seizure onset around their due date. Why the seizures occur at such an age specific period is unknown but is likely to be due to developmental gene expression of the gene itself or modifying genes. A recent study has suggested an increase in KCNQ2 and KCNQ2 gene expression in several brain regions including hippocampus in late fetal life and a decrease after birth (Takaumi et al 2008). Several families have been reported worldwide with mutations in KCNQ2 and KCNQ3. The vast majority >90% have mutations in KCNQ2 (Singh et al. 2003, Steinlein et al 2007).

7.2 Methods

7.2.1 Clinical studies

The probands from both families were referred to the Royal Hospital for Sick Children with onset of seizures in the neonatal period. The families were assessed as detailed in the methods section. The key part of the evaluation was the family history. In family A, case II:5, the grandmother of the proband provided the extensive family history. Several of her children were clinically affected. A
videotaped description of the family history was taken. Interictal EEG was available for several cases. Video telemetry captured seizures in one patient.

### 7.2.2 Molecular genetic study

DNA samples from the probands and close affected relatives were forwarded to John Mulley’s laboratory at the Women and Children’s Hospital, Adelaide. Sarah Heron, Kathleen Cox and Bronwyn Grinton undertook the molecular genetic work. The probands were tested by direct sequencing for mutations in all coding exons of KCNQ2 and exons 5 and 6 of KCNQ3. DNA from family A was subjected to multiplex ligation probe analysis (MLPA) to look for deletions and duplications which would not be detected by sequencing. This is a recently developed DNA dosage technique which can look at copy number variations in the genome and is therefore able to detect deletions or duplications within a specific region. With informed consent DNA was tested in 4 affected members of family A. In family B DNA was available in two affected individuals and two obligate carriers.

### 7.3 Results

#### 7.3.1 Clinical Study of family A

The pedigree of family A is detailed in figure 7.1. The pedigree showed that the benign familial neonatal seizures were inherited in an autosomal dominant fashion. 21 individuals from a kindred comprising 75 individuals had a history of neonatal (19) or infantile onset (2) seizures. 7 individuals had neonatal onset and offset of their seizures. 6 cases had neonatal onset and offset in the first 6 months of life. 6 cases had onset of their seizures in the neonatal period with further seizures into childhood in four cases and into adult life in another 2 cases. 2 had onset outside the neonatal period and 1 of these cases has had seizures continuing into adult life. Both individuals with onset in infancy were interviewed and DNA was available for testing.
7/21 (33%) of individuals had seizures outside of infancy with three persisting into adult life. None of the affected individuals had learning disability.

Illustrative Case Histories

Case IV:15
The proband presented on day 5 with a cluster of epileptic seizures. In between seizures her behaviour was normal as was the neurological examination. She became stiff, apnoeic and blue with a few clonic jerks of limbs. The episodes lasted 90 seconds to 3 minutes. She had a single dose of phenobarbitone but the seizures continued on day 6 and carbamazepine commenced. At 4 weeks carbamazepine was stopped due to a rash. She didn’t take regular medication after this. Up to 4 months she had four brief clusters of seizures each lasting a minute. Following this she had clusters of brief seizures when she was unwell with viral infections once or twice a year up until 4 years of age. In the seizures outside of infancy she goes stiff, and has clonic movements of face, upper and lower limbs lasting 1-2 minutes. Her development is normal. Interictal EEG was normal.

Case III:17
The mother of the proband had a single generalised convulsion during a febrile illness aged 10 months. Her mother said she had an abscess in her groin at the time. Her development is normal.

Case III:12
The aunt of the proband had no neonatal seizures. Her events started at 4 months within 24h of diphtheria, tetanus and pertussis (DPT) immunisation. She was admitted to hospital with a cluster of frequent seizures, every hour or two, over 2-3 days. She was commenced on phenobarbitone. The seizures were described as generalised clonic lasting 1-2 minutes. She had no further events until her teenage years. She had a cluster of 3 focal seizures with secondary generalisation in 24h at the age of 16. She described a tingling feeling in arm and jaw which then evolved
into a secondary generalised clonic seizure. She had two events in her first pregnancy aged 20y. At 35y and 40y she had similar clusters in a 24h period. She is currently taking oxcarbazepine. Interictal EEG was normal.

Case II.9
Events started as neonate within first few days of life. Convulsions described as generalised. Occasional seizures throughout childhood. Treated with phenobarbitone until 16 years of age. Has had one or two epileptic seizures as an adult but no longer on medication.
Figure 7.1 Pedigree of Family A
7.3.2 Clinical Study of family B

The pedigree of family B is shown in figure 7.2. The pedigree demonstrates autosomal dominant inheritance of the neonatal seizures. The kindred has 37 individuals with 10 affected by neonatal seizures. No individuals had onset of seizures outside the neonatal period. None of the cases had seizures beyond 3 months of age. None of the affected individuals have learning disability.

Illustrative case histories
Case IV6 – Proband
The proband presented aged 4 days with clusters of seizures. She had several seizures per day. CT brain scan was normal. Interictal EEG was normal. Video-EEG captured two epileptic seizures. The first episode was accompanied by sharp and slow wave complexes over the left hemisphere, spreading to involve the right hemisphere (Figure 7.3). The second episode had onset over the right hemisphere with sharp and slow waves restricted to the right hemisphere. In between the seizures she fed well and behaved normally with a normal neurological examination. She was commenced on carbamazepine. She had no further seizures after the neonatal period. Age 11 her development is normal.

Case IV:5
The father of the proband had no history of neonatal seizures or any other neurological problems.

Case IV:7
The aunt of the proband had seizures with onset and offset in the neonatal period. She had further seizures in infancy, childhood or adult life.

Case V:1
The granddaughter of case IV:7 had four seizures in total the first on day 2 of life and the last on day 4. In the events she stared forward and then had twitching of the left side of her body for 10-30 seconds. In between the events her behaviour, feeding
and neurological examination was normal. She had a single dose of phenobarbitone. She had a lumbar puncture, MRI of brain and interictal EEG which were all normal. She has had no further events and has normal development. Her father, the son of case IV:7, had no neonatal seizures or other neurological problems.

Case IV:3
Onset of seizures was day 2 with offset at 3 months. In total she had 8 or 9 events. She became rigid, neck was extended, her eyes rolled back and the whole event lasted about 3 minutes. Interictal EEG was normal. Development has been normal with no seizures throughout childhood and adult life to the age of 30.

7.3.3 Molecular genetic study

Family A
DNA was available from 4 affected family members. Sequencing of KCNQ2 and KCNQ3 detected no coding or splice site mutations. MLPA was used to test for copy numbers of each exon of KCNQ2. MLPA detected a duplication of exons 3-12 in all affected individuals tested.

Family B
DNA was available from the probands father (III:5), the probands aunt and her cousin the father of case V:1. Sequencing of KCNQ3 in all individuals tested revealed a mutation in exon 6 of the gene, c.1019G>T (p.G340V).
Figure 7.2 Pedigree of Family B with benign familial neonatal seizures.
Figure 7.3
7 channel Medilog EEG recording of proband from family B showing focal onset of epileptic seizure with spike and slow wave abnormalities developing over the left hemisphere and spreading to the right. A concomitant video recording showed an epileptic seizure as described in the text.
7.4 Discussion

Family A and family B both have clinical features in keeping with benign familial neonatal seizures however there were several important differences between the two families illustrating variable presentation in the syndrome and suggesting that the different genetic aetiologies influenced the phenotype. 10/10 individuals in family B had onset of seizures in the neonatal period, as did 19/21 individuals in family A. The pedigrees indicated an autosomal dominant pattern of inheritance in both families. All individuals had a normal developmental outcome.

The family history was the key to making the diagnosis however it was notable that either due to variable penetrance (family B), or a lack of family knowledge about the history, obtaining a history of neonatal seizures, even in families with so many affected, was not always straightforward.

The major difference in the two families was that in family A one of the oldest living female relatives, the grandmother of the proband, had a clear overview of the family history. This individual had 2 younger siblings, 2 children, 7 nieces and nephews, and 5 grandchildren with seizures. The oldest living female relative is usually the most important person to speak to in the early stages of constructing a pedigree and family history. In family B two individuals presented in the 1970s to my supervisor Professor John Stephenson. He was aware of the family history of neonatal seizures and normal development and diagnosed benign familial neonatal seizures. In an EEG report on case IV:1 he commented, “this family should be published”. More than 30 years later the paper is being written.

As is the case in these families they will continue to present to medical services every generation. In the late 1990s the proband presented. There was a vague history at the time of neonatal seizures in an aunt. The more extensive family history was unearthed by direct questioning and cross referencing to a diagnostic coding system established in the neurosciences unit in the 1970s. DNA was taken from the proband’s father who would have been an obligate carrier of the genetic mutation.
The mutation in KCNQ3 was identified several years later. By this time the family were no longer under follow up. They had moved house and the GP and local health board had no record of where they had gone to. I was making further efforts to trace them when case IV:10 presented to another hospital in the Glasgow area with neonatal seizures. Her case was mentioned to me by a colleague at a coffee break in an academic meeting with, again, a mention of a vague family history of neonatal seizures. I arranged to see the family in clinic and was able to establish a link to the proband’s family. This allowed me to contact the proband’s father and inform him and the rest of the family of the genetic diagnosis. The re-presentation of the family every few years with another neonate going through extensive and at times invasive investigations is typical of BFNS. Knowledge of the genetic defect in family B would probably save neonates extensive investigations and prolonged treatment with anti-epileptic medication as all family members to date stop having seizures in the neonatal period.

The presentation of two cases from family A with their first seizures later in infancy demonstrates that there can be some overlap between BFNS and two other benign epilepsy syndromes, benign familial neonatal infantile seizures (BFNIS) and benign familial infantile convulsions (BFIC). BFNIS is associated with missense mutations in a sodium ion channel gene SCN2A (Heron et al 2002, Herlenius et al 2007). BFIC has been linked to chromosome 16 but no gene has been identified (Caraballo et al 2001). The mean age of onset of seizures in BFNIS is 11 weeks (range 2d – 6m) against 2-3 days in BFNS (range 1d-6m) and 6 months (range 2-20m) in BFIC. This demonstrates that the diagnosis of a family should be based on the age at presentation of the majority of the individuals. In small families or isolated cases it would be justified to test KCNQ2, KCNQ3 and SCN2A.

It is of note that there was a variable outcome in the two individuals with onset of seizures outside the neonatal period. The mother of the proband had a single seizure in the context of an intercurrent illness. Her sister had her first seizure within 24h of her first DTP immunisation. This further emphasises the point that seizures in proximity to this immunisation are more likely to be genetic in nature rather than a
specific consequence of the immunisation (Berkovic et al 2006). This individual has had infrequent clusters of focal seizures throughout her life suggesting that the KCNQ2 mutation alters neuronal excitability to a degree that is only significant given certain environmental circumstances. It is likely that other genetic factors determine the significant difference in seizure frequency between close relatives.

33% (7/21) of individuals in the family had seizures outside the neonatal and infantile period. This is a much higher percentage than reported in other BFNS families. As this the first family reported in the literature with a KCNQ2 duplication it remains to be seen whether other families with similar genotypes have such a high incidence of epilepsy later in life. It seems that the risk of seizures in older individuals is related to the specific mutation found in each family. Case III:12 had seizures as an adult with tingling in her mouth at onset. This has a similarity to the focal seizures seen in benign rolandic epilepsy (benign childhood epilepsy with centrottemporal spikes- BCECTS). In Rett and Teubel’s original family with BFNS, 2 of nine affected neonates had seizures with semiology suggestive of BCECTS (Rett and Teubel 1964). This family has been re-evaluated and found to have a KCNQ2 mutation (Zimprich et al 2006). The same mutation has been found in another family with BFNS who had one individual with centrottemporal spikes at 3 years (Coppola et al 2003). A Japanese BFNS family had two affected children with centrottemporal spikes and rolandic or sylvian seizures to the age of 9 years (Maihara et al 1999). Benign rolandic epilepsy (BRE) is the commonest idiopathic focal epilepsy of childhood. The importance of KCNQ2 mutations or variants in BRE is yet to be clarified though one preliminary study found only one functionally significant mutation in 53 pedigrees with BRE (Neubauer et al 2008).

In all of the four KCNQ3 families reported previously only two of the individuals had seizures reported beyond the neonatal period (Charlier et al 1998, Hirose et al 2000, Singh et al 2003, Li et al 2007). These were the proband his sister from Hirose et al. who had seizures at 6 months and three years. Family B is the fifth family reported with a KCNQ3 mutation and has a similar relatively mild and benign
phenotype. The finding of a KCNQ3 mutation should therefore confer a relatively good prognosis.

2 individuals have been reported with refractory neonatal seizures an epileptic encephalopathy and KCNQ2 mutations. One child had refractory seizures to 15 months which are now controlled, but has severe learning disability aged 4 years. This child had a de novo KCNQ2 mutation (Schmitt et al 2005). One familial case had refractory seizures to 13 weeks, which are now controlled, but she is left with developmental delay at two years. This child had a familial KCNQ2 mutation (Dedek et al 2003). Steinlein et al. reported that in 4 out of 10 BFNC families with KCNQ2 mutations in which follow up was available, each family had one individual with significant developmental problems (Steinlein et al 2007). The condition when associated with a KCNQ2 mutation may not be as benign as previously thought. A KCNQ2 mutation in the voltage sensor region has been associated with BFNS and myokymia in one family (Dedek et al 2001). There is emerging evidence that KCNQ3 mutations are associated with “pure” BFNS whereas KCNQ2 mutations are associated with BFNS “plus” other features. KCNQ2 mutations are about ten times more frequent than KCNQ3 mutations. I have identified two further small families with BFNS who have KCNQ2 mutations identified within John Mulley’s lab (Figure 7.4a and b).

KCNQ2 and KCNQ3 can exist as homomeric potassium ion channels but the typical M channels are heteromeric, tetrameric channels with a mixture of KCNQ2 and KCNQ3 subunits. The M current is thought to be important in controlling the resting potential of many neurons and limits repetitive firing. When functional studies are performed mutant channels need to be expressed alongside wild type channel subunits of the same and different types. When KCNQ2 mutants are studied on their own there is a significant reduction in current compared to wild type. When the mutant channel subunit is expressed with wild type KCNQ2 and KCNQ3 subunits the reduction in current is much smaller. It may be that this relatively modest reduction in current is only significant in the majority of individuals in the neonatal period. This may be related to the developmental expression of these channels
around term or it may be that the switch from GABA acting as an excitatory neurotransmitter to it acting as an inhibitory neurotransmitter lessens the physiological impact of the KCNQ2 and KCNQ3 mutations (Kanaumi et al. 2008; Reid et al. 2009).

As loss of function mutations seem to underlie BFNS, medications that stabilise the M current and open the M channel could therefore be candidates as anti-epileptic medications. The compound retigabine acts by opening M channels and is currently undergoing clinical trials as an anti-epileptic medication (Conte Camerino et al. 2007). Retigabine acts on all the KCNQ subunits except for KCNQ1 which is a cardiac and not a neuronal channel subunit.

The study of families A and B emphasises the variability in phenotype and genotype in BFNS. Family A would not have had a genetic diagnosis without the use of the relatively new technique MLPA. Using this technique our collaborators in Australia also detected KCNQ2 exon deletions in 3 families who were negative for KCNQ2, KCNQ3 and SCN2A mutations on sequencing (Heron et al. 2007). Family A remains the only one reported worldwide with a KCNQ2 exon duplication. This report emphasises the importance of a two tier strategy in testing families with BFNS with the use of MLPA in sequencing negative cases.
Figure 7.4a Family C with BFNS

- BFNS
- +/- - KCNQ2 c.1732A>G (M578V)
- +/- - negative for KCNQ2 c.1732A>G (M578V)

Figure 7.4b Family D with BFNS

- BFNS
- +/- - KCNQ2 c.1855-1885del31bp (S618fsX631)
- +/- - Negative for KCNQ2 c.1855-1885del31bp (S618fsX631)
8.1 Introduction

In this chapter I report the clinical study of two large Scottish families with an uncommon genetic epilepsy called autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE). The two families “D” and “S” have featured in four publications which I will refer to in the chapter. Family D was the third reported worldwide with a mutation in the gene for the neuronal nicotinic acetylcholine receptor α4 subunit (CHRNA4) and family S was one of the first two families reported worldwide with a mutation in the neuronal nicotinic acetylcholine receptor β2 subunit. The principal neuronal nicotinic acetylcholine receptor in the brain is made up of 5 subunits, 3 β2 subunits and two α4 subunits, which assemble together to form the ionic pore (Bertrand D, 2002). The clinical study of family D emphasises the major consequences that incorrectly phenotyping even one individual in a family can have on molecular genetic studies. The study of families D and S was the first in which families with a single epilepsy syndrome (ADNFLE), caused by mutations in different genes, had their phenotypes compared (McLellan et al. 2003).

ADNFLE is a familial partial epilepsy syndrome characterised by clusters of frontal lobe motor seizures in sleep (Scheffer et al. 1994; Scheffer et al. 1995). ADNFLE was the first idiopathic epilepsy to have a defined genetic cause with a Ser248Phe mutation in the CHRNA4 gene identified in a large Australian kindred. CHRNA4 codes for a subunit of a ligand gated ion channel. The clinical study of a family
comprising 25 affected individuals by Ingrid Scheffer allowed linkage to chromosome 20 and the subsequent discovery of the CHRNA4 mutation through work done by teams led by John Mulley and Ortrud Steinlein (Scheffer et al. 1995; Steinlein et al. 1995). Subsequently different CHRNA4 mutations were detected in a Norwegian family and a Spanish family with ADNFLE (Steinlein et al. 1997; Saenz et al. 1999).

The clinical syndrome of ADNFLE is distinctive but there is considerable variability in severity between families and between individuals within families. In the seminal paper by Scheffer et al. five families from Australia, Britain and Canada are described with 47 affected individuals (Scheffer et al. 1995). Many affected individuals had been misdiagnosed as parasomnias, pseudo-seizures or nocturnal paroxysmal dystonia. The key features of the syndrome as described in 1995 were the childhood onset of clusters of partial seizures in sleep that persist into adult life. At seizure onset there was often a vocalisation and a non-specific aura, sometimes including a sensation of breathlessness. Consciousness was retained by several individuals with some having a sensation of fear. A tonic stiffening of the body sometimes with dystonia of a limb could be followed by hyperkinetic features with clonic jerking of limbs. The neurological examination was normal, cognitive abilities were normal, neuroimaging was normal, interictal EEG was normal and ictal EEG often showed no surface abnormalities. The recognition of the family history was often the key to diagnosis. Carbamazepine monotherapy was helpful in many individuals.

8.2 Methods

8.2.1 Clinical Study

Two families, originally from central Scotland, were identified and their pedigrees established (Figures 8.1 & 8.2). All living affected individuals underwent clinical evaluation comprising personal or telephone interview, clinical examination and
medical records review as detailed in Chapter 5. Both these families are very large and have had clinical evaluations in several centres.

8.2.1a Family D

The proband from family D (case IV7) was referred to Professor John Stephenson’s clinic in Glasgow. The diagnosis of ADNFLE was made by him. The initial evaluation of the family was undertaken by John Stephenson and John Tolmie (Clinical Geneticist, RHSC, Glasgow) in collaboration with Professor Sam Berkovic in Melbourne. I followed case IV in clinic and undertook the clinical evaluation of other family members. Two brothers from family D (cases IV1 and IV2) were independently referred to my clinic for evaluation of paroxysmal events. I arranged molecular genetic testing on them in collaboration with Professor Sam Berkovic (Neurologist) in Melbourne and Dr John Mulley (Molecular Geneticist) in Adelaide. I re-evaluated the clinical histories in family D and interviewed the affected family members. The proband from family D had several video-EEG studies performed in Glasgow as well as home videotape of events. EEG studies and home videotape was available for several other family members as detailed in the results.

8.2.1b Family S

The proband from family S case was referred to Dr Martin Kirkpatrick, Paediatric Neurologist, Ninewells Hospital, Dundee for evaluation of nocturnal events. Dr Kirkpatrick brought a video-EEG of the child and details of the family history for discussion with Professor Stephenson. I reviewed the case with Professor John Stephenson and we felt the diagnosis was ADNFLE. I travelled to Dundee and saw the family and reviewed case records. I contacted colleagues in Australia who agreed to study DNA from the family. DNA collection in Dundee was supervised by Dr David Goudie (Consultant Clinical Geneticist). The family subsequently attended my clinic in Glasgow for a further opinion. I supervised the paper on the phenotypic comparison of the two ADNFLE families and wrote it with Dr Ailsa McLellan, Specialist Registrar in Paediatric Neurology, Dundee. Dr McLellan and I made
Figure 8.1 Pedigree of family D with ADNFLE.

Figure 8.2 Pedigree of family S with ADNFLE.
contact with members of family D and S requesting further clinical information during the preparation of this paper. One branch of family S live in Sheffield. They have been seen by Dr Chris Rittey, Paediatric Neurologist, who forwarded clinical information to me and DNA to Australia.

8.2.2 Molecular Genetic and expression studies

8.2.2a Family D

Linkage and subsequent sequencing studies of CHRNA4 were undertaken in Dr John Mulley’s laboratory at the Women & Children’s Hospital, Adelaide, Australia. Dr Daniel Bertrand, University of Geneva performed expression studies on the mutation identified in a Xenopus oocyte model. DNA was available on 11 individuals, 6 affected, 1 obligate carrier and 4 unaffected.

8.2.2b Family S

Sequencing studies of CHRNA4 and CHRNB2 were performed on 8 individuals in this family, 6 affected, one obligate carrier and one unaffected by Dr John Mulley’s team in Adelaide. The M2 domains of the two receptor subunits were sequenced as they form the wall of the ion channel and All previous mutations in ADNFLE had been found in this region of the α4 subunit. Expression studies of the mutation identified were performed by Dr Daniel Bertrand in Xenopus oocytes.

8.3 Results

8.3.1 Clinical study

The clinical details for families D and S are summarised in tables 8.1, 8.2, 8.3 and 8.4.
8.3.1a Family D clinical study & initial molecular genetic data

Illustrative case studies

Case IV:7 (Proband)
The 21 year old proband (IV:7) presented to the neurology service aged 8 years. His seizures started aged 4 and arose from sleep both at night and during daytime naps. A video camera and tripod was lent to the family from the neurology clinic. Typically he would sit bolt upright in bed and his left arm and hand would twist into what was described as a “flamingo” posture for several seconds, shown clearly in videotape recordings taken in the home. After the dystonic limb movements had ceased he would have rhythmic kicking movements of his lower limbs and grasp at the bedclothes with both hands. He would scream loudly as if frightened, occasionally calling for his father. Rarely the seizure generalised. Each individual episode lasted from a few seconds to half a minute but he could have multiple episodes a night sometimes only separated by 15 minutes. Neurological examination was normal. A clinical diagnosis of a right mesial frontal lobe epilepsy was made after referral to neurology and viewing of the home videotape. He had previously been diagnosed by a paediatrician as having night terrors. Interictal EEG was normal. Video-EEG telemetry shows stereotyped events but no surface EEG changes are evident. MRI of the brain was normal. For two years he responded well to carbamazepine but subsequently his seizures have been poorly controlled on various antiepileptic medications. The diagnosis of ADNFLE was made after recognition of the family history.

Case III:10
The 50 year old, mother of the proband recalls having recurrent episodes from sleep when she would wake and was aware of a sense of fear during childhood. She described a funny feeling in her left arm, turning on to her left side and curling up with trunk flexion for up to a minute. She remembered having to sleep in her parents’ bed even into teenage years. As an adult she continues to have brief recurrent arousals at night time of which she is not aware.
Case III:11
The 47 year old, aunt of the proband has had nocturnal seizures since aged 8 years. The episodes last only a few seconds and involve her rousing from her sleep, sitting up and raising her right arm above her head. She is fully aware during a seizure. Sometimes she will bite down on the blanket. They continue to occur on most nights between one to three times despite carbamazepine and lamotrigine therapy. This is much less frequent than during her childhood. They typically occur in the hour before waking and she may have them if she has a daytime nap.

Case IV:9
This 14 year old boy, a cousin of the proband, presented aged 6 years with a two year history of episodes of arousal between one or rarely two times in a night, about once a week. His mother would go through to find him sometimes quietly crying, other times in an agitated state, looking frightened. He did not appear to recognise his mother. Sometimes he would talk unintelligibly. There were no abnormal motor postures during these episodes. There were no other abnormal motor features during these episodes. They lasted two to five minutes and occurred about two hours into sleep. At the end of the episode he would fall back into sleep. He has no recollection of any of the events. They now occur about once every two weeks. At the age of 14 years he still has one or two episodes per month which his mother feels happen more frequently when he has a “cold”. She describes him as looking terrified and “jumping about the room” not recognising her. He will occasionally sleepwalk and perform automatic behaviours. Recently he walked into his sisters room picked up her duvet and folded it neatly at the foot of her bed before walking back to his room to sleep. He was initially diagnosed as having ADNFLE. However when I reviewed the full history with his mother it was clear that he is having parasomnias which can be clinically classified as partial arousals arising from non REM sleep, mostly night terrors or somnambulism. He was not commenced on medication.
Initial Molecular Genetic Studies
DNA samples were taken from affected and unaffected members from family D. Molecular genetic studies were performed in Adelaide. Case IV9 was initially misclassified as having ADNFLE and he was screened for the two known mutations in CHRNA4 on chromosome 20 and for novel mutations in the gene. The proband did not have CHRNA4 sequencing at this stage. Linkage studies showed that individual IV:9 and the proband inherited different copies of CHRNA4. This was felt to give further evidence that this locus was not involved in the Scottish family. Studies also appeared to show weak linkage to chromosome 15.

Identification of Cases IV:1 and IV:2

Case IV: 2
This nineteen year old boy presented at the age of thirteen with a recent onset of nocturnal events. He had no past history of note apart from a 1–2 minute generalised clonic seizure associated with a febrile illness aged 2 years. Initially the nocturnal events occurred only once per night. He would sit bolt upright from sleep, would stare forwards and was described as having irregular flapping movements of his arms. He would seem to be intermittently responsive and the episode could go on for several minutes. He had no recollection of them in the morning.

The mother of cases IV:1 and IV:2 would have arousals from sleep and if she was in the dark would get a sensation that “everything was moving fast”. She would then get up and walk as she felt this took away the unpleasant sensation. She retained a memory of these events. These may be partial arousals from non REM sleep with preserved memory. On one occasion aged ten she walked into the street. She always sleeps with the light on. The maternal grandmother of case IV:2 had sleepwalking as a child.

Based on the history obtained case IV:2 was initially given a diagnosis of a parasomnia – a non REM partial arousal disorder.

A month after presentation he began to have up to three episodes a night and therefore the family were lent a video camera from the hospital. The video recording shows him suddenly sitting upright and then he has slow upper limb jerks for a few
seconds. This is followed by a period of confused, agitated, hyperactivity including irregular flapping of his arms and a fall from the bed. The episode lasted several minutes during which he was able to do things in a semi-directed fashion such as move objects around his room. He has no recollection at all of any of his seizures. A diagnosis of frontal lobe epilepsy was made, and after questioning, a link to family D was revealed through the estranged father of the child. Events in this individual have been controlled well by carbamazepine therapy however they do recur when he is up late at night, has drunk alcohol, or is under emotional stress.

Case IV:1
The 22 year old brother of case IV:2 presented aged 15 with nocturnal episodes occurring 3-6 times per night in which he would sit bolt upright, drum his arms legs irregularly on the bed. This lasts a few seconds, he then becomes aware that he is awake and has an unpleasant sensation of difficulty breathing which lasts several seconds. Often he will get up to put a fan on. He would have similar events in daytime naps. When he was first seen by a paediatrician these events were thought not to be epileptic however following the diagnosis of his brother’s events this boy was reviewed in the neurology service and a diagnosis of ADNFLE was made. His events were controlled by slow release carbamazepine at night. The events are now relatively infrequent and he takes a night time dose of carbamazepine only at times when he thinks seizures are likely to be more frequent such as around exam time.
Table 8.1 Family D - clinical features

<table>
<thead>
<tr>
<th>Case number</th>
<th>Sex</th>
<th>Age</th>
<th>Age at seizure onset</th>
<th>Years between onset &amp; diagnosis</th>
<th>Psychological complications</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>III:3</td>
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<td>50</td>
<td>13</td>
<td>3 months</td>
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<tr>
<td>III:6</td>
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<td>12</td>
<td>Never diagnosed as ADNFLE</td>
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<td>None</td>
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<tr>
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<td>43</td>
<td>8</td>
<td>1</td>
<td>None</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Carbamazepine</td>
</tr>
<tr>
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<td>2</td>
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<td>14</td>
<td>6 months</td>
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<tr>
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<td>18</td>
<td>6</td>
<td>3</td>
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<td>Valproate</td>
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<td>Phenobarbitone</td>
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Table 8.2 Family D - seizure semiology & EEG features

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Seizure semiology</th>
<th>Natural history</th>
<th>EEG findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>III:3</td>
<td>Arousal, aware, sensation left arm, turns onto left side, curls up, &lt; 1 minute</td>
<td>Remission age 15</td>
<td>Not available</td>
</tr>
<tr>
<td>III:6</td>
<td>Sudden awakening, aware, thrashing limbs, &lt; 2 minutes</td>
<td>Remission aged 14</td>
<td>Interictal normal</td>
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<tr>
<td>III:7</td>
<td>Sudden awakening, aware, bites blanket, tonic extension, thrashing limbs, &lt;2 minutes. Up to 30 /night</td>
<td>Improved</td>
<td>Interictal normal</td>
</tr>
<tr>
<td>IV:1</td>
<td>Arousal, drums arms, looks awake, gets up, turns on fan, amnesic to event. Up to 6 / night. More if anxious</td>
<td>Improved</td>
<td>Interictal normal</td>
</tr>
<tr>
<td>IV:2</td>
<td>Arousal, slow rhythmic upper limb jerking, agitated, amnesic to event. Home video.</td>
<td>Improved</td>
<td>Interictal normal</td>
</tr>
<tr>
<td>IV:7</td>
<td>Sudden awakening, screams, thrashing limbs, falls out of bed, heavy breathing, aware. 30+/night at times. Home and hospital video.</td>
<td>Improved Not controlled</td>
<td>Interictal normal Ictal – muscle artefact</td>
</tr>
<tr>
<td>Case no.</td>
<td>Sex</td>
<td>Age</td>
<td>Age At onset</td>
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<td>M</td>
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**Table 8.3 Family S - clinical features.**
<table>
<thead>
<tr>
<th>Case no.</th>
<th>Seizure semiology</th>
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<th>EEG findings</th>
</tr>
</thead>
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<tr>
<td>II:1</td>
<td>Sudden awakening, tenses up, cries, heavy breathing, remains aware, &lt; 1 minute.</td>
<td>Improved</td>
<td>Interictal normal</td>
</tr>
<tr>
<td>II:7</td>
<td>Sudden awakening, curls up, heavy breathing, &lt;30 seconds</td>
<td>Remission Age 14</td>
<td>Not performed</td>
</tr>
<tr>
<td>III:7</td>
<td>Curls up, heavy breathing, thrashes legs, 2-20 minutes</td>
<td>Remission Age 15</td>
<td>Interictal non specific changes</td>
</tr>
<tr>
<td>IV:1</td>
<td>Not aware, stiffens body, holds breath then heavy breathing, scratches self</td>
<td>Seizures not controlled</td>
<td>Interictal normal</td>
</tr>
<tr>
<td>IV:2</td>
<td>Sudden awakening, cries, aware, tenses up, dystonia of limbs, bites thumb</td>
<td>Improved</td>
<td>Interictal normal</td>
</tr>
<tr>
<td>IV:5</td>
<td>Violent thrashing of limbs, unresponsive, fell out of bed</td>
<td>Remission Age 13</td>
<td>Not Performed</td>
</tr>
<tr>
<td>IV:7</td>
<td>Violent thrashing of limbs, unresponsive</td>
<td>Remission Age 12</td>
<td>Interictal L fronto-temporal slow waves</td>
</tr>
<tr>
<td>IV:9</td>
<td>Dystonic posture upper limbs, bilateral jerking limbs. Aware, couldn’t speak. 10/night</td>
<td>Recurrence when treatment withdrawn</td>
<td>Interictal normal. Ictal – muscle artefact</td>
</tr>
<tr>
<td>IV:10</td>
<td>Groans, tonic extension limbs, jerking all limbs. &lt;30 secs. Up to 70 / night.</td>
<td>Improved on treatment</td>
<td>Interictal- rhythmic frontal slow. Ictal – muscle artefact</td>
</tr>
<tr>
<td>V:1</td>
<td>Sudden awakening, difficulty breathing, cries. Tonic extension left arm. Truncal flexion. &lt;3 minutes. Up to 50/night</td>
<td>Improved on treatment</td>
<td>Interictal normal. Ictal muscle artefact</td>
</tr>
</tbody>
</table>
8.3.1b Family S

Illustrative case studies

Case V:1 (Proband)
This girl with no learning disability presented age 11 with nocturnal events. She woke from sleep with a sensation of difficulty breathing. After a few seconds she would appear to hold her breath and grunt. Sometimes she would recover quickly and cry or scream. On other occasions she had tonic extension of her left arm with flexion of her trunk. Seizures could last from a few seconds to a few minutes. Stereotyped attacks could occur every 15 minutes through the night. She had clear recollection of her events. Seizures also occurred in daytime naps. Video-EEG captured several clinical events but EEG showed only muscle artefact. Seizures were initially well controlled on carbamazepine however relapse occurred and further control was difficult. She became seizure free on a combination of topirimate and phenytoin.

Case IV:10
His first events occurred aged 6 when he had recurrent arousals at night with tonic stiffening of upper limbs with extension lasting a few seconds followed by clonic movements of all limbs for up to a minute. He could have up to ten events in one night. He retained awareness throughout the events but was unable to speak. There was no post-ictal confusion. The day after multiple seizures he is very tired. Interictal EEG was unremarkable. Ictal video-EEG showed a run of rhythmic slow waves prior to the event with muscle artefact obscuring the EEG during the events. He initially responded to carbamazepine but suffered unacceptable side effects. Valproate was ineffective but vigabatrin controlled the events. A trial of withdrawal of vigabatrin produced recurrence of the nocturnal seizures. An MRI was reported as showing scattered non-specific white matter changes.
Case IV:11
The younger brother of case IV:10 presented with nocturnal seizures aged 9 years. He would have a loud groan, brief tonic stiffening of his limbs followed by clonic jerking of upper and lower limbs for 5-20 seconds. He often fell out of bed. He had no recollection of the events. An interictal EEG showed occasional runs of slow waves and low amplitude spikes at the surface. An ictal EEG was largely obscured by muscle artefact but showed fast activity at the vertex during the event. An MRI is normal. He has some specific learning difficulties with poor verbal and memory skills. His seizures were controlled on a combination of lamotrigine and vigabatrin. He has had one episode of clonic status epilepticus lasting 40 minutes.

8.3.2 Molecular Genetic and expression studies

8.3.2a Family D

DNA studies in case IV:1 and IV:2 failed to show linkage to the chromosome 15 markers that had been reported in family D. Both boys were then tested for the two known CHRNA4 mutations on chromosome 20. They both had a C to T transition in amino acid position 248 leading to the replacement of serine by phenylalanine (S248F) in the second transmembrane segment of the receptor channel. This mutation had previously been reported in the first Australian family with ADNFLE. The rest of family D were then tested for the same mutation. All affected members and the one obligate carrier tested (II:6) were found to have the mutation except for case IV:9. All unaffected cases (IV:6, IV:8, III:1) do not have the mutation.

Haplotype analysis shows that in family D the gene is embedded in the same three marker haplotype as the Australian family, raising the possibility of a founder effect. Intragenic CHRNA4 and KCNQ2 markers show an identical haplotype segregating with the mutation in family D and the original Australian kindred. However, the table of allele frequencies shows that the most common allele of each marker is the one present in the haplotype. This data is compatible with either independent mutation or founder effect.
Functional studies on the S248F mutation were described by Daniel Bertrand and colleagues following the identification of the same mutation in the Australian family (Bertrand et al 2002). Initial studies on the S248F mutation produced conflicting results however co-expression of normal and mutant receptors showed a gain in acetylcholine sensitivity.

8.3.2b Family S

Sequencing of the second transmembrane domain (M2) of CHRNA2 revealed a G→A transition. This c1025G→A mutation replaces a highly conserved valine with methionine at position 287 (V287M) (Figure 8.3). The CHRNA2 M2 domain amino acid sequence is fully conserved between different species (Figure 8.4). Expression studies show that the mutant receptor demonstrates an increase in acetylcholine sensitivity when compared to wild type. This is present in the homozygous state and when expressed with wild type β2 and α4 subunits.
**Figure 8.3** Electropherogram showing DNA sequence and position of the mutation in CHRN2 in family S with ADNFLE in upper panel, with control in the lower panel.

```
c1025G>A
TCCAAGATCNTGCCTCCCACCT
```

**Figure 8.4** The M2 domain amino acid sequence of the β2 subunit of the neuronal nicotinic acetylcholine receptor shows evolutionary conservation.

<table>
<thead>
<tr>
<th>Mutated allele</th>
<th>Human CHRN2</th>
<th>Mouse CHRN2</th>
<th>Rat CHRN2</th>
<th>Chick CHRN2</th>
<th>Goldfish CHRN2</th>
<th>Human CHRN4</th>
<th>Monkey CHRN4</th>
<th>Rat CHRN4</th>
<th>Chick CHRN4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T LCISVLLALTVFLLLISKIVP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2 domain amino acid sequence</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
8.4 Discussion

The initial clinical and molecular study of family D demonstrates the importance of detailed history taking and knowledge of all the paroxysmal disorders that can mimic epilepsy. Case IV:9 was misdiagnosed as having frontal lobe epilepsy when he had the much commoner disorder of non-REM partial arousals from sleep manifesting as night terrors and sleep walking. This error in phenotyping was further compounded by genetics colleagues in Australia as they chose to screen just one case, IV:9, for the two known mutations in CHRNA4 on chromosome 20 and for novel mutations in the gene. They did not screen the proband. Linkage studies showed that individual IV:9 and the proband (IV:7) inherited different copies of CHRNA4. This was felt to give further evidence that this locus was not involved in the Scottish family. Studies also appeared to show weak linkage to chromosome 15. This was an attractive proposition as there is known to be a cluster of three nicotinic acetylcholine receptor genes on this chromosome. Family D were therefore published in 1998 as not having a CHRNA4 mutation (Phillips et al. 1998). This has been corrected with a letter to the journal.

When case IV:2 was referred he too was suspected as having a parasomnia because of his mother's history. The importance of video recording and particularly home video recording was emphasised as it was the video of a nocturnal event taken by his mother that helped make the diagnosis of frontal lobe epilepsy. Direct questioning then revealed unreported events in his brother, case IV:1 and further questioning revealed a link to family D through the estranged father of the boys who was clinically not affected by ADNFLE. This link led to the correct genetic diagnosis in all affected members of family D.

The genetic study on family S was submitted for publication at the same time as a study of an Italian family with ADNFLE who were found to have a CHRNB2 mutation (De Fusco et al 2000; Phillips et al 2001). These reports were the first to report CHRNB2 as a new idiopathic epilepsy gene. The Italian family had a
Val287Leu mutation at exactly the same position in the gene as the Scottish Val287Met mutation. This suggests this is a functionally important part of the gene.

In Family S 10 (6F;4M) living affected individuals (aged 13 to 82 years) with the clinical phenotype of ADNFLE were identified. Eight had the Val287Met mutation in CHRNB2 as does obligate carrier III:4. In family D, 6 (3F:3M) family members aged between 16 and 50 years had ADNFLE. The median age of onset was 10 years in family S (range 6 – 18 years, mean 11.1 years) and 12.5 years in family D (range 6 – 15 years, mean 11.3 years). All affected individuals were normal intellectually. In both families there was a delay (of up to 9 years) between onset of seizures and diagnosis of epilepsy and the diagnosis of ADNFLE was only made after the presentation of a child with a severe phenotype to a paediatric neurologist.

Psychological morbidity featured prominently in both families (McLellan et al, 2003). Psychological complications had not been specifically mentioned in previous reports of families with ADNFLE. Individuals from both families experienced psychological problems including depression, low self-esteem, a hysterical gait disorder and school refusal. Two children had been thought to have a psychiatric disorder prior to the diagnosis of epilepsy being established. However, only a few families with ADNFLE have been reported in detail to date and therefore it is not possible to know definitely whether the psychological difficulties in these 2 families are a non-syndrome specific consequence of the delayed diagnosis and impact of the epilepsy on the individual, are unrelated to the epilepsy or indeed a behavioural phenotype influenced by the CHRN mutations. Since then three further papers have reported memory deficits, psychotic disorders, mood disorders and incapacitating apathy in families with ADNFLE (Magnusson et al, 2003; Bertrand et al., 2005; Derry et al., 2008). It is therefore highly likely that these symptoms are related to receptor function in the brain.

The natural history of the disorder in both families revealed remission in teenage years, persisting after withdrawal of AEDs, or improvement after puberty. However, an 81 year old lady from family S recalls nocturnal seizures since teenage years. She had 2 seizures
in her 7th decade and one in her 8th. Her cranial CT scan and EEG were normal at the age of 77 years. The probands from both families had the most severe phenotype. The proband from family S presented at the age of 11 years and had up to 50 seizures each night which are now well controlled on clobazam and topiramate. The proband from family D has up to 70 seizures at night and these persist at the age of 18 years, 12 years after his initial presentation.

All reported and videotaped seizures except for one isolated occasion in case IV:8 of family S, arose in sleep and typically occurred in clusters. In both families the seizures were brief and of frontal lobe semiology. These were stereotyped in each individual at a particular age but the seizure semiology was influenced by age. For example the proband from family D presented aged 6 years with stereotyped seizures characterised by dystonic posturing of the right upper limb followed by screaming and irregular thrashing movements of all limbs. Aged 18 his seizures comprise of arousal with a partial motor seizure affecting either side of the face and sometimes either upper limb. Video tape recordings of the changing semiology over time are available in this individual. Respiratory symptoms or signs comprising hyperventilation or a subjective sensation of difficulty in breathing during the seizure was present in 5/10 of family S and 2/6 of family D. In 3/10 in family S and 1/6 in family D there were seizures with reported secondary generalisation. 5/10 members of family S had episodes of facial grimacing sometimes associated with stereotyped vocalisation.

5/6 members of family D and 7/10 individuals from family S were treated with antiepileptic drugs (AEDs). Carbamazepine was the most frequently used (4/7 in family S and 4/5 in family D) and was the most effective AED. 3/7 members of family S and 1/5 from family D had been on three AEDs or more.

Neuroimaging was performed in 9/16 individuals and was normal except for non-specific, probably unrelated white matter abnormalities in one case. Inter-ictal EEGs were performed in 13 and was abnormal on one occasion (left fronto-temporal slow waves) in
one individual (IV:8 family S). Ictal EEG was performed on 4 individuals revealed muscle artefact only.

Comparison of the two families shows a very similar epilepsy phenotype, with mean age of onset of 11.1 years in family S and 11.3 years in family D. This compares to 8.5 years in the Australian family and 8.6 years in the Norwegian family (Scheffer et al. 1995; Steinlein et al. 1997). In the Australian family the worst affected individuals had onset at <1 year, however in family S and D the probands had onset at 11 and 6 years respectively. Therefore onset in mid or late childhood does not necessarily imply a less severe epilepsy phenotype. Although the disorder tends to remit or improve following puberty, one member of family S continued having seizures into her 8th decade emphasising that ADNFLE should be considered in the differential diagnosis of nocturnal events in adults as well as children.

Initial in vitro studies of these mutations simulating the homozygous state, using oocyte expression systems, led to some conflicting data (Bertrand et al 1998; Phillips et al. 2001). However, co-expression of normal and mutant receptors, to simulate the heterozygous state, showed increased acetylcholine sensitivity for both mutations (Ser248Phe of CHRNA4 and Val287Met of the CHRNB2) (Bertrand et al 2002). This suggests that a gain of receptor function is the basis for the epileptic seizures and their common clinical phenotype.

A third nicotinic acetyl choline receptor subunit, the α2 subunit (CHRNA2), has been reported in a Sardinian family who have ADNFLE characterised by nocturnal wandering and epileptic fear (Aridon et al, 2006). Functional studies showed this mutation increased sensitivity to acetylcholine.

A question often posed when considering ADNFLE is why should a genetic abnormality produce a focal epilepsy? Two individuals from family D, cases IV:1 and IV:2,
participated in a study in Paris coordinated by Fabienne Picard using positron emission tomography (PET) to look at nicotinic acetylcholine receptor (nAChR) density (Picard et al 2006). 8 individuals with ADNFLE were compared to a group of controls. A significant increase in nAChR density was found in the cerebellum and diencephalon of ADNFLE cases. A decrease in receptor density was found in the right dorso pre-frontal cortex. Fluorodeoxyglucose PET studies in the ADNFLE patients showed reduced glucose uptake in the right orbitofrontal cortex. This in vivo study supports regional expression of the receptors as one of the reasons for the focal semiology.

This comparative study of two Scottish families with ADNFLE from similar ethnic and geographical backgrounds shows that despite having mutations in genes coding for different subunits of the neuronal nicotinic acetylcholine receptor, the phenotype is largely identical in both families. This is likely to be due to the fact that the overall consequence of each mutation on the function of the receptor is similar. This study adds further evidence that the phenotype of ADNFLE is due to the functional consequences of mutations on the nicotinic acetylcholine receptor.
9.1 Introduction

Dravet Syndrome, also known as severe myoclonic epilepsy in infancy (SMEI), is a severe form of infantile onset epilepsy first described in 1978 by Charlotte Dravet, a French paediatric neurologist working in Marseille (Dravet 1978). The Marseille group and others went on to report further series of patients and it became clear that this was an epilepsy syndrome seen by all child neurologists with a recognisable pattern of seizure types, temporal evolution and EEG features (Dravet 1992). In 2001 Claes et al. were the first to show that the syndrome is associated with de novo mutations in the gene encoding for the α1 subunit of the neuronal sodium channel (SCN1A) (Claes et al, 2001).

Voltage gated sodium channels are essential for the initiation and propagation of action potentials in the nervous system. They are made up of more than one subunit type (heteromultimeric), consisting of a single large pore forming α subunit and one or two small auxiliary β subunits. The α subunit alone may be sufficient for channel function (George, 2005). The β subunits influence levels of cell surface expression, voltage dependence and kinetics of the channel (Meisler & Kearney, 2005). The α subunits are large transmembrane proteins consisting of approximately 2,000 amino acids. They have 4 homologous domains which are highly conserved through
evolution. Each domain contains 6 transmembrane segments (S1-6). The 4 domains make up the channel through which sodium ions can pass down a concentration gradient during the generation of the action potential. The S4 segment has a voltage sensor role and the S5-6 loop region controls ion selectivity and permeation (Figure 9.2).

Mutations in the SCN1A gene are associated with a variety of epilepsy phenotypes. The familial syndrome of generalised epilepsy with febrile seizures plus (GEFS+) was first described by Scheffer and Berkovic in 1997 (Scheffer & Berkovic, 1997). The syndrome is now in the process of being renamed genetic epilepsy with febrile seizures plus. The term describes a family and not an individual. The word “plus” refers to the variety of other epilepsy phenotypes in a family where the predominant seizure type is simple febrile seizures. These include febrile seizures beyond 6 years, febrile and afebrile generalised tonic clonic seizures, febrile seizures plus absences, focal epileptic seizures and myoclonic astatic epilepsy. The presence of focal and generalised seizures and their sometimes artificial distinction in epileptology has led to “genetic” being preferred to “generalised” in GEFS+ (Livingston et al. 2009).

Figure 9.1 shows the pedigree of a Scottish family with GEFS+ that I studied and wrote up in 1999 in an essay submitted for the ILAE Millenium Gowers Prize with the title “Ion channels and epilepsy: an exciting future” but which has not been published elsewhere. This illustrates the variety of phenotypes and bilineal inheritance that frequently occurs in these families. This family does not have a mutation in a gene known to be associated with the syndrome. GEFS+ was first associated with a mutation in the β1 subunit in a large Australian family (Wallace et al. 1998). However β1 subunit mutations are uncommon causes of GEFS+ as are mutations in the α2 subunit (SCN2A) and the γ2 subunit of the GABA_A receptor (GABRG2) (Wallace et al. 2001, Baulac et al. 2001). The vast majority are related to mutations in SCN1A but even then mutations are only found in about 10% of large families with GEFS+ (Escayg et al. 2000). The variety of phenotypes in GEFS+ is most likely related to other modifying genetic factors in each individual. The Scottish family illustrated in figure 9.1 has one individual with severe myoclonic epilepsy in infancy / Dravet Syndrome. The extension of the GEFS+ spectrum to Dravet
Syndrome was first reported by Singh et al. and subsequently Claes and colleagues went on to identify de novo SCN1A mutations in Dravet Syndrome (Singh et al. 2001, Claes et al 2001).

Identification of the genetic basis of Dravet Syndrome has allowed the study of the phenotypic spectrum of epilepsies associated with mutations in SCN1A. In this chapter I will summarise my contribution to this field, including work which I have done in collaboration with clinical and molecular genetic colleagues in Australia prior to being able to analyse the gene in Glasgow.

Dravet syndrome typically presents in the first year of life, usually in children with no pre-existing developmental problems who develop prolonged febrile generalised clonic or hemiclonic epileptic seizures. There is an evolution into other seizure types including myoclonic, focal, atypical absence and atonic seizures between the ages of 1 and 4 (Dravet 1978). The epilepsy is usually not responsive to standard anti-epileptic medication and the children develop an epileptic encephalopathy, which means that the epileptic activity in the developing brain alters brain development and leads to cognitive and behavioural impairment (Engel, International League Against Epilepsy 2001). Carers see their child with normal development suffer uncontrollable seizures, develop a significant learning disability and sometimes a motor impairment with pyramidal signs and ataxia. Seizure types within Dravet Syndrome such as status epilepticus may be life threatening and sudden unexpected death in epilepsy can occur. Borderline or borderland subtypes of Dravet Syndrome have been described by several authors and refer to children who lack some of the key features of Dravet Syndrome / SMEI including myoclonic seizures, generalised spike wave on EEG, have later onset and who may have a relatively good cognitive outcome (Ohmori et al 2003; Fukuma et al 2004). These phenotypes have been given a variety of names including SMEB and intractable childhood epilepsy with generalised tonic clonic seizures (ICEGTC) (Fujiwara et al 2003).

In conversations with Professor Ingrid Scheffer from Melbourne relating to the phenotypic spectrum of SCN1A mutations, I discussed the clinical observation of
my supervisor Professor John Stephenson that cases of so called "whooping cough vaccine damage" or "vaccine encephalopathy" were in fact Dravet Syndrome. I suggested that with the recognition of a genetic basis for Dravet Syndrome this observation could be tested. This discussion was one of the reasons that the Australian group decided to re-open their study of SCN1A related epilepsies (Ingrid Scheffer – personal communication).
Figure 9.1
Pedigree of Scottish family with GEFS+ illustrating the variety of phenotypes including SMEI / Dravet syndrome and bilineal inheritance.

- Febrile seizures and absences
- Severe myoclonic epilepsy of infancy
- Febrile seizures to 7 or 8 years
- Febrile seizures and afebrile seizures after 6 years
- Typical febrile seizures
9.2 Methods

9.2.1 Phenotypic spectrum of SCN1A related disorders

Cases of infantile onset epileptic encephalopathy including cases with a clinical diagnosis of Dravet Syndrome were identified from my own practice and that of my colleagues Dr Mary O’Regan and Dr Robert McWilliam. All individuals were under continuing follow up in the Fraser of Allander Neurosciences Unit. The definition of epileptic encephalopathies as “disorders in which there is a temporal relationship between deterioration in cognitive, sensory and motor function and epileptic activity comprising frequent seizures and/or extremely frequent ‘interictal paroxysmal activity” was used to define cases. (Nabbout and Dulac, 2003). Case records including neuroimaging, EEG studies and other investigations were reviewed. Special attention was given to the onset of epileptic seizures, their semiology over time including video recordings, and EEG features over time. Early developmental history and trajectory were recorded with details of any regression in abilities and nature of any learning disability. Cases were included if neuroimaging was normal and there was no underlying diagnosis identified. Cases were classified as Dravet Syndrome / SMEI, borderline Dravet Syndrome / SMEB or unclassified epileptic encephalopathy. The classification of Dravet Syndrome was based on onset of generalised or hemiclonic seizures in the first year of life in a developmentally normal infant who went on to develop a variety of other seizure types including myoclonus in association with an epileptic encephalopathy and generalised spike and wave on EEG. SMEB was defined as the features of Dravet Syndrome without certain features such as generalised spike and wave and myoclonus or onset in the second year.

With informed consent DNA samples were collected and forwarded to Professor John Mulley’s molecular genetic laboratory in Adelaide. All 26 exons of SCN1A were amplified by polymerase chain reaction (PCR) and analysed by denaturing high
performance liquid chromatography (DHPLC). Regions in which abnormalities on DHPLC were detected underwent bi-directional sequencing.

The clinical and molecular genetic data were included in a collaborative study with other cases primarily from Australia, New Zealand and Canada. The evaluation of the larger group was coordinated by Professor Ingrid Scheffer in Melbourne.

9.3 Results

15 cases with infantile onset epileptic encephalopathy were included in the study. 6 cases had a diagnosis of Dravet Syndrome, 1 with SMEB and there were 8 with unclassified epileptic encephalopathies. The 7 cases with Dravet Syndrome and SMEB had a range of age of onset from 3 months to 12 months. The group comprised four females and one male. All had normal development before their first seizure and none had a family history of epilepsy or febrile seizures.

6/7 had their first seizures in the context of a febrile illness. 2/7 cases had their first seizure within 24h of a DPT immunisation. In one case this was the first set of triple vaccine in the other the third set. 4/7 had febrile status epilepticus (30 minutes) as their first seizure. Of these 2 were hemi-clonic status epilepticus. All 4 were admitted to the intensive care unit. 2 cases had clusters of recurrent seizures on the first day of seizures, one several brief 1-2 minute generalised convulsions and the other recurrent myoclonic seizures causing limb jerks and head drops. All 6 Dravet Syndrome cases had hemi-clonic epileptic seizures at some point in infancy. One case who presented with a brief febrile seizure at 7 months began having episodes of status epilepticus at 1 year of age.

All 6 cases of Dravet Syndrome had different types of epileptic seizures with myoclonic and focal epileptic seizures from the second year. Initial interictal EEG studies were normal in 5 patients but all patients had abnormal EEGs with time with generalised spike and wave in 5 cases. Focal spike and wave abnormalities were seen in all cases.
4/7 developed movement problems including the case with SMEB. This individual had a mild degree of ataxia with an intermittent exercise induced movement disorder characterised by brief limb dystonia. 4 cases developed upper motor neuron signs with one having severe truncal hypotonia and three developing a diplegia with toe walking.

All cases had refractory epilepsy with between 8 and 22 medications prescribed to each child (mean 14). However all patients did show significant improvement with certain medications. Three improved with triple bromide elixir (one stopped due to side effects) and five have had significant improvement in seizure control with stiripentol.

All cases had an epileptic encephalopathy with acquired learning disability. This varied from mild to profound (see case summaries). One child had sudden unexpected death in epilepsy.

6/7 cases had mutations in the SCN1A gene. Details of the mutations are listed in table 9.1. One case of Dravet Syndrome did not have an SCN1A mutation.

**9.3.1a Case Summaries**

**Case # Clinical Diagnosis mutation amino acid change**

Case 1 Dravet Syndrome c.3714A>C p.Glu1238Asp

This 11y old female first presented aged 3 months on the day of her first DPT immunisation. That day she fed poorly, became febrile at 40 degrees celsius and had a prolonged generalised clonic seizure requiring intensive care unit admission, intubation and ventilation. As an infant she had prolonged generalised clonic seizures, clusters of brief generalised clonic seizures particularly with febrile illnesses and myoclonic seizures particularly in the morning. In the 2nd year she had prolonged hemi-clonic seizures. Development was normal in early infancy but by 18 months to 2 years developmental problems were evident.
Initial EEG was normal. At 1 year, episodes of right sided spike and slow with photic stimulation were present on EEG. Frequent generalised spike and slow wave bursts occurred in later interictal recordings. Generalised spike wave bursts were associated with myoclonic jerks. A focal epileptic seizure with secondary generalisation was captured on video-EEG telelemetry with spike and slow wave commencing on the right and spreading to all areas. Later in life there was no response to photic stimulation. Age 3 years she saw Jean Aicardi who said “By certain aspects it looks like severe myoclonic epilepsy but the onset was a bit early.” She has had several episodes of obtundation status lasting a few days characterised by markedly reduced responsiveness, some peri-oral myoclonus and a diffusely slow background EEG with very few associated sharp waves.

Multiple medications were tried - lamotrigine, topirimate, sodium valproate, clonazepam, phenobarbitone, levetiracetam. She did respond to triple bromide but this had to be stopped because of severe rash and irritability. Her seizures are now much better controlled on a combination of stiripentol and clobazam. She may go several weeks without a seizure. She has truncal ataxia and pyramidal tract signs with a diplegia. She can walk independently for short distances. She has severe global learning disability with no speech. She smiles responsively to her family members and vocalises but has autistic features with active gaze avoidance of strangers.

A novel missense mutation was detected in SCN1A causing a significant amino acid change in the S1-S2 linker in domain three of the channel. Parents did not wish to be tested.

**Case 2  Dravet Syndrome  c.3733C>T  p.Arg1245X**

This 10 year old female had her first seizures at age 7 months. She had five generalised clonic seizures 1-2 minutes all in the context of a viral upper respiratory tract infection. Early interictal EEG was normal. She continued to have clusters of brief generalised seizures mostly with febrile illness every month or so. At 1 year she began to have prolonged febrile convulsions with post ictal hemiparesis. At 22
months she was noted to have normal development. Myoclonic seizures were present by two years at which time interictal EEG showed generalised spike/polyspike and wave as well as multifocal independent spike and wave complexes. Ictal EEG during a bout of brief generalised clonic seizures showed right or left hemisphere spiking which then generalised. MRI in 1998 was normal. She was unresponsive to sodium valproate, pyridoxine, lamotrigine, clonazepam, carbamazepine and acetazolamide.

She has developed a motor disorder with toe walking and a diplegic gait. She has had cognitive regression with a severe learning disability and an acquired autistic phenotype. Last year she was commenced on stiripentol in combination with clobazam. Her seizures for the first time since infancy are now controlled. She is seizure free but her behaviour is now very difficult to manage. Her parents prefer her to be seizure free and difficult to control. She is now on risperidone.

She has a truncating mutation in the S1 region of domain 4 of the SCN1A channel. The mutation has been reported in two other cases of Dravet syndrome (Nabbout et al 2003)

**Case 3  Dravet Syndrome  c.1687delC  p.Lys563TyrfsX60**

This 14 year old girl presented age 5 months with a 40 minute hemi-clonic epileptic seizure during an upper respiratory tract infection with a fever of 38 degrees celcius. Over the next 6 months she had about 2 hemi-clonic seizures per week affecting her right or left side lasting about 20 mins. with post ictal weakness. Seizure types from the second year have been complex partial, myoclonic, bouts of generalised clonic seizures, generalised and hemi-clonic status epilepticus. Until recently she had seizures on most days with the longest time being 12 days between seizures. The initial EEG at 5 months was normal. By one year her EEG showed generalised polyspike and wave bursts associated with myoclonic jerks. Medications have included vigabatrin, clonazepam, pyridoxine, carbamazepine, triple bromide, clobazam, lamotrigine, acetazolamide, primidone, sodium valproate, ketogenic diet, tiagabine, and topirimate. She has had a dramatic response to stiripentol. This was initially commenced in combination with valproate and clobazam. Seizures reduced
in frequency however she had significant side effects. I stopped the valproate and was able to increase the stiripentol further with disappearance of the side effects. She went 6 months without a seizure.

Now she has brief complex partial seizures once every two weeks or less. She has moderate to severe global learning disability with an acquired autistic phenotype. Since her epilepsy has been better controlled she is less passive, more determined and her behaviour is more difficult to manage.

She has a novel de novo frameshift mutation in the SCN1A gene predicted to cause truncation of the protein and sited in the domain I to domain II linker region.

**Case 4**  **SMEB**  **c.1876A>G**  **p.Ser626Gly**

This 11 year old boy had normal development prior to his first seizure aged 1 year. Between 12 and 18 months he had 12 febrile seizures some of which were prolonged to 30 minutes. He then went on to have generalised seizures about every two weeks. He began to have focal seizures at about 2½ years. The longest he ever went between seizures was about 2-3 weeks. At age 5 he had febrile seizures and development was said to be normal. Between age 5 and 6 years he developed myoclonic seizures and atypical absences. EEG initially was normal but at age 6 years showed high voltage polyspike bursts with a generally slow background. EEG showed photosensitivity age 10 years though this had not been present earlier. From the age of 5 years he had cognitive decline and had mild to moderate learning disability. He developed ataxia and a diplegic gait and an intermittent dystonic movement disorder triggered by exercise. He has been treated with 11 medications with a partial but not sustained response to bromide and stiripentol. The trials of both these medications may not have been for sufficient time. This boy died with a diagnosis of sudden unexpected death in epilepsy. He has a novel missense SCN1A mutation sited in the domain I to domain II linker region.

**Case 5**  **Dravet Syndrome**  **c.3462delT**  **p.Ser1155AlafsX9**

This 14 year old male presented age 7 months with generalised clonic status (45 min) associated with a febrile illness and upper respiratory tract symptoms. After
discharge from the ICU he was noted to have myoclonic seizures. He had further admissions with status epilepticus in first year some clearly hemi-clonic as well as myoclonic, atonic and complex partial seizures. EEG at 7 months captured myoclonic jerks associated with generalised polyspike bursts. He had a myoclonic jerk during photic stimulation Intercital EEG has persistently shown generalised polyspike bursts. Several seizures captured on EEG over the last ten years including serial myoclonic evolving into generalised tonic and then clonic seizures (Figure 9.3a,b & Figure 9.4). He has had episodes of non-convulsive status. Medications used included sodium valproate, vigabatrin, nitrazepam, carbamazepine, clonazepam, ethosuximide, lamotrigine, phenobarbitone, acetazolamide, ketogenic diet and triple bromide. His seizures improved with triple bromide to about one per week however nocturnal seizures increased in frequency and he is now on stiripentol and clobazam with a good response.

He has moderate global cognitive disability with an acquired autistic phenotype, poor attention and concentration. Behavioural problems include aggressive outbursts. He has a de novo, novel, frameshift mutation in the domain II to domain III linker region predicted to cause truncation of the protein.

**Case 6 Dravet Syndrome No mutation identified**

This 15 year old male presented with a cluster of myoclonic seizures and generalised clonic seizures on the day of his third DPT immunisation aged 11 months. He was febrile (38.5 degrees celcius). Development was normal to this age. He was cruising around furniture and knew 2 body parts. Video- EEG at presentation showed clusters of myoclonic seizures associated with generalised spike and wave (Figure 9.5). Over the next year he developed generalised tonic clonic, hemi-clonic seizures and atypical absences. He had an epileptic encephalopathy with developmental regression and loss of sitting ability. He was treated with 21 anti-epileptic medications. Aged 15 he was having daily epileptic seizures with an EEG characterised by polyspike and slow spike and wave. He had a trial of stiripentol which reduced seizure frequency for several months however the effect was not sustained. He has profound global neurodisability.
Case 7  Dravet Syndrome  c.596C>G  p.Thr119Arg

This 13 year old girl presented age 7 months with a prolonged hemi-clonic epileptic seizure, lasting 20-30 minutes, mainly affecting her right side and not associated with fever. She was admitted to ICU. EEG in week after seizure was normal. She had further hemi-clonic seizures lasting 10 to 40 minutes at about monthly intervals thereafter with subsequent weakness of right or left side. She had myoclonic seizures and brief partial seizures, 3-5 per week with secondary generalisation at times.

Interictal EEG at presentation was normal. Age 1 year runs post central theta with unusual stereotyped complexes comprising low amplitude sharp, followed by high amplitude slow wave (figure 9.6). Post ictal EEG has been slow however we have never captured generalised polyspike and wave and there has been no abnormal response to photic stimulation.

Medications have included rectal diazepam, paraldehyde, carbamazepine, sodium valproate, phenytoin, pyridoxine, vigabatrin, phenobarbitone, lamotrigine and clonazepam. Prolonged hemi-clonic seizures continued every few weeks to every three months until she was started on triple bromide elixir aged 4 years. The frequency decreased thereafter. In the last 4 years she has had only one seizure. She has a general learning disability with full scale IQ in 1997 assessed at 48 using WISC III. As her epilepsy became better controlled on bromide there was an identifiable cognitive improvement with a full scale IQ of 59 and no major discrepancy between verbal and performance tasks.

She has a novel, de novo, missense mutation in the N – terminal region of SCN1A.
Figure 9.2

Cartoon of the α1 subunit of the neuronal sodium channel with two auxiliary β subunits. There are four homologous domains with 6 transmembrane segments (S1-6). The S4 regions are involved in the voltage sensor and the S5-6 loop forms the ion selectivity pore.

- Positions of mutations detected in Dravet syndrome cases 1,2,3,4,5, & 7.

Table 9.1 Mutations detected in the SCN1A gene

<table>
<thead>
<tr>
<th>Case</th>
<th>Mutation</th>
<th>Domain</th>
<th>Exon</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>c.3714A&gt;C; p.Glu1238Asp</td>
<td>DIII-S2 linker</td>
<td>19</td>
<td>Missense</td>
</tr>
<tr>
<td>2</td>
<td>c.3733C&gt;T; p.Arg1245X</td>
<td>DIVS1</td>
<td>18</td>
<td>Frameshift/Nonsense</td>
</tr>
<tr>
<td>3</td>
<td>c.1687delC; p.Lys563TyrfsX60</td>
<td>DI-DII linker</td>
<td>11</td>
<td>Frameshift</td>
</tr>
<tr>
<td>4</td>
<td>c.1876A&gt;G; p.Ser626Gly</td>
<td>DI-DII linker</td>
<td>11</td>
<td>Missense</td>
</tr>
<tr>
<td>5</td>
<td>c.3462delT; p.Ser1155AlafsX9</td>
<td>DI-DIII linker</td>
<td>17</td>
<td>Frameshift</td>
</tr>
<tr>
<td>7</td>
<td>c.596C&gt;G; p.Thr119Arg</td>
<td>N-terminal</td>
<td>2</td>
<td>Missense</td>
</tr>
</tbody>
</table>
Figures 9.3a & b. EEG of case 5. Focal epileptic seizure commencing with right hemisphere sharp and slow evolving into generalised seizure with fast spiking over all areas.
Figure 9.4 Interictal sleep EEG of case 5 showing runs of focal spike and slow wave over the right hemisphere.
**Figure 9.5** EEG in case 6 age 1 year. Generalised spike and wave in association with a myoclonic seizure

**Figure 9.6.** EEG in case 7 age 5 showing a diffuse poorly organised slow background with focal spikes over the left hemisphere
9.4 Discussion

The seven Glasgow patients discussed in detail here have been reported in a paper describing the spectrum of SCN1A related infantile epileptic encephalopathies (Harkin et al 2007). The two individuals with seizure onset within 24h of DPT immunisation have also been reported in another paper on SCN1A related phenotypes (Berkovic et al 2006).

9.4.1 Molecular genetics

Dravet Syndrome has an estimated prevalence of 1 in 20,000 to 1 in 40,000 however no large epidemiological studies have been performed and the identification of increasing numbers of genetically proven cases suggests that these figures may be underestimates (see Chapter 10 and Hurst 1990, Yakoub 1992, Lossin 2009). 5/6 (83%) of the classical Dravet syndrome cases in the Glasgow series had SCN1A mutations and this reflects the 52/66 (79%) mutations detected in the larger study group. Mutations were detected throughout the gene (Figure 9.1). In the 3.5 years that we have been sequencing SCN1A in Glasgow we have detected a further 9 mutations in patients from the West of Scotland (Chapter 10). All the mutations in the first report of SCN1A in association with Dravet Syndrome were truncating in nature (7/7) and this was suggested as the reason why the phenotype was more severe than missense mutations in GEFS+ (Claes et al 2001). However we detected 3/6 missense mutations and 3/6 truncating mutations. The figure of 50% missense mutations in Dravet Syndrome is in accordance with other larger subsequent studies including the infantile encephalopathy study where the figure was 20/52 (40%) (Harkin et al 2007). 5/6 (83%) of the mutations in the Glasgow series were novel with 80% being novel in the wider study.

Nonsense, frameshift, splice site mutations and gross rearrangements such as deletions or duplications would be expected to cause truncation of the protein and haplo-insufficiency. The case for a missense mutation being pathogenic is based on several pieces of evidence. A de novo mutation in a de novo disease is more likely to
be significant. If the mutation affects a residue that is conserved within different sodium channel subtypes and between different species (evolutionary conservation) then it is likely to be significant. If the mutation causes a significant amino acid change in a functionally important part of the gene it is more likely to be significant. Functional studies are the gold standard for showing that a mutation is pathogenic but they are not practical at present outside the research environment and studies in different models can produce differing results. Work on different mutations in SCN1A originally has produced conflicting results with some showing gain of function and others showing loss of function (Spampanato et al 2003; Mantegazza et al 2005). More recent work, particularly on mouse models, has suggested that the epileptic phenotype is caused because the sodium channel has a critical role in the function of inhibitory GABA interneurons (Yu et al 2006; Ragsdale 2008). This model suggests that mutations cause reduced excitability of GABAergic neurons and widespread loss of brain inhibition. The extensive distribution of these GABAergic interneurons, including in the cerebellum, might explain some of the motor manifestations seen in SCN1A related encephalopathies. There are likely to be more than one mechanism through which mutations cause epilepsy and a great deal of translational research work is ongoing. In an individual the rest of the genetic background is likely to influence the clinical expression of the gene mutation.

9.4.2 Clinical features

One of the Glasgow cases (case 4) was classified as SMEB because he had onset of seizures outside of the first year of life. His phenotype in infancy and early childhood was of febrile seizures, short and prolonged and generalised tonic clonic seizures. His cognitive abilities were reported as normal until age 5 when his seizures became more difficult to control. He didn’t have myoclonic seizures until 5 years. Despite what may be regarded as a milder phenotype he died at the age of 13 with sudden unexpected death in epilepsy (SUDEP).

There is a significant mortality associated with Dravet Syndrome and its subtypes. Some authors have suggested that it may be as high as 10-20% in childhood due a
variety of causes including status epilepticus, sudden unexpected death and accidental drowning (Dravet 1992, Dravet 2002). The incidence of SUDEP in adults is unknown.

We recently reported a family with GEFS+ and a novel missense mutation in SCN1A in which two individuals died of SUDEP (Hindocha et al 2008). SCN1A is expressed in cardiac muscle and the sino-atrial node but it has as yet not been shown to be implicated in cardiac arrhythmias. This family suggest their may be shared mechanisms for the epilepsy and sudden death. The prevention of SUDEP is of course a major clinical and research goal therefore any clues to molecular mechanisms are important.

The presentation of 6/7 of our cases in a febrile illness is typical of Dravet syndrome, with large series suggesting presentation with febrile seizures in 60-70% or by elevated temperature such as immersion in a hot bath, as is often the case in Japan (Ohki 1997). The presentation with febrile status epilepticus and ICU admission is also typical. Recurrent hemi-clonic status in infancy is strongly suggestive of Dravet Syndrome.

4/7 of our cases developed a progressive movement disorder in addition to the epileptic encephalopathy. All had a degree of ataxia. This is a common finding in Dravet Syndrome and may be difficult in early infancy to distinguish from side effects of medication. Case 6 who did not have an identifiable mutation had the most profound neurological decline both cognitive and motor. He was completely dependant as a teenager, gastrostomy fed, with no sitting balance, central hypotonia and pyramidal signs in his limbs. The 3 cases who developed a diplegia with pyramidal signs all had epilepsies which followed different courses and had variable cognitive outcomes. Refractory epilepsies of any aetiology may be associated with movement problems most likely due to the involvement of cortical – basal ganglia and cortical-cerebellar neuronal networks in the abnormal electrical activity. However movement disorders and pyramidal signs are frequently reported in Dravet syndrome and the features in our three patients suggest that regional expression of
the mutated channel, much as in episodic ataxias and epilepsy, may play a role in some individuals that is independent of the severity of the epilepsy. Paroxysmal dystonia was recognised in case 4, a feature that was reported in a series of 3 Japanese patients (Ohtsuka et al 2003). These authors noted that the movement disorders were worse when their patients were treated with the sodium channel blocker phenytoin but that it was still present even off this medication.

9.4.3 Medication response

All our patients had highly refractory epilepsies, with the use of multiple medications, however all showed improvement with specific medications. In some cases this was quite dramatic. The most effective medication in the group was stiripentol. This was used in combination with clobazam with or without sodium valproate. In one child who had seizures every day of her life since infancy, introduction of this drug as a teenager stopped all seizures for 6 months. Stiripentol appears to have two potential mechanisms of action. It is a cytochrome P450 inhibitor therefore it can raise levels of other AEDs such as clobazam (Perez et al 1999). It also has direct effects enhancing central GABA transmission and this, given the concept of Dravet Syndrome as a GABA interneuronopathy, may be why it appears to be specifically helpful in this syndrome when the vast majority of medications fail (Quilichini et al 2006). There have been two randomised controlled trials showing the effectiveness of stiripentol in Dravet syndrome however both had a short duration of follow up of only 2 months (Chiron et al 2000; Kassai' et al 2008). My own clinical experience and that of colleagues is that stiripentol is helpful in the long term however well designed studies have not been performed. As stiripentol is a potent cytochrome P450 inhibitor it needs to be commenced with caution. Concomitant medications may need to be reduced significantly and the children should be monitored closely with regular adjustments to doses as demonstrated in case 3. My clinical experience of the use of stiripentol in several Dravet syndrome patients has been of a significant decrease, sometimes cessation, in seizures for several months followed by a return of occasional seizures but never as frequent as prior to the introduction of the medication.
Bromides were the first antiepileptic medication available from the 19th century however they were no longer used in most centres because of the high rate of side effects. There have been case series reporting efficacy of bromides in Dravet Syndrome. Bromides have been the treatment of choice in Japan with one study showing moderate to excellent improvement in convulsive seizure frequency in 70% of patients (Oguni et al, 1994). 3 of the Glasgow cases using bromides showed reduction in seizure frequency with case 7 seizure free. Bromide levels need to be monitored closely to reduce the risk of side effects.

Topirimate has been shown to be effective in reducing seizure frequency in several open label or retrospective studies (Nieto-Barrera et al, 2000a; Kröll-Seger et al, 2006). Certain medications notably Lamotrigine and carbamazepine can increase seizure frequency, particularly myoclonic and generalised clonic seizures (Guerrini et al, 1998).

Better control of epileptic seizures should reduce the impact of the epileptic encephalopathy and improve long term cognitive and behavioural outcomes however this has not been demonstrated as yet. In the cases described one had an improvement in IQ demonstrated as the seizures improved and several were said by parents to be more alert, more confident and more focused in their actions. This resulted in challenging behaviour in some children but this was far preferable to families than their children having frequent seizures. The cognitive outcome in Dravet syndrome has been regarded as universally extremely poor however the potential to treat the epileptic encephalopathy effectively gives the hope that the cognitive outcomes may improve. At the American Epilepsy Society Meeting in 2008 Chipaux et al reported cognitive and behavioural outcome in 64 patients. This work is as yet unpublished but they reported that 42/57 cognitive evaluations in children over 4 showed mild or moderate delay with only 7 showing severe delay. They concluded that their study showed better cognitive and behavioural outcome than previously reported and that this may be because of changing patterns of treatment of the syndrome (Chipaux et al 2008).
The most important factor in the therapy of Dravet syndrome will be early treatment before the epileptic encephalopathy is established and cognitive decline occurs. This will necessitate diagnosis as early as possible in the first or second year of life before cognitive decline begins. Even three to four months of seizure freedom at this critical developmental age, when infants are beginning to develop speech, use imagination and develop their own independent personalities may have a major positive impact on development. The role of molecular genetics in aiding early diagnosis is discussed in chapter 9.

9.4.4 “Vaccine encephalopathy” and SCN1A mutations

Case 1 and case 6 had their first epileptic seizures within 24h of a DPT immunisation. The family of case 6 had received “vaccine damage” compensation and the family of case 1 were undertaking legal proceedings for “vaccine damage” compensation. My supervisor Professor John Stephenson had taught me that in his view children with so called “vaccine encephalopathy” or “whooping cough vaccine damage” in fact had Dravet syndrome. The genetic characterisation of Dravet syndrome allowed this hypothesis to be tested.

Professor Stephenson had been involved in the at times heated debate relating to “vaccine encephalopathy” in the 1970s and had been an expert witness in court. As this issue was of such importance to public health policy, wider society, and the politics of the day, much of this debate was carried out in the national press rather than in the scientific literature. Figures 9.2a and b are cuttings from the Sunday Times letters pages in May of 1977. In his letter John Stephenson makes the point that it is the seizures that are damaging rather than the vaccine, and that it is also possible that congenital abnormalities that produce epilepsy may present for the first time at around the time of immunisation. He suggested that a long convulsion brought on by the fever produced by the immunisation was important rather than anything specific about the vaccination. This was a year before Charlotte Dravet’s first description of the syndrome.
John Stephenson's Sunday Times Opinion piece dated 22/05/1977

"Whooping cough. Why the vaccine-damage campaign is misguided"

The continuing campaign for compensation to 'whooping cough vaccine-damaged' children seems to me to be completely misguided. In the first place we do not have any sound evidence that the whooping cough vaccine is ever a direct cause of brain damage.

This is quite unlike the situation with certain other vaccines (such as that against smallpox) where rarely an allergic inflammation of the nervous system may result in permanent injury. What is known is that very occasionally after whooping cough vaccine (as after any other cause of fever) a child may take a convulsive seisure with fever. A febrile convolution, which does not stop quickly like most febrile convulsions but continues for more than half an hour.

We call this medical emergency "febrile status epilepticus," and its danger is that it may result in a brain-ear from which an epilepsy can arise in later life. Or induce permanent weakness of one side of the body, so-called hemiplegia. The more widespread such brain damage, the more likely is there to be mental handicap in addition.

The important point is that although this long convulsion may have been sparked off by the whooping cough vaccine, it is the convulsion itself and not the vaccine which is damaging. It is vital, because we now know how to prevent damage from these febrile convulsions, using injections which stop them early and glucose infusions in hospital which allow brain function to repair. Except by this indirect mechanism of febrile status epilepticus, which is preventable without altering vaccination policy, we know no means whereby whooping cough immunisation leads to actual physical brain damage.

What then about the allegations that severe mental handicap can be induced by the vaccination, without severe convulsions having taken place? Can the function of the brain be seriously impaired, without there being any structural damage?

For several years there has been concern about this question, chiefly in relation to so-called "infantile spasms," otherwise known as the West syndrome because Dr West first observed and described the condition in his own son (long before the whooping cough vaccine).

Infants with West syndrome lose interest and regress in development at about the age of six months; shortly afterwards they begin to have frequent brief forward-bending spasms, easily confused with simple colic. The brain waves in a child with West syndrome are totally disorganised ("hyparrhythmia") and it seems to be this disorganisation of the electrical activity of the brain, rather than a physical damage, which underlies the mental handicap that usually follows.

For a long time it was uncertain whether whooping cough vaccination could lead to West syndrome in infants without any other identifiable cause, but at last there is excellent evidence from Denmark that it does not.
Whooping cough: it's the victims who matter

Barbara Hanson
Harley, York

CONTRAINDICATIONS: in his opinion upon the safety of whooping cough vaccine Dr. J. B. Stephenson ignores most of the facts and discounts all published evidence except for a solitary and — in our opinion — questionable article from Denmark supporting his own viewpoint.

We have both attempted in recent years to investigate the relationship between whooping cough vaccine and brain damage, one of us as an epidemiologist, the other as a paediatric neurologist. That there are difficulties and possibilities of alternative explanations in many cases, we readily admit. Allowing for these difficulties, we have independently identified and jointly examined cases of severe and permanent brain damage where it seems to us impossible to explain this damage except as a consequence of the injection of the whooping cough (pertussis) component of the triple vaccine (DTP) or of pertussis vaccine given by itself.

The action of the vaccine in this respect may be indirect: it may simply trigger a sequence of events which, by interfering with breathing or by convulsion, damages the brain in a manner common to other conditions such as the infantile spasms (West's syndrome) mentioned by Dr. Stephenson; it may affect infants with a latent genetic defect; or it may aggravate the effects of another, coincidental infection or illness.

For these reasons, even the most enthusiastic supporters of the vaccine recognize a long and growing list of absolute contraindications to its use. But, in other ways, the damaging effect is very direct and is incontrovertible, as when a child reacts almost immediately and much more violently to a second injection. Such reactions are admitted but are not, we believe, reported to the Committee on the Safety of Drugs. As we know, Dr. Stephenson has not published his evidence, as opposed to his opinion. We hope that he will not hesitate to do this. As paediatric neurologist in sole charge of the main assessment centre serving a population of about three million people at the Royal Hospital for Sick Children in Glasgow, he is in a unique position to do so.

Professor Gordon T. Stewart,
Chairman, Parents' Committee.

Dr. John Wilson, London.
Prior to routine childhood immunisation pertussis (whooping cough) was a major cause of mortality and morbidity. Between 1922 and 1932 there were about 73,000 deaths from about 17 million cases of whooping cough (Shorvon & Berg 2008). Whooping cough itself is known to be associated with an encephalopathy in between 1 in 1,200-12,000 infections. The DPT vaccine was very effective but frequently caused a brief febrile reaction and in some cases a febrile seizure. This febrile reaction is thought to be largely related to the endotoxin of the whole cell vaccine. Acellular vaccines are not as effective but have largely replaced the whole cell vaccine as there are fewer adverse reactions. A small number of children after an initial febrile seizure subsequently went on to develop a severe encephalopathy associated with frequent epileptic seizures and cognitive regression. These children were left with severe global neurodisability and total dependence on their carers. The reports of “vaccine encephalopathy” provoked a great deal of public concern in the United Kingdom and uptake of vaccination declined to 30% in the late 1970s leading to an increase in cases of whooping cough and deaths related to the infection in the early 1980s (Figure 9.3). Sweden and Germany stopped their vaccination programmes for a period (Shorvon & Berg 2008).

Several epidemiological studies in the United Kingdom and several other countries have failed to demonstrate evidence of a causal relationship between pertussis immunisation and a severe encephalopathy. Despite this their remains a strong belief of a causal relationship for many parents, lobby organisations and some physicians. Nieto-Barrera et al in the Spanish literature had recognised the coincidence of the first seizures in Dravet syndrome with the DTP immunisation (Nieto-Barrera et al 2000).

As well as the two Glasgow cases a further 12 cases who had an infantile onset encephalopathy who had suspected “vaccine encephalopathy” were identified from Australia, Canada and New Zealand. 8 had SMEI, 4 had SMEB and 2 had Lennox-Gastaut Syndrome. 11 out of 14 of these cases had SCN1A mutations. There were 6 missense, 3 frameshift and 2 nonsense mutations (Berkovic et al 2006). All the
mutations were found in the SMEI or SMEB cases (Dravet Syndrome). Two mutations had been previously reported in association with SMEI and the other missense mutations affected highly conserved amino acids in areas where SMEI mutations had been previously reported. Case 1 from Glasgow had a mutation. Case 6 did not have a mutation. He has been tested by MLPA and doesn’t have a gross rearrangement. We have not looked at GABRG2 or SCN2A as yet.

These findings are important in that they provide genetic confirmation for the original hypothesis that “vaccine encephalopathy” is truly Dravet Syndrome in a large proportion of cases. This has public health implications and medico-legal implications for families wishing to seek vaccine damage compensation including the family of case 1. In a further study, presented at the American Epilepsy Society Meeting in 2008, the phenotypes of Dravet syndrome cases who presented with their first seizure within 48h were compared to the phenotypes of Dravet Syndrome cases that presented more than 4 days from an immunisation (Berkovic et al 2008). The phenotypes were not significantly different. This suggests that the vaccination may trigger the fever related to the first seizure but that this genetically determined epilepsy would have presented even without the immunisation in the next febrile illness. There was no difference in cognitive outcome between the vaccine proximate and vaccine distant group.
Figure 9.8
9.4.5 Phenotypes associated with Dravet Syndrome

The larger encephalopathy study in which the Glasgow patients featured described other novel phenotypes associated with SCN1A mutations (Harkin et al 2007). A phenotype called severe infantile multifocal epilepsy (SIMFE) was described in 5 patients, 3 of whom had SCN1A mutations. I have not seen a case of this disorder. Ingrid Scheffer feels it can be distinguished from Dravet Syndrome and SMEB due to the lack of generalised spike and wave on EEG, lack of myoclonic seizures, and normal development with later cognitive decline than in Dravet Syndrome. Infants have focal epileptic seizures including focal myoclonus as well as prominent multifocal epileptiform discharges. Whether this is on the Dravet Syndrome / SMEB spectrum or a clearly defined phenotype is uncertain until more cases are reported.

The complex genotype phenotype relationships in SCN1A related epilepsies makes their classification difficult and a variety of names have emerged which sometimes cause more confusion than illumination.

These include:

Dravet syndrome

Severe myoclonic epilepsy in infancy (SMEI)

Borderland (borderline) severe myoclonic epilepsy in infancy (SMEB)

SMEB – SW  SMEI without spike wave
SMEB – M  SMEI without myoclonus
SMEB-O  SMEI with >1 feature not in keeping with SMEI
ICEGTC  Intractable childhood epilepsy with generalised tonic clonic seizures

I believe that all the phenotypes described above should be regarded as forms of Dravet syndrome and should be described as such. Classical Dravet syndrome has an onset at less than 12 months and is associated with prolonged febrile generalised and hemiclonic seizures. In the second and third years other seizure types emerge including myoclonus, atypical absences and focal seizures. Cognitive decline is noted in the second year in association with an epileptic
encephalopathy. EEG may be normal initially but with time shows generalised spike and wave. I think that classical Dravet syndrome need not feature prominent myoclonus. This seizure type may take years to emerge. Borderland types of Dravet Syndrome include those listed above but principally where the onset is after one year, there is little or no cognitive decline and only refractory generalised seizures.

For classification and research purposes our group is going to suggest a simplified classification for SCN1A related infantile epileptic encephalopathies. They should be all termed Dravet syndrome unless they have a clearly distinct phenotype such as SIMFE or rare cases of infantile spasms or Lennox –Gastaut. For research purposes the division into classical and borderland forms is helpful to a degree as it acknowledges that the edge of any syndromic classification is blurred and that within this group there may be other genetic aetiologies. I suggest calling these groups Dravet-C and Dravet-B and not dividing this latter group further.
10.1 Introduction

In chapter 9 I discussed the emergence of SCN1A as the most clinically relevant epilepsy gene and the spectrum of infantile encephalopathies associated with the gene. There is increasing evidence supporting specific treatment regimes for the epileptic encephalopathy associated with Dravet Syndrome (Oguni et al 1994; Chiron et al 2000; Nieto-Barrera et al 2000; Kassai et al 2008). As the encephalopathy is associated with cognitive decline and permanent neurological impairment aggressive focused therapy should be commenced as soon as possible (Mullen & Scheffer 2009). However the clinical diagnosis of Dravet Syndrome is based on recognition of seizure types, clinical course and EEG features and is often not made until 2-4 years of age, even by experienced clinicians (Hattori et al 2008). Most children with Dravet Syndrome will present to general paediatricians and distinction between the first seizures in Dravet syndrome and common febrile seizures is not easy to make. The gradual clinical evolution of the syndrome also makes early diagnosis difficult. For these reasons and with the possibility of a treatable epileptic encephalopathy there is likely to be an important role for a
molecular genetic diagnostic service that can aid clinicians in making an earlier diagnosis of SCN1A associated encephalopathies.

In late 2005 I established a joint clinical and molecular genetic SCN1A diagnostic service in Glasgow. In this chapter I will detail how this clinical diagnostic service was developed, report the nature of the mutations we have identified and the emerging evidence that SCN1A genetic diagnosis can influence patient management. I was successful in obtaining a grant from the Muir Maxwell Trust to purchase a genetic sequencer based at the Duncan Guthrie Institute of Medical Genetics, RHSC, Glasgow dedicated to analysis of the SCN1A gene. The proposal was supported by all the paediatric neurologists in Scotland and Su Stenhouse, head of molecular genetics in Glasgow. The aim was to develop a joint clinical and molecular service. I review all the clinical referral forms to the service, prioritise cases for testing and discuss results on at least a weekly basis with Rachael Birch the clinical molecular geneticist who does most of laboratory work. We then issue a joint clinical and molecular genetic report. The service was originally established with charitable funds, it then received National Services Division support in Scotland and the test now has UK Genetic Testing Network approval. This is the only diagnostic service for SCN1A in the United Kingdom and one of the few worldwide. As well as taking referrals from the UK we now do clinical testing for several countries including Australia and Ireland.

The principal focus of this study was to describe the nature of the mutations detected in individuals with Dravet Syndrome (classical and borderline) related infantile epileptic encephalopathy, to describe the age at onset of the first epileptic / febrile seizure in this group and to audit the age of genetic diagnosis. We hypothesised that genetic diagnosis would allow early diagnosis of SCN1A related epileptic encephalopathies. This study was done on samples analysed within a clinical service in a Clinical Pathology Accredited (CPA) laboratory therefore it reflects clinical diagnostic standards rather than research standards.
10.2 Methods

From early 2006 The Glasgow SCN1A service began to accept DNA samples. All referrals are accompanied by a clinical form which I developed with external review by Professor Ingrid Scheffer (Appendix 1). I review all referral forms and assign each case a priority score (1 -5) and clinical diagnosis if possible. The score is based on the diagnosis and the age of the child at time of referral. Scores 4 & 5 are given for high degrees of clinical confidence that the child has either classical Dravet Syndrome or Borderline Dravet Syndrome. Classical Dravet Syndrome (Dravet-C) is defined as in chapter 9. Borderline Dravet Syndrome (Dravet-B) includes all classes of SMEB as detailed in chapter 9 including intractable childhood epilepsy with generalised tonic clonic seizures. Less severe phenotypes associated with GEFS+, and in the context of a family history, are assigned a 3/5 priority score. Scores of less than three were given when there was little evidence from the referral form that the case had an epilepsy known to be associated with SCN1A gene mutations. If cases were less than 2 years old and could have evolving Dravet Syndrome phenotypes they were scored as 4 or 5. The scoring system was not used to reject samples for testing but to prioritise testing particularly for young infants. Cases with scores of 3 or greater automatically went on to MLPA analysis if sequencing studies were negative.

SCN1A genetic sequencing and MLPA studies were performed by Rachael Birch and colleagues. Molecular analysis was performed on genomic DNA extracted from patients’ venous blood. All 26 exons of the SCN1A gene were PCR amplified with 28 specific primer pairs and standard PCR conditions. Sequence products were run on an ABI 3130 automated sequencer (Applied Biosystems) and data were analysed using mutation surveyor software package (SoftGenetics V3.24). In cases where an SCN1A mutation was found, available parental DNA was analysed by DNA sequencing to determine whether a mutation occurred de novo or was inherited. Sequence changes were distinguished from coding single nucleotide polymorphisms and specific missense mutations were further validated by comparing them to a panel of anonymous blood donors as a control population.
In point mutation negative cases, when the phenotype suggested an SCN1A related epilepsy, multiplex ligation-dependent probe amplification (MLPA) was performed to detect large scale rearrangements in the gene. We used the commercially available MLPA kit from MRC-Holland (P137) and tests were conducted according to the manufacturer’s instructions. Electrophoresis of PCR products was performed using an ABI 3130 sequencer and MLPA data were analysed using the GeneMarker software SoftGenetics V1.8). The relative peak height of each exon was first normalised by dividing the average relative peak heights of the internal control probes. The normalised data for each peak was then quantified by dividing by the average relative peak height of the corresponding exons of the control samples.

We analysed the clinical and molecular genetic data for those children with an infantile onset epileptic encephalopathy, Dravet-C or Dravet-B, with particular reference to nature of mutation, age at first epileptic or febrile seizure and age at genetic diagnosis. The date of final reporting of the mutation was used as the age of genetic diagnosis. Statistical analysis was performed using SPSS version 15.0 (SPSS Chicago). The study has been approved by the NRES UK.

10.3 Results

A total of 800 patients underwent SCN1A mutation screening from early 2006 to mid 2009. 280 fulfilled the diagnostic criteria for Dravet Syndrome with 200 classed as Dravet-C and 80 as Dravet-B. We identified a total of 202 patients with SCN1A mutations or deletions from these two groups. (Appendix). 191 mutations were detected on sequencing and a further 11 had gross rearrangements of the gene detected on MLPA. Mutations were detected throughout the gene (Figure 10.1). Figure 10.2a shows the nature of the mutations in the whole group.

171/200 Dravet-C patients had a SCN1A mutation (85%). 77 (45%) were non-truncating missense mutations, 4 (2%) were inframe insertions or deletions, 18 (11%)
were splice site mutations, 29 (17%) were nonsense mutations, 32 (18.7%) were frameshift mutations and there were 11 (6.4%) gross rearrangements (Figure 10.2b).

31/80 (39%) of the Dravet-B patients had a SCN1A mutation. 17 (55%) were non-truncating missense mutations, 1 (3%) was an inframe insertions or deletions, 2 (6%) were splice site mutations, 8 (26%) were nonsense mutations and 3 (9%) were frameshift mutations (Figure 10.2c).

There was no significant difference in the frequency of truncating mutations (all types apart from missense) between the Dravet-C and Dravet-B groups although all the gross rearrangements detected by MLPA were all found in the Dravet-C group.
Figure 10.1. Cartoon of SCN1A channel illustrating mutations found throughout the gene.

Figure 10.2a. Nature of mutation in 202 individuals with Dravet Syndrome.
Figure 10.2b

Dravet-C

Mutation type
- missense
- nonsense
- frameshift
- splice
- inframe insdel
- gross rearrangement

6.43%
2.34%
18.71%
16.96%
45.03%
10.03%

Figure 10.2c

Dravet-B

Mutation type
- missense
- nonsense
- frameshift
- splice
- inframe insdel

54.84%
25.81%
6.45%
9.58%
3.23%
The age of the first epileptic seizure was available for 194 / 202 mutation positive cases (Figure 10.3). The age of first seizure ranged from 2 months to 26 months with the mean at 6.2 months and median at 6 months with a standard deviation of 3.2. 94% of cases had seizure onset before their first birthday.

The median age at genetic diagnosis among all mutation positive patients was 4.5 years (Figure 10.4). The range was 7 months to 42 years. 13/202 patients were over 16 years of age at time of genetic diagnosis. 10 (5%) genetic diagnoses were made at under the age of 1 year and 20% of genetic diagnoses were made under the age of 2 years. The highest number of genetic diagnoses (31) were made in the second year of life.
Figure 10.3
Age at first epileptic / febrile seizure in individuals with Dravet Syndrome & SCN1A mutations.
Figure 10.4
Age at SCN1A genetic diagnosis to 18 years
10.4 Discussion

We have identified a wide spectrum of mutation types in the SCN1A gene associated with Dravet Syndrome in the 3½ years that our service has been functioning. This is one of the largest series of SCN1A mutations reported. Only a French group with 243 mutation positive Dravet Syndrome cases collected over 5 years have reported more cases (Depienne et al 2008).

The proportion of positive mutations in the whole Dravet Syndrome group was 202/280 (72%). This is similar to the report from France where the figure was 242/333 (73%). This figure has varied from 30-100% in the literature. The figure of 100% relates to the original important but small study of 7 patients with Dravet syndrome / SMEI in which all the cases had truncating mutations (Claes et al 2001). The figure of 33% was from a study of 24 patients classified as Dravet Syndrome / SMEI from several centres where 8 mutations were detected (Wallace et al. 2003). The percentage of mutation positives will be related to the degree of phenotypic detail available to classify the cases, the criteria used for classification and the consistency of classification. Our referral form appears to be able to allow us to classify patients appropriately and consistently. It is possible that classification by a single clinician further promotes consistency. We identified mutations in 85% of Dravet-C cases. Depienne et al. reported mutations in 78% of cases they termed Dravet Syndrome. 2 clinicians classified all cases in Depienne et al. The lower proportion of positive results in the Dravet-B group in our series is not surprising as this is a more clinically heterogenous group.

46% of our cases had missense mutations which is very similar to the 42% described by Depienne et al. The remainder detected on sequencing had nonsense, frameshift, splice site and small deletions and insertions all predicted to cause premature stop codons and truncation of the protein. We detected missense mutations throughout the gene with no major “hot spots”. The mutations are listed by exon in Table 10.1. The missense mutations we identified in SCN1A tend to cause significant amino acid changes in functionally important parts of the gene. We are currently analysing the
Grantham score, a measure of the significance of the amino acid change, in all our cases. The Grantham score is determined by composition, polarity and molecular volume (Grantham R 1974). It is possible that the degree of change and the site of change are major determinants of the pathogenicity of a specific mutation. Rarely a missense mutation is associated with Dravet Syndrome in one individual and a benign epilepsy syndrome in another within a GEFS+ family. In this situation other modifying genetic factors are likely to be important.

We identified a further 11 cases with gross rearrangements of the gene detected by MLPA (Figure 10.5; Table 10.1). All these cases had Dravet-C and represented 5.5% of the total positive cases and a positive yield in 11/91 (12%) of Dravet Syndrome patients who were negative on sequencing. 7 had whole gene deletions with 4 partial gene deletions. We have not identified any exon duplications to date. The 3 partial exon deletions in which parental samples were available were de novo. Depienne et al (2008) reported that 14/234 (6%) of their 234 mutation positive cases had gross rearrangements in the gene detected by MLPA. This represents 13% of their sequencing negative Dravet Syndrome cohort. These figures are remarkably consistent with our own and those of Marini et al (Marini et al 2009). This group found a frequency of 12.5% MLPA detected gross rearrangements in Dravet syndrome patients negative on sequencing.

Sequencing of the SCN1A gene was able to detect mutations in 68% of our Dravet Syndrome referrals. MLPA will detect a further 5% therefore our results support utilising MLPA as a second tier test in a clinical service. The finding that all cases with gross rearrangements in SCN1A had Dravet-C, supports offering MLPA to cases with a phenotype consistent with Dravet Syndrome.
Figure 10.5

Multiplex ligation probe amplification of the SCN1A gene. The upper panel shows reduced peak sizes in a patient with a deletion including exon 2, exon 11 and exon 20. The lower panel is from a control panel showing normal peak sizes as two copies of each exon are present.
The age of first epileptic or febrile seizure was under one year in 94% of cases emphasising that onset in the first year is a strong diagnostic indicator for Dravet Syndrome. It also emphasises the developmental expression of the gene lesion in early infancy. The median and mean age of onset are both around 6 months. 29% of cases had onset at 2, 3 and 4 months which is exactly the time that children in the United Kingdom have their first three sets of routine immunisations. This is typically a period of parental concern and anxiety so it is therefore little surprise that parents frequently link the onset of their child’s problems with their immunisations. In the 1970s the first three immunisations were frequently spread out over a longer period in infancy (2, 4 and 6 months) therefore encompassing the same period as 65% of first seizures in Dravet Syndrome.

Dravet-C by definition has onset in the first year of life. There were only 12 mutation positive children with epileptic encephalopathies who presented after the age of 12 months. The latest presentation of Dravet-B was 26 months. Though uncommon the diagnosis of Dravet Syndrome must be considered even if there is onset of epileptic or febrile seizures in the second year or early into the third year of life.

Although the onset of the first seizure was in the first year in 94% of cases the median age of genetic diagnosis was 4.5 years, 4 years after the median age of onset. At first analysis this appears a significant delay when faced with an infantile epileptic encephalopathy in which developmental problems emerge in the second year of life and the first few years are characterised by a refractory epilepsy. The picture may not be altogether bleak as there is emerging evidence from our data that a significant proportion of cases are being diagnosed early.

There are several reasons for the diagnosis to be delayed. Febrile seizures are common in infancy and even febrile status epilepticus is regarded as a common general paediatric problem. It is usually only when the seizures change in nature or there are repeated admissions to ICU that the diagnosis of Dravet Syndrome is considered. The full syndrome does not emerge until well into the second or third
year of life though there can be a strong suspicion much earlier in many cases. Most febrile seizures and febrile status epilepticus present to general paediatricians and ICU specialists who are unlikely to have detailed knowledge of Dravet Syndrome.

The prevention or amelioration of the epileptic encephalopathy should be the primary goal in management of Dravet syndrome and it is now widely accepted that early aggressive therapy may have the potential to do this (Chipaux et al 2008; Mullen & Scheffer 2009). To date this has not been shown in prospective studies but is one of the research themes underway in Glasgow. My own clinical experience is that controlling epileptic seizures in individual patients may lead to cognitive improvement as detailed in chapter 9. Inherent in the concept of an epileptic encephalopathy is that controlling the seizures should improve or abolish the encephalopathy. Early diagnosis is important to allow treatment before there has been significant cognitive impairment.

Children referred before 18 months usually do not have a clinical diagnosis of Dravet Syndrome but a suspicion of the diagnosis. The youngest case was 7 months at genetic diagnosis and we have made a diagnosis in 9 more infants under a year of age. What is encouraging is that the highest number of diagnoses is being made in the second year of life (31). 20% of children are diagnosed before their second birthday and 30% prior to their 3rd birthday.

We have an ongoing project looking at age at clinical diagnosis in the pre-genomic era in Glasgow. This work is incomplete but early results suggest that with good knowledge of the syndrome it was possible to make a clinical diagnosis late in the second year in Dravet-C cases but that the diagnosis was delayed significantly in Dravet-B. The diagnoses were made by experienced paediatric neurologists working in a tertiary centre.

It is important to offer MLPA to all cases with onset in the first year of life and who are not old enough to have developed sufficient features diagnostic of Dravet Syndrome if they are negative on sequencing. All cases detected on MLPA had Dravet-C phenotypes with onset in the first year.
If a detailed history of the semiology and evolution of the seizures is available Dravet Syndrome is recognisable in adults with learning disability and epilepsy (Jannsen et al 2006). We have made a genetic diagnosis of Dravet Syndrome in 13 adults. It would be nice to think this reflects increasing awareness of paediatric onset epilepsy syndromes amongst adult neurologists however most of the adult referrals come from two UK centres.

In a retrospective study Hattori et al. compared the characteristics of seizures in the first year of life in 46 patients with Dravet Syndrome (38/46 SCN1A mutations) and 50 patients with febrile seizures (Hattori et al 2008). From their analysis they proposed several factors which could predict a diagnosis of Dravet Syndrome. The Dravet syndrome group were more likely to have >5 seizures in the first year, onset <7 months, hemiclonic seizures, focal seizures and seizures longer than ten minutes. They proposed a scoring system based on these factors that general paediatricians could use to decide whether to request SCN1A gene analysis. The referral form developed in Glasgow contains all these clinical details and we are currently analysing the data to see which elements best predict the presence of a mutation in our large sample.

Making a genetic diagnosis early in a child with an infantile onset encephalopathy may lead to appropriate treatment controlling the epileptic encephalopathy but it may have many other benefits. In the short term better seizure control may prevent episodes of status epilepticus and the morbidity and mortality associated with this medical emergency. With better control of the seizures long term cognitive and behavioural outcome may improve with benefits for the individual, family and wider society. Infants with epileptic encephalopathies would normally be subject to multiple invasive and costly biochemical, electrophysiological, metabolic and imaging investigations. A genetic diagnosis has the potential to save the health service significant amounts of money. Having a definitive diagnosis is important for many families. It provides them with an explanation for their child's problem and may lessen any feelings of guilt. Families frequently report that having a diagnosis to
put on forms makes it easier for them to access certain services. A genetic diagnosis allows genetic counselling. Many families with a severely disabled child will decide not to consider having more children unless they understand the recurrence risks. The vast majority of Dravet syndrome cases are due to de novo mutations in the gene however somatic and germline mosaicism has been reported (Depienne et al 2006; Gennaro et al 2006; Morimoto et al 2006). There is therefore a very small recurrence risk even if both parents do not appear to carry the mutation on standard testing. In a few selected cases we have agreed to prenatal diagnostic testing. This included two cases in which an older sibling had a severe epileptic encephalopathy and had died due to status epilepticus. The benefits to patients and physicians of genetic testing, is one of the areas of ongoing and future research that I will discuss in chapter 11.

There has been much recent debate about the importance of genetic testing in epilepsy. Some authors have acknowledged the major advances that have taken place in our understanding of the genetic mechanisms that underlie epilepsy but still do not regard genetic testing as contributing significantly to patient care (Delgado-Escueta & Bourgeois 2008). I would argue that SCN1A genetic analysis has now become established as the first genetic diagnostic test in epilepsy that can directly influence patient management.
Table 10.1 SCN1A Gene Mutations detected in Glasgow, ordered by exon.

<table>
<thead>
<tr>
<th>EXON</th>
<th>SEX</th>
<th>PHENOTYPE</th>
<th>Onset in months</th>
<th>Age at Diagnosis yrs/ms</th>
<th>NA level</th>
<th>AA level</th>
<th>REFERENCE</th>
<th>TOPOLOGY</th>
<th>TYPE</th>
<th>Inheritance</th>
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NA = Not Available
That the channelopathies have emerged in the last 15 years as an important class of neurological disorder is not surprising given the critical role of ion channels in electrical signalling throughout the central and peripheral nervous system and muscle. In excess of 400 genes are known to encode ion channels, making up 1-2% of the human genome (Venter et al, 2001). The protein products of these genes, the channel subunits, can combine in a variety of different combinations to produce channels with distinct functions. Ion channels may be widely distributed or only expressed in certain tissues. Alternative splicing of channel genes can lead to a further variation in channel subtypes (Gargus, 2003). This enormous potential diversity of ion channels helps explain the number and variety of neurological channelopathies and the distinct nature of conditions caused by mutations in the same gene.

The phenotypic description of these disorders has led to genetic characterisation which in turn has allowed further phenotypic analysis of the variability and boundaries of individual syndromes. The clinician has a key role in these advances in knowledge. The discovery that common neurological disorders such as the epilepsies are largely a family of ion channelopathies has led to major advances in
our understanding of the neurobiology of disease and the hope that improved more targeted therapies can be developed. Although the major discoveries in the epilepsies have been in monogenic disorders it is likely that ion channel variation will be a major risk factor in common epilepsies with complex inheritance (Heron et al 2007b). Similarly the commonest neurological disorder, migraine, may be caused in a significant proportion of patients by ion channel variation.

11.1 Epilepsy and movement disorders

The studies of individuals and families with episodic ataxias type 1 and 2 (EA1 and EA2) with epilepsy have broadened the phenotypic spectrum of these disorders and provided insights into the relationships between epilepsy and movement disorders. We have shown that the epilepsy and the movement disorder cannot be separated at the molecular level and that the clinical distinction is likely to be influenced by the following factors:

- The regional expression of the mutant ion channels.
- The neuronal network involved
- The nature / functional consequences of a specific mutation
- The stage in development the mutant ion channel is expressed

The same is true of the other features in these disorders including myokymia and neuromyotonia in EA1 and migraine in EA2.

These findings suggest that other disorders in which epilepsy and movement disorders are associated may also be caused by ion channel dysfunction. I have studied a large Scottish family with benign familial infantile convulsions (BFIC) and paroxysmal dyskinesia in collaboration with John Mulley in Adelaide (Figure 11.1). This family links to the pericentromeric region of chromosome 16 (Figure 11.2). As yet despite sequencing many candidate genes we have not found a mutation that segregates with the disease. This syndrome in which benign epileptic seizures, which usually remit in infancy, are followed by a paroxysmal kinesigenic dyskinesia in late
childhood or teenage years is a strong candidate for an ion channel disorder (Rochette et al. 2008).

The differential diagnosis or what has been termed the "Border-land of Epilepsy" by Gowers in 1907 and more recently by Crompton & Berkovic is an area of great complexity and diagnostic challenges for the clinician (Gowers 1907; Stephenson & Zuberi 2005; Crompton & Berkovic 2008). Molecular genetic studies allied to detailed clinical descriptions are revealing that many of these borderland paroxysmal disorders share an underlying pathophysiology in ion channel dysfunction.
Figure 11.1 Benign familial infantile convulsions and paroxysmal dyskinesia in a Scottish family

[Diagram of a family tree showing the inheritance pattern of benign familial infantile convulsions and paroxysmal dyskinesia.]
Figure 11.2. Scottish family with benign familial infantile convulsions and paroxysmal dyskinesia and other reports with linkage to pericentromeric region of chromosome 16.
11.2 Ion channel associated proteins

Phenotypes of paroxysmal disorders resembling or identical to ion channelopathy phenotypes may be caused by mutations in genes encoding structurally or physiologically related proteins which influence channel function rather than in an ion channel itself. This may be the case in BFIC and dyskinesia.

Ion channel subunits interact with a variety of proteins in the cell membrane. Kv1.1 channel function may be affected by mutations in the leucine-rich glioma inactivation gene (LGI1) producing the phenotype of autosomal dominant partial epilepsy with auditory features (Schulte et al 2006). Congenital myasthenias are caused by presynaptic, synaptic and postsynaptic genetic defects in neuromuscular transmission. A variety of mutations have been described in different acetylcholine receptor subunits causing congenital myasthenia (Table 1.1). The receptor associated protein of the synapse (Rapsyn) is important in localising and maintaining the acetylcholine receptor in the post synaptic cell membrane. Mutations of the Rapsyn gene produce acetylcholine receptor deficiency and therefore a congenital myasthenia responsive to pyridostigmine (Ohno et al 2002).

Hyperkplexia or startle disease is a rare disorder associated with mutations in the α1 or rarely the β2 subunit of the glycine receptor (Table 1.1). This disorder may present in a dominant fashion in large families but is more commonly sporadic due to compound heterozygosity. In this condition individuals have an excessive startle response due to failure of the inhibitory functions of glycine in the brainstem and spinal cord. Infants may have hypertonia with attacks of apnoea and sudden deaths have been reported. It can respond to treatment with clonazepam. We have recently participated in a study which has been the first to report a glycine transporter mutation in a new form of hyperekplexia (Rees et al 2006). This is the first human neurological disease caused by mutations in a transporter for a neurotransmitter. We have identified two individuals with this disorder. Mutations in the SLC6A5 gene encoding the glycine transporter (GlyT2) were detected in both these individuals by Mark Rees and colleagues in Swansea. In one case there is a dominantly inherited
mutation and the other case is homozygous for the nonsense mutation C1315T which results in a truncated protein R439X (Figure 11.3). This child has unrelated parents. GlyT2 related hyperekplexia is characterised by severe life threatening attacks of apnoea in early infancy but with time, in the dominantly inherited cases, the tendency to startle disappears and they can come off medication. Our dominant case now plays competitive rugby. In our homozygous case the tendency to startle has persisted and she has remained on a small dose of clonazepam. The remarkable feature about this individual, which is not noted in other genetic forms of hyperekplexia, is that she has a learning disability. This is as yet unreported but developmental delay is a feature of the other two cases worldwide with homozygous mutations in the glycine transporter gene (Mark Rees personal communication). This suggests that this gene may be important more widely in cognitive processes.

11.3 Cognitive impairment & behaviour problems associated with channelopathies

The study of families with ADNFLE revealed frequent behavioural psychiatric problems, a finding confirmed in other publications. The phenotype of the ion channelopathies is commonly characterised by a paroxysmal disorder however there is increasing evidence of chronic symptoms related to channel dysfunction. These may be the relatively subtle features seen in the ADNFLE families or may be the severe encephalopathy seen in infants with Dravet syndrome. The subtle memory deficits reported in families with the potassium channelopathy EA1 deserve further investigation especially as it is known that Kv1.1 potassium antibody related limbic encephalitis is associated with severe acquired memory impairment.
**Figure 11.3** Molecular evidence for SLC6A5 homozygous mutation in child with hyperekplexia (Courtesy of Professor Mark Rees).

Molecular evidence for SLC6A5 R439X

**Sequence results**

<table>
<thead>
<tr>
<th>130</th>
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<tbody>
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Normal  C1315

C1315T - R439X (Heterozygous)

C1315T - R439X (Homozygous)

RFLP test: Gain of Ddel

<table>
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<tr>
<th>R439X*</th>
<th>R439X</th>
<th>R439X</th>
<th>N</th>
<th>N</th>
<th>N</th>
</tr>
</thead>
</table>

* = Homozygous
N = Normal
11.4 Genetic diagnosis in SCN1A related infantile epileptic encephalopathies

The severe encephalopathy associated with Dravet syndrome is devastating for the infant and their family. The prospect of early aggressive therapy offers some hope to families and it will be important that this is followed up with prospective studies. We are currently undertaking such studies in the large cohort of children referred to our genetic service for which we have ethical approval for long term follow up. We are looking at measures of cognition, behaviour and quality of life.

Genetic diagnosis is aiding clinicians in making an early diagnosis of SCN1A related encephalopathies but it will be important to know whether physicians and families regard the genetic diagnosis as helpful and whether genetics causes management to change. We have ongoing studies looking at physician and parental attitudes to genetic testing.

We have had DNA from a large number of children with infantile epilepsy, most who do not have Dravet syndrome, and who do not have mutations in the SCN1A gene. With appropriate consent we are able to look at other potential epilepsy genes in this group. In collaboration with Professor Mark Rees in Swansea we are looking at GABA receptor and transporter genes in this cohort.

A new gene with a phenotype that has some similarities to Dravet syndrome is the protocadherin 19 gene. This was first described in families in which there was epilepsy in females with mental retardation (EFMR) but more recently has been found in a significant proportion of sporadic females resembling Dravet syndrome (Dibbens et al 2008; Depienne et al 2009). We are planning to set up a service to sequence this gene in selected SCN1A negative cases.
11.5 Therapy for ion channel disorders

Many of the medications developed for the treatment of ion channel disorders such as epilepsy in the pre genomic era have modes of action that influence ion channel function. For example the anti-epileptic medication carbamazepine blocks voltage gated sodium channels and can be used to treat a variety of epilepsies as well as the neuromyotonia and paroxysmal ataxia in the potassium channelopathy EA1.

Ion channel pharmacology is an important area of research in CNS and peripheral nerve drug development. Ion channels are the targets of many therapeutic compounds whether they be for epilepsy, muscle disease, anaesthesia or pain relief. The recognition that a rare inherited disorder of pain transmission, paroxysmal extreme pain disorder (PEPD), is associated with gain of function mutations in the sodium channel gene SCN9A has providing an important clue to the underlying cause of pain syndromes (Fertleman et al 2009).

Genotype phenotype studies can suggest that a particular medication may be helpful in a particular syndrome such as stiripentol in Dravet syndrome. This can then be studied in double blind placebo controlled studies. Knowledge of the mode of action of a drug linked to knowledge of the genetic defect and its functional consequences may help to explain why a particular medication is helpful and promote development of allied compounds. The knowledge that stiripentol enhances central GABA transmission and that Dravet syndrome can now be conceptualised as a GABA interneuronopathy may explain the effectiveness of this medication in this particular syndrome. In chapter 7 I discussed the development of retigabine a medication which specifically enhances the function of the M channel which is made of subunits that are mutated in benign familial neonatal seizures. The new field of pharmacogenomics will seek to develop medications tailored to an individual’s genotype with the hope that more targeted therapy will be more effective and have fewer side effects.
Gene therapy is not an immediate prospect for the neurological channelopathies. Techniques such as exon skipping or upregulation of associated non mutant proteins are under trial in genetic neuromuscular disorders such as Duchenne muscular dystrophy however CNS disorders will present even greater challenges (Muir & Chamberlain 2009).

Ten years ago I wrote an essay with the title “Ion Channels & Epilepsy; an exciting future”. In the field of the neurological channelopathies that future has arrived.


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cause the ADNFLE epilepsy. Epilepsia 43 supp5:112-22

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distinct memory deficits. Neurobiology of Disease. 20:799-804

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Episodic ataxia/myokymia syndrome is associated with point mutations in 
the human potassium channel gene, KCNA1. Nature Genetics. 8:136-40

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continuous myokymia. Brain 113:1361-82

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(Cacnb4(Lh)) and tottering (Cacna1atg) mouse thalami. Journal of 
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Appendix 1. SCN1A referral form.

SCN1A mutation screening for infantile onset epilepsies

This is a joint clinical epileptology and molecular genetic service providing sequencing of all 26 exons of SCN1A and MLPA (looking for large scale rearrangements) in selected sequencing negative cases. The test is performed within a National Health Service Clinical Pathology Accredited (CPA) Lab and reporting times conform to national standards.

Testing is undertaken when a completed referral form accompanies the DNA/blood sample. The clinician managing the individual’s epilepsy may be best placed to complete the form. Completion of the form allows us to prioritise samples, this is necessary for quality control purposes, audit and is essential for the provision of a joint clinical and molecular genetic service. The likelihood of the laboratory identifying a mutation is dependent upon the phenotype of the affected individual. In the first 18 months of the service the overall mutation detection rate has been approximately 30% and in classical SMEI / Dravet Syndrome cases between 80-90%.

Prenatal testing is not offered routinely. Cases can be discussed on an individual basis.

Referring Clinician Name & Institution:

<table>
<thead>
<tr>
<th>Name:</th>
<th>Institution Address:</th>
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</table>

(Please circle one of the following)
- Child Neurologist
- Adult Neurologist
- General Paediatrician
- Other Epilepsy Specialist (specify)
- Clinical Geneticist

Affected Individuals Name and Date of Birth:

| Is the test for: Affected individual or carrier status? (Please indicate name and mutation of affected relative) |

Epilepsy Phenotype: Please delete as appropriate and provide details where necessary:

<table>
<thead>
<tr>
<th>Age at first epileptic / febrile seizure</th>
<th>Details:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any factors precipitating first seizure?</td>
<td>Details:</td>
</tr>
<tr>
<td>Prolonged febrile seizures</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Hemi-clonic or focal febrile seizures</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Hemi-clonic or focal afebrile seizures</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Did different focal seizures affect the opposite side</td>
<td>YES / NO Details:</td>
</tr>
<tr>
<td>Myoclonic seizures</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Generalised tonic-clonic / clonic seizure</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Focal seizures with impairment of awareness</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Atypical absences</td>
<td>YES / NO Age of onset:</td>
</tr>
<tr>
<td>Status epilepticus</td>
<td>YES / NO Details:</td>
</tr>
<tr>
<td>Question</td>
<td>YES / NO Details:</td>
</tr>
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<td>------------------------------------------------------------------------</td>
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<tr>
<td>Obtundation status</td>
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<tr>
<td>Has there been a prolonged seizure free period?</td>
<td>YES / NO If yes, how long for?</td>
</tr>
<tr>
<td>Epileptic encephalopathy</td>
<td>YES / NO Details:</td>
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<tr>
<td>Any other comments on seizure types:</td>
<td>Details:</td>
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</table>

**DEVELOPMENT:**

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<tbody>
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<td></td>
</tr>
<tr>
<td>Cognitive decline following epilepsy onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquired autistic features</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behaviour problems</td>
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Acquired motor / movement disorder? *(Please circle one of the following)* hypotonia, ataxia, spasticity, dyskinesia.

Is the movement disorder static or paroxysmal? Details:

Current developmental status: *(please circle one of the following)*

Normal  Mild  Moderate  Severe  Profound  Learning disability

Age at which development noted to be abnormal

Continued development regression or plateau at a certain age?

**EEG FEATURES:** *(Please provide details)*

<table>
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<tr>
<td>Normal interictal EEG</td>
<td></td>
</tr>
<tr>
<td>Generalised spike &amp; wave</td>
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</tr>
<tr>
<td>Photosensitivity (any EEG with date)</td>
<td></td>
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<tr>
<td>Focal / multifocal EEG abnormalities</td>
<td></td>
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<tr>
<td>Additional comments: Did EEG change over time?</td>
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</table>

**MRI:** *(Please provide details)*

**MEDICATION:**

Have any medications increased seizure frequency?

Any medications reduced seizure frequency?
**FAMILY HISTORY:** (Please provide details/pedigree of family history of febrile seizures or epilepsy in space provided)

**SYNDROMIC DIAGNOSIS:** (Please circle one of the following)
- Severe myoclonic epilepsy in infancy / Dravet's Syndrome
- Borderline severe myoclonic epilepsy (SMEB)
- Intractable childhood epilepsy with generalised tonic clonic seizures
- Specific epilepsy syndrome within a family with generalised epilepsy and febrile seizures plus (GEFS+). (Please draw pedigree and state syndromes in space above)
- Other Syndrome - please specify
- Unclassified epilepsy

**COMMENTS:** (Please provide any other relevant details in space provided below)

**INVOICE DETAILS:** (Samples from outside Scotland should provide details of billing address below)

*Clinical queries can be directed to Dr Sameer Zuberi, Consultant Paediatric Neurologist (sameer.zuberi@ggc.scot.nhs.uk)*

*Queries relating to technical aspects of the mutation analysis can be directed to Rachael Birch (rachael.birch@ggc.scot.nhs.uk).*

DNA or whole blood (2-5ml in an EDTA tube) should be forwarded to Rachael Birch, DNA Lab, Duncan Guthrie Institute of Medical Genetics, Royal Hospital for Sick Children, Yorkhill, Glasgow, United Kingdom, G3 8SJ. Tubes must be labelled with name and date of birth.
Appendix 2. Example of fictional SCN1A report

Dr Finlay
Consultant Paediatric Neurologist
St Elsewhere
Borchester
BDU 9ZX
Borchester
cc: DNA Laboratory,

<table>
<thead>
<tr>
<th>Surname</th>
<th>Smith</th>
<th>Pedigree No.</th>
<th>Lab Number</th>
<th>Type of Specimen</th>
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<tbody>
<tr>
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<td>John</td>
<td>Date of Birth</td>
<td>10/11/2004</td>
<td>DNA</td>
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<tr>
<td>Hospital No.</td>
<td>33333333</td>
<td>Date Received</td>
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</tr>
<tr>
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<td>Date Activated</td>
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Reason for Referral: Affected by Severe Myoclonic Epilepsy of Infancy (SMEI)
Test Required: Multiplex Ligation-dependant Probe Amplification (MLPA) was undertaken to detect deletions or duplications of the SCN1A gene.

MOLECULAR GENETIC ANALYSIS REPORT

RESULT:

John Smith
Deletion of exons 1-16 of the SCN1A gene

CONCLUSION:
Previous genetic testing performed on this patient involved direct sequencing of the SCN1A gene. This test did not identify a pathogenic mutation.

Due to the reported phenotype we went on to carry out MLPA analysis of the SCN1A gene. This test has identified a large deletion that includes exons 1 to 16 of the SCN1A gene.

Patients with SMEI and associated infantile onset epilepsies may have large scale rearrangements of the SCN1A gene (Mulley et al 2006). The mutation identified is very likely to be associated with the epilepsy phenotype in this patient.

The P137 probe mix contains probes for 25 of the 26 exons of the SCN1A gene. The NM_006920.2 sequence was used to design the MLPA kit.

References:
http://www.mlpa.com

Molecular Geneticist: Rachael Birch
Checked by: [Signature]

Consultant Paediatric Neurologist: Dr Sameer Zuberi
Date reported: 10/10/2007

A CPA (UK) Ltd fully accredited laboratory