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A study to find novel genetic abnormalities associated with epilepsy in affected Irish families.

Gerard O'Connor
Royal College of Surgeons in Ireland

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A study to find novel genetic abnormalities associated with epilepsy in affected Irish families.

Ged O' Connor, MB, BCh, BAO, BMedSc, MRCPI

A thesis submitted to the School of Postgraduate Studies, Faculty of Medicine and Health Sciences, Royal College of Surgeons in Ireland, in fulfilment of the degree of Doctor of Medicine

Supervisors
Dr. Norman Delanty, Consultant Neurologist, Beaumont Hospital
Dr. Gianpiero Cavalleri, Royal College of Surgeons in Ireland

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I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher degree (Doctor of Medicine) is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Date ___ 29/10/2014 ___

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Table 21. Top 10 candidate variants filtered from whole exome sequencing in affected members of Family H.

Summary

Background; Epilepsy is one of the commonest neurological illnesses. There is considerable evidence that, in many cases, epilepsy is partly or completely genetic in aetiology. However, in spite of progress in certain Mendelian epilepsy syndromes, the genetic cause of epilepsy within families often remains unclear. With the advent of techniques such as whole exome sequencing, it may now be possible to identify the novel genetic variations underlying epilepsy in affected families. The aim of this study is to use whole exome sequencing to identify novel genetic abnormalities associated with epilepsy in affected Irish families. These novel genetic abnormalities will act as candidate causal variants for further study.

Methods; We sought to identify Irish families with strong histories of epilepsy. Familes were considered for inclusion if the index case, one of their first degree relatives and one other individual in the family were affected by epilepsy. In each family identified, we clinically phenotyped individuals who were affected and unaffected by epilepsy syndromes. From each participant, we obtained a tissue sample (blood or saliva) for DNA analysis. In a number of selected families, we performed genetic analysis using whole exome sequencing looking for novel genetic variations which could be the cause of epilepsy in these families.

Results; We identified 96 families who met the criteria for inclusion on initial assessment, of whom 11 were recruited, representing a total of 110 individuals. There was considerable variability of phenotypes within the families identified. Affected individuals from four families had whole exome sequencing, with one other family having candidate gene analysis based on the results of previous screening of a family member. We identified novel genetic variations which could be candidates for further study as a possible cause for the epilepsy syndromes seen in three of the five families analysed. In one family, we identified a novel variation in a known epilepsy gene (A348Y in the *SLC2A1* gene). In two other families, novel mutations were identified in genes not previously demonstrated to be associated with epilepsy (*CHRNB3* and *NARG2*)

Conclusion; Our study demonstrates that whole exome sequencing can be used to characterise novel genetic variations in Irish families with a strong history of epilepsy. Further studies are necessary to demonstrate that these abnormalities are causal for the epilepsy syndromes seen.

Introduction

Epilepsy is a common neurological disorder, affecting tens of thousands individuals in Ireland alone. The causes (aetiology) of epilepsy are very varied, but a familial or genetic component to epilepsy has been recognised since at least the time of Hippocrates of Cos. However, in spite of many advances in the last decades, the nature of the genetic causes of epilepsy in many cases continues to elude us. It is hoped that the use of newer genetic techniques, such as whole exome sequencing, will reveal the genetic mechanisms underlying common epilepsy syndromes.

In this study, we have attempted to find novel genetic abnormalities associated with epilepsy in affected Irish families.

In chapter one, I have reviewed the literature in relation to the genetics of epilepsy. I have divided this chapter into a number of different sections –

In section one, I have reviewed the current classification system for seizures and for epilepsy syndromes. An understanding of this system is needed for the phenotyping of participants in this study. In this section, I have outlined also some of the difficulties with this classification system.

In section two, I have outlined the epidemiological features of epilepsy as a disease. I reviewed the prevalence of the condition in Europe and in Ireland, and have explored briefly aspects of the burden this condition places on individuals and on healthcare systems. To place research on any aspect of epilepsy in the proper context, it is necessary to understand the potential impact of such research.

In section three, I have reviewed the evidence for epilepsy being a disease with a genetic component to its aetiology. I have reviewed different study models such as twin studies, studies of affected multiplex kindreds, familial aggregation studies and (in brief) animal models of epilepsy. I explored different aspects of these study models, especially their strengths and weaknesses.

In section four, I reviewed some of the genetic analysis techniques used thus far in the study of the genetic basis of epilepsy. I have reviewed four different

areas – linkage analysis, association analysis, genome wide association studies and next generation sequencing (emphasising the use of whole exome sequencing). In this way, I hope to have introduced the strengths and weaknesses of previously used techniques, and to have explained in more depth about whole exome sequencing, the technique used in analysing the genotypes of screened participants in this study.

In section five, I have commented on known genetic mutations reported thus far in the literature in association with epilepsy. In each case, I have given a brief summary of the clinical phenotypes seen in association with mutations in these genes. The purpose of this review is to summarise the genetic causes of epilepsy reported to date, and to demonstrate the variability in the phenotypes associated with these mutations. Such a review will allow the reader to comment on the novelty of the genetic findings in this study, as appropriate.

In chapter two, I have outlined the methodology used in the study. I have explained the process of obtaining ethical consent for the study. I have explained the process of recruitment of participants and obtaining clinical phenotypes and tissue samples. I have given outlines of the methods used for obtaining DNA for analysis from blood and saliva samples, and for performing whole exome sequencing based on the protocols in the laboratories involved.

In chapter three, I have given the results of the study. The initial section of this chapter has outlined the broad results of the study, including such aspects as the rate of recruitment, number of participants, etc. After this, the families recruited have been presented. For each family, the family tree has been presented first followed by the detailed phenotypes of each participant from that family. At the end of this section, I have presented the novel genetic findings from those families who have had genetic analysis performed.

In chapter four, I have discussed the results of the study. I have commented on the recruitment process and the issues encountered in this and on the process of phenotyping participants and the reliability thereof. I have commented on the potential implications of the novel genetic findings in the families assessed. I have

also commented on the use of whole exome sequencing for identifying novel mutations in the families analysed.

The final conclusions of the study have been presented in chapter five.

A glossary of terms has been provided after these chapters to further explain some of the terms used in the course of the study. A bibliography for works referenced has been provided after the glossary of terms.

1. Review of the literature

1.1 Definition of seizures and epilepsy

In the assessment of patients with epilepsy and the descriptions of their clinical presentations, numerous terms have been used to describe different seizure types and different forms of epilepsy. The use of such terms should, ideally, serve a number of purposes, such as ensuring the accurate and consistent description of events, facilitating communication between clinicians, and helping in the selection of investigations and treatments. In spite of much effort, older and less accurate phrases such as "petit mal" and "grand mal" are still commonly used by patients and by non-specialists in this area. Other terms, such as "absence", are used in an inconsistent manner, leading to inaccuracy in the description of events.

To clarify many of the descriptions used in the literature on epilepsy and later in this study, it is necessary to give an outline of the current classification systems used to describe different types of epilepsy and seizures. The relevance of this will become apparent in later chapters when discussing the nature of epilepsy syndromes which have been shown to have a genetic basis and in describing the nature of epilepsy in the affected family kindreds included in this study.

1.1.1 Epileptic seizures

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (Fisher et al, 2005). This abnormal activity is a specific dysfunction, characterised by abnormal synchronisation, excessive excitation and inadequate inhibition (Fisher et al, 2005).

Epileptic seizures can be classified in a number of ways. Currently, the most widely used classification system is that of the International League Against Epilepsy (ILAE) (Commission on Classification and Terminology of the International League Against Epilepsy, 1981), although other systems have been developed for particular situations such as video EEG reporting (Luders et al, 1998). In the ILAE

classification, seizures are initially separated into generalised seizures and focal (also called partial) seizures.

1.1.1.1 Generalised seizures

Generalised seizures are felt to originate from some point within, and to rapidly engage, bilaterally distributed networks in both cerebral hemispheres (Berg et al, 2010). Such networks can be cortical or subcortical, but do not necessarily have to involve the entire cerebral cortex. There is no evidence of a focal onset found for these seizures on investigation. Motor manifestations are bilateral and EEG patterns initially are bilateral, although it is recognised that the manifestations of such generalised seizures can be asymmetric (Berg et al, 2010).

In the ILAE classification system for generalised seizures, the recognised types are –

- Absence seizures
  - Typical
  - Atypical
  - Absence with special features
    - Myoclonic absence
    - Eyelid myoclonia
- Myclonic seizures
  - Myoclonic
  - Myoclonic atonic
  - Myoclonic tonic
- Tonic seizures
- Clonic seizures
- Tonic-clonic seizures
- Atonic seizures

1.1.1.2 Focal seizures

In contrast to the generalised seizures described above, focal (or partial) seizures are felt to arise out of networks limited to one hemisphere (Berg et al, 2010). They may

remain localised to that hemisphere, or they can propagate from there to involve other areas. In these events, seizure onset is consistent from one seizure to another. Although the latest update of the seizure classification system by the ILAE eliminates the terms from the 1981 guidelines which differentiated between different focal seizures, these terms are given below as they are still in widespread use clinically. In the 1981 classification, events where consciousness is not impaired are called simple partial, and events where consciousness is impaired are referred to as complex partial (Commission on Classification and Terminology of the International League Against Epilepsy, 1981).

The recognised types of partial seizure under the older definitions were –
- Simple partial
  1. with motor signs
  2. with somatosensory or special sensory symptoms
  3. with autonomic symptoms or signs
  4. with psychic symptoms

- Complex partial
  1. simple partial seizure at onset progressing to loss of consciousness
  2. with loss of consciousness at onset

- Partial seizures evolving to secondarily generalised seizures
  1. simple partial seizures evolving to secondarily generalised seizures
  2. complex partial seizures evolving to secondarily generalised seizures
  3. simple partial seizures evolving to complex partial evolving to secondarily generalised seizures

1.1.1.3 Difficulties with the definitions of seizures

Although the definitions given above seem clear-cut, the International League Against Epilepsy has acknowledged that there are limitations in this classification system.

The terms focal and generalised are used to represent a dichotomy in the classification of seizures and of epilepsy syndromes. However, it has been long recognised that focal lesions can cause both focal and generalised seizures (Nguyen et al, 2006; Tezer and Saygi, 2009). Similarly, generalised seizures can be associated with focal features at onset (Boylan et al, 2006; Jayalakshimi et al, 2010), making the distinction between the focal and the generalised very unclear. There are also individuals in whom focal and generalised epilepsy syndromes co-exist (Jeha et al, 2006). These terms still have a pragmatic utility, and are maintained, but it is felt that the dichotomy between these groups may not be very clear. Many of the terms used in describing focal seizures are imprecise or misleading. However, the Commission also acknowledges that such terms or descriptions, although not very scientific, can have clear practical uses (for example, preservation or loss of awareness has considerable implications for driving).

As there is no broad acceptance of any new classification system at this point, in the course of describing seizures in this study, we have maintained the use of the older terms listed above.

1.1.2 Epilepsy and epilepsy syndromes

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure (Fisher et al, 2005). As a condition, epilepsy can be further divided into different epilepsy syndromes, many of which are markedly different in their clinical manifestations, severity and prognosis.

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An epilepsy syndrome is defined as an epileptic disorder characterised by a cluster of signs and symptoms customarily occurring together. These include such items as the type of seizure, aetiology, anatomy, precipitating factors, age of onset, severity, chronicity, diurnal and circadian cycling and sometimes prognosis (Engel, 2001; Berg et al, 2010). However, it should be noted that a specific epilepsy syndrome does not imply a common aetiology or prognosis (Wolf, 2006; Berg et al, 2010).

The most widely used classification scheme for epilepsy syndromes is that of the International League Against Epilepsy (ILAE). Again, this has undergone a revision in recent years. Prior to 2010, the classification of the epilepsy syndromes had been last fully updated in 1989 (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). This older system broadly divides epilepsy into generalised epilepsies and focal, also called localisation related (arising from one area and possibly spreading to others) epilepsies (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). In this classification, syndromes are felt to be symptomatic (secondary to a recognisable cause), idiopathic (presumed to be secondary to a genetic cause) or cryptogenic (of no clear cause but assumed to be secondary to a lesion or focus which has not been proven on investigation).

A brief description is given below of each of these broad classifications, as these terms are still in widespread use. The newer classification system will be presented in brief afterwards, as it is of relevance in discussing some of the specific syndromes mentioned in later chapters. The rationale for the change in definitions will be briefly discussed, including the potential implications for the definitions of phenotypes in our study.

1.1.2.1 Idiopathic generalised epilepsies

The idiopathic generalised epilepsies (IGE) are a group of epilepsy syndromes whose cause is felt to be purely or predominantly genetic (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). These epilepsies often have specific clinical features such as their age of onset, clinical course and specific seizure types or EEG findings associated with them (Duron et al,

2005). Imaging studies such as MRI are usually normal in individuals with this type of epilepsy (Duncan, 2005). Some of these syndromes are often associated with a good response to anti-epileptic medications (Szaflarski et al, 2010; Faught, 2004). Overall, these syndromes represent up to 40% of all epilepsy syndromes in certain populations, although this figure may be higher in the developed world (Duron et al, 2005).

Within the spectrum of IGE, there is significant variability in the syndromes seen. The spectrum ranges from juvenile myoclonic epilepsy (JME) (Alfradique and Vasconcelos, 2007) to age-specific syndromes such as benign neonatal familial seizures (Heron et al, 2004) to syndromes such as genetic epilepsy with febrile seizures plus (GEFS+) (Scheffer and Berkovic, 1997). A full description of each of these syndromes is beyond the scope of this study, but specific idiopathic generalised syndromes will be described further in later sections where they are relevant to a specific genetic aetiology.

1.1.2.2 Localisation related epilepsies

Localisation related epilepsies (LRE) (also termed focal epilepsies) form the majority of epilepsy syndromes. These are disorders in which seizure semiology or findings at investigation disclose a focal or localised origin for the seizures (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). These syndromes include those patients with small circumscribed constant epileptogenic lesions (such as anatomical lesions like tumours or mesial temporal sclerosis) (Menzler et al, 2011; Parvizi et al, 2011) but also those with less well-defined lesions or with particular genetic conditions such as autosomal dominant lateral temporal lobe epilepsy (Michelucci et al, 2009).

As a means of differentiating pathophysiology, the 1989 ILAE guidelines divided LRE into cases which are symptomatic, idiopathic or cryptogenic (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). In this case, idiopathic is used to indicate, not a genetic cause as in the idiopathic generalised epilepsies, but that the cause of the LRE is unknown and that no other cause is suspected. Symptomatic is a term used to describe LRE in which the cause is

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known and can be demonstrated on investigation, with a good example being the mesial temporal sclerosis which can be demonstrated on magnetic resonance imaging (MRI) in some cases of epilepsy arising from the temporal lobe. Cryptogenic, when used in LRE, denotes an epilepsy syndrome which is felt to be symptomatic but the cause of which cannot be demonstrated on investigation (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). This latter term in LRE can be difficult to clarify, and is a difficulty with this classification system.

1.1.2.3 Symptomatic generalised epilepsies

Symptomatic generalised epilepsies are a diverse group of epilepsy syndromes, many of which are associated with developmental delay or regression. They are felt to be secondary to a global dysfunction of brain development (Commission on Classification and Terminology of the International League Against Epilepsy, 1989), and are often associated with intellectual impairment. They are a diverse group of disorders, and can have clear causes such as tuberous sclerosis (Thiele, 2004) and other inherited conditions, or may be of unclear origin. Included in the category of symptomatic generalised epilepsies are the "epileptic encephalopathies", epilepsy syndromes associated with developmental regression after the onset of seizures (Engel, 2001; Berg et al, 2010). Examples of such syndromes include Lennox-Gastaut syndrome (Arzimanoglou et al, 2009). Investigations in these cases are often non-specific, but the EEG pattern seen with seizures in these syndromes usually shows generalised changes rather than focal ones.

1.1.3 Proposed new classification system for epilepsy

It has been proposed that the classification of epilepsies and of seizures presented above will be superseded by an updated version published in 2010 by the ILAE Commission. This updated classification was proposed to address a number of limitations noted in the older classification system, as well as developments in our understanding of the aetiology of various epilepsy syndromes (Berg et al, 2010).

Instead of the terms idiopathic, symptomatic and cryptogenic, under the proposed new system the terms genetic, structural / metabolic, and unknown would now be favoured for considering the aetiology of an epilepsy syndrome (Berg et al, 2010).

1.1.3.1 Genetic epilepsies

Genetic epilepsies are the direct result of a known or presumed genetic defect or defects, in which seizures are the core symptom of the disorder. Designation of the fundamental nature of the disorder as genetic does not exclude the possibility that environmental factors may contribute to the expression of the disease. The reason for the use of this term is the increasing recognition of genetic influences in the different types of epilepsy under the older classification, especially the localisation-related epilepsies.

1.1.3.2 Structural / metabolic epilepsies

Structural / metabolic epilepsies are secondary to a distinct structural or metabolic condition or disease which has been shown to be associated with a substantially increased risk of developing epilepsy. Such lesions can be obvious and acquired (such as neurological infections or traumatic brain injury) but can also be of genetic origin (such as tuberous sclerosis or Angelman syndrome). However, distinct from the genetic epilepsies, there is felt to be a separate disorder interposed between the genetic defect and the epilepsy.

1.1.3.3 Epilepsies of unknown aetiology

The category of epilepsies designated as unknown is those in which the cause of the epilepsy syndrome is unclear. This is to replace the older term cryptogenic, which was unclear and often felt to imply a cause without sufficient evidence.

1.1.3.4 Electroclinical syndromes and epilepsy constellations

The updated classification system also eliminates the previously recognised more general categories for epilepsy syndromes, i.e., the differentiation into idiopathic

generalised epilepsy, localisation related epilepsy and symptomatic generalised epilepsy. These have now been replaced by the concept of different electroclinical syndromes. An electroclinical syndrome is a complex of clinical features, signs and symptoms which together define a distinctive, recognisable clinical disorder. These are identifiable on the bases of age of onset, specific EEG characteristics, seizure types and other features (Berg et al, 2010).

Related to this is the concept of a constellation rather than a distinct electroclinical syndrome. Epileptic constellations are entities which do not fulfil exactly the criteria to be recognised as electroclinical syndromes, but which represent clinically distinct constellations on the basis of specific features or lesions. Examples include mesial temporal epilepsy with hippocampal sclerosis or gelastic epilepsy with hypothalamic hamartoma (Parvizi et al, 2011; Menzler et al, 2011). Although not meeting the current criteria for electroclinical syndromes, these entities are sufficiently distinct to be recognised as diagnostic entities in their own right.

1.1.4 Classification in clinical practice and conclusions

Both the older and newer classification ILAE classification systems have their limitations, something which the Commission readily acknowledge (Berg et al, 2010) and which others have commented upon (Gómez-Alonso and Bellas-Lamas, 2011). It should also be borne in mind that the newer classification system is designed as a scientific, rather than a clinical, classification and that the older 1989 classification system for epilepsy syndromes remains in widespread clinical use.

As the proposed new classification system for epilepsy syndromes has not yet been broadly accepted, for the classification of epilepsy syndromes for patients in this study, we have persisted with the use of the 1989 ILAE classification.

Before leaving this chapter, it should be noted that, in the classification of an epilepsy syndrome in the case of an individual patient –

1. Every attempt should be made to classify the patient's epilepsy into a specific syndrome or constellation, for diagnostic clarity and to guide therapy.

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2. It may not be possible to accurately classify a patient's epilepsy into a single syndrome (Serrano-Castro et al, 2001; Avanzini et al, 1996)
3. Similar epilepsy syndromes may have different aetiologies, and genetic factors may be implicated both in those epilepsies felt to be purely genetic and in those felt to be structural / metabolic in origin (Berg et al, 2010).

The definition of an epilepsy syndrome in an individual as part of a pedigree can have significant impact on the results of genetic studies. The classification of seizures or epilepsy syndromes in individuals in genetic studies is the process of phenotyping these individuals. It is this process which will dictate the relevance of the results of genetic testing, as accuracy of phenotyping is essential for interpreting these results. These difficulties have been mentioned by a number of commentators as a potential reason for failure to replicate studies (Greenberg and Suburan, 2011).

1.2 Epidemiology of epilepsy

In clinical medicine, the epidemiology of a disease may be defined as the study of the distribution, determinants and control of health-related states and events in populations. In this section, I hope to provide a brief overview of the epidemiology of epilepsy and also the overall impact the disease has on healthcare services.

Most of the studies to date on the epidemiology of epilepsy have reported on either the incidence or prevalence of the disease (Sander and Shorvon, 1987; Forsgen et al, 2005). The incidence of a disease is the measure of the rate of occurrence of new cases of a given disease per unit time, within a specified population. In contrast, the prevalence of the disease is the proportion of the population with a given disease at a specified time.

There are many studies of epilepsy prevalence reported in the literature, and the particular methods used or populations studied have varied greatly. Most papers focus either on defined populations (such as children, those with mental retardation or those aged over 65 years) (Forsgren at al, 1990; de la Court et al, 1996; Beilmann et al, 1999) or occasionally on countries or particular geographical regions as a whole (Rocca et al, 2001; Granieri et al, 1983; Linehan et al, 2010). Studies of the incidence of epilepsy are rarer in the literature. Such studies should ideally be prospective and are thus more difficult to perform accurately (Forsgren et al, 2005).

Because of the differences in methodologies and populations studied, direct comparisons between studies can be difficult to make. However, some comparisons can be made, based on the figures available from studies performed in numerous countries. It should also be noted that the incidence and prevalence of epilepsy has been broadly similar in similar populations when similar methodologies have been used (Sander and Shorvon, 1987; Forsgren et al, 2005).

1.2.1 Prevalence of epilepsy worldwide

Epilepsy is one of the most common neurological disorders. Worldwide, it has been estimated that approximately 50 million people have epilepsy (Duncan et al, 2006).

The lifetime prevalence of active epilepsy has been estimated at four per 1,000 members of the population, making epilepsy the third commonest neurological condition after stroke and transient ischaemic attack (Commission on Epidemiology and Prognosis, International League Against Epilepsy, 1993). The majority of people with epilepsy are felt to be resident in the resource-poor countries (Duncan et al, 2006), in part due to different environmental factors (Sander, 2003) which may be contribute to the aetiology of epilepsy. It is felt that the focal epilepsies are more common than the generalised epilepsies, accounting for approximately 70% of all cases of epilepsy (although this percentage can vary between populations).

1.2.2 European prevalence and incidence of epilepsy

When considering the prevalence and incidence of epilepsy in Europe, studies have been performed in a number of different European countries, and different methodologies have been used (Fosgren et al, 2005). The methodologies used have included analyses based on hospital records (Granieri et al, 1983), primary healthcare records (Crombie et al, 1960; Goodridge and Shrovon, 1983), recorded use of anti-epileptic drugs (AEDs) (Purcell et al, 2002; Giuliani et al, 1992) and analyses of certain subgroups by age, profession, other health conditions or availability (Forsgren et al, 1990; Tidman et al, 2003; Sangrador and Luaces, 1991).

In Europe as a whole, it is estimated that the prevalence of active epilepsy is 3.3 – 7.8 cases per 1,000 of the population. Active epilepsy in this sense is defined as any epileptic seizure of any type in the last 5 years. The overall prevalence of active epilepsy varied between age groups. There are 4.5 – 5.0 per 1,000 children and adolescents affected, 6 per 1,000 adults and 7 per 1,000 adults over 64 years. The estimated overall figure for the prevalence of active epilepsy in Europe is comparable to that in Australia (7.5 per 1,000) (Beran et al, 1985) and in the USA (6.8 per 1,000) (Hauser et al, 1991).

Incidence of new cases of epilepsy per year, again based on a review of multiple studies, has been estimated at 70 per 100,000 in children and adolescents, 30 per 100,000 adults and 100 per 100,000 in adults aged 65 years and older. The overall annual incidence rate in Europe is approximately 50 – 55 cases per 100,000

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population. Again, this figure is comparable to that in the USA of 52 per 100,000 population (Zarelli et al, 1999).

1.2.3 Irish prevalence of epilepsy

The most recent – and comprehensive – study on the prevalence of epilepsy in Ireland calculated that 38,000 people in Ireland have active epilepsy (Linehan et al, 2010). It is notable that this study is one of the few to address the issue of prevalence for a whole country. The lifetime prevalence of self-reported epilepsy in Ireland among adults was calculated at 10 per 1,000 population. For individuals aged 5 years and older, the prevalence of treated epilepsy was 9 per 1,000. Based on this study, the prevalence of epilepsy in Ireland is felt to be similar to that in Europe as a whole.

1.2.4 Response to therapy in epilepsy

In the population of people with epilepsy, there are differences in the response to therapy and in the course of the disease. Although estimates vary, it is widely accepted that approximately 60 – 70% of those with epilepsy will become free of seizures in the longer term (Schmidt, 2009; Duncan et al, 2006). This includes those who have had a remission of their seizures without active therapy and those who are well controlled on therapy. The exact figure for refractory epilepsy (epilepsy which does not respond to medication therapy) varies, as definitions can vary between studies (Schmidt, 2009) but figures as high as one-third of all patients with epilepsy have been found in different studies (Beleza, 2009; Schmidt, 2009).

1.2.5 Morbidty and mortality in epilepsy

There is a higher rate of mortality in those with epilepsy (Lhatoo et al, 2001) when compared to matched controls in the general population. This can be due to the underlying cause of the epilepsy (as is the case in symptomatic epilepsy from cerebral malignancies), to a poorer prognosis for those with epilepsy after certain other medical conditions (Janszky et al, 2009) or features more specific to the occurrence of seizures themselves (as is the case with sudden unexpected death in epilepsy (SUDEP)) (Duncan et al, 2006). There is also an appreciable morbidity

from such issues as injuries from seizures (Nonato and Borges, 2011; Asadi-Pooya et al, 2011) or the side effects of medications (Petty et al, 2007; Schmidt, 2009; Singh, 2011).

Those with active epilepsy are more likely to require intervention from multiple health care services, and face a higher rate of unemployment than matched controls or the general population (Holland et al, 2009).

1.2.6 Cost of epilepsy

The economic burden to healthcare systems of active epilepsy is considerable. Different figures for this burden have been advanced from different countries or regions. The reasons for these differences are principally to do with differences in study methodology, models of healthcare provision between regions, and the specific populations studied (i.e., all epilepsy versus refractory epilepsy, all patients versus specific groups, etc) (Strzelczky et al, 2008).

A previous study (Pugliatti et al, 2007) estimated the cost of epilepsy in Europe for the year 2004, and arrived at a figure of €15.5 billion, the majority of which was accounted for by indirect costs of the disease (such as sick leave from work). The same paper gives an estimated cost of epilepsy of €146 million for Ireland for that year. Different figures for the cost of treating an individual with epilepsy per year have also been published (Sancho et al, 2008; Beecham et al, 2010) indicating the significant impact of epilepsy on healthcare and social budgets.

1.2.7 Conclusions

There are numerous factors which contribute to the risk of developing epilepsy. Genetic factors, such as a family history of epilepsy, are one such factor (Lennox, 1951). Given the high prevalence of epilepsy, any investigation to identify the factors which can predict the development of the disease or which can clarify the appropriate therapy has the potential to avoid significant morbidity and possibly mortality for individuals at risk. Given the cost of treating epilepsy on the overall healthcare

budget and on loss of individuals to the workforce, then identifying these factors also has considerable potential for society as a whole.

1.3 Epilepsy as a genetic condition

The aetiology of epilepsy can vary greatly, and the predominant aetiological factor in any group affected will differ in the populations studied (Preux and Druet-Cabanac, 2005; Mac et al, 2007; Arroyo and Kramer, 2001). Genetic factors are known to be a risk factor for the development of epilepsy (Annegers et al, 1996). In this chapter, I hope to outline the evidence behind the concept of epilepsy as a genetic disease. In this way, I hope to demonstrate the importance of research into the genetic causes of epilepsy. Also, the discussion on the methods involved in genetic studies to date will have direct relevance in the discussion of the methodology of our study, i.e., the assessment of multiple family kindreds.

From when epilepsy began to be assessed as a medical condition, there have been references to the notion of epilepsy as a heritable condition. Even Hippocrates commented on cases where epilepsy appeared to be hereditary in origin (Riggs and Riggs, 2005). Indeed, the "heritability" of epilepsy was a source of the legal prohibition against marriage and in favour of the forced sterilisation of people with epilepsy in some countries (Kendregan, 1966; Garver and Garver, 1991). However, it is only from the middle of the twentieth century that well-conducted trials began to assess the extent of heritable / genetic factors in the development of epilepsy with the work of William Lennox (Lennox, 1951). In a study of 20,000 relatives of 4,231patients with epilepsy, he found that the prevalence of epilepsy in near relatives of those with epilepsy was significantly higher than that in the general population. He also found that the prevalence was higher in relatives of those with "essential" epilepsy (felt to be genetic in origin) when compared to the relatives of those with symptomatic epilepsy.

Since Lennox's initial work, a number of different study designs and clinical observations have been used to investigate the notion of a genetic aetiology for epilepsy. The principal study designs which have been used are –

- Clinical descriptions of multiplex families
- Familial aggregation studies
- Twin studies

- Animal models of epilepsy

In the following sections, I hope to expand on the advantages and limitations of each of these study designs in turn.

1.3.1 Clinical descriptions of multiplex affected families

Studies of this type have been used in many diseases to demonstrate the heritability of these conditions (Villano et al, 2009; Michel et al, 2001). Several studies have described the clinical manifestations of epilepsy in single families with well described seizure types and syndromes, and repeated findings in different families are leading to the development of newer epilepsy syndromes (Marini et al, 2004; Gourfinkel-An et al, 2004; Helbig et al, 2008). Indeed, these studies may be said to have an attraction for the clinician, as they reflect the types of families which are met in clinical practice.

1.3.1.1 Examples of studies of mutiplex families

In those cases where the mode of inheritance has fitted a clear Mendelian pattern, results of studies in these families have proven extremely useful in eliciting the underlying genetic cause of epilepsy for some of the specific epilepsy syndromes (Crompton et al, 2010; Di Bonaventura et al, 2011; Rosanoff and Ottman, 2008;Striano et al, 2009; Gourfinkel-An et al, 2004). Specific examples of conditions in which particular genes have been identified by further genetic studies on extended epilepsy pedigrees include GEFS+ (Scheffer and Berkovic, 1997), benign familial neonatal convulsions (Dedek et al, 2003) and autosomal dominant lateral temporal lobe epilepsy (Michelucci et al, 2009).

The applicability of the results of this type of study to the population as a whole is often unclear, as these Mendelian epilepsies are felt to account for only 1% of the total of all epilepsies (Kjelsden et al, 2001) and only a small number of patients with epilepsy have a family pedigree with a clear Mendelian pattern (Crino, 2007; Ottman et al, 1996).

1.3.1.2 Limitations of studies of multiplex families

The study of multiplex families certainly indicates that epilepsy can have a genetic aetiology, but in themselves such studies cannot tell us the nature of the genetic abnormality underlying the condition under study. For this, all such pedigees will need further study, and some authors have commented that the clinical syndromes identified by the study of such families should be verified by methods such as linkage analysis (Ottman, 2001), which are discussed in later sections.

The study of multiplex familes to demonstrate a genetic aetiology for epilepsy is limited in a number of ways. The status of individuals as affected by epilepsy or not can be difficult to clarify in these pedigrees. Due to the stigma which can still surround the diagnosis of epilepsy, there may be active concealment of family members with the disease, which can mask a possible inheritance pattern in a family and thus suggest a non-genetic aetiology for the epilepsy syndrome. Conversely, when attempting to classify individuals in extended pedigrees as being affected by a specific epilepsy syndrome or not, it can be difficult to avoid the bias which comes from knowledge of the proband's diagnosis. In this manner, individuals can be misclassified as having epilepsy and a genetic aetiology assumed where none exists.

These studies can demonstrate the co-occurrence of epilepsy syndromes of different types within families. However, this co-occurrence is difficult to interpret. Such different epilepsy syndromes can occur in the same family by chance, rather than directly due to a genetic abnormality (Ottman, 2001; Gourfinkel-An et al, 2004). A simple example would be the sibling of a patient with idiopathic generalised epilepsy developing epilepsy after meningitis in early life. The possibility of random chance occurrence is difficult to exclude in studies of this type and can confound the analysis of common conditions, such as epilepsy (Gourfinkel-An et al, 2004).

It should be noted that findings from these studies may not be applicable to the population as a whole. As mentioned above (see section 1.3.i.a), these large pedigrees are seen in only a small minority of those with epilepsy. Given this, whether the findings of particular syndromes or supposed modes of inheritance in these families can be applied to the general epilepsy population is not clear.

1.3.2 Familial aggregation studies

These studies use an epidemiological approach to assess the degree of increased risk in relatives of individuals with epilepsy (Ottman, 2001). It is important to emphasise that in these studies, it is more useful to consider the epidemiological sense of familial aggregation (that there is a greater frequency of disease in close relatives with the disease than in relatives of those without the disease) rather than the more usual familial clustering in the clinical sense (extended families that happen to have multiple cases of a disease or syndrome of interest). Usually in these studies, the prevalence of epilepsy is compared between certain individuals related to an individual with epilepsy (such as siblings or second-degree relatives) to individuals in the general population. If the trait under investigation is one which can be termed a binary trait (i.e., that a patient either has the disease in question or not, as is the case with epilepsy), the ratio of these prevalence rates is the recurrence risk ratio (Ottman 2001; Helbig et al, 2008).

These studies have been used to demonstrate an increased risk of epilepsy in first degree relatives of patients with both localisation related and idiopathic generalised epilepsy (Bianchi et al, 2003; Annegers et al, 1982; Ottman et al, 1991; Ottman et al, 1998; Hemminki et al, 2006), with the risk for epilepsy in first degree relatives being two- to four-fold that of the general population (Annegers et al, 1982; Ottman et al, 1996). These results would indicate a genetic factor as a possible causative mechanism behind such an increased risk.

1.3.2.1 Limitations of familial aggregation studies

One of the principle limitations of these studies is that they cannot distinguish between genetic and non-genetic causes for the aggregation seen in families (Ottman, 2001). Clustering of a disease within a family can occur from non-genetic causes such as shared exposure to an environmental risk or shared behaviours which put the family members at risk for the disease.

Earlier familial aggregation studies had errors in methodology such as the failure to include a control group and selection bias of the included probands (Ottman, 2001).

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Both of these factors can give rise to a misleading ascertainment bias. As with many other forms of earlier genetic study, some of these studies failed to clarify adequately definitions of affection status (e.g., occurrence of any seizure as opposed to a recognised epilepsy syndrome or particular endophenotype) (Ottman et al, 1998; Bianchi et al, 2003). Failure to adequately define the phenotype of the disease under investigation can lead to false positive or false negative results in many different study designs, and earlier aggregation studies were no exception to this.

Some familial aggregation studies have failed to control for the confounding effect of the cause of the epilepsy in the proband (Ottman, 2001). This is particularly relevant in the study of focal epilepsies. Focal epilepsy frequently results from identified acquired insults to the central nervous system, and the genetic contribution to the aetiology of the epilepsy syndrome in such individuals is much smaller. Inclusion of such individuals as probands in familial studies would make it more difficult to identify a genetic contribution.

1.3.2.2 Examples of familial aggregation studies

Familial aggregation studies have been performed in a number of different populations from different countries (Hemminki et al, 2006; Ottman et al, 1989; Bianchi et al, 2003; Jain et al, 2004). They have also been used to assess specific aspects of epilepsy syndromes, such as seizure types within families (Winawer et al, 2005; Winawer et al, 2003) or epilepsy severity (Ottman et al, 1991).

In contrast to older studies, more recent studies have tried to control for the limitations mentioned above, and the results have shown a higher rate of epilepsy in first degree relatives of probands (Annegers et al, 1982; Hemminki et al, 2006; Bianchi et al, 2003). Some of these studies have also shown a higher risk recurrence rate for specific subtypes of epilepsy within first-degree relatives of probands (Bianchi et al, 2003; Ottman et al, 1998), where others have studied other aspects such as the severity of epilepsy (Ottman et al, 1991) Aggregation studies have been largely consistent in demonstrating a higher recurrence risk in first-degree relatives of patients with epilepsy, with a greater risk for those with idiopathic generalised epilepsy than localisation-related. However, the degree of this increased risk and

whether this is common to all epilepsy syndromes or specific to the epilepsy subtype has been reported differently in different studies (Winawer et al, 2003; Ottman et al, 1998; Ottman et al, 1989; Berkovic et al, 1998).

1.3.3 Twin studies

These have been used in a number of conditions as a means of ascertaining the degree to which hereditable factors are responsible for a particular disease (McGregor et al, 2000; Kjelsden et al, 2001). Twin studies usually compare monozygotic and dizygotic twins with each other to see if the concordance rates differ between the different types of twins. The idea behind such studies is to avoid the difficulty which familial aggregation studies have in differentiating between genetic and environmental causes of the results found.

For these studies, it is assumed that monozygotic twins are genetically identical. Each twin pair should have been exposed to the same environment. For both monozygotic and dizygotic pairs, the concordance rate is calculated. Concordance refers to the presence of the disease in both twins (Kjelsden et al, 2001). The differences in disease concordance between the different types of twins (monozygotic versus dizygotic) should be due to genetic factors only (Kjelsden et al, 2001; Hawkes, 1997). In a disease with a genetic contribution, the concordance rate will be higher in monozygotic twins than in dizygotic (Johnson and Sander, 2001).

Twin studies, as well as eliminating the potential difficulties of environmental factors seen in familial aggregation studies, also allow for significant results to be achieved with smaller sample sizes. Consistent and detailed phenotyping is easier in such smaller sample sizes.

1.3.3.1 Limitations of twin studies

As in any study model, there are problems associates with twin studies. There is the difficulty that twin studies may not be representative of the general population. They are also prone to specific ascertainment biases (Bundey, 1991). Earlier reports of twin studies also tended to be unrepresentative, being more case studies than

population-based studies. There are also likely to be unappreciated biases, such as different environmental factors applied to twins included in studies (e.g., dizygotic twins of different gender) (Greenberg and Pal, 2007; Evans et al, 2002), bearing in mind that one of the principal assumptions of twin studies is that the twins share the same environment. Interactions between genetic and environmental factors may also give rise to difficulties in this type of study, which makes the assumption that such factors are independent of each other (Greenberg and Pal, 2007; Evans et al, 2002).

1.3.3.2 Examples of twin studies

Population-based twin studies have had a major role to play in the study of epilepsy genetics. Studies have been successful in demonstrating the relative contribution of genetic factors in the aetiology of epilepsy, both of the generalised and focal epilepsies (Lennox, 1951; Berkovic et al, 1998). The concordance rate has been found to be consistently higher in monozygotic twins than in dizygotic, in a number of different populations (Kjelsden et al, 2001; Berkovic et al, 1998; Kjelsden et al, 2003). Studies have also shown a high concordance rate for specific epilepsy syndromes in monozygotic twin pairs (Berkovic et al, 2006; Kjelsden et al, 2003; Lennox, 1951), suggesting the influence of distinct genetic traits for these syndromes rather than more general susceptibility traits to epilepsy.

Specific examples of twin studies used in the study of epilepsy overall and different epilepsy syndromes include the original work of William Lennox in the mid-twentieth century (Lennox, 1951). The high concordance rate found in his work in monozygotic twins was strongly supportive of a genetic factor in the aetiology of epilepsy. Later re-assessment of Lennox's study (Vadlumadi et al, 2004) demonstrated that these findings are reliable even using the current classification system of epilepsy syndromes. More recent studies have demonstrated similar findings in different populations (Berkovic et al, 1998; Kjelsden et al, 2003; Miller et al, 1999; Kjelsden et al, 2005; Sharma, 2005), again showing a higher concordance rate amongst monozygotic twins. Such studies have also been used to indicate possible genetic factors at work in other aspects of epilepsy, such the occurrence of status epilepticus (Corey et al, 1998) and overall outcome (Johnson et al, 2003). The

have also been used to assess the genetic aetiology of different specific epilepsy syndromes (Berkovic et al, 1996; Vadlamudi et al, 2006).

Overall, twin studies in epilepsy have suggested a heritability of 70 – 80% for the condition (Berkovic et al, 1998; Kjelsden et al, 2003). The heritability of a phenotypic trait is defined as the proportion of variation in the phenotype which can be attributed to genetic effects (Wilson, 2008). A greater value for the heritability of a trait indicates that genetic factors are more important in the development of that trait rather than environmental ones.

1.3.4 Animal models

The use of animal models of disease states is a well-established tool in research for the study of various diseases. These in vivo models (as opposed to in vitro models such as cell lines or brain slices) are generally considered the best experimental model for the study of disease as they should reproduce the kind of conditions and complexity seen in human disease (Mantegazza et al, 2010).

1.3.4.1 Phenotype-driven animal models

Several different species of animal can present with convulsive behaviour or with decreased threshold of seizure induction (Rubio et al, 2010; Löscher, 2011). Thus, such animals are spontaneous models of epilepsy. These spontaneous models can be used as models of human epilepsy in a manner which is referred to as a phenotype-driven approach (Mantegazza et al, 2010). The animals in question are observed for a particular phenotype (such as the occurrence of convulsions) without prior knowledge of the genetic basis of the mutation responsible for the phenotype. These animals are usually inbred strains and their phenotype is usually homogenous and well described. The most commonly used species are rodents, due to their small size, ease of maintenance and well characterised phenotypes.

Phenotypes can also be generated by strategies such as chemical mutagenesis (Mantegazza et al, 2010). In this animal model, certain substances are used to induce random mutations in rodents. These rodents are then screened for certain phenotypes,

such as the occurrence of seizures, and mutations are then sought in these selected rodent lines. Chemicals which can be used for this approach include ethylating agents such as N-ethyl-N-nitrosurea, a potent mutagen. This approach is more useful for the identification of possible candidate genes for further study.

Both of the above approaches are also termed phenotype-driven, as the animals for study are chosen on the basis of an observed phenotype.

1.3.4.2 Transgenic and gene-targeted animal models

Transgenic models are generated by introducing additional copies of a gene into the genome of the animal (Ristevski, 2005). Although this model does not reproduce the pathophysiology of conditions as the gene targeted model, it does have a number of advantages. Transgenic models are relatively easy to generate and relatively inexpensive, allowing for small-scale screening of mutations.

The alternative method to generating transgenic models is the use of gene targeted models. In this method, a gene is replaced with a completely inactive version of the same gene ("knock out" model) or one carrying a missense mutation ("knock in" model) (Capecchi, 1989). The mouse models resulting from this alteration are accurate reproductions of the genetics of the human disease under study, and can be used to study the pathogenic mechanisms and phenotypes in animals in which a human mutation has been accurately reproduced. This approach is time-consuming and expensive (Collins et al, 2007), and also faces the same challenges of the phenotype-driven approaches in relating the animal pathophysiology to the human phenotype (Mantegazza et al, 2010).

1.3.4.3 Examples of animal models of epilepsy

In spite of the limitations of these studies, in vivo models have been of considerable benefit in the study of the genetic aspects of human epilepsy. Transgenic mouse models have been used as models for GEFS+ (Tang et al, 2009), benign familial neonatal convulsions (Peters et al, 2005) and autosomal dominant nocturnal frontal lobe epilepsy (Zhu et al, 2008). Knock-in and knock-out mice have been generated as

models of GEFS+ (Martin et al, 2010), autosomal dominant nocturnal frontal lobe epilepsy (Teper et al, 2007) and childhood absence epilepsy with febrile seizures (Chiu et al, 2008). Well-recognised phenotype-driven models used in the study of human epilepsy include the GAERS (genetic absence epilepsy rat from Strasbourg) rat (Powell et al, 2009), the WAG (Wister Albino Glaxo) / Rij rat (van Luijtelaar and Coenen, 1986) and different ENU induced mutation models (Papale et al, 2009; Mashimo et al, 2010).

1.3.5 Conclusions

Based on these study types, there is clear evidence of a genetic contribution to the aetiology of epilepsy across a broad range of epilepsy syndromes. Estimates of the extent to which epilepsy is genetic in origin vary in the literature, and can be different based on the methodology used, the populations studied and the epilepsy type or syndrome studied. Mendelian epilepsy syndromes are estimated to account for about 1% of all cases of epilepsy (Kjelsden et al, 2001), but idiopathic generalised epilepsies (which are felt to be largely or wholly genetic in origin) account for about 30% - 40% of all epilepsy (Duron et al, 2005; Berkovic et al, 2006) and some papers have estimated a genetic contribution to the aetiology of epilepsy in 40% of all cases of epilepsy (Gardiner, 2000).

1.4 Genetic analysis techniques

There are a number of different techniques used to identify particular genes or genetic loci in epilepsy. In this section I hope to give broad outlines of the methods of greatest relevance to this study. The four categories I hope to discuss are –

- Linkage analysis
- Association analysis
- Genome wide association studies
- Next generation sequencing and whole exome sequencing

1.4.1 Linkage analysis

Linkage analysis is usually the first stage in the process of positional cloning. This type of analysis aims to identify a particular chromosomal region associated with a particular phenotype under study (Pulst, 1999). As such, it is often the first stage in the genetic analysis of particular conditions, as it can direct further studies to particular sites of interest (Dawn Teare and Barrett, 2005).

1.4.1.1 Linkage and recombination

The usual human cell contains 23 homologous pairs of chromosomes (22 autosomal chromosomes and one pair of sex chromosomes). Thus, it is termed diploid. However, in the process of meiosis, reproductive cells called gametes are formed which are haploid, i.e., contain only one member of each homologous pair (Olson et al, 1999). In reproduction, the fusion of these gametes (one from each parent) allows for the formation of offspring with diploid cells containing genetic material from each parent. In this process, different genetic loci are transmitted from parent to offspring. Two genetic loci on a chromosome are described as being linked if they are transmitted together from parent to offspring more often than would be expected than independent segregation (Schulz and McMahon, 2003; Dawn Teare and Barrett, 2005).

During meiosis, the pair of chromosomes being separated into different gametes can crossover and exchange material at certain points along the chromosomes before being finally separated. This process of the exchange of material is called recombination (Burton et al, 2005; Williams et al, 2011). Recombination is a process which can break down the association between particular alleles on a chromosome, leading to different genetic loci being transmitted to the offspring from the parent. If we define linkage in light of this, we can say that two loci are linked if the probability of recombination between the two loci is less than 50% (Schulz and McMahon, 2003; Dawn Teare and Barrett, 2005).

The extent to which genetic loci are linked at meiosis is expressed as the recombination fraction, which is a measure of the probability of recombination occurring between the loci (Burton et al, 2005; Williams et al, 2011). This fraction is zero for two genes which are perfectly linked (i.e., no recombination is possible between the two loci), and is 0.5 for two genes which are completely unlinked (i.e., which have independent segregation).

1.4.1.2 Linkage disequilibrium

The term linkage disequilibrium is one which was coined by population geneticists. It is used to describe the degree to which alleles of one SNP are correlated to an allele of another in a population. As a term, it refers to the association between alleles within and across populations of 'unrelated' individuals rather than within particular pedigrees (Olson et al, 1999; Schulze and McMahon, 2003). Two loci are felt to be in linkage disequilibrium if, across the population as a whole, they are found together on the same haplotype more often than would be expected than by chance alone (Olson et al, 1999; Dawn Teare and Barrett, 2005). When the term linkage disequilibrium is used in this way, the qualifying term chromosomal linkage can be used to differentiate it from linkage as described above. In contrast to linkage disequilibrium, chromosomal linkage may be relevant to particular families, but may not show a strong enough association at a population level. It should be noted that chromosomal linkage can extend over much larger regions of the genome than linkage disequilibrium, which often involves loci which are much more closely related (Dawn Teare and Barrett, 2005).

Although these different terms are used in the literature, it should be borne in mind that there is no real difference between chromosomal linkage and linkage disequilibrium. The terms refer to the same phenomenon.

1.4.1.3 Linkage analysis – model-based and model-free

Linkage analysis studies can make use of this phenomenon of linkage in the study of genetic causes of human disease (Palmer and Cardon, 2005; Botstein and Risch, 2003). The goal of such studies is to identify a marker – in these cases, a particular allele – which consistently segregates, i.e., is linked, with a disease or phenotype of interest (Pal et al, 2008). Because of the tendency for shorter haplotypes to be passed on intact without recombination at meiosis, if such a marker can be identified then it suggests that a gene with a functional effect is close to it (Dawn Teare and Barrett, 2005; Schork et al, 2007). The implication of this is that, once linkage has been established between the marker in question and the phenotpype of interest, then sequencing the region in which the marker is found may reveal the potentially disease-causing variation. The most commonly used markers in such studies at the moment are single nucleotide polymorphisms (SNPs) (Weiss and Clark, 2002), although other markers such as microsatellites have also been used (Botstein and Risch, 2003).

The phenotypes studied by linkage analysis can be a disease itself (such as epilepsy or particular epilepsy syndromes, i.e., juvenile myoclonic epilepsy) (Weissbecker et al, 1991; Pinto et al, 2004) or a particular endophenotype of that disease (such as a characteristic EEG pattern or MRI finding) (Rommelse et al, 2008). Linkage studies have been employed in more traditional genetic studies (analysis of pedigrees, analysis of candidate genes in certain conditions) (Ratnapriya et al, 2010; Siren et al, 2010; Michelucci et al, 2008; Izzi et al, 2003) and also as part of larger cohort studies of families drawn from specific populations (EPICURE consortium et al, 2012).

There are a number of different modes of inheritance of genetic traits. Two approaches to linkage analysis have developed to account for Mendelian and non-

Mendelian segregation / inheritance patterns in genetic analysis. These are the model-based (or parametric) and model-free (or nonparametric) approaches.

Parametric linkage analysis studies may be thought of as the analysis of co-segregation of loci in pedigrees. This form of linkage analysis requires prior specification of a genetic model for the trait being studied (Olson et al, 1999; Dawn Teare and Barrett, 2005). For a simple Mendelian disease, this genetic model amounts to a mode of inheritance and frequency of disease allele. The parameters must be chose before the particular linkage analysis, and are preferably based on previous population studies of the disease.

From parametric linkage analyses, the degree of linkage is often reported as a LOD (logarithm of the odds) score (Chotai, 1984; Ott, 2001). This is a function of the recombination fraction or of the chromosomal location, and is different depending on which value of the recombination fraction is being considered. The greater the positive value for the LOD score, the greater the strength in favour of linkage, with the converse being true for negative values. A LOD score of +3 is significant for evidence of linkage (being equivalent to a p-value in normal statistical analysis of 0.0001), with values of -2 being sufficient to reject linkage between any of the loci tested and the phenotype in question (Chotai, 1984). The usual practice currently in such studies is to calculate LOD scores for different values of the recombination fraction and to reject all such values of -2 or less (Dawn Teare and Barrett, 2005).

Nonparametric linkage analysis does not assume a specific mode of inheritance in analysis (Dawn Teare and Barrett, 2005). As a result, it is often more useful in cases when the genetic model cannot be specified (i.e., when the mode of inheritance is non-Mendelian) (Schulze and McMahon, 2003) or where the disease is felt to be multifactorial. These methods rely more on the sampling of relatives or family members, as it would be expected that, between these relatives, there would be an excess sharing of haplotypes which are identical by descent (IBD) in the region of the gene or genes of interest (Olson et al, 1999). This excess sharing would be expected to occur regardless of the actual mode of inheritance within that family. In nonparametric analysis, statistical methods are then used to test whether the sharing of such IBD regions in individuals who are affected by the disease or trait under

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study is greater than would be expected if there were no linkage between these regions (Dawn Teare and Barrett, 2005).

A number of different models of sampling have been used in such nonparametric studies. Affected sibling pairs have been used, which rely on the recruitment of numerous pairs of siblings affected by the condition of interest (Olson et al, 1999). If there is no linkage (the null hypothesis for such studies), then at any given locus, the number of identical by descent alleles which are shared between the siblings is none (probability of 25%), one (probability of 50%) or two (probability of 25%) (Burton et al, 2005; Dawn Teare and Barrett, 2005). Linkage is suggested by significant deviation from these probabilities, i.e., if the pair share significantly more alleles than would be suggested.

These studies have been expanded beyond sibling pairs, to allow for the recruitment of multiple siblings or whole families. However, certain corrections must be introduced to allow for the different probabilities of inheriting IBD alleles in different degrees of relationship (Whittemore and Halpern, 1994).

1.4.1.4 Limitations and confounders in linkage analysis

Linkage analyses – both parametric and nonparametric – are powerful tools in the analysis of conditions with a genetic aetiology, such as epilepsy, but they have a number of limitations. This has been evident in the failure to replicate many of the findings between groups and in different populations. These limitations may be in the methods of the analyses themselves or they may be factors which influence linkage.

In linkage analyses, the definition of phenotype is crucial (Greenberg and Pal, 2007; Dawn Teare and Barrett, 2005). Any lack of clarity in the definition of the phenotype will lead to difficulties in the interpretation of results and failure of replication due to the inappropriate inclusion or exclusion of individuals from the analysis. Similarly, phenocopies can confound analysis (Schork et al, 2007).

The statistical analysis of the results of linkage analysis i.e., the calculation of a LOD score and the level of significance attached to it in each particular case, is something

which has been of particular interest as studies have moved from sibling pair models to genome wide analysis. The usual level of statistical significance is felt to be achieved with a LOD score of +3 or greater. However, there are theoretical calculations which show that this may be too lenient. There are also models drawn from practical examples which show that, in particular cases, this may be too stringent (Landers and Kruglyak, 1995). There are corrections which can be applied to attempt to correct for this, such as the use of simulation (Dawn Teare and Barrett, 2005), but such issues may also account in part for the failure of replication of results between groups.

Although extremely useful, linkage analysis allows for the identification of cosegregating genetic loci, but not for the identification of specific genes (Williams et al, 2011). In monogenic diseases with a simple Mendelian inheritance pattern, linkage analysis has shown itself to be extremely useful (Altmüller et al, 2001; Botstein and Risch, 2003). However, in a condition such as epilepsy, the multiple genetic variants felt to be responsible are more likely to be higher in frequency and incompletely penetrant. The identification of such variants requires genotyping at a resolution which is beyond that of linkage analysis. For the analysis of the genetic causes of such diseases, other approaches must be used.

1.4.2 Association studies

Family-based linkage analysis has been successful in identifying genes of large effect in Mendelian diseases. However, such an approach has a low power for the detection of relevant genes in complex disorders (Pearson and Manolio, 2008), and for diseases with complex inheritance patterns, linkage approaches have generally been unsuccessful (Altmüller et al, 2001). A different approach to search for genetic abnormalities in such diseases is provided for by genetic association studies.

In epidemiology, the question which is often asked is whether or not exposure to a particular factor (such as a toxin, water source or other environmental feature) has a statistically significant association with a particular disease or trait. In genetic epidemiology, genetic association studies ask an equivalent question (Burton et al, 2005). The goal of an association study is to see if there is a statistical relation

between genomic variation at one or more sites and phenotypic variation within a certain population (Hattersley and McCarthy, 2005), and such association studies are designed to look for differences in the frequencies of specific alleles between patients with the disease or trait of interest and others in the population who do not have this disease or trait (Williams et al, 2011; Abou-Sleiman et al, 2006).

Association studies are different from the linkage studies described above in a number of important and fundamental ways. In association studies, as they focus on population frequencies, the goal is demonstrate if an allele is associated with (or enriched in) a disease or trait. Linkage analysis allows for different alleles of large effect to be associated with the same trait in different families (Cordell and Clayton, 2005). Linkage studies (especially in the sense of chromosomal linkage decribed above) are usually based on pedigrees, but association studies are usually based on populations of unrelated individuals.

Association studies have greater power than linkage studies to detect small effects (Williams et al, 2011) and can detect an allele which has a more indirect effect on disease expression (Greenberg and Pal, 2007). Although association studies need to examine more markers as part of the analysis than linkage analysis, when applied at genome-wide level they have a correspondingly greater resolution in terms of mapping the actual causal variant. Linkage analysis of complex diseases typically points to large genomic regions, typically between two and twenty centimorgans in size (Abou-Sleiman et al, 2006; Dawn Teare and Barrett, 2005; Greenberg and Pal, 2007) (a centimorgan is approximately 1 million base pairs in length), which may harbour the gene of interest. By contrast, association analysis can identify a region which is either the gene itself or one which is in linkage disequilibrium with the gene. Given the density of markers used in association studies, such a region is likely to be within a few tens of kilobases of the area identified (Greenberg and Pal, 2007).

1.4.2.1 Direct association

Association analyses can be broadly classified as studying direct or indirect association (Cordell and Clayton, 2005). Tests of association are termed direct when analysing a potentially functional genetic variant. In these, the target of genetic

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analysis – a particular polymorphism – is felt to be a possible causal variant, i.e., one which occurs within a coding region of the DNA sequence and which can lead to an amino acid change. There are different classes of genetic polymorphism which can be studied, such as microsatellites (Botstein and Risch, 2003), but the commonest used variant in both direct and indirect association studies is a single nucleotide polymorphism (SNP) (Greenberg and Pal, 2007).

The choice of which genetic variant to study in direct association tests is critical and it requires the specification of a candidate gene or genes for analysis. Assessing candidacy is "a notoriously imprecise art" (Hattersley and McCarthy, 2005). However, there are a number of factors which may indicate that a particular gene may be an appropriate target for assessment. The protein encoded by the gene may be known to be involved in the biological pathway underlying the disease of interest. There may be information from animal models implicating the gene in the aetiology of the disease. Previous linkage or association studies may have identified the locus containing the gene in individuals with the disease. Finally, there may be previous studies demonstrating an association between the gene and the disease of interest (Hattersley and McCarthy, 2005).

The particular variant chosen for direct association analysis can be any polymorphism which is a potential causal variant (Cordell and Clayton, 2005). The choice of variant for study is not restricted to coding variants and is based on the assumed functional effect of the variant. If a direct association is demonstrated between the variant and the disease in question, it is a powerful indicator that that specific variant is directly involved in the aetiology of the disease. Although direct association studies have considerable power, their usefulness is limited. Many mutations which affect the heritability of a condition may lie in non-coding regions, and these cannot be predicted with sufficient accuracy for use in traditional direct association analysis. Thus, this approach only has the potential to discover some of the genetic causes of disease (Cordell and Clayton, 2005). Some authors (Greenberg and Stewart, 2012; Greenberg and Subaran, 2011) have pointed to the assumptions made about loci in these studies as a further difficulty with this approach. In specifying a candidate locus for analysis, the choice of such a locus is based on the information available. An example would be the choice of a locus associated with the

function or structure of an ion channel when studying epilepsy. However, assuming such loci are associated with the trait under investigation may be inaccurate due to incomplete information. This becomes especially problematic when positive results are used to justify the initial assumption in the absence of other data.

1.4.2.2 Indirect association

Indirect association analysis assesses the associations with the disease or trait of a genetic polymorphism which is a surrogate for the real causal locus (Cordell and Clayton, 2005). This approach relies on the polymorphism being assessed being in linkage disequilibrium (LD) with the true causal variant (Greenberg and Pal, 2007; Palmer and Cardon, 2005). In contrast to direct association analysis, no assumption is made about the polymorphism studied – demonstrating an association between it and the disease of interest does not implicate the polymorphism in the aetiology of the disease, merely that it is in LD with the true causal variant. Previously, this indirect association approach was often used to investigate candidates in a region previously shown to be linked with the disease of interest by linkage analysis (Greenberg and Pal, 2007; Abou-Sleiman et al, 2006). However, with improvements in SNP mapping and haplotype definition, indirect association testing is now more commonly used in genome-wide association studies. Indirect association analysis allows for finer mapping than conventional linkage analysis (Greenberg and Pal, 2007).

It should be noted that analysis does not have to be limited to one particular candidate gene, or to only one genetic variant within that gene, in association analysis, and studies have been published which have assessed both single and multiple variants in candidate genes (Cavalleri et al, 2007; Cavalleri et al, 2007; Tan et al, 2004).

1.4.2.3 Study designs in genetic association studies

The issue of optimum study design for association studies of either type is one which has been much debated. In deciding which design to use, an investigator must take account of a number of factors including practical matters such as study budget, the

ease of recruitment of patients and controls, etc. The use of prospectively recruited cohorts remains the "gold standard" (Hattersley and McCarthy, 2005), but it suffers from a number of significant limitations. The time and expense of recruiting and following individuals in such a cohort make these studies prohibitive, and the loss of patients on follow-up can limit the information from such studies (Cordell and Clayton, 2005). Unless the cohort recruited is extremely large, or the disease under investigation very common, it is likely that the number of affected individuals in the cohort will be relatively small, thus limiting the power of the study both for gene discovery and for statistical significance (Hattersley and McCarthy, 2005).

Principally because of the issue of difficulties in recruitment and long-term follow-up of cohorts, one of the most commonly performed methods has been the use of a case-control study design (Hattersley and McCarthy, 2005). Cases (those individuals with the disease or trait of interest) and controls (who are unaffected) are analysed for the presence of particular markers at certain alleles, and a statistically significant difference indicates the presence of a disease-causing variant at or around the sampled polymorphisms.

Other forms of design are more specific for genetic studies, and include analysis of case-parent triads, case-parent-grandparent septets, particular family pedigrees and the assessment of isolated cases only (Cordell and Clayton, 2005; Hopper et al, 2005). All of the family-based designs have been proposed largely in an attempt to overcome issues with population stratification (Schulze and McMahon, 2003), as the unaffected family members act as matched controls for the affected individuals (having been exposed to the same environmental factors). Depending on the inheritance pattern of the allele under assessment, selection of individuals with a family history may also decrease the number of individuals required for inclusion in a study (Teng and Risch, 1999; Antoniou and Easton, 2003). Although useful, in association analysis such designs are difficult to power properly, given the difficulties in recruiting enough affected families (Cordell and Clayton, 2005; Williams et al, 2011). Other authors have commented that the recruitment of pedigrees with multiple affected individuals is susceptible to ascertainment bias (Ottman, 2001).

1.4.2.4 Limitations and confounders in association studies

Association studies must, in their design, take account of a number of factors, all of which can limit the accuracy of their results. Many of these factors are also seen as confounding the results of other forms of genetic study, and some are mentioned above as possible sources of error for linkage analysis (see section 1.4.1 above).

The differences between different populations are a factor which may account for some of the difficulty in replicating the results of linkage analyses (Pal et al, 2008). The difference in linkage for particular genes in different populations is termed population heterogeneity (Palmer and Cardon, 2005) or population structure (Abou-Sleiman et al, 2006). Allele frequencies can vary widely between different populations, and there is likely to be a high degree of linkage disequilibrium between populations of different origins. Within populations, there may be population stratification (Abou-Sleiman et al, 2006), whereby the frequency of certain alleles varies across subpopulations. Population structure / stratification can give rise to both false positive and false negative results (Abou-Sleiman et al, 2006). To avoid this, approaches such as genomic control and the use of particular genetic markers to identify individuals from populations of different genetic background can be applied (Abou-Sleiman et al, 2006). Population stratification is also an issue for genome wide association studies (see section 1.4.3.2 below).

The accuracy of case ascertainment and phenotype definition are paramount, as heterogeneity in cases will lessen the power of the study. The notion of a phenotype, and the difficulties inherent in defining it, has already been discussed (see sections 1.1.4 and 1.4.1.4 above) (Greenberg and Pal, 2007; Teare and Barrett, 2005).

Sample size calculations are usually carried out prior to starting recruiting individuals for any study, as this has a direct effect on the power of a study to identify a significant association (Ioannidis et al, 2003; Hattersley and McCarthy, 2005). Many early association studies were underpowered due to inadequate numbers recruited. Given that the effect of many of the genetic variants assessed is assumed to be modest, association studies require large numbers for adequate power. Association studies are prone to difficulties with sample size, as large numbers

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would be needed to ensure adequate statistical power for these studies. Indeed, although an assumption is usually made that the SNPs of interest for such conditions as epilepsy and other disorders occur with a frequency of 1 % or more in the population as a whole, it is also possible that there are many more rare alleles occurring with a much lower frequency which are responsible for such diseases. If this is the case, then the sample size required for adequate statistical power becomes huge. Although this is less problematic in family studies, it is a major issue when using genome wide analysis (Altmüller et al, 2001).

Although the quality and accuracy of genotyping has been mooted as another possible cause for error in these studies (Abou Sleiman et al, 2006), improvements in technology have made this much less of a concern than in older studies. Many studies incorporate specific reviews and protocols to detect and control against any possible genotyping error.

Specifically in relation to the study of epilepsy, association studies (especially of candidate genes) have been affected by a lack of replication (Helbig et al, 2008). There are a number of unknown variables which can affect this approach, such as the numbers of susceptibility genes involved and the size of the effect of each of these genes (Helbig et al, 2008). Given this lack of replication, a different approach was felt to be required, which was less reliant on the difficulties inherent in identifying appropriate candidates. With improvements in SNP mapping and haplotype identification, genome-wide association studies were the next method used to elucidate the genetic basis for many common diseases with complex genetics.

1.4.3 Genome-wide association studies (GWAS)

1.4.3.1 Definition of GWAS

A GWAS is defined by the National Institute for Health in the United States as a study of common genetic variations across the entire genome designed to identify genetic associations with observable traits (Pearson and Manolio, 2008). Such genome wide studies have been made possible by a number of developments.

The sequencing of the human genome (Sachidanandam et al, International SNP Map Working Group, 2001) and later work to identify reliable SNP variants in different populations (Durbin et al, 2010) and reliable mapping of haplotypes in humans (International HapMap Consortium, 2005) have greatly increased what is known of genetic variation and the frequency of certain SNP variants at particular loci. GWAS rely on the fact that genetic variance at one locus can predict with high probability variance at an adjacent locus (Pearson and Manolio, 2008; Mullen et al, 2009). The markers of genetic variance in these studies are referred to as tag SNPs, and they are in linkage disequilibrium with adjacent SNPs. If information is known about a tag SNP which is representative of a particular block of alleles or SNPs which are in linkage disequilibrium (i.e., a haplotype), then an idea of total genomic variation can be obtained if information is gathered on all such tag SNPs (Altshuler et al, 2008; Mullen et al, 2009). Given this haplotypic nature of the human genome, it follows that it is possible to survey the entire genome for common variability by typing 500,000 – 1,000,000 well-chosen markers in a study population of sufficient of cases and controls (Hardy and Singleton, 2009; Visscher et al, 2012).

GWAS have been made possible by the recent improvements in the technology and processing speeds of genetic analysis. Such studies use pre-prepared probes (chips) to scan the entire genome for presence of tag SNPs (Mullen et al, 2009), and rely on high-throughput genotyping technologies to assay hundreds of thousands if SNP variants and relate these variants to diseases or health-related traits.

GWAS rely on the "common disease, common variant" hypothesis. This suggests that genetic influence on many common diseases will be at least partly attributable to a limited number of variants present in more than 1 – 5% of the population overall (Becker, 2004; Mullen et al, 2009; Schork et al, 2009; McClellan and King, 2010). Although this hypothesis has been extremely useful in such studies, GWAS are very unlikely to detect multiple rare variants which may also be responsible for such diseases under the "common disease multiple rare variants" hypothesis (McClellan and King, 2010).

Genome-wide association studies have been increasingly used in recent years in an attempt to unravel the genetic basis of many common diseases, including diabetes

mellitus (Sladek et al, 2007), asthma (Himes et al, 2010) and rheumatoid arthritis (Wellcome Trust Case Control Consortium, 2007). Such diseases are believed to result from the interactions of multiple different alleles of multiple genes, possibly interacting with environmental factors (Hardy and Singleton, 2009), and are felt to have complex rather than Mendelian inheritance.

1.4.3.2 Stages in GWAS

A typical GWAS has four elements, and the most commonly used study design thus far has been the case-control model (Mullen et al, 2009). Other designs which have been employed include the use of trios (in which DNA is analysed from an affected individual and both of their parents) (Spielman et al, 1993) and the use of prospective cohorts (Cordell and Clayton, 2005). Each of these study designs has advantages and limitations particular to the assumptions made about the cases included, the ease of recruitment and the expense of the study (Pearson and Manolio, 2008).

The first element in a GWAS is the selection of appropriate cases and controls for study. For the selection of cases, this refers to the difficulties with phenotyping which have been mentioned previously – specifically in relation to epilepsy, this refers to classifiying individuals into particular epilepsy syndromes (see section 1.1.4). It should be noted that many cases for such studies are often drawn from particular settings (such as hospital out-patient clinics), and that this can introduce a selection bias in the choice of cases (as those who are most severely affected and who are only very mildly affected are unlikely to be included).

The choice of controls for such studies can also lead to inaccuracy in a number of ways. For common diseases, it must be clear that controls are truly unaffected by the disease under investigation. If this is not the case, then the statistical power of the study could be markedly reduced (Pearson and Manolio, 2008). Important differences between the groups in other factors should also be considered, as these may act as confounding factors in the analysis of the study results. If not addressed, then a spurious association could be identified between a genetic variant and the confounding factor rather than the disease or condition of interest (Pearson and Manolio, 2008; The Wellcome Trust Case Control Consortium, 2007). Finally, cases

and controls should be drawn from the same population, to avoid the potential difficulties with population stratification (Tan et al, 2004; Cordell and Clayton, 2005; The Wellcome Trust Case Control Consortium, 2007).

Population stratification is the presence of a systematic diffence in allele frequencies between subpopulations within a population. The acceleration in the growth of the human population over the last few millennia has meant that rare variants in the human genome have not had time to be subjected to the full effects of natural selection (Goh and Choi, 2012), and these rare variants can have different frequencies in different populations. This can arise for a number of different reasons, including migration patterns, founder effects and population bottlenecks (Tan et al, 2004; Laird and Lang, 2006). If the cases and controls in an association study are not drawn from the same population, i.e, if the two groups are chosen from different subpopulations within a population, then it may be that the two groups in an association study would have different allele frequencies at particular loci without this difference representing a true association with the disease (Tan et al, 2004). Although there is some uncertainty about how important a factor population stratification is in large-scale studies, there have been instances where it has led to the results of some studies being questioned at a later stage (Wang et al, 2009; McClellan and King, 2010), and it is often suspected when groups have been unable to replicate the findings of association studies (Cardon and Palmer, 2003). Most GWAS now attempt to control for this by excluding subjects with outlier genotypes and by taking into account differences between populations when designing studies (McClellan and King, 2010).

After the isolation of DNA from cases and controls, the second major element in a GWAS is genotyping DNA and then reviewing the data to avoid (in as much as is possible) genotyping error. Genotyping error can be an important cause of spurious associations in GWAS (Pearson and Manolio, 2008) and there are a number of steps in the genotyping process which a robust study will employ to minimise this (The Wellcome Trust Case Control Consortium, 2007). The accuracy of analysis may also be affected by the study population from which the DNA samples are drawn – currently, there are more complete genotyping platforms available for populations of

European and Asian ancestry than those of recent African origin (Pearson and Manolio, 2008).

The third element in a GWAS is an appropriate statistical analysis. GWAS generate large amounts of data which can be analysed in a number of different ways. The simplest way in which the data can be analysed is by direct comparison – the frequency of an allele is compared between cases and controls. However, such an analysis is overly simplistic. It has been calculated that, at the usually accepted significance level of a less than 5% chance of the results being due to chance alone ($p < 0.05$) used in studies such as randomised controlled trials of medications, a GWAS of 1,000,000 SNPs would show that 50,000 of these SNPs would be statistically associated with the disease under investigation (Pearson and Manolio, 2008). In the vast majority of cases, such associations would be spurious. Because GWAS involves multiple comparisons, the analysis must include statistical correction for this (Altshuler et al, 2008; Mullen et al, 2009). The most commonly used method thus far in GWAS has been the use of the Bonferroni correction (Farrall and Morris, 2005; Marian, 2012), although other methods of statistical correction have been advanced (Tan et al, 2004). Use of the Bonferroni correction sets the p-value for statistical significance at stringently low levels, and ensures that false-positive results are minimised (Hunter and Kraft, 2007). Although there has been some debate about the validity of the use of such corrections and the potential for inadvertently dismissing as spurious some true associations (Palmer and Cardon, 2005; Hattersley and McCarthy, 2005; Hunter and Kraft, 2007), the use of such correction methodology ensures that findings which have been demonstrated are statistically robust (Marian, 2012).

The fourth element of a robust GWAS is a replication of the findings of the study itself. For many studies, this step is performed either by another group as part of a collaboration or by the same group as part of a follow-up study for their original findings (Pearson and Manolio, 2008; Mullen et al, 2009). Replication of the findings of a particular GWAS should include a study of the same phenotype or one which is very similar, a demonstration of a similar magnitude of effect and of statistical significance, and an association with the same SNP / allele as the original study (Pearson and Manolio, 2008). Replication remains the gold-standard for the

demonstration a robust biological association between an allele and a disease or health-related trait. Lack of replication has been reported for a number of associations (Murk et al, 2011; Sinner et al, 2011), and been ascribed to a number of factors. These potentially include factors such as population stratification, phenotype differences between the studies, selection bias and genotyping errors (Tan et al, 2004; Chanock et al, 2007; Kraft et al, 2009). Other authors have also described alternative reasons for failure to replicate findings between studies, such as the age at which participants are recruited into the study (Lasky-Su et al, 2008), insufficient statistical power of the studies (Ioannidis et al, 2001) and random chance and a lack of appreciation of the likelihood of non-replication (Gorroochurn et al, 2007).

1.4.3.3 Examples of GWAS

The most frequently used design to date in GWAS has been the case-control design, in which the frequency of an allele in a population of individuals with the condition or disease under investigation is compared to a population of individuals without it (Bush and Moore, 2012). An example of this was the first published GWAS in 2005, which was investigating the aetiology of the condition age-related macular degeneration (ARMD) (Klein et al, 2005). This took samples from cases (a group of individuals with ARMD) and controls (unaffected individuals matched for age and other factors). The identification of two alleles associated with ARMD demonstrated the potential of this approach for the identification of alleles contributing to human disease.

Many other case-control GWAS studies have been published since then (for examples, see Wellcome Trust Case-Control Consortium, 2007; Scott et al, 2007; Barrett et al, 2008), with many of these being the product of large international consortia to ensure that these studies are adequately powered. As of 2012, more than 1,200 GWAS had been completed and thousands of loci identified as being associated with complex traits (Marian, 2012). However, a number of issues arose when considering the results of these studies. The effect sizes of the common variants identified through GWAS are typically very small and clinically negligible (Marian, 2012).

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Other GWAS have made use of family-based designs. Family-based association studies have been proposed as a way around the difficulties of population stratification (Laird and Lange, 2006; Ott et al, 2011). The use of extended family pedigrees was initially not widespread in GWAS but came to be considered again when much of the variability in human inheritance seemed to be unexplained by the use of case-control studies (Ott et al, 2011). In families, the unaffected individuals can act as controls for those affected, without the risk of population stratification (Marian, 2012).

Different models of association studies incorporating families exist, but of the most widely-used is the case-parent trio (Infante-Rivard et al, 2009; Laird and Lange, 2009; Mirea et al, 2012). In this model, an affected individual (the case) is phenotyped and DNA extracted for analysis from this individual and his parents. The interpretation of the results of this model relies on the use of the transmission disequilibrium test (TDT) (Laird and Lange, 2006). This test compares the observed number of alleles transmitted to an individual to those expected in Mendelian transmission. Based on Mendelian inheritance, each parental allele has a 50% probability of being transmitted to the offspring. An excess of alleles of one type in the affected individual suggests that the locus for a particular trait or disease is associated with the marker allele (Infante-Rivard et al, 2009). The principal advantage of the case-parent trio design is that it is quite robust to population stratification. It has limitations also, especially in relation to missing a parent from the trio and in the analysis of complex phenotypes (Laird and Lange, 2006). In an attempt to address such issues, other family-based association study designs were developed, studying discordant sibling pairs (one affected and one unaffected sibling from the same family) (Boehnke and Langefeld, 1998) and discordant sibling trios (at least one discordant sibling pair and one other sibling) (Laird and Lange, 2009; Mirea et al, 2012). There are different strengths and weaknesses for each design, depending on the nature of the condition or trait being studied (Laird and Lange, 2009). Family-based study designs in GWAS have been advanced as a way of improving the accuracy of the results from case-control studies (Laird and Lange, 2009).

However, family-based study designs have the same inherent difficulties as others using extended families for genetic research, i.e., selection bias, inability to control for shared environmental confounding factors, presence of phenocopies and the uncertainty of the applicability of the results to the general population (Schork et al, 2007; Kazma and Bailey, 2011). There have been strenuous efforts used to minimise these potential difficulties and to improve the statistical methods in family-based GWAS (Hiekkalinna et al, 2012) and some GWAS have integrated family-based and case-control methods to good effect (Herbert et al, 2006; Himes et al, 2010). It remains to be seen if the use of family-based study designs will further our knowledge of the genetic basis of human disease.

Results of GWAS in conditions studied thus far have been varied. However, in a number of conditions, some results have been found to be reliable on the basis of replication in further studies. These results have been surprising and extremely useful in expanding our understanding of these conditions. . Many of the demonstrated associations have been in genes which had previously not been suspected to be involved in the disease studied, and have generated new research into the mechanisms underlying these diseases (Altshuler et al, 2008; Hirschorn, 2009; Visscher et al, 2012). Many of the loci associated with diseases have been shown to lie in non-coding regions of the genome, and the exact significance and pathogenic implications of these loci are in many cases still unclear (Altshuler and Daly, 2007; Hardy and Singleton, 2009; Visscher et al, 2012). For many of the demonstrated alleles associated with diseases, the overall increase in risk is quite low (risk ratio of 1.5 or less), although a number of much more significant associations have been demonstrated with risk ratios of up to 12 (Marian, 2012; Manolio, 2010; Klein et al, 2005; Barrett et al, 2008).

To date, GWAS in epilepsy specifically has proven more disappointing. A large collaborative looking for susceptibility variants in patients of European ancestry failed to find any significant associations, in spite of recruiting nearly 5,000 patients and having strict methodology to avoid such errors as population stratification and genotyping error (Kasperavičiūte et al, 2010). However, it may be that population-specific factors are responsible for some of the lack of success of this model in epilepsy thus far, as a study of a Chinese population suggested a number of

susceptibility loci in this population not reported in those of European ancestry (Guo et al, 2012). Many of the GWAS in epilepsy thus far have focussed on focal epilepsy syndromes. Although focal epilepsy syndromes might be expected to have a lesser genetic component to their aetiology, this is less of a limitation in these studies than might be expected, as there are well-defined focal epilepsy syndromes with genetic aetiologies and certain genetic abnormalities can cause both focal and generalised epilepsy syndromes (Kullmann, 2002). Issues of sample size have also been problematic (Mullen et al, 2009).

1.4.3.4 Limitations of GWAS

GWAS have a number of limitations, some of which have already been touched upon. GWAS can identify loci associated with diseases, rather than genes directly (Altshuler et al, 2008; Pearson and Manolio, 2008). The study methodology has the potential to generate false positive results (Pearson and Manolio, 2008) and the stringent statistical criteria for association needed to avoid such false positive results leads to a requirement for large sample sizes (Mullen et al, 2009). As a design, GWAS are relatively insensitive to rare variants, and cannot identify loci at which there are many rare risk alleles in any given population (Casals et al, 2012; Marian, 2012). They are also relatively insensitive to other genetic variants, such as copy number variants (Casals et al, 2012).

In spite of initial enthusiasm, there has been much comment about the failure of GWAS to identify fully the genetic basis for many common conditions (McClellan and King, 2010; Goldstein, 2009). Although some of the lack of progress may be somewhat disappointing, such criticisms have been replied to (Visscher et al, 2012) by those who point out the strengths of GWAS. Much more is now known about the pathways involved in many common diseases with a genetic basis (Visscher et al, 2012), and the identification of unsuspected novel mechanisms in these diseases has opened new avenues of research (Marian, 2012). In spite of the debate on this issue, it seems to be widely acknowledged that a different approach is needed to advance further our understanding of the genetic basis of common diseases, such as epilepsy. One such approach is discussed in the next section.

1.4.4 Next generation sequencing (NGS) / whole exome sequencing (WES)

1.4.4.1 Description of NGS

Whole exome sequencing relies on so-called "next generation sequencing". This phrase is used to describe the change in base pair sequencing techniques which became available from 2005 onwards (Marian, 2012). Prior to this, sequencing was carried out by the process which has become known as "Sanger sequencing", after one of the authors of the original description of the process in 1977 (Sanger et al, 1977). In this process, once a DNA sample has been obtained, a complimentary DNA strand for the sequence of interest is formed from a sequencing oligonucleotide. This process forms fragments of DNA which are 700 – 800 base pairs long. Once these fragments are obtained, they are separated by capillary electrophoresis. Although time-consuming and laborious, Sanger sequencing has a lower error rate than NGS (Majewski et al, 2011) and is still viewed as the "gold standard" for DNA sequencing (Casals et al, 2012). It was also the process used in the initial sequencing of a human genome, a process which took approximately 13 years to complete and cost somewhere in the region of US$ 2.7 billion (Majewski et al, 2011).

The first description of next generation sequencing (also known as second generation sequencing or high-throughput sequencing) using a parallel sequencing platform was published in 2005 (Margulies et al, 2005), and the techniques involved have continued to improve since then. Although a number of different methods are used, all have the common feature of high throughput sequencing of DNA molecules that are spatially separated in a flow cell (Singleton, 2011). The process can be performed on the genome as a whole (whole genome sequencing, or WGS) (Teer and Mullikin, 2010) or on specific regions of the genome such as the protein-coding regions (whole exome sequencing, or WES) (Singleton, 2011; Casals et al, 2012).

Next generation sequencing has a number of advantages over Sanger sequencing. It is both faster and more cost-effective. In comparison to the length of time taken to sequence the first human genome, laboratories can now sequence a whole human genome in a matter of days, and the cost of WES in some laboratories has now fallen

to less than US$ 1500 per sample (Singleton, 2011). The amount of information produced in next generation sequencing is also a number of magnitudes greater than that produced by Sanger sequencing. A single run of next generation sequencing can produce up to several million bases, depending on the region under analysis (Bamshad et al, 2011).

1.4.4.2 Stages in a NGS / WES study

The process of next generation sequencing begins with the genomic DNA sample from an individual, which is used to construct a library (Bamshad et al, 2011; Casals et al, 2012). In this step, the genomic DNA is sheared into short fragments either by physical or enzymatic reactions. The length of these fragments can vary depending library construction method. After these fragments have been created, they are ligated to short DNA sequences called adaptors. These adaptors are complimentary to the oligonucleotides used for sequencing. Once this is done, the library is enriched for the regions of interest – in the case of WES, this is the exome, the protein-coding regions of the genome. This enriching can be performed in a number of ways (including polymerase-mediated capture and regional capture) (Teer and Mullikin, 2010) but is usually by either solid-phase hybridisation or liquid-phase hybridisation (Teer and Mullikin, 2010). In the former, the probes complimentary to the sequences of interest are affixed to solid supports, such as microarrays (Albert et al, 2007; Hodges et al, 2007). In the latter, the probes are biotinylated, and after hybridisation they are extracted by being bound to magnetic streptavidin beads (Gnirke et al, 2009; Teer and Mullikin, 2010). When the DNA library has been enriched for the sequences of interest, then these sequences are amplified. After this, massively parallel sequencing can be performed on the amplified DNA library to generate sequences for the regions of interest. These sequences are then compared to a reference genome (a process known as alignment), where one is available, and the variants in the sequence can be detected (Casals et al, 2012).

1.4.4.3 Rationale for use of WES

The process of NGS has decreased the cost of sequencing exponentially, and has made it possible to cost-effectively determine nearly all the variation in a particular

region of a genome. Any subset of the genome can be targeted in the process, but one of the most common is the exome in WES.

There are a number of reasons why work to date has focussed on whole exome sequencing (Bamshad et al, 2011). Previous studies using other techniques on the protein-coding region of the genome have, when adequately powered, proven highly successful in identifying variants for monogenic diseases, and there is no reason to suspect that this not be the case for WES (Antonarakis and Beckmann, 2007). Furthermore, based on our knowledge to date, the majority of variants and mutations known to underlie Mendelian diseases have been found to disrupt protein coding sequences, making these regions the one most likely to give results when analysed (Kryukov et al, 2007; Choi et al, 2009). Compared to the overall size of the genome as a whole, the exome is comparatively small (comprising only c. 1% of the overall human genome) (Casals et al, 2012), so both overall cost and sequencing effort are considerably less than sequencing the whole genome, with the amount of information generated by WES being considerably easier to analyse and filter than WGS.

Compared to the previously used methods outlined in sections 1.4.1, 1.4.2 and 1.4.3 above, NGS (and by extension WES) has a number of advantages.

As mentioned above, most of the GWAS performed thus far have focussed on alleles with a frequency of 5% or greater in the populations studied and low frequency (1 – 5%) or rare variants typically remain undetected by such studies (Casals et al, 2012; Marian, 2012). Although GWAS have been successful in identifying large numbers of loci which contribute to the genetic basis of complex traits, they have largely failed to explain the heritability of these traits (McClellan and King, 2010). Low frequency and rare variants are known to be enriched for functional variations which alter the amino acid sequence, and results of previous studies have suggested that common diseases may be more Mendelian in character than would be suggested by the common disease common variant hypothesis which has usually been applied to diseases with complex genetics and which underlies GWAS (i.e., that such conditions may be more in keeping with the common disease multiple rare variants hypothesis) (Mulley et al, 2005; Schork et al, 2009). Such rare variants, which were largely not detectable by GWAS, can now be detected by NGS (Casals et al, 2012).

Indeed, WES has the potential to identify such variants and allow definite connections to be made between specific genes and common diseases (Majewski et al, 2010).

In terms of the older gene-discovery techniques, such as linkage analysis, a number of factors limit their usefulness, many of which are outlined in previous sections above (see sections 1.4.1, 1.4.2 and 1.4.3). Such techniques encounter great difficulty when faced with conditions with decreased penetrance, locus heterogeneity (the appearance of phenotypically similar characateristics resulting from mutations at different genetic loci), and small number of cases or families to study. Such limitations are much less problematic when NGS is used (Bamshad et al, 2011). High-penetrance variants have a strong physiological effect and thus have usually been identified in monogenic Mendelian disorders using linkage studies, but low-penetrance variants have a much weaker effect on the phenotype and are thus much more difficult to identify outside of large case – control cohorts (Majewski et al, 2011). The amplification and massively parallel sequencing used in NGS mean that WES allows for DNA samples collected from small families or from isolated individuals to be used to discover mutations which cause disease (Choi et al, 2009; Sobreira et al, 2010; Singleton, 2011). Unlike other techniques, the ability of WES to identify a disease-causing mutation relies only on the mutation being present in the captured portion of the genome and our ability to recognise the pathogenic mutation among the thousands of new variants each WES identifies (Majewski et al, 2011). This allows for the identification of novel genetic mutations using this technique in individuals with novel or atypical phenotypes, or phenotypes that are difficult to differentiate into specific categories (Bamshad et al, 2011).

Generating such quantities of data brings with it considerable difficulties in analysis and one of the major challenges in WES is the accurate identification of pathogenic mutations among the background of other non-pathogenic variants. It has been estimated that each WES will identify approximately 20,000 single nucleotide variants in individuals of European descent, with a higher number again for those of African descent (Bamshad et al, 2011; Singleton, 2011). Identifying one variant as the causal mutation amongst this vast number requires extensive analysis, and a number of techniques have been developed thus far to facilitate this.

The process of discrete filtering uses a comparison between the variants found in the WES and those from controls or from a common database (Casals et al, 2012; Bamshad et al, 2011) (such as the 1,000 Genomes Project or the Single Nucleotide Polymorphism Database) (Via et al, 2010; Day, 2010). The assumption underlying this approach is that the variants which are also found in the controls / common database cannot be responsible for the disease. It has been estimated that filtering the variants from a WES against the two common databases mentioned can decrease the number of potential causative mutations from over 20,000 to approximately 700 (Singleton, 2011). One limitation of this approach is that it risks eliminating truly pathogenic candidate variants which are also found in the control population at a low frequency (Casals et al, 2012).

After discrete filtering, candidate variants can be stratified on the basis of their predicted impact or their potential for a deleterious effect (Bamshad et al, 2011). Filtering can also be performed using tests of association, an approach which can also be used to give an estimate of the number of individuals needed to adequately power a study (Banshad et al, 2011).

Filtering can also make use of pedigree information. Given what is known about the extent of variants which are identical by descent in a family between varying degrees of relative, in a given family, sequencing the two most distantly related individuals with the same phenotype of interest can substantially narrow down the number of candidate variants when searching for very rare alleles (Cirulli and Goldstein, 2010; Bamshad et al, 2011). Another example of a pedigree-based analysis is the use of parent – affected child trios, which have proven extremely useful in identifying de novo mutations as a cause of disease (Bamshad et al, 2011). Studies have already shown how combining WES with linkage studies within pedigrees can identify the genetic cause for Mendelian diseases (Sobreira et al, 2009), and this method has been advanced as one way in which possible rare variants underlying human genetic disease with familial segregation can be identified (Cirulli and Goldstein, 2010).

1.4.4.4 Limitations in WES

The use of WES is already proving a powerful tool in the genetic analysis of human disease (Ku et al, 2011), but as with the other conditions already mentioned, there are a number of caveats and limitations with the use of this technique.

The initial stage of targeting particular segments of the genome for analysis and capturing them by hybridisation techniques has a number of inefficiencies. Due to uneven capture efficiency, there may be inadequate coverage of particular exons (Casals et al, 2012). If one of these happens to contain the causal variant, then WES may not identify this.

Specifically in relation to WES, there is still a degree of uncertainty about which parts of the human genome comprise the exome, i.e., are truly protein coding regions (Bamshad et al, 2011; Majewski et al, 2011). Current exome capture methods use data based on those exons which are already known. As such, exons which are currently not known will not be captured and the resulting WES will be missing some of the exome (Bamshad et al, 2011).

Current capture techniques are also inefficient in capturing non-coding sequences which are evolutionarily conserved and regulator and promoter regions for genes (Singleton, 2011; Majewski et al, 2011; The Encode Project Consortium, 2012). There can be difficulties with the process of alignment of the sequences from WES with a comparison genome, such that it may not be possible to identify the base sequence (Casals et al, 2012). WES also has a higher error rate in identifying the base sequence than Sanger sequencing, so key / potentially causal variants identified by WES should be re-validated by follow-up sequencing using the older (e.g. Sanger) method (Marian, 2012). The process of NGS is inefficient for the identification of repeat mutations (such as trinucleotide repeats), and copy number variants are more difficult to detect with WES (Singleton, 2011), although this is less of an issue with whole genome sequencing.

Copy number variants have been reported in association with epileptic encephalopathies (Mefford et al, 2011), idiopathic generalised and focal epilepsy

syndromes (Mefford et al, 2010), and it is still unclear how much their prevelance in epilepsy will limit the use of NGS as a genetic technique. However, it still unclear how such copy number variants contribute to the aetiology of epilepsy, and studies identifying copy number variants in patients with epilepsy have also identified unaffected carriers, giving rise to the idea of other genetic or non-genetic factors playing a role in the clinical presentation (Mefford et al, 2011). Although such variants may be a reason why NGS was unsuccessful in identifying the particular genetic aetiology for epilepsy in a given individual, they do not exclude NGS as a genetic investigation method.

For all of these difficulties, the advent of NGS / WES represents a considerable step forward in the genetic analysis of human disease. As the cost of NGS continues to decrease and the capturing and base-calling methods involved improve, many commentators are already hoping to replace WES with whole genome sequencing (WGS), in the hope of analysing non-coding regions and CNVs more fully (Majewski et al, 2011). Whether NGS (of whatever type) will reveal the elusive genetic mechanisms underlying many common conditions, or whether we face another round of enthusiasm followed by disappointment, remains to be seen.

1.4.5 Conclusions

As our understanding of human genetics has improved, so the techniques being used to identify the basis for human genetic traits and disease have improved. It should by no means be assumed that any one technique will represent the acme of analysis, and the rate of investigation and innovation in this area shows no signs of slowing. The advent of common comparison genomic data now allows for reference controls for studies, and the decreasing cost associated with such studies means that their use in clinical medicine is drawing nearer, particularly with regards to conditions such as mental retardation, psychiatric illness and epilepsy.

However, to use the newer sequencing techniques to their optimum degree will require information on the common variants in the populations and the role such variants may have in relation to human disease. Given this, it is opportune to review

what is currently known about genes implicated in the aetiology of epilepsy in humans.

1.5 Previously identified genetic abnormalities associated with epilepsy

In this section, I hope to give some idea of the known genetic causes of epilepsy. The emphasis will be on single gene mutations which can lead to epilepsy, rather than the known chromosomal and mitochondrial genetic abnormalities associated with epilepsy.

Given the nature of on-going research in this area, any comprehensive list of "epilepsy genes" is likely to be incomplete. I have paid particular attention to those genes previously analysed by the ILAE as possible candidates for genetic screening in those with epilepsy (Ottman et al, 2010). I have also supplemented this list with other genes whose effects have been demonstrated on other studies (Tan and Berkovic, 2010). This is not meant as an exhaustive list, as on-going work will expand and refine our notions of "epilepsy genes".

In each section, I have attempted to give a brief outline of the normal function of the gene prior to describing the effects of mutations in the gene. As mentioned in the previous sections, many of the genes which have been found to be associated with epilepsy thus far have controlled the synthesis or function of ion channels. For this reason, the discussion of these genes will group them loosely by the ion channel to which they contribute.

1.5.1 Sodium channel gene mutations

Sodium channels are located throughout different tissue types in the body. The principal channels of interest in human epilepsy are voltage-gated (where the channel is opened in response to an action potential). In neurons, the movement of sodium through voltage-gated sodium channels is responsible for the rising phase of the neuronal action potential (Leterrieret al, 2010), and thus plays a critical role in the initiation of the action potential in response to a stimulus.

The human voltage-gated sodium channel in the brain is composed of a larger α subunit in association with two smaller β subunits (Hahn and Neubauer, 2009). The α subunit contains the voltage sensors and sodium ion conducting channel. It is

composed of four repeat domains, each of which contains six distinct regions which span the cell membrane (Isom, 2002). The α subunit is functional on its own (Goldin et al, 1986), but the β subunits which are associated with the α subunit can alter the properties of the channel further (Meisler et al, 2010). There are nine distinct types of α subunit, each of which is coded for by a different SCNA gene. There are four different SCNB genes which code for the β subunits (Meisler et al, 2010).

The sodium channel genes are responsible for the synthesis of different components of the voltage-sensitive sodium channel. Each gene codes for a different part of the sodium channel, and different types of sodium channel predominate in different tissues in the body but also within different areas of the nervous system itself (Meisler et al, 2010).

1.5.1.1 SCN1A

One of the first described and best characterised genes involved in human epilepsy, this gene maps to the chromosomal location 2q24.3 (Malo et al, 1994; Escayg et al, 2000). Mutations in this gene have been associated with a number of different presentations, and the gene remains one of the most clinically relevant in human epilepsy genetics (Mulley et al, 2005).

The most widely recognised epilepsy syndromes associated with mutations in this gene is that of generalised epilepsy with febrile seizures plus (GEFS+). Typically, febrile convulsions are confined to early childhood (between the ages of 6 months and under 6 years of age), are less than 15 minutes in duration, do not have focal features associated with them and are associated with a low overall risk for the later development of epilepsy (Subcommittee on Febrile Seizures, American Academy of Pediatrics, 2011; Baulac et al, 2004). However, individuals with febrile seizures plus suffer febrile seizures extending beyond the age of 6 years, which may be prolonged or have focal features, and may or may not have associated afebrile generalised tonic-clonic seizures (Scheffer and Berkovic, 1997). Individuals with GEFS+ go on to develop generalised epilepsy at a later age. However, intellectual development is unaffected by the development of epilepsy. Many families have been described with

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this syndrome in different populations (Scheffer and Berkovic, 1997; Escayg et al, 2000; Guerrini et al, 2010).

A more severe phenotype associated with mutations in the SCN1A gene is that of Dravet's syndrome, also referred to as severe myoclonic epilepsy of infancy (SMEI) (Claes et al, 2001). Dravet's syndrome is associated with complex febrile seizures (as in GEFS+) but is also associated with a generalised epilepsy syndrome with both myoclonic and generalised tonic-clonic seizures. Typically, onset of the syndrome is in the first year of life (Suls et al, 2010; Harkin et al, 2007) but the timing of the onset of different seizure types within the syndrome can vary (Dravet, 2011) . Affected individuals also demonstrate some degree of developmental regression after the onset of the generalised epilepsy, with loss of previously attained milestones or intellectual abilities (Dravet, 2011). Dravet's syndrome patients have an increased rate of mortality at younger ages (Oguni et al, 2011; Genton et al, 2011), but cases have been reported of milder phenotypes allowing for individuals to reach adulthood and reproduce (Suls et al 2010). The majority of individuals who reach adult life are dependant (Genton et al, 2011).

Apart from its role in the aetiology of epilepsy, mutations in the SCN1A gene have also been investigated as potential explanations for the response to certain therapies in epilepsy. There are recognised cases of patients with mutations in this gene having worsening of their seizure control as a result of being treated with sodium-channel blocking agents (Liao et al, 2010; Chiron and Dulac, 2011). Particular genotypes within the SCN1A gene have been associated with differing severities of the clinical course of the associated epilepsy syndrome and the response of the individual to therapy (Heinzen et al, 2007). This is an example of how knowledge of the underlying genetic mechanisms of a disease may lead to a more rational choice of therapy, a situation which is still comparatively rare in clinical epilepsy

1.5.1.2 SCN1B

This gene maps in humans to chromosome 19q13.1 (Makita et al, 1994), and codes for the beta subunit of the voltage-gated subunit of the sodium channel (Reid et al, 2009). Thus far, it is the only example identified in human disease of a gene which

codes for this beta subunit and which has been associated with epilepsy. Evidence for high expression of the gene has been detected in different brain regions, but expression of the gene has also been detected in skeletal muscle and cardiac tissue (McClatchey et al, 1993).

Mutations in SCN1B have been associated with GEFS + principally, and have been reported with cases which been negative for mutations in SCN1A (Wallace et al, 1998; Audenaert et al, 2003). However, and possibly reflecting the distribution of the gene, it has also been reported in association with cases of cardiac dysrhythmias such as Brugada syndrome (Watanabe et al, 2008). This particular syndrome is associated with abnormalities of electrical conduction within the heart, and can be associated with sudden cardiac death (Lippi et al, 2012).

<u>1.5.1.3 SCN2A</u>

This gene, which maps in humans to chromosome 2q24.3 (Ahmed et al, 1992), codes for a subunit of the α receptor of the voltage-gated sodium channel (Heron et al, 2007). Clinically, this gene has been reported in association with benign familial neonatal – infantile seizures and with early infantile epileptic encephalopathy.

Benign familial neonatal – infantile seizures (BFNIS) are characterised by starting between day 2 and 7 months of age, and remitting within the first year (Herlenius et al, 2007; Deprez et al, 2009). The seizures in these children have been reported as largely being secondarily generalised tonic-clonic seizures (Berkovic et al, 2004). Development is normal in these children. EEGs in these children during the period while they are affected have been reported as being normal or as showing focal interictal abnormalities (Berkovic et al, 2004).

Early infantile epileptic encephalopathy with suppression-bursts, also termed Ohtahara syndrome, is a severe illness (Yamatogi and Ohtahara, 2002; Korff and Nordli, 2006). It forms part of the spectrum of the early onset epileptic encephalopathies. There are a number of types of early onset epileptic encephalopathy, which differ on the basis of the age of onset, predominant seizure type and associated EEG pattern. However, the common features in these conditions

are that they have particular ages of onset, that they are associated with frequent minor generalised seizures, that the aetiology for these conditions is usually heterogenous and that the EEG shows a severe and continuous epileptic pattern (Ohtahara and Yamatogi, 2006).

Early infantile epileptic encephalopathy with suppression-bursts is associated with an onset of seizures in the first three months of life (Yamatogi and Ohtahara, 2002). The characteristic seizure type is the tonic spasm, but other seizure types are seen in up to half of all cases (Yamatogi and Ohtahara, 2002). The aetiology of this condition is very variable, and cases have been described in association with structural brain lesions (Fusco et al, 2001) as well as mutations in a number of different genes (Mastrangelo and Leuzzi, 2012). Prognosis is poor, with a high rate of early mortality and severe intellectual disability (Yamatogi and Ohtahara, 2002).

1.5.1.4 SCN8A

This gene, which maps to chromosome 12q13, has been much more extensively studied in mouse models of epilepsy rather than in human studies. It was first clearly identified in human tissue by Plummer et al in 1998. It has been shown to be widely distributed in the human central and peripheral nervous system. Studies of mouse models with mutations in this gene have suggested that mutations in the SCN8A gene are associated with a number of neurological presentations in mice (Meisler et al, 2002), and have also suggested that the presence of mutations in SCN8A can act as modifiers for the phenotype associated with SCN1A mutations (Hawkin et al, 2011) or of the phenotype of SMEI (Martin et al, 2007).

However, there is very limited information on the occurrence of this mutation in humans. A previous study identified a family affected by a mutation in the SCN8A gene with the phenotype characterised by ataxia, cerebellar atrophy and cognitive impairment in the most severely affected individual. Phenotypes in other individuals were milder, and epilepsy was not reported in this family (Trudeau et al, 2006). One whole exome sequencing study of a family kindred revealed a mutation in SCN8A in the affected individual but not in the other family members (Veeramah et al, 2012).

The mutation was felt to be a de novo mutation. Thus far, this is the only reported definite association of a mutation in this gene with human epilepsy.

1.5.1.5 SCN9A

This gene, which maps in humans to chromosome 2q24.3, was first identified in 1995 (Klugbauer et al, 1995). Analysis of the sequence of the gene found that it was very similar to the sodium channels found in the brain and in skeletal tissue. It has also been demonstrated that this is the principal sodium channel expressed in the smooth muscle cells of the normal human bronchus, main pulmonary artery and coronary arteries (Jo et al, 2004).

Clinically, this gene has been associated with conditions such as primary erythromelalgia or paroxysmal extreme pain disorders (Dabby et al, 2011; Lampert et al, 2010). The clinical features of these syndromes differ, but they are characterized by excruciating painful attacks in the extremities (in the case of primary erythromelalgia) or the cranial nerves or deep abdominal nerves (in the case of paroxysmal extreme pain disorder) that begin in childhood and progress over life (Lampert et al, 2010). Triggers for attacks of pain in these conditions are usually daily activities, and the pain can be extremely resistant to standard treatments (Lampert et al, 2010).

However, mutations in this gene have been associated with the occurrence of different epilepsy syndromes. The gene has been found to be associated with isolated febrile seizures, GEFS + and refractory epilepsy (Pfeiffer et al, 1999; Singh et al, 2009) in a single large family. It has been suggested that the presence of SCN9A mutations may act as a genetic modifier for the condition, as it was found in conjunction with mutations in other genes such as the SCN1A gene (Singh et al, 2009) in a further screening study. As is the case with many of these genes, the association has been reported in a single family kindred, and there is poor correlation between genotype and phenotype.

1.5.2 Potassium channel gene mutations

Like sodium channels, potassium channels are found in most cell types and play a major role in setting the resting membrane potential and shaping the action potential (Ravens and Cerbai, 2008). Potassium channels have also been classified into four different groups – voltage-gated, calcium-gated, inwardly-rectifying and tandem-pore types (Gutman et al, 2005). A different classification scheme for potassium channels also exists, where the channels are termed 2TM (also known as inward-rectifying), 4TM and 6TM (which are principally voltage-gated but include the calcium-activated type) (Sandhiya and Dkhar, 2009). All of these are composed of a primary pore forming α subunit in association with a regulatory subunit (Sandhiya and Dhkar, 2009). These channels are the most abundant of all ion channels, and in humans the subunits of these channels are coded for by at least 75 genes (Sandhiya and Dkhar, 2009).

In humans, the most common types of potassium channel are largely divided into two groups – the voltage-gated type and the inwardly-rectifying type. These channels have slightly different actions and roles. Mutations in different genes coding for particular components of the potassium channel have been described in association with a number of different disease states in humans (Ravens and Cerbai, 2008; Riant et al, 2011; Dedek et al, 2001). However, a number of mutations within these genes have been described in association with epilepsy.

1.5.2.1 KCNJ11

This gene, which has been mapped to genomic locus 11p15.1 (Inagaki et al, 1995), codes for subunits of the pore of an ATP-sensitive potassium channel. The KCNJ11 channel is a member of the inwardly-rectifying potassium channel group (the other type of potassium channel recognised is the voltage-gated type, for which see other examples in this section). It has been commented that, as a class, the inwardly-rectifying potassium channels have a role in a number of physiological functions, such as the maintenance of the resting membrane potential in excitable cells, the generation of the action potential in neurons and the alteration of cell excitability.

Channels containing the KCNJ11 subunits are found in muscle cells, neurones and beta cells in the pancreas (Girard et al, 2009). Thus, abnormalities in the structure or function of this type of channel would be expected to be associated with abnormalities of neuronal function and glucose regulation.

Clinically, mutations in the KCNJ11 gene have been associated with such conditions as hyperinsulinaemic hypoglycaemia (Pinney et al, 2008) and permanent neonatal diabetes mellitus (Yorifuji et al, 2005). More specific to our study, there have been descriptions of permanent neonatal diabetes mellitus with neurological features. These neurological features have included seizures (including myoclonic seizures and hypsarrhythmia), developmental delay and dysmorphic features (Gloyn et al, 2006). Some reports have emphasised a genotype – phenotype correlation for specific mutations (Flanagan et al, 2006), but others have shown less convincing association between the neurological illness and the specific mutations found (Gloyn et al, 2006; D'Amato et al, 2008).

1.5.2.2 KCNQ2

This gene, which has been mapped to the genomic locus 20q13.3 (Biervert et al, 1998; Singh et al, 1998), codes for a voltage gated potassium channel. The channel is expressed in brain, and previously has been shown to have its highest concentration in the cerebellar cortex, amygdala, caudate nucleus and the hippocampus (Yang et al, 1998).

The role of the KCNQ2 channel is, in association with the KCNQ3 channel, to form the M channel, a form of channel which was identified prior to the discovery of the KCNQ2 channel. The M channel is a slowly activating and deactivating potassium channel (Brown and Yu, 2000). This current helps to determine the response of neurons to inputs from other neurons. It is expressed in many neurons in the central nervous system, and it has been previously demonstrated that the KCNQ2 and KCNQ3 channel subunits can assemble to form a channel which has the properties of the M channel, and thus that these genes encode the M channel (Wang et al, 1998).

Clinically, mutations in the KCNQ2 gene have been identified in families with benign familial neonatal seizures (Singh et al, 1998; Dedek et al, 2001; Heron et al, 2007). Seizures in this condition begin usually on the second or third day of life, but later presentations have been described. The seizures are usually partial with or without secondary generalisation. The normal clinical course sees seizures resolve within weeks or months of onset, with a minority going on to develop seizures later in life (Deprez et al, 2009). There is usually no developmental delay associated with the onset of seizures (Specchio and Vigevano, 2006). The risk for the development of subsequent epilepsy has been reported as 16% (Ronen et al, 1993). However, many authors have again commented on the phenotypic variability within families with the same genotype (Borgatti et al, 2004).

There have been more severe phenotypes reported with mutations in the gene. Previous reports (Dedek et al, 2003; Borgatti et al, 2004) have identified families in which the KCNQ2 mutation was associated with drug-resistant epilepsy and epileptic encephalopathy. There is an increasing recognition that the phenotype associated with mutations in this gene may be more severe than was previously reported (Weckhuysen et al, 2012).

Myokimia, a rippling or continuous undulating movement of muscles associated with hyperexcitiability of the lower motor neurons innervating skeletal muscles, has also been reported with mutations in this gene (Dedek et al, 2001). Myokimic discharges on electromyographic studies are brief bursts of single motor unit potential firing with a following period of electrical silence (Gutmann and Gutmann, 2004). There are multiple possible causes of myokimia, many of which are associated with acquired dysfunctions of the peripheral nerve axon rather than a genetic cause (Gutmann and Gutmann, 2004). The occurrence of myokimia in the setting of potassium channel gene mutations emphasises the importance of the potassium channel in the aetiology of myokimia in certain conditions.

1.5.2.3 KCNQ3

Another of the family of voltage-gated potassium channel genes, KCNQ3 has been mapped to the 8q24 genomic locus (Charlier et al, 1998). It has been demonstrated

that, like KCNQ2, the KCNQ3 channel is expressed in brain, and previous studies have demonstrated localisation of the protein products of this gene in certain neurons in the cortex and hippocampus in the human brain (Cooper et al, 2000). As mentioned above in the discussion on KCNQ2, the KCNQ3 channel is responsible for the generation of the M current and thus the determination of the reaction of certain neurons to synaptic inputs.

A mutation in the gene was identified in a family with a phenotype of benign familial neonatal seizures (Charlier et al, 1998) and other families have been reported with a similar phenotype from other populations (Li et al, 2008). Screening studies of patient cohorts have identified mutations in the KCNQ3 gene in patients with epilepsy syndromes such as benign familial neonatal seizures, benign rolandic epilepsy (Neubauer et al, 2008) and juvenile myoclonic epilepsy (Vijai et al, 2003).

Benign rolandic epilepsy, more properly termed benign childhood epilepsy with centrotemporal spikes (BECTS), is the most common focal epilepsy syndrome of children of school-going age (Ericksson and Koivikko, 1997). Onset is usually around the age of 8-9 years (Kramer et al, 2002). Seizures in this condition are typically nocturnal, and usually have either focal sensory of motor phenomena at onset which can progress into a secondarily generalised tonic-clonic seizure (Ma and Chan, 2003). The EEG pattern classically shows epileptiform discharges over the mid-temporal and central regions (Chahine and Mikati, 2006). Most cases of BECTS resolve by mid-adolescence, and most patients remain in remission after the age of 18 years (Callenbach et al, 2010).

Juvenile myoclonic epilepsy (JME) is one of the commonest idiopathic generalised epilepsy syndromes (Durón et al, 2005), and in some series has been found to account for up to 11% of all cases of epilepsy (Panayiotopoulos et al, 1991). It has a variable age of onset, but typically occurs in adolescence (Alfradique and Vasconcelos, 2007). As a syndrome, it is characterised by the occurrence of myoclonic jerks, absence seizures and generalised tonic-clonic seizures, although the age of onset and frequency of occurrence of each seizure type can vary amongst affected individuals (Durón et al, 2005). The EEG typically shows generalised, frontally-predominant, symmetrical epileptiform disharges with a frequency of 3-5

Hz (Hrachovy and Frost, 2006). Although JME is felt to be exclusively genetic in origin, different loci and particular genes have been reported as being linked or in association with this syndrome (Alfradique and Vasconcelos, 2007), and no one single genetic locus or allele has been consistently associated with it.

1.5.2.4 KCNJ10

Another of the inwardly-rectifying potassium channel genes (similar to KCNJ11), this gene has been mapped to genomic locus 1q23.2 (Tada et al, 1997).

Clinically, mutations in the KCNJ10 gene have been associated with SeSAME syndrome. This syndrome consists of seizures, sensorineural deafness, ataxia, mental retardation and electrolyte imbalance. A report describing 5 affected patients from 4 unrelated families (Scholl et al, 2009) was followed soon afterwards by a report of a similar clinical picture from a different single family (Bockenhauer et al, 2009). However, in this latter family, the clinical condition was described as EAST syndrome (epilepsy, ataxia, sensorineural deafness and tubulopathy). The affected individuals in the family reported all had abnormalities of electrolyte balance affecting potassium and magnesium levels, seizures and a later onset of sensorineural deafness as well as epilepsy. The mutations identified in the KCNJ10 gene in the individuals studied were felt to lead to abnormal function of the KCNJ10 channel, leading to accumulation of potassium in the extracellular space in the brain (and thus lowering the seizure threshold) as well as affecting the renal recycling of potassium (thus leading to the metabolic abnormalities seen).

Previous screening studies have reported particular variation in the KCNJ10 gene as being associated with seizure resistance in IGE cohorts (Lenzen et al, 2005), but subsequent functional assays of the effect of some of these variants seemed to exclude this (Shang et al, 2005).

1.5.2.5 HCN2

This gene has been mapped to genomic locus 19p13.3 (Ludwig et al, 1999), and codes for one of the voltage-gated potassium channels. This channel belongs to the

group of hyperpolarisation-activated cation channels which contribute to spontaneous rhythmic activity in both the brain and the heart.

In studies of human populations comparing individuals with febrile seizures and GEFS+, a particular variant was found in 2.4% of patients compared to 0.2% of controls (Dibbens et al, 2010). Functional analysis suggested that the variant found caused a gain of function in the HCN2 channel and the authors concluded that this may be a contributor to polygenic epilepsy. More recently, a report of an individual with idiopathic generalised epilepsy and a homozygous mutation in the HCN2 gene was published (DiFrancesco et al, 2011). However, in this latter case, the individual was the only one identified after screening multiple families for mutations in this gene, and extended family pedigrees with this mutation have yet to be identified.

1.5.2.6 KCNA1

Mutations in this gene, which maps to genomic locus 12p13 (Albrecht et al, 1995), have been associated with the disorder episodic ataxia with or without myokimia (Browne et al, 1994).

Episodic ataxia as a term is used collectively for a group of conditions which are inherited in an autosomal dominant manner and which are characterised by paroxysmal attacks of imbalance and poor co-ordination (Riant et al, 2011). There are different types of episodic ataxia, but they differ in the age of onset of the clinical symptoms, the durations of each attack, the findings on physical examination between attacks and the other features associated with those described above (Jen, 2008). The mutations associated with episodic ataxias vary in the different subtypes (Jen, 2008).

However, in one previously reported family (Zuberi et al 1999), 2 of 5 affected individuals also had focal epilepsy as well as episodic ataxia. In the same paper, a review of previously reported families with episodic ataxia suggested an over-representation of epilepsy in these families. Subsequent reports of affected families have also confirmed the association with mutations in this gene with phenotypes of combined movement disorders and epilepsy (Demos et al, 2009), and mutations in

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genes other than KCNA1 have also been shown to be associated with different types of episodic ataxia and epilepsy. Based on expansion of our knowledge of the underlying mechanisms of autosomal dominant lateral temporal epilepsy, this gene was one of number previously investigated for an association with this condition. However, no proven association has been shown at this time (Diani et al, 2008).

1.5.2.7 KCNMA1

This gene codes for the alpha subunits of the BK channel. The BK channel is slightly different to many of the other potassium channels, in that it can be activated by cell membrane depolarisation (hence its classification in the voltage-gated category) and also by intracellular calcium ions (N'Gouemo, 2011). The gene has been mapped to the genomic locus 10q22 (Du et al, 2005).

One family has been reported in which there was the co-occurrence of paroxysmal dyskinesia and idiopathic generalised epilepsy in association with a mutation in this gene (Du et al, 2005). By a genome wide linkage scan, a locus associated with the phenotype observed was found at 10q22. Subsequent analysis revealed the KCNMA1 gene within this locus and a mutation within this gene. The group reporting this family hypothesised that a mutation within this gene was responsible for the clinical picture, given the previous reports of potassium channelopathies in association with epilepsy and movement disorders. However, it should be noted that, thus far, the above reported family is the only report of epilepsy in humans associated with a mutation in the KCNMA1 gene.

Paroxysmal dyskinesia is a term used for a group of rare neurological disorders, which are clinically and aetiologically heterogenous (Rochette et al, 2008). All of these conditions are characterised by the occurrence of episodic involuntary abnormal movements (dyskinesia), and although there are well described cases secondary to other causes, most cases are idiopathic (Rochette et al, 2008). The attacks in the idiopathic paroxysmal dyskinesias can be triggered by a number of different stimuli, including exercise, sudden movements, hyperventilation and stress (Rochette et al, 2008). The co-occurrence of epilepsy syndromes of different types and paroxysmal dyskinesias is well recognised (Swoboda et al, 2000; Guerrini et al,

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2002), and has been described in both linkage and familial segregation studies (Tomita et al, 1999; Hattori et al, 2000). However, in spite of the replication of linkage studies implicating a locus on chromosome 16 (Rochette et al, 2008), the mutation in the KNCMA1 gene described above is the only case thus far which has been localised to a specific gene on this chromosome. More recently, mutations in the SLC2A1 gene, which is located on a different chromosome, have been associated with paroxysmal exercise-induced dyskinesia in some families (Suls et al, 2008).

Other groups have screened for mutations within this gene on the basis of linkage studies implicating a locus on chromosome 10q21 being associated with hot water epilepsy, but no mutations were identified and no association was proven (Ratnapriva et al, 2009).

1.5.3 GABA receptor gene mutations

GABA (gamma-aminobutyric acid) is a known inhibitor of neuronal activity and plays a role throughout the central nervous system in the modification of neuronal activity (Planells-Cases and Jentsch, 2009). GABA activates two major types of receptors, $GABA_A$ (and $GABA_C$, a closely-related group) and $GABA_B$. The former group function as chloride channels and the latter are baclofen-sensitive (Galanopoulou, 2010).

The $GABA_A$ receptors are pentameric in structure, being composed of five different subunits (two α, two β and one other subunit). The GABA binding site is formed by the two α and two β subunits. The $GABA_A$ receptors mediate the principal inhibitory function in the brain (Galanopoulou, 2010), principally by allowing the influx of chloride into the cell, thus causing hyperpolarisation and preventing the neuron from generating an action potential. GABA-A receptors have been shown to be the targets for medications such as benzodiazepines and neurosteroids (Planells-Cases and Jentsch, 2009). Changes in the state of phosphorylation of the GABA-A receptor have also been demonstrated in cortical tissue from patients with epilepsy compared to controls (Laschet et al, 2007).

Given the role of GABA in inhibitory activity, abnormalities of GABA-ergic systems were long suspected of playing a role in the aetiology of epilepsy, and mutations in the genes which code for GABA receptors have been shown to have such role. Although some initial work implicated mutations coding for elements of the chloride channel itself as being involved in the aetiology of epilepsy (Haug et al, 2003), this initial study was later retracted (Haug et al, 2009) and further studies failed to replicate this finding. Thus far no convincing abnormalities in genes coding for the chloride channel itself have been identified in human epilepsy (Planells-Cases and Jentsch, 2009).

1.5.3.1 GABRD

This gene codes for a subunit of the ligand-gated chloride channel for GABA. It has been mapped to the genomic locus 1p36 (Emberger et al, 2000; Windpassinger et al, 2002). Evidence for its expression has been demonstrated in the human cerebral cortex, cerebellum, and frontal, occipital and temporal lobes (Windpassinger et al, 2002).

The role of the GABRD channel lies in the modification of tonic inhibition of neuronal excitability. Previous studies (Brickley et al, 2001) concluded that the loss of such inhibition (for example, through abnormal function of the GABA receptor) leads to a change in normal neuronal behaviour due to a change in the magnitude of potassium conductance of the cell.

Clinically, mutations in the GABRD have been reported in association with GEFS+ and with idiopathic generalised epilepsy (Dibbens et al, 2004). However, although the original descriptions of this association showed that 8.3% of those with IGE had mutations in this gene (compared to 4.2% of controls) (Dibbens et al, 2004), a later study (Lenzen et al, 2005) did not show any association of the same mutation with IGE in a German population.

1.5.3.2 GABRA1

Unlike the GABRD receptor discussed above, the product of this gene is a GABA$_A$ receptor, a ligand-gated chloride channel. The GABRA1 gene lies at locus 5q34 (Johnson et al, 1992). Evidence for the gene's expression has been demonstrated in the human cortex (Garrett et al, 1988).

There have been reports of mutations in this gene being associated with epilepsy in extended families (Cossette et al, 2002; Lachance-Touchette et al, 2011) and in isolated individuals (Maljevic et al, 2006). Particular SNP variants within the gene have been reported in an Indian population as being associated with a susceptibility to epilepsy and drug resistance (Kumari et al, 2010).

1.5.3.3 GABRG2

This gene is another which codes for part of the GABA-A receptor. It has been mapped to the genomic locus 5q34 (Wilcox et al, 1992).

The involvement of mutations in this gene in the aetiology of human epilepsy was first described in 2001 (Baulac et al, 2001; Wallace et al, 2001). The former group reported a mutation in the GABRG2 gene in a family with a phenotype of GEFS+. The latter group reported a mutation in the gene in a single family with phenotypes of childhood absence epilepsy and febrile convulsions. Subsequently, mutations in the GABRG2 gene have been reported in association with phenotypes such as Dravet's syndrome (Harkin et al, 2002), GEFS+ (Fendri-Kriaa et al, 2009) and familial febrile seizures (Audenaert et al, 2006), in families from different populations. Screening studies have demonstrated an association between particular SNP variants in the gene and simple febrile convulsions (Salam et al, 2011) in an Egyptian population, but in contrast to this, no mutations were found in a screening study of an Italian cohort with childhood idiopathic generalised epilepsies (Orrico et al, 2009).

1.5.4 Acetylcholine receptor gene mutations

Acetylcholine (ACh) is a receptor perhaps best known for its role in the peripheral system and especially in the actions at the motor end-plate of the neuromuscular junction. However, it also has a significant role as a neurotransmitter in the central nervous system.

There are two main types of ACh receptor in the central nervous system (Graef et al, 2011). Muscarinic ACh receptors (mAChR) mediate their effects by coupling to particular G proteins. Different subtypes of mAChR have been identified. M1 is the most abundant type within the cortex and hippocampus, and M2 can be mainly found in the thalamus, brainstem and cerebellum (Graef et al, 2011).

The nicotinic ACh receptors (nAChR) are different to the mAChR described above, as they are part of ligand-gated ion channels which do not need a second messenger (such as the G proteins mentioned above) to mediate their effects (Graef et al, 2011). As such, they are a class of ion channel, similar to many of the others mentioned above. Each nAChR is composed of 5 subunits (belonging to α or β classes) arranged around a central pore, which is the ion-conducting part of the receptor (Steinlein and Bertrand, 2010). Again, numerous different nAChR subtypes have been identified in the central nervous system, and the composition of these varies. nAChR can be either homomeric (i.e., all subunits in the receptor are of the α type) or heteromeric (i.e., a combination of α and β subunits) (Graef et al, 2011; Steinlein and Bertrand, 2010).

Numerous genes have been identified which code for muscle and neuronal nAChRs (Albuquerque et al, 2009). Abnormalities in the genes controlling the synthesis of some classes of nAChR have been associated with particular epilepsy syndromes and other neurological illnesses.

1.5.4.1 CHRNA2

This gene has been mapped to the genomic locus 8p21.2 (Wood et al, 1995). Later work showed that this gene has a high degree of expression in the thalamus in humans (Aridon et al, 2006). Dysfunction of thalamic function has been implicated

in general with the initiation and progression of the abnormal synchronous electrical discharges associated with epileptic seizures in work in both animal models of epilepsy and some studies of human subjects (Meeren et al, 2005; Tyvaert et al, 2009; Chang et al, 2011).

The nicotinic ACh receptor coded for by this gene facilitates fast signal conduction at neuronal synapses, and it is clear that dysfunction of such neuronal conduction could be associated with disruption of normal cortical – subcortical networks associated with epilepsy (Marini and Guerrini, 2007).

There has been only one family reported where a mutation in this gene was found in association with an epilepsy syndrome (Aridon et al, 2006). The phenotype in this Sardinian family was compatible with autosomal dominant nocturnal frontal lobe epilepsy (Scheffer et al, 1995).

Autosomal nocturnal frontal lobe epilepsy (ADNFLE) is a syndrome which usually has an onset in childhood. Seizures usually arise out of sleep, and are characterised by a marked motor component (i.e, wandering, thrashing movements of limbs) (Gourfinkel-An et al, 2004). Imaging is usually normal in these individuals, and EEG during the events (although difficult due to the muscle activity) shows that the seizures have a focal onset (Scheffer et al, 1995), although the exact site can vary between different regions (Ryvlin et al, 2006). Seizures often respond to treatment with carbamazepine (Scheffer et al, 1995). The syndrome can show marked heterogeneity (Ryvlin et al, 2006), and seizures can persist into adult life. Although not a prominent feature of this condition, intellectual impairment has been reported as a part of the phenotype in individual cases or small series (Ryvlin et al, 2006).

In the isolated family reported thus far to have ADNFLE in association with a mutation in CHRNA2, not all individuals with the genetic mutation were affected, with 10 affected members of the pedigree manifesting the condition and one carrier remaining unaffected, indicating incomplete penetrance. The rarity of mutations in this gene as causes of nocturnal frontal lobe epilepsy has been emphasised by screening studies in cohorts from different populations (Gu et al, 2007; Combi et al, 2009), which have identified very few mutations associated with epilepsy.

1.5.4.2 CHRNA4

This gene has been mapped to the genomic locus 20q13.33 (Steinlein et al, 1994) after previous work had demonstrated that the gene was located on chromosome 20 (Anand and Lindstrom, 1992). It codes for a nicotinic ACh receptor.

An association between a mutation in this gene and autosomal dominant nocturnal frontal lobe epilepsy was demonstrated in a large Australian kindred initially (Steinlein et al, 1995). Mutations have since been identified in association with nocturnal frontal lobe epilepsy in families from multiple different populations (Hirose et al, 1999; Cho et al, 2003; Saenz et al, 1999; Steinlein et al, 1997). As in the mutations of CHRNA2, mutations in this gene probably result in disruption of normal cortical – subcortical networks, but mouse models have been created to investigate this particular aspect of the effects of mutations further (Klaassen et al, 2006).

1.5.4.3 CHRNB2

Coding for another nicotinic ACh receptor, this gene has been mapped to genomic locus 1q21.3 (Lueders et al, 1999) expanding on previous work which had refined the chromosome on which the gene was located (Anand and Lindstrom, 1992; Rempel et al, 1998).

Clinically, families have been described where the presence of a mutation in the CHRNB2 gene was associated with autosomal dominant nocturnal frontal lobe epilepsy (De Fusco et al, 2000; Phillips et al, 2001; Diaz-Otero et al, 2008). Some of these reports have identified unaffected carriers of these mutations, indicating an incomplete penetrance in these families. Other reported families have emphasised the phenotypic variability between affected carriers (Cho et al, 2008), including verbal memory deficits (Bertrand et al, 2005). There has been one previous report of a potential association between a mutation in this gene and non-familial nocturnal frontal lobe epilepsy in an individual identified in a screening study (Liu et al, 2011), although no other similar cases have been reported to date.

1.5.5 Calcium channel gene mutations

Calcium channels are widespread in numerous systems, including the central nervous system. There are a number of different subtypes of calcium channel, but the voltage-gated calcium channels are key in allowing changes in intracellular calcium levels in response to changes in membrane potential (Catterall, 2011). Such changes in calcium levels help to initiate many different physiological events, including the contraction of skeletal muscle in response to nerve stimulation (Tanabe et al, 1993), the secretion of hormones (Yang and Berggren, 2006) and synaptic transmission in neurons (Catterall and Few, 2008). There are ten different types of voltage gated calcium channel in mammals (Catterall, 2011), and these can be grouped into different classes. CaV1 and CaV2 channel types are activated by strong depolarisations, whereas CaV3 types are activated by weaker depolarisations (Minor and Findeisen, 2010).

The voltage-gated calcium channel is made up of multiple subunits, the pore of the channel being formed by the α-1 subunit (Minor and Findeisen, 2010). There are numerous different types of α-1 subunit, but also the function of this unit can be altered by other subunits which can be associated with it, including α2δ, β and γ (Catterall, 2000). All of these are coded for by different genes, and disruption of channel function due to mutations in these genes has been associated with different clinical presentations (Streissnig et al, 2010; Pietrobon, 2010).

1.5.5.1 CACNA1A

This gene has been mapped to the genomic locus 19p13.2 (Diriong et al, 1995), and has also been given the nomenclature CACNL1A4. It codes for the α-1A form of the alpha subunit in the voltage-gated calcium channel

Mutations in this gene have been described in families associated with familial hemiplegic migraine (Ophoff et al, 1996; Kors et al, 2003), episodic ataxia (Ophoff et al, 1996; Denier et al, 2001), and spinocerebellar ataxia type 6 (Craig et al, 2008).

Familial hemiplegic migraine is a rare disorder, with one previous study from a Danish population estimating the prevalence as 0.003% (Thomsen et al, 2002). The onset of attacks in this condition is usually in childhood. The frequency of attacks per year is variable and usually relatively low (three per year) (Terwindt et al, 1996). The attacks may have a preceding aura (which can be variable in character between individuals (Thomsen et al, 2002)), and are characterised by the gradual onset of motor deficit (varying in severity from clumsiness to full paresis) involving one or both sides of the body (Russell and Ducros, 2011). The attacks are usually associated with a migrainous headache. Studies of hemiplegic migraine (both sporadic and familial) have shown that the condition can occur in association with episodes of impaired consciousness, typical migraine with or without aura and epilepsy (Russell and Ducros, 2011).

Spinocerebellar ataxia (SCA) is not one condition in isolation but is a term used to refer to a group of heterogenous disorders which are of genetic origin and which are characterised by progressive loss of motor co-ordination, either in isolation or associated with other features (Solodkin and Gomez, 2012). There are numerous different subtypes of spincerebellar ataxia, and SCA type 6 has been associated with the expansion of a trinucleotide CAG repeat in the CACNA1A gene (Gazulla and Tintoré, 2007). Clinically, SCA type 6 is marked by onset usually in adult life and is associated with a slow progressive truncal and limb ataxia (Gazulla and Tintoré, 2007).

A previous study demonstrated that the presence of a particular SNP within the CACNA1A gene is significantly associated with idiopathic generalised epilepsy (Chioza et al, 2001). However, there are also well-described cases in which focal seizures occurred as part of the spectrum of the phenotype of individuals with familial hemiplegic migraine (Kors et al, 2004; Stam et al, 2008) and episodic ataxia associated with mutations in this gene. A review of the available cases of episodic ataxia with proven mutations in CACNA1A (Rajakulendran et al, 2010) demonstrated that 7% of these individuals also have epilepsy, a much higher risk than that of the background population.

Phenotypes associated with mutations in this gene are extremely variable. Sporadic cases have been well documented in which mutations in this gene have been associated with hemiconvulsion-hemiplegia-epilepsy syndrome (Yamazaki et al, 2011), hemiplegic migraine with epilepsy (Zangaladze et al, 2010) and the syndrome of early seizures and cerebral oedema after trivial head trauma (ESCEATHT) (Stam et al, 2009).

Hemiconvulsion-hemiplegia-epilepsy syndrome (Auvin et al, 2012) is a rare childhood syndrome. It is characterised by prolonged focal motor seizures in the context of a febrile illness, followed by the development of hemiplegia on the side affected. The age of onset is usually before the age of 4 years. After the resolution of the initial phase, most patients go on to develop epilepsy with spontaneous recurrent seizures. The exact pathological mechanisms underlying this condition, and most cases are sporadic (Auvin et al, 2012), but there have been cases reported in association with mutations in the CACNA1A gene (Yamazaki et al, 2011) and the SCN1A gene (Sakakibara et al, 2009), as well as cases in which it has been associated with mutations in genes for conditions such as L-2-hydroxyglutaric aciduria (Lee et al, 2006) and factor V Leiden (Scantlebury et al, 2002). Outcome is variable, but epilepsy surgery can be of benefit in controlling the seizures (Auvin et al, 2012; Kim et al, 2008).

ESCEATHT is a descriptive term, and a similar syndrome in children is recognised as juvenile head trauma syndrome (Haas et al, 1975). Both terms refer to the clinical phenomenon of a delayed deterioration after an apparent mild head trauma, often associated with seizures and unilateral cerebral oedema (Stam et al, 2009). The prognosis is usually good (Oka et al, 1977; Haas et al, 1975), although not universally so, and deaths have been reported (Snoek et al, 1984). The exact aetiology remains unknown, but a previous study examined the prevalence of mutations in the CACNA1A gene in those with ESCEATHT, given the relationship between this gene and familial hemiplegic migraine and that seemingly trivial head trauma can trigger attacks in those with familial hemiplegic migraine (Stam et al, 2009). Out of 13 patients included, three had the full ESCEATHT phenotype, with others having early post-traumatic seizures or cerebral oedema on imaging. This

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paper concluded that specific mutations in the CACNA1A gene could be responsible for a variable phenotype, including ESCEATHT.

1.5.6 Mutations in other classes of genes

Although the majority of genes which have been implicated in the aetiology of epilepsy to date have been involved in coding for ion channels, it would seem unlikely that this is the only way in which genetic mutations can cause epilepsy. The notion of epilepsy being exclusively a channelopathy has drawn criticism from some authors (Greenberg and Subaran, 2011), and genetic mutations which do not affect ion channel structure have been implicated in the aetiology of certain epilepsy syndromes. Some of these genes encode channels which are specific for the transportation of particular compounds into or out of the central nervous system, or proteins which are important in the development of particular parts of the central nervous system.

1.5.6.1 SLC2A1

This gene is perhaps more commonly known by its alternate name, GLUT1 (standing for glucose transporter 1). It was first cloned in 1985 (Mueckler et al, 1985) and is found at the genomic locus 1p34.2 (Wang et al, 2005).

The function of this gene is in the transport of glucose across the relatively impermeable blood-brain barrier into the central nervous system. The central nervous system accounts for a large amount of total body glucose utilisation, out of proportion to its overall size (Fehm et al, 2006). Because of the high requirements for glucose to meet this demand, specialised systems are needed to transport glucose to the central nervous system, such as the GLUT1 transporter. This transporter has also been shown to facilitate the transport of galactose, glucosamine and mannose (Augustin, 2010). The transporter is found in cells outside of the nervous system, having been shown to be expressed in erythrocytes (Augustin, 2010) and thyroid tissue, including thyroid carcinomas (Matsuzu et al, 2005).

Specifically in relation to epilepsy, a number of families have been reported in which mutations in the SLC2A1 gene are associated with epilepsy. The phenotypes reported have included seizures such as generalised absence, complex partial, myoclonic and generalised tonic-clonic (Klepper et al, 2001; Rotstein et al, 2010). More recently, mutations in this gene have been identified in families with idiopathic generalised epilepsy of different syndromes (Striano et al, 2012). It has also been associated with developmental delay and with movement disorders such as cerebellar ataxia, dystonia, paroxysmal exercise-induced dyskinesia and paroxysmal choreoathetosis / spasticity (Suls et al, 2008; Klepper et al, 2001; Weber et al, 2011).

The role of the SLC2A1 mutations in the aetiology of epilepsy gives a good example of how such genetic knowledge can give rise to changes in therapy. There are a number of reports of patients with such mutations whose seizures have shown a beneficial response to changes in diet, such as the ketogenic diet (Verrotti et al, 2012).

1.5.6.2 ATP1A2

This gene has been mapped to the 1q23.2 locus (Oakey et al, 1992). The gene codes for the alpha subunit of the ATP1A2 receptor (De Fusco et al, 2003). This class of receptors, the sodium – potassium – adenosine triphosphatases (Na^+, K^+, ATPases), are present in all tissue types in the body and help in maintaining the normal gradient between sodium and potassium across the cell membrane (Morth et al, 2009). During the usual function of this protein, three sodium ions are transported out the cell for every two potassium ions transported in (Morth et al, 2009). The Na^+, K^+, ATPases are composed of two subunits, alpha and beta. There are four different isoforms of alpha subunits (Shull et al, 1986; Shamraj and Lingrel, 1994), and isoform 2 predominates in skeletal muscle, and is also found in brain, heart and adipose tissue (Morth et al, 2009).

Most of the reported families with mutations in this gene have had a phenotype of particular migraine syndromes such as familial hemiplegic migraine (De Fusco et al, 2003) or alternating hemiplegia of childhood (Swoboda et al, 2004). However, there is considerable variability in the phenotypes within these families, and in the reports

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of these families, some individuals have had benign familial infantile convulsions (Vanmolkot et al, 2003), developmental delay or intellectual impairment (Jurkat-Rott et al, 2004) and epilepsy. A previous screening study in a series of patients with sporadic hemiplegic migraine revealed clinical features such as ataxia, epilepsy and intellectual impairment in individuals with mutations in ATP1A2 and CACNA1A (Riant et al, 2010).

Alternating hemiplegia of childhood (AHC) is a rare condition with an incidence of approximately 1 case per million in the population (Neville and Ninan, 2007). Clinically, the condition is marked by onset prior to the age of 18 months, with repeated episodes of hemiplegia and occasionally of quadriparesis (Mikati et al, 2000). It is associated with epilepsy, with up to half of all children affected by AHC also affected by epilepsy (Neville and Ninan, 2007; Kanavakis et al, 2003).

1.5.6.3 ARX

This gene is an example of an X-linked syndrome in human epilepsy, located as it is on the X chromosome at genomic locus Xp21.3 (Strømme et al, 2002). The product of this gene – aristaless-related homeobox (ARX) protein – is one of the homeodomain proteins (Bienvenu et al, 2002), which are known to play an important role in cerebral development (Gécz et al, 2006). Functional assessment of the effects of this gene has shown that, in the absence of the ARX protein, numerous genes cannot function properly (Fulp et al, 2008).

Clinically, mutations in the ARX gene have been associated with different epilepsy phenotypes. Abnormalities of cortical formation and neuronal migration have been described with these mutations, and are a good example of how a genetic abnormality can give rise to focal structural abnormalities.

The best characterised structural abnormalities in these cases have been lissencephaly (Kitamura et al, 2002) and agenesis of the corpus callosum (Kato et al, 2004). Both of these terms refer to structural abnormalities of the brain. Lissencephaly is a term used to cover a group of rare malformations which have in common the abnormal appearance if the brain associated with abnormal cortical

organisation, both of which are due to abnormalities in the migration of neurons during brain formation (Verloes et al, 2007). Two general types of lissencephaly are recognised; classical and cobblestone (Verloes et al, 2007). In variants of classical lissencephaly, the abnormality may be accompanied by abnormalities in other structures, such as agenesis of the corpus callosum. Classical lissencephaly has been reported in association with mutations in genes such as ARX, DCX and LIS1 (Liu, 2011). Most patients with lissencephaly have poor clinical outcomes, with many of them having severe intellectual impairment and dependency for daily activities (de Wit et al, 2011). Epilepsy associated with lissencephaly and other neuronal migration disorders is often intractable (Aicardi, 1994; Verloes et al, 2007; Liu, 2011).

Other phenotypes reported in association with ARX mutations have included epileptic encephalopathy (Kato et al, 2007), West syndrome (Strømme et al, 2002) and X-linked mental retardation (Bienvenu et al, 2002).

X-linked mental retardation is a general term used to describe significant intellectual impairment associated with abnormalities of function of genes located on the X chromosome or of the structure of the X chromosome itself. In total, approximately 300 genes have been associated with mental retardation (Inlow and Restifo, 2004), and over 60 X–linked mental retardation genes have been described (Ropers and Hamel, 2005), although the overall contribution of X-linked changes in the aetiology of mental retardation and human cognition is still debated (Humeau et al, 2009). Many of the genes on the X chromosome associated with mental retardation syndromes have been shown to have a function in brain development, synaptic function and neuronal migration (Vaillend et al, 2008; Humeau et al, 2009).

Movement disorders such as dystonia or chorea have occurred in specific families. There are also reports of abnormal development in structures other than the central nervous system, especially of the external genitalia (Kitamura et al, 2002; Kato et al, 2004). Some families carrying mutations in the ARX gene clearly show X-linked patterns of inheritance, i.e., males are affected but females are either mildly affected or are asymptomatic carriers (Kato et al, 2004; Marsh et al, 2009).

1.5.6.4 PCDH19

Another gene whose locus is on the X chromosome (at Xq22.1) (Dibbens et al, 2008), this gene codes for one of the protocadherin class of molecules. These are a class of cell-cell adhesion molecules (Dibbens et al, 2008; El-Amraoui and Petit, 2010), and they are primarily found in the nervous system (Halbleib and Nelson, 2006; Kim et al, 2011). Protocadherins, or more specifically, non-clustered protocadherins (Kim et al, 2011), have particular patterns of expression in the brain which seem to involve regions involved in circuit formation (Kim et al, 2011) and have critical roles in the formation of synapses between neurons (El-Amraoui and Petit, 2010). This specific gene was first cloned in 2000 (Nagase et al, 2000). Expression of this gene has been demonstrated in the developing human cortex (Dibbens et al, 2008).

To date, mutations in the PCDH19 gene have only been described in heterozygote females who have been affected with the syndrome of female-restricted mental retardation and epilepsy (also termed epileptic encephalopathy 9) (Hynes et al, 2010; Dibbens et al, 2008). In this syndrome, affected females have generalised epilepsy and developmental delay, but for some reason male carriers seem unaffected (there is a report of one affected male, but analysis of this individual revealed genetic mosaicism) (Depienne et al, 2009). The reasons for this are unclear, although some authors have advanced theories in this regard (Dibbens et al, 2008). Since the PCDH19 gene is subject to the phenomenon of X inactivation, the affected females are likely to be mosaics comprising PCDH19-negative and PCDH-19 wildtype cells. This tissue mosaicism may scramble cell-cell communication, which then manifests as epileptic encephalopathy. In males, a functional rescue was considered to occur by a gene on the Y chromosome, PCDH11Y (Dibbens et al, 2008).

1.5.6.5 LGI1

The product of this gene was first isolated from a glioblastoma cell line in 1998, (Chernova et al, 1998) but has since been shown to have a role in regulating glutamatergic synapse development (Anderson, 2010). The gene is located at the genomic locus 10q23.33 (Nobile et al, 2009). Although originally isolated from a

tumour cell line, a more recent review (Gu et al, 2005) concluded that evidence for the involvement of LGI1 in the aetiology of malignant gliomas was weak. Expression of the LGI1 gene has been demonstrated in all layers of the frontal and temporal cortices of the brain (Gu et al, 2002).

Mutations in this gene are well associated with the syndrome of autosomal dominant lateral temporal lobe epilepsy (ADLTE, also termed autosomal dominant partial epilepsy with auditory features). The term "lateral temporal lobe epilepsy" is used to emphasise that the seizures are felt to arise from the cortical layers on the lateral aspect of the temporal lobe, as distinct from seizures from the mesial temporal regions which can be associated with hippocampal sclerosis (Florindo et al, 2006). In seizures arising from the lateral temporal lobe, features such as auditory auras, vertiginous symptoms and aphasia are frequently seen (Michelucci et al, 2009). In temporal lobe epilepsy overall, lateral temporal lobe epilepsy accounts for a small percentage of all cases. Since the first description of ADLTE (Ottman et al, 1995), cases in different populations have been described, but the exact prevalence of ADLTE remains unknown.

At least 35 families with this condition have been reported from various populations (Kalachikov et al, 2002; Chabrol et al, 2007; Michelucci et al, 2009; Nobile et al, 2009), and mutations in LGI1 have been found in approximately 50% of reported families with this clinical syndrome. A previous review of the pathogenic mutations identified thus far in this gene did not show any obvious genotype / phenotype correlations (Nobile et al, 2009), and families have been reported in which there have been no identified mutation in the LGI1 gene, demonstrating the genetic heterogeneity of this syndrome (Chabrol et al, 2007).

1.5.6.6 CDKL5

This gene (an alternative title for which in some earlier papers was STK9) has been mapped to the Xp22.13 genomic locus (Montini et al, 1998). The protein product of this gene is widely distributed in all tissues, with particularly elevated levels in the brain and thymus (Lin et al, 2005). Although not entirely clear as yet, this gene is felt to play a role in the regulation of developmental processes.

Mutations in this gene have been associated with a number of clinical syndromes. Mutations have been associated with atypical Rett syndrome (Bienvenu and Chelly, 2006; Scala et al, 2005), severe infantile spasms (Tao et al, 2004; Weaving et al, 2004) and epileptic encephalopathy (Tao et al, 2004; Rosas-Vargas et al, 2008; Mastrangelo and Leuzzi, 2012). There has also been a report of a mutation in this gene occurring in an individual with Angelman syndrome (Russo et al, 2009).

Rett syndrome is a neurodevelopmental disorder which primarily affects females (Samaco and Neul, 2011). It is characterised by an intial stage of normal development, with the clinical features manifesting usually from the age of 6 months onwards. Over time, the affected child shows developmental regression, losing spoken language and hand skills, and subsequently developing hand stereotypies, autistic features and gait dysfunction (Weng et al, 2011). Although reported in association with mutations in CDKL5, the most common genetic association is with mutations in MeCP2 (Gadalla et al, 2011; Samaco and Neul, 2011). Given the usual finding of smaller brain volumes in these patients on pathological examination (Weng et al, 2011), when epilepsy arises in these individuals it is felt to be a symptomatic generalised syndrome.

The most frequent phenotype associated with mutations in this gene is that of early-onset seizures with intractable epilepsy, and often with a degree of intellectual impairment. The majority of cases reported thus far have been female, although male cases have also been reported (Elia et al, 2008; Weaving et al, 2004). Although the overall numbers of cases reported remain small, it has already been noted that the spectrum of epileptic disorders associated with this mutation is quite broad (Archer et al, 2006).

Angleman syndrome is more commonly seen in the setting of a chromosomal disorder such as deletion of a portion of the maternally-derived copy of chromosome 15 (Battaglia and Guerrini, 2005). However, 20 – 25% of cases are felt to be due to single gene mutations. The clinical condition is characterised by developmental delay, marked impairment of speech, ataxia of gait and behaviour marked by frequent laughter and excitability (Battaglia and Guerrini, 2005). Epilepsy is very

common in this condition, affecting up to 90% of all those with the syndrome (Zori et al, 1992).

1.5.6.7 STXBP1

This gene is located at the genomic locus 9q34.11 (Swanson et al, 1998). It encodes for a protein called Munc18-1, and studies both in rat models and in humans seem to suggest that this protein is needed for the release of vesicles containing neurotransmitters from the presynaptic membrane and that it also plays a role in facilitating vesicle docking in the postsynaptic membrane (Weimer et al, 2003; Wierda et al, 2007).

In humans, there have been reports that mutations in this gene are associated with early infantile epileptic encephalopathy (Saitsu et al, 2008; Hamdan et al, 2009), variably characterised by mental retardation, seizures of different types and spastic quadriparesis. However, the overall number of patients reported with such mutations has been small and no large pedigrees with this mutation have been reported in the literature.

1.5.6.8 EFHC1

This gene is located at the 6p12.2 locus (Suzuki et al, 2004), and has a widespread distribution within the central nervous system in an animal model. Experiments suggested that the protein encoded for by this gene has a role in enhancing calcium flow through particular channels (Suzuki et al, 2004). Individuals have been identified with mutations in this gene associated with idiopathic generalised epilepsy syndromes such as juvenile myoclonic epilepsy (Suzuki et al, 2004; Medina et al, 2008) and juvenile absence epilepsy (Stogmann et al, 2006).

However, it has been noted that these mutations have a reduced penetrance, as unaffected carriers have been reported in the affected families (Suzuki et al, 2004). It has also been noted that, for some of the mutations identified in these families, they have also been identified in control populations, leading to questions about whether such mutations are truly pathogenic (Stogmann et al, 2006). A previous screening

study for the presence of particular SNPs in the EFHC1 gene was carried out on a mainly Hispanic population of cases of juvenile myoclonic epilepsy and controls and did not show any evidence of association of the SNPs chosen with epilepsy (Bai et al, 2009).

A report of a family studied by whole exome sequencing has suggested that, contrary to the more benign epilepsy syndromes in the cases above associated with heterozygous mutations in the EFHC1 gene, homozygous mutations can be associated with intractable seizures and early mortality (Berger et al, 2012).

1.5.7 Genes with unconfirmed associations with epilepsy syndromes

Many candidate genes have been screened for possible associations with epilepsy. Unfortunately, replication of many initial promising studies has often been lacking, giving considerable difficulty in interpreting the significance of previous results. The following genes are three which had a strong initial association with epilepsy, but which was not replicated on subsequent tests.

1.5.7.1 PDYN

This gene (prodynorphin) has been mapped to the genetic locus 20p13 (Litt et al, 1988), and has also been termed enkephalin B or preproenkephalin B. Although more recognised in association with spinocerebellar ataxia type 23 (Bakalkin et al, 2010), a previous case-control study demonstrated an association between the frequency of certain alleles of this gene and non-lesional temporal lobe epilepsy (Stogmann et al, 2002). However, this was not confirmed on a further study (Tilgen et al, 2003).

1.5.7.2 ME2

This gene, which maps to locus 18q21.2 (Yanaihara et al, 2001), is a nuclear gene which codes for a mitochondrial enzyme. One previous study of this gene demonstrated that the presence of a particular SNP homozygously was associated with idiopathic generalised epilepsy (Greenberg et al, 2005). However, this finding

was not replicated in a case-control study in a different population (Lenzen et al, 2005) and the previously demonstrated association is awaiting replication from other sources and populations.

1.5.7.3 GRIK1

This gene is located at 21q21.3 (Eubanks et al, 1993). It codes for a subunit of the glutamate receptor, GLUR5. A previous study on the association of this gene with epilepsy suggested that particular allelic variants of the gene were significantly associated with juvenile absence epilepsy (Sander et al, 1997). However, subsequent sequencing of the gene in patients with juvenile absence epilepsy (including those whose families had shown linkage to the GRIK1 locus) did not demonstrate any significant difference in the frequency of the polymorphisms studied between cases and controls (Izzi et al, 2002). There has not been any replication of the original study demonstrating an association of this gene with any particular epilepsy syndrome.

1.6 Conclusions from review of the literature

As mentioned at the start of this chapter, the study of genetics in epilepsy is in a state of rapid and exciting expansion. It is likely that any list or textbook will quickly become outdated in light of the new information which is becoming available. Considerable work is on-going into the genetics underlying neuronal migration disorders (Spalice et al, 2009) and early-onset epileptic encephalopathies (Mastrangelo and Leuzzi, 2012), blurring the notion of "symptomatic" and "idiopathic" epilepsies (see section 1.1) further. After reviewing the available literature, we can conclude that –

1. Epilepsy is a common neurological condition with significant morbidity for individuals and significant costs for health care services worldwide

2. Epilepsy frequently has a genetic aetiology

3. Numerous different techniques have been used in an attempt to identify the exact nature of the genetic aetiology of epilepsy. However, many of these techniques have had significant limitations, either in methodology or in the results which have been obtained by their use.

4. Many of the genes identified thus far as being causative for epilepsy have been involved in the structure of ion channels, but an increasing number of "non-channel" genes are being recognised.

5. The use of the newest genetic assessment techniques such as whole exome sequencing seems to have great potential to identify the previously unknown genetic factors in the aetiology of epilepsy.

It is likely that previously unknown genetic abnormalities are causative for epilepsy in Irish families affected by epilepsy. The aim of this study is to use whole exome sequencing to identify such novel genetic abnormalities associated with epilepsy in

affected Irish families. These novel genetic abnormalities will act as candidate causal variants for further study. This will be achieved by –

- Identifying families with a strong family history of epilepsy from centres in Ireland
- Phenotyping in detail members of these families, both those who are affected by epilepsy and those who are unaffected
- Obtaining tissue from these individuals (either blood or saliva) for DNA extraction
- Using whole exome sequencing in particular families to search for novel genetic variantions which may be associated with epilepsy in these families

The methods used in this study are outlined in the next chapter (chapter 2).

2. Methodology

In this chapter, I outline the methods used to collect and analyse the data in this study.

2.1 Ethical approval

Recruitment for the study took place in three different centres in Ireland. The three centres were Beaumont Hospital and St. James's Hospital in Dublin and Cork University Hospital in Cork. Ethical approval was needed for the study in each of these centres.

- Ethical approval for the study in Beaumont Hospital was sought in 2005. This application was made to the local ethics committee and ultimately ethical approval was granted in January 2006 (Beaumont Hospital Ethics Committee application number 05/56 – "Genetic studies of familial forms of epilepsy in an Irish population"). Given the length of time between the initial granting of ethical approval and the start of the study, clarification was sought and received from the Beaumont Hospital Ethics Committee that the original ethical approval was still valid and that a new application was not needed.

- Ethical approval for the study in St. James's Hospital was sought after a consultant Neurologist in that institution suggested that some of the patients attending his clinic would be interested in participating. Ethical approval was sought from the joint Adelaide and Meath / St. James's Hospitals Ethics Committee. Ethical approval was sought on the basis of the original ethical application to the Beaumont Hospital Ethics Committee. The study protocol submitted was the same as that submitted to the Beaumont Hospital Ethics Committee (application number 05/56 – "Genetic studies of familial forms of epilepsy in an Irish population"). After review of this documentation, ethical approval was granted by chairperson's decision to expand the study to St. James's Hospital in January 2011.

- Ethical approval for the study in Cork University Hospital was sought after a consultant Neurologist there suggested that some of his patients would also be interested in participating. A new application for ethical approval was needed for the Cork University Hospital Ethics Committee. This was submitted in December 2010, and after initial review and a number of minor alterations, ethical approval was granted to expand the study to Cork University Hospital in April 2011 (Clinical Research Ethics Committee of the Cork Teaching Hospitals protocol "Genes and familial forms of epilepsy in the Irish population").

2.2 Identification of candidates

In an attempt to obtain the most robust results from the study, we sought to recruit families with a strong history of epilepsy. We defined a strong family history as being a family with two first degree relatives and one other individual (including relatives of the second or third degree) affected by epilepsy. We defined an epilepsy syndrome as any type of epilepsy. For recruitment, individuals who had febrile convulsions only were classed as having an epilepsy syndrome but individuals who had had acute symptomatic seizures only (such as may occur shortly after acute alcohol withdrawal or head trauma) were not. Family members did not have to have the same type of epilepsy syndrome as the proband for inclusion.

The recruitment protocol was different in Beaumont Hospital compared to the other centres. Potential index cases were identified by interrogating the epilepsy-specific Electronic Patient Record (EPR) system used in that hospital for individuals with a family history of epilepsy. From this list, individuals whose family history of epilepsy would be strong enough for inclusion in the study were identified. Given that the EPR is a new system and is not yet recognised as the record of note for patients attending Beaumont Hospital, these individuals then had to be contacted – either by meeting them in the Epilepsy Clinic or the Epilepsy Monitoring Unit in Beaumont Hospital or by telephoning them – to confirm that the family history in the EPR was correct. This was not part of the study per se, but formed part of the clinical care of the patients in that the emphasis was on correcting potential errors in the data contained in the EPR.

In both St. James's Hospital and Cork University Hospital, potential index cases were identified by the local consultant Neurologist. In these cases, the consultant Neurologist discussed participation in the study with the individual in question, and consent to be contacted was obtained from the patient at that stage. Contact details for the individual were forwarded to the investigator by the consultant Neurologist. We set out to recruit ten families who met the inclusion criteria.

2.3 Recruitment protocol

In all cases, once the family history had been confirmed with the individual by the investigator, the nature of the study was explained to the individual by the investigator and consent was sought to send the individual some background information about the project. If people agreed to receive this information, then a number of information leaflets were sent out to the individual. These were –

- A "Patient Information" leaflet. This was for the index case. This leaflet outlined the nature of the study, the goals behind it and what was involved in participating in the study. It also included the contact details for the principal investigator for the study.
- Three "Family Member Information" leaflets. These were for the other family members who may consider participating. Again, this leaflet outlined the nature of the study, the goals behind the study, why family members would need to participate, and what was involved in participating in the study.
- Three "Consent To Be Contacted" forms. These forms were for individuals who wanted to speak to one of the investigators more about the study, and were designed to allow interested individuals to ask the investigator to contact a particular contact number.

Once this information was sent out to the individual, a period of at least two weeks was allowed to elapse. This was designed to allow the index case and members of their family to consider whether or not they would agree to participate in the study

and to allow the investigators to contact any other individuals who wished to discuss the study further.

After this period, the investigators would contact the index case again. At this stage, if the index case and their family agreed to participate, then a date was fixed to meet the family in a location which was convenient for them.

2.4 Phenotype data

Although it was originally envisioned that the location to meet participants would be a local general practice surgery or similar healthcare building, in the course of the study it became apparent that the easiest place to meet families was in the house of one of the participants. This was more convenient for participants, as it was easier to find, involved less travelling and was a more relaxed and familiar environment. In all cases, separate rooms were available to ensure that confidentiality was maintained for all participants.

The investigator met all participants at a previously specified time and date. Prior to interviewing individuals, the investigator first clarified the family tree to identify all affected individuals, both participating and non-participating. Once this was done, the investigator would interview individuals in a separate room.

Prior to beginning the interview, the investigator explained to all individuals again the nature and goals of the study, what was involved in participating and how the information obtained by the investigator would be handled. In all cases, the investigator emphasised the voluntary nature of participation and that individuals were free to withdraw from the study at any stage. The consent form for the study was then explained to the potential participant by the investigator and time given for questions.

If the individuals were happy to participate, they were asked to sign a consent form, which the investigator then signed and dated in the participant's presence. In the case of children or where there was any potential doubt about an individual's ability to give valid consent, parents or guardians were asked to witness the explanation of the

study also and were invited to ask any questions they may have had. In all such cases, the parents or guardians were also asked to sign the consent form on the child's behalf.

All participants were then given a copy of the relevant information leaflet again with the contact details for the principal investigator. At all times, it was emphasised that individuals could withdraw from the study at any stage, without having to give a reason, by contacting the investigators and asking for their sample to be withdrawn. After this, the investigator conducted a focussed interview with each individual. This was conducted by means of a clinical history, and the information was collected in a semi-structured manner. Certain details in particular were sought from each individual, but the investigator was free to ask for other details if other issues emerged in the course of the interview. All individuals who participated were asked about the following –

1. Age

2. Handedness

3. Past medical history. All participants were asked about previous hospitalisations, previous operations and previous clinic attendances for any reason. All participants were also asked about any history of chronic or on-going health issues. If not specifically mentioned, all participants were asked about a history of –

- hypertension

- hypercholesterolaemia

- arthritis (of any type),

- diabetes mellitus

- migraine

- ischaemic heart disease

- stroke

- psoriasis and chronic skin conditions

- depression and mental health issues

All participants were asked about these conditions in particular because they are common medical conditions which are often not volunteered spontaneously by patients in interviews. Also, by seeking such details, participants were encouraged to think as thoroughly as possible, allowing for in-depth phenotypic detail to be collected. All participants were also asked about early risk factors for the development of epilepsy – previous head trauma with loss of awareness, meningitis, encephalitis, neonatal complications and febrile convulsions.

4. Allergies. The question was asked in an open manner to encourage participants to volunteer information about any allergies of any sort. If none were reported, participants were specifically asked about drug allergies.

5. Medications. A list of all regular medications being taken by the participant at the time of recruitment was taken. If doses were available, these were also recorded.

6. Previous anti-seizure medications. If the participants had been on such medications and could provide the information, the response to these medications and the reasons for discontinuation were also sought. Information was sought about any adverse effects of these medications also.

7. Family history. This information was particularly relevant for older participants and for the parents of index cases to complete the family pedigree.

8. Epilepsy history. For those individuals with an epilepsy syndrome, details were collected on the age of onset of the syndrome, possible precipitants for onset, and how the syndrome had evolved from that time. Collateral details

were sought from other family members if the participant could not remember relevant details, but only if the participant had agreed to this.

9. Seizure semiology. Details for clinical events were obtained from each affected participant and, when possible, the events were classified according to the ILAE classification for seizures.

10. Specific symptoms. In case individuals were having seizures which they did not recognise themselves, a number of symptoms were asked about specifically in all individuals, both those affected and seemingly unaffected by an epilepsy syndrome. All individuals were asked about –

- photosensitivity

- myoclonus

- isolated aura symptoms – unusual smells, unusual tastes, rising epigastric sensations, prolonged déjà vu, any other unusual experiential phenomena

- nocturnal tongue / cheek biting or eneuresis of unclear cause

- absence episodes

11. Previous investigations. All participants were asked about any previous imaging or EEG studies they may have had, and where and when these studies had been done.

2.5 Phenotyping

All phenotyping was performed by a clinician trained in the diagnosis and management of medical and neurological illnesses in general and epilepsy specifically (G.O'C.). Initial phenotypes were based on the clinical information available from study participants. Refinements of phenotypes or corrections in classification were made in light of other investigation results, when it was

appropriate to do so and when such information was available. All participant phenotypes were reviewed by a Consultant Epileptologist (N.D) to ensure accuracy and consistency.

2.6 Tissue sampling

All participants had samples taken for genetic analysis. Samples were either of saliva or of blood. Blood samples were drawn by the investigator using an aseptic technique. At least 3 mls of blood were collected in an EDTA blood collection bottle. Blood samples were transported on the day of collection to the RCSI laboratories in Beaumont Hospital. Samples were stored at +4°C prior to further processing for DNA extraction. Saliva samples were taken in the Oragene collection tube and 4 mls of saliva was collected in each case. Saliva samples were transported to laboratory on the day of collection and stored at room temperature prior to further processing.

2.7 DNA extraction

The extraction of DNA from saliva and blood samples was carried out as per the protocols of the RCSI Biobanking service.

2.7.1 Acknowledgement and accreditation

I am indebted to Dr. Mark McCormack, PhD in genetics in the RCSI, for information on the laboratory protocols used for DNA extraction. Work on the families in this study was performed in conjunction with Dr. McCormack. Gene mapping in pedigrees requires both clinical and genetic expertise, and this was the case in this study. Dr. McCormack provided the genetic / basic science lead whereas I provided the clinical / applied lead. The following sections are based on the protocols of the Biobanking laboratory in the RCSI.

2.7.2 DNA extraction from blood samples
For manual extractions of DNA from blood, the following two-stage protocol was used:

Stage 1:

- The blood sample was poured into a 50ml tube which was then filled to capacity with standard laboratory reagent (SLR) and left on ice for 15 minutes.
- This mixture was centrifuged at 3500 rpm for 15 minutes at 4°C.
- When complete, the supernatant was poured off and the pellet remaining was resuspended in 50ml of SLR once more. This mixture was then centrifuged for 10 minutes at 3500 rpm at 4°C.
- The supernatant from this second round of centrifuging was poured off and 3ml of Tris-EDTA 10:1/NaCl (0.4M) was added to the pellet of white blood cells. This was mixed by vortexing until the aggregates disappeared.
- At this point the sample contained buffy coat and could be separated into two aliquots. One aliquot of buffy coat was stored as a precautionary measure.

Stage 2:

- 500 μl of Proteinase K buffer solution (1mg / ml) was added to the white blood cell mixture.
- This was incubated at 65°C for 10 minutes in a shaking water bath. After this, the tube was transferred to an ice/water mix for 10 minutes.
- When chilled, 1 ml of 5M NaCl was added to the mixture and vortexed thoroughly.
- The tube was then placed back in the centrifuge for 15 minutes at 3000 rpm at 4°C.
- The supernatant was transferred to fresh 50ml tubes containing 5ml of isopropanol. The sample was centrifuged again for 15 minutes at 3500 rpm at 4°C, the supernatant was discarded and 1ml of 70% ethanol was added to the pellet.
- Both the pellet and the ethanol were carefully transferred into a new and labelled DNA storage tube and centrifuged for a final time at 10,000 rpm for 15 minutes.
- When complete, the supernatant was carefully discarded the and the pellet was left to dry for 30 minutes before resuspending in 1ml TE buffer. The DNA concentration was obtained using a Nanodrop spectrophotometer

device (ThermoFisher). The DNA solution was then stored in a -20°C freezer.

2.7.3 DNA extraction from saliva samples

- The Oragene/ saliva sample was mixed by inversion and gentle shaking for a few seconds.
- The sample was then incubated at 50°C in a water incubator for a minimum of 1 hour. After this, the entire sample was transferred to a 15 ml centrifuge tube.
- 1/25th volume Oragene Purifier was then added to each tube and mixed by vortexing for a few seconds. The volume of Oragene Purifier to be added was calculated by dividing the volume of the sample by 25.
- This sample was incubated on ice for 10 minutes and then centrifuged at room temperature for 10 minutes at a minimum of 2,500 g.
- The majority of the clear supernatant resulting from this step was transferred with a pipette to a fresh 15 ml centrifuge tube.
- An equal volume of room-temperature 95-100% ethanol was added to the supernatant and mixed by inversion.
- The sample was allowed to stand at room temperature for 10 minutes to allow the DNA to fully precipitate then centrifuged at room temperature for 10 minutes, again at a minimum speed of 2,500 g.
- After this step, the supernatant was discarded, leaving behind a DNA pellet.
- The DNA pellet was then dissolved in 1 ml of DNA solvent (TE buffer) and transferred to a 1.5 ml microcentrifuge tube to allow complete rehydration.
- The rehydrated DNA was centrifuged at room temperature for 15 minutes at 15,000 g

The supernatant was then transferred to a fresh 1.5 ml microcentrifuge tube without disrupting the pellet.

2.8 Whole exome sequencing

After the DNA extraction process, samples were sent for further analysis. Whole exome sequencing was performed for all selected samples in the Genomic Analysis Facility, Duke University Center for Human Genome Variation, Duke University, North Carolina, USA.

2.8.1 Acknowledgement and accreditation

Again, I am indebted to Dr. Mark McCormack, PhD in genetics, RCSI, for information on the protocols for whole exome sequencing and for the information provided on the methods followed in selection of candidate variants. The following sections are based on the laboratory protocols in the Duke University Center for Human Genome Variation, which in turn evolved from those supplied by Illumina Inc., San Diego, and on the work carried out by Dr. McCormack in this study. I have drawn on work from Dr. McCormack's PhD thesis in this section, with his permission.

2.8.2 Summary of protocol

In each family in which we performed whole exome sequencing, the DNA from the proband in that family and at least one other distant affected family member were selected for assessment. This strategy reduces the possible candidate disease loci. Samples from family A, D, F and H were selected for whole exome sequencing. Different methods were used for analysis in family A compared to the other families.

- Samples from Family A were enriched with Agilent's SureSelect All Exon 38Mb capture array. This system captures genomic material of interest for analysis in a next generation sequencing system (Asan et al, 2011).
- Samples were subsequently sequenced on an Illumina Genetic Analyzer IIx. This kit captures >37Mb of the genome (approximately 1.22%) and includes NCBI Consensus CDS database (CCDS) regions, >700 human miRNAs from

the Sanger v13 database and >300 additional human non-coding RNAs such as snoRNAs and scaRNAs.

- The NCBI Consensus CDS database is a collaborative effort to identify a core set of human protein coding regions that are consistently annotated and are of high quality (Harte et al, 2012). Annotation of genes is provided by multiple public resources, using different methods, and resulting in information that is similar but not always identical. The human genome sequence is sufficiently stable to identify those gene placements that are identical, and to make those data public and supported as a core set by public genome browsers. The long term goal is to support convergence towards a standard set of gene annotations.

Samples from Families D, F and H were sequenced on an updated array.

- Samples from these families were sequenced on the Agilent SureSelect All Exon 50Mb capture array. This kit builds upon the previous version (used in the analysis of family A samples) by encompassing coding exons annotated by the GENCODE project, all exons annotated in the consensus CCDS database (as of March 2009) as well as 10 base pairs of flanking sequence for each targeted region. In addition, the content contains small non-coding RNAs from the miRBase and Rfam projects.
- The resulting genomic material was then analyzed on the Illumina HiSeq2000 next generation sequencer (Lim et al, 2012).
- The GENCODE project is a subproject of the ENCODE consortium (Harrow et al, 2012). The aim of the GENCODE project is to annotate all evidence-based gene features in the entire human genome at high accuracy.

After enrichment, the genomic samples were further analysed as follows.

- One microgram of genomic DNA was fragmented by nebulization. In this process, a fine mist is created by forcing a DNA solution through a small hole in the nebulizer unit and then collected. Nebulisation requires only small

amounts of DNA (0.5-5µg), and the resulting DNA fragments are distributed over a narrow range of sizes (700-1330 bp).

- The fragmented DNA was repaired. Because DNA fragmentation does not result in homogeneous, blunt-ended fragments, end repair is needed to ensure that each molecule is free of overhangs, and contains 5' phosphate and 3' hydroxyl groups.
- An 'A' was ligated to the 3'end of the DNA. For Illumina libraries, incorporation of a deoxyadenosine 5'-monophosphate (dAMP) onto the 3' end of blunted DNA fragments, a process known as dA-tailing, is necessary.
- Illumina adapters were then ligated to the fragments.
- The sample was size selected aiming for a 350–400 base pair product.
- The size selected product was PCR amplified.
- The final product was validated using the Agilent Bioanalyzer. This platform provides quantitation and quality control for DNA, RNA and proteins from next generation sequencing studies (Panaro et al, 2000; Sodowich et al, 2007).

Samples were then amplified on the flow cell and sequenced using the Genome Analyzer IIx, following the Illumina supplied protocols. The majority of sequence runs were paired end with 75 base reads in preparation for variant calling.

2.8.3 Variant calling

As described in the earlier section on next generation sequencing and whole exome sequencing (see section 1.4.4), once the sequence of a particular sample has been determined, this must then be further analysed to see how the called sequence compares to the public reference. Such variants compare to reference genotypes. In this study, the identification of specific variants – variant calling – was performed with SAMtools software.

The Sequence Alignment / Map (SAM) format is a generic alignment for storing read alignments, such as those produced by the Illumina Infinium II assay, against reference sequences (Li et al, 2009). The SAM format has been used in international

efforts such as the 1,000 Genomes Project (Li et al, 2009) and other large-scale sequencing projects, and the accompanying SAMtools software is well recognised for the purposes of analysing whole exome sequencing results.

2.8.4 Selection of candidate variants

After identification of the variants in the samples analysed by WES, further analysis is needed to identify candidate variants which may be associated with the disease under investigation (in this study, epilepsy).

SVA (SequenceVariantAnalyzer) was used to identify variants of interest. SVA is a software tool that assigns a predicted biological function to variants identified in next-generation sequencing studies and provides a browser to visualize the variants in their genomic contexts (Ge et al, 2011). The filtering criteria used were –

- single nucleotide variant (SNV) quality (a measure of the probability that the variant identified is an accurate call)
- SNV consensus score (a measure of the likelihood of a particular variant being present at a site from the alignment of multiple sequences)
- insertion-deletion (Indel) consensus score (a measure of the likelihood of a particular variant being present from the alignment of multiple sequences)
- Indel quality (a measure of the probability that the indel identified is an accurate call)
- number of reads supporting SNV or Indel ≥3 as standard (i.e. a minimum of three reads should support the SNV or Indel call).

All called variants with less than 10x coverage were eliminated, to ensure that the variants included were as accurate as possible. The term 10x coverage means that the genome at that site was sequenced at least ten times, which improves the consensus between sequencing runs and makes miscalling at that site less likely.

To establish that the variants identified were novel, previously genotyped populations were needed for comparison. Dr. Erin Heinzen, PhD, Assistant Research

Professor in the Duke Centre for Human Genome Variation, provided a set of 395 screened neurologically normal controls for the computation of minor allele frequency (MAF). This was enhanced by population sequence data from 5,400 unscreened individuals of European-American (n=3,500) and African-American (n=1,900) ethnicity on the Exome Variant Server (EVS) database, maintained by the University Of Washington National Heart, Lung, and Blood Institute (NHLBI) Grand Opportunity (GO) Exome Sequencing Project (ESP), (Seattle, WA) (*http://evs.gs.washington.edu/EVS/*, figures accurate as of April 2011). In this dataset all variants had site coverage of at least 10x (i.e., the site had been sequenced at least ten times) and a quality score of at least 30 to ensure confident calling. The Phred quality score is a tool used to assess the quality of the sequences at a site, and in this case utilies PhyloP, a freely-availaible computer programme which employs four different methods of measuring phylogenetic conservation to generate this score (Pollard et al, 2009). The quality score is logarithimic. A score of 10 means that there is a one in ten chance of a miscall and 90% base calling accuracy, but a score of 30 means that there is a one in one thousand chance of a miscall and 99.9% base calling accuracy.

A threshold of <1% MAF from the EVS data was applied to remove common variants. Where the EVS database reported zero or unavailable genotype data for a variant, the coverage at this location was checked on the EVS website. If a site was covered sufficiently (i.e., coverage of 10x or greater) then the variant was considered novel (0% EVS MAF). However, if the site was not covered sufficiently, the variant frequency could not be established from EVS. If this was the case, the MAF in the other screened controls was considered.

Based on a hypothesis of damaging mutations (i.e., "functional" variants) impacting upon protein-coding genes, all "non-functional" variants were removed (including those in promoter, enhancer and intronic regions). Ensembl Variant Effect Predictor (VEP) was used to predict the effect of amino acid changes on a protein and the impact of individual insertions and deletions (McClaren et al, 2010) (*http://www.ensembl.org/info/docs/variation/vep/index.html*). VEP can be used to determine the effect of the variants identified on genes, transcripts, and protein sequence, as well as regulatory regions. The co-ordinates of any variants and

nucleotide changes found can be assessed to find out the genes affected by these variants, the location of the variants and the consequence of these variants on protein sequence. This tool also analyses for known variants which match those found, and the associated minor allele frequencies. Priority was given to variants that were predicted to be damaging, i.e., variants known to disrupt an essential splice site, variants that targeted the transcript for nonsense decay, and nonsynonymous variants predicted by Polyphen-2 to disrupt the protein-coding sequence with high confidence. Polyphen-2 is a program incorporated into Ensembl VEP that computationally predicts the impact of amino acid substitutions on protein function (Adzhubei et al, 2010).

In short, the steps involved in the selection of candidate variants can be summarised as follows –

1. The variants which are shared between affected individuals are identified
2. The non-functional variants are removed, leaving shared functional variants for analysis
3. Common variants are then removed
4. Rare novel variants are identified by comparison with screened controls and with unscreened population controls
5. The novel functional rare variants selected by this process can be then assessed for possible causative effects in epilepsy.

For all variants identified as potentially causal in families, Sanger sequencing was performed subsequently to confirm the findings of whole exome sequencing.

3. Results

The results of the data collected in the course of the study are presented in this section. The first section will deal with the overall results. This will be followed by the data for each family recruited. The family data will be presented as the family pedigree, followed by the detailed phenotypic information for each individual recruited from that family, and then details given about individuals who did not participate but for whom some phenotypic information is available.

3.1 Overall results

3.1.1 Rate of recruitment

A total of 96 families were identified over the course of the study which met the criteria for inclusion. At the end of recruitment, a total of 12 families had participated in the study. Thus, 12.5% of families identified had been included in the study.

In the case of one family (family J), at the end of the recruitment period for the study only one individual from the family has been recruited. As this did not meet the criteria for inclusion, data from this family were excluded from the subsequent analysis.

3.1.2 Reasons for non-participation

There were a number of reasons for families being excluded or excluding themselves from the study (see figure 1 on page 111 for summary).

The family history was inaccurate or out of date in 28 cases (29.2%). In most cases, this related to the index case not having an affected first degree relative, or to the affected first-degree relatives being dead.

In 8 of the 96 families (8.3%), the index case was unwilling to participate. The reasons for unwillingness were not systemically recorded. Very few individuals mentioned the genetic aspect of the research, and most did not volunteer the reasons for not participating. In two cases, index cases changed their minds after receiving the information leaflets about the study but without offering any further reasons.

Unwillingness of family members to participate in the study was the reason for failure to recruit a further 9 families (9.4%). Some of this reflected conflict within

families for different reasons, but in most cases a specific reason was not given. In two of these nine cases, index cases who were willing to participate specifically mentioned that their relatives did not wish to take part in the study as there was an unwillingness to discuss epilepsy within the larger family. However, in the absence of talking to other family members, it cannot be verified that the stigma attached to a diagnosis of epilepsy was definitely to blame in these or other families.

Initial contact or follow-up contact could not be made with 22 of the 96 identified index cases identified (22.9%). In the majority of these cases (18 of the 22), initial contact could not be made due to inaccuracy of the contact details for index cases in the official medical records. The other four cases were contacted initially and agreed to receive information leaflets about the study but did not reply to further enquiries and did not return any information to the investigators.

Index cases were found to be unable to consent to involvement in the study in six cases (6.3%). In all cases, this was due to an undocumented learning disability in these individuals which impaired their ability to understand the purpose of the study and the need for procedures such as phlebotomy.

Other causes were found for failure to recruit the remaining 11 (11.5%) identified index cases. In three cases, the index case and their family were agreeable to participate, but an appointment for data collection could not be arranged. In five cases, the index case had received the information leaflets for the study and follow-up contact had yet to be made. In the remaining three cases, the index case or their family members were abroad and, although agreeable to participate, could not travel for an appointment to be recruited.
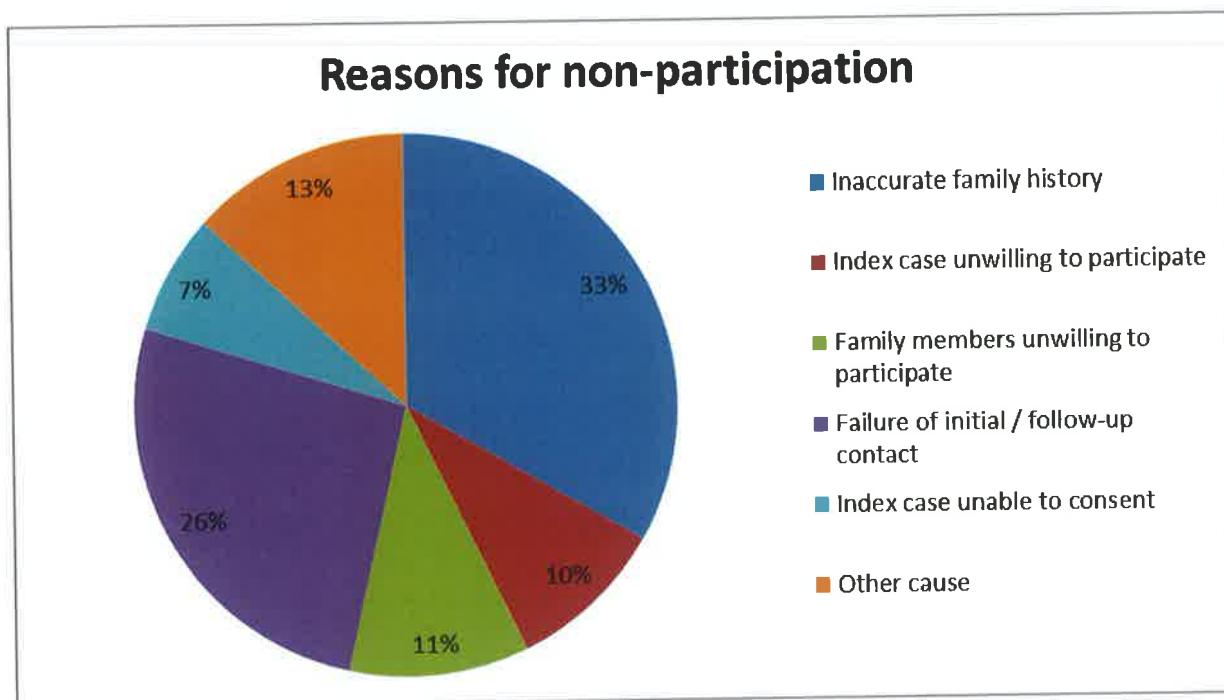
Figure 1. Reasons for non-participation of individuals in the study

78 index cases were contacted about recruitment during the course of the study. This figure excludes those with whom initial contact could not be made (18) but includes those with whom initial contact was made but not subsequent contact (4). The overall rate of completed recruitment for contacted families was 15.4%.

3.1.3 Number of participants

A total of 110 participants were recruited from the 11 families, with a mean number of 10 individuals per family. There was considerable variation in the numbers recruited in each family, from 4 to 21. The modal number of participants from each family was seven.

3.1.4 Epilepsy syndromes in participants

Of the total number of participants, 46 (41.8%) were affected by an epilepsy type or seizure type (i.e., one of the following - localisation related epilepsy, idiopathic generalised epilepsy, acute symptomatic seizures only, febrile convulsions only) (see figure 2, page 112). Of these, all could be classified into one of these four phenotypes apart from two individuals (4.3% of those affected with an epilepsy syndrome). Two participants had acute symptomatic seizures only and seven had

febrile convulsions only at the time of recruitment. Twenty-five individuals had a form of localisation related epilepsy and ten had some form of idiopathic generalised epilepsy.
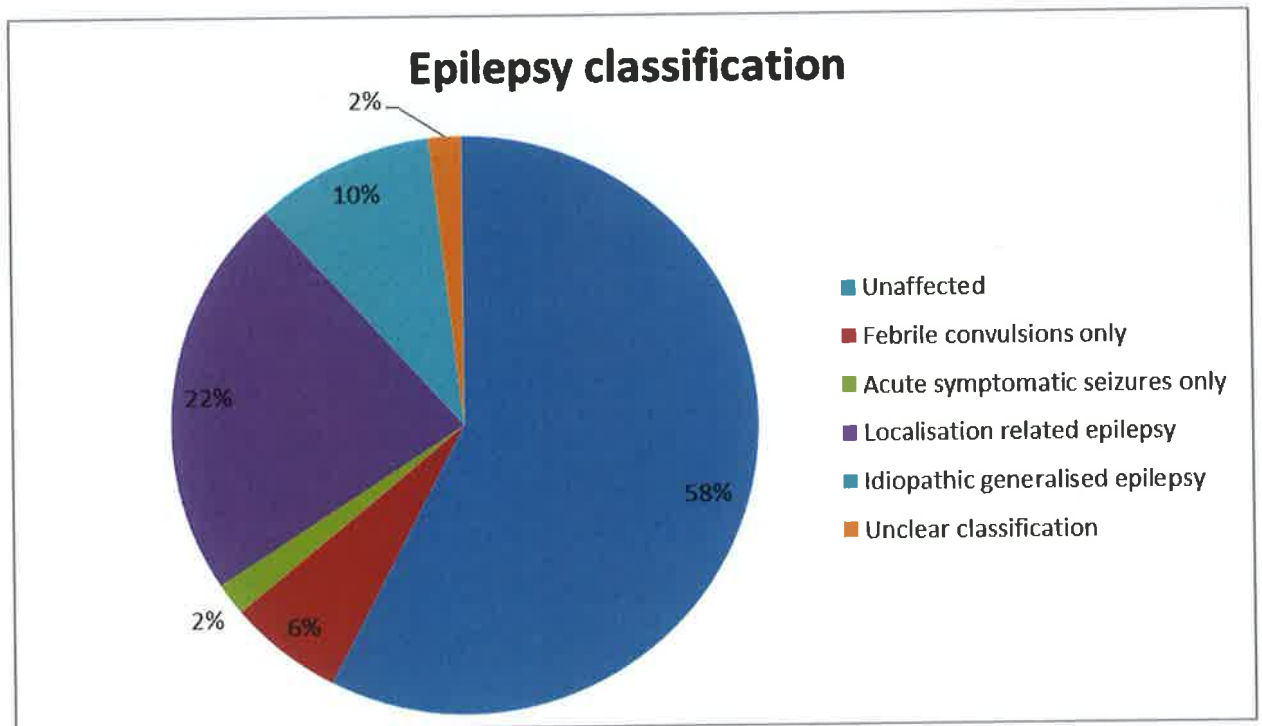
Figure 2. Syndromic classification of study participants

It was considerably more difficult to classify affected individuals into specific epilepsy subtypes (such as the different forms of idiopathic generalised or localisation related epilepsy mentioned in section 1). Of the affected individuals, only 24 could be definitely assigned into specific ILAE subtypes. Of these, temporal lobe epilepsy was the commonest, with 8 individuals affected (see figure 3, page 113). Other specific syndromes encountered included frontal lobe epilepsy (1 individual) and juvenile myoclonic epilepsy (5 individuals) (see figure 4, page 113). However, as shown below, in many cases the specific epilepsy syndrome could not be clarified.
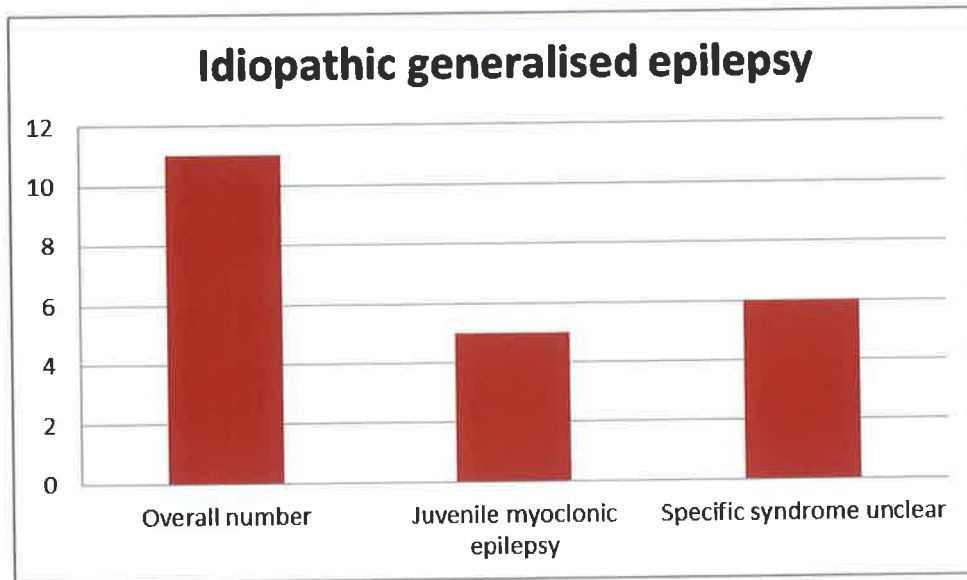
Figure 3. Specific idiopathic generalised epilepsy syndromes seen in participants
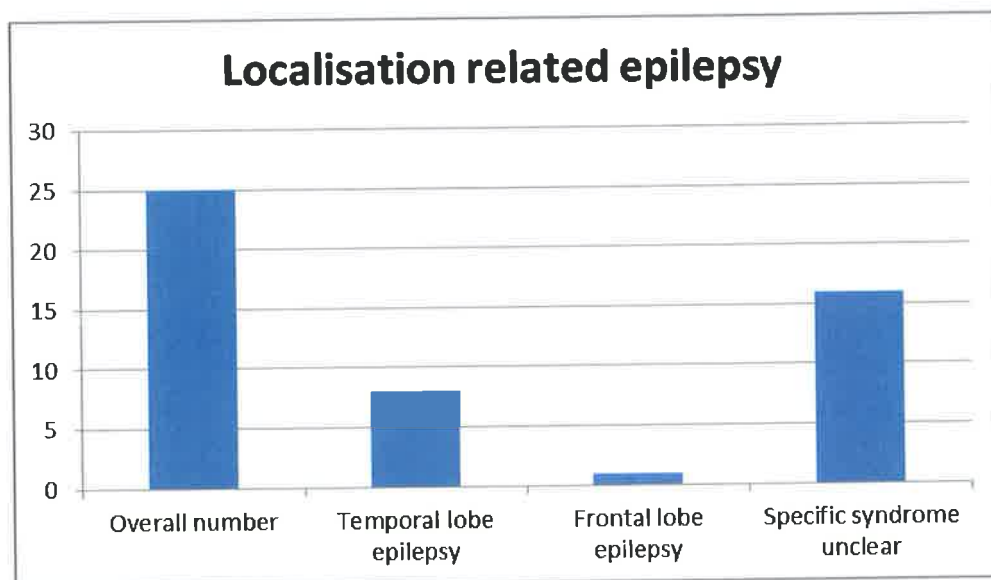
Figure 4. Specific localisation related epilepsy syndromes seen in participants

3.1.5 Early risks for epilepsy in participants

In the course of interviewing participants, information was collected on the presence or absence of risks factors for developing epilepsy. These risks factors were previous meningitis, previous encephalitis, previous concussion with loss of awareness, occurrence of febrile convulsions and the occurrence of neonatal complications. All individuals had a family history of epilepsy, and this was not classed as a separate risk factor for the participants in this study.

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Of the 110 participants, 25 (22.7%) had early risks for the later development of epilepsy. It should be noted that of these 25, at the time of recruitment 8 had not developed any form of epilepsy syndrome (32% of those with one of these early risks).

The commonest early risk factor in the participants in this study was the occurrence of febrile convulsions (16 individuals). Of these 16 individuals, 7 had had febrile convulsions only at the time of recruitment without any further seizure types. The other early risks identified were previous concussion (7 individuals) and previous meningitis (2 individuals).

3.1.6 Gender, handedness and age of participants

57 of all participants (51.8%) were female. There was a difference in the percentage of males and females affected – of 57 females, 30 (52.6%) were affected, compared to 17 (32.1%) out of 53 males.

Handedness was assessed in all participants. Four participants were ambidextrous (3.6%), six were left handed (5.4%) and 93 (83.8%) were right handed. Handedness in the remaining seven individuals was either unclear or was probably but not yet to be definitely lateralised, due to the age of the participants.

The age of participants varied greatly, with the youngest participant being 1 year and 8 months old, and the oldest being 91 years at the time of recruitment. The mean age of participants was 35.8 years.

3.1.7 Concordance of syndromes and seizures

Families are defined as concordant if all affected relatives have the same clinical features (Kinirons et al, 2009). To assess the degree of concordance in the families included, assessment was made on the principal epilepsy type in the proband – idiopathic generalised epilepsy (IGE) or localisation related epilepsy (LRE). After this, we assessed for concordance in specific epilepsy syndrome (juvenile myoclonic epilepsy, temporal lobe epilepsy, etc.), seizure type (myoclonic seizures, secondarily generalised tonic – clonic seizures, etc.) and in the clinical features of the epilepsy syndromes in families (such as age of onset, particular triggers for seizures, co-morbidities, etc.).

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3.1.7.1 Concordance in families – proband with IGE

In three families, the proband's epilepsy type was an IGE syndrome. However, none of these families were concordant in the epilepsy type amongst other affected individuals, with family A having 28.6% (2/7) with an IGE syndrome, family B having 66.7% (6/9) and family E 40% (2/5) of affected members having an IGE syndrome also.

In family A (see figure 6, page 130), the proband and the other sibling with IGE both had juvenile myoclonic epilepsy, and both had the same seizure types (absence, myoclonic and generalised tonic-clonic). The EEG patterns in these two individuals were also concordant, in that they showed generalised changes with some focal features in the frontal regions. However, this is very different to the other 5 affected individuals, all of whom had LRE. In these individuals, there was no concordance in the specific syndrome, the seizure types, the clinical features such as the age of onset, or the EEG patterns in the three individuals from whom the results were available.

In family B (see figure 7, page 133), the proband, her siblings and her mother all had IGE, all were female and all had had simple febrile convulsions. However, although the proband had juvenile myoclonic epilepsy, it was not possible to classify the IGE type in the other individuals. Although all these individuals had generalised tonic-clonic seizures, the seizure types were otherwise discordant (3 had absence seizures, 2 myoclonic, 1 atonic) and the clinical features (such as age of onset) were also discordant. The proband's father had LRE, and two of the third generation of the family had febrile convulsions only. However, of the nine individuals affected in this family, eight (88.9%) had had simple febrile convulsions.

In family E (see figure 10, page 142), the proband's niece was the only other of the five affected individuals with IGE. The EEG in the proband and this individual showed generalised 3Hz epileptiform discharges. However, in all nearly all points, these two individuals were discordant, with different IGE types, ages of onset, and seizure types. The other affected individuals were discordant in all of the points of analysis.

3.1.7.2 Concordance in families – proband with LRE

In eight families, the proband's epilepsy type was a LRE syndrome. The concordance rate in families was more marked in this group. Family C (3/3), family F (3/3), family G (2/2), family H (3/3) and family L (2/2) are all concordant in that all affected individuals had LRE. For other families with a proband with LRE, in family D 40% (2/5) of affected individuals also had LRE, in family I the figure was 66.6% (2/3) and in family K it was 25% (1/4).

In family C (see figure 8, page 136), the concordance is the most marked of all families in the study, with the proband and other affected individuals having the same epilepsy syndrome, same seizure semiology, same trigger factors for seizures, same age of onset and same gender. The clinical details provided by the proband's parents on the deceased sibling allowed us to infer that he also had the same features.

Family H (see figure 13, page 150) is similar to family C in that the epilepsy type is the same in all the affected individuals. However, the age of onset is more variable (6 months – 3 years) and the clinical semiologies differ between individuals (all of whom have simple or complex partial seizures with secondarily generalised tonic-clonic events). The EEG available in two affected individuals shows the same pattern of focal changes in an area to the right of the midline.

Although all affected individuals in family F have LRE (see figure 11, page 145), the clinical features are more discordant on closer inspection, with the affected individuals having different ages of onset, different seizure types (complex partial, secondarily generalised tonic-clonic versus secondarily generalised tonic-clonic only) and different epilepsy severity. As only one of the individuals had an EEG, electrophysiological criteria cannot be used to distinguish the reason for this difference.

Only one individual in family K has LRE (see figure 15, page 155). However, all four affected individuals in this family have had simple febrile convulsions, and it unclear if the differences between the proband and his children will be eliminated over time, as the onset of epilepsy in the proband was at an age older than the participants at their recruitment and the proband was also affected by febrile convulsions in childhood.

Family D (see figure 9, page 139) and family I (see figure 14, page 153) show considerable intrafamilial discordance in epilepsy types, seizure types and clinical features of the conditions seen. Family G (see figure 12, page 147) shows concordance in the epilepsy type and seizure type in the affected individuals, but the clinical features (age of onset, clinical course, etc.) are considerably different between individuals.

Table 1 below shows the concordance rates within the families in this study for a number of features assessed.

Table 1. (page 118) Concordance within study families. In each family, the percentage figures (in brackets) refer to the percentage of affected individuals within the particular family. LRE: localisation related epilepsy, IGE: idiopathic generalised epilepsy, TLE: temporal lobe epilepsy, FLE: frontal lobe epilepsy, JME: juvenile myoclonic epilepsy, SPS: simple partial seizure, CPS: complex partial seizure, GTC: generalised tonic-clonic seizure, 2°GTC: secondarily generalised tonic-clonic seizure.

Family	Epilepsy type	Number (%)	Epilepsy syndrome	Number (%)	Seizure types	Number (%)3	EEG features	Number (%)	Age at onset	Number (%)
A	IGE	2 (28.6%)	JME	2 (28.6%)	Absence	2 (28.6)	Generalised and focal epileptiform changes	2 (28.6)	1 - 10 years	2 (28.6)
	LRE	5 (71.4%)	LRE - type unclear	5 (71.4%)	Myoclonic	2 (28.6)	Normal	1 (14.3)	11 - 18 years	2 (28.6)
					GTC	2 (28.6)	Focal epileptiform changes	2 (28.6)	19 - 24 years	2 (28.6)
					SPS	2 (28.6)	Not performed	2 (28.6)	25 - 50 years	1 (14.3)
					2° GTC	3 (42.9)				
					Unclear	2 (28.6)				
B	Febrile convulsions only	2 (22.2)	Febrile convulsions only	2 (22.2)	Simple febrile convulsions	8 (88.9)	Not performed	5 (55.6)	<1 year	1 (11.1)
	IGE	6 (66.7)	JME	1 (11.1)	Myoclonic	2 (22.2)	Result unavailable	1 (11.1)	1 - 10 years	3 (33.3)
	LRE	1 (11.1)	IGE - type unclear	5 (55.6)	Absence	3 (33.3)	Generalised epileptiform discharges	1 (11.1)	11 - 18 years	4 (44.4)

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C	LRE	3 (100)	TLE	1 (11.1)	Atonic	1 (11.1)	Generalised slowing	1 (11.1)	> 50 years	1 (11.1)
					GTC	6 (66.7)	Non-specific temporal slowing	1 (11.1)		
					SPS	1 (11.1)				
					2° GTC	1 (11.1)				
			LRE - type unclear	3 (100)	CPS	3 (100)	Normal	1 (33.3)	11 - 18 years	3 (100)
					2° GTC	3 (100)	Result unavailable	2 (66.7)		
					Simple febrile convulsions	1 (33.3)				
D	Febrile convulsions only	1 (20)	Febrile convulsions only	1 (20)	Simple febrile convulsion	1 (20)	Not performed	1 (20)	1 - 10 years	2 (40)
	Symptomatic seizures only	1 (20)	Acute symptomatic seizure	1 (20)	GTC	1 (20)	Result unavailable	2 (40)	11 - 18 years	2 (40)

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Classification	n (%)	Subtype	n (%)	Seizure type	n (%)	EEG	n (%)	Age	n (%)
LRE	2 (40)	TLE	1 (20)	SPS	2 (40)	Bilateral focal epileptiform discharges	1 (20)	25 - 50 years	1 (20)
Unclear	1 (20)	LRE - type unclear	1 (20)	CPS	2 (40)	Normal	1 (20)		
		Unclear	1 (20)	2° GTC	1 (20)				
				Unclear	1 (20)				
E IGE	2 (40)	JME	1 (20)	Simple febrile convulsion	2 (40)	Generalised epileptiform discharges	2 (40)	1 - 10 years	3 (60)
LRE	1 (20)	IGE not otherwise specified	1 (20)	Absence	1 (20)	Result not available	1 (20)	11 - 18 years	1 (20)
Febrile convulsions only	1 (20)	LRE - type unclear	1 (20)	Myoclonic	1 (20)	Not performed	2 (40)	> 50 years	1 (20)
Unclear	1 (20)	Febrile convulsions only	1 (20)	GTC	3 (60)				
		Unclear	1 (20)	CPS	1 (20)				
				2° GTC	1 (20)				

F	LRE	3 (100)	Unclear	2 (66.7)	2° GTC	3 (100)	Result unavailable	2 (66.7)	<1 year	1 (33.3)
			TLE	1 (33.3)	CPS	1 (33.3)	Focal epileptiform discharges	1 (33.3)	1 - 10 years	1 (33.3)
									25 - 50 years	1 (33.3)
G	LRE	2 (100)	TLE	2 (100)	CPS	2 (100)	Bilateral focal epileptiform discharges	1 (50)	<1 year	1 (50)
					2° GTC	2 (100)	Result unavailable	1 (50)	25 - 50 years	1 (50)
H	LRE	3 (100)	FLE	1 (33.3)	SPS	2 (66.7)	Result unavailable	1 (33.3)	<1 year	1 (33.3)
			LRE - type unclear	2 (66.7)	CPS	1 (33.3)	Focal epileptiform discharges	2 (66.7)	1 - 10 years	2 (66.7)
					2° GTC	3 (100)				

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Group	Syndrome	n (%)	Classification	n (%)	Seizure type	n (%)	EEG	n (%)	Age group	n (%)
I	LRE	2 (66.7)	TLE	1 (33.3)	SPS	1 (33.3)	Normal	2 (66.7)	19 - 24 years	2 (66.7)
	Symptomatic seizures only	1 (33.3)	LRE - type unclear	1 (33.3)	CPS	1 (33.3)	Result unavailable	1 (33.3)	25 - 50 years	1 (33.3)
			Acute symptomatic seizures	1 (33.3)	2° GTC	2 (66.7)				
					GTC	1 (33.3)				
K	LRE	1 (25)	TLE	1 (25)	Simple febrile convulsion	4 (100)	Bilateral focal epileptiform discharges	1 (25)	1 - 10 years	3 (75)
	Febrile convulsions only	3 (75)	Febrile convulsions only	3 (75)	CPS	1 (25)	Not performed	1 (25)	25 - 50 years	1 (25)
					2° GTC		Normal	2 (50)		
							Normal	1 (25)		
L	LRE	2 (100)	TLE	1 (50)	SPS	1 (50)	Focal epileptiform discharges	1 (50)	1 - 10 years	1 (50)
			LRE - type unclear	1 (50)	2° GTC	2 (100)	Normal	1 (50)	11 - 18 years	1 (50)

3.1.8 Co-morbidities seen in participants

A considerable amount of detail was collected from all participants about their medical background and previous medical history. 72 of all participants (65.5%) had some other clinical condition or medical issue in their past medical history apart from an epilepsy syndrome of any sort. 30 of the 46 participants who had an epilepsy syndrome (65.2% of this group) had some other feature of note in their past medical history, as opposed to 43 of the 64 participants unaffected by an epilepsy syndrome (67.2%). 35 of the 53 males who participated (66.0%) had some form of other medical condition, as did 37 of the 57 female participants (64.9%).

A broad range of different medical conditions were reported by the participants. These could be broadly divided into conditions which were unlikely to have a genetic contribution (such as once-off infections, surgeries such as tonsillectomies or appendicectomies and acquired malignancies in adult life) and conditions which may have a genetic contribution to their aetiology (such as psoriasis, ischaemic heart disease or autoimmune arthritides). The most commonly seen medical condition in the participants in the study was hypercholesterolaemia, with 16 of the 111 participants (14.4%) affected. The next most commonly reported medical conditions were anxiety / depression (8 participants – 7.2%), migraine (7 participants – 6.3%), hypertension (7 participants – 6.3%), previous concussion (7 participants – 6.3%) and psoriasis (6 participants – 5.4%) (see figure 5, page 124).

Medical conditions reported by participants

(Please note - figures refer to the percentage of the total number of medical co-morbidities rather than the percentage of participants with the condition

- Alcohol dependance syndrome
- Ankylosing spondylitis
- Anxiety / depression
- Arthritis (gouty / psoriatic / rheumatoid)
- Arthritis (osteoarthritis)
- Asthma (current or in childhood)
- Autism / developmental delay of unclear type
- Cardiac arrhythmia
- Carpal tunnel syndrome
- Colitis (type unclear)
- Concussion
- Congenital thumb tendon shortening
- Diabetes mellitus
- Essential tremor
- Facial haemangioma
- Foetal valproate syndrome
- GORD / peptic ulcer disease
- Gout
- Haemochromatosis
- Heart murmur
- Hepatitis (type unclear)
- Hypercholesterolaemia
- Hypertension
- Hypertension / toxaemia in pregnancy
- Ischaemic heart disease
- Migraine (with / without aura)
- Multiple sclerosis
- Other surgery (various)
- Polycystic ovary syndrome
- Previous appendicectomy
- Previous cholecystitis / cholecystectomy
- Previous fracture
- Previous meningitis
- Previous osteomyelitis
- Previous syncope
- Previous tonsillectomy
- Prostate adenocarcinoma
- Psoriasis
- Stevens-Johnson syndrome
- Testicular cancer
- Thyroid dysfunction
- Urticaria

Figure 5. Medical conditions reported in participants in the study.

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3.1.9 Availability of ancillary data for phenotyping participants

For all individuals with an epilepsy syndrome, we attempted to obtain copies of previous investigations performed and any relevant clinical opinions. There were three particular types of information we sought – previous imaging studies (either CT or MRI brain), previous EEG studies (of any type) and previous clinical opinion, if available. These data were used along with the clinical impression from structured interview to phenotype affected participants as accurately as possible. The availability of these data varied.

Clinical opinions

For all affected individuals, copies of the opinions from specialists who had seen these individuals as part of their usual care were sought. 30 of the 47 affected individuals gave a history of previously attending a Neurologist for the investigation and management of their events. However, the extent to which clinical opinion could be obtained was variable, with medical records or clinical notes available for review in 20 of these 30 individuals. All of the index cases in the families recruited had medical records which could be assessed.

Imaging

Imaging results were available in 20 affected individuals. A further 5 individuals had had imaging tests performed but the results were not available. Of the 20 individuals with imaging reports available, 2 had had CT scans only. The other 18 individuals had either 1.5 Tesla or 3 Tesla MR imaging. Both CT scans and 14 of the 18 MRI scans were reported to be normal. Two of the MRI scans showed hippocampal sclerosis, one showed evidence of previous infarcts and one showed transient non-specific white matter lesions which resolved fully on repeat imaging.

EEG

Of the 46 affected individuals in the study, 10 had never had an EEG performed. Of these, 3 individuals had idiopathic generalised epilepsy, 1 had localisation related epilepsy, 1 had an epilepsy syndrome of unclear classification and the other 5 had had febrile convulsions only.

In 12 cases, the participants reported having had EEGs but the results were not available. In one case, the record in the centres in question had been destroyed but in all other cases there were no records of the individuals in question having attended the centre they mentioned.

EEG records were available for the remaining 24 affected individuals. 15 of these studies were routine tracings, 2 were sleep-deprived studies and 7 were periods of video EEG monitoring. Of these 24 studies, 16 showed definite epileptiform abnormalities or captured clinical events.

Overall, of the 46 affected individuals in the study, all ancillary information was available for 19 individuals (41.3% of all affected individuals).

3.2 Recruitment from individual centres

Recruitment was from the three centres in the project – Beaumont Hospital in Dublin, St. James's Hospital in Dublin and Cork University Hospital.

3.2.1 Beaumont Hospital

The identification of families in Beaumont Hospital was by means of interrogating the Electronic Patient Record system. Regular re-interrogation of the EPR was carried out up to February 2012 from the time of the start of recruitment. A total of 88 potential families for inclusion in the study were identified in this way. These included a total of 92 individuals attending the Epilepsy Service in Beaumont Hospital, as these 88 families included 4 sibling pairs.

At the time of the last interrogation in February 2012, the EPR had details on epilepsy syndrome for 1,739 patients. Of these patients, 92 met the criteria for inclusion in the study (5.29% of all patients recorded on the EPR). The percentages of individuals with particular epilepsy syndrome who met the criteria varied between the different epilepsy syndromes, as shown in table 2 below (page 127). Although the relatively low percentage of individuals who qualified for inclusion may seem to indicate that inherited factors have a role in the aetiology of a minority of epilepsy cases, it should be noted that 24.84% of the total number of patients have a family history of epilepsy, and that the overall numbers on the EPR include many cases of epilepsy which are clearly not genetic in aetiology, e.g., those secondary to

infections, head trauma and brain tumours. Furthermore, the criteria for inclusion in the study aimed to identify families with the strongest history of epilepsy who were always likely to be a minority of the patient population.

Table 2. Number of patients attending the Epilepsy service in Beaumont Hospital who would meet criteria for inclusion in the study. IGE: idiopathic generalised epilepsy, LRE: localisation related epilepsy, SGE: symptomatic generalised epilepsy.

	Total	Positive family history	%	1st degree family history	%	Meets criteria for inclusion	%
IGE	256	110	42.97	45	17.58	26	10.16
LRE cause known	869	176	20.25	71	8.17	32	3.68
LRE cause unknown	360	111	30.83	51	14.17	26	7.22
Multifocal	41	10	24.39	3	7.32	1	2.44
SGE cause known	89	7	7.87	3	3.37	2	2.25
SGE cause unknown	124	18	14.52	9	7.26	5	4.03

The overall recruitment rate of families from those identified from Beaumont Hospital over the course of the study was 12.5%, with eleven out of the possible 88 families recruited.

3.2.2 St. James's Hospital

The identification of patients in St. James's Hospital was by Dr. Colin Doherty, a consultant neurologist in that centre. A total of 6 families were identified from this centre after the granting of ethical approval. The proband of one family was recruited during the course of the study (16.7% of families identified in St. James's Hospital).

3.2.3 Cork University Hospital

The identification of patients in Cork University Hospital was by Dr. Daniel Costello, a consultant neurologist in that centre. A total of 2 families were identified

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from this centre after the granting of ethical approval. No families were recruited from this centre.

3.3 Families recruited

The pedigrees of the families recruited are presented here. In creating these pedigrees, freely available pedigree-drawing software was used from an on-line source, i.e., Progeny Genetics (http://www.progenygenetics.com/online-pedigree/). Although a very useful tool, there are limits in the variability of symbols which can be used to denote certain medical conditions.

For each pedigree, we have used the following standard annotations.

1. The numbered boxes in each pedigree represent individuals who provided informed consent to participate in the study

2. Unnumbered boxes in each pedigree represent individuals who did not participate in the study

3. The proband in each pedigree is denoted by a black arrowhead to the lower left of the symbol

4. Individuals with localisation related epilepsy are denoted by a solid red left upper quadrant

5. Individuals with idiopathic generalised epilepsy are denoted by a solid blue right upper quadrant

6. Individuals who have had febrile convulsions are denoted by a solid green right lower quadrant

7. Individuals who have had acute symptomatic seizures only are denoted by a dark blue marker in the right lower quadrant

8. Individuals with epilepsy of unclear classification are denoted by a solid yellow left lower quadrant

9. A numbered individual with an empty box has no medical conditions.

A legend is provided with each pedigree. For all individuals, detailed phenotypic data are to be found in Appendix A.

3.3.1 Family A Recruited June 2010

Figure 6. Family A family tree

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Table 3. Phenotypic data for members of family A (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
A	i 1	Female	No	Unaffected	n/a	n/a	Nil	Hypertension, hypercholesterolaemia, hyperthyroidism of unclear type (previous radio-iodine treatment), arthritis (probable rheumatoid arthritis)
A	i 2	Female	No	Unaffected	n/a	n/a	Nil	Hypertension, asthma
A	i 3	Female	No	Unaffected	n/a	n/a	Nil	Nil
A	i 4	Male	Yes	LRE	Unclear	< 20 years	Nil	Colitis of unclear type
A	ii 1	Male	Yes	IGE	JME	23 years	Nil	Essential tremor
A	ii 2	Female	Yes	LRE	Unclear	28 years	Nil	Migraine without aura, previous appendicectomy
A	ii 3	Male	Yes	LRE	Unclear	15 years	Nil	Nil
A	ii 4	Male	No	Unaffected	n/a	n/a	Nil	Nil
A	ii 5	Male	No	Unaffected	n/a	n/a	Nil	Nil
A	ii 6	Female	Yes	IGE	JME	12 years	Nil	Nil
A	ii 7	Male	No	Unaffected	n/a	n/a	Nil	Nil
A	iii 1	Female	No	Unaffected	n/a	n/a	Nil	Previous chronic daily headache, cause unclear
A	iii 2	Female	Yes	LRE	Unclear	4 years	Nil	Nil
A	iii 3	Female	Yes	LRE	Unclear	6 years	Nil	Asthma in childhood
A	iii 4	Male	No	Unaffected	n/a	n/a	Previous concussion	Asthma
A	iii 5	Male	No	Unaffected	n/a	n/a	Nil	Nil
A	iii 6	Male	No	Unaffected	n/a	n/a	Nil	Nil
A	iii 7	Male	No	Unaffected	n/a	n/a	Nil	Nil
A	iii 8	Female	No	Unaffected	n/a	n/a	Nil	Nil
A	iii 9	Female	No	Unaffected	n/a	n/a	Nil	Nil
A	iii 10	Female	No	Unaffected	n/a	n/a	Nil	Nil

A.   The father of the proband, this individual developed epilepsy in adult life after a fall from a tree with associated head trauma and loss of consciousness. His events were characterised by loss of awareness, oral automatisms, and subsequent generalised convulsions. As far as family members are aware, he never had events prior to his episode of head trauma, and they cannot remember him having any other types of event.

3.3.2 Family B Recruited August 2010

Figure 7. Family B family tree

Table 4. Phenotypic data for members of family B (for in-depth phenotypes, please see Appnedix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
B	i 1	Female	Yes	IGE	Unclear	9 months	Febrile convulsions	Arthritis of unclear type, previous hysterectomy
B	i 2	Male	Yes	LRE	TLE	59 years	Nil	Hypercholesterolaemia
B	ii 1	Female	Yes	IGE	Unclear	13 years	Febrile convulsion	Nil
B	ii 2	Female	Yes	IGE	Unclear	7 years	Febrile convulsions	Developmental regression after onset of epilepsy
B	ii 3	Female	Yes	IGE	Unclear	12 years	Febrile convulsions	Previous cholecystectomy, hypertension in one pregnancy, known +ve for SCN1A mutation
B	ii 4	Female	Yes	IGE	Unclear	18 years	Febrile convulsions	Congenital shortened tendon in R thumb
B	ii 5	Female	Yes	IGE	JME	13 years	Febrile convulsions	Depression, hypercholesterolaemia, known +ve for SCN1A mutation
B	ii 6	Male	No	Unaffected	n/a	n/a	Nil	Heart murmur investigated aged 15 years, felt to be benign
B	ii 7	Male	No	Unaffected	n/a	n/a	Nil	Cardiac arrhythmia in childhood, nature of same unclear, one previous episode of convulsive syncope (fully investigated)
B	iii 1	Female	No	Unaffected	n/a	n/a	Nil	Nil
B	iii 2	Male	No	Unaffected	n/a	n/a	Nil	Foetal valproate syndrome, previous operation for correction of hypospadias
B	iii 3	Male	No	Unaffected	n/a	n/a	Nil	Nil
B	iii 4	Male	No	Unaffected	n/a	n/a	Nil	Nil
B	iii 5	Male	Yes	Febrile convulsions	Febrile convulsions	1 year	Febrile convulsions	Benign heart murmur, known +ve for SCN1A mutation

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B		Male	Yes	Isolated febrile convulsion	Febrile convulsions only	1 year	Febrile convulsion	Known +ve for SCN1A mutation
B	iii 6	Male	Yes				Nil	
B	iii 7	Male	No	Unaffected	n/a	n/a	Nil	Nil
B	iii 8	Male	No	Unaffected	n/a	n/a	Nil	Nil
B	iii 9	Male	No	Unaffected	n/a	n/a	Nil	Receptive speech difficulty, cause unclear (under investigation)
B	iii 10	Male	No	Unaffected	n/a	n/a	Nil	Autism, Wolff-Parkinson-White syndrome, cardiac arrest aged 11 days
B	iii 11	Female	No	Unaffected	n/a	n/a	Nil	R facial haemangioma

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3.3.3 Family C Recruited September 2010

Figure 8. Family C family tree

Table 5. Phenotypic data for members of family C (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
C	i 1	Female	No	Unaffected	n/a	n/a	Nil	Nil
C	i 2	Male	No	Unaffected	n/a	n/a	Nil	Hypertension, hypercholesterolaemia, psoriasis, arthritis of unclear type
C	ii 1	Female	No	Unaffected	n/a	n/a	Nil	Previous correction of strabsimus in childhood
C	ii 2	Female	Yes	LRE	Unclear	13 years	Febrile convulsions	Psoriasis, previous toxaemia in pregnancy
C	ii 3	Female	Yes	LRE	Unclear	14 years	Nil	Polycystic ovarian syndrome, depression
C	ii 4	Female	Yes	LRE	Unclear	15 years	Nil	Nil
C	ii 5	Female	No	Unaffected	n/a	n/a	Nil	Psoriasis
C	ii 6	Female	No	Unaffected	n/a	n/a	Nil	Psoriasis
C	ii 7	Female	No	Unaffected	n/a	n/a	Nil	Previous fracture of L arm
C	ii 8	Female	No	Unaffected	n/a	n/a	Nil	Previous fracture of ankle
C	ii 9	Male	No	Unaffected	n/a	n/a	Nil	Nil
C	ii 10	Male	No	Unaffected	n/a	n/a	Nil	Migraine without aura
C	iii 1	Male	No	Unaffected	n/a	n/a	Nil	Asthma in childhood

A.   This individual developed epilepsy in mid-adolescence (aged 14-15 years) without any obvious precipitants of which his family are aware. He had events characterised by loss of awareness, staring and subsequent abnormal motor behaviour. His family specifically mention that he had episodes of "walking around to the right". He would occasionally proceed to presumably secondarily generalised tonic clonic seizures. He never had status epilepticus. He also suffered from depression and died of suicide at the age of 25 years.

B.   This individual developed epilepsy in her teenage years without any obvious precipitant. The family could not give a direct description of this individual's events but commented that they were "just the same" as the proband.

C.   The family are certain that this individual has epilepsy. However, there are no details available on the age of onset, types of events or other features.

D.   This individual has had a single febrile convulsion, according to his mother. This occurred at the age of 1½ years. There were no focal features associated with this event, which lasted for only "a few minutes". He recovered to normal within 20 – 30 minutes, and he has not had any other events of any other type. He was assessed at the time of his event, but has never had any imaging or EEG studies. He has never been on any anti-seizure medications.

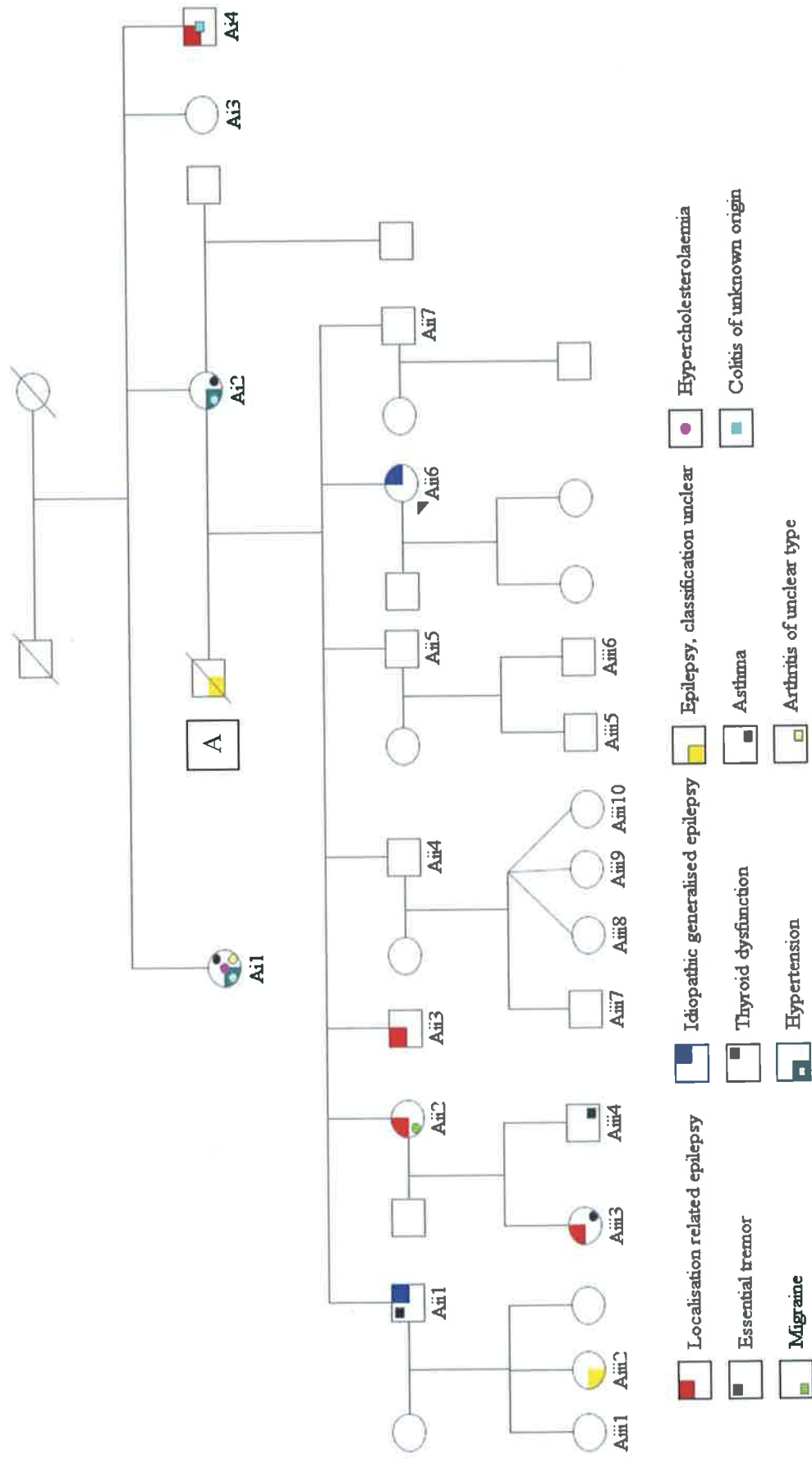## 3.3.4 Family D Recruited October 2010

Figure 9. Family D family tree

Table 6. Phenotypic data for members of family D (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
D	i 1	Female	No	Unaffected	n/a	n/a	Nil	Hypertension, hypercholesterolaemia
D	i 2	Male	No	Unaffected	n/a	n/a	Nil	Hypercholesterolaemia, previous R knee osteomyelitis, previoous R eye retinal detachment
D	i 3	Female	Yes	Isolated febrile convulsion	Febrile convulsions only	1 year	Febrile convulsion	Hypertension, meningitis aged 10 years
D	ii 1	Male	Yes	LRE	TLE	15 years	Nil	Hypercholesterolaemia
D	ii 2	Female	Yes	Isolated symptomatic	Acute symptomatic only	29 years	Nil	Generalised anxiety disorder
D	ii 3	Female	Yes	LRE	Unclear	13 years	Nil	Benign heart murmur, previous wisdom tooth extraction
D	iii 1	Male	Yes	Unclear	Unclear	2 years	Nil	Nil

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Non-participating individuals with phenotypic information available

A. This individual developed epilepsy in late adolescence. According to his mother, his first seizure occurred after an episode with head trauma with loss of awareness (he was concussed in the course of a football game, but did not require hospitalisation or other medical intervention). His first event occurred within the following few days after this, but his mother is unsure of the exact timing. He has events characterised usually by loss of awareness and upper limb automatisms, but more exact details were not available.

B. This individual had frequent convulsions. Details of other events, age of onset and response to medications could not be provided by her family. She died in her early thirties, and her family suspect that this was related to a seizure but are unsure of this.

C. The exact nature of this individual's events is unclear. The proband's mother remembers that he "used to have funny attacks" which would usually occur out of sleep, but could also occur more rarely out of clear consciousness. She did not witness one of these events, as she was always asked to leave the area if one occurred and she mentions specifically that "people didn't talk about things like that". She feels that he had epilepsy, but cannot clarify this further.

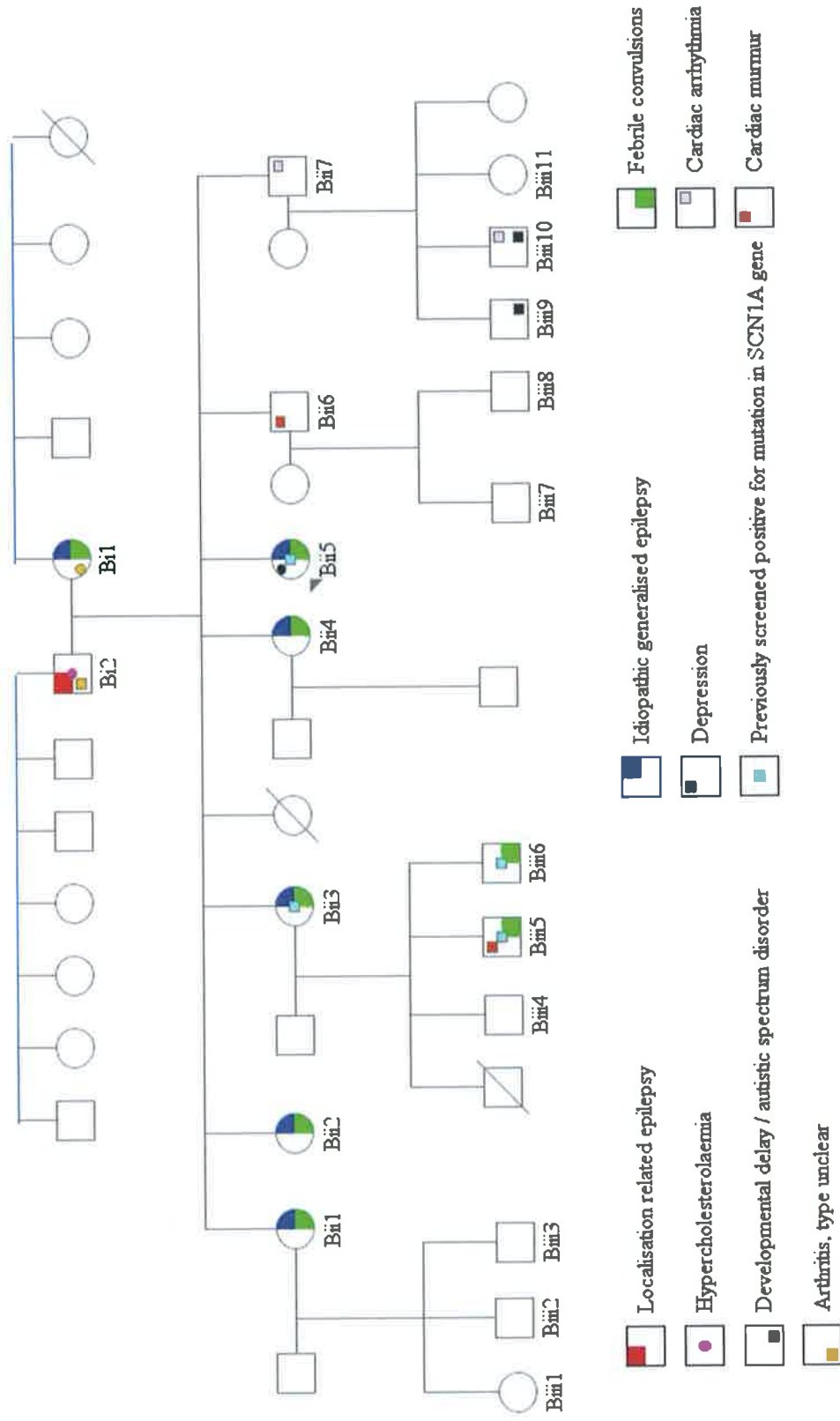## 3.3.5 Family E Recruited December 2010

Figure 10. Family E family tree

Table 7. Phenotypic data for members of family E (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
E	i 1	Male	Yes	IGE	Not otherwise specified	42 years	Nil	Type 2 diabetes mellitus, previous fracture of left ankle, hypercholesterolaemia
E	i 2	Female	No	Unaffected	n/a	n/a	Nil	Osteopaenia, previous R carpal tunnel release, migraine without aura
E	i 3	Female	Yes	Unclear	Unclear	1 year	Nil	Nil
E	ii 1	Male	Yes	LRE	Unclear	9 years	Nil	Ankylosing spondylitis
E	ii 2	Female	Yes	Isolated febrile convulsion	Febrile convulsions only	1 year	Febrile convulsion	Nil
E	ii 3	Male	No	Unaffected	n/a	n/a	Nil	Previous appendicectomy
E	ii 4	Male	No	Unaffected	n/a	n/a	Nil	Previous fracture of jaw
E	ii 5	Female	Yes	IGE	JME	16 years	Febrile convulsions	Depression

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<u>Non-participating individuals with phenotypic information</u>

A.   This individual had epilepsy in adult life, according to the proband. He can remember this individual having generalised tonic clonic seizures, but there are no details about lateralising features, other types of seizure, age of onset or other features.

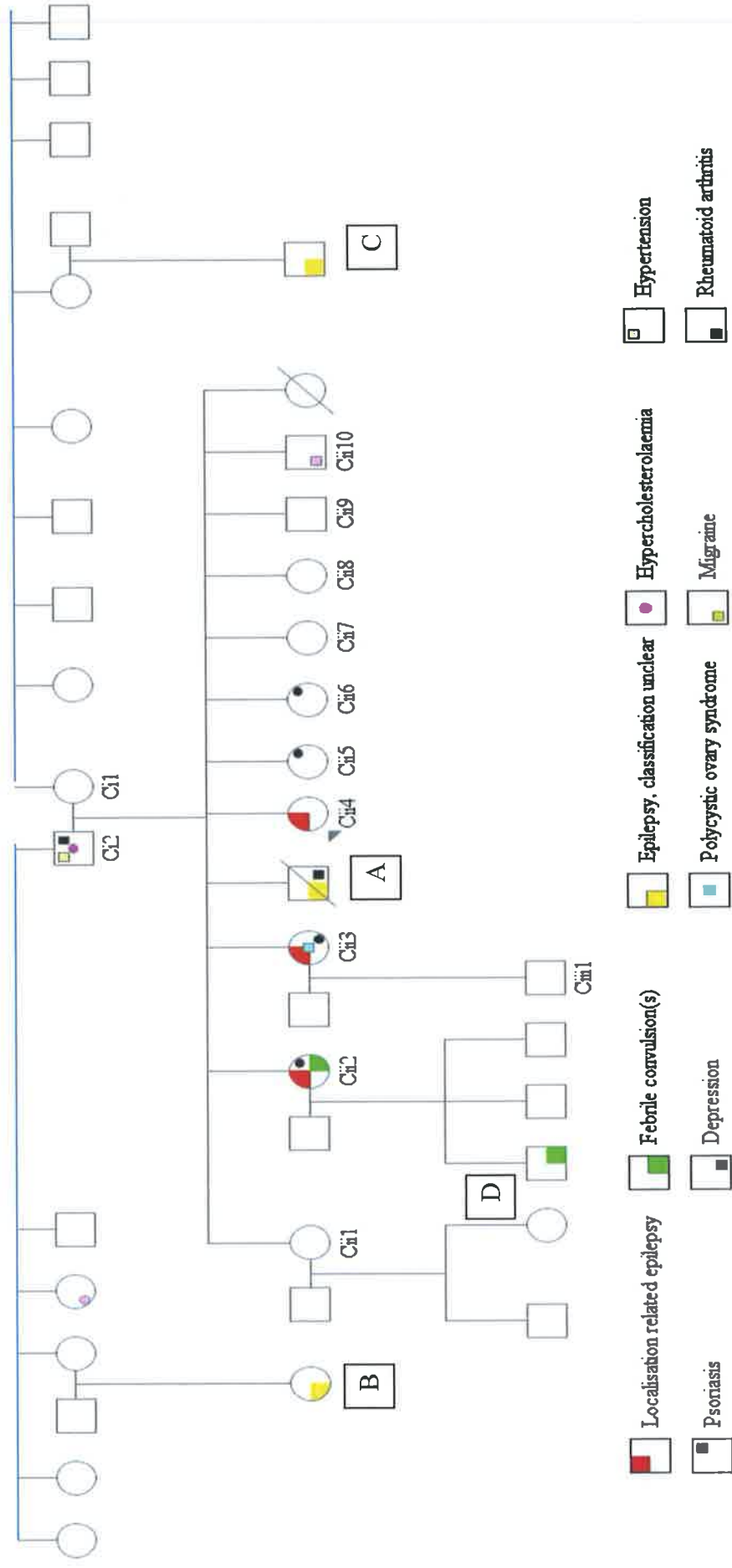3.3.6 Family F Recruited December 2010

Figure 11. Family F family tree

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Table 8. Phenotypic data for members of family F (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
F	i 1	Female	No	Unaffected	n/a	n/a	Nil	Previous appendicectomy, previous tonsillectomy, 2 previous total hip replacements
F	i 2	Female	Yes	LRE	Unclear	30 years	Nil	Hypercholesterolaemia, hypothyroidism, ischaemic heart disease with previous CABG, osteoarthritis, 2 previous hip replacements
F	ii 1	Male	Yes	LRE	Unclear	10 years	Nil	Hypercholesterolaemia, testicular cancer (previous surgery and chemotherapy for same)
F	ii 2	Male	No	Unaffected	n/a	n/a	Previous concussion	Previous concussion
F	ii 3	Female	No	Unaffected	n/a	n/a	Nil	Hypercholesterolaemia, previous cholecystectomy, asthma in childhood
F	ii 4	Female	Yes	LRE	TLE	6 months	Nil	Nil
F	ii 5	Male	No	Unaffected	n/a	n/a	Previous concussion	Hypercholesterolaemia, previous concussion

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3.3.7 Family G Recruited February 2011

Figure 12. Family G family tree

Table 9. Phenotypic data for members of family G (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
G	i 1	Female	No	Unaffected	n/a	n/a	Nil	Hypercholesterolaemia, multiple sclerosis
G	ii 1	Female	Yes	LRE	TLE	29 years	Previous concussion	Nil
G	ii 2	Female	Yes	LRE	TLE	3 months	Nil	Nil
G	iii 1	Male	No	Unaffected	n/a	n/a	Nil	Previous suspected meningitis

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A.   This individual had epilepsy from early life, according to the proband's mother. She is not aware of any precipitants for the development of epilepsy. She can remember him having frequent generalised convulsions but cannot remember any lateralising features. She is unaware of any other types of seizure. He did have a number of episodes of status epilepticus. He died at the age of 32, but she is unsure if this was related to a seizure or not.

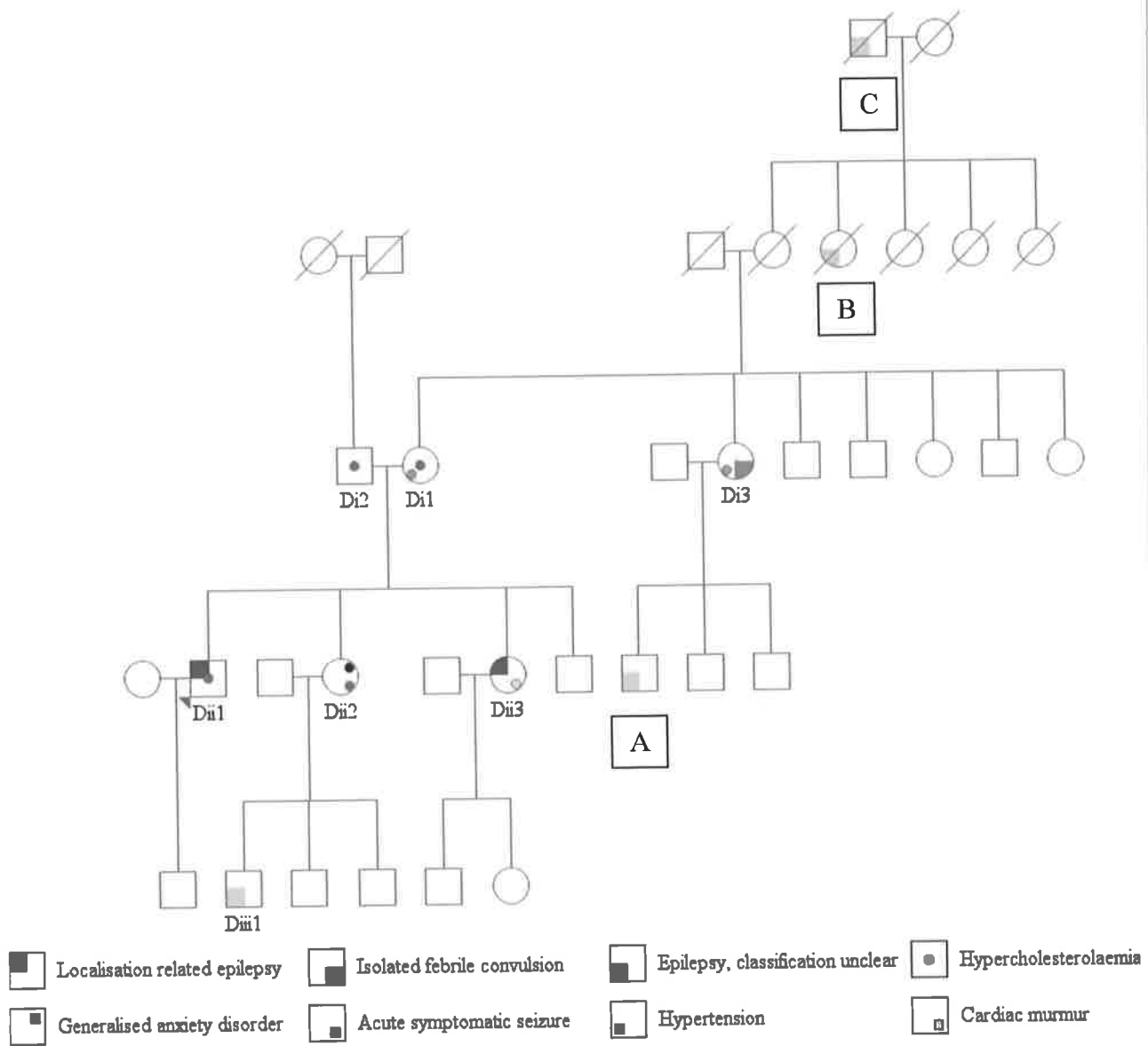3.3.8 Family H Recruited February 2011
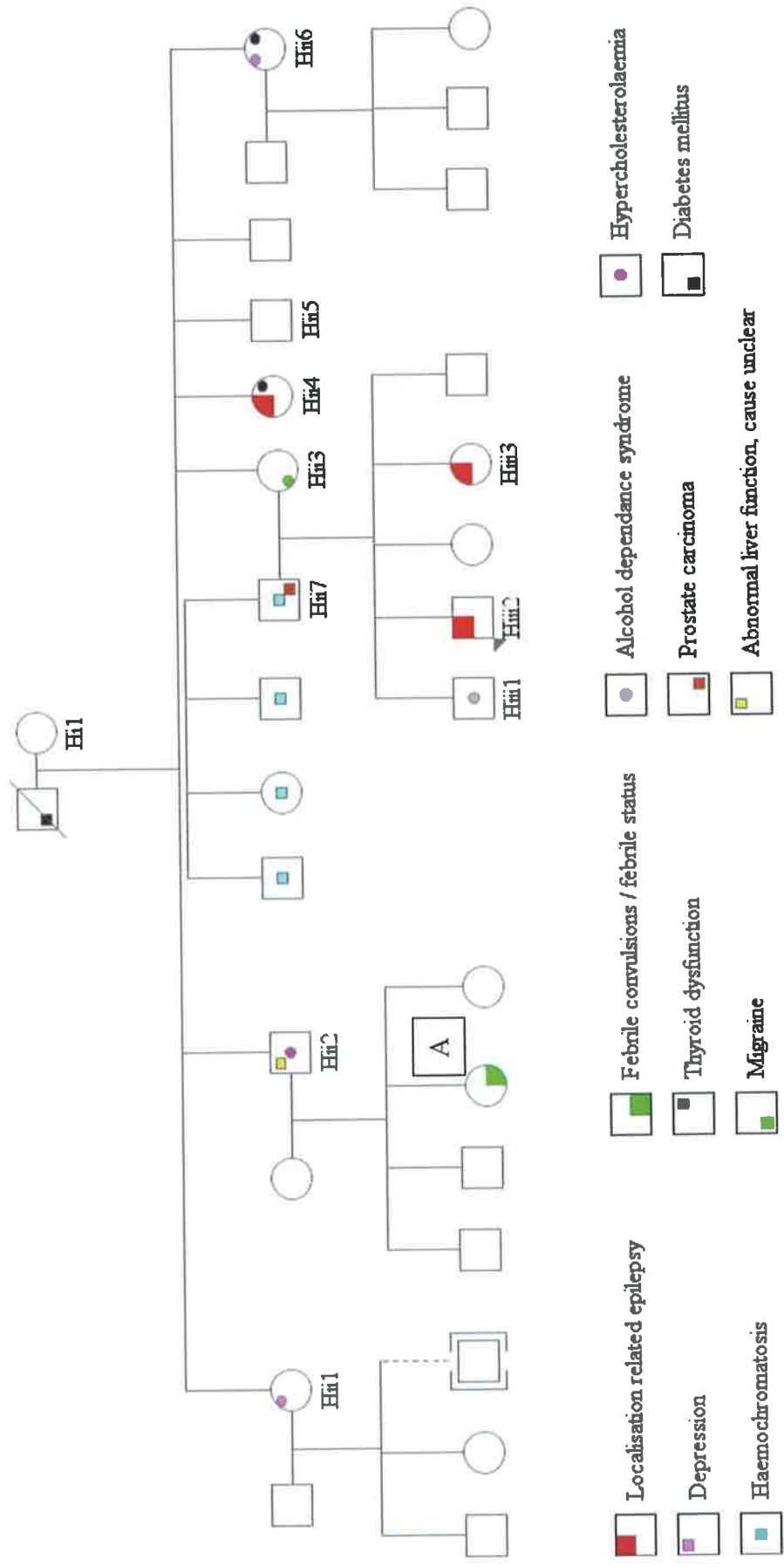
Figure 13. Family H family tree

Table 10. Phenotypic data for members of family H (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
H	i 1	Female	No	Unaffected	n/a	n/a	Nil	Nil
H	ii 1	Female	No	Unaffected	n/a	n/a	Nil	Depression, peptic ulcer disease, previous cholecystitis
H	ii 2	Male	No	Unaffected	n/a	n/a	Nil	Hypercholesterolaemia, abnormal liver function tests of unclear cause
H	ii 3	Female	No	Unaffected	n/a	n/a	Nil	Migraine without aura
H	ii 4	Female	Yes	LRE	Unclear	3 years	Nil	Hypothyroidism, iron-definiency of unknown cause
H	ii 5	Male	No	Unaffected	n/a	n/a	Nil	Nil
H	ii 6	Female	No	Unaffected	n/a	n/a	Nil	Hypothyroidism, depression
H	ii 7	Male	No	Unaffected	n/a	n/a	Nil	Haemochromatosis, urticaria, adenocarcinoma of prostate
H	iii 1	Male	No	Unaffected	n/a	n/a	Previous concussion	Gastro-oesophageal reflux disease, alcohol dependance syndrome
H	iii 2	Male	Yes	LRE	Frontal	6 months	Nil	Nil
H	iii 3	Female	Yes	LRE	Unclear	14 months	Nil	Nil

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A.   This individual's father was able to provide details on this individual's events. She first began to have events at the age of 2½ years. All of her events were associated with an increased temperature, with the first event occurring in the setting of croup. There were no focal features with any of her events and she never had seizures without an increase in body temperature. All of the events were consistent with generalised tonic clonic seizures. However, her father did mention that attacks could occur at relatively low temperatures and were very prolonged, going onto febrile status on a number of occasions. He also mentions that she had events on being put into a hot bath. She attended a Paediatric Neurologist and was on phenobarbitone for some years. Her last event occurred at the age of 5½ years. She has been free of events in adulthood and has never had any other types of event of which her family are aware.

3.3.9 Family I Recruited July 2011
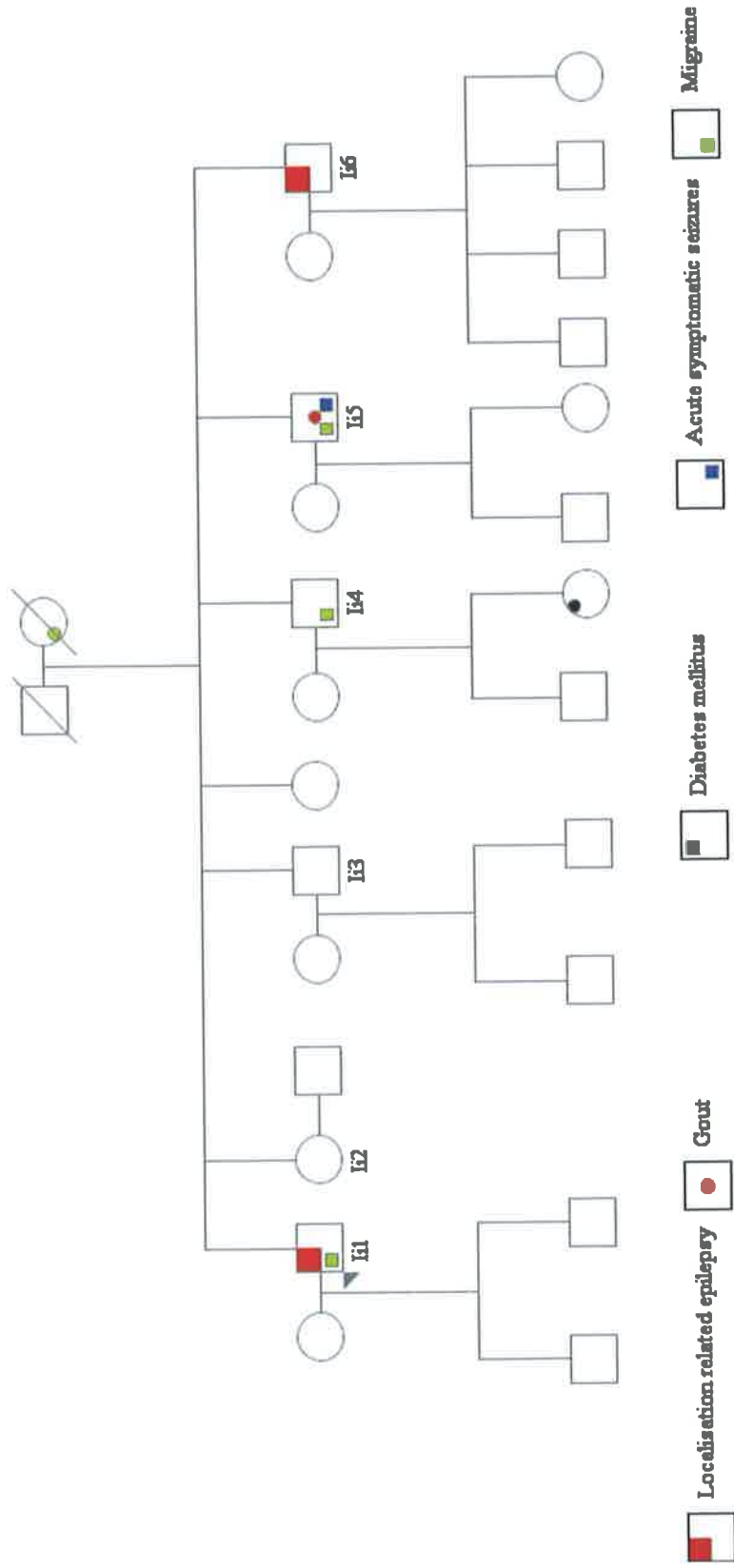
Figure 14. Family I family tree

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Table 11. Phenotypic data for members of family I (for in-depth phenotypes, please see Appendix A)

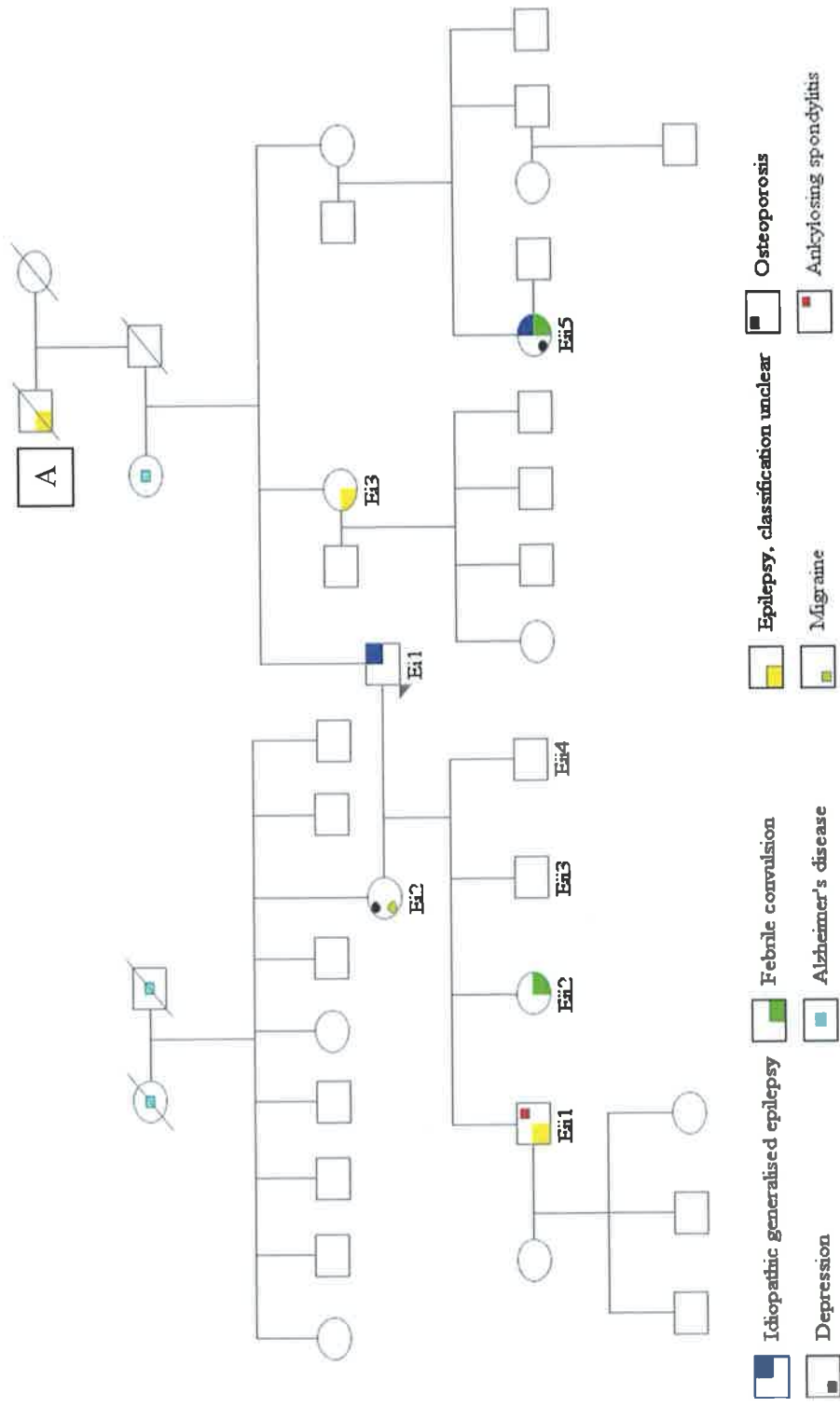| Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
I	i 1	Male	Yes	LRE	TLE	20 years	Nil	Migraine without aura
I	i 2	Female	No	Unaffected	n/a	n/a	Nil	Depression, hepatitis in childhood
I	i 3	Male	No	Unaffected	n/a	n/a	Nil	Nil
I	i 4	Male	No	Unaffected	n/a	n/a	Nil	Migraine without aura, previous tonsillectomy
I	i 5	Male	Yes	Acute symptomatic seizures	Acute symptomatic only	n/a	TB meningitis	TB meningitis, secondary hypopituitarism, gout, secondary arthritis from gout
I	i 6	Male	Yes	LRE	Unclear	35 years	Nil	Nil

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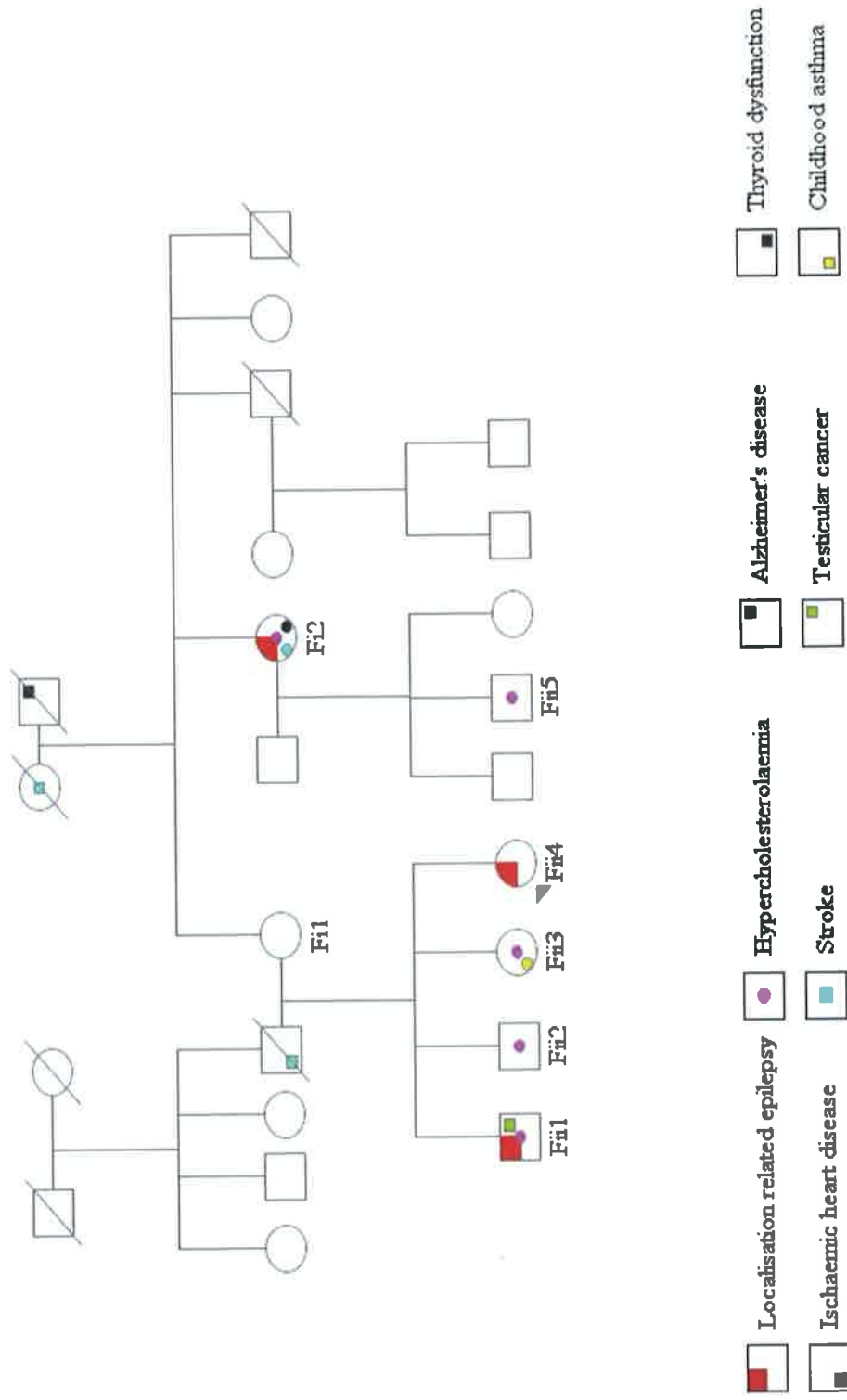3.3.10 Family K Recruited October 2011
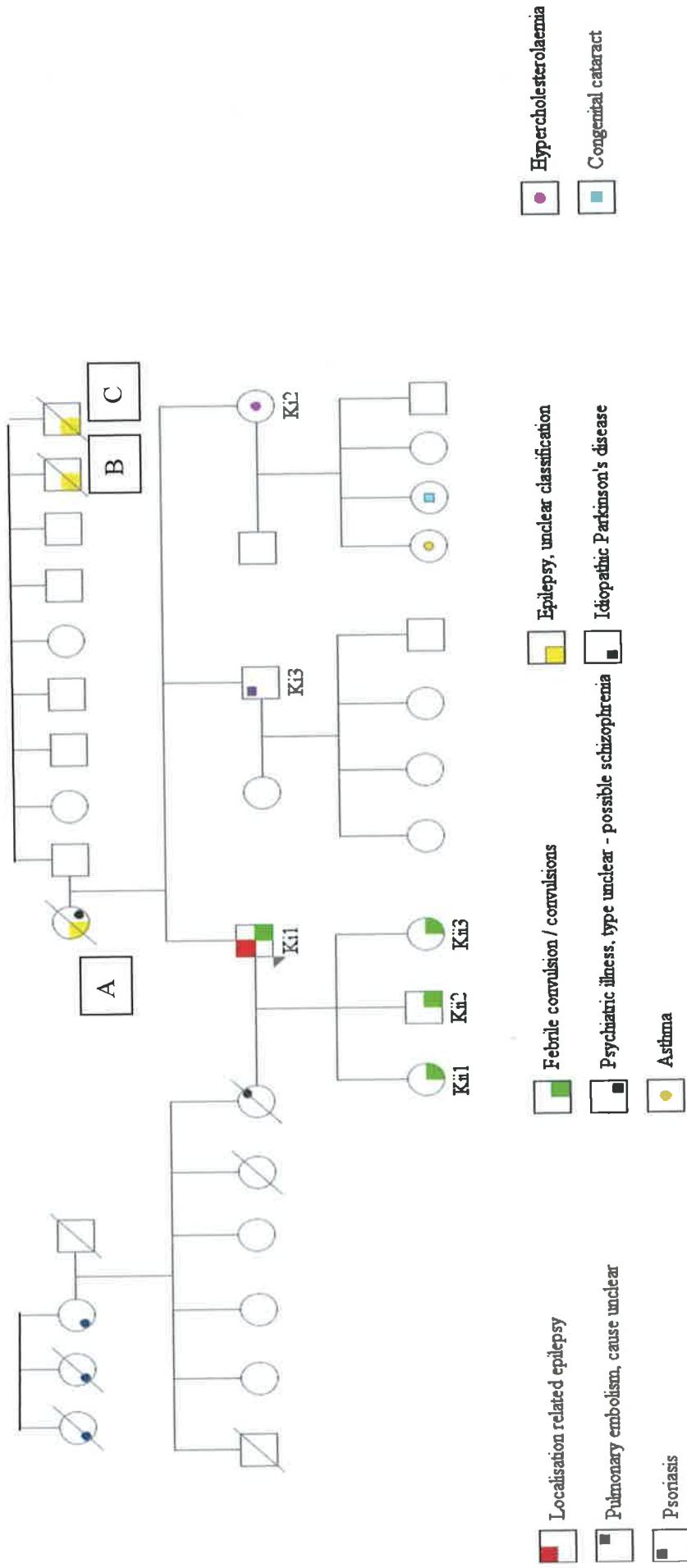
Figure 15. Family K family tree

Table 12. Phenotypic data for members of family K (for in-depth phenotypes, please Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
K	i 1	Male	Yes	LRE	TLE	30 years	Nil	Anxiety / depression, hypertension
K	i 2	Female	No	Unaffected	n/a	n/a	Nil	Hypothyroidism, hypercholesterolaemia, previous hip replacements, previous appendicectomy, uncomplicated syncopal events in childhood
K	i 3	Male	No	Unaffected	n/a	n/a	Previous concussion	Psoriasis, psoriatic arthritis
K	ii 1	Female	Yes	Two simple febrile convulsions	Febrile convulsions only	< 3 years	Febrile convulsions	Nil
K	ii 2	Male	Yes	Isolated febrile convulsion	Febrile convulsions only	< 3 years	Febrile convulsion	Two uncomplicated syncopal events in adolescence
K	ii 3	Female	Yes	Two simple febrile convulsions	Febrile convulsions only	< 3 years	Febrile convulsions	Previous cholecystectomy

<u>Non-participating individuals with phenotypic information</u>

A. The proband can remember this individual having generalised convulsions. However, there was no information available on any lateralising features or preceding auras associated with these. The onset of these events appears to have been after the onset of this individual's unspecified psychiatric illness, but no specific precipitants for attacks were recalled by the proband. This individual died in her forties of liver failure, the cause of which was unclear.

B. The proband is certain that this individual had epilepsy. However, no other details in relation to this were available.

C. The proband is certain that this individual had epilepsy. However, no other details in relation to this were available.

3.3.11 Family L Recruited December 2011

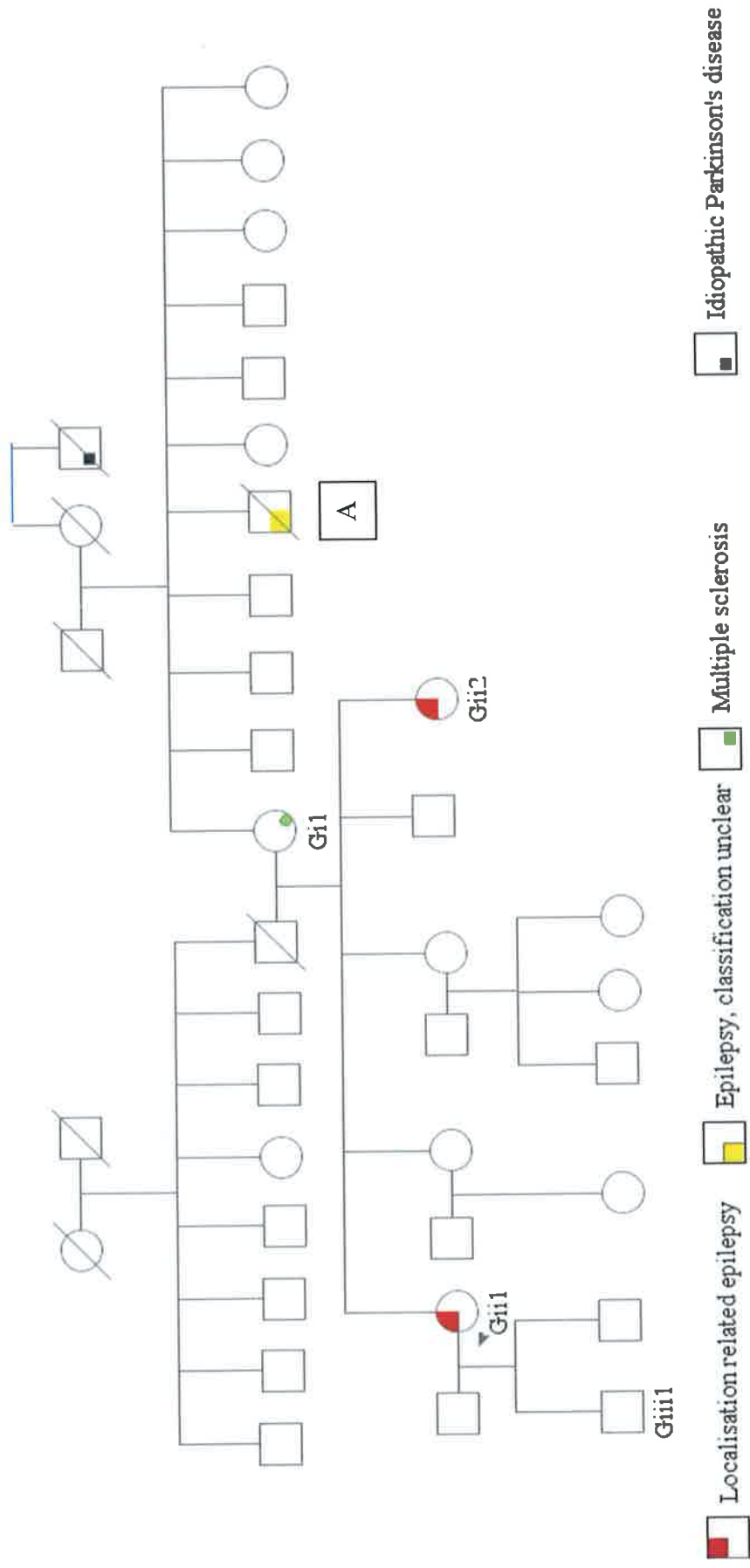

Figure 16.Family L family tree

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Table 13. Phenotypic data for members of family L (for in-depth phenotypes, please see Appendix A)

Family	Number	Sex	Epilepsy / seizures	Classification	Syndrome	Age at onset / occurrence	Epilepsy risk factors	Co-morbidities
L	i 1	Female	No	Unaffected	n/a	n/a	Meningitis	Psoriasis, previous appendicectomy, previous meningitis in childhood
L	i 2	Male	No	Unaffected	n/a	n/a	Nil	Hypercholesterolaemia, peptic ulcer disease
L	ii 1	Male	No	Unaffected	n/a	n/a	Nil	Nil
L	ii 2	Female	Yes	LRE	TLE	< 2 years	Nil	Nil
L	ii 3	Female	No	Unaffected	n/a	n/a	Nil	Stevens-Johnson syndrome after HSV re-activation
L	ii 4	Male	No	Unaffected	n/a	n/a	Previous concussion	Previous concussion
L	ii 5	Female	Yes	LRE	Unclear	12 years	Nil	Nil

A.   This individual has had epilepsy since his teenage years, according to the proband's father (this individual's brother). He has had generalised tonic clonic seizures, but no further information is available regarding lateralising features or preceding auras. He has not had any episodes of status epilepticus. The proband's father was unaware of any other types of seizure that this individual may have had and is unsure of there were any precipitants for this individual's epilepsy.

B.   This individual developed epilepsy at the age of the age of 12 years. She had her first seizure within 1 – 2 weeks of receiving her MMR vaccination booster. The proband's father is unsure of her seizure semiology, but knows that she has generalised tonic clonic seizures. She has never had any episodes of status epilepticus. He is unaware of any other types of seizure that this individual may have had.

C.   This individual developed epilepsy at the age of 11-12 years, according to the proband's mother (this individual's sister). The onset of her epilepsy did not have any obvious precipitant. Her initial events were characterised by loss of awareness, staring and oral automatisms, usually lasting for 1 – 2 minutes and associated with post ictal drowsiness and disorientation. She was formally diagnosed with epilepsy after a presumed secondarily generalised tonic clonic seizure. She has had one episode of status epilepticus at the age of 14-15 years.

D.   This individual developed epilepsy at the age of 11-12 years, according to the proband's mother (this individual's aunt). There is no information about any possible precipitants for this individual's epilepsy. He has had generalised tonic clonic seizures, but there is no information available about lateralising features or preceding auras. The proband's mother was unaware of any other types of seizure this individual has had, but she is certain that he has never had any episodes of status epilepticus.

E.   This individual developed epilepsy before he was one year old. There were no obvious precipitants, but he also has a diagnosis of "cerebral palsy", according to the proband's mother. He has generalised tonic clonic seizures and has been hospitalised as a result of these, although there is no information available about whether or not he has had status epilepticus. There is no information available about any other types of seizure which this individual has had, and the cause of his developmental delay is unclear.

3.4 Results of genetic analysis of families screened

Genetic screening was performed on a subset of the families described above, specifically families A, D, E, F and H. This was done in order to utilise access to whole exome sequencing to the optimum, as these families were felt to be the best candidates in which to identify novel genetic variants. The reason these families were chosen were due to the number of individuals in each family from whom DNA was available for analysis, the number of affected individuals in these families, and the distance between affected individuals within the family. Individuals from families A, D, F and H were analysed by whole exome sequencing.

Individuals selected for whole exome sequencing were as follows –

- In Family A, the proband (Aii6) and two of her siblings (Aii1 and Aii2).

- In Family D, the proband (Dii1) and his nephew (Diii1).

- In Family F, the proband (Fii4), her brother (Fii1) and their aunt (Fi2).

- In Family H, the proband (Hiii2), his sister (Hiii3) and their aunt (Hii4).

In each instance, the proband and at least one other affected family member were selected. Where possible, screening was performed on the proband and the most distant affected family member, although in some families the most distant members were still siblings. In general, this strategy of selecting based on genetic distance minimizes the shared genomic interval and thus reduces the possible candidate disease loci.

Individuals from family E were analysed by candidate SNP analysis, as an individual had been recruited previously into a different study of apparently sporadic idiopathic generalised epilepsy.

3.4.1 Acknowledgement and accreditation

Once again, I would like to acknowledge the work of Dr. Mark McCormack, PhD, RCSI, in the sequencing of DNA and candidate variant selection from the individuals selected for whole exome sequencing. I have drawn on work from Dr. McCormack's PhD thesis for the results presented below, with his permission.

Initial whole exome sequencing was performed on three individuals (Aii1, Aii2 and Aii6). Among the three sequenced siblings, there were 20,919 shared variants across 8,512 genes. The results of the filtering steps applied in selecting candidate variants (see section 2.8.4 above) are shown below in table 14.

Table 14. Flow-chart of filtering steps on shared variants in family A. MAF: minor allele frequency.

	Total variants	
Shared variants	20919	Subsequent removal of non-functional variants
Functional variants	5151	Subsequent removal of common variants (> 3% MAF)
Functional and rare variants	556	Subsequent focus on novel variants in screened controls
Functional and very rare (i.e., not identified in screened controls)	14	Subsequent focus on novel in all controls (minor allele frequency = 0)
Novel functional and rare variants	2	

The final 14 candidate variants (mentioned in table 14 above) are outlined below in table 15 (page 164).

Table 15. Top 14 candidate variants filtered from whole exome sequencing in three affected members of Family A. Variant ID; chromosome:base position in NCBI Build 36, EVS MAF; minor allele frequency reported in EVS database for 3500 European-Americans. Conservation scores calculated from PhyloP range from -4 (not conserved) to +4 (highly conserved) across vertebrates while the effect of amino acid substitution on protein function is predicted by Polyphen2 (see section 2.8.4).

Variant ID	Gene	Function	EVS MAF	PhyloP	Polyphen
chr1:43394634	SLC2A1	Non-synonymous	0	3.09	probably-damaging
chr1:43394635	SLC2A1	Non-synonymous	0	2.558	Benign
chr5:160115036	ATP10B	Non-synonymous	0.000096	-0.0503	Benign
chr16:70917931	HYDIN	Non-synonymous	0.000097	1.113	n/a
chr8:124811888	FAM91A1	Non-synonymous	0.000105	0.8693	Benign
chr5:156346497	TIMD4	Non-synonymous	0.000186	2.497	probably-damaging
chr3:62063888	PTPRG	Non-synonymous	0.000186	0.467	Benign
chr17:78023998	CCDC40	Non-synonymous	0.00019	-1.2448	probably-damaging
chr6:149700359	MAP3K7IP2	Non-synonymous	0.000279	0.8246	probably-damaging
chr11:55563402	OR5D14	Non-synonymous	0.000279	1.1034	possibly-damaging
chr11:65320416	LTBP3	Non-synonymous	0.000279	-0.0833	n/a
chr17:20768788	CCDC144NL	Non-synonymous	0.000279	0.088	n/a
chr19:14499616	CD97	Non-synonymous	0.000372	-2.3524	probably-damaging
chr3:73433112	PDZRN3	Non-synonymous	0.000465	2.0265	probably-damaging

This process identified a novel genetic mutation which was present in all of three affected individuals. Screening in other family members was carried out then to assess for the presence of this mutation.

A novel mutation was discovered in the SLC2A1 gene. This mutation – A348Y – caused a substitution at the 348 position of tyrosine for alanine. This mutation was heterozygous in the family members in which it was present. As well as the initial family members screened (Aii1, Aii2 and Aii6), this mutation was found in two of the other four affected individuals in this family (Ai4 and Aiii2), and in two of the fourteen unaffected individuals (Ai2 and Aii7). Two of the affected individuals (Aii3 and Aiii3) did not carry the mutation.

This mutation was not found in any controls screened from Irish of over 900 individuals or in over 1,100 samples from sporadic epilepsy cases, indicating that the mutation is extremely rare and may be private to this family.

The other mutations found in the three affected members of this family were not screened for in the other family members. This was because these mutations were predicted to be benign or because the genes in which these mutations were found did not have any known role in ion channel or central nercvus system formation or function.

3.4.3 Family D

Whole exome sequencing was performed on DNA samples from two individuals, the proband (Dii1) and his nephew (Diii1). Amongst these individuals, there were a total of 741 shared variants. Applying the filtering criteria (outlined in table 16 below), 13 candidate disease-causing mutations were identified.

Table 16. Flow-chart of filtering steps on shared variants in family D. MAF: minor allele frequency.

	Total variants	
Shared variants	741	Subsequent removal of non-functional variants
Functional variants	213	Subsequent removal of common variants (>

		3% MAF)
Functional and rare variants	123	Subsequent focus on novel variants in screened controls
Functional and very rare (i.e., not identified in screened controls)	13	Subsequent focus on novel in all controls (minor allele frequency = 0)
Novel functional and rare variants	4	

The final 13 candidate variants identified by this process are listed in table 17 below.

Table 17. Top 13 candidate variants filtered from whole exome sequencing in affected members of Family D. Variant ID; chromosome:base position in NCBI Build 36, EVS MAF; minor allele frequency reported in EVS database for 3500 European-Americans. Conservation scores calculated from PhyloP range from -4 (not conserved) to +4 (highly conserved) across vertebrates while the effect of amino acid substitution on protein function is predicted by Polyphen2 (see section 2.8.4).

Variant ID	Gene	Function	EVS MAF	PhyloP	Polyphen
chr16:169169	NPRL3	Stop-Gain	0	2.53138	n/a
chr13:73547814	PIBF1	Essential splice-site	0	2.42052	n/a
chr4:2901103	ADD1	Non-synonymous	0	2.40333	benign
chr21:42762618	MX2	Non-synonymous	0	2.00333	probably damaging
chr12:107937882	BTBD11	Non-synonymous	0.000093	2.65824	probably damaging
chr16:46729580	ORC6	Non-synonymous	0.000186	-0.64642	benign
chr8:10622935	PINX1	Non-synonymous	0.000207	1.32079	benign
chr9:123215882	CDK5RAP2	Non-synonymous	0.000279	-0.52395	benign
chr16:16101793	ABCC1	Non-synonymous	0.000305	2.22145	probably damaging
chr1:15888644	DNAJC16	Non-synonymous	0.000372	2.7156	probably

					damaging
chr12:122862168	CLIP1	Non-synonymous	0.001208	0.762063	benign
chr22:16449076	OR11H1	Non-synonymous	0.001488	0.033858	benign
chr9:123586913	PSMD5	Non-synonymous	0.002789	0.564354	benign

Based on this analysis, we concluded that there were no shared rare variants in the individuals screened which were of likely deleterious effect and functionally causal. The mutations found in the screened individuals affected genes with no known role in ion channel or central nervous system function or formation, and thus could not contribute to the development of epilepsy in this family.

3.4.4 Family E

This family were recruited into the study in the same manner as other families. However, after recruitment, it became clear that initial screening had been performed on one individual in this family – Eii5 – as part of a previous study. The proband in this family – Ei1 – was screened as part of this study into idiopathic generalised epilepsy, and when he was found to carry the same mutation as Eii5 the rest of the family were screened also as a candidate gene analysis.

Whole exome sequencing was not performed in this family, but given that this family was recruited as part of the study and shows the benefits of accurate family history and phenotypic data in genetic studies, it was felt to be appropriate to include this family in the results presented.

All affected individuals (Ei1, Ei3, Eii1, Eii2 and Eii5) were found to have a heterozygous glutamine to a stop-gain mutation (a nonsense mutation) at position 134 in the CHRNB3 gene. This was not seen in any of the unaffected individuals in this family.

The same variant was seen in two out of 6,500 individuals screened from the control population on the Exome Variant Server database. The status of these individuals in relation to being affected by an epilepsy syndrome or not is unclear. However, it has also been seen in an unaffected individual screened as part of another study (private correspondence from Dr. Mark McCormack).

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3.4.5 Family F

In this family, DNA from the proband (Fii4), her brother (Fii1) and their aunt (Fi2) was analysed. Amongst these individuals, there were a total of 559 shared variants across 428 genes. Using the filtering criteria, 13 possible candidate disease-causing variants were identified, as outlined in table 18 below.

Table 18. Flow-chart of filtering steps on shared variants in family F. MAF: minor allele frequency.

	Total variants	
Shared variants	559	Subsequent removal of non-functional variants
Functional variants	167	Subsequent removal of common variants (> 3% MAF)
Functional and rare variants	90	Subsequent focus on novel variants in screened controls
Functional and very rare (i.e., not identified in screened controls)	11	Subsequent focus on novel in all controls (minor allele frequency = 0)
Novel functional and rare variants	4	

The final 11 candidate variants are shown in table 19 (see page 169).

Table 19. Top 11 candidate variants filtered from whole exome sequencing in affected members of Family F. Variant ID; chromosome:base position in NCBI Build 36, EVS MAF; minor allele frequency reported in EVS database for 3500 European-Americans. Conservation scores calculated from PhyloP range from -4 (not conserved) to +4 (highly conserved) across vertebrates while the effect of amino acid substitution on protein function is predicted by Polyphen2 (see section 2.8.4).

Variant ID	Gene	Function	EVS MAF	phyloP	Polyphen
chr17:73844703	WBP2	Essential splice-site	0	-0.97541	n/a
chr2:120362458	PCDP1	Non-synonymous	0	2.72044	possibly damaging
chr6:30545887	ABCF1	Non-synonymous	0	2.71969	possibly damaging
chr6:161010654	LPA	Non-synonymous	0	-0.50747	benign
chr6:24447202	FUCA2	Non-synonymous	0.000093	2.5098	probably damaging
chr15:28443533	GPLD1	Non-synonymous	0.000279	1.24239	probably damaging
chr6:36107131	CALCRL	Non-synonymous	0.000279	1.03572	benign
chr18:67794783	HERC2	Non-synonymous	0.000372	0.161898	benign
chr15:40678625	MAPK13	Non-synonymous	0.001394	0.57048	benign
chr20:33581222	RTTN	Non-synonymous	0.004027	0.20578	probably damaging
chr6:36168072	BRPF3	Essential splice-site	0.005857	n/a	n/a

Based on this analysis, we concluded that there were no shared rare variants in the individuals screened which were of likely deleterious effect and functionally causal. The mutations found in the screened individuals were either located in genes with no known role in ion channel or central nervous system formation or function, or were not identified as being probably damaging.

3.4.6 Family H

Three of the affected individuals in this family – Hii4, Hiii2 and Hiii3 – had whole exome sequencing performed on their DNA samples. Among the three sequenced siblings in this family, there were 547 shared variants. Applying the filtering criteria, 10 candidate disease-causing mutations were identified, as outlined in table 20 below.

Table 20. Flow-chart of filtering steps on shared variants in family H. MAF: minor allele frequency.

	Total variants	
Shared variants	547	Subsequent removal of non-functional variants
Functional variants	154	Subsequent removal of common variants (> 3% MAF)
Functional and rare variants	100	Subsequent focus on novel variants in screened controls
Functional and very rare (i.e., not identified in screened controls)	10	Subsequent focus on novel in all controls (minor allele frequency = 0)
Novel functional and rare variants	6	

The final 10 candidate variants identified by this process are listed in table 21 below.

Table 21. Top 10 candidate variants filtered from whole exome sequencing in affected members of Family H. Variant ID; chromosome:base position in NCBI Build 36, EVS MAF; minor allele frequency reported in EVS database for 3500 European-Americans. Conservation scores calculated from PhyloP range from -4 (not conserved) to +4 (highly conserved) across vertebrates while the effect of amino acid substitution on protein function is predicted by Polyphen2 (see section 2.8.4).

Variant ID	Gene	Function	EVS MAF	PhyloP	Polyphen
chr15:60724149	NARG2	Non-synonymous	0	1.28493	probably damaging
chr16:14340831	MKL2	Non-synonymous	0	2.758	probably damaging
chr2:233393299	CHRND	Non-synonymous	0	0.500646	benign
chr16:14340770	MKL2	Non-synonymous	0	0.731283	benign
chr8:22137074	PIWIL2	Non-synonymous	0	-0.13493	benign
chr1:231403442	GNPAT	Non-synonymous	0	2.431	benign
chr16:2161264	PKD1	Non-synonymous	0.000094	-1.24709	probably damaging

chr2:233398659	CHRND	Non-synonymous	0.000279	2.67098	probably damaging
chr4:123664026	BBS12	Non-synonymous	0.000465	0.38615	benign
chr2:220239603	DNPEP	Non-synonymous	0.0081	2.71131	benign

This process revealed a novel mutation which was present in all three of the screened individuals. Subsequent screening was performed on the samples from the other family members. A novel mutation – L712V – was identified in the NARG2 gene in the three affected individuals and in Hii1, Hii2 and Hii3. The individuals were heterozygous for this mutation. The mutation was not present in Hii5, Hii6, Hii7 and Hiii1.

The other mutations found in the screened individuals were not screened for in other family members. This was for two reasons. Either these mutations were not predicted to be damaging, or because the mutations affected genes which have no plausible role in the aetiology of epilepsy.

4. Discussion of results

In this section I hope to explore themes which have arisen in course of the study and to expand on the significance of the results obtained.

4.1. Recruitment

4.1.1 Rate of recruitment

At 16.2% of invited participants (11 probands and their families out of 68 who could be contacted), the overall rate of recruitment in the study would seem low. Of the families identified in course of recruitment, only one-eighth were included in the study.

The principal reason for this lies in the accuracy of the family history supplied to the investigators. This was inaccurate in 28 of a possible 96 potential candidates, and was the single biggest factor in limiting recruitment. Although difficult for the investigators, such inaccuracies are reflective of the usual difficulties in clinical medicine in clarifying aspects of patient histories.

It is interesting to reflect on the other causes for the low level of recruitment. There seemed to be little concern amongst those contacted about the notion of genetic testing per se, once they were reassured about the confidential nature of the research. Most individuals who did not wish to take part declined to volunteer specific reasons, which restricted our ability to draw firm conclusions explaining non-participation. Only two individuals mentioned the notion of epilepsy as a stigmatising disease (Varley et al, 2010) as a reason why family members did not wish to participate, but again no firm conclusions can be made without a further investigation.

The initial goal for the study was the recruitment of ten families for analysis, with at least three affected individuals. More than ten families were phenotyped and sampled during the course of the study, and others had been agreeable to take part but could not be recruited for logistical reasons. Although not all families were

recruited in full, this still demonstrates that such genetic studies can be carried out in the Irish context. With the increasing emphasis on epilepsy as an area for further investment by the HSE (as outlined in "The National Epilepsy Clinical Care Programme in Ireland",

http://www.hse.ie/eng/about/Who/clinical/natclinprog/epilepsy.pdf) and the expansion of epilepsy services and electronic patient records, the identification of potential candidates for inclusion in genetic mapping studies in families is likely to improve, making these studies more feasible than they currently are.

4.1.2 Mechanisms for identifying potential participants

The majority of patients were recruited by interrogating the epilepsy-specific Electronic Patient Record (EPR) in Beaumont Hospital. This system has been newly developed over the last number of years under the auspices of the Epilepsy Service in Beaumont Hospital, and is used in the main as a clinical tool for the management of epilepsy patients attending this service.

Given the wealth of detail on the EPR system, there is great potential for the use of such a system as a research tool (Perna, 2012). However, the accuracy of the information on the system must be of the highest calibre to realise its full potential. It should be pointed out that, in many ways, this problem is not new and the issue of inaccuracy of records is not unique to electronic record systems (Walther et al, 2011; Co et al, 2010). A good example of this is seen in the numbers of individuals who could not be contacted by the investigators to discuss participation. Contact numbers were provided either by the consultant who had met the patient themselves or by interrogating the EPR. In the latter case, these numbers are automatically populated into the EPR from the main hospital system. In spite of having access to such sources, the contact details were not sufficiently accurate to allow the investigators to contact the patients in 18.8% of cases.

Overall, the use of the EPR allowed for the rapid identification of potential candidates for inclusion in the study. The approach is more systemic than trying to identify individuals on an ad hoc basis in an out-patient department or after they have been admitted to an acute hospital. As seen from the relatively high level of

inaccurate family histories, the investigators must confirm the family history prior to discussing participation.

4.1.3 Recruitment site

In the recruitment process, the idea of using a medical facility near to the patient's home was quickly changed to visiting a family member's home. This caused surprisingly few difficulties with sampling, ensuring confidentiality, sample transport, etc., and often helped the process in that participants felt more at ease and were more willing to volunteer information. Given the need for complete phenotyping in this study and other genetic research, any approach which maximises the possibility of obtaining relevant information should be encouraged, and recruiting in the participants own locality should be used more widely when this is feasible.

4.2 Phenotyping

4.2.1 Accuracy of phenotyping

The interpretation of genetic assays relies heavily on the accuracy of the clinical phenotyping of the individuals sampled (Greenberg and Subaran, 2011). Although the use of endophenotypes has been mooted as a means of making phenotype status clearer for genetic studies, in a study such as this it is still the issue of whether an individual has an epilepsy syndrome or not which is of greatest importance.

In this study, individuals could be classified into a form of epilepsy syndrome when affected, or could reliably be classified as unaffected, with a high degree of accuracy. There were a number of individuals whose status could only be confirmed after taking a collateral history from other family members.

The strength of collateral histories in the study can be demonstrated by a number of examples. In family C, although one of the affected individuals was deceased, a consistent epilepsy syndrome can be shown to have existed in affected siblings in this family by the details of the age of onset and clinical semiology of events

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provided by the proband's parents. In many of the other affected members in this family, the initial symptoms would be inadequate to differentiate the clinical events from syncopal attacks, but the collateral history provided by other family members allowed for the accurate classification of these events. Other examples include affected members of family E and family K, when some of these individuals had isolated febrile convulsions or other syndromes in childhood and would have been inadvertently classified as unaffected if only their own account had been available.

It is unclear to what extent this problem has been addressed in other studies of epilepsy genetics. This feature of the phenotyping of patients emphasises the importance of seeking as many clinical details as possible in the recruitment of patients. The clinical experience of the investigators in the diagnosis of epilepsy was of great benefit in this area, as the use of collateral histories in the clarification of diagnoses is a well established clinical technique. The recruitment of whole families at a time allows for the investigator in family genetic mapping studies to have access to individuals – such as parents or older siblings – who may be able to provide these collateral histories, and thus allow for the most accurate clinical phenotyping possible at the time of recruitment.

It should be noted that the use of a collateral history is not always necessary to confirm the phenotype of an individual, and that – as in some members of family E – there are occasions when a collateral history may not be available. When available, and when a participant consents to it, a collateral history should be sought to increase the accuracy of phenotyping. However, it should not be viewed as an absolute necessity in studies such as ours.

The ILAE classification scheme has been of great use in the management of patients clinically. In this study, however, some of the limitations in using a clinical classification scheme for classification of individuals for genetic testing have become apparent. This becomes more evident when considering the extent to which affected participants could be classified into specific epilepsy syndromes. In many cases, it was not possible to do so. Although in some cases this was due to the absence of ancillary information, in many cases it was because there was insufficient clinical information to be able to clearly define the specific syndrome, a

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phenomenon which has been commented on in other studies (Serrano-Castro et al, 2001; Avanzini et al, 1996). The issues around the utility of the ILAE classification system for studies such as ours have yet to be satisfactorily resolved, although alternatives are being suggested by some authors (Camfield, 2012). It has been suggested that the ILAE 1989 classification system could be refined so that epilepsy syndromes are classified as either generalised (idiopathic or symptomatic), focal (idiopathic, symptomatic or cryptogenic) or unsure if focal or generalised (Camfield, 2012). The advantage of such an approach in population studies is that it would strike a balance between the need for accurate phenotyping and the need to recruit sufficient numbers in each study.

Although a more scientific classification system, it is unlikely that the newer ILAE 2010 classification system for epilepsy (Berg et al, 2010) would provide a solution to the difficulties of classification in a study such as this. The 2010 system places more emphasis on non-clinical features (such as imaging results). In a study such as this, this may give rise to inaccuracies in classification, given the difficulties in obtaining more in-depth phenotypic information or ancillary information (such as MRI results) for participants. Although the classification scheme used may change with our increasing understanding of the genetics underlying epilepsy, for now, a classification based on a clinical assessment is more useful for studies such as this, provided that the researchers maintain an awareness of the limitations of such systems.

The difficulties in phenotyping individuals into a particular classification should be borne in mind, as some commentators have emphasised the need for more rigorous classification as a means of overcoming some of the difficulties with genetic studies in epilepsy (Greenberg and Subaran, 2011). Based on the difficulty phenotyping in this study, a more practical approach may be to allow for post study re-analysis on the basis of different classifications before commentating definitively on genotype – phenotype associations. This approach may be a better stance to take when faced with the difficulty of phenotyping individuals.

As individuals can be misclassified in usual clinical practice, we must acknowledge the possibility that some individuals in this study have been misclassified also.

Epilepsy and the diagnosis of particular epilepsy syndromes remain clinical diagnoses, and it is acknowledged that people can incorrectly be classified or may not fit into particular classifications (Seino, 2006; Kinoshita et al, 2008). We feel that we have minimised the possibility of this in this study, as the phenotypes were reviewed by the principal investigator (Dr. Norman Delanty) after assignment, and because there has been a conscious effort to avoid committing to a particular diagnostic label unless this is correct. The implications of errors in classification in genetic studies are considerable (Göring and Terwilliger, 2000; Greenberg and Subaran, 2011), and we are satisfied that we have minimised the possibility for such errors.

The phenotyping of individuals in this study was possible due to the experience of the researchers in the regular assessment and management of individuals with different types of epilepsy. Such experience is usually only gained over a considerable period of time, and allowed for a detailed approach to phenotyping to be adopted from the start of the study which required little, if any, modification as the study continued. However, such experience is not readily available, and can be daunting for individuals whose background is not in clinical medicine.

One attempt to overcome this is the use of a checklist for researchers. However, such an approach lacks flexibility. A long list of questions is off-putting for participants, and the rigidity of such a list does not allow for the unusual way in which participants may describe their symptoms or conditions. Over the course of this study, it has become clear that a collaborative approach between the scientific and clinical aspects is the most appropriate approach for large phenotyping projects such as this, with all phenotypes being reviewed by a senior clinician experienced in the treatment of the condition under investigation.

4.2.2 Availability of ancillary results for phenotyping

As part of phenotyping individuals, we sought ancillary information whenever this was possible, i.e., results of imaging or EEG studies, letters or opinions from individuals who had assessed the participants clinically. Frequently, this was

unavailable, with all three types of information (i.e., imaging results, EEG results and clinical opinions) being available in under half of affected participants (42.6%).

Two reasons were encountered in the course of the study for the unavailability of such information. Either participants had never had these tests done or they reported having the test or clinical opinion in a hospital which had no record of their attendance. All clinicians contacted for ancillary information were extremely helpful and willing to volunteer results once a copy of the appropriate consent form had been forwarded to them.

It is when trying to obtain these ancillary details that the EPR was extremely helpful. As a single site containing all relevant information for individuals with epilepsy attending Beaumont Hospital, details about previous imaging and EEG studies were immediately available with other phenotypic data. With access to such a system, investigators could rapidly obtain all necessary information.

It may be felt that the availability of such information would have a negative impact on the accuracy of phenotypes for this study. We are satisfied that the broad epilepsy classifications given for participants are accurate, but the availability of this information would certainly have helped to classify the syndromes of two of the 47 affected individuals whose classification remains unclear and may have helped to clarify specific epilepsy syndromes for participants. We acknowledge that some clinical features can be misleading, such as the presence of focal features in idiopathic generalised epilepsies (Jayalakshimi et al, 2010). However, with the use of the available information, collateral histories and specifically asking for such features as myoclonus (Panayiotopoulos et al, 1991), we are satisfied that where ancillary information is absent it does not have a significant effect on the accuracy of phenotypes in this study.

4.2.3 Variability within families – concordance of phenotypes

The families recruited into the study have been marked by significant discordance in phenotypes. Different epilepsy syndromes have been noted to co-exist within the same family in seven of the twelve families recruited. The other five families

recruited had consistent epilepsy types between different individuals, but even in these families there were marked differences in the clinical features expressed in different individuals. In none of the families recruited did all individuals within a family express a single specific epilepsy syndrome with consistent clinical features.

The importance of concordance has been stressed by some authors in the selection of families for genetic studies of epilepsy. However, different studies have calculated concordance in different ways. As a result, making exact comparisons between studies has been difficult and has given rise to differences in the reported rate of concordance in different types of epilepsy (Winawer et al, 2003). In our study, given that the number of families available for analysis was limited to eleven, we have opted to calculate concordance without correction for concordance by chance.

In discussing concordance and the variability in phenotypes seen in the study families, the different aspects studied can give different rates of concordance. This has been shown before in studies which considered the concordance in such features of epilepsy as the seizure type between individuals rather than in the exact epilepsy syndrome (Greenberg and Subaran, 2011). Such studies have demonstrated novel genetic findings when analysed for these aspects which would not otherwise have been apparent. However, in our study, we have chosen to take more narrow view of concordance, giving the apparent lack of it in the families studied.

The issue of concordance in interpreting genetic analysis remains a difficult one (Ottman et al, 1998). Examples in the literature exist of the successful use of concordance for epilepsy syndromes or for particular seizure types in genetic studies to clarify the importance of mutations found. Whether concordance is a necessity for a mutation to be considered a candidate causal variant is unclear. There are well-described families with Mendelian epilepsy (such as can be caused by mutations in the *SCN1a* gene) in which the phenotype varies between carriers of the same disease allele, to the extent that some carriers are not affected (Harkin et al, 2007; Scheffer and Berkovic, 1997). Although we cannot definitively say that the families in our study have Mendelian epilepsy, we have seen similar variability in some of the families in which candidate causal variants have been identified (family A and family E), in that phenotypes in these families are very discordant between

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individuals. It is unclear if such variability indiciates that the mutations found in family A and family E are not causative for epilepsy, or if they are a predisposing factor which requires other features to allow the development of the full clinical phenotype, or if there are unrecognised modifier factors in unaffected carriers of which we are unaware. Further study will be needed to clarify this point.

Our study would suggest that concordance is not a necessity for interpreting genetic analysis in families. Novel candidate causal mutations can be identified within families even when the phenotypes differ between individuals carrying the mutation. However, the interpretation of the role of such mutations is more difficult to study in the absence of a clear phenotype associated with such a mutation. To allow for further assessment of the impact of such mutations, phenotyping should be as detailed as possible, in case the mutation is associated with a particular seizure type or electrographic feature rather than an epilepsy syndrome per se.

The variability within families has been demonstrated in the Results chapter (section 3.1). This phenomenon has been demonstrated in other studies of family kindreds affected by epilepsy (Kinirons et al, 2008), with estimates of concordance varying from 39% to 83% within families (Winawer et al, 2003). As such, the families in this study would not be unusual in this regard. However, there was considerable variation in families where a novel genetic mutation was associated with epilepsy in that family. For example, both family A and family E had marked variation between individuals. Although it may seem to argue against the novel variants identified being causative for epilepsy in these families, this is not necessarily the case because this feature has been shown in other families with specific mutations. Indeed, variability in the phenotype of individuals has been demonstrated in families affected by Mendelian epilepsy syndromes such as GEFS+ caused by mutations in the *SCN1a* gene (Scheffer and Berkovic, 1997).

A high degree of concordance between affected family members is no guarantee that the genetic factors behind epilepsy in these families will be identified. An example of the former in this study is family E, in whom a novel, potentially causal mutation was identified in spite of a marked variation between the clinical features and epilepsy syndromes in these individuals. In contrast, the affected individuals in

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family H had high degree of concordance between each other for certain features but no novel, epilepsy-causing genetic mutation was demonstrated in this family.

This variability in phenotypes appears to be separate from any difficulties in clarifying phenotypes in these individuals. Overall, in this study we were able to phenotype 98.2% of participants into an epilepsy classification, and these phenotypes were subsequently confirmed by the opinion of an experienced consultant Epileptologist (N.D.). Thus, errors in phenotyping are unlikely to account for the variability seen.

Heterogeneity in phenotypes within families is not accounted for by obvious environmental or medical causes, such as different early risks for epilepsy between affected individuals. Indeed, in most participants who had an early risk factor for the development of epilepsy, such factors usually give rise to acute symptomatic seizures (as in the case of meningitis), or have not lead to the development of epilepsy, or (as seen in families with a history of febrile convulsions) are shared between affected individuals.

It may be the case that the age of the participants at recruitment is a factor in the variability seen. Some of the individuals who were younger at recruitment may be classified in the study as having febrile convulsions only. If these individuals were to be followed up over time, they may develop a different epilepsy syndrome in later life. However, even in adults of similar ages with the same epilepsy type, there are very good examples of different clinical features such as the presence of myoclonus, the presence of certain triggers and responses to different medications.

In a number of cases, affected individuals had events or epilepsy syndromes which were unclassifiable. It is unlikely that this gave rise to the degree of discordance seen within families in this study. The great majority of individuals could be classified and most events experienced by participants could be ascribed to a particular clinical category.

The use of particular endophenotypes to explain these differences in phenotypes within families in our study may not be possible, given the difficulty in obtaining

some data for some affected individuals (as discussed in the section above). However, even in families with the same genetic mutation, there have been reports of differences in the type of seizure experienced, the EEG findings and other features which have been proposed as suitable endophenotypes for genetic studies in epilepsy (Rommelse et al, 2008). However, it is not possible to be certain what effect on concordance the availablilty of such data would have, especially in the cases of those affected individuals who had previously had EEGs. The numbers of individuals recruited in our study and the availability of EEG and MRI data are insufficient to make too definite a statement on this issue.

Aside from these characteristics of the participants, there may be particular genetic factors which have contributed to the lack of concordance between individuals in families in our study. There may be variable penetrance of a genuinely causative mutation, a feature which has been described before with some Mendelian epilepsy syndromes (such as autosomal dominant lateral temporal lobe epilepsy) (Rosanoff and Ottman, 2008), or a variable phenotype with the mutation identified. However, the absence of a particular mutation associated with epilepsy in families D and F would argue against this being the case for all the families in this study (although it may be the case for families A and E). Also, there may be unrecognised genetic or environmental factors acting in these families, leading to the different phenotypes observed.

4.2.4 Presence of co-morbidities

Numerous co-morbidities were noted in participants in this study, with 65.8% of individuals having some form of past medical history. The nature of such co-morbidities varied. As might be expected, individual participants (both those affected and unaffected by an epilepsy syndrome) sometimes had a co-morbidity known to have a strong genetic component. Examples of such conditions included multiple sclerosis, haemochromatosis and ankylosing spondylitis, all of which are known to have a genetic predisposition (Del-Castillo-Rueda et al, 2012; Reveille, 2012; Lin et al, 2012). However, where these conditions were seen, they were limited to specific individuals and did not show an obvious co-occurrence with epilepsy in these families.

As might be expected when recruiting from families from the general population, the most frequently encountered co-morbidities were common medical conditions. The most common co-morbidities seen in the study participants were psoriasis (5.4%), thyroid dysfunction (4.5%), hypercholesterolaemia (14.4%) and hypertension (5.4%). All of these are known to have both genetic and environmental factors associated with their development (Capon and Barker, 2012; Eriksson et al, 2012; Motazacker et al, 2012) and some conditions (such as hypercholesterolaemia) are known to occur at a higher frequency in those with epilepsy (Elliott et al, 2007). In individual families in this study (such as family C and family F), certain conditions such as psoriasis and hypercholesterolaemia seem to be over-represented in individuals. It may be that such co-morbidities may indicate specific candidate loci in these families for further investigation, but more will need to be known about the genetic basis of these conditions before this is certain.

4.2.5 Ethical considerations arising from collection of phenotypic data

In the course of the study, a considerable volume of data was collected from each participating family. This raises a number of ethical issues.

In the course of obtaining collateral histories about these families, information was obtained about individuals who had not expressly consented to involvement in the study. The inclusion of phenotypic data from individuals in this manner is usual practice in obtaining a family history in clinical medicine – in such cases the individual does not consent to their data being revealed but an assumption of consent is made in the patient's best interest. However, this assumption cannot be made in the case of a research study such as this. The information leaflet approved by the Beaumont Hospital Ethics Committee (see Appendix B) did not specifically mention this issue, but that approved by the CUH Ethics Committee (see Appendix C) did specify that the investigators would be enquiring more about family history.

After discussion, a decision was made that the data from these individuals could be retained for the time being. This decision was informed by a number of factors. These data were completely anonymised, to the extent that no details were available

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on these individuals' names, dates of birth or other possible identifiers. Thus, these data were fully confidential. The retention of these data would facilitate later participitation by these individuals in other studies if they so wished. For those deceased individuals, we were satisfied that the family members could provide consent by proxy for the retention of such data. Although it could be argued that retention of such data would also allow for the identification of individuals who may be "at risk" for certain genetic conditions, we considered that such post hoc justification would not be an adequate reason to retention.

The extent of phenotypic detail available in each family could, it may be argued, allow for the identification of the participating families, especially in the event of details being published in a scientific journal. However, we thought that this was unlikely. All data in the study were fully anonymised. In the event of seeking to publish any such details, we would retain such anonymisation. We would not seek to publish any identifiers (such as family names or dates of birth), but consistent with the spirit of the consent obtained, we would publish in line with standards in the field, i.e., the pedigree would be illustrated but identifiers would not be associated with it. Unless an individual had detailed clinical knowledge of the family, they would not be identifiable.

Although we are satisfied that we have addressed these ethical issues to an appropriate standard for participating and non-participating individuals, we are aware of the need to ensure such concerns remain central in studies such as ours. We have continued to engage with the Beaumont Hospital Ethics Committee and are in the process of updating our protocols to address issues around publication.

4.3 Novel genetic findings

Of the five families from whom individuals were screened during the course of this study, potentially disease-causing (in the context of epilepsy) variations were found in three – family A, family E and family H. We will expand on the findings in each of these families in turn.

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Family A showed a previously unreported variation in the *SLC2A1* gene in the form of two deleterious non-synonymous mutations not reported in other databases. *SLC2A1* is a known "epilepsy gene", but the phenotype can be quite variable (Striano et al, 2012; Rotstein et al, 2010; Suls et al, 2008). The different epilepsy types seen with mutations in this gene have been reported previously, but within individual families the epilepsy syndrome seen has usually been concordant between individuals. This is not the case in Family A. It is interesting to note that no individual in Family A reported seizures occurring in the setting of prolonged fasting, something which has been emphasised in the previous reports of individuals with epilepsy secondary to *SLC2A1* mutations (Verrotti et al, 2012). The low prevalence of movement disorders in those with the genetic mutation in this family is also noteworthy, as many of the previous reports have described the occurrence of such disorders in families including cerebellar ataxia, dystonia and paroxysmal exercise-induced dyskinesia (Suls et al, 2008).

In this family, there is a history of epilepsy in the proband's maternal uncle and her father. It is unclear how this complicates the genetic analysis. No DNA is available from this individual or from any other members of his family (such as parents or siblings) to compare to the recruited participants. In the absence of this, it is difficult to be certain that there are no other genetic factors affecting the development of epilepsy in the proband and her siblings. However, from the collateral details obtained from family members, the proband's father seems to have developed epilepsy as a result of head trauma in later life. If these details are accurate, then the absence of DNA from this individual may not have any impact on the genetic analysis in this family. Without more phenotypic details for this individual or DNA samples from other members of this individual's family, this matter cannot be completely resolved.

There are difficulties in interpreting the results of whole exome sequencing in this family. On the one hand, two seemingly unaffected individuals carry the mutation but have no evidence of epilepsy. This may reflect reduced penetrance of the effects of the mutation, a phenomenon which has been noted with other genetic mutations

associated with epilepsy (Striano et al, 2012). An "age of onset" phenomenon is also possible, as one of the carriers is still not an adult and may develop symptoms when beyond a certain age – however, as the other carrier is an adult, this is perhaps less likely to explain our findings. To date, neither of these unaffected individuals has had an EEG, so we cannot comment on whether the use of the presence of epileptiform EEG changes as an endophenotype (common to the other affected individuals in this family) would change their status from unaffected to affected.

More problematically, two of the affected individuals in this family do not carry the mutation in the *SLC2A1*. This would imply that the mutation in the *SLC2A1* gene is not the cause of the epilepsy syndrome in these patients, indicating that they are "phenocopies". There are no features in the clinical phenotypes for these two affected individuals which could account for their epilepsy syndromes being secondary to environmental factors, making an acquired brain injury an unlikely reason for the findings. Whether there are other genetic variations not highlighted by whole exome sequencing (or our mapping strategy) in these individuals causative for their epilepsy syndromes cannot be confirmed or refuted on the basis of the information available.

To prove that the variant identified in this family is causal for epilepsy would require either a change in the phenotype of the unaffected carriers (such as demonstrating an abnormal EEG pattern or the emergence of a previously obscure history of seizures) or demonstrating the caual effect of the mutation by other means (replication in another study or the generation of an appropriate in-vitro or in-vivo model). However, mutations in the *SLC2A1* gene have been reported in epilepsy, so this mutation has biological plausibility as a cause for epilepsy in this family. For now, rather than assuming causality for this mutation, it should be regarded as a candidate causal variant deserving of further study.

The mutation seen in this family has not been seen in any other individuals from control populations (both international and Irish). This would imply that the mutation is private to this family, and would limit the utility of such a mutation as a "screening tool" for other affected Irish families.

<u>4.3.2 Family E</u>

Family E was found to have a stop-gain (nonsense) mutation in the *CHRNB3* gene. This gene codes for one of the nicotinic acetylcholine receptors, many of which have been described in association with nocturnal frontal lobe epilepsy. This particular gene has not previously been reported as a cause of epilepsy in a family before.

The *CHRNB3* gene has been mapped to the locus 8p11.2 (Koyama et al, 1994). Variations in this gene have been reported in association with a predisposition to nicotine addiction (Rice et al, 2012; Saccone et al, 2010). A role for mutations in this gene in the aetiology of idiopathic generalised epilepsy had been suggested in a previous study of affected families by linkage analysis (Durner et al, 1999). This study assessed a number of families with different types of idiopathic generalised epilepsy with onset in adolescence and suggested linkage to the area of chromosome eight encompassing the *CHRNB3* gene. The authors of that study suggested that this indicated that *CHRNB3* could be a candidate gene for adolescent-onset forms of idiopathic generalised epilepsy (Durner et al, 1999). This finding has not been reported by other groups. Other studies of the role of this gene in the aetiology of epilepsy have concentrated more on autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), given the association of other acetylcholine receptor genes with this specific syndrome. However, studies of affected families from an Italian population did not show any evidence of an association of mutations in this gene with ADNFLE (De Marco et al, 2007; Bonatti et al, 2002).

Unlike family A, the mutation in *CHRNB3* is seen only in those individuals with an epilepsy syndrome (heterozygote genotype) and not in any of the unaffected individuals. Again, the clinical phenotype seen in those carrying the mutation is very variable, ranging from isolated febrile convulsions to juvenile myoclonic epilepsy. The previous study in which the *CHRNB3* gene locus was linked to the onset of idiopathic generalised epilepsy may provide us with a pointer to the relevance of the finding in this family. Of the affected individuals, two have definite idiopathic generalised epilepsy syndromes and one has had an isolated febrile convulsion. The other two had epilepsy syndromes of unclear classification, but which had onset in

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early life. It may be that mutations in *CHRNB3* are associated only with idiopathic generalised epilepsy and not with localisation-related epilepsy. Hence, it is possible that the negative results from the screening studies in ADNFLE families may reflect the lack of involvement of this gene in these epilepsy syndromes rather than in epilepsy overall

Although initially promising as a finding, this variant in the gene has been reported at least twice in available public databases and in at least one unaffected carrier (Dr. Mark McCormack, PhD student RCSI, private correspondence). The public databases for genetic variations are not screened for the presence of epilepsy or febrile convulsions, but the presence of this variation in an unaffected individual would argue against a causative effect for this variation.

At this time, it is unclear whether the variant seen in this family could be the definite cause of the epilepsy syndromes seen. However, it is a plausible candidate for further study, especially for the generation of an animal model.

4.3.3 Family H

Affected individuals screened from family H were found to have a novel, non-synonymous mutation (predicted to be damaging) in the *NARG2* gene.

NARG2 has been mapped to the chromosomal locus 15q21.3 (Sugiura et al, 2004) and is one of number of genes which code for the N-methyl-d-aspartate (NMDA) class of glutamate receptors. It has a high degree of expression in foetal kidney, lung, brain and liver. Previous studies have shown that the gene seems to be highly expressed in dividing and immature cells and is down-regulated on terminal differentiation (Sugiura et al, 2004), indicating a possible role in early cell development and in the differentiation of neurons (Sugiura et al, 2001). A joint neuroimaging and genetic study in a Mexican American population (Kochunov et al, 2011) suggested, on the basis of SNP analysis, that cortical grey matter thickness was associated with variations in this gene along with two others, RORA and ADAM10.

With this information, it is biologically plausible that the mutations seen in this family are responsible for the epilepsy syndrome seen in affected individuals. *NARG2* dysfunction could lead to abnormalities of neuronal development and of grey matter thickness, predisposing to neuronal dysgenesis at a level which cannot be demonstrated on conventional imaging and which would lead to the localisation related epilepsy seen in these individuals. Alternatively, the possibility of dysfunction of glutamate transmission within the brain could increase the possibility of seizures, given the excitatory role glutamate has in the central nervous system. However, such conclusions are speculative, and the results will require further modelling (ideally with an animal model) or confirmation by replication in a different kindred before conclusions about the effects of such mutations can be made safely.

As in family A, not all of those individuals who are heterozygous for the mutation are affected. However, the presence of the mutation in these unaffected individuals does not exclude the variant identified from being causative for epilepsy, as this may indicate a reduced penetrance for the mutation. Whether the history of migraine in individual Hii3 is relevant is unclear. As mentioned in the review of the literature, mutations in certain genes have been associated with both migraine and epilepsy, but more usually with migraine with aura or hemiplegic migraine rather than migraine without aura. It is more likely that the occurrence of migraine in this individual is co-incidental rather than related to the novel mutation found, especially given that none of the other individuals who are heterozygous for the mutation are affected by this condition. Unlike family A, there are no obvious phenocopies in this family, which would be more supportive for a causative role for the mutation detected. However, for now, the mutation detected must be regarded as a candidate causal variant for further study, rather than being accepted as definitely causal for epilepsy in this family.

4.3.4 Use and limitations of whole exome sequencing to identify novel genetic abnormalities

Whole exome sequencing has great potential for the identification of novel genetic variations in complex genetic disease. Of the eleven families who met the inclusion

criteria for this study, four were analysed by whole exome sequencing. Of these, 2 families showed good candidates for epilepsy-causing mutations, Family A and Family H. This demonstrates the potential of this approach in identifying causal mutations in familial epilepsy. Although we cannot be definite about whether these novel variations are causative for epilepsy in these families, they are candidates for further studies to see if this is the case.

In contrast to these families, screening in Family D and Family F did not reveal any obvious epilepsy-causing mutations in affected individuals with epilepsy syndromes. When considering these families, there is no obvious reason why this is the case. Both families have histories of epilepsy which are no less clear than in Family H. There is phenotypic variability in Family D, but this is also the case in Family A, and the number of affected individuals and their relationships is very similar in Families F and H. There are no obvious environmental factors in the affected individuals in families D and F which account for the phenotypes seen being produced by non-genetic factors. The extent to which ancillary information – EEG and MRI results specifically – were available is similar in all of these families, and would not account for the failure to identify genetic variations.

The failure to identify common genetic variations in the affected individuals in Families D and F by whole exome sequencing may be due to a number of factors. It may be that the mutation is not in the exome, but may be elsewhere in the genome or may be epigenetic. Exomes consist of 1% of the genome (Rabbani et al, 2014), and if the causative mutation lies outside this, whole exome sequencing will not identify this. Other techniques (such as whole genome screening) may identify the mutation responsible for the epilepsy syndromes seen in these families.

It may also be that a different genetic mechanism is responsible for epilepsy in these families, such as a copy number variant. Copy number variants (CNVs) are deletions, duplications, rearrangements or insertions of DNA that reduce or increase copy number of certain sections of the genome (Marian, 2012; The Epi4K Consortium, 2012). They can range from 1kb up to several megabases in size. These CNVs have been identified in patients with epilepsy (Dibbens et al, 2009; Helbig et al, 2009; Mefford et al, 2010) of different types, and rare CNVs have been detected

in approximately 8% of those with epileptic encephalopathies (Mefford et al, 2011). Phenotypic variability has also been reported with CNVs (Girirajan and Eichler, 2010). Next generation sequencing techniques, such as whole exome sequencing, may not be sufficient to screen accurately for such CNVs (Majewski et al, 2011; Marian, 2012). If such a structural variation was causative for epilepsy in Families D and F, whole exome sequencing may not be able to detect it.

Whole exome sequencing also has other technical limitations. The converage of the exome can vary in different regions, and uneven capture efficiency can result in exons with low coverage frequency (Majewski et al, 2011). Further, regions which are not yet known or annotated as exonic will not be captured by whole exome sequencing. Similarly, regulatory regions such as enhancers and promoters are not captured (Ku et al, 2011). Thus, if causative variants are in these regions, whole exome sequencing will not identify them, and techniques such as whole genome sequencing may be more appropriate.

As in this study, the data generated by whole exome sequencing requires filtering before it can be analysed. Although every effort has been made to ensure that these filters do not exclude any possible causative variants, it is possible that the variants which we have deemed unlikely to be causative for epilepsy, or which were filtered out in the process of genetic analysis, may have greater roles in the aetiology of epilepsy than we have ascribed to them. When considering the results of genetic analysis in each family, we felt that variants in genes which were not associated with central nervous system or ion channel function, or which did not have a previously demonstrated association with epilepsy, were unliklely to be causative for epilepsy. This may later be proven to be incorrect if new discoveries of gene function are made. For the variants seen in the individuals from Family D and Family F, it may be that one of these may have a role in the aetiology of epilepsy of which we are currently unaware and that, to interpret the results, prior knowledge of gene function may have to be ignored or new discoveries of gene function may be needed. Although the filtering strategies applied in whole exome sequencing allow us to find promising variants in the exomic data, the possibility of important variants in the exome being discarded may be a source of error (Ku et al, 2011).

In any study, recruiting sufficient individuals to ensure that the study is adequately powered is a frequent concern. Our study is no different. The number of families recruited in this study may seem small, but compares favourably to other whole exome studies which have identified novel genetic variants in other conditions (Rabbani et al, 2014). In the families screened in this study, a relatively small number of individuals underwent whole exome sequencing (three in each family screened). It can be argued that this increases the risk of the variants identified in these families as being "false positives", i.e., that these are chance findings rather than genuinely causative variants for the epilepsy syndromes seen. We feel that this is unlikely as a source of error, given the strenuous efforts made to eliminate all variants which were felt to be anything less than biologically plausible and probably damaging. However, we are aware that this may still be a source of error in our study. This is another reason why we have been cautious to describe the variants identified as candidate causal variants and why we have emphasised the need for further studies to confirm our findings.

Alternatively, there may be unidentified environmental factors which have caused epilepsy in some the affected individuals. In the same manner that some individuals may not have recall having had febrile convulsions, it is possible that individuals may have forgotten neonatal complications, infections or head trauma which may be relevant in the aetiology of their epilepsy. This would introduce inaccuracy in phenotyping, which would in turn confound the genetic analysis.

In spite of the inability to identify clearly causative novel variations in individuals from two of the families analysed by whole exome sequencing, it should be noted that potentially epilepsy-causing mutations were identified in two of four families thus assessed. This would indicate the potential for this technique in recognising these novel variations and generating candidate genetic variants for further study. There are limitations in this technique, some of which are common to all genetic analysis techniques (such as the difficulties in phenotyping) and some of which are specific to whole exome sequencing itself (such as the interpretation of the quantities of information generated).

5. Final conclusions

The aim of this study was to find novel genetic abnormalities associated with epilepsy in affected Irish families. After performing this study, we can now draw a number of conclusions.

1. Recruitment for family-based genetic studies such as this in Ireland is possible. However, the rate of recruitment can be quite low. Further studies should allow for this in the study design.

2. The use of an electronic patient record system greatly assists in the identification of potential participants for a study such as this. It also allows for more accurate phenotyping of participants. The expansion of this system will facilitate further studies. If possible, access to this system should be given to investigators performing such studies, although we recognise that this will require discussion and approval by the relevant clinical services.

3. Clinical phenotyping of participants in studies of epilepsy must include a collateral history from other family members if possible to ensure accurate defnintions of affected status. However, even in cases where collateral details and ancillary information are available, clinical phenotyping can still be limited.

4. Irish families affected by epilepsy show considerable intrafamilial phenotypic variability. This should not preclude their inclusion in genetic studies.

5. Whole exome sequencing can identify potentially disease-causing mutations in affected Irish epilepsy kindreds.

6. We have identified a novel abnormality in a gene known to be associated with epilepsy (A348Y in *SCL2A1*) and novel abnormalities in genes not previously associated with epilepsy (*CHRNB3* and *NARG2*).

6. Glossary of terms

Below are some of the terms used in the text. Some of the terms used in this study are specifically discussed in the course of the review of the literature, and as such are not included in this section.

Allele – one member of a pair (or any of the series) of genes occupying a specific spot on a chromosome that controls the same trait. In organisms with diploid cells, an organism usually has two alleles of each gene (Feero et al, 2010). Diploid cells whose chromosomes have the same allele of a given gene at a particular locus are called homozygous with respect to that gene, while those that have different alleles are called heterozygous.

Autosomal dominant – a form of Mendelian inheritance. In this pattern, an individual inherits only one copy of a particular gene or mutation, but that this single copy is sufficient for that individual to manifest the phenotype of that gene. A good example of a condition with such inheritance is Huntington's chorea (Kingston, 1989).

Autosomal recessive – a form of Mendelian inheritance. In this pattern, an individual must have two copies of the same gene or mutation for the particular phenotype to manifest itself. Examples include metabolic disorders or neurocutaneous disorders such as tuberous sclerosis (Kingston, 1989).

Channelopathy – a disease process caused by an inherited mutation causing an abnormality in all or part of an ion channel (Kass, 2005). The resulting abnormal function of the ion channel in the neuronal cell membrane is felt to account for the clinical condition. The idea of epilepsy as a channelopathy is probably best demonstrated by those idiopathic epilepsy syndromes which are felt to be purely monogenic, i.e., caused by an abnormality in the function of one gene only (Jurkatt-Rott et al, 2010). Although most mutations have been found to result in a loss of function of the channel affected, some have been shown to result in a gain of function or an increase in the normal rate of ion flux (Escayg and Goldin, 2010). The idea of epilepsy as a channelopathy has been extremely useful in the study of the

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aetiology of epilepsy (Catterall et al, 2008), although other mechanisms for the aetiology of epilepsy have been emphasised by some commentators (Greenberg and Subaran, 2011).

Common disease common variant (CDCV) hypothesis – a hypothesis in genetic analysis of common diseases. In the study of epilepsy, it has also been termed the common variant complex epilepsy model. This hypothesis states that the genetic risk for common diseases will often be due to disease-producing alleles found at relatively high frequencies in the population under analysis. In this context, a common variant is felt to be one which occurs at a frequency of greater than 1% (Visscher et al, 2012). In essence, this means that the reason for the high frequency of certain diseases in a population is due to the high frequency of the underlying disease-influencing alleles (Hemminki et al, 2008).

Common disease rare variant (CDRV) hypothesis – this hypothesis argues that rare variations, which are specific to the individual or the family studied, are responsible for the disease seen. Thus, in each case, a unique or very rare set of alleles combine to give rise to a disorder (such as epilepsy) which acts as a 'final common pathway' for these multiple variants. The disease is common because variants are common in the human genome, but these variants are much less common and much more individual than the CDCV hypothesis states. The variants can differ, but are often SNPs or CNVs. An expansion of this hypothesis has been used in the study of epilepsy genetics and has been termed the multiple rare variant complex epilepsy model. This hypothesis is a useful one to explain a number of factors which have been noted in the study of epilepsy genetics thus far, especially the failure to replicate findings in different populations.

Common variant multiple disease (CVMD) hypothesis – an expansion of the CDCV hypothesis which seeks to explain the overlapping linkage results and multiple genetic associations which have been ascribed to a number of alleles. This expansion of the original hypothesis states that the common alleles which contribute to a certain disease may act in other genetic backgrounds resulting in different clinical outcomes.

Copy number variants or polymorphisms (CNVs) – a structural genomic variant that results in confined copy number changes in a specific chromosomal region. If its population allele frequency is less than 1%, it is referred to as a variant; if its frequency exceeds 1%, the term polymorphism is used (Beckmann et al, 2007). CNVs can be deletions, insertions, duplications or larger scale variants (Feuk et al, 2006). It has been estimated that up to 12% of the human genome contains CNVs (Redon et al, 2006).

Endophenotype – a particular trait or aspect of a condition which is less affected by subjective assessment and may be present in those without overt disease (Cannon and Keller, 2006). The accepted criteria that a biomarker must fulfill to be called an endophenotype include: that the endophenotype is associated with illness in the population: that it can be inherited: that it can manifest itself in an individual whether that individual is affected by a particular disease or not, i.e., that it is state-independent: that it and the condition being studied will co-segregate within affected families: and that the endophenotype in affected individuals is found in non-affected family members at a higher rate than in the general population (Zitman, 2008).

Epigenetic inheritance – inherited alterations that they are not due to alterations in DNA sequence but to other factors. Epigenetic alterations were first recognised as contributing to disease states in the study of certain cancers, and have been shown to have a contributory role not only in the development of certain cancers (Dawson and Kouzarides, 2012) but also on the response to certain therapies (Popovic and Licht, 2012). Epigenetic gene alterations are associated with certain neurodevelopmental conditions, such as Rett syndrome (LaSalle and Yasui, 2009) and ATRX syndrome (Baker et al, 2008), which can have seizures as part of the phenotype of the conditions.

Exon – the portion of a gene that codes for amino acids and are expressed in the protein product of the gene.

Expressivity – the variability with which basic patterns of inheritance are modified, both in degree and in variety, by the effect of a given gene in people of the same

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genotype. For example, polydactyly may be expressed as extra toes in one generation and extra fingers in another.

Frame shift mutation – a mutation which changes the reading frame of the gene downstream from it, often leading to a premature stop codon (Guttmacher and Collins, 2002).

Gain-of-function mutation – a form of mutation which alters the phenotype by increasing the quantity or the functional activity of a protein, causing the protein to take on a toxic function (Guttmacher and Collins, 2002). Mutations of this type are often dominantly inherited. Examples include trinucleotide repeat disorders such as Hungtington's disease (Jacobsen et al, 2011).

Gene – the basic unit of inheritance, the definition of a gene has undergone considerable revision since the initial use of the term 1909 (Gerstein et al, 2007). It is often defined as the entire nucleic acid sequence that is necessary for the synthesis of a functional polypeptide. A more complete definition is that a gene is a locatable region of genomic sequence, corresponding to a unit of inheritance, which is associated with regulatory regions, transcribed regions and/or other functional sequence regions (Pearson 2006). However, other definitions have been proposed (Gerstein et al, 2007).

Genetic heterogeneity – a phenomenon in which a single phenotype may be caused by any one of a number of mutations in different alleles or loci. Heterogeneity can be classified as one of two types. Allelic heterogeneity means that different mutations within a single gene locus cause the same phenotypic expression, such as mutations within the CFTR gene which cause cystic fibrosis (Castellani et al, 2008). Locus heterogeneity is used to describe variations in unrelated gene loci which can cause a particular phenotype, for example, the clinical condition of osteogenesis imperfecta, which can have autosomal dominant and autosomal recessive inheritance (van Dijk et al, 2012).

Genetic pleiotropy – refers to the phenomenon in which a single locus affects two or more apparently unrelated phenotypic traits and is often identified as a single

mutation that affects two or more traits (Stearns, 2010). In contrast to genetic heterogeneity, in this case a single gene may control or influence multiple (and possibly unrelated) phenotypic traits (Sivakumaran et al, 2011). The condition phenylketonuria is often used as an example of pleitropy.

Genotype – the genetic makeup, as distinguished from the physical appearance, of an organism. In some instances, the term is used in relation to a particular locus. When used in this sense, it refers to the combination of alleles located on homologous chromosomes that determines a specific characteristic or trait.

Genotyping – the process of determining the genotype of an individual by examining that individual's DNA sequence using biological assays and comparing it to another individual's sequence or to a reference sequence.

Haplotype – a term used to refer to a series of alleles at linked loci along a single chromosome (as in the haploid gametes) (Burton et al, 2005).

Heritability – the proportion of phenotypic variance which is attributable to variance in genotype (Hasler et al, 2004).

Indel – a structural genetic variation arising from either an insertion or deletion event involving <1 kb of DNA (Freeman et al, 2006).

Intron – the parts of the gene sequence that are not expressed in the protein

Locus – the specific location of a gene or DNA sequence on a chromosome.

Loss-of-function mutation – a form of mutation which alters the phenotype by decreasing the quantity or the functional activity of a protein (Guttmacher and Collins, 2002).

Mendelian inheritance – an inheritance pattern for autosomal gene pairs, this is a collective term for the concepts of autosomal dominant, autosomal recessive and X-

linked inheritance patterns. These patterns of inheritance were first elucidated by Brother Gregor Mendel (Kingston, 1989).

Missense mutation – a mutation in which one base in a codon is substituted for another such that it leads to the production of an alternative amino acid to the normal (Guttmacher and Collins, 2002).

Mitochondrial – a disease caused by mutations in the mitochondrial genes and which, if inherited, can only be inherited from the individual's mother (Guttmacher and Collins, 2002). Mitochondria are organelles within every cell of the body. An individual's mitochondria are inherited from the maternal side only, as sperm cells do not contribute any mitochondria to the developing gamete. Mitochondria have a separate DNA to that in the cell nucleus (Wallace, 1999). Mutations in the mitochondrial DNA lead to abnormalities of synthesis of proteins in the respiratory electron chain and subsequent abnormalities of energy generation and utilisation.

Mutation – a modification in the sequence of a gene that is clearly identified as, or strongly believed to be, the cause of a disease (Feero et al, 2010). Mutations can be classified by the causative mechanism or by their functional effect (Guttmacher and Collins, 2002).

Non-Mendelian inheritance – also called complex inheritance. The current concept of a genetically complex disease implies that multiple genes and environmental factors contribute to the aetiology of the disease under study, and that none of these factors in isolation has a major effect of disease risk (Ottman, 2005). Complex genetic conditions include factors such as height, weight and IQ, as well as common disease such as diabetes mellitus, ischaemic heart disease, asthma and cancer. These traits are normally distributed in the population, and show a pattern consistent with the influence of multiple genetic contributions and environmental factors (Ottman, 2005). Complex genetic conditions often exhibit familial clustering and concordance amongst relatives (Winawer et al, 2005), both of which are often seen in studies of epilepsy. Complex diseases tend to show a more rapid decrease in the risk to relatives per degree of relationship to the proband than Mendelian diseases (Pal et al, 2006).

Nonsense mutation – a mutation in a codon which changes an amino acid codon into a premature stop codon. This causes the termination of the protein instead of producing an amino acid (Guttmacher and Collins, 2002).

Penetrance – the proportion of individuals carrying a particular variation of a gene which also express the phenotype of that gene. Complete penetrance occurs if all individuals with the particular gene express the phenotype of that gene. Penetrance is felt to be low or incomplete if some individuals with the allele fail to express the phenotype. Penetrance can be difficult to determine accurately, given that onset of symptoms from a particular disease-causing mutation can be age dependent or can be affected by environmental factors (Rochette et al, 2010). Thus, a gene may be inherited in an autosomal dominant fashion but some individuals with the gene may not manifest the disease as the penetrance may be incomplete.

Phenocopy – a similar phentoypes to the disease or condition under assessment but having a different aetiology. Phenocopies can confound genetic analysis (Schork et al, 2007) as the individuals affected will be classified as being affected but may not have a genetic aetiology for their status.

Phenotype – the observable structural and functional characteristics of an organism determined by its genotype and modulated by its environment (Schulze and McMahon, 2004). Phenotypes result from the interaction of an organism's genotype with its environment. As such, similar phenotypes can be caused by environmental or genetic causes, or both. For example, lateral temporal lobe epilepsy can occur as a result of genetic inheritance or acquired factors (Michelucci et al, 2009). The phenotype of an individual can change during the course of their life, principally due to the influence of environmental factors.

Segmental duplications – also termed "low-copy repeats", these are duplicated genomic regions between 1 and 400 kb in length, occurring at more than one site within the genome, with 90% or greater sequence identity among the duplicates (Sharp et al, 2005; Bailey and Eichler, 2006; Kim et al, 2008).

Silent mutation – also known as synonymous mutation, a mutation in which one base in a codon is replaced with another, so that the resultant codon still codes for the same amino acid (Guttmacher and Collins, 2002).

Single nucleotide polymorphism (SNP) – a class of DNA variant. It represents a variation in a single nucleotide base in the DNA sequence. A common SNP can be defined as a locus at which two SNP alleles are present, both at a frequency of 1% or more in the population. Although the amount of information carried by individual SNPs can be limited, SNPs are relatively easily typed by molecular mechanisms. It is estimated that there may be as many as 10 million common SNPs in the human genome. For these reasons – ease of identification and large number – SNPs are widely used as markers in genetic studies.

Sporadic – spontaneous occurrence of a genetic mutation in an individual which is causative of a disease state. Sporadic mutations can cause difficulty in family genetic studies, as the occurrence of such mutations in a pedigree may imply a mode of inheritance which is inaccurate. A good example of this in epilepsy is Dravet syndrome, in which most of the causative mutations in the SCN1A gene are felt to be sporadic rather than inherited (Meisler and Kearney, 2005).

Structural genetic variation – refers to a class of genomic alterations of DNA that usually span more than 1000 bases (Freeman et al, 2006; Feuk et al, 2006). It is a broad term for variants which alter chromosomal structure (Hurles et al, 2008). It includes quantitative (unbalanced) changes such as copy-number variants (CNVs), and less common balanced variations involving chromosomal inversions and translocations.

Translocation – a chromosome abnormality caused by rearrangement of parts between different chromosomes or normally non-contiguous segments of a single chromosome (Lengauer et al, 1998).

Variant – a modification in a gene which contributes to the susceptibility for the disease but is not proven to be causative. This is more in keeping with polygenic epilepsies, where the phenotype can be generated by the interaction of a number of

different alleles – in these cases, modifications of gene sequences contribute to the susceptibility to develop epilepsy, and the association with epilepsy is inferred from the functional effects of these variants in experimental models and the fact that the variants are much more commonly found in individuals with epilepsy.

X-linked – a special form of Mendelian inheritance resulting from the differences between sex chromosomes in the two genders. Every female inherits two X chromosomes (one from each parent). In the usual form of X-linked inheritance, if a gene on one X chromosome carries a disease-causing mutation, it is unlikely to manifest itself in a female due to the effect of the normal gene on the other X chromosome. However, every male inherits only one X chromosome, with the Y chromosome being inherited from his father. In this case, the gene carrying the disease-causing mutation is expressed and gives rise to a particular phenotype. A good example of a disease inherited in such a manner is haemophilia A (Kingston, 1989). The usual pedigree in X-linked conditions will show that males are affected by the conditions, whereas females act as carriers which are usually unaffected. X-linked dominant conditions are also well recognised (Jais et al, 2003) although their occurrence is rarer. Occasionally, females can be affected by such conditions when there is spontaneous deactivation of one of the two X chromosomes, allowing for the mutation-carrying gene to express itself – this is referred to as Lyonisation (Sun and Tsao, 2008; Jeon et al, 2012).

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Appendices

Appendix A – Detailed phenotypic information for study participants

<u>Individual family members – family A</u>

A i 1

Age at recruitment; 63

Sex; Female

Handedness; Right

Diagnoses;

1.   Previous appendicectomy

2.   Hyperthyroidism of unclear cause (has had radio-iodine therapy previously)

3.   Hypercholesterolaemia

4.   Hypertension

5.   Arthritis of unclear type – probable rheumatoid arthritis.

Medications;

1.   Atorvastatin 20mg od

2.   Eltroxin 100mcg od

3.   Antihypertensive therapy, unsure of name of same

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A i 2

Age at recruitment; 61

Sex; Female

Handedness; Right

Diagnoses;

1.   Asthma

2.   Hypertension

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A i 3

Age at recruitment; 57

Sex; Female

Handedness; Right

Diagnoses; none

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A i 4

Age at recruitment; 54
Sex; Male
Handedness; Right

Diagnoses;

1.   Colitis of unclear cause

2   Epilepsy

Medications;

1.   Phenytoin 150mg bd

2.   Treatment for colitis, unsure of name or dose of same

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Localisation related epilepsy

Seizure classification; Simple partial, simple partial progressing to secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
Although unsure when he first started having seizures, he was diagnosed with epilepsy at the age of 20 years. He says that he has never been seen by a specialist for his epilepsy but that he had investigations done in Port Laoise General Hospital.

He has about 1 – 2 events per month. A typical seizure begins with a sensation of getting distant from his surroundings, described as being like "you could be talking to me and I wouldn't know what you're saying". After this, he can develop an unpleasant taste in his mouth. Most events stop after this, but rarely he will go on to lose awareness and then have a generalised tonic-clonic seizure. He has not had any focal features reported to him for these events, which last for c. 2 minutes. He is usually drowsy for c. 15 minutes afterwards.

He has never had status epilepticus. He does not have myoclonus. He has no other types of event.

Imaging results; never performed

EEG results; never performed

A ii 1

Age at recruitment; 40

Sex; Male

Handedness; Right

Diagnoses;

1. Essential tremor – diagnosed by a consultant Neurologist
2. Epilepsy

Medications;

1. Valproate chrono 600mg bd
2. Levetiracetam 1000mg bd

Previous anti-seizure medications;

1. Phenytoin
2. Carbamazepine – caused a rash

Allergies; Carbamazepine

Early risks for epilepsy; none

Epilepsy classification; Idiopathic generalised epilepsy

Epilepsy syndrome; Juvenile myoclonic epilepsy

Seizure classification; Generalised absence, generalised myoclonic, generalised tonic-clonic

Summary of epilepsy history and seizure semiology;
He is unsure at what age he began to have seizures, but was diagnosed at the age of 23 years after his first generalised tonic clonic seizure. He has noticed that he has photosensitivity. He feels that his first (and so far only) generalised tonic clonic seizure was precipitated by sunlight coming through tree branches when he was cycling, and he mentions that "flickering lights drive me mad".

During his absence seizures, he loses awareness without a warning or a preceding aura. He has not been noticed to have any automatisms and has been described as "staring a bit" but nil else. These events last for 10 – 15 seconds. He has a variable frequency of events, but when they occur they tend to occur in clusters of 3 – 4 per day.

He has brief has myoclonic seizures on a daily basis, saying that "the arm or leg can fly out".

He has never had status epilepticus or febrile convulsions. He has no other types of event.

Imaging results; 1.5T MRI was normal

EEG results; Routine study showed bilateral bursts of generalised spike and slow wave activity, with occasional focal spike and wave activity over the right frontal area.

A ii 2
Age at recruitment; 40
Sex; Female
Handedness; Right

Diagnoses;
1.   Previous appendicectomy
2.   Migraine without aura
3.   Epilepsy

Medications;

1.   Carbamazepine 300mg mane, 200mg nocte

Previous anti-seizure medications;
1.   Lamotrigine – caused anorexia and insomnia
2.   Carbamazepine retard – caused anorexia and insomnia

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Localisation related epilepsy

Seizure classification; Simple partial, simple partial progressing to secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She is unsure when she started having seizures, but was diagnosed at the age of 28. She has noticed that events can be triggered by sleep deprivation and stress but has not noticed any other precipitants.

Her seizures begin with a "funny feeling", "like I wasn't there". She describes this feeling as a sensation of being very disconnected from her surroundings. After this, she will have sensation of "seeing double", followed by a sensation of her eyes "moving" or flickering. All of this lasts for c. 10 minutes, and then she may lose awareness. If she loses awareness, she has a generalised tonic-clonic seizure, which usually lasts for c. 2 minutes. After events with loss of awareness, she says that she is "out of it" for 30 – 60 minutes.

She has simple partial seizures (i.e., the "funny feeling") c. 3 times per week without progression. Her last convulsion was 2 years prior to recruitment.

She has never had status epilepticus. She does not have myoclonus. She has no other types of event.

Imaging results; unavailable

EEG results; Routine study was reported as a normal waking EEG

A ii 3

Age at recruitment; 39

Sex; Male

Handedness; Right

Diagnoses;

1.   Epilepsy

Medications;

1.   Carbamazepine 400mg mane, 500mg nocte

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Localisation related epilepsy

Seizure classification; Presumed secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;

He is unsure when his seizures started, but he was diagnosed with epilepsy at the age of 15 years. He has one or two events on average per year. His first seizure was precipitated by fasting, but he has not noticed any consistent triggers for his seizures.

For his usual events, he has no definite preceding aura. On the last two occasions, he has had a preceding brief (lasting only seconds) "chalky taste in the mouth", followed by loss of awareness. More usually, he loses awareness at onset. He has a generalised tonic clonic seizure and does not have any focal features according to the descriptions he has received of his events. His events usually last for 1-2 minutes and he recovers to baseline over 15 minutes.

He has never had status epilepticus. He does not have myoclonus. He has no other types of seizure.

Imaging results; never performed

EEG results; A routine study showed one burst of sharp and slow wave activity over the left frontal and temporal region. During photic stimulation, one further burst of sharp wave activity was noted over the left posterior temporal and occipital area followed by a generalised seizure discharge

A ii 4
Age at recruitment; 37
Sex; Male
Handedness; Right

Diagnoses; Nil

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A ii 5
Age at recruitment; 36
Sex; Male
Handedness; Ambidextrous, right predominant

Diagnoses; Nil

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A ii 6

Age at recruitment; 35
Sex; Female
Handedness; Right

Medical diagnoses;
1.   Epilepsy

Medications;
1.   Zonisamide 500mg nocte
2.   Folic acid 5mg od

Previous anti-seizure medications;
1.   Carbamazepine – caused a rash
2.   Clobazam
3.   Lamotrigine
4.   Levetiracetam – worsened her seizures
5.   Topiramate – caused difficulty with cognition and behaviour
6.   Valproate chrono

Allergies;

1. Carbamazepine

Early risks for epilepsy; None

Epilepsy classification; Idiopathic generalised epilepsy

Epilepsy syndrome; Juvenile myoclonic epilepsy

Seizure classification; Generalised absence, generalised myoclonic, generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She began having seizures in early adolescence, about the age of 12 years. However, her initial events were described as "staring spells" and she did not come to medical attention until the age of 13 years when she had her first generalised tonic clonic seizure.

Her generalised absence attacks ("staring spells") occur out of clear consciousness. They are characterised by behavioural arrest and loss of awareness. There are no associate automatisms, although others have noticed that her respiratory rate increases during the events. Each event lasts for c. 30 seconds and then spontaneously resolves.

She has generalised tonic clonic seizures. These occur without any preceding aura or warning. She loses awareness at onset. A collateral history from family members describes a generalised convulsion without any focal features. Attacks are usually associated with tongue biting, urinary incontinence and excess salivation. They usually resolve within three minutes, and are associated with post ictal drowsiness.

She has generalised myoclonic jerks out of clear consciousness. There are no focal features with most of these events, which usually occur in the mornings, but she can occasionally have isolated jerks of the right arm only.

She has never had status epilepticus. She has no history of photosensitivity, but has noticed that sleep deprivation and stress increase the likelihood of having an event of any type. She has no other seizures other than those described above.

Imaging results; Never performed

EEG results; During video EEG monitoring, several bursts of generalised frontally maximal polyspike and wave activity were seen. In addition, focal discharges were seen independently over the left and right temporal regions. Findings were felt to be in keeping with a generalised epilepsy syndrome.

A ii 7
Age at recruitment; 34
Sex; Male
Handedness; Right

Diagnoses; none

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 1
Age at recruitment; 16
Sex; Female
Handedness; Right

Diagnoses;
1.   Headaches, clinically consistent with chronic daily headache – was assessed by a consultant Neurologist

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; 1.5T MRI was normal

EEG results; Previous routine and sleep-deprived studies were normal with no epileptiform features.

A iii 2
Age at recruitment; 11
Sex; Female
Handedness; Right

Diagnoses;
1   Epilepsy

Medications; Nil

Previous anti-seizure medications;
1.   Valproate liquid 6mls bd

Allergies; Nil

Early risks for epilepsy; none

Epilepsy classification; Unclear

Seizure classification; Unclear

Summary of epilepsy history and seizure semiology;

A history from the patient and a collateral history from her parents were obtained. She has only had one type of event, consistent in semiology and on-going since she was four years of age. These have been described as seizures to the individual and her family.

The events begin with the onset of a headache. This is initially of a mild intensity and slowly increases in severity over time. It is not lateralised but involves "the whole head". After this has been present for a while, she begins to have "numbness" of the left arm and left leg, which spreads to involve the left side of her body over a matter of some minutes. She has no loss of awareness during the course of the event and does not exhibit uncontrolled movements or posturing of her limbs. All events last for 30 – 50 minutes before resolution. According to her parents, she "looks like she'd had a stroke" during events. Both the patient and her parents are clear that she has never had a secondarily generalised convulsion or generalised absence seizure.

The diagnosis of epilepsy was reached by the paediatric services on the basis of EEG findings. Previous EEG request details mention features such as unilateral clonic jerking, aphasia, and two generalised convulsions, but these were not confirmed by the clinical history.

At the time of recruitment, she had been off all medications for some years and was free of all events. She has never had status epilepticus. She does not have myoclonus. She has not had any other types of seizure.

Imaging results; 1.5T MRI was normal

EEG results; A routine study showed independent focal epileptiform discharges in the right parietotemporal and left centroparietal areas

A iii 3

Age at recruitment; 22

Sex; Female

Handedness; Right

Diagnoses;

1. Asthma in childhood

2.   Epilepsy

Medications;
1.   OCP

Previous anti-seizure medications;
1.   Carbamazepine

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Localisation related epilepsy

Seizure classification; Unclear

Summary of epilepsy history and seizure semiology;
According to the patient, she was diagnosed with epilepsy in childhood. She had seizures between the ages of 6 and 9 years and has not had any since then. She was on anti-seizure medications until aged 11, when they were weaned off.

She has never had absence events, myoclonus or generalised tonic-clonic seizures. According to her mother, events would occur when she was excited or rushing, she would "let out an over the top laugh", which was very odd and unsettling in nature. After this, her eyes would roll back and she would collapse to the ground. She was described as being "floppy" during these events and not moving limbs or face. She would regain awareness within a minute and would be "dazed" for a few minutes afterwards. She had no further events after being started on carbamazepine.

She has never had status epilepticus. She has not had any other types of seizure.

Imaging results; Never performed

EEG results; Unavailable

A iii 4
Age at recruitment; 12

Sex; Male

Handedness; Right

Diagnoses;

1.   Asthma

Medications;

1.   Becotide inhaler 2 puffs PRN

2.   Salbutamol inhaler 2 puffs PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; One previous concussion, nil else

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 5

Age at recruitment; 9

Sex; Male

Handedness; Left

Diagnoses; none

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 6
Age at recruitment; 2
Sex; Male
Handedness; Unclear, possibly left

Diagnoses; none

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 7
Age at recruitment; 4
Sex; Male
Handedness; Right

Diagnoses; None

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 8
Age at recruitment; 3
Sex; Female
Handedness; Unclear, probable right

Diagnoses; None

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 9
Age at recruitment; 3
Sex; Female
Handedness; Unclear, probable right

Diagnoses; None

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

A iii 10
Age at recruitment; 3
Sex; Female
Handedness; Unclear, probable left

Diagnoses; None

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; None

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

<u>Individual family members – family B</u>

B i 1
Age at recruitment; 65
Sex; Female
Handedness; Right

Diagnoses;
1.   Previous hysterectomy
2.   Arthritis (probable osteoarthritis)
3.   Received anti-D therapy in 1977, 1978 – not on treatment for hepatitis, has been investigated for same
4.   Epilepsy

Medications;
1.   Phenobarbitone 30mg bd

Previous anti-seizure medications; nil

Allergies; previous reaction to "an injection" – she is unsure of the name of the product but says that it caused swelling and paresis of the injected arm. She is certain that this product is now "off the market".

Family history;
1.   Mother died in her 60s of a stroke
2.   Father died aged 76 of Alzheimer's disease
3.   One sister died aged 5 of meningitis
4.   None of her siblings have epilepsy or febrile convulsions

Early risks for epilepsy; none definite. She feels that the events in early childhood were epileptic seizures, rather than febrile convulsions, as some of them occurred without an increase in temperature.

Epilepsy classification; Idiopathic generalised epilepsy

Seizure classification; Generalised tonic clonic seizures

Summary of epilepsy history and event semiology;
According to the patient, she was diagnosed with epilepsy in infancy – she says of her seizures that "I had them when I was a baby in the pram". Notes from other family members estimate the age of onset of her seizures as 9 months. Initially seizures only occurred in the setting of a high temperature. Due to recurrent events, she was put on anti-seizure medication (she is unsure of which one but thinks it was phenobarbitone) in early childhood. She was taken off this medication when she was 7 years old as her seizures were well controlled. Events began to recur at age of 14 years.

Seizures could be triggered by exercise – she gives the example of events in adolescence often occurring after vigorous dancing. She has no aura prior to events and loses awareness at onset. Collateral history reports that she gives a deep inhalation and will then collapse, with stiffening and subsequent shaking of all limbs. She may have had occasional urinary incontinence. Each seizure lasts for 1- 2 minutes. She is profoundly tired after events with a generalised headache.

Her last event occurred at the age of 27, after which she was put on regular phenobarbitone.

She has no events of any other type.

Imaging results; Never performed

EEG results; Never performed

B i 2
Age at recruitment; 68
Sex; Male
Handedness; Right

Diagnoses;
1.   Arthritis (probable osteoarthritis)
2.   Hypercholesterolaemia
3.   Epilepsy

Medications;
1.   Clonazepam 1mg od
2.   Pravastatin 40mg od
3.   Aspirin 75mg od

Previous anti-seizure medications; nil

Drug allergies; nil.

Family history;
1.   Mother died aged 66 of a MI
2.   Father died aged 87 of Alzheimer's disease
3.   None of his siblings have epilepsy or febrile convulsions

Early risks for epilepsy; None

Epilepsy classification; Localisation related epilepsy

Seizure classification; Simple partial progressing to secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He was diagnosed in adulthood, with his first event occurring in 2001 at the age of 59. He has had only two events in total, both of which had the same semiology. There are no obvious precipitatnts for his seizures.

He remembers having an unusual epigastric sensation initially, which he described by saying "I felt the stomach was sick". This sensation ascended through him and he then lost awareness. He cannot remember details of the seizures after this, but he was subsequently told in hospital that he "had a seizure". His next clear memory after losing awareness in both instances is of lying on the ground feeling sore.

After investigations in 2004, he was started on anti-seizure medications. He has not had any events since starting treatment.

He has no other events of any type.

Imaging results; Unavailable – patient was told his MRI showed a "shadow on the brain"

EEG results; Routine study showed independent disturbances in each temporal region but no epileptiform discharges during the recording.

B ii 1
Age at recruitment; 41
Sex; Female
Handedness; Right

Diagnoses;
1.   Epilepsy
2.   Isolated febrile convulsion

Medications;
1.   Sodium valproate 1,000mg mane, 1,100mg nocte

Previous anti-seizure medications;
1.   Previously on phenobarbitone on a PRN basis at times of febrile illnesses
2.   Carbamazapine – ineffective

Allergies; nil.

Early risks for epilepsy; Febrile convulsion, nil else

Epilepsy classification; Idiopathic generalised epilepsy

Seizure classification; Simple febrile convulsion, generalised tonic clonic, possible generalised absence

Summary of epilepsy history and seizure semiology;
She had one convulsion in the setting of a febrile illness at the age of 4 years (according to her mother she had "a sore throat" at the time). Neither she nor her mother could recall the duration of the event, but her mother feels that it did not have any focal features and was not particularly prolonged. She did not have any further febrile convulsions.

Her next definite seizure occurred without warning at the age of 13 years. She did not have a febrile illness at the time. She had a loss of awareness without warning when walking to school and awoke in a ditch with cuts on her face and having been incontinent of urine.

She has had generalised tonic clonic seizures since then. Events are usually associated with loss of awareness at onset with jerking of all four limbs, tongue biting, urinary incontinence and a generalised post ictal headache.

She herself does not recall any absence events, but a collateral history from family members mentions that she had "petit mals" in early teenage years. These were characterised by staring and being unresponsive without any automatisms.

She has noticed some possible triggers such as playing sports, and feels that seizures were more likely to occur prior to meals.

Imaging studies; Never performed

EEG results; Unavailable

B ii 2

Age at recruitment; 40

Sex; Female

Handedness; Right

Diagnoses;
1. Febrile convulsions
2. Epilepsy
3. Previous fracture of left ankle

Medications;
1. Lamotrigine 150mg bd
2. Clonazepam 0.5mg nocte
3. Levetiracetam 500mg bd
4. Phenytoin 200mg bd

Previous anti-seizure medications;
1. Carbamazepine – very fatigued, mentally slowed, rash
2. Sodium valproate

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3. Clobazam
4. Ethosuximide
5. Gabapentin
6. Phenobarbitone
7. Primidone

Allergies; she had an adverse reaction previously to "an injection". She is unsure of the name of this, but says that it caused swelling of the injected arm and paresis. Her mother says that this was the same preparation to which she had an adverse reaction. She developed a rash when on carbamazepine.

Early risks for epilepsy; febrile convulsions

Epilepsy classification; Idiopathic generalised epilepsy

Epilepsy syndrome; Not otherwise specified

Seizure classification; Simple febrile seizures, absence, generalised tonic clonic, generalised atonic, status epilepticus

Summary of epilepsy history and seizure semiology;
She had her first febrile convulsions three weeks prior to her third birthday. She was admitted to her local hospital at that time for three weeks. She was well until the age of 7 years, when she had her first afebrile generalised convulsions. She was admitted to her local hospital and subsequently transferred to a Paediatric Neurology unit. She had an EEG at that stage and was commenced on regular AED therapy. According to her parents and sisters, she had a definite loss of abilities after the onset of her epilepsy at this age. Her mother gives the example of her being "top of the class" prior to the onset of epilepsy, and subsequently needing to attend special schooling.

She has had, and continues to have, a number of different types of event. In childhood, she had generalised absence seizures characterised by an acute behavioural arrest, staring and being unresponsive. There were no automatisms associated with these events. They have been well controlled in adult life.

She has had generalised atonic seizures characterised by sudden loss of awareness and collapse. These have occurred in adult life, but seem well controlled on her current meidcations. They have occasioned a number of injuries, the most severe of which was a fractured left ankle.

She has also had generalised tonic-clonic seizures. These are not associated with any obvious aura or warning and usually occur out of clear consciousness. There are no associated lateralising features with these events according to collateral histories fro, family members. She usually has urinary incontinence and tongue bioting with these events and occasionally has cyanosis. She has had two episodes of status epilepticus arising out of this type of seizure.

She has no other types of seizure of which her family are aware, and dose not have myoclonus. Her parents have noticed that fasting for prolonged periods, stress and menses can increase the liklihood for her to have a seizure of any type.

Imaging results; 1.5T MRI was normal

EEG results; In a routine study, the record seemed to be excessively slow but no epileptiform features were noted

B ii 3

Age at recruitment; 38

Sex; female

Handedness; Right

Diagnoses;

1. Febrile convulsions
2. Epilepsy
3. Three previous Caesarean sections
4. Previous stillbirth at term – took 2 weeks to deliver remains of placenta
5. Previous cholecystectomy

6.   Previously screened positive for mutation in SCN1A gene

Medications;
1.   Valproate chrono 400mg bd

Previous anti-seizure medications;
1.   Phenytoin – switched to this during pregnancy
2.   Carbamazepine – felt "fatigued", "slowed"
3.   Phenobarbitone – in childhood

Allergies; nil

Early risks for epilepsy; febrile convulsions

Epilepsy classification; Idiopathic generalised epilepsy

Seizure classification; Simple febrile convulsions, generalised tonic clonic, unclear

Summary of epilepsy history and seizure semiology;
She had numerous febrile convulsions in her youth, all of which were below the age
of five. None were associated with focal features or particularly prolonged course,
according to a collateral history. She was investigated and put on phenobarbitone
therapy for a number of years as a preventative. She was weaned off this
subsequently after she had been free of events for some years.

She has had afebrile convulsions from the age of 12 – 13 years. With her initial
events, she would lose awareness without warning. Only in adult life has she noticed
a preceding feeling of being "lightheaded and shakey". When she loses awareness,
she collapses and has a generalised tonic clonic seizure without any reported
lateralising features. She has tongue biting, urinary incontinence and has injured her
teeth. Post ictally, she has a generalised headache.

In adult life, she has had occasional episodes of unclear semiology, described by her
as "petit mals". These begin with her usual "shaky feeling" and are followed by a

state of altered awareness where she "could see and hear everything but couldn't respond". She has no significant sequelae from these events.

She has no other types of event.

She has noticed particular precipitants for her attacks, and specifically mentions stress, sleep deprivation, exercise and fasting (many of her events in school happened prior to lunch). In adult life, she has noticed that she can abort attacks if she eats when feeling "lightheaded and shakey".

Imaging results; Never performed

EEG results; Never performed

B ii 4
Age at recruitment; 36
Sex; Female
Handedness; Right

Diagnoses;
1.   Febrile convulsions
2.   Epilepsy

Medications;
        4.   Phenobarbitone 30mg od

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; febrile convulsions

Epilepsy classification; Idiopathic generalised epilepsy

Seizure classification; Simple febrile convulsion, generalised tonic clonic, generalised myoclonic

Summary of epilepsy history and seizure semiology;
She had two febrile convulsions in childhood without atypical features at the ages of 18 months and 4 years. Given her family history, she was placed on phenobarbitone therapy at the age of 18 months, and was on this until she was 18 years. At that stage, she was weaned off treatment.

Her first afebrile convulsion occurred at the age of 18 years. She was markedly sleep deprived at the time. There was no preceding warning, and she lost awareness at the onset of the event. A direct collateral history was not available for the event, but she was told that she was shaking all four limbs "like a fit". She did not have any investigations performed at that stage. She had one further convulsion one year later in the setting of sleep deprivation. She was re-started on phenobarbitone and has been free of these events since.

She has generalised myoclonic events which worsened during a recent pregnancy. She has been seen by a consultant Epileptologist to confirm her diagnosis.

Imaging results; Never done

EEG results; Never done

B ii 5
Age at recruitment; 34
Sex; Female
Handedness; Right

Diagnoses;
1.   Epilepsy
2.   Febrile convulsions
3.   Depression

4.   Previously screened positive for mutation in SCN1A gene

Medications;
1.   Clobazam 10mg mane, 15mg nocte
2.   Zonisamide 100mg nocte
3.   Folic acid 5mg od

Previous anti-seizure medications;
1.   Carbamazepine – marked fatigue
2.   Gabapentin
3.   Lamotrigine – worsened severity and frequency of seizures
4.   Levetiracetam – caused agitation, mood disturbance
5.   Phenobarbitone
6.   Phenytoin
7.   Topiramate – rash
8.   Valproate chrono – weight gain, gum hypertrophy
9.   Vigabatrin

Allergies; Topiramate caused her to develop a rash

Early risks for epilepsy; Febrile convulsions

Epilepsy classification; Idiopathic generalised epilepsy

Epilepsy syndrome; Not otherwise specified

Seizure classification; Simple febrile convulsions, generalised absence, generalised myoclonic, generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She had her first events of any type at the age of 2 years when she "had the whooping cough", according to her parents. She has no recollection of these events herself, but the collateral history describes generalised convulsions of less than five

minutes duration. These resolved themselves and there were no focal features associated with them. She had two of these events in the course of this illness.

Her next events occurred at the age of 13 years. She had no febrile illnesses at that time and neither she nor any of her family can recall any obvious precipitants for her these events. She was noted to have "petit mals", i.e., generalised absence seizures.

She continues to have rare absence seizures. These are characterised by behavioural arrest, staring, unresponsiveness and loss of awareness. There are no associated automatisms.

She has occasional generalised tonic-clonic seizures. These are characterised by an initial inhalation, followed by stiffening of all four limbs and a subsequent convulsion. There is no associated aura and no lateralising features were reported in the collateral history obtained. Each event lasts for less than three minutes and then spontaneously resolves. She is usually tired and disorientated afterwards.

She has generalised myoclonic jerks out of clear consciousness.

She has no events of any other type. At the time of recruitment, with the withdrawal of lamotrigine, the frequency and severity of all seizure types had improved considerably.

Imaging results; 1.5T MRI was normal

EEG results; A routine study captured paroxysmal episodes of generalised spike and wave activity with a frequency of 2.5 - 4.5 Hz, enhanced by hyperventilation. The findings were felt to be consistent with a generalised epileptiform disorder.

B ii 6
Age at recruitment; 33
Sex; Male
Handedness; Right

Diagnoses;

1.   Heart murmur – was unsure of nature of same, previously investigated medically and not felt to require any intervention.

2.   One previous episode of syncope, cause unclear

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B ii 7

Age at recruitment; 32

Sex; male

Handedness; Right

Diagnoses;

1.   "Irregular heart beat" – nature of same unclear, was previously medically investigated, present in childhood only according to the participant

2.   Previous episode of convulsive syncope – had full medical investigations and opinion from consultant Epileptologist

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Unavailable

EEG results; Unavailable

B iii 1
Age at recruitment; 8
Sex; Female
Handedness; Right

Diagnoses; Nil

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B iii 2

Age at recruitment; 7

Sex; Male

Handedness; Right

Diagnoses;

1. Foetal valproate syndrome
2. Previous correction of hypospadias

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never done

EEG results; Never done

B iii 3

Age at recruitment; 4

Sex; Male

Handedness; Right

Diagnoses;

1.   Possible asthma

2.   Respiratory difficulties in immediate neonatal period – exact diagnosis unclear, required admission to SCBU

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Neonatal complications

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B iii 4

Age at recruitment; 11

Sex; Male

Handedness; Right

Diagnoses;

1.   Respiratory difficulties in immediate neonatal period – exact diagnosis unclear, did not require SCBU admission

2.   Previously screened negative for mutation in SCN1A gene

Medications; nil

Previous anti-seizure medications; nil

Allergies; Nil

Early risks for epilepsy; Neonatal complications

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B iii 5
Age at recruitment; 9
Sex; Male
Handedness; Ambidextrous

Diagnoses;
1.   Previously screened positive for mutation in SCN1A gene
2.   "Heart murmur" – exact nature of same unclear, previously medically investigated, not felt to require any intervention
3.   Febrile convulsions

Medications; nil

Previous anti-seizure medications; Nil

Allergies; Has allergy to dust mite allergen

Early risks for epilepsy; febrile convulsions

Epilepsy classification; Febrile convulsions only

Seizure classification; Simple febrile convulsions

Summary of epilepsy history and seizure semiology;
He has had a number of febrile convulsions, between the ages of 1 and 6 years.
Temperatures with these events have ranged from 100.3 – 102.8 degrees F (37.9 –
39.3 °C), according to his mother. There were no atypical features noted with these
attacks, and he has been investigated by a consultant Paediatric Neurologist for
these.

He has not had any definite afebrile seizures at any stage.

Imaging results; Never done

EEG results; Never done

B iii 6
Age at recruitment; 4
Sex; Male
Handedness; Unclear, probably right

Diagnoses;
1.   Previously screened positive for mutation in SCN1A gene
2.   One previous febrile convulsion at age 1½ years

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Febrile convulsion

Epilepsy classification; Febrile convulsion only

Seizure classification; Simple febrile convulsion

Summary of epilepsy history and seizure semiology;
He has only had one febrile convulsion at the age of 18 months, and has not had any since that time in spite of other febrile illnesses. There were no atypical features noted in this single febrile convulsion.

He has never had any events of any other type.

Imaging results; Never performed

EEG results; Never performed

B iii 7
Age at recruitment; 3
Sex; Male
Handedness; Unclear, probably right

Diagnoses;
1.   Possible asthma – awaiting investigations

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B iii 8

Age at recruitment; 20 months

Sex; Male

Handedness; unclear

Diagnoses;

1.   Possible asthma

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B iii 9

Age at recruitment; 6

Sex; Male

Handedness; Right handed

Diagnoses;

1. Developmental delay – characterised as "receptive speech difficulty" by parents, exact diagnosis unclear, has been medically investigated

Medications; nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Unavailable

EEG results; Never performed

B iii 10
Age at recruitment; 5
Sex; Male
Handedness; Right handed

Diagnoses;
1. Autistic spectrum disorder
2. Wolff-Parkinsonism-White syndrome – had cardiac arrest aged 11 days

Medications;
1. Digoxin
2. Propranolol

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Cardiac arrest in neonatal period

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

B iii 11
Age at recruitment; 3
Sex; Female
Handedness; Unclear, probably right

Diagnoses;
1.   Right facial haemangioma in isolation – previously medically assessed

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

<u>Individual family members – family C</u>

C i 1

Age at recruitment; 52

Sex; Female

Handedness; Right

Diagnoses; nil

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C i 2

Age at recruitment; 65

Sex; Male

Handedness; Right

Diagnoses;

1.   Hypertension
2.   Hypercholesterolaemia
3.   Previous gunshot wound to left arm
4.   Psoriasis

Medications;
1. Atorvastatin 20mg od
2. Enalapril 5mg od

Previous anti-seizure medications; nil

Allergies; nil

Family history;
1. Epilepsy
2. Psoriasis
3. Rheumatoid arthritis – sister and father

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 1

Age at recruitment; 33
Sex; Female
Handedness; Right

Diagnoses;
1. Previous eye operation in childhood for correction of strabismus

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 2

Age at recruitment; 31

Sex; Female

Handedness; Right

Diagnoses;
1. Psoriasis
2. Seven months pregnant at the time of recruitment
3. Epilepsy
4. "Toxaemia" after delivery in a previous pregnancy – details unclear, was admitted to hospital with same

Medications;
1. Carbamazepine Retard 400mg mane, 200mg nocte

Previous anti-seizure medications;
1. Was on "a little white tablet" in childhood – nature of same unclear

Allergies; nil

Early risks for epilepsy; febrile convulsions

Epilepsy classification; Localisation related epilepsy, febrile convulsions

Seizure classification; Simple febrile convulsions, simple partial, complex partial, secondarily generalised tonic clonic.

Summary of epilepsy history and seizure semiology;

She is certain that she had febrile convulsions. She cannot remember what age she was with these, how high her temperature was or whether there were any focal features associated with them. A collateral history did not identify any atypical features with these events. She began having afebrile events from the age of 13 – 14 years.

Her current seizures have a definite preceding warning of being "able to hear but not see" and being unable to respond to others. There are no other more specific features with this aura. These seizures can resolve at this stage or proceed to a loss of awareness.

After losing awareness, she has been told by others that she can have unusual motor behaviour (she gives examples of wandering out of classroom, pulling up handbrake of car and turning off engine when she had meant to drive). She has not had lateralising automatisms.

More usually, she will have a convulsion after losing awareness, possibly with head and eye deviation to the right initially and subsequent generalised tonic clonic movements. She can bite either side of her tongue. She recovers spontaneously, but is very tired after events and has to sleep for an hour. She has no other significant sequelae.

She feels that tiredness or stress can trigger events. She was admitted to ITU once with seizures, but this was in the setting of "toxaemia" after delivery.

She has no other seizures of any other type.

Imaging results; Never performed

EEG results; Unavailable

C ii 3

Age at recruitment; 29

Sex; Female

Handedness; Right

Diagnoses;

1.   Epilepsy

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2. Depression

3. Polycystic ovarian syndrome

Medications; nil

Previous anti-seizure medications;

1. Valproate Chrono 500mg bd – failed weaning once in teenage years, weaned herself off in adult life

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Complex partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She says that her first seizure occurred at the age of 14 years. She had not had any events for a number of years at the time of recruitment. All events has the same semiology.

Her seizures began with a sensation of "noise fading into the background". She described herself as feeling "all hot" and had difficulty with expressive speech (she "can't say anything"). After this, she would always lose awareness after this. When she is unaware, others have noticed that she has unusual motor activity, and collateral descriptions include activities such as "walking in a trance" and that she will "keep walking around to the right". No lateralising automatisms were described by witnesses. Events can stop at this stage.

If she does not regain awareness at this stage, she will go on to have a generalised convulsion. She has not been told of any focal features at the onset of this and collateral history does not suggest any. She can bite her tongue and have urinary incontinence. She is fatigued afterwards but has no other sequelae.

She has never had any other types of seizure.

She has noticed that stress, sleep deprivation and possibly flashing lights (they give her "a really bad headache") can trigger events.

Imaging results; Never performed

EEG results; Unavailable

C ii 4
Age at recruitment; 24
Sex; Female
Handedness; Right

Diagnoses;
1.   Epilepsy

Medications;
1.   Lamotrigine 100mg bd

Previous anti-seizure medications;
1.   Valproate Chrono 600mg bd – weaning off same under medical direction at the time of recruitment

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She had her first seizure at the age of 15 years. Her initial event occurred out of clear consciousness, in the evening, without any warning. Collateral history from family members describes a generalised tonic clonic seizure with tongue biting but without obvious lateralising features.

Her semiology changed slightly at the age of 17. She began to have an aura prior to her seizures, described as a "sick feeling" rising from the epigastric area. Collateral history for these events described initial left head version at the onset of her tonic clonic seizures.

After age of 21, developed "staring episodes" – no more information available on same.

She has not had any other seizure types apart from these.

She feels that events are more likely to happen around menses and that stress and sleep deprivation may be triggers for events, but has not noticed any photosensitivity.

Imaging results; Never performed

EEG results; Routine study was normal, with no epileptiform features.

C ii 5

Age at recruitment; 22
Sex; Female
Handedness; Right

Diagnoses;
1. Psoriasis
2. Six months pregnant at the time of recruitment

Medications;
1. Iron supplements, exact type unclear

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 6

Age at recruitment; 20

Sex; Female

Handedness; Right

Medical diagnoses;

1. Psoriasis

Medications;

1. Topical steroids PRN

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 7

Age at recruitment; 18

Sex; Female

Handedness; Right

Diagnoses;

1. Previous fracture of left arm

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 8
Age at recruitment; 17
Sex; Female
Handedness; Right

Diagnoses;
1.   Previous fracture of ankle

Medications; nil

Previous anti-seizure medications; Nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 9

Age at recruitment; 14

Sex; Male

Handedness; Right

Diagnoses; nil

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C ii 10

Age at recruitment; 13

Sex; Male

Handedness; Right

Diagnoses;
1.   Migraine without aura
2.   Previous hospital admission for tonsillitis

Medications;
1.   Migraleve PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

C iii 1

Age at recruitment; 12

Sex; Male

Handedness; Right

Diagnoses;

1.   Asthma in early childhood

Medications; nil

Previous anti-seizure medications; nil

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

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<u>Individual family members – family D</u>

D i 1

Age at recruitment; 63

Sex; Female

Handedness; Right

Diagnoses;

1.   Hypercholesterolaemia

2.   Hypertension – previous hospitalisation with acute exacerbation of same

3.   Vasovagal syncope in adolescence – not medically investigated

Medications;

1.   Clopidogrel 75mg od

2.   Bisoprolol 5mg od

3.   Asarel one tablet od

Previous anti-seizure medications; Nil

Allergies; Nil

Family history;

1.   Epilepsy – maternal aunt and possibly her maternal grandfather ("he had something funny in his sleep", she was never told of the details)

2.   Ischaemic heart disease – father died of MI

3.   Stroke – three of her brothers (aged 57, 50 and 67)

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

D i 2

Age at recruitment; 70

Sex; Male

Handedness; Right

Diagnoses;

1.   Hypercholesterolaemia
2.   Previous partial detachment of retina in right eye
3.   Right knee osteomyelitis – required admission to Orthopaedic unit
4.   Previous appendicectomy

Medications;

1.   Atorvastatin 20mg od

Previous anti-seizure medications; Nil

Allergies; Nil

Family history;

1.   Ischaemic heart disease – mother died of myocardial infarct

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

D i 3

Age at recruitment; 57

Sex; Female

Handedness; Right

Diagnoses;

1. Hypertension
2. Previous left breast biopsy of benign lump (exact nature of same unclear)
3. "Meningitis", aged 10 years – exact nature of this illness is unclear. She was treated by her GP and can remember getting "two injections a day" for some weeks but was never hospitalised and had no specific investigations.
4. Isolated febrile convulsion

Medications;
1. Bisoprolol 5mg od

Previous anti-seizure medications; Nil

Allergies;
1. Septrin
2. Penicillin

Family history;
1. IHD – father died of MI
2. Epilepsy – maternal aunt, possibly her maternal grandfather, and one of her sons

Early risks for epilepsy; Febrile convulsion, possible meningitis

Epilepsy classification; Febrile convulsions only

Seizure classification; Simple febrile convulsion

Summary of epilepsy history and seizure semiology;
She reports having a single febrile convulsion at the age of 1 year. She can remember being "put into a bath of ice" as a result of having had "a turn". No other details are available. She had one event only and did not have any significant sequelae from this event of which she is aware.

She has not had any afebrile events or seizures of any other type.

Imaging results; Never performed

EEG results; Never performed

D ii 1

Age at recruitment; 37

Sex; Male

Handedness; Right

Diagnoses;

1.   Epilepsy

2.   One episode of post ictal psychosis

3.   Hypercholesterolaemia

Medications;

1.   Zonisamide 200mg nocte

2.   Carbamazepine retard 400mg bd

3.   Clobazam 10mg nocte

Previous anti-seizure medications;

1.   Levetiracetam

2.   Phenobarbitone

3.   Valproate chrono

4.   Lamotrigine

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Epilepsy syndrome; Temporal lobe epilepsy

Seizure classification; Simple partial, complex partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;

He began to have events at the age of 15 years. Initially, all of his events occurred out of sleep. According to a collateral history, his parents noted that he would make "really odd noises" when asleep and would be unrousable when they went into him. There were no

obvious precipitants for these events. He was diagnosed with epilepsy when his parents managed to witness a nocturnal convulsion.

He has a number of different types of event. He has frequent simple partial seizures, characterised by an intense feeling of déjà vu and of an unpleasant rising epigastric sensation. These events last for up to one minute then resolve spontaneously.

Occasionally, he will have episodes with loss of awareness. With these events, he does not have any aura which he can recall after the event. He stares straight ahead during the events. He does not respond to others and is often noted to be sweating heavily. He can rock back and forth, but has no other automatisms associated with the attacks. These seizures last for approximately 1 – 2 minutes then resolve. He usually feels quite tired after these events.

Rarely, his events with loss of awareness will progress to a generalised tonic clonic seizure. There are no obvious lateralising features for these on collateral history. He occasionally bites his tongue and has urinary incontinence with these events. They last for 2 – 3 minutes then resolve. He is profoundly sore and usually sleeps for a prolonged period after these events. However, as most of these events occur out of sleep, he is aware of them only by how he feels the following morning.

He has never had any episodes of status epilepticus. He has not had any other types of seizure of which he is aware. He has noticed that stress and sleep deprivation increase the likelihood of having a seizure.

Imaging results; 3T MRI was normal.

EEG results; Video EEG monitoring showed bilateral interictal epileptiform discharges in sleep, more prominent over the left temporal region. Six seizures were captured during monitoring, with four arising from the left temporal region and one from the right temporal region (lateralisation could not be determined in one event). Some events were associated with ictal bradycardia.

D ii 2
Age at recruitment; 35
Sex; Female
Handedness; Right

Diagnoses;
1. Generalised anxiety disorder
2. Previous Caesarean section
3. Single symptomatic seizure – was hospitalised and medically investigated, has an opinion from a consultant Neurologist

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Isolated symptomatic seizure

Seizure classification; Generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She had a single seizure, which occurred within 24 hours after discharge home following a C/section for the delivery of her first child. She had no specific aura prior to that event – she says that she was "sick all day" prior to it, but had no other prodrome. She has no recollection of the event itself, and lost awareness at the onset. A collateral history mentions that she was "frothing" and that she was shaking all four limbs. There did not appear to be any unusual behaviour or definite automatisms associated with the event. She was not put on anti-seizure therapy, and has been event free since then.

She has not had any other events of any type.

Imaging results; 1.5T MRI initially showed multiple areas of hyperintensity of unclear aetiology, and follow-up imaging showed progressive resolution of these changes.

EEG results; Unavailable

D ii 3
Age at recruitment; 30
Sex; Female

Handedness; Right

Diagnoses;
1. Epilepsy
2. Previous wisdom tooth extraction
3. Heart murmur – exact nature of same unclear, previously medically investigated
and not felt to require intervention

Medications; Nil

Previous anti-seizure medications;
1. Carbamazapine retard – dose unknown

Allergies; She reports that she has a severe reaction to alcohol characterised by flushing, malaise and a sensation of respiratory discomfort

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Complex partial

Summary of epilepsy history and seizure semiology;
She had her first seizure at the age of thirteen. She had events predominantly at night, but would also occasionally have events during the day.

For the daytime events, she would have an aura characterised by "feeling really panicky", a sensation of feeling that "I have to get out of here". After this, she would feel "zoned out", and describes a sensation that she was very disconnected from her surroundings. People told her that during these events she was "staring", but a collateral history did not report any actions suggestive of automatisms. She feels that these events lasted for "a minute or two", and that she was very tired after them.

She has no recollection of the nocturnal events, and had no aura for these of which she is aware. Collateral history did not describe any preceding vocalisation and she was often happened upon sitting up in bed, staring. She would return to sleep after these events, but would feel extremely tired the following morning.

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She last had seizures when aged 15 years, and weaned herself off medications after she had been seizure-free for some years. At the time of recruitment, she was still seizure-free.

She has never had any other seizures of any type.

Imaging results; CT brain was normal.

EEG results; Routine and sleep-deprived studies were normal, with no epileptiform features.

D iii 1
Age at recruitment; 5
Sex; Male
Handedness; Right

Diagnoses;
1.   Epilepsy

Medications;
1.   Sodium valproate – dose and preparation unclear

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unclear

Seizure classification; Unclear

Summary of epilepsy history and seizure semiology;
His seizures first started at the age of 2 years. According to a collateral history, there were no obvious precipitants for his seizures.

All events were stereotypical. His parents describe brief events occurring out of clear consciousness. He has behavioural arrest, and then stares ahead of him. He then has forward

flexion of his head and roving eye movements in both lateral directions. After this, he recovers rapidly to normal, with no awareness of the event and without any obvious sequelae. Each event lasts for about 10 – 15 seconds. There were no oral or limb automatisms with these events, and he did not seem to have any obvious aura prior to their onset.

His diagnosis was confirmed by a consultant Paediatric Neurologist. He has been seizure-free for two years on valproate therapy.

He has not had any other events of any sort.

Imaging results; Never performed

EEG results; Unavailable

Individual family members – family E

E i 1

Age at recruitment; 67

Sex; Male

Handedness; Ambidextrous

Diagnoses;
1. Previous fracture of left ankle
2. Previous back injury
3. Type 2 diabetes mellitus – diet controlled
4. Hypercholesterolaemia

Medications;
1. Atorvastatin 10mg od
2. Valproate Chrono 1000mg mane, 1200mg nocte

Previous anti-seizure medications;
1. Levetiracetam
2. Phenytoin
3. Primidone

Allergies; Nil

Family history;
1. Epilepsy
2. Alzheimer's disease – mother

Early risks for epilepsy; Nil

Epilepsy classification; Idiopathic generalised epilepsy not otherwise specified

Seizure classification; Generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He had the onset of his events in adult life only, with no events in childhood. His events have had a consistent semiology since onset. He has generalised tonic clonic seizures without any preceding aura or automatisms. His events occur out of wakefulness and are not related to sleep. At the time of recruitment, he had been seizure free for some years on therapy.

He had one episode of non-convulsive status after a significant head injury.

Imaging results; 1.5T MRI was normal

EEG results; Routine study showed generalised polyspike and wave 3-4 Hz activity, consistent with a diagnosis of idiopathic generalised epilepsy.

E i 2
Age at recruitment; 66
Sex; Female
Handedness; Right

Diagnoses;
1. Previous right carpal tunnel release
2. Migraine without aura
3. Previous removal of vein from right side of neck – unsure of the exact nature of this operation
4. Osteopaenia

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Medications;

1.   Alendronic acid 35mg once / week

Previous anti-seizure medications; Nil

Allergies; Nil

Family history;

1.   Alzheimer's disease – both parents

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

E i 3

Age at recruitment; 61
Sex; Female
Handedness; Right

Diagnoses;

1.   Epilepsy

Medications; Nil

Previous anti-seizure medications;

1.   Unclear – was given "an injection" on a PRN basis from her treating physician in childhood, but cannot remember the name of this.

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unclear

Seizure classification; Unclear

Summary of epilepsy history and seizure semiology;
She had epilepsy in childhood only. She developed epilepsy when she was under 1 year of age and her last event was when she was seven years old. She cannot remember any of the events themselves, but was told by others that she had generalised convulsions. She cannot remember any auras or focal features, but admits that she doesn't remember the events well. She never had status epilepticus and did not have seizures with high temperatures.

As far as she is aware, she was never formally investigated for these events, and cannot remember having an EEG.

She has not had any other events apart from these.

Imaging results; Never performed

EEG results; Never performed

E ii 1
Age at recruitment; 42
Sex; Male
Handedness; Right

Diagnoses;
1.   Epilepsy
2.   Ankylosing spondylitis

Medications; Nil

Previous anti-seizure medications;
1.   Phenobarbitone – unsure of dose

Allergies; nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Complex partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He first had a seizure at the age of nine years. The events were characterised by an initial sensation of becoming distant from environment, followed by loss of awareness. Collateral history describes him as having left lip twitching, unilateral automatisms (which may have been right sided, although his parents were unsure) and occasional progression to a generalised convulsion.

He had an opinion form a consultant Paediatric Neurologist to confirm his diagnosis. His last event occurred when he was 12 years old.

He has never had any other seizures of any type apart from those described above.

Imaging results; CT brain was normal

EEG results; Unavailable

E ii 2
Age at recruitment; 41
Sex; Female
Handedness; Right

Diagnoses;
1.   Previous laparoscopy for investigation of infertility

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Febrile convulsion

Epilepsy classification; Febrile convulsion only

Seizure classification; Simple febrile convulsion

Summary of epilepsy history and seizure semiology;
By her own account, she never had any febrile convulsions, seizures, myoclonus or aura symptoms. However, her mother volunteered a collateral history that she had a single febrile convulsion at the age of 1 year, when she had a temperature. There were no focal features associated with this, it lasted for less than one minute and she made an uneventful recovery.

She has not had any other events of any type.

Imaging results; Never performed

EEG results; Never performed

E ii 3
Age at recruitment; 38
Sex; Male
Handedness; Ambidextrous

Diagnoses;
1.   Previous appendicectomy

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

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Imaging results; Never performed

EEG results; Never performed

E ii 4
Age at recruitment; 32
Sex; Male
Handedness; Right

Diagnoses;
1. Previous lumpectomies – nil of note found
2. Previous fracture of jaw

Medications; nil

Previous anti-seizure medications; nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

E ii 5
Age at recruitment; 34
Sex; Female
Handedness; Right

Diagnoses;
1. Epilepsy

2.   Depression

Medications;

1.   Clobazam

2.   Zonisamide

Previous anti-seizure medications;

1.   Gabapentin

2.   Levetiracetam

3.   Oxcarbazepine

4.   Pregabalin

5.   Topiramate

6.   Valproate chrono

Early risks for epilepsy; Febrile convulsion

Epilepsy classification; Idiopathic generalised epilepsy

Epilepsy syndrome; Juvenile myoclonic epilepsy

Seizure classification; Simple febrile convulsion, generalised absence, generalised myoclonic, generalised tonic clonic.

Summary of epilepsy history and seizure semiology;
She had her first event of any sort at the age of 4 years. This occurred in the setting of a febrile illness. She had a simple febrile convulsion without any focal or atypical features. This lasted for "a few minutes", according to collateral history, without any obvious significant sequelae.

She began to have afebrile seizures at the age of 16 years. At the time of recruitment, she had a number of different types of seizure. She describes having generalised absence seizures. These are characterised by a loss of awareness at onset with no preceding aura. She is noted by others to be staring straight ahead without any automatisms. Each of these events last for 30 – 60 seconds then spontaneously resolves.

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She has generalised myoclonic seizures. These usually occur out of clear consciousness and are more common in the mornings. She has not noticed any lateralising features for these events.

More rarely, she has generalised tonic clonic seizures. She has no preceding aura for these events. She loses awareness at onset. Collateral history describes a generalised convulsion without any lateralising features. Each of these events lasts for less than 3 minutes, then spontaneously resolves.

She has never had any other types of seizure of which she is aware. She has never had any episodes of status epilepticus. She has noticed that seizures are more likely to occur in the setting of sleep deprivation, stress of menses.

Imaging results; Never performed

EEG results; A routine study captured an isolated six second burst of generalised spike / polyspike slow wave activity, with a frequency of 3 Hz. This was consistent with a clinical diagnosis of primary generalised epilepsy.

Individual family members – family F

F i 1
Age at recruitment; 68
Sex; Female
Handedness; Right

Diagnoses;
1.   Rheumatic fever
2.   Two previous total hip replacements
3.   Lower limb venous ligation
4.   Previous appendicectomy
5.   Previous tonsillectomy

Medications;
1.   Calcichew D3 forte two tablets od
2.   Alendronate 70mg once per week

Previous anti-seizure medications; Nil

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Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

F i 2

Age at recruitment; 76

Sex; Female

Handedness; Right

Diagnoses;

1.   Ischaemic heart disease – previous coronary artery bypass graft operation

2.   Osteoarthritis

3.   Hypothyroidism

4.   Hypercholesterolaemia

5.   Two previous total hip replacements

6.   Epilepsy

Medications;

1.   Esomeprazole 20mg od

2.   Atorvastatin 40mg od

3.   Alprazolam 0.25mg tds

4.   Frusemide 20mg od

5.   Lisinopril 20mg od

6.   Eltroxin 25mcg od

7.   Nebivolol 7.5mg od

8.   Lercanidipine 10mg od

9.   Aspirin 75mg od

10.   Sertraline 100mg od

11.   Carbamazepine 100mg od

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Presumed secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She was first formally diagnosed with epilepsy at the age of 35 in 1969. However, she had
been having episodes of nocturnal enuresis for some years prior to that, which she assumes
now were due to unrecognised seizures.

She is unsure of the exact details for most of her events, as she says that they almost all
arose out of sleep. She cannot remember any definite aura for them, and has no recollection
of events themselves. She was told by others that she had jerking off all four limbs, urinary
incontinence and frothing at the mouth. She had one out of clear consciousness, which was
associated with a preceding warning characterised by a compelling feeling that she "had to
walk off the footpath" to her right side.

She has had her diagnosis confirmed by a consultant Neurologist.

She had one episode of status epilepticus at the age of 48 which required admission to an
Intensive Care Unit. At the time of recruitment, she had been seizure free for over 20 years.
She has never had any other types of seizure apart from those described above.

One of her maternal uncles had epilepsy and died at the age of 28, but she is unsure of other
details.

Imaging results; Unavailable

EEG results; Unavailable

F ii 1

Age at recruitment; 44

Sex; Male

Handedness; Right

Diagnoses;

1. Hypercholesterolaemia
2. Testicular cancer – diagnosed aged 21, treated with surgery and chemotherapy
3. Epilepsy

Medications;

1. Phenytoin 400mg od

Previous anti-seizure medications; nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Presumed secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He was first formally diagnosed with epilepsy in his early teenage years by a consultant Neurologist. As far as he can remember, he had seizures between the ages of 10 and 14 years.

He remembers very little of his seizures, saying that they all occurred out of sleep. He did not recognise any triggers for the events, and has no recollection of any associated aura / prodrome. He can remember trying to arise out of sleep and feeling as if everything was "like in a dream", but has no recollection of events after that. Collateral history suggests that he had a generalised tonic clonic seizure, but cannot specify if there were any particular focal features. He would be extremely tired afterwards.

He has never had any other types of seizure and has not had any events from clear consciousness. At the time of recruitment, he had been seizure-free since the age of 14 years.

Imaging results; Unavailable

EEG results; Unavailable

F ii 2

Age at recruitment; 42
Sex; Male
Handedness; Left

Diagnoses;
1. Previous right knee arthrospcopy
2. Previous fractured finger
3. Previous traumatic tooth injury
4. Episodes of swelling of feet, lower limbs and lips when "run down" – will develop over 12 – 18 hours, then slowly resolve. This has occurred intermittently since the age of 18 years. The exact diagnosis remains unclear, and it has been medically investigated.

Medications; nil

Previous anti-seizure medications; Nil

Allergies; Possible allergy to penicillin, as he had marked nausea after one dose of augmentin

Early risks for epilepsy; Previous head trauma with loss of awareness

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

F ii 3

Age at recruitment; 36

Sex; Female

Handedness; Right

Diagnoses;

1. Previous cholecystectomy, 2010
2. Asthma in childhood
3. Hypercholesterolaemia

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

F ii 4

Age at diagnosis; 24

Sex; Female

Handedness; Left

Diagnoses;

1. Epilepsy

Medications;

1. Carbamazepine retard 400mg bd
2. Lamotrigine 200mg bd
3. Zonisamide 300mg bd
4. Clobazam 5mg mane, 10mg nocte

5.　　　Vagus nerve stimulator in situ

Previous anti-seizure medications;
1.　　　Gabapentin
2.　　　Levetiracetam
3.　　　Oxcarbazepine
4.　　　Topiramate
5.　　　Valproate chrono

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Complex partial, presumed secondarily generalised tonic clonic

Summary of epilepsy history and seizure classification;
She had her first seizure at the age of 6 months. There were no obvious precipitants for this event, and she did not have a febrile illness at the time. She has had on-going events since that time.

Her complex partial events are characterised by loss of awareness at onset. She has no preceding aura which she can recall. Collateral history describes her as "moaning", eyes rolling and bicycling movements of both legs. Later in the event, her head and eyes will deviate to the right. If she is standing when she has an event, she will stagger and can fall. Each event last for approximately one minute then resolves. If the events occur out of clear consciousness, she is tired and disorientated afterwards.

Occasionally, her complex partial events will progress into a secondarily generalised tonic clonic seizure. These events last for 2 – 3 minutes then resolve spontaneously. She has had one episode of status epilepticus previously. She has had a number of minor injuries as a result of her seizures, including lacerations and scalp haematomata.

She has not had any other types of seizure of which she or her family are aware.

Imaging results; 1.5T MRI brain was normal.

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EEG results;Video EEG monitoring was suggestive of a focal lesion involving the right frontotemporal / central region. Seizure semiology and electrographic features suggested a right temporal neocortical onset, but the seizure onset had a broad field

F ii 5
Age at recruitment; 46
Sex; Male
Handedness; Right

Diagnoses;
1.   Hypercholesterolaemia

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Previous head trauma with loss of awareness

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

Individual family members – family G

G i 1
Age at recruitment; 60
Sex; Female
Handedness; Right

Diagnoses;

1.   Multiple sclerosis
2.   Hypercholesterolaemia

Medications;
1.   Clonazepam 5mg PRN
2.   Betahistine 16mg tds
3.   Diazepam 2.5mg PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Family history;
1.   Idiopathic Parkinson's disease – uncle
2.   Epilepsy – brother
3.   Stroke – brother

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; 1.5T MRI showed white matter hyperintensities in a pattern consistent with demyelination

EEG results; Never performed

G ii 1
Age at recruitment; 32
Sex; Female
Handedness; Right

Diagnoses;
1.   Epilepsy

2. Previous concussion secondary to a road traffic accident, aged 18 years

Medications;
1. Lamotrigine 100mg bd

2. Folic acid 5mg od

Previous anti-seizure medications;
1. Valproate chrono
2. Carbamazepine retard

Allergies; Nil

Early risks for epilepsy; Previous head trauma with loss of awareness

Epilepsy classification; Localisation related epilepsy

Epilepsy syndrome; Temporal lobe epilepsy

Seizure classification; Simple partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She had her first seizure at the age of 29 years. She is unaware of any obvious precipitants for this event. All of her events have had the same semiology since the onset of her epilepsy.

Her events usually occur out of clear consciousness. In most of her events, she has a pronounced sensation of déjà vu without other features. She maintains awareness, and the events resolve spontaneously within one minute. She has no significant sequelae.

She has had rare events with secondary generalisation. She has no recollection of these events, but a collateral history was obtained. In each event, she had initial right arm posturing followed by collapsing to the ground and a generalised convulsion. She has had tongue biting and urinary incontinence in these events. All of these events lasted for less than three minutes, and were followed by a brief period of disorientation.

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She has never had any other seizures of any other type. At the time of recruitment, she had been seizure free for some years.

Imaging results; 1.5T MRI was normal.

EEG results; A routine study showed numerous bursts of sharp waves occurring over both hemispheres independently, maximal over the central and temporal head regions.

G ii 2

Age at recruitment; 21

Sex; Female

Handedness; Left

Diagnoses;
1. Epilepsy
2. "Stroke in the womb" according to her mother – nature of same unclear, has mild learning impairment secondary to same

Medications; Nil

Previous anti-seizure medications; She is unsure of the details for these

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Simple partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
According to a collateral history, she began to have seizures from shortly after birth. Initial events were not associated with any obvious precipitants. She was diagnosed with epilepsy by a consultant Paediatrician.

She says that she used to have an olfactory aura prior to events which she described as "the smell of the hospital". After this, she would lose awareness. According to a collateral

history, her "head would shake" first, but without definite head version. After this, she would fall to the ground and have a convulsion. There were no obvious lateralising features in these events. Each event would last for 2 – 3 minutes and then resolve. There were no significant sequelae for these events.

She has never had any other types of seizure. She has been seizure-free since she was seventeen. After being seizure-free for some years, she successfully weaned herself off anti-seizure medications.

Imaging results; 1.5T MRI showed changes consistent with an old infarct in the left pre and post central gyrus.

EEG results; Unavailable

G iii 1
Age at recruitment; 8
Sex; Male
Handedness; Left

Diagnoses;
1.   Bilateral grommet insertion

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

<u>Individual family members – family H</u>

H i 1

Age at recruitment; 91

Sex; Female

Handedness; Right

Diagnoses; Nil

Medications;

1. Alprazolam PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H ii 1

Age at recruitment; 66

Sex; Female

Handedness; Right

Diagnoses;

1. Cholecystitis – 2 previous episodes
2. Depression
3. Gastritis / peptic ulcer disease
4. Arthralgia – cause unclear, has been investigated medically

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Medications;
1. Seroxat 20mg od
2. Omeprazole 20mg od

Previous anti-seizure medications; Nil

Family history;
1. Diabetes mellitus – grandfather, grandmother, father
2. Epilepsy
3. Hypothyroidism

Allergies;
1. Iron infusion – is unsure of the name of the preparation

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H ii 2
Age at recruitment; 65
Sex; Male
Handedness; Right

Diagnoses;
1. Hypercholesterolaemia
2. Abnormal liver function tests, cause unclear – has been medically investigated, tests for haemochromatosis negative
3. Intermittent skin rash, nature and cause unclear – has been medically investigated, had opinion from consultant Dermatologist

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; He reports that he consistently develops a skin rash if he takes caffeine

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H ii 3
Age at recruitment; 61
Sex; Female
Handedness; Right

Diagnoses;
1.   Migraine without aura

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H ii 4

Age at recruitment; 58

Sex; Female

Handedness; Right

Diagnoses;

1. Epilepsy
2. Iron-deficiency anaemia, cause unclear – has been medically investigated
3. Hypothyroidism

Medications;

1. Levetiracetam 1000mg mane, 1500mg nocte
2. Carbamazepine retard 400mg mane, 600mg nocte
3. Clobazam 10mg bd, 15mg nocte
4. Lamotrigine 200mg bd
5. Calcichew D3 forte one tablet bd
6. Eltroxin – unsure of dose

Previous anti-seizure medications;

1. Phenobarbitone
2. Phenytoin
3. Primidone
4. Tiagabine
5. Valproate Chrono
6. Zonisamide

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Simple partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
She had her first seizure at the age of 3. There were no obvious precipitants for this first event. A collateral history was not available for this event.

She has on-going seizures, all of which have a similar semiology. All seizures have an aura of a sensation of intense fear, followed by loud vocalisation. After this she loses awareness. According to a collateral history, she has a generalised convulsion without lateralising features. Each event usually lasts for 2 – 3 minutes. She is usually fatigued for some time afterwards, but has no other significant sequelae.

She has had her diagnosis confirmed by a consultant Neurologist and a consultant Epileptologist. She has never had any other types of seizure.

Imaging results; 1.5T MRI was normal

EEG results; Unavailable

H ii 5
Age at recruitment; 54
Sex; Male
Handedness; Right

Diagnoses;
1.   Possible irritable bowel syndrome – has been medically investigated

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H ii 6
Age at recruitment; 52
Sex; Female
Handedness; Right

Medical diagnoses;
1.   Mastitis
2.   Hypothyroidism
3.   Depression

Medications;
1.   Eltroxin – unsure of dose
2.   Seroxat 20mg od

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H ii 7
Age at recruitment; 61
Sex; Male
Handedness; Right

Diagnoses;

1. Urticaria – cause of same unclear, has been medically investigated and has had a Dermatology opinion
2. Adenocarcinoma of prostate – previous transurethral resection for same
3. Haemochromatosis

Medications;

1. Cetirizine PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Family history;

1. Haemochromatosis – two brothers, one sister, possibly his father

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

H iii 1

Age at recruitment; 34
Sex; Male
Handedness; Right

Diagnoses;

1. Alcohol dependence syndrome
2. One previous episode of deliberate self-harm
3. Gastro-oesophageal reflux disease
4. One previous hospitalisation after head trauma

Medications;

1.   Esomeprazole 40mg od

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Unavailable

EEG results; Never performed

H iii 2

Age at recruitment; 32

Sex; Male

Handedness; Right

Diagnoses;

1.   Epilepsy

Medications;

1.   Clobazam 10mg od
2.   Levetiracetam 1000mg bd
3.   Phenytoin 300mg od
4   Topiramate 200mg bd
5.   Valproate chrono 1000mg mane, 1500mg nocte

Previous anti-seizure medications;

1.   Carbamazepine retard
2.   Clonazepam
3.   Lamotrigine
4   Phenobarbitone

5.   Vigabatrin
6.   Lacosmaide – increased the frequency of seizures

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Epilepsy syndrome; Frontal lobe epilepsy

Seizure classification; Simple partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He had his first seizure at the age of six months. This was not associated with a febrile illness or any other obvious precipitant. He has had recurrent events since the age of fourteen months.

His events usually begin shortly after awakening from sleep and can occur in clusters. At onset, he has a rising epigastric sensation and an abnormal sensation in his head (described as "like an electric current"). Occasionally, his events will not progress beyond this. If this is the case, the events last for less than thirty seconds and then resolve without any sequelae.

If his events progress from this, he will lose awareness. A collateral history describes him as extending both arms and subsequently having a generalised convulsion. There are no associated lateralising features. Each of these events lasts for approximately one minute, then resolves. He is quite tired and disorientated afterwards.

He has never had status epilepticus. He has never had any other types of seizures at any stage.

Imaging results; 3T MRI was normal

EEG results; On video EEG monitoring, the interictal EEG points to a lesion with epileptogenic potential close to the midline, with a maximum at the vertex and to the right of

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the midline. The seizure semiology is consistent with a frontal lobe focus, but the events are difficult to lateralise on surface EEG. Some events are associated with ictal bradycardia.

H iii 3

Age at recruitment; 24

Sex; Female

Handedness; Left

Diagnoses;

1. Epilepsy

Medications;

1. Zonisamide 100mg mane, 400mg nocte
2. Carbamazepine 400mg bd
3. Folic acid 5mg od

Previous anti-seizure medications;

1. Clonazepam
2. Gabapentin
3. Levetiracetam
4. Phenytoin
5. Valproate Chrono

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Complex partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;

She had her first seizure at the age of 14 months. There were no obvious precipitants for this first event. Collateral history describes a generalised convulsion without lateralising features.

Currently, she has events in which she will lose awareness at onset. The seizures are characterised by an initial phase of behavioural arrest and staring with subsequent abdominal pain. After this, others notice that she has impairment of expressive speech. Usually, seizures will stop at this stage and usually only last for 1 – 2 minutes. More rarely, she will have progression to a generalised convulsion. She has no significant sequelae for her events.

She has had her diagnosis confirmed by a consultant Epileptologist.

Imaging results; 1.5T MRI was normal

EEG results; Video EEG monitoring showed interictal right parietal and frontal spike discharges. The seizures captured during captured showed a right central seizure focus with spread to the right frontal region.

Individual family members – family I

I i 1
Age at recruitment; 67
Sex; Male
Handedness; Right

Diagnoses;

1. Epilepsy

2. Migraine without aura

Medications;
1. Levetiracetam 1000mg bd
2. Carbamazepine 200mg mane, 400mg nocte

Previous medications;
1. Sodium valproate
2. Phenobarbitone
3. Vigabatrin

Drug allergies; Nil

Family history;

1. Migraine – mother

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Epilepsy syndrome; Temporal lobe epilepsy

Seizure classification; Simple partial, complex partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He is unsure when he first began to have seizures but "became aware of them" in his early twenties. All of his events have had a consistent semiology since the onset of his epilepsy.

His events usually begin with an aura characterised by an abnormal sensation ("tingling") beginning in both lower limbs and spreading superiorly. With this, he has a change in mental state, described as a "loss of interest" in his surroundings.

Although his events can stop at this stage, more usually they proceed to a loss of awareness. A collateral history describes oral automatisms with these events but no other clinical features. These events last for about thirty seconds, and are followed by a relatively rapid recovery to baseline within one minute.

He has had rare secondarily generalised tonic clonic seizures. However, he has no recollection of these events, and a collateral history was unavailable to characterise them further.

He has never had any other types of seizure at any stage.

Imaging results; A 1.5T MRI showed left hippocampal sclerosis with associated atrophy.

EEG results; A routine study was normal.

I i 2
Age at recruitment; 65

Sex; Female

Handedness; Right

Diagnoses;

1. Scarlet fever in childhood
2. Hepatitis in childhood
3. Reactive depression

Medications;

1. Hormone replacement therapy
2. Fluoxetine

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

I i 3

Age at recruitment; 63

Sex; Male

Handedness; Right

Diagnoses;

1. Isolated episode of chest pain, aged 18 – medically investigated, cause unclear

Medications; nil

Previous anti-seizure medications; nil

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

I i 4
Age at recruitment; 57
Sex; Male
Handedness; Right

Diagnoses;
1.   Chronic back pain secondary to degenerative disc disease
2.   Previous tonsillectomy
3.   Migraine without aura

Medications;
1.   PRN analgesia – solpadol, diclofenac, reliflex

Previous anti-seizure medications; nil

Allergies; nil

Family history;
1.   Migraine
2.   Epilepsy
3.   Type 1 diabetes mellitus – daughter

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

I i 5
Age at recruitment; 52
Sex; Male
Handedness; Right

Diagnoses;
1.   Gout – secondary arthritis from same
2.   Previous left ankle arthrodesis
3.   "TB in the brain" – exact nature of same unclear. Had 2 episodes of same, 32 and 27 years prior to recruitment. He required ventriculoperitoneal shunt insertion on the first occasion. His clinical course was complicated by "two strokes" which caused left hemiparesis, and he was told that "it affected the pituitary gland". He was hospitalised on both occasions and was seen by a consultant Neurologist.
4.   Pituitary dysfunction – secondary to the above
5.   Headaches – cause unclear. He has been medically investigated and has had a cerebral angiogram. He has been diagnosed with migraine but has not seen a consultant Neurologist for this complaint.
6.   Epilepsy

Medications;
1.   Eltroxin
2.   Venlafaxine
3.   Lansoprazole
4.   Testerone and GH injections
5.   Arthrotec

Previous anti-seizure medications; He is unsure of the name of the medication he was on – possibly valproate

Allergies; Nil

Early risks for epilepsy; Possible meningitis

Epilepsy classification; Acute symptomatic seizures only

Seizure classification; Generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He says that he had seizures at the time of his second presentation with "TB in the brain" at the age of 25. He has no recollection of the nature of even, but has no definite recollection of any aura or prodrome. No recollection of injuries from same. He has never received a description of the seizures other than being told he had "a fit". No collateral history was available.

He was on anti-seizure therapy for 3 – 4 years after the onset of his seizures. After this, he was weaned off this under the guidance of a consultant Neurologist. He has not had any events of any type since then.

He has not had any other events of any type.

Imaging results; 1.5T MRI was normal

EEG results; Unavailable

I i 6
Age at recruitment; 47
Sex; Male
Handedness; Right

Diagnoses;
1.   Epilepsy

Medications;
1.   Valproate chrono 700mg bd

Previous anti-seizure medications;

1.   Carbamazepine – ineffective

Allergies; Nil

Early risks for epilepsy; Nil

Epilepsy classification; Localisation related epilepsy

Seizure classification; Presumed secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;
He was diagnosed with epilepsy after his initial presentation at the age of 35. He was hospitalised after an event occurring out of sleep. No collateral history was available for this.

He has had events out of sleep only and has never had events out of wakefulness. He has never been given a detailed description of his events, and a collateral history was unavailable. However, he has no preceding vocalisation and has been told that all of his limbs are shaking during events. He has no recollection of any aura or prodrome for his events, but admits that he has no recollection of his seizures himself.

His diagnosis has been confirmed by a consultant Neurologist. He has been seizure-free for some years on valproate monotherapy.

He has never had any other types of seizure at any stage.

Imaging results; Unavailable

EEG results; A routine study did not show any focal or epileptiform features

Individual family members – family K

K i 1
Age at recruitment; 65
Sex; Male
Handedness; Right

Diagnoses;
1. Epilepsy
2. Anxiety / depression
3. Previous right temporal lobe resection for control of epilepsy

Medications;
1. Clobazam 10mg od
2. Levetiracetam 1500mg bd
3. Phenytoin 150mg mane, 200mg nocte

Previous anti-seizure medications;
1. Diazepam
2. Lamotrigine
3. Phenobarbitone
4. Pregabalin
5. Valproate chrono
6. Zonisamide

Allergies; Nil

Family history;
1. Psoriasis
2. Thyroid dysfunction
3. Febrile convulsions
4. Epilepsy – his mother had a mental illness (type unclear, although he feels that it may have been schizophrenia) and he also feels that she had "convulsions"

Early risks for epilepsy; Nil definite. He is unsure if he had febrile convulsions or not, and a collateral history was unavailable.

Epilepsy classification; Localisation related epilepsy

Epilepsy syndrome; Temporal lobe epilepsy

Seizure classification; Complex partial, secondarily generalised tonic clonic

Summary of epilepsy history and seizure semiology;

He had his first unprovoked event in his late twenties (he is unsure of the exact age). All events have had a characteristic semiology.

He loses awareness at the onset of his events. He has no definite aura which he can recall after the events. By the collateral history obtained, the events are characterised by an initial phase of staring straight ahead and breathing more heavily than normal. After this, he tries to wander around his environment. If the events do not secondarily generalise, they will stop after less than three minutes, with a relatively quick recovery.

Occasionally, his seizures will progress from this to a secondarily generalised tonic clonic seizure. During these, he usually bites his tongue. Each secondarily generalised event lasts for 1 – 2 minutes with a slower recovery.

He has had one episode of status epilepticus after his temporal lobe surgery. However, he has been seizure free since then.

He has never had any events of any other type.

Imaging results; 1.5T MRI showed right mesial temporal sclerosis

EEG results; Video EEG monitoring showed bilateral independent interictal epileptiform abnormalities, more marked in the left temporal lobe than the right. All seizures captured during monitoring were localised to the right temporal lobe.

K i 2
Age at recruitment; 60
Sex; Female
Handedness; Right

Diagnoses;
1. Two previous total hip replacements
2. Breast biopsy 35 years ago – benign lump
3. Previous appendicectomy
4. Hypercholesterolaemia
5. Hypothyroidism
6. Vasovagal syncope in adolescence – not medically investigated

Medications;

1. Atorvastatin 10mg od
2. Aspirin 75mg od
3. Eltroxin – unsure of dose

Previous anti-seizure medications; Nil

Allergies; She is "intolerant" of kiwi fruit

Family history;

1. Epilepsy
2. Asthma – daughter
3. Congenital cataract – daughter

Early risks for epilepsy; Nil

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

K i 3

Age at recruitment; 72
Sex; Male
Handedness; Right

Diagnoses;

1. Psoriasis – has psoriatic arthritis
2. Previous concussion
3. Previous circumcision

Medications;

1. Methotrexate
2. Neurofen PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Early risk factors for epilepsy; One episode of head trauma with loss of awareness

Epilepsy classification; Unaffected

Seizure classification; Unaffected

Imaging results; Never performed

EEG results; Never performed

K ii 1
Age at recruitment; 28
Sex; Female
Handedness; Right

Diagnoses;
1. Previous elective surgery on teeth, aged 9
3. Previous febrile convulsions

Medications;
1. Diclofenac PRN

Previous anti-seizure medications; Nil

Allergies; Nil

Family history;
1. Epilepsy
2. Febrile convulsions
3. Psoriasis
4. Pulmonary embolus – mother

Early risks for epilepsy; Febrile convulsion

Epilepsy classification; Febrile convulsions only

Seizure classification; Simple febrile convulsion

Summary of epilepsy history and seizure semiology;
By her own account, she had never had any seizures of any type. Her father gave a collateral history which describes two febrile convulsions. He is unsure how old she was when she began having them, but is definite that her last one was when she was younger than three years. There were no associated focal features and recovery was rapid from both. She was medically assessed but she was not seen by a Paediatric Neurologist and was not felt to require any investigations.

Imaging results; Never performed

EEG results; Never performed

K ii 2
Age at recruitment; 26
Sex; Male
Handedness; Right

Diagnoses;
1. Previous isolated asthma attack – not formally diagnosed with asthma
2. Two previous syncopal episodes – cause unclear, medically investigated
3. Isolated febrile convulsion

Medications; Nil

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Febrile convulsion

Epilepsy classification; Febrile convulsions only

Seizure classification; Simple febrile convulsion

Summary of epilepsy history and seizure semiology;
By his own account, he has never had any seizures of any type. A collateral history clarified that he has has had one febrile convulsion. He was not medically investigated at the time. He did not have any particular focal features he can recall, and the event did not seem particularly prolonged.

He has never had any other types of seizure at any stage.

Imaging results; Never performed

EEG results; A routine study showed bilateral theta and delta slowing which was a little in excess of what was normal for age, but was of uncertain significance. No focal or epileptiform abnormalities seen.

K ii 3
Age at recruitment; 23
Sex; Female
Handedness; Right

Diagnoses;
1.   Previous cholecystectomy
2.   Febrile convulsions
3.   Has been told that she "tends to have low blood pressure" – not medically investigated

Medications;
1.   Oral contraceptive pill

Previous anti-seizure medications; Nil

Allergies; Nil

Early risks for epilepsy; Febrile convulsions

Epilepsy classification; Febrile convulsions only

Seizure classification; Simple febrile convulsions

Summary of epilepsy history and seizure semiology;
She is aware that she had febrile convulsions. She cannot remember any particular details of the attacks, except that they began when she was 3 years old and did not persist beyond the age of 5. A collateral history confirms that she had at two febrile convulsions, and that the first was at the age of 3 years. There were no unusual or focal features for her events, and she recovered rapidly in each case. She was medically assessed, but did not have a Paediatric Neurology opinion.

She has never had any other type of seizure.

Imaging results; Never performed

EEG results; Routine study was normal.

Appendix B

Letter of ethical approval from the Ethics (Medical Research) Committee of Beaumont Hospital

Ethics (Medical Research) Committee - Beaumont Hospital

Notification of ERC/IRB Approval

Investigator: Dr. N. Delanty

Protocol No.: 05/56

Protocol Title: Genetic Analysis of Familial Forms of Epilepsy in an Irish Population

Ethics Committee Meeting Date: 6th December 2005

Final Approval Date: 9th January 2006

From: Ethics (Medical Research) Committee - Beaumont Hospital, Beaumont, Dublin 9

Documents Reviewed

Document and Date	Date Reviewed	Approved
Protocol No. 05/56 - v2, 23/12/05	9/1/06	Yes
Index Cases:		
Cover letter, v2, 23/12/05	9/1/06	Yes
Information Leaflet, v2 23/12/05	9/1/06	Yes
Family Members:		
Cover letter, v2, 23/12/05	9/1/06	Yes
Information Leaflet, v2, 23/12/05	9/1/06	Yes
Consent to be Contacted, v2, 23/12/05	9/1/06	Yes
Consent Form, v2, 23/12/05	9/1/06	Yes
Genetic Consent Form, v2, 23/12/05	9/1/06	Yes
Parents/Guardians/Legal Representatives:		
Cover letter, v2, 23/12/05	9/1/06	Yes
Information Leaflet, v2, 23/12/05	9/1/06	Yes
Consent to be Contacted, v2, 23/12/05	9/1/06	Yes
Consent Form, v2, 23/12/05	9/1/06	Yes
Genetic Consent Form, v2, 23/12/05	9/1/06	Yes
Minors:		
Information Leaflet, v2, 23/12/05	9/1/06	Yes
GP letter, v2, 23/12//05	9/1/06	Yes
Protocol Amendment N/A		

_____

ERC/IRB – Convenor's Signature
9/1/06

Forms for recruitment protocol for Beaumont Hospital and St. James's Hospital

Beaumont / SJH – Information leaflet for family members

FAMILY MEMBER INFORMATION LEAFLET

Genetics of Epilepsy

What is the purpose of this study?

Epilepsy affects about 40,000 people in Ireland. The cause of epilepsy is often unknown. However it is clear that epilepsy runs in some families and therefore genetic or 'in-built' factors are likely to play a role. We are carrying out a genetic study of epilepsy in the Irish population. To identify these genes it is necessary to collect a blood sample from as many relatives as possible in each family. This information will improve our understanding of epilepsy in Ireland and elsewhere and may lead to the development of better treatments.

Why are we contacting you?

One of your relatives has epilepsy and has given a blood sample to Beaumont Hospital as part of a research project into epilepsy. This relative has indicated to us that he/she has family members who might be willing to become involved in an epilepsy research project also.

What will happen next?

Take your time to read this information leaflet and to discuss it with relatives and friends and/or your GP. If you want to take part, please tick the appropriate box in the enclosed form at the end of this Leaflet and return it in the envelope provided. We will telephone you in 2-3 weeks time to discuss further whether you would be interested in taking part in this study.

If you decide not to take part, simply tick the appropriate box on the response form and return it in the enclosed envelope. You will not receive any further contact from us.

What happens next to me next?

If you consent to be contacted, we will telephone you to discuss this study further with you. Should you agree we can arrange for you to meet with a doctor in person to discuss this study. At this meeting, he/she will explain the study and answer any of your questions. Should you agree to take part in the study, he /she will ask you to sign a consent form.

If you agree to participate, you will need to give a blood sample. From your point of view it will involve a once-off visit to Beaumont Hospital for a blood test. If this is inconvenient we can arrange to visit you at your home or at your GP's surgery. We can arrange a time for you to give this blood sample during this telephone call.

What happens to my blood sample?

We will analyse your blood sample for genetic and biochemical factors associated with epilepsy. (i.e. we will be looking at genes and DNA in these blood samples.) The blood samples will be coded with

a number and stored at the Royal College of Surgeons in Ireland, at SurGen Limited. The stored sample will not contain information by which you could be identified.

What are the risks?

Blood taking may cause some discomfort and superficial bruising may occur at the puncture site. Care will be taken to avoid such complications.

What are the benefits?

In recent years, identification of genes that predispose to epilepsy has allowed doctors and researchers to better understand how epilepsy is caused. Taking part in this study may help us to understand why more than one person in the same family has epilepsy and this research may be of benefit to future epilepsy patients.

Will I get the results of the genetic test back?

This is a research study and therefore you will not get individual results back from the genetic tests, except in very rare cases. Only families identified with rare hereditary forms of epilepsy will be contacted and referred for genetic counselling (ie. Information and support for people with an inherited condition and screening of family members if appropriate). All samples will be coded and therefore the genetic information will be studied anonymously. It is hoped that this type of research may help allow genetic tests for epilepsy to be developed.

Confidentiality:

All findings from this study will be kept confidential. The information obtained from the blood samples given by your family members is studied anonymously. A special code linking your family members' names to their samples will be held by the main investigator Dr. Delanty, to allow updating of clinical information only in respect of the current research project. It will not be used for identification for any other purpose. The results of our study may be published in a medical journal at a later date. However, your family's name or number will not appear in the publication.

Further Information: You will be able to keep this Patient Information Document. If you have any further questions please contact:

Dr. Norman Delanty, Consultant Neurologist, Beaumont Hospital.

Telephone: 01-8092055

Beaumont Hospital

Dublin 9

Consent Form for Adults Capable of Giving Informed Consent:

Title of the project: Genetics of Epilepsy
Principle Investigator: Dr. Norman Delanty

Name of Participant:

Name of Researcher:

Contact details for research team: Dr Norman Delanty, Beaumont Hospital, Dublin 9.

Tel: 01 8092055

Please tick appropriate boxes Yes/No

I am an adult taking part in this study.   Yes ☐   No ☐

I have read the information leaflet about this research project, and have been given a copy to keep. The information has been fully explained to me and I have been able to ask questions. I understand why the research is being done and any risks involved.   Yes ☐   No ☐

I agree to donate a sample (blood//DNA) for this research project. I understand that giving a sample for this research is voluntary and that I am free to withdraw my approval at any time without my medical treatment being affected.   Yes ☐   No ☐

I give permission for research personnel to look at my medical records to obtain information. I have been assured that information about me will be kept confidential.   Yes ☐   No ☐

Future uses of Biological Material

I give permission for my sample and information collected about me to be stored for possible future research related to this study (including DNA studies) but only if my consent is obtained at the time for this future research and the research is approved by a Research Ethics Committee.

    Yes ☐   No ☐

Signed:.. Date:...

Name of research participant

Signed:.. Date:...
Name of person taking consent

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Consent Form 1a

Study Title: **Genetics of Epilepsy**

Name of Principle Investigator: **Dr. Norman Delanty**

1. The research project has been explained to me and what will be expected of me. I have read the Patient Information Document or have had it read to me. The person named below has explained any possible problems that might arise as a result of my participation. I have had time and opportunity to ask questions, and I have received satisfactory answers.

2. I understand that I am free to not participate in this research. I understand that if I refuse to participate, it will not in any way affect my future treatment or medical management.

3. I understand that I am free to withdraw my name at any time, without giving any reason and without my medical care or legal rights been affected.

4. I understand that my name will not be used in any report that comes from the blood samples. In turn, I understand that I cannot restrict the use of the information that comes from studying the blood samples. I accept that the information may be given to medical regulatory authorities in Europe or elsewhere. I am aware that the study resource will be owned and used by both academic and commercial researchers. These include, but are not limited to, the Royal College of Surgeons in Ireland and SurGen Limited.

5. I have been made aware of my rights concerning the collection, processing and use of the data for this procedure. I agree to the collection, processing and use of personal data as described in this document.

I, the undersigned, hereby consent to participate as a subject in the above project.

SIGNATURE: _____ DATE: _____

PRINT NAME: _____

Name of Person Explaining   Signature   Date

Informed Consent

_____   _____   _____

RE: Genetics of Epilepsy

Consent to be Contacted Form:

Patient Name:

Address:

Phone number:

I wish to be contacted about the Genetics of Epilepsy study.   ▢

I do not wish to be contacted about the Genetics of Epilepsy study.   ▢

SIGNED: ....

DATED: ....

Please return to:

Dr. Norman Delanty
Consultant Neurologist
Beaumont Hospital
Beaumont,
Dublin 9
Ireland

Forms for recruitment protocol for Cork University Hospital

CUH – Information leaflet for family members

Family member information leaflet

Protocol Title:

Genes and familial forms of epilepsy in the Irish population

Principal Investigator's Name: Dr. Daniel Costello

Principal Investigator's Title: Consultant Neurologist

Telephone No. of Principal Investigator: 021 - 4922461

You are being invited to take part in a clinical research study carried out at Cork University Hospital. Before you decide whether or not you wish to take part, you should read the information provided below carefully and should you wish, discuss it with your family, friends or GP. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Consent.

You may change your mind at any time (before the start of the study or even after you have commenced the study) for whatever reason without having to justify your decision and without any negative impact on the care your family member will receive from the medical staff at Cork University Hospital.

WHY IS THIS STUDY BEING DONE?

The goal of this study is to identify genes or regions of DNA that are contributing to epilepsy in Ireland. By identifying these genes we hope to learn more about the development of epilepsy and how best to treat it.

Epilepsy affects about 40,000 people in Ireland. The cause of epilepsy is often unknown. However it is clear that epilepsy runs in some families and therefore genetic or 'in-built' factors are likely to play a role. We are carrying out a genetic study of epilepsy in the Irish population. To identify genes involved in epilepsy we need to first recruit families with a strong history of epilepsy. By studying the DNA of family members we can look to see if particular genes are always inherited with epilepsy in a family

WHO IS ORGANISING AND FUNDING THIS STUDY?
This work is being run by Cork University Hospital together with the Royal College of Surgeons in Ireland and St. James' Hospital. It is being funded by the Health Research Board of Ireland. It is being organised by Dr. Daniel Costello and Dr. Brian Sweeney, Consultant Neurologists (Cork University Hospital), Dr. Norman Delanty, Consultant Neurologist (Beaumont Hospital) and Dr. Gianpiero Cavalleri, The Royal College of Surgeons.

HOW WILL IT BE CARRIED OUT?
You have been contacted because several members of your family have epilepsy. We are seeking to recruit family members – both affected and unaffected by seizures. Researchers will look closely at the DNA of family members to see if they can identify regions of DNA that appear to be correlated with epilepsy – for example a piece of DNA that is always present in family members affected by seizures but always absent from those who do not experience seizures. The analysis will be carried

out by researchers in Ireland (led by the Royal College of Surgeons) as well as by collaborating researchers in other groups around the world.

WHAT WILL HAPPEN NEXT?

1) If you wish to learn more about this study please tick the appropriate box in the enclosed "consent to be contacted" form and return it in the envelope provided. Returning this form does not mean you wish to take part, rather you are interested in learning more about the study.

2) Should you return the "consent to be contacted" form we will contact you by phone to discuss the study. If you are still interested after this conversation we will arrange a time to meet you and members of your family at a location of your choice. This could be at the hospital, at your home or perhaps a local GPs office. At the meeting we will provide you with a further chance to ask questions about the study and ask you some questions in relation to epilepsy in your family and whether or not you experience seizures. We will also ask you and each of your family members to donate around a teaspoon of blood – this allows us to isolate DNA.

3) Should you participate in the study your sample will be processed and stored by the RCSI Biobanking Facility in Dublin. During processing we will assign a code to your sample. Dr. Costello will hold the link between this code and your name. We assign this code so that researchers working on your sample are unable to identify who it belongs to. In order to keep our records complete and up to date, members of our team may wish to contact you via telephone or email in the future.

4) We will use your DNA to determine your genetic profile. This genetic profile is used to gain further understanding of how genetics might be able to predict the development of epilepsy. In order to generate this profile we may on occasion be required to send your DNA to another institute with whom we have a formal collaborative agreement. Similarly we may wish to send your genetic profile to a collaborating centre. This would come under similar formal agreement and protection.

WHAT WILL HAPPEN TO ME IF I CHANGE MY MIND?
If you agree to take part in this study, and later on decide you would like to withdraw you may do so without justifying your decision and the future treatment of your family member with epilepsy will not be effected. You can withdraw from this study at any point in time.

BENEFITS:
Taking part in this study may help us to understand why more than one person in the same family has epilepsy and this research may be of benefit to future epilepsy patients. This research will not cure epilepsy in your family. However, through the identification of genetic predictors of these conditions, it may lead to the development of improved treatments or predictive tests.

RISKS:
Blood taking may cause some discomfort and superficial bruising may occur at the puncture site. Care will be taken to avoid such complications.

Will I get the results of the genetic test back?

This is a research study and therefore you will not get individual results back from the genetic tests, except in cases where we have found a clear cause of epilepsy in your family and/or a genetic signature that we deem to be of clinical relevance and importance to lifestyle decisions. In such cases

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we will offer you details/results together with genetic counselling (ie. information and support for people with an inherited condition and screening of family members if appropriate).

CONFIDENTIALITY ISSUES:
All information and data handled in the course of this study is strictly confidential and will not be made available by us to anyone other than those who are directly involved in the study. In addition, any results from the study will be presented only in a way that does not allow you, your sibling or anyone who takes part in the study, to be identified.

If you are currently being treated for epilepsy or a related disorder we will request details from your clinical records from the relevant hospitals. These will be reviewed by epilepsy researchers who work under the direction of Dr. Daniel Costello.

The results of our study may be published in a medical journal at a later date. However, your family's name will not appear in the publication.

Information from your sample could be used for research involving the commercial sector including pharmaceutical companies. Any involvement of the commercial sector will be through collaboration/partnership between the academic and commercial partners.

IF YOU REQUIRE FURTHER INFORMATION
If you have any questions about the study, please do not hesitate to contact either Dr. Gerard O'Connor or Dr. Danny Costello (see contact details below)

For additional information now or any future time please contact:

1. Name: Dr. Gerard O'Connor, SpR in Neurology, Beaumont Hospital

Address: Department of Neurology, Beaumont Hospital, Dublin 9

Phone No: 01 809-2210
Email: gerarddanieloconnor@physicians.ie

2. Name: Dr. Daniel Costello

Address: Department of Neurology, Cork University Hospital, Wilton, Cork.

Phone No: 021 - 4922461
Email: Daniel.Costello@hse.ie

Patient Information Leaflet

Protocol Title:

Genes and familial forms of epilepsy in the Irish population

Principal Investigator's Name: Dr. Daniel Costello

Principal Investigator's Title: Consultant Neurologist

Telephone No. of Principal Investigator: 021 - 4922461

You are being invited to take part in a clinical research study carried out at Cork University Hospital. Before you decide whether or not you wish to take part, you should read the information provided below carefully and should you wish, discuss it with your family, friends or GP. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Consent.

You may change your mind at any time (before the start of the study or even after you have commenced the study) for whatever reason without having to justify your decision and without any negative impact on the care you will receive from the medical staff.

WHY IS THIS STUDY BEING DONE?

The goal of this study is to identify genes or regions of DNA that are contributing to epilepsy in Ireland. By identifying these genes we hope to learn more about the development of epilepsy and how best to treat it.

Epilepsy affects about 40,000 people in Ireland. The cause of epilepsy is often unknown. However it is clear that epilepsy runs in some families and therefore genetic or 'in-built' factors are likely to play a role. We are carrying out a genetic study of epilepsy in the Irish population. To identify genes involved in epilepsy we need to first recruit families with a strong history of epilepsy. By studying the DNA of family members we can look to see if particular genes are always inherited with epilepsy in a family

WHO IS ORGANISING AND FUNDING THIS STUDY?

This work is being run by Cork University Hospital together with the Royal College of Surgeons in Ireland and St. James' Hospital. It is being funded by the Health Research Board of Ireland. It is being organised by Dr. Daniel Costello and Dr. Brian Sweeney, Consultant Neurologists (Cork University Hospital), Dr. Norman Delanty, Consultant Neurologist (Beaumont Hospital) and Dr. Gianpiero Cavalleri, The Royal College of Surgeons.

HOW WILL IT BE CARRIED OUT?

Patients with a strong family history of epilepsy will be invited to take part. We are seeking to recruit family members – both affected and unaffected by seizures. Researchers will look closely at the DNA of family members to see if they can identify regions of DNA that appear to be correlated with epilepsy – for example a piece of DNA that is always present in family members affected by seizures but always absent from those who do not experience seizures. The analysis will be carried out by researchers in Ireland (led by the Royal College of Surgeons) as well as by collaborating researchers in other groups around the world.

WHAT WILL HAPPEN NEXT?

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If you wish to learn more about this study please tick the appropriate box in the enclosed "consent to be contacted" form and return it in the envelope provided. Returning this form does not mean you wish to take part, rather you are interested in learning more about the study.

Should you return the "consent to be contacted" form we will contact you by phone to discuss the study. If you are still interested after this conversation we will arrange a time to meet you and members of your family at a location of your choice. This could be at the hospital, at your home or perhaps a local GPs office. At the meeting we will provide you with a further chance to ask questions about the study and take a clinical history of your epilepsy. We will also ask you and each of your family members to donate around a teaspoon of blood – this allows us to isolate DNA.

Should you participate in the study your sample will be processed and stored by the RCSI Biobanking Facility in Dublin. During processing we will assign a code to your sample. Dr. Costello will hold the link between this code and your name. We assign this code so that researchers working on your sample are unable to identify who it belongs to. In order to keep our records complete and up to date, members of our team may wish to contact you via telephone or email in the future.

We will use your DNA to determine your genetic profile. This genetic profile is used to gain further understanding of how genetics might be able to predict the development of epilepsy. In order to generate this profile we may on occasion be required to send your DNA to another institute with whom we have a formal collaborative agreement. Similarly we may wish to send your genetic profile to a collaborating centre. This would come under similar formal agreement and protection.

WHAT WILL HAPPEN TO ME IF I CHANGE MY MIND?
If you agree to take part in this study, and later on decide you would like to withdraw you may do so without justifying your decision and your future treatment will not be effected. You can withdraw from this study at any point in time.

BENEFITS:
Taking part in this study may help us to understand why more than one person in the same family has epilepsy and this research may be of benefit to future epilepsy patients. This research will not cure your epilepsy. However, through the identification of genetic predictors of these conditions, it may lead to the development of improved treatments or predictive tests.

RISKS:
Blood taking may cause some discomfort and superficial bruising may occur at the puncture site. Care will be taken to avoid such complications.

WILL I GET THE RESULTS OF THE GENETIC TEST BACK?

This is a research study and therefore you will not get individual results back from the genetic tests, except in cases where we have found a clear cause of epilepsy in your family and/or a genetic signature that we deem to be of clinical relevance and importance to lifestyle decisions. In such cases we will offer you details/results together with genetic counselling (ie. information and support for people with an inherited condition and screening of family members if appropriate).

CONFIDENTIALITY ISSUES:
All information and data handled in the course of this study is strictly confidential and will not be made available by us to anyone other than those who are directly involved in the study. In addition, any results from the study will be presented only in a way that does not allow you, your sibling or anyone who takes part in the study, to be identified.

For the purposes of this study your clinical information will be reviewed from your hospital chart by epilepsy researchers who work under the direction of Dr. Daniel Costello. We may request your files from other hospitals or clinics you have been treated at around the country.

The results of our study may be published in a medical journal at a later date. However, your family's name will not appear in the publication.

Information from your sample could be used for research involving the commercial sector including pharmaceutical companies. Any involvement of the commercial sector will be through collaboration/partnership between the academic and commercial partners.

IF YOU REQUIRE FURTHER INFORMATION
If you have any questions about the study, please do not hesitate to contact either Dr. Gerard O'Connor or Dr. Danny Costello (see contact details below)

For additional information now or any future time please contact:

1. Name: Dr. Gerard O'Connor, SpR in Neurology, Beaumont Hospital

Address: Department of Neurology, Beaumont Hospital, Dublin 9
Phone No: 01 809-2210
Email: gerarddanieloconnor@physicians.ie

2. Name: Dr. Daniel Costello

Address: Department of Neurology, Cork University Hospital, Wilton, Cork.
Phone No: 021 - 4922461
Email: Daniel.Costello@hse.ie

<u>**Family member information leaflet**</u>

Protocol Title:

Genes and familial forms of epilepsy in the Irish population

Principal Investigator's Name: Dr. Daniel Costello

Principal Investigator's Title: Consultant Neurologist

Telephone No. of Principal Investigator: 021 - 4922461

Your relative is invited to participate in a research study carried out at Cork University Hospital. As they are under the legal age of consent for participation, you as a parent/guardian are being asked to consent on their behalf. Before you decide whether or not you wish for them to take part, you should read the information provided below carefully and should you wish, discuss it with your family, friends or GP. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Consent.

You may change your mind at any time (before the start of the study or even after you have commenced the study) for whatever reason without having to justify your decision and without any negative impact on the care your family member will receive from the medical staff at Cork University Hospital.

WHY IS THIS STUDY BEING DONE?

The goal of this study is to identify genes or regions of DNA that are contributing to epilepsy in Ireland. By identifying these genes we hope to learn more about the development of epilepsy and how best to treat it.

Epilepsy affects about 40,000 people in Ireland. The cause of epilepsy is often unknown. However it is clear that epilepsy runs in some families and therefore genetic or 'in-built' factors are likely to play a role. We are carrying out a genetic study of epilepsy in the Irish population. To identify genes involved in epilepsy we need to first recruit families with a strong history of epilepsy. By studying the DNA of family members we can look to see if particular genes are always inherited with epilepsy in a family

WHO IS ORGANISING AND FUNDING THIS STUDY?
This work is being run by Cork University Hospital together with the Royal College of Surgeons in Ireland and St. James' Hospital. It is being funded by the Health Research Board of Ireland. It is being organised by Dr. Daniel Costello and Dr. Brian Sweeney, Consultant Neurologists (Cork University Hospital), Dr. Norman Delanty, Consultant Neurologist (Beaumont Hospital) and Dr. Gianpiero Cavalleri, The Royal College of Surgeons.

HOW WILL IT BE CARRIED OUT?
You have been contacted because several members of your family have epilepsy. We are seeking to recruit family members – both affected and unaffected by seizures. Researchers will look closely at

the DNA of family members to see if they can identify regions of DNA that appear to be correlated with epilepsy – for example a piece of DNA that is always present in family members affected by seizures but always absent from those who do not experience seizures. The analysis will be carried out by researchers in Ireland (led by the Royal College of Surgeons) as well as by collaborating researchers in other groups around the world.

WHAT WILL HAPPEN NEXT?

1) If you wish to learn more about this study please tick the appropriate box in the enclosed "consent to be contacted" form and return it in the envelope provided. Returning this form does not mean you wish to take part, rather you are interested in learning more about the study.

2) Should you return the "consent to be contacted" form we will contact you by phone to discuss the study. If you are still interested after this conversation we will arrange a time to meet you and members of your family at a location of your choice. This could be at the hospital, at your home or perhaps a local GPs office. At the meeting we will provide you with a further chance to ask questions about the study and ask you some questions in relation to epilepsy in your family and whether or not you experience seizures. We will also ask you and each of your family members to donate around a teaspoon of blood – this allows us to isolate DNA.

3) Should you participate in the study your sample will be processed and stored by the RCSI Biobanking Facility in Dublin. During processing we will assign a code to your sample. Dr. Costello will hold the link between this code and your name. We assign this code so that researchers working on your sample are unable to identify who it belongs to. In order to keep our records complete and up to date, members of our team may wish to contact you via telephone or email in the future.

4) We will use your relatives DNA to determine their genetic profile. This genetic profile is used to gain further understanding of how genetics might be able to predict the development of epilepsy. In order to generate this profile we may on occasion be required to send their DNA to another institute with whom we have a formal collaborative agreement. Similarly we may wish to send their genetic profile to a collaborating centre. This would come under similar formal agreement and protection.

WHAT WILL HAPPEN TO ME IF I CHANGE MY MIND?
If you agree for your relative to take part in this study, and later on decide you would like to withdraw them, you may do so without justifying your decision and the future treatment of your family member with epilepsy will not be effected. You can withdraw from this study at any point in time.

BENEFITS:
Taking part in this study may help us to understand why more than one person in the same family has epilepsy and this research may be of benefit to future epilepsy patients. This research will not cure epilepsy in your family. However, through the identification of genetic predictors of these conditions, it may lead to the development of improved treatments or predictive tests.

RISKS:

Blood taking may cause some discomfort and superficial bruising may occur at the puncture site. Care will be taken to avoid such complications.

Will I get the results of the genetic test back?

This is a research study and therefore you will not get individual results back from the genetic tests, except in cases where we have found a clear cause of epilepsy in your family and/or a genetic signature that we deem to be of clinical relevance and importance to lifestyle decisions. In such cases we will offer you details/results together with genetic counselling (ie. information and support for people with an inherited condition and screening of family members if appropriate).

CONFIDENTIALITY ISSUES:

All information and data handled in the course of this study is strictly confidential and will not be made available by us to anyone other than those who are directly involved in the study. In addition, any results from the study will be presented only in a way that does not allow you, your sibling or anyone who takes part in the study, to be identified.

If you are currently being treated for epilepsy or a related disorder we will request details from your clinical records from the relevant hospitals. These will be reviewed by epilepsy researchers who work under the direction of Dr. Daniel Costello.

The results of our study may be published in a medical journal at a later date. However, your family's name will not appear in the publication.

Information from your sample could be used for research involving the commercial sector including pharmaceutical companies. Any involvement of the commercial sector will be through collaboration/partnership between the academic and commercial partners.

IF YOU REQUIRE FURTHER INFORMATION

If you have any questions about the study, please do not hesitate to contact either Dr. Gerard O'Connor or Dr. Danny Costello (see contact details below)

For additional information now or any future time please contact:

1. Name: Dr. Gerard O'Connor, SpR in Neurology, Beaumont Hospital

Address: Department of Neurology, Beaumont Hospital, Dublin 9
Phone No: 01 809-2210
Email: gerarddanieloconnor@physicians.ie

2. Name: Dr. Daniel Costello

Address: Department of Neurology, Cork University Hospital, Wilton, Cork.
Phone No: 021 - 4922461
Email: Daniel.Costello@hse.ie

Telephone: 01 8092210
email: gerarddanieloconnor@physicians.ie

Date:

Dear _____,

I am writing to you on behalf of Dr. Daniel Costello, to invite you to take part in a clinical research study we are carrying out at Cork University Hospital.

This study is seeking to identify genes that are involved in the development of epilepsy. You were identified as a potential participant from your records at the Epilepsy Service at Cork University Hospital which indicate you have a strong family history of epilepsy.

Enclosed with this letter is:

1. An information sheet explaining the study.

2. A "consent to be contacted" form should you wish to learn more.

3. A stamped addressed envelope to return the "consent to be contacted" form.

4. A separate envelope for your family members. This envelope contains separate information sheets and "consent to be contacted" forms for them to read and decide if they would also like to learn more.

Please read the information sheet carefully and do not hesitate to contact me if you have any questions at all.

Yours sincerely,

_____
Dr. Gerard O'Connor
SpR in Neurology
Beaumont Hospital