Genetics in Epilepsy

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I, Chien Ning Lo, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

This thesis reports a series of investigations to identify the disease loci of three distinct epilepsy syndromes, and also a pilot study to assess the feasibility of using saccadic eye movements as a biomarker of potential interest in genetic studies. The fact that some epilepsy syndromes have a strong genetic tendency has been known for decades, but very few susceptibly genes have been found. I sought to identify disease loci in three distinct epilepsy syndromes: familial temporal lobe epilepsy (2 families), photosensitive epilepsy (1 large family), and Kohlschütter-Tönz syndrome (5 families). Medical histories, seizure patterns and investigations were gathered through interview and case record reviews. The DNA samples were collected and underwent an eight-cM whole genome analysis. Parametric and non-parametric linkage analyses were carried out to these pedigrees. One of the families with mesial temporal lobe epilepsy was considered to have single gene inheritance and three regions of interest were identified. Five genes were sequenced in 5 individuals in this pedigree but no pathological mutations were found. The other family with mesial temporal lobe epilepsy had more complex inheritance pattern and no disease loci were identified. In the photosensitive epilepsy pedigree, our analysis showed polygenic inheritance with one region of interest on chromosome 16, but with no specific genes identified. In the Kohlschütter-Tönz pedigree, a disease locus was identified on chromosome 16 in a region of 30 known genes but no causative gene was identified. These results, both positive and negative are discussed. In the pilot study to assess the feasibility of the methodology of using saccadic eye movement as a biomarker (as biomarker in pharmocodynemic studies), a system for measurement was devised and a control group and patients with widely varied antiepileptic drug measurements were examined (including one patient with phenytoin toxicity). The experiment showed the intra-individual variability of saccadic eye movements was too large to allow small changes to antiepileptic measurements to be easily identified. That seems that the measurement of main sequence will not be useful as a biomarker for pharmacogenomic or pharmacodynamic studies. It is possible that other measurements will be more reliable and these are discussed.

Aims

This thesis reports studies in two broad areas. The first is a series of investigations in families using advanced linkage analysis; and the second is a pilot investigation assessing the potential of saccadic eye-movements to be a biomarker of the pharmacodynamic actions of antiepileptic drugs, for future use in genetic (pharmacogenomic) studies.

1. Family Linkage Analyses (8 families)

In this body of work, I studied 8 families within three epilepsy syndromes (familial temporal lobe epilepsy, photosensitive epilepsy, Kohlschütter-Tönz syndrome). I collected appropriate clinical phenotypic data (from clinical examination, note review and hospital inquiry), carried out the laboratory genetic analysis of the DNA from individuals in this family (preparation, genotyping and sequencing), and devised and conducted the computerised analysis of this DNA using various linkage programmes. The aims of the study were:

i. Familial temporal lobe epilepsy (two families). To identify the genetic basis of the familial epilepsy, to determine whether there was a similar genetic cause in the two families, to determine the mode of inheritance in the families, to identify linked regions, to sequence candidate genes within the linked regions and to determine the clinical phenotype associated with any linkage discovered.

ii. Photosensitive epilepsy (one large family): To identify the genetic basis of the Photoparoxysmal response (PPR) in a large family with photosensitive epilepsy, to determine the mode of inheritance, and to identify linkage.

iii. Kohlschütter-Tönz Syndrome: To identify the genetic basis (linkage) of this rare syndrome (in five families), to determine the mode of inheritance, to identify linkage, to define the clinical phenotype associated with the linked area, and to if possible identify the causative gene.

2. To conduct a pilot study as part of a programme to investigate the potential of saccadic eye movements (main sequence, peak saccadic velocity) to act as a biomarker of value in pharmacogenomic studies of epilepsy.

The long-term aim of this work is to determine whether saccadic eye-movements were useful as a measure of the pharmacological effects of antiepileptic drugs – in other words to determine whether they would prove a useful *pharmacogenomic biomarker.* . The hypothesis was that pharmacological effectiveness on epilepsy would be mirrored by pharmacological effects on eye-movements. The specific objective is to determine whether effects on saccadic velocity due to drugs correlated with drug effects on epilepsy in terms of side-effects or efficacy. If the AED effects on saccades did indeed correlate with clinical AED effect, a second phase of the programme was planned to identify whether polymorphic variation in SCN1A (or other candidate genes) could be identified as underpinning the saccadic variation – thus potentially leading eventually to the use of saccadic eye-movements as a biomarker, and identification of genotypes which correlate to clinical response. The first phase of the project reported in this thesis, the pilot (or scoping) phase, which had the following narrower aims:

1. to devise a clinically-applicable method for measuring saccadic main sequence and peak saccadic velocity.
2. to carry out measurements in a suitable control group, to identify mean values and the range of normality.
3. to carry out a pilot study in patients with epilepsy, to determine whether changes in blood levels were reflected by measurable changes in saccadic velocity in a reliable fashion, and to determine the extent of this effect
4. In a preliminary manner, to whether the there is an association between the magnitude of the effect of saccadic variation and clinical effects (side-effects/efficacy).

Personal contribution to the work

1. Contribution to design, methodology, laboratory, analysis and writing up

a.. The linkage analysis

In the genetic analysis project, I was responsible for the overall design of the study and the design of the analytical strategy (in collaboration with my supervisors), the literature review, the recruitment of subjects, the interviews, obtaining consent and collection of blood samples of the two pedigrees with familial mesial temporal lobe epilepsy (B pedigree and the W pedigree). This involved travelling to Scotland (Glasgow), East London (Chingford, Walthamstow, Romford, Hornchurch) and East coast of England (Norfolk), and South Wales (Swansea); taking consents; and interviewing every subject in person to take medical histories, seizures patterns and details; constructing family pedigrees; drawing blood samples; and the phenotyping. I also personally carried out all the laboratory based molecular genetics studies including polymerase chin reactions (PCR), genotyping (for fine mapping part) and gene sequencing. I selected the linkage analysis software in carrying out the linkage analysis, based on the study design of Professor Newton Morton, the sequential tests of linkage analysis. I carried out the computation linkage analysis, including software installation, files formatting, and the linkage analysis of the 8 families involved in this project. I searched the bio-informatics database online including several websites to retrieve information of the genes within the disease loci identified, and performed the literature reviews to identify possible candidate genes. I formulated the findings and results to write up the experiments. In the work I was assisted by discussions with my supervisors and also Dr Mary Davies (chief technician in the genetics laboratory).

b. The saccadic eye movement pilot study

I was responsible for the overall design of the study (in collaboration with my supervisors and also Prof Linda Luxon and Dr Doris Bamou, who advised my on the technical side) and the literature review, the design of the testing schedules and methodology including computerized electro-nystagmography and the video-based tracking eye movement recording facilities, and later on the infra-red limbal trackers for this pilot

study. I devised the methodology and the experiments in the eye movement recording. I recruited subjects from the Epilepsy out patient clinic as well as organized the appointment for the eye movement experiments and conducted the measurements on the patient and control group. I reviewed medical notes of the subjects, carried out the data conversion to re-construct the saccadic main sequence. I did the statistical analysis, and formulated the findings and results to write up the experiments.

B. Skills Training

I sat in the epilepsy out patient clinic with Professor Simon Shorvon, assisted in identifying probands for genetic analysis studies, as well as recruiting subjects for both the genetic linkage study and the saccadic eye movement study. I sat in the Neuro-otology clinic with Professor Linda Luxon and was trained in the Neuro-otology unit in the NHNN the skills in recording and assessing saccadic movement, as well as in the eye movement laboratory in the Institute of Child Health, Great Ormond Street Hospital in operating the IRIS 6500 infrared limbal tracker (Skalar Medical, Delft, The Netherlands) with Dr. Richard Clement, as well as learning to use the software Mathematica in carrying out the conversion and analysis of saccadic eye movement measurements. I learned the laboratory skills in carrying out the laboratory works on molecular genetics with the help from Dr Mary Davis, Dr Vaneesha Gibbons, Dr Andrea Haworth, and Dr Richa Sud. I learned more skills in primer designs and PCR with Professor Sheau Yu Teddy Hsu in Stanford University, California, USA where I spent several weeks during 2008.

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Introduction

Section 1: Familial Mesial Temporal Lobe Epilepsy and Its Genetics

1.0 Background

Epilepsy is one of the oldest recorded diseases. The description of epilepsy can be traced back as early as 1067 B.C on two tablets known as part of the medical textbook in Assyrian-Babylonian, the *Sakkiku,.*

One of the characteristics of epilepsy is the heterogeneity of its clinical presentations as well as of aetiology (Berg, 2007). Some epilepsies are mild and some are severe, some (especially in childhood) conform to syndromic categories, but others do not, and clinical seizure type varies. The aetiology of epilepsy is commonly divided into three main categories, idiopathic, cryptogenic and symptomatic (Commission on Epidemiology and Prognosis and International League Against Epilepsy, 1993). Idiopathic epilepsies often refer to epilepsies that their causes are primarily genetic in origin, and symptomatic epilepsy refers to the epilepsies that occur in the presence of a causative insult or condition. Almost any grey matter disease can result in the development of epilepsy, common examples being stroke, head injuries, and cerebral palsy. One important consideration is the fact that epilepsy is often multi-factorial and can be the result of both external and internal (usually polygenic) influences.

The term "cryptogenic epilepsy" is used to describe seizures that are "probably symptomatic" but their aetiologies cannot be identified through available medical investigation (Commission on Classification and Terminology of the International League Against Epilepsy., 1989; Commission on Epidemiology and Prognosis and International League Against Epilepsy, 1993). Some of the epilepsies that were classified as cryptogenic have been found to be genetic in origin through rigorous twin studies (Johnson *et al.* 2001; Johnson *et al.* 2003), and appeared to have its influences on epilepsy outcome. Other studies revealed that a frontal lobe epilepsy syndromes [Autosomal Dominant Nocturnal

Frontal Lobe Epilepsy (ADNFLE)] is also genetic in origin, that the gene mutations on acetylcholine receptor subunits (CHRNA4, CHRNB2, and CHRNA2) appear to be the susceptibility gene and have been identified in several different families. (Aridon *et al.* 2006; Beck, 1994; Fusco *et al.* 2000). These mutations cause gain of function of the acetylcholine receptors and reduce the 2 mM Ca^{2+}-induced increases in the peak 30 uM acetylcholine response (Rodrigues-Pinguet *et al.* 2003; Steinlein and Bertrand, In press). These findings show that epilepsies which tend to cluster in families and failed to have a clear pathogenesis identified may be genetic in origin.

Familial epilepsies can be inherited in an autosomal dominant (AD), AD with an incomplete penetrance, or autosomal recessive (AR) pattern (Baulac *et al.* 2001a; Berkovic *et al.* 2004; Cossette *et al.* 2002; Phillips, 1995; Tournev *et al.* 2007). The majority are autosomal dominant (Shahwan *et al.* 2005). Epilepsy can also be the result of mitochondrial inheritance in such case it usually is part of a wider syndrome, such as in "Mitochondrial Encephalopathy, Lactic Acidosis and Stroke-like Episode Syndrome" (MELAS), and "Myoclonus Epilepsy with Ragged-Red Fiber Syndrome" (MERRF) (Hirano *et al.* 2007). Alpers-Huttenlocher syndrome is another mitochondria disease in which intractable seizures are a part of the clinical presentation, and is caused by mutations in the nuclear POLG gene (encoding mitochondria polymerase γ), which causes multiple mitochondria DNA deletion (Davidzon *et al.* 2005; Nguyen *et al.* 2005). Besides these mutations, aberration of chromosome structure is another cause of epilepsy syndromes, such as ring chromosome 20 syndrome, (Ville *et al.* 2006) and ring chromosome 17 (Ricard-Mousnier *et al.* 2007). Patients with ring chromosome 20 syndromes and ring chromosome 17 syndrome have refractory seizures and delayed of psychomotor development.

Familial genetic linkage studies have been carried out to explore the genetic of many familial epilepsy syndromes. In some of the studies, the gene mutations have been successfully identified, the results were replicated in families with the same epilepsy syndromes and the mechanisms were further elucidated with subsequent functional studies of these gene mutations. Such epilepsy syndromes include familial lateral temporal epilepsies, febrile seizures, Generalized Epilepsy with Febrile Seizures Plus (GEFS+), and Severe Myoclonic Epilepsy of Infancy (SEMI, or Dravet disease) (Audenaert *et al.* 2003;

Berkovic *et al.* 2004; Kalachikov *et al.* 2002; Wallace *et al.* 2002; Wallace *et al.* 1998). However, for many familial epilepsies, their genetic basis has not yet been fully understood. Such epilepsy syndromes include familial mesial temporal lobe epilepsy (fMTLE) (Striano *et al.* 2008b), photosensitive epilepsy (PhE)(Kasteleijn-Nolst Trenité, 2006; Pinto *et al.* 2005), and familial partial epilepsy with variable foci (FPEVF) (Scheffer *et al.* 1998). In each of these familial epilepsies, one or more disease loci may have been reported without replications, and in some studies a few candidate genes were sequenced without disease causing mutations found. (Andermann *et al.* 2005; Pinto *et al.* 2007; Vadlamudi *et al.* 2003).

1.1 Familial Temporal Lobe Epilepsy (fTLE)

For decades, partial epilepsies have been recognized to have genetic predispositions (Andermann, 1982), but not until recently was familial Temporal Lobe Epilepsy (fTLE) recognized as a well defined epilepsy syndrome (Engel, 2001). Familial TLE was first described by Berkovic in a twin study (Berkovic *et al.* 1996). It was first described as a relatively benign syndrome with late onset seizures without history of prolonged febrile seizures. However, in subsequent families indentified, some had a less benign course and a higher portion of hippocampal atrophy or sclerosis. In some resective surgery was necessary to control their intractable seizures (Cendes *et al.* 2007; Kobayashi *et al.* 2001).

In terms of diagnosis, it is not possible to differentiate familial temporal lobe epilepsies from sporadic temporal lobe epilepsies solely by clinical presentation. In many studies, familial TLE has been considered to be present when at least 2 members in the same family are diagnosed with temporal lobe epilepsy. This criterion however has not been consistently employed, and particular studies have varied as to whether the term should be restricted to first degree relatives or whether the familial tag can also be applied if there are affected second or third degree relatives (Picard *et al.* 2000).

The classification of familial temporal lobe epilepsy is the same as sporadic temporal lobe epilepsy. Based on the origin of seizure discharge, it is divided into familial mesial temporal lobe epilepsy (fMTLE) and familial lateral lobe epilepsy (fLTLE). A strand of evidence supporting the view that fLTLE is different from fMTLE is the fact that the gene mutation in Leucin-rich Glioma Inactive 1 gene (LGI1) which causes fLTLE has never

been found in families identified with fMTLE (Berkovic *et al.* 2004; Vadlamudi *et al.* 2003). Although the symptoms of fLTLE and the symptoms of fMTLE often overlap, there are differences in their clinical presentations.

1.1.1 Familial Mesial Temporal Lobe Epilepsy

Epidemiology

A preliminary hospital based study found that familial MTLE represented 7% of all MTLE patients (Kobayashi *et al.* 2001). However, the true incidence and prevalence of familial mesial temporal lobe epilepsy is still unknown as there are not enough representative studies (Cendes *et al.* 2007).

Clinical presentation

The clinical presentation of familial temporal lobe epilepsy cannot be differentiated from sporadic temporal lobe seizures. Typical seizures of mesial temporal lobe take the form of simple partial seizure, complex partial seizures, and secondary generalized seizures. A typical complex partial seizure has three components: aura, absence, and automatism. Simple partial seizures comprise motor, autonomic, somatosensory, and/or psychic manifestation. Complex partial seizures have an indistinct onset and may last for 2-10 minutes while simple partial seizure is often brief and typically evolves within seconds. The details of the symptoms in mesial temporal lobe epilepsy are presented in Table 1.1 and Table 1.2.

Diagnosis and differential diagnosis

The diagnosis of familial mesial temporal lobe epilepsy is largely history-based. High resolution Magnetic Resonance Imaging (MRI) and Electroencephalography (EEG) are used to assist in diagnosis, but it is not uncommon for both investigations to be normal in those patients. Typical EEG finding of mesial temporal lobe epilepsy include interictal epileptiform discharge over mid-and infero-mesial temporal regions, and a similar ictal EEG findings to sporadic mesial TLE. MRI may show hippocampal atrophy or hippocampal sclerosis (other pathologies would exclude a diagnosis of idiopathic familial

TLE) which are also similar to sporadic TLE. It is worth noting that normal MRI and EEG findings do not exclude the diagnosis of fMTLE.

The differential diagnosis of fMTLE include lateral temporal lobe epilepsy, GEFS+, febrile seizures (FS), familial partial epilepsy with variable foci (FPEVF), and a type of partial epilepsy recently identified in a large Brazil family with several family members consistent with MTLE, which showed linkage to chromosome 4p15 (Kinton *et al.* 2002). These epilepsies can have seizures that have the characteristics of temporal lobe epilepsy.

Treatment

Most of the patients can achieve seizure remission either with adequate medical control or with surgical intervention. The majority of patients with fMTLE are usually well controlled by anti-epileptic drugs indicated for partial epilepsies based on individual responses and tolerability. For those with hippocampal sclerosis or atrophy and those who are refractory to medical treatment, surgery may be necessary. In a seizure outcome study of fMTLE, around 24% of patients were refractory to medical treatment (Kobayashi *et al.* 2001).

Table 1.1 Complex partial seizure of mesial temporal lobe origin (Shorvon, 2005)

		Duration	Descriptions of seizures
Complex partial seizures	Aura	Brief, usually a few seconds	Aura are simple partial seizures, can be in any form of the simple partial seizure in Table 1.2
	Absence	Seconds	Dreamy state. Arrest of the activities. Sometimes speech continues but may just be only repetitive vocalization. Absence could also be seen in extra-temporal lobe epilepsy
	Automatism	Can last for minutes	1. Oro-alimentary: such as lip smacking, chewing, and swallowing. 2. Gestural: such as fumbling, fidgeting, repetitive motor action, undressing, walking or running. 3. Automatism of temporal lobe origin is much less violent than those of frontal lobe origin. (often with contralateral spasm of tonic posturing in unilateral hippocampal seizures)

Table 1.2 Simple partial seizures and secondary generalized seizures of mesial temporal lobe origin (Engel and Williamson, 2007; Shorvon, 2005)

Seizures type	Duration	Description
Simple partial seizures	Brief, usually seconds	***Motor*****:** speech arrest, or aphasia (dominant lobe)
		Autonomic: epigastric raising sensation, flushing, palpitation, tachycardia, bradycardia, hyperventilation
		Somatosensory**:** tingling, paresthesia, pain
		Psychic: 1. Perceptual hallucination: could be visual, or olfactory 2. Dysmnestic: such as déjà vu, panoramic experience, jamais vu. 3. Cognitive impairment: such as change in reality, depersonalization, dreamy states or forced thinking. 4. Affection: fear, anger, distress, or pleasure.
Secondary generalized seizures	Minutes, usually less than 5 mins).	Generalized seizures, usually tonic clonic type. The aura is indicative of the partial onset.

1.1.2 Familial lateral temporal lobe epilepsy (fLTLE)

History

Familial lateral temporal lobe epilepsy is also known as familial TLE with auditory features or "Autosomal Dominant Partial Epilepsy with Auditory Features". It was first described by Ottman in a gene mapping study (Ottman, 1995), which identified the disease locus on chromosome 10q. Subsequent studies mapped the gene (Leucine-rich Glioma Inactivate 1, LGI1) in this locus either the same gene mutation or new mutations (Kalachikov *et al.* 2002; Rosanoff and Ottman, 2008; Striano *et al.* 2008a). Since its clinical manifestation is similar but different from mesial temporal lobe epilepsy, and the site of discharge can be located at lateral temporal lobe, it is now recognized as a well established epilepsy syndrome that is considered to be distinct from (familial) mesial temporal lobe epilepsy (Cendes *et al.* 2007).

Epidemiology

Accurate figures of the prevalence and incidence of both fMTLE and fLTLE are not available, but it is roughly estimated that fMTLE is more common than fLTLE (Cendes *et al.* 2007). There is currently no figure showing any preponderance in either gender or in any ethnic group for both types of familial TLE. The prognosis of fLTLE is quite benign. A detailed family history is necessary to confirm the diagnosis; the current estimated prevalence of familial LTLE may be underestimated.

Clinical presentation

The clinical presentations of both types of familial TLE are similar, but there are subtle clues to distinguish one from the other. The cardinal feature of fLTLE is the auditory aura which is comprised of roaring, buzzing, and distortions in sounds or words (Shorvon, 2005). This suggests that the discharge origin is located at superior or lateral temporal lobe, involving neocortex. Occasionally cephalic symptoms or visual hallucinations may occur in lateral temporal lobe epilepsy but are less common. Many of the manifestations of mesial temporal lobe epilepsy can also be seen in lateral temporal epilepsy, which may suggest anatomical overlap of the epileptogenic area or the discharge may rapidly spread from lateral temporal lobe to mesial temporal lobe. Symptoms suggestive of fLTLE that may help in differentiating it from fMTLE are summarized in Table 1.3.

Table 1.3 Symptoms suggestive of Familial Lateral Temporal Lobe Epilepsy (Shorvon, 2005)

Auditory	Roaring, buzzing, music like, radio or motor-like broadcasting sound, or distorted sounds of words, suggesting involvement of Herschel gyrus.
Cephalic aura	Headache, light headedness, non-vertigineous dizziness, pulling pressure of head (Although these are more common in frontal lobe seizures)(Palmini and Gloor, 1992)
Hallucination or illusion	More complex hallucinations than in occipital lobe epilepsy. These can be either visual or auditory, such as complex visual pattern, illusion of size, shape, and distance
Trigger factor	can be triggered by environmental sound or noise (although this feature is more common in seizures of central origin)

Diagnosis and differential diagnosis

The diagnosis of familial lateral TLE is largely history based. Often the EEG is normal, but posterior temporal epileptiform discharge can be seen. The MRI findings are usually normal, but recently there are studies indicating the volume of lateral temporal lobe in individuals with LGI1 mutations are larger than healthy controls, in particular with protrusion and small gyri (Kobayashi *et al.* 2003a; Tessa *et al.* 2007). In contrast to Familial MTLE, there is no hippocampal atrophy or sclerosis.

The differential diagnosis of fLTLE includes fMTLE, GEFS+, FS, and other types of partial epilepsies.

Treatment

The control of familial lateral TLE is usually quite satisfactory by low dose anti-epileptic drugs indicated for partial epilepsies, based on individual response. If seizures relapse after adequate control, usually these are induced by precipitating factors, such as sleep deprivation or alcohol. For those individuals that the gene mutations of LGI1 have been confirmed, it is advised to stay on medication rather than coming off even if seizure free for years.

1.2 Genetics of familial temporal lobe epilepsy

The genetics of familial temporal lobe epilepsy are little understood, especially in fMTLE. From most of the published family studies, the inheritance mode is usually autosomal dominant with incomplete penetrance for both types of familial temporal lobe epilepsies (Chabrol *et al.* 2007a; Gambardella *et al.* 2000; Hedera *et al.* 2007; Picard *et al.* 2000; Rosanoff and Ottman, 2008), but it is possible that other types of inheritance may be found in the future.

There are currently no genes identified for fMTLE, but several possible loci have been reported. In recent genetic studies of fLTLE, mutations in Leucine-rich Glioma Inactivated 1 gene (LGI1) have been identified both in familial and sporadic type of lateral temporal lobe epilepsy, but only half of such families identified had these particular gene mutations (Ayerdi-Izquierdo *et al.* 2006; Michelucci *et al.* 2007). This suggests that more than one susceptible gene or unidentified non-genetic factors are responsible in developing fLTLE.

1.2.1 Genetics of mesial temporal lobe epilepsy

The susceptibility genes in mesial temporal lobe epilepsy in human being have yet to be discovered. Several animal studies have indicated that mutations of potassium channel (KCN) may contribute to mesial temporal lobe epilepsy (Wenzel *et al.* 2007) but this has not been confirmed in human epilepsy.

1.2.1.1. Genetic association studies in MTLE

There are a few candidate genes based association studies, some have reported position associations but most did not identify any associations. Even in studies which positive associations have been reported, most findings have not been replicated in independent studies, which are mandatory in confirming the association.

1.2.1.1.1. Association studies with positive results

The polymorphism of the gene encoding Apolipoprotein ε 4 (APOE 4) has been suggested to have its impact on temporal lobe epilepsy, especially on memory and cognitive functions (Yeni *et al.* 2005). Whether this gene also has a causative role in MTLE or its existence merely influences the memory and cognitive function of MTLE patients has yet to be clarified (Kumar *et al.* 2006). A case control study and meta-analysis of mesial temporal lobe epilepsy with hippocampal sclerosis showed that the polymorphism of prodynorphin (an opioid peptide precursor) with the allele *L* is associated with mesial temporal lobe epilepsy with a $p\ value < 0.0001$, in comparison to another allele *H* (Kauffman *et al.* 2008a). In another meta-analysis of association studies in mesial temporal lobe epilepsy with hippocampal sclerosis, a positive association was shown with Interleukin 1 β polymorphism (IL -1β) with an odds ratio of 1.92 (95% CI 1.11-2.08), when comparing 2 genotypes (TT vs CT) of the IL-1β gene (Kauffman *et al.* 2008b). This might suggest that the variants of IL-1β gene could contribute to hippocampal sclerosis but more epidemiological evidence is needed to support this finding.

Other candidate genes based genetic association studies that reported positive findings include GABBR1 polymorphism G1465A (Gambardella *et al.* 2003b; Johnson, 2003), CHRNA4 polymorphism (Ser543Ser)-C/T (Chou *et al.* 2003), prion protein (PrP^C) gene

PRNP (Walz *et al.* 2003), and a 5-Htt polymorphism (Manna *et al.* 2007). These studies are summarized in Table 1.4. Only in the case of the IL1-β polymorphism 511C>T has replications been performed to confirm the association with mesial temporal lobe epilepsy. Furthermore, none of the genes (including IL1-β) identified through genetic association studies have been identified in genetic linkage studies of familial mesial temporal epilepsy.

1.2.1.1.2. Association studies with negative results

The majority of the candidate gene based association studies in temporal lobe epilepsy reported negative association results. Such studies are summarized in Table 1.5. Cavalleri et al (Cavalleri *et al.* 2005) investigated 752 patients with temporal lobe epilepsy. These patients were stratified according to the criteria used in several original candidate gene association studies which investigated interleukin 1- β, PDYN, GABBR1, PRNP, CHRNA4, and GABRG2 and showed associations with mesial temporal lobe epilepsy. The stratified population were genotyped accordingly (and in some of the patients might be genotyped more than twice for different candidate genes), the results were compared to previous studies which reported the associations. However, Cavalleri and colleagues failed to replicate any of the reported positive association results. Other candidate genes that had been investigated in other association studies of temporal lobe epilepsy with negative association results include 5-HTT polymorphisms, BDNF, and ATP1A2 (Table 1.5).

Table 1.4 Association studies with positive results in temporal lobe epilepsy

Gene (polymorphism)	Reference	TLE subtype Case and control Number	Allele frequency Case (%): Control(%)	Genotype frequency Case (%): Control (%)	
IL-1 β (511)	Kanemoto (Kanemoto *et al.* 2000)	TLE and FS with and without HS	N/A	HS(+) : control 1/1 9(18):31(27.7) 1/2 19(38):58(51.8) 2/2 22(44):23(20.5) [*P*=0.085 compared with control, 0.0017 with HS(-)]	HS(-) : control 1/1 13(24.5):31(27.7) 1/2 30(56.6):58(51.8) 2/2 10(18.9):23(20.5) (*P*=NS compared with control
	Kanemoto (Kanemoto *et al.* 2003)	TLE and FS with and without HS	N/A	HS(+) : control 1/1 12(18.2):44(27) 1/2 24(36.4):82(50.3) 2/2 30(45.5):37(22.7) (*P*=0.0028 compared with control)	HS(-) : control 1/1 16(24.5):44(25) 1/2 36(56.6):82(56.3) 2/2 12(18.9):37(18.7) (*P*=0.072 compared with control
PRNP(M129V)	Labate (Labate *et al.* 2007)	Non-lesional mild MTLE Case: 289 Control:272	M 382(66.1):391(71.9) V 196(33.9):153(28.1) (*P*=0.036)	MM 127(43.9):139(51.1) MV 128(44.3):113(41.5) VV 34(11.8):20(7.4) (*P*=0.101)	
PRNP residue 171	Walz (Walz *et al.* 2003)	MTLE with HS post lobectomy	N/A Case: 100 Control: 180	Asn/Ser 23(100):0(0) *(P<0.0001)*	
PDYN	Stögmann (Stögmann *et al* 2002)	Control: 404 Familial TLE: 86 sporadic TLE: 204	Control: Familial: Sporadic L 124(30.7): 43(50) :58(28.4) H 280 (69.3) : 43(50): 146(71.6) p=0.002	Control: Familial: Sporadic LL 18 (8.9): 10(23.3) :6(5.9) LH 88(43.6):23(53.5):46(45.1) HH 96(47.5):10(23.3):50(49) P=0.005	
GABBR1	Gambardella (Gambardella *et al.* 2003b)	Non-lesional TLE and TLE with HS, AD familial type was excluded	A 24(8.5):2(0.3) G 258(91.5): 742(99.7) *(P<0.0001)*	AA 0(0):0(0) AG 24(17):2(0.5) *(P<0.0001)* [OR:38.0, C.I:8.82-163.73] GG 117(83): 370(99.5)	
5-HTTVNTR	Manna (Manna *et al.* 2007)	TLE with and without HS	10 188(26.2):252(40.8) *(P=NS)* 12 364(73.9): 366(59.2) *(P=0.0107)*	10/10 38(5.2):58(18.8) *(P=0.0019)* 10/12 112(46.2):136(44.0) *(P=NS)* 12/12 126(48.6):115(37.2) *(P=NS)*	

Table 1.5 Association studies with negative results in temporal lobe epilepsy

Gene (polymorphism)	Reference	TLE subtype	Allele frequency Case No (%): Control No (%) *P value*	Genotype frequency Case No (%): Control No (%) *P value*
GABBR1 (rs29259)	Wang (Wang *et al.* 2008b)	MTLE	N/A	TT 244(76.73) : 220(69.84) CT 62 (19.50) 84 (26.67) CC 12 (3.77): 11(3.49) *(P=0.118)*
GABBR1 (rs29261)				TT 269(84.59): 264(83.81) CT 49(15.41): 47(14.92) CC 0(0): 4(1.27) *(P=0.604)*
GABBR2 (rs3780428)				GG 226(71.07): 215(68.25) GA 76(23.90): 90(28.57) AA 16(5.03): 10(3.17) *(P=0.908)*
GABBR2 (rs1999501)				CC 75(23.58): 98(31.11) CT 182(19.18): 156(49.52) TT 61(25.79): 61(36.83) *(P=0.191)*
GABBR2 (rs967932)				GG 82(25.79): 116(36.83) GA 164(51.57):136(43.17) AA 72(22.64): 63(20) *(P=0.018)*
GABBR2 (rs944688)				CC 279(87.74): 255(80.95) CT 32(10.06): 54(17.14) TT 7(2.20): 6(1.09) *(P=0.056)*

(continued)

Table 1.5 Association studies with negative results in temporal lobe epilepsy (continued)

Gene (polymorphism)	Reference	TLE subtype	Allele frequency Case No (%): Control No (%) *P value*		Genotype frequency Case No (%): Control No (%) *P value*	
GABBR1 (G1465A)	Stögmann (Stögmann *et al.* 2006)	TLE (middle European Descendent)	A 2(0.5): 0(0) G 374(99.5): 518(100) *(P=NS)*		AA 0(0):0(0) AG 0(0):2(1.1) GG 259(100): 186(98.9) *(P=NS)*	
	Salzman (Salzmann *et al.* 2005)	both lesional and non-lesional TLE	Non-lesional TLE A 2(0.5): 2(0.3) G 218(99.09):742(99.7) *(P=NS)*	Lesional TLE A 0(0):2(0.3) G 48(100)⊗742(99.7) *(P=NS*	non-lesional TLE:control AA 0(0):0(0) AG 2(1.82):0(0) GG 108(98.18): 145(100) *(P=NS)*	Lesional TLE : control AA 0(0):0(0) AG 0(0):0(0) GG 24(100): 145(100) *(P=NS)*
	Tan (Tan *et al.* 2005)	TLE with and without HS	A 1(0.2):1(0.3) G 467(99.8):327(99.7) *(P=0.64)*		AA 0(0):0(0) AG 1(0.4):1(0.6) GG 233(99.6):163(99.4) *(P=0.93)*	
	Ma (Ma *et al.* 2005)	TLE with FS	45%:87%, *(P=0.988)*		N/A	
	Wang (Wang *et al.* 2008b)	MTLE	A 0: 0 G 120 (100)		All GG in both case and control group	
	Cavalleri (Cavalleri *et al.* 2005)	Non-lesional TLE, include HS	N/A, *(P=0.68)*		AA 0(0):0(0) AG 2(1):8(1) GG 218(99): 1061(99) *(P=NS)*	

(continued)

Table 1.5 Association studies with negative results in temporal lobe epilepsy (continued)

Gene (polymorphism)	Reference	TLE subtype	Allele frequency Case No (%): Control No (%) *P value*	Genotype frequency Case No (%): Control No (%) *P value*	
PRNP (M129V)	Cavalleri (Cavalleri *et al.* 2005)	Refractory TLE with HS	N/A, *(P=1)*	Asn/Asn 109(100): 360(99) Asn/Ser 0(0): 1(2) Ser/Ser 0(0): 0(0)	
	Wang (Wang *et al.* 2008a)	Sporadic MTLE, AD familial epilepsy was excluded	M 634(99.06):1098(98.39) V 6(0.94):18(0.61) (*P*=0.24)	MM 314(98.12):540(96.77) MV 6(1.88):18(3.23) VV 0:0 (*P*=0.24)	
IL-1 β (511T)	Cavalleri (Cavalleri *et al.* 2005)		*N/A (P=0.927)*	GG 59(45): 161(44) GA 57(44): 162(45) AA 15(11): 41(11) *(P=0.988)*	
	Ozkara (Ozkara *et al.* 2006)	MTLE with HS Case:47 Control:99	N/A	1/1 16(34): 41(41.4) 1/2 21(44.6): 41(41.4) 2/2 10(21.2): 17 (17.1) (*P*=0.66)	
	Jin (Jin *et al.* 2003)	MTLE with and without HS (Chinese population)	HS(+):HS(-):control 1 64(48):48(53):114(50) 2 70(52):42(47):116(50)	HS(+) : control 1/1 16(24):26(23) 1/232(48):62(54) 2/2 19(28):27(23) (*P*=NS compared with control)	HS(-) : control 1/1 12(27):26(23) 1/2 23(53):62(54) 2/2 9(20):27(23) (*P*=NS compared with control)
	Buono (Buono *et al.* 2000)	MTLE with HS (European population)	1 70%: 66% 2 30%: 34% (numbers not available)	1/1 31:44 1/2 24:68 2/2 6:7 (*P=0.09*)	
	Heils (Heils *et al.* 2000)	MTLE with HS after surgery (German)	N/A	1/1 34(38):57(42) 1/2 42(49):60(45) 2/2 11(13):16(12) (*P=0.840*)	
	Tilgen (Tilgen *et al.* 2002)	TLE with antecedent FS	1 61(71): 163(65) 2 25(29): 89(35) (*P=0.35*)	1/1 22(52):52(41) 1/2 17(40):59(47) 2/2 4 (9):15(12) (*P=0.53*)	

(continued)

Table 1.5 Association studies with negative results in temporal lobe epilepsy (continued)

Gene (polymorphism)	Reference	TLE subtype	Allele frequency Case No (%): Control No (%) *P value*	Genotype frequency Case No (%): Control No (%) *P value*
APOE 4	Cavalleri (Cavalleri *et al.* 2005)	Refractory TLE	ε4 frequency 13% *(P=0.078)*	Haplotype counts ε3 230(80): 469(74) ε4 36(13):108(17) ε2 20(7): 57(9) *P=0.205*
	Gambardella (Gambardella *et al.* 1999, 2005)	TLE	ε2 8(6.5): 35(8) ε3 113(89.6): 382(86.8) ε4 5(3.5): 23(5.2) *P=NS*	ε2-2 0(0): 1(0.3) ε3-3 101(73.2): 227(76.4) ε2-3 13(9.5): 38(12.8) ε3-4 21(15.2): 27(9.2) ε2-4 2(1.4): 3(1) ε4-4 1(0.7): 1(0.3) *P=NS*
	*Blumcke (Blumcke *et al.* 1997)*Study done by electrophoresis,	MTLE with HS, TLE with other pathologies but HS as control	N/A	ε2-4 and ε3-4: 56.8% in HS patients and 40.5% in TLE with other pathologies
	Yeni (Yeni *et al.* 2005)	MTLE with HS	APOE 4(+): 8(17):10(16.1) APOE 4(-): 39(83): 52(83.9) *(P>0.05)*	N/A
	Briellmann (Briellmann *et al.* 2000)	TLE with HS	at least 1 ε4: 23% no ε4: 77% (associated with early onset habitual seizures but not a general factor of TLE)	Genotype frequency in patients with chronic TLE only ε2/ ε4 2% ε2/ ε2: 0% ε3/ ε4 19% ε2/ ε3: 19% ε4/ ε4 2% ε3/ ε3: 58%

(continued)

Table 1.5 Association studies with negative results in temporal lobe epilepsy (continued)

Gene (polymorphism)	Reference	TLE subtype	Allele frequency Case No (%): Control No (%) *P value*		Genotype frequency Case No (%): Control No (%) *P value*	
ATP1A2 (4 base pair insertion)	Buono (Buono *et al.* 2000)	MTLE with HS post temporal lobectomy	N/A 56 cases, 56 control		ATP1A2 (4 base pair insertion) 15(26.7): 16(28.5) *(P=0.9)*	
5-HTTLPR	Manna (Manna *et al.* 2007)	TLE with and without HS	L 300(51): 322(52.1) *(P=NS)* S 252(49): 296(47.9) *(P=NS)*		L/L 77(21.1): 90(29.1) L/S 146(60.2): 142(46.0) S/S 53(18.3): 77(24.9)	
BDNF (position 240)	Lohoff (Lohoff *et al.* 2005)	TLE	C 94.9%: 93.4% T 5.1%: 6.6% *(P=0.436)*		CC 90.4%: 86.7% CT 8.8%: 13.3% TT 0.7%: 0 *(P=0.247)*	
BDNF (Val66Met)			Val 83.8%: 80.7% Met 16.2%: 19.3% *(P=0.297)*		Val/Val 72.2%: 65.6% Val/Met 23.2%: 30.2% Met/Met 4.6%: 4.2% *(P=0.355)*	
PDYN	Gambardella (Gambardella *et al.* 2003a)	TLE(90% mild form) European ancestry	non-familial risk TLE L 58(25.2): 137(26.4) H 172(74.8):381(73.6) *(P=0.72)*	familial risk TLE L 39(32.5): 137(26.4) H 81(65.5): 381(73.6) *(P=0.18)*	non-familial risk TLE LL 9(7.8): 16(6.2) LH 40(34.8): 105(40.5) HH 66(57.4): 138(53.3) *(P=0.53)*	familial risk TLE LL 7(11.6): 16(6.2) LH 25(41.7): 105(40.5) HH 28(46.7): 138(53.3) *(P=0.29)*
	Cavalleri (Cavalleri *et al.* 2005)	TLE(90% mild form) European ancestry	N/A *(P=0.0429)*		non-familial risk TLE LL 8(17): 30(8) LH 22(47): 160(44) HH 17(36): 175(48) *(P=0.53)*	

1.2.1.2 Genetic Linkage study in fMTLE

Although the association studies of sporadic mesial temporal lobe epilepsy, suggests that the Interleukin-β gene might be the susceptibility gene, this has not been identified in any familial linkage studies, whether with or without hippocampal sclerosis. Even in the genetic linkage studies of familial MTLE, no single study has replicated the results of the others.

1.2.1.2.1Genetic Linkage analysis with positive findings

Baulac (Baulac *et al.* 2001b) suggested that the there could be more than one disease locus in familial TLE. Recently in 2 genetic linkage studies which reported a LOD score > 3 mapped 2 different new loci that linked to familial temporal lobe epilepsy independently, but no genes have been mapped so far in these two regions. Claes *et al* (Claes *et al.* 2004) in the linkage analysis of a family with familial TLE associated with FS mapped the disease locus to 12q22-q23.3 based on a 2 point LOD score of 6.9 and a multipoint LOD score of 7.8 at the same marker. No disease gene was found in this locus, and the result has not been replicated. In the familial linkage study of Hedera (Hedera *et al.* 2007), a LOD score of 4 was obtained at 4q13.2-q21.3. Two candidate genes were identified and sequenced even if no known ion channel genes exist in this region. The sodium bicarbonate co-transporter (*SLC4A*) gene is in the linked region and plays an important role in tissue excitability. The cyclin I (*CCNI*) gene is also in the region and plays a role in the cell migration which may contribute to subtle cortical abnormalities. These 2 genes were sequenced but no disease causing mutations were found. A whole genome wide scan was carried out in both linkage analysis studies, with an average 10 cM distance in Claes's study and an 8cM average distance in Hedera's study.

1.2.1.2.2 Genetic Linkage analysis with negative findings

Other recent linkage analyses reporting negative linkage findings include Santo's familial linkage study on several candidate loci (Santos *et al.* 2003), Strianos' linkage analysis of 15 Italian family studies (Striano *et al.* 2008b), and Maurer -Morelli's genetic linkage study of 2 families with fTLE (Maurer-Morelli *et al.* 2006). In Depondt's linkage analysis of a large Belgium family with familial TLE, the phenotyping in this study was not specific to mesial temporal lobe epilepsy but it seemed that there were no lateral temporal

epilepsy subjects included (Depondt *et al.* 2002). This study did not show positive disease linkage. In Picard's autosomal dominant partial epilepsy family series, 7 families with temporal lobe seizures were included however he did not specified whether these were fMTLE (Picard *et al.* 2000). His genetic analysis did not show positive linkage, either. Whole genome wide scan was not carried out in these genetic linkage studies, but only several candidate microsatellite markers were investigated.

1.2.2 Genetics of familial lateral temporal epilepsy

1.2.2.1 LGI1 gene and familial lateral TLE

In contrast to mesial temporal lobe epilepsy, mutations of a glioma suppressor gene, the Leucine–rich Glioma Inactivated 1 (LGI1) gene, have been found in lateral temporal lobe epilepsy, or "autosomal dominant partial epilepsy with auditory features" (ADPEAF) (Morante-Redolat *et al.* 2002; Staub *et al.* 2002). The details of these studies are presented in Table 1.4. Until 2008, twenty two different mutations causing fLTLE have been published (Brodtkorb *et al.* 2002; Chabrol *et al.* 2007b; Fertig *et al.* 2003; Hedera *et al.* 2004; Kalachikov *et al.* 2002; Kobayashi *et al.* 2003a; Kobayashi *et al.* 2004; Michelucci *et al.* 2000; Ottman, 1995; Ottman *et al.* 2004; Pizzuti *et al.* 2003; Poza *et al.* 1999; Striano *et al.* 2008a; Winawer *et al.* 2002). In these 22 mutations, nine provoke a truncation of the protein either by altering the reading frame of the mRNA due to the existence of insertions (1 mutation), or deletions causing a nonsense mutation (4 mutations), or by a single-base mutation affecting a consensus splice site (4 mutations) (Table 1.6). Another 13 mutations are amino acid exchanges found in different domains of the protein (Table 1.7), which either caused the function loss of the LGI1 protein, or decreases its secretion. However, polymorphisms in around 50% of families identified with fLTLE no such mutations of LGI1 were identified (Ayerdi-Izquierdo *et al.* 2006). This suggests that either there are other genetic mutations yet to be found or that there are nongenetic factors involved in familial lateral temporal epilepsy.

1.2.2.2 LGI1 Gene and sporadic lateral TLE

Sporadic (non-familial) cases with apparently idiopathic partial epilepsy with auditory features (IPEAF) have been described (Bisulli *et al.* 2004). These patients appear to be

clinically indistinguishable from ADPEAF cases with the only difference being that they lack a family history. Gene sequencing analysis of LGI1 exons in IPEAF patients have revealed two de novo LGI1 mutations (Bisulli *et al.* 2004; Michelucci *et al.* 2007); providing a link between familial and sporadic patients partial epilepsy with auditory features. The percentage of LGI1 mutations in sporadic lateral TLE is not yet known, one study investigated 16 patients with sporadic lateral TLE but did not find any mutations on LGI1(Flex *et al.* 2005). The value and the application of this gene in screening sporadic types of patients awaits more study.

Table 1.6 Truncation mutation of LGI1[adapted and modified from Gu with permission (Gu *et al.* 2005)]

Ref.	Mutation	Mean age of onset (range, years)	Aura (auditory, visual, aphasic)	Seizure types	Seizure precipitant	EEG	Severity/course
(Hedera *et al.* 2004; Kalachikov *et al.* 2002; Ottman, 1995; Winawer *et al.* 2002)	c329delC	13 (10–15)	4/7 Auditory	7/7 GTC 5/7 CP 3/7 SP	2/7 Sudden noise/voice 1/7 Flashing light	7/7 Normal	1 Status epilepticus Several poorly controlled
	IVS33C>A	17 (11–23)	6/9 Auditory 1/9 Aphasic	3/3 GTC	N/A	N/A	N/A
(Hedera *et al.* 2004; Kalachikov *et al.* 2002; Ottman, 1995; Winawer *et al.* 2002)	c611delC	10 (9–10)	4/4 Auditory	4/4 GTC	1/4 surprising noise	Mild unspecific slowing in one	N/A
(Michelucci *et al.* 2000; Morante-Redolat *et al.* 2002; Poza *et al.* 1999)	c758delC	24 (11–40)	4/11 Auditory 6/11 Visual	10/11 GTC 2/11 CP 8/11 SP	N/A	2/7 Epileptiform Left temporal-occipital 5/7 Normal	Long remissions under treatment
(Michelucci *et al.* 2003)	c758delC	15–20	10/11 Auditory	11/11 GTC	None	3/3 Normal	Controlled with treatment
(Berkovic *et al.* 2004; Fertig *et al.* 2003)	IVS72A>G	19 (10–35)	12/18 Auditory 6/18 Aphasic 3/18 Visual	14/18 GTC	N/A	N/A	"Benign"

GTC generalized tonic–clonic, CP complex partial, SP simple partial, R right, L left, N/A not reported (continued)

Table 1.6 Truncation mutation of LGI1(continued)

Ref.	Mutation	Mean age of onset (range, years)	Aura (auditory, visual, aphasic)	Seizure types	Seizure precipitant	EEG	Severity/course
(Hedera *et al.* 2004; Kalachikov *et al.* 2002; Ottman, 1995; Winawer *et al.* 2002)	c1050_1051delCA	17 (13–21)	2/3 Auditory 1/3 Visual	3/3 GTC	N/A	N/A	N/A
(Michelucci *et al.* 2000; Morante-Redolat *et al.* 2002; Poza *et al.* 1999)	c1420C>T	7 (6–8)	3/3 Auditory	3/3 GTC	N/A	3/3 Either normal or unspecific temporal L or R	Improvement with age
(Michelucci *et al.* 2003)	c1420C>T	15–23	2/3 Auditory	3/3 GTC 1/3 partial	Unspecific	3/3 Normal	Controlled with treatment
(Hedera *et al.* 2004; Kalachikov *et al.* 2002; Ottman, 1995; Winawer *et al.* 2002)	c1639insA	12 (8–19)	6/11 Auditory 2/11 Visual 1/11 Aphasic	11/11 GTC 7/11 CP 2/11 SP	1/11 Speech	8/11 Normal	7/11 Seizure free for >3 years
(Chabrol *et al.* 2007b)	c431 + 1G>A	19(11-24)	2/4Auditory 1/4 Aphasic	4/4 GTC 1/4 SP 1/4CP	Sound telephone	N/A	Adequate control

GTC generalized tonic–clonic, CP complex partial, SP simple partial, R right, L left, N/A not reported

Table 1.7 Amino acid mutation of LGI1 [adapted and modified from Gu with permission (Gu *et al.* 2005)]

Ref.	Mutation	Mean age of onset (range, years)	Aura (auditory, visual, aphasic)	Seizure types	Seizure precipitant	EEG	Severity/course
(Ottman *et al.* 2004)	C42R 1/2	11 (10–12)	2/2 Auditory 2/2 Visual 2/2 Aphasic	2/2 GTC 1/2 CP 2/2 SP	1/2 Sound	Unspecific L fronto-temporal 1/2 Normal	N/A
(Kobayashi *et al.* 2003b)		13 (8–19	2/3 Auditory 2/3 Visual	1/3 GTC 3/3 CP ? SP	N/A	N/A	Easily controlled, Improvement with age
(Gu *et al.* 2002) (Brodtkorb *et al.* 2002)	C46R	18 (4–42)	8/12 Aphasic 6/12 Auditory	12/12 GTC 11/12 SP	7/12 Speech	3/12 Epileptiform L temporal 1/12 Epileptiform side shifting 2/12 Unspecific L-sided	Improvement with age GTCs controlled 6/12 persistent auras
(Pizzuti *et al.* 2003)		13 (9–15)	3/4 Auditory 2/4 Aphasic	3/4 GTC	N/A	2/4 Epileptiform L temporal 1/4 Unspecific Bitemporal 1/4 Normal	3/4 persistent auras
(Ottman, 2005)	A110D	9 (3–13)	2/3 Auditory 1/3 Visual	3/3 GTC, 2/3 CP 2/3 Myoclonic, 1/3 Absence	None	2/3 Epileptiform Generalized 1/3 Epileptiform bilateral, R>L	N/A
(Winawer *et al.* 2002)	S145R	18 (11–35)	3/5 Auditory	5/5 GTC 2/5 CP 3/5 SP	1/5 Unexpected Sound 1/5 Flashing light	5/5 Normal	Mild in most cases

GTC generalized tonic–clonic, CP complex partial, SP simple partial, R right, L left, N/A not reported

(continued)

Table 1.7 Amino acid mutation of LGI1(continued)

Ref.	Mutation	Mean age of onset (range, years)	Aura (auditory, visual, aphasic)	Seizure types	Seizure precipitant	EEG	Severity/course
(Michelucci *et al.* 2003)	C200R	18–50	3/4 Auditory 1/4 Aphasic	4/4 GTC 3/4 Partial	3/4 Sudden noises	3/3 Mild unspecific temporal	Controlled with treatment
(Ottman, 2005)	I298T	15 (10–28)	5/6 Auditory 3/6 Aphasic	6/6 GTC 3/6 CP 3/6 SP	N/A	1/2 Epileptiform L temporal 1/2 Epileptiform generalized	N/A
(Kobayashi *et al.* 2004; Kobayashi *et al.* 2003b)	F318C	25 (8–42)	7/7 Auditory	7/7 GTC 2/7 CP	N/A	1/6 Epileptiform L temporal 5/6 Normal	Excellent response to anticonvulsant therapy
(Hedera *et al.* 2004; Kalachikov *et al.* 2002; Ottman, 1995; Winawer *et al.* 2002	E383A	18 (12–30)	2/3 Auditory	3/3 GTC 2/3 CP	N/A	1/3 Interictal: Epileptiform R posterior; ictal: onset L mid-anterior temporal	N/A
(Winawer *et al.* 2002)	V432E	12–19	0/3 Auditory 2/3 Aphasic	3/3 GTC 3/3 Partial	N/A	3/3 Mild unspecific temporal	Controlled with treatment

GTC generalized tonic–clonic, CP complex partial, SP simple partial, R right, L left, N/A not reported

(continued)

Table 1.7 Amino acid mutation of LGI1(continued)

Ref.	Mutation	Mean age of onset (range, years)	Aura (auditory, visual, aphasic)	Seizure types	Seizure precipitant	EEG	Severity/course
(Winawer *et al.* 2002)	S473L	23 (10–51)	6/8 Auditory 2/8 Visual	8/8 CP 6/8 GTC	N/A	N/A	Mild
(Michelucci *et al.* 2007)	A136T (c,406 C-->T)	25	1/1Auditory 1/1Aphasic	1/1CP 1/1GTC	Auditory stimili. Telephone	1/1 normal	Seizure free
(Chabrol *et al.* 2007b)	Leu232Pro	18(9-36)	1/7 Auditory	1/3 GTC 1/3 Partial 1/3 CP	Auditory stimili. telephone	N/A	Not controlled
(Striano *et al.* 2008a)	Ile122Lys (c365T>A)	23(14-26)	3/5 auditory 2/5 none	2/5 GTC 3/5 SP	1/4 stress sleep deprivation	2/5 normal 2/5 unremarkable	2/5 seizure free 2/5 continue SP

GTC generalized tonic–clonic, CP complex partial, SP simple partial, R right, L left, N/A not reported

1.2.2.3 Structural and functional studies of LGI1 Gene

The structure of LGI1 has been well studied (Staub *et al.* 2002). the protein contains a hydrophobic segment representing a putative transmembrane domain with the amino terminus located outside the cell. It also contains leucine-rich repeats with conserved cysteine-rich flanking sequences. This gene is predominantly expressed in neural tissues and its expression is reduced in low grade brain tumours and significantly reduced or absent in malignant gliomas (Gu *et al.* 2005), and is rearranged as a result of translocations in glioblastoma cell lines (Chernova *et al.* 1998).

The function of the gene LGI1 in relation to ion channel has been investigated (Schulte *et al.* 2006). In their study by proteomic analysis, it showed that LGI1 is tightly associated with Kv1.1-containing channel complexes when the complexes were affinity purified from rat brain. The LGI1-mediated modulation of A-type K^+ currents in hippocampal presynapses may provide a reasonable explanation for both epileptogenesis and inheritance in autosomal dominant lateral temporal lobe epilepsy (ADTLE, or fLTLE). This research showed that the LGI1 protein could be a subtype of Kv1.1 channel associated protein, and the mechanism underlies LGI1 product in causing fLTLE may be related to potassium channel dysfunction. Based on this study and the fact that potassium channel had been reported to cause epilepsy (Singh *et al.* 1998; Singh *et al.* 2003), Diani *et al* (Diani *et al.*) screened several potassium channel genes on patients with either sporadic or familial types of lateral TLE, including KCNA1, KCNA4, and KCNAB1 which encode potassium ion channel Kv1.1, Kv 1.4 and Kv β1, respectively, but no mutations were found.

Fukata (Fukata *et al.* 2006) demonstrated that a transmembrane protein ADAM22 serves as a receptor for LGI1 in rat brain. When mutated itself, ADAM22 may cause seizures by altering the regulation of the glutamate-AMPA neurotransmission. LGI1 may enhance AMPA receptor-mediated synaptic transmission in hippocampal slices but the mutated form of LGI1 fails to bind to ADAM22. Based on this study, Chabrol (Chabrol *et al.* 2007a) sequenced the ADAM22 receptor gene on several fLTLE families, and Diani (Diani *et al.*) sequenced the ADAM22 gene on both sporadic and familial LTLE patients. No disease-causing mutations were found in either study, although several synonymous and non-synonymous polymorphisms were identified.

1.2.2.4 Temporal lobe structural anomaly caused by LGI1

One report (Tessa *et al.* 2007) showed that in LGI1 mutated individuals, both with sporadic and familial LTLE, there were abnormalities of the left middle temporal gyrus. This brain imaging study was performed by using voxel-based analyses of T1-weighted, diffusion-tensor, and magnetization transfer (MT) images in 8 patients (3 women and 5 men, mean age 49 ± 13 years) with both fLTLE and LGI1 mutations and compared to 24 healthy control subjects (14 women and 10 men, mean age 43 ± 7 years). Another study showed that in one family, among those who underwent MRI investigation, 53% of those who had LGI1 mutations showed a temporal lobe anomaly, either malformation with enlargement of temporal lobe or a combination of temporal lobe enlargement and abnormal shape of fusiform gyrus, temporal lobe enlargement and parahippocampal gyrus malformation, or abnormal hippocampal shape. In contrast to fMTLE, none of them had hippocampal atrophy or sclerosis. Another remarkable finding was that all the temporal lobe anomalies found and reported in these fLTLE patients were located on the left side.

1.3 Febrile seizures and its genetics in temporal lobe epilepsy

Febrile seizures have long been noticed to have an important role in the causation of mesial temporal lobe epilepsy and probably hippocampal sclerosis. A history of antecedent febrile seizures is often found in those who developed hippocampal sclerosis and refractory seizures. Whether genes causing febrile seizures (which have been identified) may also be implicated in the causation of familial mesial temporal lobe epilepsy has not been fully clarified.

1.3.1 Definitions of febrile seizures, febrile seizures plus, and generalized epilepsy with febrile seizures plus (GEFS+)

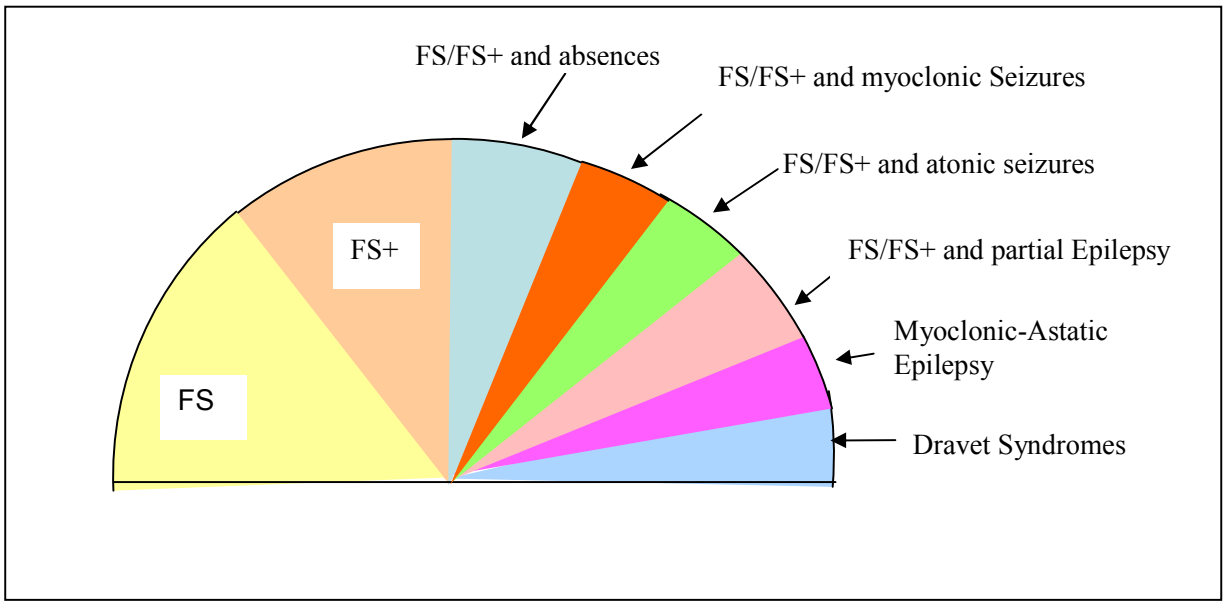
Febrile seizures (FS) are defined as convulsive seizures which occur with fever above 38°C between three months and 6 years of age at their broadest limits. The term febrile seizures plus (FS+) refers to a numbers of different presentations: febrile seizures which continue to take place either after the age of 6 or rarely, before three months old; and the situation where afebrile seizures occur with febrile seizures during the typical age range (within 3 moths to 6 years) (Scheffer and Berkovic, 2007). FS+ may occur with afebrile

generalized or partial seizures, such as absence, myoclonic seizure, atonic seizures or generalized tonic-clonic seizures.

Generalized epilepsy with febrile seizures plus (GEFS+) is defined as a "familial epilepsy syndrome", which is diagnosed when more than one individual within a family has a history of seizures that fit into the category of febrile seizures or febrile seizures plus. In other words, GEFS+ is a spectrum of phenotypes seen within a family ranging in severity from mild to severe seizure disorders. Recently, the spectrum of GEFS+ has been expanded to include febrile seizures only, FS+, FS/FS+ and absences, FS/FS+ and myoclonic seizures, FS/FS+ and atonic seizures, FS/FS+ and partial Epilepsy, Myoclonic-Astatic Epilepsy, and Dravet's Syndromes (Scheffer and Berkovic, 2007; Mulley *et al* 2005) (Figure.1). According to another review of 21 families with GEFS+ which the underlying genetics defects had been identified (Baulac *et al.* 2004), 42% of them had FS, 48% had FS+, and only 10 % of them had afebrile seizures. Generalized tonic-clonic seizures were the most common types of seizures, followed with absence, myoclonic seizure, atonic seizures and partial seizures.

Figure 1.1 Spectrum of GEFS+ Syndromes

[Adapted from (Mulley *et al* 2005) with permission]



1.3.2 The genetic basis of GEFS+

GEFS+ is a complex disease with polygenic involvement, although the discovery of its genetic basis was through linkage analysis of multiplex families with autosomal dominant inheritance (Scheffer and Berkovic, 2007). Current studies showed that GEFS+ are related to sodium channel and GABA receptor mutations: SCN1A and SCN1B (Colosimo *et al.* 2007; Wallace *et al.* 2002), and $GABA_A$ receptor γ 2 subunit gene (GABRG2) (Baulac *et al.* 2001a). The mutations in these susceptibility genes have been repeatedly found in many genetic studies of families with GEFS+ and the functional studies of these genes have demonstrated that they can contribute to epileptogenesis. More recently, mutations in the $GABA_A$ receptor δ subunit gene (GABRD2) (Lenzen *et al.* 2005) and calcium channel subunit gene (CACNA1H) have also been reported (Heron *et al.* 2007), but have not been replicated in other studies and await functional confirmation. A new locus on chromosome 8p23-p21 was recently identified in a few French families, but within this locus there are no ion channel genes and no susceptibility genes have been identified (Baulac *et al.* 2008).

Not all GEFS+ can be explained by the gene mutations described above. Bonanni (Bonanni *et al.* 2004) investigated 7 Italian families with GEFS+ pedigrees, but did not find any mutations of SCN1A, SCN1B and GABRG2. Sun et al (Sun *et al.* 2008) reported the combine frequency of SCN1A, SCN1B and GABRG2 in GEFS+ was only around 8.7% in a Chinese GEFS+ families study. Overall, the genes currently known for GEFS+ account for < 20% of large families studied (Meisler and Kearney, 2005; Wallace *et al.* 2001). These facts suggest that there are unknown susceptibility genes or as equally likely there are unknown environmental factors which may contribute to GEFS+.

1.3.3 Familial mesial temporal lobe epilepsy and febrile seizures

Epidemiological research has demonstrated that febrile seizures are associated with the development of temporal lobe epilepsy during teens or adulthood. Around 25% of temporal lobe epilepsy patients were found to have antecedent febrile convulsions (Hamati-Haddad and Abou Khalil, 1998). In refractory mesial temporal lobe epilepsy, the percentage with antecedent febrile convulsion during infancy can be as high as 50-80% (French *et al.* 1993; Sagar and Oxbury, 1987). In families with history of febrile seizure, or febrile seizures plus (GEFS+), it is common to find individuals with temporal lobe epilepsy, with or without

hippocampal sclerosis. It is also common to find individuals in families with familial mesial temporal lobe epilepsy who had history of febrile seizures (Claes *et al.* 2004; Kobayashi *et al.* 2003a; Striano *et al.* 2008b). In one recent report, SCN1A mutation was found in a family with febrile seizures, and three of the family members went on to develop temporal lobe epilepsy (Colosimo *et al.* 2007). In another study which investigated SCN1B gene mutations in 4 families, 5 individuals with temporal lobe epilepsy were found to have C121W mutation on SCN1B (Scheffer *et al.* 2007).

However, none of the genes identified in GEFS+ has been found in families with familial temporal lobe epilepsy. Furthermore, the extent to which these genes also contribute to temporal lobe epilepsy has not been fully investigated. Besides, many patients with temporal lobe epilepsy or hippocampal sclerosis do not have history of febrile seizures.

1.3.4 Genetic relationship between FS and TLE

1.3.4.1 Sporadic MTLE and FS

Association studies of sporadic temporal lobe epilepsy with hippocampal sclerosis, found that the homozygous state of Interleukin 1 –β gene to be more frequent with patients who had antecedent febrile seizures than those patients who did not (Kanemoto *et al.* 2000; Kanemoto *et al.* 2003). Another association study reported that the low-expression L-alleles of the prodynorphin gene (PDYN) promotor confer an increased risk for temporal lobe epilepsy in patients with a family history for febrile seizures, and irrespective of the familial background, L-homozygote display a higher risk for secondarily generalized seizures and status epilepticus as well as in the TLE patients with febrile seizure, especially those with more severe complex seizures (Stögmann *et al.* 2002). However, except for the IL1-β polymorphism which has shown weak association with sporadic temporal lobe epilepsy through meta-analysis, there has no replication of the PDYN gene polymorphism in TLE genetic studies.

1.3.4.2 Familial MTLE and FS

Subtle hippocampal abnormality could lead to febrile seizures and subsequent hippocampal sclerosis and temporal lobe epilepsy, as described in 2 pedigrees (Fernandez *et al.* 1998). Kobayashi (Fernandez *et al.* 1998) further confirmed that individuals in families with fMTLE who were not epileptic could have subtle hippocampal abnormalities. Such abnormalities might be caused by a combination of genetic predisposition and subsequent environmental influences, and could have contributed to febrile seizures in some individuals, leading to later development of temporal lobe epilepsy.

In contrast to GEFS+ in which several susceptibility genes were identified, the familial genetic studies of mesial temporal lobe seizures have not identified any susceptibility genes. Nonetheless, the fact that febrile seizures are closely related to mesial temporal lobe epilepsy may suggest that they share genetic mechanisms or perhaps interactions between susceptibility genes.

1.4 Susceptibility genes in other epilepsy syndromes

For other idiopathic generalized epilepsy syndromes, several genes have been identified. Most of them are ion channel genes or receptor genes. Several sodium channel genes have been frequently reported as the major susceptibility genes in IGEs, especially GEFS+. Only a few non–ion channel genes have been reported in epilepsy genetic studies, and so far not much is understood about how these non-ion channel genes contribute to the susceptibility of epilepsy. Table 1.8 is a brief summary of these genes, their possible mechanism, and their corresponding epilepsy syndromes.

Table 1.8 Susceptibility genes in other types of epilepsy (adapted form Avanzini *et al* 2007 with permission)

Human epilepsy	Affected gene	Affected current	Effect on the current	Main functional mechanism	Reference
BFNC	KCNQ2 KCNQ3	M-current	Loss of function	Decreased expression or modifications of gating kinetics that reduce K^+ M-current induced hyperpolarisation	(Biervert *et al.* 1998) (Castaldo *et al.* 2002)
Focal familial seizures and myokymia	KCNA1	Delayed rectifier	Loss of function	Decreased delayed rectifier K^+ current by various mechanisms	(Eunson *et al.* 2000)
Generalized epilepsy & paroxysmal dyskinesia	KCNMA1	IK_{Ca} (BK)	Gain of function	Enhanced Ca^{2+} sensitivity (cell hyperexcitability may be due to rapid action potential repolarization and enhanced recurrent firing)	(Du *et al.* 2005)
BFNIC	SCN2A	I_{Na}	Gain of function	Increase of current by various modifications of voltage dependence of gating	(Scalmani *et al.* 2006)
GEFS+ type 1	SCN1B	I_{Na}	Gain of function	Variable according to the expression system, often loss of modulation of I_{NaT} inactivation	(Wallace *et al.* 1998) ; (Meadows *et al.* 2002)

(continued)

Table 1.8 Susceptibility genes in other types of epilepsy (continued)

Human epilepsy	Affected gene	Affected current	Effect on the current	Main functional mechanism	Reference
GEFS+ type 2	SCN1A	I_{Na}	Gain or loss of function	Variable according to the mutation, expression system and cDNA used Slowed time course of I_{NaT} inactivation and faster recovery from inactivation	(Alekov *et al.* 2000)
				Decreased use dependent inactivation	(Spampanato *et al.* 2001)
				Enhanced I_{NaP} fraction and decreased fast inactivation of I_{NaT}	(Lossin *et al.* 2002)
				Hyperpolarizing shift of both I_{NaT} activation and inactivation causing a hyperpolarizing shift of window current Depolarizing shift of I_{NaT} activation	(Spampanato *et al.* 2003)
				Reduced current and enhanced recovery from slow I_{NaT} inactivation Complete loss of function (no current)	(Lossin *et al.* 2003)
				Depolarizing shift of I_{NaT} steady-state inactivation due to altered interaction with beta1 subunit	(Spampanato *et al.* 2004)
SMEI	SCN1A	I_{Na}	Gain or loss of function	No current	(Lossin *et al.* 2003)
				Enhanced I_{NaP} fraction, No current	(Rhodes *et al.* 2004)
ICE(GTC)	SCN1A	I_{Na}	Gain or loss of function	No current, Various effects on gating properties according to the mutation	(Rhodes *et al.* 2005)
FS	SCN1A	I_{Na}	Loss of function	Decreased current, positive shift voltage dependence of activation	(Rhodes *et al.* 2005)
Absence epilepsy and episodic ataxia	CACNA1A	I_{Ca} (P/Q)	Loss of function	Decreased P/Q Ca^{2+} current by reduced membrane targeting	(Imbrici *et al.* 2004)

(continued)

Table 1.8 Susceptibility genes in other types of epilepsy (continued)

Human epilepsy	Affected gene	Affected current	Effect on the current	Main functional mechanism	Reference
CAE	CACNA1H	I_{Ca} (T)	Gain or loss of function	Various effects on gating properties of T type Ca^{2+} channels	(Imbrici *et al.* 2004)
IGE and episodic ataxia	CACNB4	I_{Ca}	Gain of function	Decrease in the fast inactivation time-constant when coexpressed with alpha subunit	(Escayg *et al.* 2000)
IGE with absences and convulsions	CLCN2	I_{Cl}	Loss of function	Complete loss of function causing decreased transmembrane Cl^- gradient (and GABA inhibition)	(Haug *et al.* 2003)
				Changes in voltage-dependent gating (membrane depolarization?)	
GEFS+ type 3	GABRG2	I_{GABA}	Loss of function	Decreased current amplitude by reduced membrane targeting and receptor assembly	(Baulac *et al.* 2001a); (Hales *et al.* 2005; Kang and Macdonlad, 2004)
CAE & FS	GABRG2	I_{GABA}	Loss of function	Loss of benzodiazepine sensitivity	(Wallace *et al.* 2001)
JME	GABRA1	I_{GABA}	Loss of function	Reduced GABA sensitivity and altered channel gating	(Cossette *et al.* 2002; Fisher, 2004)
ADNFLE type 1	CHRNA4	nAChR	Loss of function	Various effects	(Bertrand *et al.* 2002) (Combi *et al.* 2004)
ADNFLE type 3	CHRNB2	nAChR	Gain of function	Slower desensitization	(De Fusco *et al.* 2000)
				Increase in acetylcholine sensitivity	(Phillips *et al.* 2001)

Section 2: Photosensitive Epilepsy and Its Genetics

2.0 Overview and terminology

The term photosensitive epilepsy commonly refers to epilepsies that can be evoked by flickering light. It is not usually considered to constitute an epilepsy syndrome on its own, because such phenomenon can be found on many well established epilepsy syndromes, such as Juvenile Myoclonic Epilepsy, and Childhood Absence Epilepsy (De Bittencourt, 2004). The term photosensitivity, strictly applied, refers exclusively to the electroencephalographic phenomenon of a photoparoxysmal response (PPR) on EEG recording.

Of all the photosensitive epilepsies, only one form of focal idiopathic photosensitive epilepsy, the Idiopathic Photosensitive Occipital Lobe Epilepsy (IPOLE) is currently classified as a unique entity under the category of reflex epilepsy according to the ILAE classification proposed in 2001 (http://www.ilae-epilepsy.org/Visitors/Centre/ctf/CTFtable1.cfm). Other photosensitive epilepsies are included within existing well-defined epilepsy syndromes, such as the Idiopathic Generalized Epilepsies, or as other visual sensitive epilepsies.

The use of terminology in photosensitive epilepsy can be confusing as there is yet no officially standardized terminology scheme. Different authors and researchers in the past were (and still are) using different terms to define the same phenomenon, or have different definitions. Examples are described in later sections (2.1.3). To avoid misunderstanding, there are a few terms that need to be clarified before considering photosensitive epilepsy. Some different terms are summarized in Table 2.1.

In this section, the ILAE classification is applied, and the term "photosensitive epilepsies" is used to include both IPOLE and other forms of visual sensitive epilepsies.

Table 2.1 Terminology of Photosensitive Epilepsy

Term	Definition
Photic induced-seizure (Visual sensitivity, or Visually induced seizures)	A seizure provoked by visual stimulation. The usual stimulus is a flashing light, but can be patterns of lines, gratings, checkerboards or other configurations. The seizures can be in any form by the majorities are generalized tonic-clonic epilepsy, Occurrence of Seizures provoked by visual stimili in daily life (Kasteleijn-Nolst Trenité, 2006) (Verrotti *et al.* 2002).
Photosensitive epilepsy	A reflex epilepsy that is evoked by flickering light, TV, video games, or moving water under sunshine. It does not constitute an epilepsy syndrome by its own.
Iiopathic Photosensitive Occipital Lobe Epilepsy	A typical disorder of adolescence, with female predominance characterized by occipital seizures provoked by visual stimuli (http://www.ilae-epilepsy.org/ctf/gloss_frame.html).
Photoparoxysmal response (PPR)	An abnormal EEG response to light or pattern, consisting of spikes, spike-waves, or intermittent slow waves. The spikes are not confined to occipital regions only and should not be confused with the normal visual evoked response that is phase-locked to the flash.
Photosensitivity	An abnormal response of the EEG to light or pattern stimulation, consisting of a PPR. Photosensitivity does not constitute an epileptic syndrome on its own, because it can be found in all the main categories of epileptic disorders.
Photomyoclonic response = Orbifrontal photomyoclonus response	1. Forehead and muscle twitching in response to light flash, disappearing with eye opening. 2. The intermittent photic stimulation (IPS) can trigger in adult patients and, in particular in elderly, rapid myoclonic jerking of the periorbital muscles, which produce eyelid fluttering and blinking, synchronous with the flashes (Kasteleijn-Nolst Trenite´ et al., 2001). This normal response to photic stimuli is called orbifrontal photomyoclonus (or *frontopolar, recruiting, photomyogenic responses)*

2.1 Photosensitive Epilepsies

2.1.1 History

The first reliable record and description of photosensitive epilepsy can be traced back in 1885 (Gowers, 1885), with a reference of a girl whose seizures was induced by walking

into bright sunshine, and a man whose aura and subsequent fit could be evoked by looking into bright sunlight. Subsequent records include absence seizures and eyelid myoclonus induced when looking into bright light with the effect of eye closure (Rodovic *et al.* 1932). Goodkind (Goodkind, 1936) later demonstrated a method that induced fits in a photosensitive patient by using sunlight coming through a wire window screen. The effect of intermittent photic stimulation (IPS) during EEG recording was described by Adrian and Matthew (Adrian and Matthew, 1934), but its clinical relevance in inducing seizures was not described until Cobb reported that flickering light can be a precipitant of seizures (Cobb, 1947). The application of IPS as an activating technique in EEG recording dates back to Grey Walter (Walter *et al.* 1946), who introduced the electronic stroboscope.

With the invention and availability of television during the 1950s, the first case of seizures induced by television was reported by Livingston (Livingston, 1952). Since then, such cases have been documented in many reports (Binnie and Wilkins, 1998; Jeavons and Harding, 1975; Zifkin and Kasteleijn-Nolst Trenité, 2000). The first case study of seizures induced by TV was reported by Gastaut and colleagues (Gastaut *et al.* 1962), who studied 35 patients with seizures induced by watching television. They distinguished patients who had seizures from those who had fainting episodes as well as those who had frequent seizures and likely to be coincidentally related to watching TV and not induced by watching TV. In the remainder, they believed that some relation existed between evoking seizures and watching TV. In late 1990, several TV programs that contained flashing light of certain frequencies induced seizures in children and some adults who watched those programs, such as Pocket Monster that was broadcast in Japan in 1996, and an advertisement shown on commercial TV in the United Kingdom (Harding and Harding, 1999). The latter led to an official guideline solicited by the *Independent Television Commission (ITC)* for advertisements and TV programs in the UK. Besides television, video games and flashing computer screens can also induce epilepsy (Fish *et al.* 2005).

After video games and computers became widely available, the flickering light coming from the screen and monitors has triggered seizures in cases with photosensitivity. The first case of such epileptic seizures was reported by Rushton (Rushton, 1981). Such cases were documented in may other subsequent reports (Graf *et al.* 1994; Kasteleijn-Nolst Trenité, 1994; Quirk *et al.* 1995).

Pattern sensitivity in photosensitive subjects was first reported by Bickford, who noticed that patterns such as stripes also induced seizures (Bickford *et al.* 1953). Such cases were documented in several reports but little is understood about their relation to photosensitivity. Radhakrishnan (Radhakrishnan *et al.* 2005) studied 73 patients diagnosed with pattern sensitive epilepsy, evaluated their EEG both with intermittent photic stimulation and different patterns. Their data showed that only 11% of patients did not have epileptiform discharge during IPS, but all of them had epileptiform discharge during pattern stimulation and those epileptiform discharges were similar. This suggests that pattern-induced epilepsy is closely related to photosensitive epilepsy and both may share similar pathogenesis.

2.1.2 Epidemiology

The Epidemiology of photosensitivity can be more thoroughly described in three different ways: the epidemiology of photosensitivity itself, its epidemiology in relation to epilepsy syndromes, and the epidemiology in the newly-defined IPOLE.

2.1.2.1 Epidemiology of Photosensitivity

The prevalence of photosensitivity in non-epileptic subjects ranges from 0.5 to 8.9% (Quirk *et al.* 1995; Takahashi *et al.* 2001; Verrotti *et al.* 2002). Doose and Gerken (Doose and Gerken, 1973) found PPR in 7.6% of 662 normal children, including those children with headache or had a family history of epilepsy. Several studies showed the visual sensitivity is age dependent and is highest in late childhood and early adolescence ((Jeavons and Harding, 1975; Kasteleijn-Nolst Trenité, 1989). Some authors (Shiraishi *et al.* 2001; Wolf and Goosses, 1986) reported a higher prevalence of PPR in the age group of 11–15 years and noted that the incidence suddenly decreased after the age of 20. The same age dependency was also found in studies in patients with common epilepsy syndromes, showing that an epileptiform response to IPS is found in about 10–20% of children and 5–10% of adults, and that response is more common in females at any age group compared to males, in a female to male ratio of approximately 2:1 (Kasteleijn-Nolst Trenité, 1998; Kasteleijn-Nolst Trenité, 2006).

Ethnic factors may be contributory to photosensitivity. In a study, Africans were shown to be more sensitive than Indians, and Europeans were the least sensitive (Familusi *et al.* 1998). This finding is in contrast with another research carried out approximately in the same time (De Graaf, 1992). In the latter, amongst 1493 epileptic patients in Namibia with PPR were found in only 0.4% of Africans, 4% of Indians, and 5.2% of Europeans subjects. These findings may indicate that visual sensitivity depends primarily on genetic rather than on environmental factors.

2.1.2.2 Epidemiology of Photosensitivity in different Epilepsy Syndromes

Photosensitivity can be found in patients presented with various epilepsy syndromes, but especially in Idiopathic Generalized Epilepsy (IGE). Wolf and Goosses (Wolf and Goosses, 1986) found that in the generalized epilepsies group, 15% of patients were photosensitive and in the localization- related epilepsies group, only 3% were photosensitive. Among the different epilepsy syndromes, the highest prevalence of a PPR was found in Juvenile Myoclonic Epilepsy (JME) (30%), followed by childhood absence epilepsy (CAE) (18%), and West and Lennox syndromes (17%). In Juvenile Absence Epilepsy (JAE) only, 8% showed PPR. Shiraishi et al. (Shiraishi *et al.* 2001) studied a similar type of cohort using the same criteria, among a total of 2187 unselected patients from a Japanese epilepsy centre (age range 1–81; mean 24.2 years; 56% male, 44% female), thirty seven patients (1.7%) were found to have a generalized PPR. Most of the PPR-positive patients were found among IGE patients (5.6%), compared to 0.7% in localization-related epilepsies. Within the IGE group, both JME (17.4%) and grand mal on awakening (7.6%) had a significant higher percentage than other types of IGE, while in the group of localization related epilepsies, occipital lobe epilepsy (6.1%) had the highest percentage.

Beside IGEs, PPR can also be found in progressive myoclonic epilepsy (Rubboli *et al.* 1999), and very rarely in mesial temporal lobe epilepsy (Fiore *et al.* 2003). PPR is found in other less common epilepsy syndromes, such as Neuronal Ceroid Lipofuscinoses (Berkovic *et al.* 1988), Lafora Disease (Tinuper *et al.* 1983), Unverricht Lundborg Disease (Guerrini and Genton, 2004; Kasteleijn-Nolst Trenité, 2001), and also mitochondria disease, such as

MERRF (So *et al.* 1989). The prevalence and incidence of PPR in each of these epilepsy syndromes is unknown.

2.1.2.3 Idiopathic photosensitive occipital lobe epilepsy (IPOLE)

The Idiopathic Photosensitive Occipital Lobe Epilepsy (IPOLE) is a type of reflex epilepsy that is exclusively triggered by visual stimuli with a peak incidence around puberty. The syndrome is now recognized as an independent entity within the reflex epilepsy category by the ILAE force task on classification and terminology in 2001**.** The diagnosis of this photosensitive epilepsy can be quite challenging, as its symptoms largely overlap with other epilepsy syndromes, especially Juvenile Myoclonic Epilepsies (Taylor *et al.* 2004), and also many other photosensitive epilepsies (Parra, 2006).

The epidemiology of this photosensitive epilepsy is much less understood. One study estimated that patients with idiopathic photosensitive occipital lobe epilepsy represented 0.4% of 2,447 consecutive epilepsy patients seen in 2 specialized centres (Guerrini *et al.* 1995). In these series, there was girl preponderance in a ratio of 4:1. Age- and sex-related trends overlap with those seen overall in photosensitive patients, with a peak at around puberty to adolescence.

2.1.3 Classification

In 2001, the ILAE has proposed a new diagnostic scheme for "people with epileptic seizures and epilepsy" (http://www.ilae-epilepsy.org/Visitors/Centre/ctf/CTFtable1.cfm) to replace the old classification. In this classification, visual sensitive epilepsies are categorized under Reflex Epilepsies, and within these visual sensitive epilepsies the "pure photosensitive reflex epilepsy" which is exclusively triggered by visual stimuli is isolated and is named as "Idiopathic Photosensitive Occipital Lobe Epilepsy" (IPOLE), which is now considered an independent syndrome, within the category of reflex epilepsy.

Before this classification was introduced, there were several different classifications on photosensitive epilepsies, either based on the clinical manifestations, or based on the IPS-induced PPR patterns on electroencephalography. Even after this new classification proposal was introduced, most researches have not yet adopted this classification and old terminologies are still in use.

2.1.3.1 Classification by clinical manifestation

Initially, Harding and Jeavons in their large case study of photosensitive epilepsy in Birmingham classified their patients into three separate classes, based on the severity of the patient's sensitivity to light: (1) "a clinically sensitive group in which light of the intensity encountered in daily life is capable of inducing clinical attacks"; (2) "a less sensitive group in which clinical seizures can be induced only under conditions of high intensity of illumination and rapid flicker" and (3) "a group in which the only evidence of sensitivity is the occurrence of epileptiform discharges on IPS, unaccompanied by any detectable clinical evidence of a seizure" (Kasteleijn-Nolst Trenité *et al.* 2001).

More recently Kasteleinjn et al (Harding and Jeavons, 1994a) proposed a relatively more comprehensive classification, based on the clinical presentations of visual sensitivity (Table 2.2).

Table 2.2 Classification by clinical symptoms (Kasteleijn-Nolst Trenité *et al.* 2001)

1. Mild subjective symptoms (SS)
2. Orbitofrontal photomyoclonus (OPM)
3. Eyelid myoclonus (EM) a. Eyelid myoclonus with absences (EMA) b. Self-inducing behavior (SI)
4. Focal, asymmetrical, myoclonus (FM)
5. Generalized myoclonus (GM) a. Without loss of consciousness, often isolated b. With impairment of consciousness
6. Tonic, versive phenomena (TVP)
7. Absence seizures (A)
8. Generalized tonic–clonic seizures (GTCS)
9. Partial seizures (PS) a. With simple visual symptoms b. With complex visual symptoms c. With limbic symptoms

2.1.3.2 Classifications by EEG findings

EEG findings have been applied to the classification of photosensitive epilepsy and are widely used. A widely used EEG classification system was proposed by Waltz and Doose, who classified the PPR into 4 different types based on the distribution of the slow waves and spikes induced by IPS (Waltz *et al.* 1992). This classification has been widely used as

the basis for phenotyping in the genetic study of photosensitivity and photosensitive epilepsies. This classification is summarized in Table 2.3

Table 2.3 PPR classification by Doose (Waltz *et al.* 1992)

Type	Description
1	Spike within the occipital rhythm,
2	Parietal-occipital spikes with biphasic slow waves
3	Parieto-occipital spikes with biphasic slow waves and spread to the frontal region
4	Generalized spike and waves or polyspikes

Another classification introduced by Jeavons and Harding was also based on the PPR on EEG (Harding and Jeavons, 1994a). In their classification, occipital spikes were not considered as a PPR response or epileptiform discharge as was classified in the Waltz and Doose classification. Their classification is summarised in Table 2.4 and Table 2.5.

Table 2.4 PPR classification by Jeavons and Harding (Harding and Jeavons, 1994a)

Classification 1: Photomyoclonic responses (PMRs)	Responses seen only in the anterior regions
Classification 2: Photoparoxysmal responses (PPR)	Widespread, involve anterior and posterior, bilateral response
Classification 3: Photic driving, (visual evoked potentials, occipital spikes)	Response seen only in the posterior region

Classification 1 Photomyoclonic responses (PMRs) were extremely rare. The PPR are confined to the anterior region only. Classification 2 (PPR) of Jeavons and Harding's described above were further divided into six types (Table 2.5). In contrast to the scheme of Doose classification, Photic driving was considered normal in the Jeavons and Harding's classification, included occipital spikes that did not persist or spread. Although occipital spikes may not be epileptiform and are considered normal in the Jeavons and Harding classification, they have been considered as a phenotype in some studies and could useful as biomarkers for genetic studies.

Table 2.5 Sub-classification of Classification 2 in Table 2.4 by Jeavons and Harding

Type	description
1	Spike-wave bursts, usually with a slow component around 3/sec
2	Theta spike-waves burst at 4-7 (theta) frequencies
3	Burst of polyspikes or polyspike-waves
4	Spikes coinciding with the flash rate, but extending widely to the anterior region
5	Spike-waves at 3Hz lasting at least 5 seconds after the flashing has ceased, and associated with a clinical absence
6	Bilateral, diffuse high-amplitude slow waves without spikes, seen in all channels

Besides the PPR classifications of Doose and Jeavons, Kasteleijn-Nolst Trenité et al (Kasteleijn-Nolst Trenité *et al.* 2001) have proposed another 2 classifications, based on the EEG response to IPS (Table 2.6) and the electro-clinical phenomena (Table 2.7). The classification based on the electro-clinical phenomena takes into account the different phenotypes which are often seen clinically, and has been used as the basis of phenotyping in several unpublished genetic studies. However, there is currently no evidence of which classification is superior to the others in clinical or genetic studies.

Table 2.6 Classification by EEG responses to IPS by Kasteleijn-Nolst Trenité

(Kasteleijn-Nolst Trenité *et al.* 2001)

1. Photic following a. At flash rate b. At harmonics
2. Orbitofrontal photomyoclonus (OPM)
3. Posterior-stimulus-dependent response
4. Posterior-stimulus-independent response a. Limited to the stimulus train b. Self-sustaining
5. Generalized photoparoxysmal response a. Limited to the stimulus train b. Self-sustaining
6. Activation of pre-existing epileptogenic area

Table 2.7 Classification of electro-clinical phenomena by Kasteleijn-Nolst Trenité

(Kasteleijn-Nolst Trenité *et al.* 2001)

1. Individuals with a photoparoxysmal response (PPR) in the EEG, and no history of epileptic seizures
2. Patients with spontaneous seizures, but with a PPR in the EEG
3. Patients with an isolated visually induced seizure in special circumstances, with or without a PPR in the EEG
4. Recurrent visually induced seizures and no spontaneous seizures, with or without a PPR in the EEG
5. Visually induced and spontaneous seizures, with or without a PPR in EEG

2.1.4 Assessment of Photosensitivity

Photosensitivity or PPR are usually assessed by EEG with IPS. Different facilities and protocols in applying photic stimulation may result in different responses, as some are more effective in triggering PPR and some are not. As discussed below, lack of standardisation in IPS frequency, strength of illumination, position of equipment (and so on) can result in significant variations in findings and in sensitivity. As more diagnostic tools become available, criteria for photosensitivity may change. Positron Emission Tomography (PET), visual evoked potential (VEP), magnetoencephalography (MEG), and other functional imaging such as fMRI have been recently applied to the assessment of photosensitivity and photosensitive epilepsies, and the importance of a universal standard protocol in assessing photosensitivity has been discussed (Kasteleijn-Nolst Trenité *et al.* 2001).

2.1.4.1 Assessment by IPS and EEG

Visual sensitivity (VS) can be assessed with different diagnostic procedures, but the most common method is intermittent photic stimulation (IPS). This procedure can provide information on seizure susceptibility of the individual exposed to intermittent lights, as well as the degree of photosensitivity and the relation between VS and epileptic syndromes (Rubboli *et al.* 2004). The effectiveness of IPS is indicated by the capability of inducing an abnormal EEG response in the highest number of patients in whom visual stimulation can potentially precipitate a seizure, but reducing to a minimum the chance of obtaining such responses in normal subjects (Rubboli *et al.* 2004; Zifkin and Kasteleijn-Nolst Trenité, 2000). This effectiveness depends largely on the methodology. Since in different

laboratories different protocols and photic stimulators are used, it is difficult to evaluate and compare the IPS effects across different laboratories. Therefore, a standardized protocol may be necessary to provide a fair evaluation and comparison of researches across different laboratories as well as to facilitate collaboration. In 1999, recommendations for a standardized protocol to perform and to report results of IPS were made in a European expert panel (Kasteleijn-Nolst Trenité, 1998). In this proposal, it was suggested that photostimulator, montage and the procedure of IPS during EEG could be standardized and at the same time to provide the best result in testing. A 13 cm circular Grass lamp is preferred as it has the best potency compared to other products. At least 16 channel montages are necessary to record sufficient data.

According to the standard protocol proposed in 1999 and later to some adjustments, the IPS should be performed in the EEG laboratory, preferably in an artificially dimmed environment and artificially lit at a constant level. The IPS must be done ≥ 3 min after hyperventilation with the patient placed 30 cm from the photic stimulator. This distance provides a sufficiently large visual field with the13-cm circular Grass lamp, and enables the examiner to detect subtle clinical phenomena. Ten-second trains of flashes are given for each frequency with intervals of at least 7 seconds between stimulus trains. Eyes should be kept open for the first 5 seconds of each train of flashes, fixing the center of the lamp (Rubboli *et al.* 2004). The patient should then close the eyes and remain in the eyes-closed condition for another 5 seconds of stimulation. Stimuli start at 1 and progresses to 20 flashes/s; recommended frequencies and their order of delivery are 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, and 20 Hz ((Zifkin and Kasteleijn-Nolst Trenité, 2000). Should a generalized epileptiform discharge occur, the stimulator must be switched off and the IPS procedure should be stopped? If PPR does not appear, a second sequence of stimulation is resumed at 60 Hz, decreasing to 20 Hz with the same precautions (60, 50, 40, 30, and 20 Hz). Total screening time should be 6 min or less. For clinical purposes, it may be important to report (1) if the EEG response can outlast the stimulus and, if yes, whether it is blocked by monocular occlusion; (2) whether clinical signs/symptoms are observed; (3) alertness and eventual sleep deprivation of the patient; and (4) antiepileptic drugs (AED) or other medicaments ((Rubboli *et al.* 2004).

Photosensitivity can be expressed at a range of frequencies which induce a PPR (Harding and Jeavons, 1994a); with lower and upper limits being usually between 10 and 30 Hz. Around 90% of patients are sensitive at 16 Hz, whereas 49% are sensitive at 50 Hz, only around 15% are sensitive to 60 Hz, which are also the frequencies of TV in Europe and North America, respectively (Harding and Harding, 1999). About 3% of the photosensitive population with some degenerative disorders is sensitive to IPS at 1–3 Hz (Zifkin and Kasteleijn-Nolst Trenité, 2000). Therefore, a flash rate ranging from 1 to 60 Hz during EEG recording should be able to detect almost all patients with photosensitivity.

It is worth mentioning that many different factors (individual factors and external factors) have a marked influence on photosensitivity. Therefore, it is incorrect to conclude that a patient is not sensitive to IPS from a single test session. Higher light intensity, lower background illumination, longer duration of photic stimulation, and grid pattern stimulation will enhance PPR (Harding and Jeavons, 1994a). Also the properties of the stimulator, how it is used, and the frequencies and intensity of the flashes influence photosensitivity. For example, the flashes must be sufficiently bright and the photostimulator must be able to deliver consistently bright flashes throughout the required frequency range of 1–60 flashes/s to have the best effectiveness (Kasteleijn-Nolst Trenité,, 1999).

2.1.4.2 Other diagnostic methods

Several new diagnostic tools have been tested to detect photosensitivity and visual sensitive epilepsies, both in diagnosis or in localization of the epileptogenic zone. Some tools have shown good results, but their validity and accuracy have not yet been fully verified.

MEG

Magnetoencephalography (MEG) has been used to assist EEG recording in picking up abnormal signals. A study combining EEG and MEG in detecting signal change on photosensitive patients has shown that MEG was able to detect the epileptiform discharges during IPS before they were shown on the EEG, that an enhancement could be seen on the phase clustering index at the gamma frequency band, compared with that at the driving frequency (Kalitzin *et al.* 2002). Another study used EEG, MEG and functional MRI

(fMRI) in evaluating the focally stimulated visual cortex to validate the use of MEG in assisting EEG (Sharon *et al.* 2007). In this study, the author concluded that combining MEG and EEG data is important for high-resolution spatiotemporal studies of the human brain and would improve the localization accuracy.

Visual evoked potential (VEP)

A recent study investigated whether VEP could predict if a subject could be photosensitive and whether it can be a non- provocative test for photosensitivity (Vermeulen *et al.* 2008). VEP was carried out in 17 patients who had photosensitivity but with different epilepsy syndromes (including 2 individuals whose photosensitivity had disappeared), 2 patients with epilepsy only without photosensitivity, and 3 controls. Based on their analysis and results, the authors concluded that VEP may be able to provide more information than just EEG with IPS, and it may be possible to predict whether a patient is likely to be photosensitive or not from the analysis of VEP. In the future, this may assist the diagnosis of photosensitivity and may provide more information about the physiology of photosensitivity (Wilkins *et al.* 2004).

Imaging studies

PET

Positron Emission Tomography (PET) has been tested on detecting photosensitivity on baboons in a case-control study (Akos Szabó *et al.* 2007). The PET was performed on both photosensitive baboons as well as non-photosensitive baboons. This preliminary study showed that PET can pick up signals in anterior cingulate gyrus and orbitofrontal cortex, especially at 25 Hz of IPS, but failed to pick up any significant signal change from the occipital cortex. Whether PET can be used in the study of photosensitivity and photosensitive epilepsies in human awaits further investigations.

SPECT

One study has used SPECT to detect the changes of regional cerebral blood flow (rCBF) when PPR was induced during IPS in photosensitive epilepsy patients. The results did not demonstrate any significant signal change in the occipital area, but did so in the

frontal cortex (Kapucu *et al.* 1996). Another 2 studies used SPECT to localize the epileptogenic zone on refractory occipital lobe epilepsy patients (Kim *et al.* 2001; Sturm *et al.* 2000), but both studies showed that SPECT was not helpful in picking up signals of PPR or in localization.

Functional Magnetic Resonance Imaging (fMRI)

fMRI has been used to assist the diagnosis and localization of epilepsy by detecting regional blood flow changes during ictal or post ictal period. However there are no reports yet specifically on visual sensitive epilepsies or IPOLE.

2.1.5 Clinical presentation of photosensitive epilepsies

Currently available information for IPOLE was largely adopted from Guerrini's studies and review (Guerrini *et al.* 1995; Guerrini and Genton, 2004) and cases study from Destina-Yalçin (Destina Yalçin *et al.* 2000). In other studies, although information regarding the clinical presentations in photosensitive epilepsies was provided, it is difficult to tell whether other visual sensitive epilepsies were included as the symptoms of these syndromes often overlap.

2.1.5.1 Idiopathic photosensitive occipital lobe epilepsy

Idiopathic photosensitive occipital lobe epilepsy is characterized clinically by partial seizures beginning around puberty, and which are exclusively triggered by visual stimuli. Television and video games are the most commonly reported triggers. Secondary generalized seizures are common, and these may be difficult to differentiate this from photosensitive IGEs (Guerrini *et al.* 1995). Other environmental stimuli have been reported less often, such as flickering or bright sunlight, sunlight reflected by water or other surfaces discotheque lighting, and computer screens. Precipitation by visual patterns is reported by some patients. Unlike photosensitive generalized epilepsy, there is no clear evidence for self-induction of seizures (Guerrini *et al.* 1995).

Visual phenomena or hallucinations are the initial ictal manifestation in most patients, which are usually described as bright and colourful, in the shapes of rings or spots that are fixed or flashing in the periphery of the visual field, rotating or moving slowly to the

opposite half-field, vomiting were common following the visual phenomena (Destina Yalçin *et al.* 2000). Following visual aura, head and eye deviation without lost of consciousness were often, either toward the ispilateral or contralateral side of the ictal discharge (Guerrini *et al.* 1995). If the seizures progress, some patients may have paroxysms of sharp or piercing cephalic pain during their seizures. Epigastric discomfort or nausea is reported in about half of the patients (Walker *et al.* 1995).

From the available EEG reports in studies and case reports (Destina Yalçin *et al.* 2000; Guerrini *et al.* 1995; Walker *et al.* 1995), it can be concluded that the majority of cases have normal background EEG, with unilateral or bilateral occipital spikes or spike and wave discharges which may or may not be reactive to eye opening.

2.1.5.2 Other visual sensitive epilepsies

For all other visual sensitive epilepsies (photosensitive epilepsies excluding the IPOLE), earlier studies revealed that the majority seizure types were tonic-clonic generalized seizures (84%), followed with absence seizures (6%), partial motor seizures (possibly asymmetric myoclonus in some cases) in 2.5%, and myoclonic seizures in 1.5% of patients (Jeavons and Harding, 1975). However, as this study was done before IPOLE was classified as a specific form of reflex epilepsy, and it is possible that several IPOLE cases were included in the analysis.

Because photosensitivity and visual sensitive seizures are frequently seen as part of the symptoms in epilepsy syndromes, such as IGE, some researchers classified this entity (visual sensitive epilepsies) according to the epilepsy syndromes that the photosensitivity is related to. These include epilepsy syndromes in the IGEs, progressive myoclonic epilepsies (PMEs), mitochondrial epilepsies, some focal epilepsies and undetermined epilepsies (Kasteleijn-Nolst Trenité *et al.* 2001).

2.1.6 Differential diagnosis

The main challenge in the differential diagnosis is to differentiate IPOLE from all other visual sensitive epilepsies. Based on currently available information, the comparison of IPOLE and other visual sensitive epilepsies are summarized in Table 2.8. To differentiate

visual sensitivity from other non-visual-sensitive epilepsies should be a less problem through careful history taking and if optimal EEG testing can be obtained.

2.1.7 Prognosis and treatment

2.1.7.1 Anti-epileptic drugs (AEDs) treatments

The prognosis of both visual sensitive epilepsies and IPOLE is in general quite good (Harding and Jeavons, 1994b). In the past, sodium valproate was frequently prescribed as a monotherapy, and favourable control were reported especially in cases with pure photosensitive epilepsy in Hardings' study (Harding and Jeavons, 1994c). A less favourable outcome was reported by Covanis et al (Covanis *et al.* 2004) in patients presented with eyelid myoclonia and absence.

Table 2.8 Comparison of IPOLE and other visual sensitive epilepsies

Epilepsy syndrome	IPOLE	Other visual sensitive epilepsies
Trigger factors	Mostly television and video games, less frequent from flickering lights or bright light	Flickering light, bright light on running rivers or water, shadows under bright sunshine, reflections from glasses, or patterns, less TV and video games
Aura	Visual hallucination or phenomena, in the shape of dots or ring, could be in multiple colour	depends on the type of epilepsy syndrome the visual sensitivity is involved
Clinical presentations	Partial epilepsies with frequent secondary generalization, conscious eye and head deviations are common. Myoclonus is not a feature. All seizures are exclusively induced by photic stimili	Mostly generalized seizures, less frequent partial seizures or absence. Seizures can be induced by photic stimili but not exclusively, other triggering factor may also contribute.
EEG findings	Normal back ground EEG, with spikes or spikes and wave discharge at unilateral or bilateral occipital area	Depends on the epilepsy syndrome detected with photosensitivity

The efficacy of Lamotrigine in the treatment of photosensitive epilepsies was evaluated by Binnie (Binnie *et al.* 1986) and Covanis (Covanis *et al.* 2004). Their preliminary data showed that lamotrigine abolished PPR in many patients and also improved seizures

control in most. All Binnie's subjects showed reduced photosensitivity and reduced seizures frequency. In Covanis report, 62.5% of his patients with photosensitive generalized tonic-clonic epilepsy and intolerant to the adverse effects of valproate became seizure free on treatment with lamotrigine monotherapy.

Benzodiazepines, such as clonazepam and clobazam, are also effective according to Harding's study (Harding and Jeavons, 1994c). Phenytoin and phenobarbital have been proved to have little or poor effect in reducing photosensitivity or to control the photosensitive epilepsies, and ethosuximide had only intermediate effect (Jeavons and Harding, 1975).

Some new generation AEDs such as Zonisamide, Levetiracetam, and Topiramate have been reported to have good effect on visual sensitive epilepsies (Covanis *et al.* 2004). Levetiracetam has been reported effective in suppressing both photosensitivity and myoclonic jerks. Some case reports noted that withdrawing levetiracetam resulted in increased photosensitivity and relapse of myoclonic seizures (Kasteleijn-Nolst Trenité *et al.* 1996). Other new or candidate AEDs, such as brivaracetam, a novel SV2A ligand, and carisbamate, a novel carbamate antiepileptic drug, have been studied in patients with photosensitive epilepsy and both showed good effect in controlling photosensitive seizures in these small series (Kasteleijn-Nolst Trenité *et al.* 1996; Kasteleijn-Nolst Trenité *et al.* 2007).

Some AEDs have been shown no or negative effect on photosensitive epilepsies, such as carbamazepine. Although carbamazepine may reduce PPR, it can exacerbate myoclonus in photosensitive epilepsies and so should be avoided in such patients (Genton, 2000).

Although there is a lack of large scale controlled long-term prospective studies, it is clear from the existing accumulative reports, that sodium valproate is the most effective AED in monotherapy of photosensitive epilepsies (Covanis *et al.* 2004; Harding and Jeavons, 1994c). It often abolishes both photosensitivity and seizures very effectively. Other new AEDs have promising effects, but more experience and evidence is needed before their role in this indication, relative to valproate and other conventional therapy, is fully established.

2.1.7.2 Non-AEDs treatment

Besides drug treatments, there are a few general protective rules that can effectively prevent evoking seizures in patients with photosensitive epilepsies. These are summarized in Table 2.8. Most of these are ways to avoid encountering flickering light, and adequate suppression of triggering factors when engaged in certain activities, such as watching TV, using computers or walking under the sun.

Table 2.9 Non-AED treatment of photosensitive epilepsy (Covanis *et al.* 2004; Verrotti *et al.* 2002)

1. Avoid relevant stimuli, such as discotheque lighting, striped clothing, and other striped patterns; sunlight on water; flickering sunlight; sudden changes in light and contrast; and flashing TV programs and video games
2. Use small TV, 12-inch set
3. Use 100-Hz, LCD, or thin film transistor (TFT) screens
4. Use remote control
5. Do not adjust the controls or fast-run the video
6. Avoid getting close to the screen: keep a distance of ≥3 times the width of the TV screen
7. Cover one eye if exposed to provoking stimuli
8. Wear spectacles: polarized lenses, dark glasses, or colored glasses (dark blue/green or precision tints)
9. Avoid stress, extreme fatigue, sleep deprivation

2.2 Genetic of photosensitive epilepsies

Since late 1940s (Walter *et al.* 1946), family and twin studies have clearly shown that there is a genetic predisposition for PPR. Case reports of monozygotic twins have shown high concordance between identical twins, and family studies have indicated a sibling recurrence risk of 20–30%, and this increased to 40% if one of the parents was also affected. This risk increased to almost 50% if a restriction of age was made to 5-15 years old in the study group (Doose and Waltz, 1993; Rabending and Klepel, 1970; Waltz and Stephani, 2000; Waltz, 1994). Several factors however make the mode of inheritance difficult to evaluate, such as the dependence of age, preponderance of female gender, and the relevance of different PPR patterns on EEG. However most have believed the heritability figures from the above reports suggest that the mode of inheritance is likely to be an autosomal dominant (AD) inheritance with age-related reduced penetrance (Davidson

and Watson, 1956; Herrlin, 1960; Waltz and Stephani, 2000). In spite of this, it has proved difficult to map the disease locus of photosensitivity.

Recently, several case reports, familial linkage studies, and genetic association studies have identified a few candidate loci, however, no susceptibility genes of photosensitivity or photosensitive epilepsy have been mapped within those loci.

2.2.1 Case report

A locus in chromosome 2 has been suggested to be a susceptible locus based on a single case report of a child showing refractory myoclonic photosensitive epilepsy and a complex chromosomal rearrangement (Van Esch *et al.* 2002). However, subsequent studies have not yet successfully replicated the results nor have any genes been identified from this locus.

2.2.2 Genetic linkage analysis studies

A candidate loci based, familial genetic linkage study of 37 PPR families failed to find any evidence for linkage to the dopamine receptors gene regions DRD1 to DRD5, located on loci EPM1 (21q22.3) and EPM2 (6q24); nor was there any evidence suggesting linkage with the idiopathic generalized epilepsy (IGE) loci previously reported, including 1p, 2q36, 3p14, 3q26, 4p, 6p11, 6p21.3, 8p11 and 15q14 (Neubauer *et al.* 2005). Another linkage study located a candidate locus at 6p21.2 in 19 families with PPR and photosensitive seizures, and at 13q31.3 in 25 PPR/IGE families (Tauer *et al.* 2005). However, no genes in those 2 regions have been mapped or proposed as candidate genes, nor have the results been replicated. Pinto et al (Pinto *et al.* 2005) reported another 2 newly found loci through linkage analysis in 16 PPR multiplex families with a prominent myoclonic seizure back ground, including their proband and first degree relatives. The two loci were 7q32 at D7S1804 (Doose classification PPR class 1, P=0.004) and 16p13 at D16S3395 (Doose classification PPR class 2, and P=0.01) (P represent genome wide significance). These data were re-analyzed in a two locus linkage analysis using 2 models, the multiplicative (MULT) epistasis model and the heterogeneity (HET) model. The multiplicative (MULT) epistasis model encompasses the situation where disease is only caused by a combination of alleles at both loci, and the heterogeneity (HET) model assumed that disease can result from the presence of alleles at either single locus. However, these two locus linkage

analyses did not substantially give greater evidence for linkage than did the single-locus analyses, nor did they improve the location estimates (Pinto *et al.* 2007).

2.2.3 Genetic association studies

Based on the finding of linkage analysis of Juvenile Myoclonic Epilepsy (JME), a genetic association study of the photoparoxysmal response was carried out focusing on the BDR2 gene and its polymorphisms in a German population (Lorenz *et al.* 2006b). The preliminary results showed that BDR2 could be an underlying susceptible gene for both JME and PPR (including Doose type 1-4), based on the comparison of 666 healthy control cases. Another candidate gene based association study, which included 218 JME and 95 photosensitive IGE patients, 78 PPR probands without IGE, and 662 healthy German population as controls, however failed to show any association evidence with the gene succinic semialdehyde dehydrogenase gene (ALDH5A1) in either IGE syndromes or visual sensitive epilepsies (Lorenz *et al.* 2006a).

2.2.4 Other genetic studies in photosensitive epilepsies

Grosso (Grosso *et al.* 2006) studied photosensitivity in children with epilepsy and chromosome anomalies. He found that 4 in 28 children with chromosome anomalies had photosensitivity that all of them had Waltz and Doose type 4 PPR. The chromosome anomalies were: one trisomy13, one with 45,XY, t(14–21) (q31.1–q22.11), one 46,X,t(10;X)(qter;p11.3)+del(X)(p11.3;qter), and one 46,XY,del(3)(p25-pter). However this study did not look at molecular genetic data and did not seek possible loci for photosensitivity. Taylor (Taylor *et al.* 2007) investigated the genetic aspects of the female preponderance in photosensitive epilepsies by studying the photo pigment gene on chromosome X. The comparison of the allele frequency made between 38 males with photosensitive epilepsies and 84 healthy male control cases did not support the hypothesis that this photo pigment gene is the main cause of the female preponderance.

A proposed susceptible gene of epilepsy, the NEDD4-2 (Neuronally Expressed Developmentally Down-regulated 4) gene was screened in 80 families with photosensitive epilepsies (Dibbens *et al.* 2007). They found that 4 (5%) out of these 80 families carried three different variants (S233L, E271A, and H515P) of this gene. This gene encodes an

ubiquitin protein ligase to regulate cell surface levels of several ion channels, receptors and transporters which are involved in regulating neuronal excitability, including voltage gated sodium channels (VGSCs). However, none proved to play a major role in causing photosensitive epilepsies, although the possibility of these variants causing interaction on an unidentified target protein was said not to be ruled out.

2.3 Inheritance of "photosensitivity only" without epilepsies

Some of the genetics of photosensitivity were investigated in families with one member presented with visual sensitive epilepsies, becuase it is impossible for an individual to realize that one is photosensitive without other accompany symptoms. One such study was done by Waltz and Stephani (Waltz and Stephani, 2000). They inspected the inheritance of photosensitivity by dividing families into two groups, group 1 were families with one photosensitive epileptic patient and a photosensitive parent, and group 2 were families with one photosensitive proband but neither of the parents were photosensitive. They concluded form the comparisons that PPR in parents is a major determinant for the risk of PPR in offspring, which is compatible with an autosomal dominant transmission. No molecular genetic study has been carried out on photosensitivity alone.

Section 3: Kohlschütter-Tönz Syndrome

3.1 History

The Kohlschütter-Tönz syndrome is a rare hereditary disease which is comprised of early onset seizures, abnormal enamel development and delay or deteriorations of the psychomotor and neuromotor development. This syndrome was first described by Kohlschütter in 1974 on 5 affected brothers in a farmer family from a small valley in central Switzerland (Kohlschütter *et al.* 1974). The seizures onset in these 5 brothers was between 11 months and 4 years of age. Their teeth were yellowish with hypoplasia of the enamel. Significant mental deterioration was noticed as they grew older and none of them survived into their teens possibly because they were severely handicapped and prone to complications, and adequate nursing was not available. In the following years, Kohlschütter observed another 2 Swiss families with similar clinical presentations. The seizure onset was between 11 month and 4 years of age in these 2 pedigrees. The neurological developmental were normal before the seizure onset, but mental deterioration and spasticity gradually developed after the onset of intractable seizures. No female patients were reported in the three pedigrees Kohlschütter reported, and he suspected that this syndrome might be an X linked disease or autosomal recessive disease (AR).

Later on, Christodoulou (Christodoulou *et al.* 1988) documented a Sicilian family which 4 male and 2 female family members were affected with early onset seizures, spasticity, mental retardation or deterioration after onset of intractable seizures with amelogenesis imperfecta (AI). The clinical presentations were similar to the kindred described by Kohlschütter. Besides the 6 affected individuals, there were another 4 maternal female relatives who had mental retardation of unknown cause. There was no consanguity in this pedigree. Several basic biochemistry and urine screen investigations were carried out on some of the affected family members and all the investigations were normal. EEG and CSF examination was carried out on one of the affected child, which showed excessive slow activities on EEG and slightly elevated lactate level and mildly elevated glycine and alanine level in CSF. Because both male and female were affected in

this pedigree and parents were normal, the inheritance pattern appeared autosomal recessive.

Zlotogora in 1993 (Zlotogora *et al.* 1993) reported a Druze family with 2 affected individuals. In this pedigree, the parents were unaffected and they had 4 children, only 2 younger children were affected. The parents were said to be first cousins. Both the affected children had intractable febrile seizures, spastic gait, increased deep tendon reflex, amelogenesis imperfecta with yellow teeth (amelogenesis imperfecta), and intellectual learning disability. The seizures onset was between 12 months and 3 years old. The EEG showed excessive slow activities and the basic laboratory investigations were all normal in these 2 subjects. In one of the affected individuals, the seizures were always febrile seizures. The inheritance mode of this pedigree was AR. Another German pedigree with similar clinical presentation was reports by Petermöller (Petermöller *et al.* 1993). In this pedigree, there were 2 affected individuals. Their clinical presentations were similar to the kindred reported before and included febrile seizures and later afebrile seizures with jerks mainly on the left side, psychomotor deterioration, and amelogenesis imperfecta with yellow teeth. The parents were unaffected unrelated healthy individuals. The inheritance pattern appeared to be AR.

A variant of Kohlschütter-Tönz syndrome was reported by Guazzi in a Sicilian family (Guazzi *et al.* 1994). In this family, the clinical presentations were different from the pedigrees described above. The neurological deficit occurred in the second decade, and seizure onset was not in early infancy or childhood, and in some individuals the seizures were well controlled. Muscle cramps and pain were the first symptoms in one of the affected individuals which started at the age of 10, with gradual psychomotor deterioration. Other symptoms in this individual were ataxia, yellow teeth, hyperactive deep tendon reflex, and degenerative muscular change of the calves in lower limbs. This pedigree was quite complicated as there were 4 different phenotypes and the parents were first cousins. Some of the individuals in this pedigree had amelogenesis imperfecta only, some of them had abnormal neurological symptoms only such as delay of psychomotor development or seizures only without amelogenesis imperfecta, and some of them had both but without signs of mental retardation. Although Guazzi concluded that the inheritance pattern of this pedigree was autosomal dominant (AD), it could also be an autosomal recessive pattern

depending on the phenotype defined. This pedigree was included in our linkage analysis and the discussion of the phenotype and its mode of inheritance is discussed in Section 10. Another variant of Kohlschütter-Tönz Syndrome was described by Musumeci in another Sicilian family (Musumeci *et al.* 1995). In this pedigree, in addition to seizures, abnormal enamel, and mental retardation which are frequently reported, these individuals also had broad thumbs, toes and enlargement of the lateral ventricles, cerebellum malformation. Similar to the pedigree reported by Guazzi, they also had ataxia and spasticity. There were consanguity in this pedigree and the mode of inheritance was AR.

More recently, an UK family with the diagnosis of Kohlschütter-Tönz Syndromes were documented by Donnai (Donnai *et al.* 2005). As better medical care became available, the cases reported by Donnai were able to live into their teens but with severe handicap. Besides seizures, mental retardation, and amelogenesis imperfecta, abnormal crystal sediments were found in the urine of these cases. The parents were healthy unrelated individuals in this pedigree and the mode of inheritance was AR. The latest case report was that documented by Haberlandt and appeared to be another variant of Kohlschütter-Tönz Syndrome (Haberlandt E *et al.* 2006). The neurological deficits in his case were milder than the previous cases reported, and the seizures in this case showed good response to medical treatment with phenobarbital and vigabatrin. His seizures onset was at 8 months and developmental delay was noted at the same times. There were no consanguity reported in this pedigree however parents were from neighbouring villages, and inbreeding was possible. Only one child in this pedigree was affected. MRI showed moderate ventricle enlargement and cerebellum hypoplasia. The mode of inheritance in this pedigree appeared to be AR, although it was not specified in the article.

Until now, according to Haberlandt, there were only 19 cases in 6 pedigrees that have been reported, including a patient described by Wygold (Wygold *et al.* 1996), who had progressive mental retardation, enamel defect of teeth, pathological activities on EEG, and cerebellar atrophy on MRI scan.

3.2 Clinical presentation

The major clinical presentation of Kohlschütter-Tönz Syndrome is early onset seizures, intractable seizures, mental retardation or deterioration, spasticity, ataxia, and amelogenesis

imperfecta. Cerebellum hypoplasia is frequent, and enlargement of lateral ventricles were seen in most of the affected subjects. The pregnancies are usually uneventful. Other minor symptoms reported include broad toes and thumbs, skull deformities, and muscle cramps. Because this syndrome is quite rare, the epidemiology and pathogenesis remain largely unknown and the differential diagnosis is quite limited. Treatment is limited to seizure control and supportive nursing care only.

3.3 Genetics of Kohlschütter-Tönz Syndrome

In Kohlschütter's report, he noticed that geographical isolation could accidentally contribute to the development of Kohlschütter-Tönz Syndrome, although no consanguinity was found. The other phenomenon he noticed was there were no female cases. He concluded that the inheritance mode could either be autosomal recessive or X-linked.

As more families were reported, it appeared that consanguinity existed in some of the affected families and most of the families exhibited autosomal recessive inheritance pattern, except the one reported by Guazzi, which was complicated by several different phenotypes. Donnai reviewed that 25 cases from 8 families have been reported (including 2 pedigrees with mainly amelogenesis imperfecta), and Haberlandt reviewed that till now 19 cases in 6 pedigrees have been documented. The genetics of this syndrome is largely unknown, except from the observations of the cases reported that the inheritance mode is likely to be autosomal recessive, and the fact that consanguinity and geographical isolation are two main factors contributing to this syndrome. No disease locus or susceptibility genes have been identified.

Section 4: Statistical aspects of linkage analysis

4.1. The beginning of genetic statistics

The first genetic statistic analysis was that of Gregor Johann Mendel, who calculated the probability of the pea offspring having different phenotypes in his peas-hybrid studies conducted during 1856 – 1863, in the garden of the Augustinian Convent in Brno. He discovered that in the pea offspring he bred, 25% had pure recessive alleles, 50% had hybrid alleles, and 25% had pure dominant allele. These findings led to the establishment of Mendel's laws. His research and publication was little appreciated at the time, and it was not until 3 decades later that the importance of his research was rediscovered by Hugo de Vries and Carl Correns in 1900 (Henig, 2000). Based on Mendel's discovery, William Bateson, a British geneticist whose research led to the discovery of genetic linkage with Reginald Punnett, first introduced the term "genetic" to describe the study of inheritance and science of variation (Harper, 2005).

4.2. Genetic linkage and recombination

Genetic linkage occurs when particular genetic loci or alleles for genes are inherited jointly, or co-segregate during meiosis. This means that the genetic loci on the same chromosome are physically connected and tend to segregate together during meiosis, thus they are genetically *linked* (Strachan and Read, 2004a). Alleles for genes on different chromosomes are usually not linked, because of independent assortment of chromosomes during meiosis. A segment of chromosome may crossover during meiosis when the chromosomes segregate, so alleles on the same chromosome may end up in different daughter chromosomes, and alleles originally located on different chromosomes may eventually segregate into the same daughter cell. The re-arrangement of these alleles during segregation at meiosis is called r**ecombination** (Strachan and Read, 2004b). The probability of recombination is greater if the alleles are far apart on the chromosome. Based on this concept, Sturtevant proposed that the relative distance between two genes can be calculated by working out the number of recombinants (Lorentz *et al.* 2002). This distance is defined as the **genetic map unit (m.u.). A centimorgan** (cM) is defined as the distance between genes for which one product of meiosis in 100 is recombinant (Tefferi *et al.*

2002). Based on the relative distance between 2 genes or loci, a genetic map can be drawn up. Examples are the Marshfield genetic map, and Decode genetic map.

Genetic linkage analysis can be used to identify regions of the genome that contain genes that predispose to disease. The application of genetic linkage has contributed to the identification of many disease genes in epilepsy syndromes (Table 1.8).

4.3. Modern genetic linkage statistics

The most widely used statistic in linkage analysis is the *logarithm of the odd score (*LOD score) of Newton Morton (Morton, 1955). It is defined as the logarithm of the likelihood ratio (odds score), and is a way of presenting the result of linkage analysis. Genetic linkage analysis can be roughly divided into parametric (model-based) and non-parametric (model-free) methods.

4.3.1 Parametric (model-based) analysis

4.3.1.1. History

Parametric linkage analysis was initially performed by direct observation and calculation. Valid scoring procedures were first applied to human linkage by Bernstein (Bernstein, 1931), who showed that each family can be assigned a score, and the sum, expected value, and variance of the score can provide a test of the null hypothesis. Bernstein's scores were further developed by Hogben (Hogben, 1934) and Haldane (Haldane, 1934). However the evolution by Fisher (Fisher, 1935) of a maximum likelihood scoring procedure (the u score) proved to be more efficient than Bernstein's scores for all linkage intensities. However, the u score has certain disadvantages. It is not feasible for pedigrees larger than 2 generations and when the parental genotypes are unknown (Smith, 1953). To overcome the disadvantages, Morton proposed the logarithm of odd score system (LOD scores), which is based on a sequential test to detect linkage (Morton, 1955).

4.3.1.2 Basic concept and application

Parametric or model-based linkage analysis is the analysis of the cosegregation of genetic loci in pedigrees. Loci that are close enough together on the same chromosome segregate together more often than do loci further apart or on different chromosomes. The

further apart the two loci are on the same chromosome; the more likely that a recombination event will occur during meiosis and this will break up the cosegregation.

In the situation where microsatellite markers are used for genotype, each genotype for one genetic marker or locus is made up of two alleles, one inherited from each parent. Based on the concept of segregation and recombination described above, if a disease locus is close enough to a microsatellite marker, it is likely that they will almost always segregate together. This tendency can be detected through linkage analysis, where ideally all affected individuals in a pedigree will exhibit a particular marker allele while the unaffected individuals will not have that marker allele. Many sets of linkage-mapping markers are available in which markers are regularly spaced across the genome. Usually the more the markers in a set, the shorter is the space in between 2 markers and the more informative is the marker.

Parametric linkage analysis is suitable for pedigrees with Mendelian inheritance. In the past two decades, many rare disease genes have been indentified by parametric linkage analysis. Defining the mode of inheritance (autosomal dominant, autosomal recessive, or X-linked) is a necessary pre-requisite in parametric analysis. Some estimation before the analysis might be necessary, such as the penetrance rate of the disease, and gene frequency. Because the mode of inheritance has to be specified before the analysis, it is also referred as model-based linkage analysis.

4.3.1.3 Heterogeneity (admixture) LOD method

The heterogeneity LOD method is a modified parametric linkage analysis. It allows locus heterogeneity to be assessed in the analysis. There have been a number of studies indicating that this modified parametric LOD-score models can be more powerful than "nonparametric" techniques (see below), as long as the mode of inheritance is at least *approximately* correct. The heterogeneity LOD scores (HLOD) are calculated under a simple dominant as well as a simple recessive model at a fixed intermediate penetrance (Greenberg *et al.* 1998; Hodge *et al.* 2002; Huang and Vieland, 2001; Vieland and Logue, 2002).

4.3.2 Non-parametric (model-free) approach

The ability of parametric linkage to detect linkage is significantly reduced in diseases where the modes of inheritance are not certain and for those pedigrees that do not exactly follow the Mendelian laws (Greenberg *et al.* 1998). For situations in which modes of inheritance cannot be defined, alternative methods of analyses have been developed. The simplest of such methods utilizes pairs of affected relatives, most commonly siblings, the so called Affected Sib Pair (ASP) analysis.

Currently, the main approach of current ASP analyses is the likelihood-ratio method of Risch (Risch, 1990). This concept was introduced as early as 1975 by Hill and Smith (Hill, 1975; Smith, 1975), who both introduced this method independently and with different mathematical methodologies. The basic concept of this method is "allele sharing", or identical by descent (IBD), which means to seek alleles shared by the affected individuals which are descended from the same founder in a pedigree. By testing whether the chance of alleles (haplotype) shared by the affected individuals are identical by descent is statistically greater than the expected values under the null hypothesis provides another way of inferring linkage. Because the mode of inheritance needs not to be specified for the analysis, it is referred as model-free linkage analysis or nonparametric linkage analysis (NPL).

4.3.2.1 Sib-pair (S_{pair}) NPL analysis

At any locus, according to the null hypothesis of no linkage, the number of alleles shared identical by decent (IBD) by a pair of siblings is none with probability 0.25, one with probability 0.5, or two with probability 0.25. If IBD sharing in the families is known, the observed proportions of pairs sharing no, one, and two alleles at a candidate locus can be compared with these expectations. Linkage would be suggested if the pairs of siblings, both of whom are affected by a disease, share significantly more alleles IBD than expected by chance. For pedigrees in which more relatives are affected, the sibling pair analysis can be extended to include more affected individuals, such as uncle-nephew pair, or two first cousins, and not necessarily confined to sibling pairs (Risch, 1990). The calculation of S_{pair} analysis has been implemented in several linkage analysis programs, such as GENEHUNTER (Kruglyak *et al.* 1996), and SimWalk2 (Sobel and Lange, 1996).

4.3.2.2 S_{all} NPL analysis

S_{pairs} measures the number of alleles shared IBD by a pair of affected relatives, whereas S_{all} captures information regarding the sharing between larger sets of affected relatives, thus can avoid the disadvantage of S_{pair} measures which is restricted to paired relatives only (Whittemore and Halpern, 1994). S_{all} calculates the observed IBD probability over all possible configurations and puts extra weight on families in which three or more affected individuals share the same allele. Therefore, for sibships with only two affected siblings, the two statistics are equal, but for S_{all}, sharply increasing weight is given to families as the number of affected individuals sharing a particular allele increases. The S_{all} statistic is best suited to analysis of dominant loci, since more affected individuals per family are likely to share the same allele with this type of inheritance (Nyholt, 2000).

4.3.3 Quantitative trait mapping

Both parametric and non-parametric linkage analysis were designed to detect single gene diseases. Non-parametric linkage analysis is less restricted in that a specific inheritance model is not necessary. However, for quantitative trait disease, the inheritance of a phenotypic characteristic varies in degree and can be attributed to the interactions between two or more genes and their environment. Therefore, neither parametric nor non-parametric analysis can be applied to map the disease genes in such diseases. Although quantitative trait locus mapping was designed to suit such circumstances, however, it is not as simple a way of detecting linkage when compared with parametric or NPL analysis.

The basic concept of quantitative trait mapping is the measured genotype approach. Supposed that a few microsatellite markers adjacent to candidate genes are presumed potential quantitative loci which may alter the gene expressions and may give rise to different phenotypes in a pedigree; for each marker, each of its genotypes is considered a class, and all of the members of the population with that genotype are considered an observation for that class. The mean value of the quantitative trait in individuals who have either specific alleles or specific genotypes are compared by either by ANOVA, regression model (Haseman and Elston, 1972), or variance component linkage method to assess the

degree of linkage. However, not all the disease models of quantitative traits are observable, and a segregation analysis is often necessary before carrying out such analysis.

4.3.3.1 Variance components linkage method

One such method to map quantitative trait is the Variance component analysis. There are another 2 methods available in quantitative trait mapping, which are the ANOVA method and the regression method. The idea of variance component analysis is to partition the trait variance into components that attribute to the genetic and environment factors, so the trait variance among a group can be analyzed via statistics (Goldgar, 1990; Schork and Guo, 1993).

The variance model can be easily expanded from a simple equation to a more complex one to include variable components, such as the effect of environmental factors on samples at the time collected, effects of individuals to each other by living together, or a component modelling a correlation among spouse. The genetic components of the model can also be expanded to incorporate dominance effect, so that gene-gene and gene-environment interaction can be taken into account (Almasy and Blangero, 2008). However, to set up the partition of variance components, certain degrees of estimation are needed. Although variance component analysis is complicated, programs such as SOLAR (Almasy and Blangero, 1998), or MERLIN (Abecasis *et al.* 2002) are available for carrying out the analysis.

4.3.4 Comparison between model-based and model-free linkage analysis

The best analysis to detect linkage in a pedigree depends on the characteristics of the pedigree (such as mode of inheritance) and the extent of the genetic effect underlying the disease. In reality, it is uncommon that an inheritance mode of a pedigree of a common disease to be discernable, which often makes NPL the favourable choice for genetic linkage analysis. However, there are exceptions. Because NPL is based on IBD, it often ignores existing information from relatives who are unaffected in a pedigree, which makes it less precise in placing the genes than parametric linkage analysis (Forabosco *et al.* 2005). Some researchers found that in some circumstances, using HLOD method based on the highest LOD-score from 2 simple model-based linkage analysis, one assuming a recessive mode

and one assuming a dominant mode of inheritance at a fixed intermediate penetrance, can be at least as powerful as NPL alone in identifying disease locus, and non-parametric analysis might equal to a parametric analysis using a simple recessive model in mapping complex disease or quantitative traits (Abreu *et al.* 1999; Hodge, 2005). However, NPL is often routinely used in linkage analysis as it has its advantages in assisting identifying the susceptibility genes in complex disease.

A parametric analysis is a powerful way of detecting a disease locus in Mendelian traits, if the inheritance and penetrance pattern can be defined correctly or nearly correctly. However the power of parametric linkage analysis in gene mapping is significantly compromised in detecting a disease locus in complex disease trait. It can be successful occasionally in finding a locus when a complex disease has the "Mendelian sub-form", in which a major disease gene is responsible for the major phenotype. Such examples include finding the breast cancer gene BRCA1. However such examples are rare. Besides, if a false disease inheritance mode is used for the analysis, it can easily result in false positive result. Other advantages of parametric analysis include the ability to accommodate complications derived from genetic heterogeneity (when the same clinical phenotype is caused by mutations in different independent genes), incomplete penetrance and presence of phenocopies (Morton, 1955) by alternating the parameters needed in the parametric analysis.

However, both influence of gene-gene and gene-environment interactions cannot be properly taken into account either in parametric or non-parametric analysis. A simple comparison of the parametric and nonparametric linkage analysis is presented in Table 4.1.

4.4 The LOD (logarithm of odd) scores

Logarithm of odd score (LOD score) is a standard way to present the results of a linkage analysis. Depending on the analysis methods used, different types of LOD score are generated and they are not equivalent to each other. The statistic threshold for declaring a positive linkage can differs from one method to another. Careful interpretation of the LOD scores is necessary to avoid false claim or rejection of linkage results (Nyholt, 2000).

Table 4.1 Comparisons of parametric and non-parametric linkage analysis

	Parametric analysis	Nonparametric analysis
Power in locus mapping	Robust and powerful if the inheritance mode can be correctly observed	Conservative in Mendelian traits, but may relatively superior in complex trait compared to model-based analysis
Mode of inheritance	Need to be specified	Model free
Application	Preferably Mendelian pedigrees, or its sub-form	Complex trait disease/ non Mendelian inheritance where a dominant gene inheritance is likely
Limitations	1. Effect of gene-gene and gene-environment interaction cannot be analyzed. 2. Unsuccessful in polygenic disease.	1. Ignores genotype information of unaffected individuals, which makes it conservative in finding disease linkage. 2. Effect of gene-gene and gene-environment interaction cannot be analyzed.

4.4.1 Maximum LOD Score

The maximum LOD score (MLOD, or LOD/MLS) is an extended application of the LOD scores method of Morton. It has been implemented in the genetic analysis programs LINKAGE which was further modified and upgraded in the latest package of FASTLINK. The calculation of Logarithm of Odd (LOD) is defined as the decimal logarithm of the likelihood ratio, and the likelihood ratio is defined as the ratio of 2 recombination fractions:

$$\frac{L(\theta_1)}{L(\theta_2)}$$

Where the likelihood of recombination = $L(\theta) = \frac{1}{2}[(1-\theta)^n + \theta^n]$

θ = recombination fraction

= observed recombination event / total recombination event,

n = the number of non-recombinants of meiosis.

Thus the LOD score can be presented as

$$Z(\theta) = \log_{10}[\frac{L(\theta)}{L(1/2)}] = \log_{10}[\frac{\frac{1}{2}[(1-\theta)^n + \theta^n]}{L(1/2)}]$$ (Terwilliger and Ott, 1994).

i.e the logarithm of the likelihood ratio when $\theta_2 = ½$ (which denotes of no linkage).

The maximum LOD score is then obtained by alternating the value of θ (from 0 to 0.5). Traditionally, a LOD score of 3 signifies a linkage while a score of -2 rejects the hypothesis of a linkage. If the value falls between -2 and 3.0, it is considered inconclusive. However, as Lander and Kruglyak (Lander and Kruglyak, 1995) pointed out, these threshold should be adjusted in the genome wide level as genotyping is usually done by a genome wide scan nowadays, and the point wise *p* value should be quoted with the corresponding LOD score to assist the interpretation. For converting the maximum LOD score to its corresponding *P*-value, times the LOD score with 4.6, the product is equal to the Chi square value when the degree of freedom is 1 (LOD score x 4.6= Chi Square value). The P values can then be looked up from the Chi square table (Ott, 1999b).

4.4.2 Heterogeneity (Admixture) LOD score (HLOD, or $MMLS_{het}$)

The heterogeneity LOD score (or Admixture) method is a modified parametric linkage analysis. It allows any heterogeneity of the locus to be taken into account during analysis. When linkage analysis is carried out allowing for more than one disease locus, the LOD score is maximized with respect to two parameters, α (= the proportions of families linked to this locus) and θ (recombination fraction). Because the LOD score obtained by this way is a mixture of X^2 distributions with 1 and 2 degrees of freedom (df), resulting from the introduction of an additional free (heterogeneity) parameter, this will increase the probability of a type I error, so the threshold for declaring positive linkage is modified by adding 0.3 (=3.3) to the traditional LOD score threshold (Hodge *et al.* 2002). The HLOD can be presented as:

$$HLOD(\alpha,\theta) = Log_{10}[\frac{L(\alpha,\theta)}{L(\alpha=1,\theta=1/2)}]$$ (Ott, 1999a; Vieland and Logue, 2002)

α = the proportions of families linked to this locus. The maximum HLOD score is then decided by alternating θ, when α is calculated and given a fixed value.

4.4.3 NPL LOD scores

4.4.3.1 LOD S_{pairs} (NPL_{pair})

The NPL LOD scores are LOD scores obtained from NPL analysis, which includes the nonparametric *sib pair* linkage analysis LOD scores (LOD S_{pairs}) and the S_{all} LOD scores. Both calculations has been implemented in GENEHUNTER (Kruglyak *et al.* 1996). The S_{pairs} calculation can be presented as followed:

$$LR(Z_0, Z_1) = 2\log_{\varepsilon} \frac{L(Z_0, Z_1)}{L(Z_0 = 1/4, Z_1 = 1/2)}$$ (Teare and Barrett, 2005)

Where Z_0 = the likelihood of IBD sharing 0 allele, and Z_1 = the likelihood of IBD sharing 1 allele. The maximum LOD score (S_{pairs}) is found by maximizing the ratio with respect to Z_0 and Z_1.

Holmans (Holman and Clayton, 1995) and Faraway (Faraway, 1993) independently showed that maximization of the likelihood ratio in this way (S_{pairs}) could result in parameter values that are not biologically plausible. Therefore, Holmans and Faraway suggested that maximization be restricted to the set of sharing probabilities consistent with these possible genetic models; denoted as the "possible triangle" (*PT*). The set is defined by $Z_1 \leq 0.5$ and $Z_0 \leq 0.5 \times Z_1$. Such NPL LOD scores are shown as NPL MLS_{PT}.

4.4.3.2 LOD S_{all} (NPL_{all})

In contrast to LOD S_{pair}, the LOD S_{all} score takes into account all the affected individuals in a pedigree and put extra weight on pedigrees with three or more affected individuals sharing the same allele IBD. The LOD S_{all} score equation of Kruglyak (Kruglyak *et al.* 1996) is presented as:

$$Z = \frac{\sum_{i=1}^{m} r_i \overline{Z}_i}{\sqrt{\sum_{i=1}^{m} r_i^2}}$$

Where *m* is the number of pedigrees, *Zi* denotes the normalized score for the *i*th pedigree, and the *r*, are weighting factors.

Kong and Cox (Kong and Cox, 1997) consider that the NPL S_{all} LOD scores of Kruglyak might be too conservative to detect linkage. They proposed that by maximization of a single parameter (δ) in the numerator on the basis of observed genotype data can avoid such disadvantage. The δ represents the degree of allele sharing in a pedigree, where, under the null hypothesis of no linkage, $\delta = 0$; where $\delta > 0$ corresponds to the alternative hypothesis of excess sharing. Therefore, these nonparametric LOD scores are asymptotically distributed in the same manner as the standard LOD score of Morton. The Kong and Cox LOD score method has been implemented in the program MERLIN.

4.5 Interpretation of the LOD scores

All LOD scores are not created equal when different methods are used. Moreover, as whole genome assessment becomes available either by the use of sets of microsatellite markers or by different single nucleotide polymorphism (SNPs) platforms, the standard for declaring linkage has to be modified. Furthermore, it is suggested that the point wise *p* value is quoted with LOD scores in the interpretation (Lander and Kruglyak, 1995). In general, based on the LOD scores obtained, the result of linkage analysis is interpreted as suggestive of linkage, significant linkage, and highly significant linkage. Table 4.2 and Table 4.3 are summaries of the interpretations of several different LOD scores obtained by different linkage analysis methods.

Table 4.2

Interpretation of different LOD scores and its corresponding *P* values

adapted from (Lander and Kruglyak, 1995; Nyholt, 2000) with permission

p value	LOD/MLS	(NPL) MLS_{PT}	$MMLS_{het}$	Interpretation
$p < 0.05$	0.59	0.74	1.09	region of potentially interest
$p < 0.01$	1.18	1.38	1.71	region of potentially interest
$p < 0.005$	1.44	1.66	1.99	region of potentially interest
$p < 0.001$	2.07	2.32	2.63	suggestive of linkage
$p < 7.4 \times 10^{-4}$ (a)	2.10	2.45	2.75	suggestive of linkage
$p < 2.2 \times 10^{-5}$ (b)	3.63	3.93	4.20	significant linkage
$p < 3 \times 10^{-7}$ occurred	5.3	5.76	5.99	highly significant linkage

a: Suggestive linkage threshold when *allele sharing methods in sib pairs* are used

b: Significant linkage threshold when *allele-sharing methods in sib pair* are used

c: Highly significant threshold when *allele-sharing methods in sib pair* are used

MLS: maximum LOD score of Morton (traditional)

NPL MLS_{PT}: NPL LOD scores, using possible triangle of Holmans.

$MMLS_{het}$: maximized-maximum LOD score, same as heterogeneity LOD scores (HLOD)

Table 4.3 Modified interpretation of LOD of Morton's based on *genome wide level*

(Lander and Kruglyak, 1995; Nyholt, 2000)

***P* value**	**LOD**	**Interpretation**
$p = 0.05$	0.5875	Region of potential interest in *human parametric linkage analysis*
$p = 1.7 \times 10^{-3}$	1.86	suggestive of linkage in *human parametric linkage analysis*
$p = 4.9 \times 10^{-5}$	3.3	confirmed of linkage in *human parametric linkage analysis*

4.6 Common Software for linkage analysis

Genetic linkage analysis involves massive amount of data and complicated statistical calculations. The best way in analyzing such data is to implement the algorithm and calculation protocol from a computing program. Some of the commonly used analytic

programs are LINKAGE, GENEHUNTER, Simwalk2 and MERLIN. Their major functions, platform of operation system, advantages and disadvantages are summarized in Table 4.4.

Many programs cover more than one analysis methods. For example, SimWalk2 provides parametric and Non-parametric linkage analysis as well as MERLIN. However, the algorithm implemented in each program varies, and the ability to handle the size of pedigree also differs in each program. It is advised that programs for linkage analysis are selected according to the characteristic of the pedigree being investigated, its inheritance pattern of the disease, and the genotyped method. If the pedigree is genotyped via SNPs, many conventional analytic programs will not be able to handle such massive amount of data.

Table 4.4 Common linkage analysis programs (Almasy and Blangero, 2008; Almasy and Warren, 2005)

Program	Linkage Methods	Platform	Advantage	Disadvantage	Reference
LINKAGE (FASTLINK)	Parametric	Microsoft Dos, UNIX	1. Can handle large size and large number of pedigrees. 2. Robust in detecting linkage in Mendelian trait (or rare disease, rare variant).	1. Limited numbers of (14) markers per analysis, could be time consuming if there is consanguity in the pedigree. 2. Not suitable for quantitative trait disease or common complex disease. 3. Time consuming in multipoint analysis	(Lathrop *et al.* 1984; Terwilliger and Ott, 1994)
GENEHUNTER	Parametric, Non-parametric, Variance component	UNIX, Mac OS X	1. Faster speed in analyzing compared to FASTLINK 2. Both parametric and NPL analysis programs are available 3. Handles large number of markers per analysis	1. Not suitable for large size pedigrees.	(Kruglyak *et al.* 1996)
MERLIN	Parametric, Non-parametric, Variance component	UNIX, Microsoft	1. Able to handle data obtained by SNPs in both linkage and association analysis 2. Good error detection in pedigrees 3. Good graph generation in output files.	1. Not suitable for large pedigrees. 2. May be complicated in preparing input files. 3. Losses information when trimming off individuals in the pedigrees to suit MERLIN	(Abecasis *et al.* 2002)
SIMWALK2	Parametric, Non-parametric, Variance component	UNIX, Solaris	1. Use Markov Chain Monte Carlo method to simulate the annealing. 2. Provides 5 different statistics in the NPL analysis.	1. Slower than MERLIN and more time consuming in the NPL analysis 2 Less precise in parametric analysis than MLINK (Linkage)	(Sobel and Lange, 1996)
SOLAR	Variance component	UNIX, Solaris	1. Suitable for quantitative trait 2. Take into account gene-gene and gene-environment interaction	1. Segregation analysis and setting disease model has to be carried out before analysis	(Almasy and Blangero, 1998)

Section 5: Saccades and Anti-epileptic Drugs (AEDs)

5.0 Introduction

Saccades are discrete ballistic ocular movements that direct the eyes toward a visual target and fix the image to the fovea within tens of milliseconds. Saccades can be classified into several subtypes, depending on the stimuli and the cortex responded to such stimuli in generating saccades (Table 5.1 and Table 5.2).

In medical practice, saccades provide important clues leading to diagnosis of various neurological disorders. Recently, different types of saccades and their unique parameters have been investigated to see whether they can be surrogate biomarkers for common diseases in determining their phenotypes or to evaluate their treatment outcome, such as in schizophrenia, bipolar disorder or Parkinson disease (Reilly *et al.* 2008).

5.1 The control pathway of saccades

5.1.1 Cortex

Cortical areas, including primarily the frontal eye fields (FEFs), the supplementary eye fields (SEFs), dorso-lateral prefrontal cortex (DLPFC), parietal eye fields (PEF) and the cingulate cortex, are the highest–level control centres of saccades (Pierrot-Deseilligny *et al.* 2004). In general, all these cortical areas are involved in generating saccades, but the characteristics of saccades generated from different areas differ slightly. For example, the frontal eye fields are especially involved in voluntary saccades while the parietal eye filed is more likely to be involved in reflexive saccades. Table 5.2 summarizes the cortical areas currently known which are responsible for different types of saccades.

The cortex controls saccades through several different pathways. First, there are direct projections to eye-movement-related structures in the brain stem and superior colliculus (SC). A less direct pathway passes through the pontine nuclei to eye movement regions of the cerebellum (mainly ventral paraflocculus and oculomotor vermis), which in turn access the output motor nuclei for eye movements, via projections to brainstem motor nuclei (Krauzlis, 2005). Secondly, there are descending pathways involving nuclei of the basal ganglia, such as the caudate nucleus and the substantia nigra pars which exert their

influence on eye movements through the SC (Hikosaka *et al.* 2000). Figure 1 shows a simple illustration of saccades control pathways. Figure 5.2 is a simplified model showing the inter-relationship between cortical areas in controlling saccades.

Table 5.1 Types of saccades, adopted form Leigh et al. (Leigh and Zee, 2006)

Types	Description
Spontaneous saccades	Seemingly random saccades that occur when the subject is not required to perform any specific behavioural task
Reflexive saccades	Saccades generated to novel stimuli (visual, auditory or tactile) that occur unexpectedly
Express saccades	Short-latency saccades that can be elicited when the novel stimulus is presented after the fixation stimulus has disappeared
Voluntary (intentional) saccades	Elective saccades made as part of purposeful behaviours
Memory-guided saccade	Saccades generated to the location in which a target has been previously present
Predictive or anticipatory saccades	Saccades generated in anticipation of the appearance of a target
Eye-head saccades	Saccades recorded without head fixed in a position (Freedman, 2008)
Anti-saccades	Saccades generated voluntarily in the opposite direction to a sudden appearance of a target

Table 5.2 Functions of cortex in saccades control

Frontal	FEF	Intentional, voluntary saccades
	DLPFC	Inhibit reflexive saccades, spatial memory saccades,
	SEF	Target selection, motor planning of intentional saccades
Parietal	PEF	Reflexive saccades
Cingulate	ACC	Intentional (voluntary) saccades
	PCC	Reflexive saccades

FEF: frontal eye field, DLPFC: dorsal lateral prefrontal cortex, SEF: supplementary eye field
PEF: parietal eye field, ACC: anterior cingulated cortex, PCC: posterior cingulated cortex

5.1.2 Superior colliculus

The superior colliculus (SC) is critically involved in generating saccades. Besides direct motor control of saccades, researchers have demonstrated that there are sensory and motor maps in the superior colliculus (King, 2004). The dorsally located superficial layers of the superior colliculus are pure sensory maps which receive direct input from retina visual stimuli and indirect input from visual cortex. The underlying deep layers respond to auditory, tactile and visual stimuli and receive converging modality-specific input that enable them with multisensory response properties. These new findings suggests that SC might act as a modulating centre of saccades according to various visual inputs in different conditions (Krauzlis, 2005).

5.1.3 Brainstem

The neuronal activities within a circuit which spreads across the brainstem are directly involved in the generation of saccades. Such areas include the paramedian pontine reticular formation (PPRF) and rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF), (Scudder *et al.* 2002). These neuronal activities arise mainly from three different types of burst neurons.

Short lead burst neurons

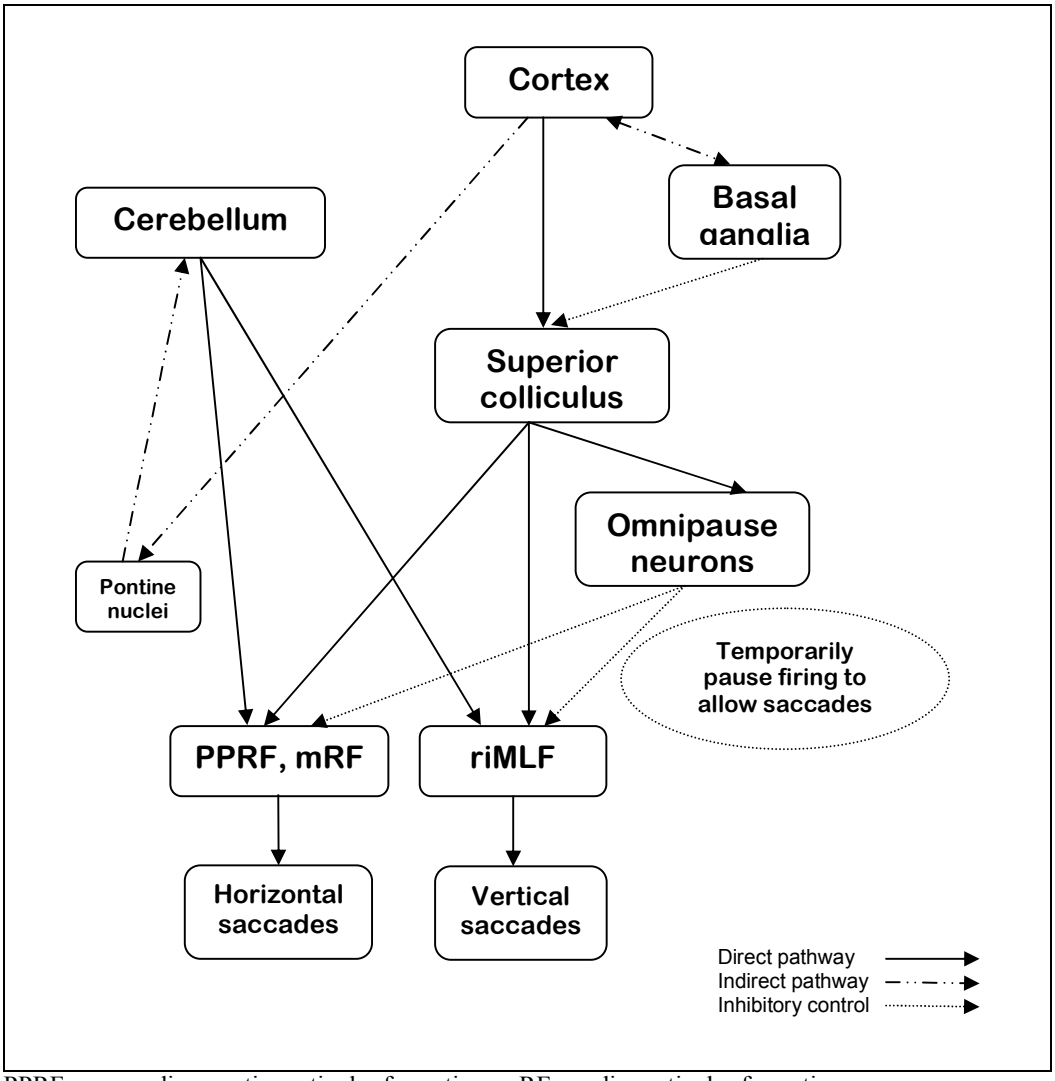
The short lead burst neurons (SLBNs) emit burst activities of which their precise timing determines the amplitude of the saccade. They include both excitatory (EBNs) and inhibitory burst neurons (IBNs). A group of SLBNs located in the PPRF and medial reticular formation (mRF) are responsible for the generation of horizontal saccades, while another group of SLBNs located in the riMLF and the interstitial nucleus of Cajal project to oculomotor and trochlear nuclei to generate vertical saccades (Moschovakis *et al.* 1991; Scudder *et al.* 2002). It is generally accepted that the EBN of the SLBNs receive inhibition from omnipause neurons (OPNs), and when OPNs cease firing, EBN will activate to generate saccades (Figure 5.1)

Long lead burst neurons

The long lead burst neurons (LLBNs) demonstrate their activities 100 ms prior to the emission of a saccade related burst of activity, and continue to fire during saccadic activity (Scudder *et al.* 1988). Observations regarding the timing of firing between omnipause

neurons (OPNs, see below) and LLBNs has led to a hypothesis that this "prelude" activity represents inhibitory signals for OPNs to cease firing (Scudder *et al.* 2002).

Figure 5.1 Model of saccades control



PPRF: paramedian pontine reticular formation; mRF: median reticular formation

riMLF: medial longitudinal fasciculus

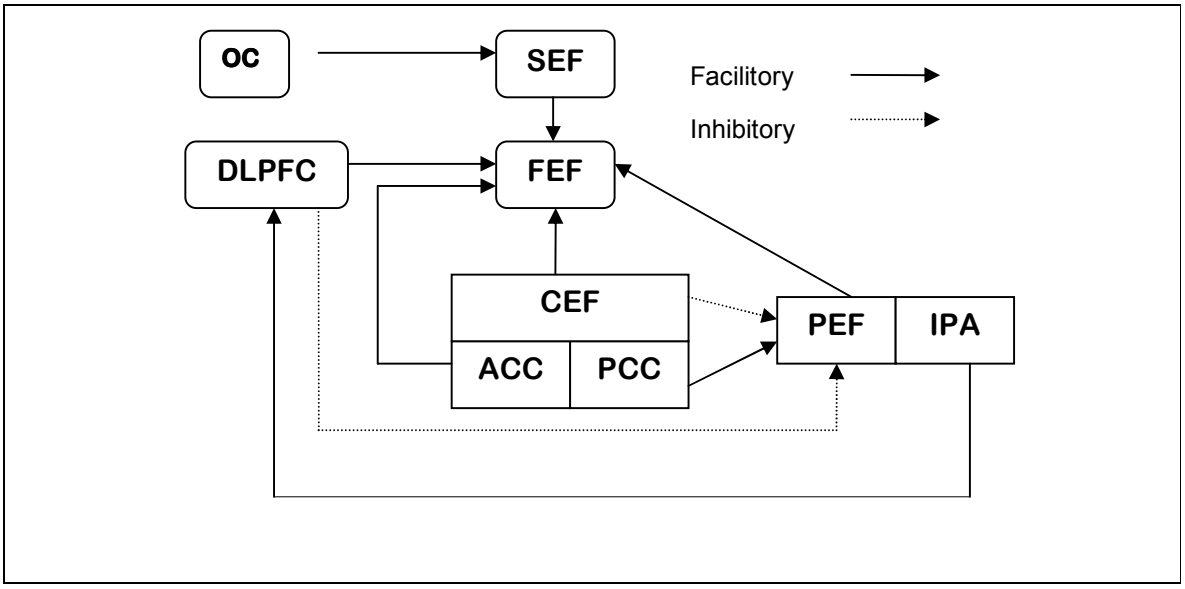


Figure 5.2. The relationship between cortical areas in controlling saccades. SEF receives impulses from visual cortex and processes motor planning, sends signal to frontal cortex to elicit intentional (voluntary) saccades. DLPFC and CEF assist FEF in target selection, inhibit unnecessary reflexive saccades signals coming from PEF. PCC assists PEF in reflexive saccades generation and ACC helps in eliciting intentional saccades in the FEF.
OC: Occipital cortex, SEF: Supplementary eye field, FEF: Frontal eye field, CEF: cingulate eye filed, comprise of ACC and PCC.
ACC: Anterior cingulate cortex, PCC: Posterior cingulate cortex, DLPFC: Dorsolateral prefrontal cortex. PEF: parietal eye filed, IPA: intraparietal area.

Pause neurons

Pause neurons, the so called omnipause neurons (OPNs), discharge steadily but stop firing during some or all saccade generation. Of particular interest, OPNs have been intensively studied recently not only because of their relationship to the superior colliculus in controlling saccades but also because they are thought to act as a gating system for both saccadic and pursuit eye movements. New models suggest that OPNs probably receive signals from the superior colliculus to cease firing momentarily and disinhibit the EBNs of the SLBNs to generate saccades (Scudder *et al.* 2002).

5.2 Modulation of saccades

There are two structures which are not directly involved in generating saccades; but act as modulating centres.

5.2.1. Cerebellum

The ventral paraflocculus (VPF), and fastigile oculomotor region (FOR) are the main structures involved in modifying saccades, especially in modulating the accuracy of the voluntary saccades (Krauzlis, 2005). Clinical observations have demonstrated that damage of the cerebellum does not abolish saccadic eye movements but makes them highly inaccurate. Damage of the VPF and FOR will interrupt the timing, accuracy, dynamics and the adaptation of saccades (Klein *et al.* 2003). Without the regulatory activity of FOR, the brainstem burst generator produces grossly dysmetric saccades that exhibit considerable variation in amplitude and deviant saccadic trajectory.

5.2.2 Basal ganglia

The key role of the basal ganglia is to send inhibitory signals to the superior colicullus (SC) after receiving stimuli from cortical areas, including FEF, IPA and DLPFC, mainly via the substantia nigra pars reticula (SNr) (Hikosaka *et al.* 2000). Hikosaka, who had studied in detail the influence of the basal ganglia on saccades, concluded that the basal ganglia may be an integration centre for saccades. As the SC receives multisensory signals from the cortex, a specific structure is expected to filter useful signals from multiple sensory inputs, so that saccades can be elicit efficiently and correctly within mini-seconds to any stimuli. Because SNr acts as the main inhibition input to the SC, and may be able to "select" relevant inputs in generating oriented movements, the basal ganglia may be the structures that have evolved to be the integration centre of saccades between the cortex and the SC.

5.3 Characteristics of Saccade and localization

Several physiological studies have demonstrated that measuring the behaviours of saccades provide good information for localization. For example, cortical lesions have complicated influences on saccades involving high cortical functions. Voluntary and visual

guided saccades are usually impaired if the visual cortex or the frontal lobe are damaged. The latency of spontaneous saccades in monkeys with hemianopsia is prolonged. During experiments, those targets falling in the impaired visual field would fail to elicit any visual guided saccades (Lynch and McLaren, 1989; Tusa *et al.* 1986). Lesions in the prefrontal cortex decrease the accuracy of memory guided saccades (Curtis, 2005), while slow and dysmetric saccades in individual with impaired cerebellar function are characteristics and are easily detected during clinical examinations. Such dysmetric saccades are found in patients with multiple sclerosis, schizophrenia, and stroke involving the deep vermis nucleus, especially the fastigile nucleus. Lesions in the basal ganglia will not abolish saccades, but alter their characteristics. Patients with Parkinson disease may have abnormal saccades which tend to be hypometric, slow with prolonged latency, and a large saccade may be broken into several smaller saccades (stepped). More detailed studies showed that the major characteristic of saccades in basal ganglia disorders are impaired memory-guided saccades and difficulties in suppressing visually guided saccades (Hikosaka *et al.* 2000).

Because different part of the brain can either demonstrate their unique impact on saccades or exert their influence on saccadic regulation, it is not only possible that observing the altered saccadic characteristics to locate the damage to the brain, but also measuring the parameters unique to each types of saccades can provide further information, such as the severity of the damage, the responses to treatment, and the functional state of the brain. In fact, voluntary saccades that involve simple psychomotor functions, such as anti-saccades or memory guided saccades have been applied to evaluate the effects of alcohol, nicotine and sleep deprivation (Khan *et al.* 2003; Larrison-Faucher *et al.* 2004; Porcu *et al.* 1998).

5.4 Methods in recording saccades

Leigh and Zee have summarized methods available for measuring eye movements in their book (Leigh and Zee, 2006). Among those, electro-oculography, infrared reflection method, video-based tracking systems and magnetic scleral search coils are frequently used in modern laboratories. Recent researches done by Träisk and colleagues had made further comparisons between infrared reflection method and magnetic scleral search (Träisk *et al.* 2005; Träisk *et al.* 2006). These findings suggest that recordings of maximum velocity of

saccades by infrared reflection exhibited higher variability compared with magnetic scleral search coil. The advantages and disadvantages of these methods are summarized in Table 3.

As expected, the parameters of saccades measured using different facilities will be different. Most of the parameters of saccades were measured by electro-oculography before other facilities were widely available, and more recent researches in saccadic eye movements are mostly done either by video-based tracking or infra-red reflection method. Such differences made comparisons of some research results difficult.

Table 5.3.Summary of eye movements recording methods [adopted from (Leigh and Zee, 2006)]

Method	Advantages	Disadvantages
Electro-oculography	Non-invasive, cheap, able to record large amplitudes of saccades up to ±40°. Widely used in clinical laboratory.	Unsteady baseline that requires repeated calibration. Affected by lighting, lid movements, electrical and electro-myographic noises. Not reliable in recording vertical saccades.
Infrared reflection	Non-invasive, little noise, resolution of 0.5°	Limited range,± 20°horizontal and ±10°vertical. Higher variability.
Video-based tracking	Non-invasive, resolution of 0.5° or better.	Bulky head gear, limited in analyzing slow saccades
Magnetic scleral search coil	Sensitive and accurate, capable in recording all directions of eye movements.	Need topical anaesthesia and wearing a scleral coil. Invasive and uncomfortable. Expensive.

5.5 Parameters of Saccades

There are several parameters that measure saccadic eye movements, such as latency, peak velocity, duration and amplitudes. Between some parameters, there are mathematical relationships. For example, the main sequence is the mathematical equation that describes the relationships between peak velocities, and amplitudes. The Q value evaluate the skewness by the ratio of maximum velocity and mean velocity. This value is approximately 1.6, even for slow saccades because of fatigue. For certain types of saccades, they have unique parameters. For example, anti-saccades and memory guided saccades can be evaluated by error rates. Saccades trajectory is a graph illustrating the track of the eye movements, although it is not considered a parameter; it has been very useful in various

neurophysiological studies. Table 5.4 is a summary of parameters in saccades and their definitions.

Table 5.4 Parameters of saccades

Parameter	Definition
Latency (Reaction time)	The time between the appearance of the stimuli and the beginning of a saccade (sec).
Duration	The time between the beginning and the end of a saccade (msec)
Peak Velocity	The maximum velocity measured during a saccade (deg/sec)
Acceleration velocity	The measurement of how fast a saccade can reach the peak velocity (deg/sec^2)
Deceleration velocity	The measurement of how fast a saccade can stop from its peak velocity (deg/sec^2)
Mean velocity	The averaged velocity of a saccade (deg/sec)
Amplitude	The magnitude of a saccade, usually in the measurement by degree (deg)
Accuracy (visual guided saccades, memory guided saccades**)**	The ratio of the amplitude of a saccade generated to the stimuli amplitude. (i.e the ratio of the actual amplitude to the amplitude that it should have to foveate the target in interest) (%).
Error rate (anti-saccades, memory guided saccades)	The percentage of errors made during in performing memory guided saccades or anti-saccades (%).
Q value	Peak velocity/ mean velocity ≈ 1.6, measure skewness.
Main sequence	Peak Velocity $=V_{max} \times (1-e^{-Amplitude})$.

5.5.1 Main Sequence of saccades

The term "main sequence" was originally used to describe the relationship between the brightness of a star and its surface temperature in Astronomy. Such relationship can be illustrated as the "Hertzsprung-Russel Diagram", using colours to represent the surface temperatures of stars. In early 1960, when accurate measurement of saccadic eye movements was successfully carried out (Cook and Stark, 1968; Robinson, 1964a;

Robinson, 1964b), this term was adopted to describe the relationships between the duration, the peak velocity, and the magnitude (amplitude) of human saccades. Since the discovery of a constant mathematical relationship between the three saccades parameters (duration, peak velocity, and amplitudes), the Main Sequence of human saccades has been used as a diagnostic tool for assessing eye movement disorders (Bahill *et al.* 1975). Recently, measuring the Main Sequence of human saccades has been extended as a measure of treatment outcome in psychomotor and neurological disorders (Chan *et al.* 2005; Griffiths *et al.* 1984). There is more than one mathematical model that describes the main sequence of human saccades. Two of the most widely used mathematical models are the exponential equation and the modified square root equation models.

5.5.1.1 The exponential model (equation)

In the exponential equation model, the saccadic duration is approximately proportional to the amplitude. The relationship between the peak velocity and the amplitude of human saccades can be fitted with an exponential function:

$$\text{Peak Velocity} = V_{MAX}(1-e^{-\text{amplitude}/c})$$ (Baloh *et al.* 1975),

Where V_{MAX} is the velocity at the point where it does not continue to increase in spite of increased amplitude (the asymptotic maximum velocity). The constant C is the amplitude value corresponding to a peak velocity equal to 63% of V_{MAX}. This equation model has been widely used in many modern studies especially those using the scleral coil method to record and analyse saccadic eye movements (Irving *et al.* 2006; Träisk *et al.* 2006; van Beers, 2007).

5.5.1.2 The square root model (equation)

In the square root equation model, the mathematical relationship between peak velocity or duration and the magnitude of saccades is presented as:

$F=F_1 * \sqrt{A}$ (Lebedev *et al.* 1996),

Where F is either the duration or the peak velocity of a saccadic eye movement, and F_1 is the corresponding parameter value when the amplitude (A) is equal to 1°. For horizontal saccades, in a range of amplitudes from 1.5° to 30°, it is possible to approximate both the saccade duration and the peak velocity in this square root form (Lebedev *et al.* 1996). In this study, the square root equation was chosen for the analysis, as it is easier to make comparison between the Main Sequence recorded under different batteries, and for horizontal saccades of small magnitude, it is as accurate as the exponential equation model.

5.6 AEDs that affect saccades

Although it has been known for decades that certain conventional AEDs affect eye movements, the exact mechanisms are not yet fully understood. Among all AEDs, benzodiazepines (mainly diazepam) were frequently studied because their psychomotor effects were easily recognized and measuring saccadic parameters was originally thought to be a good technique to monitor their effects. The second commonly studied AED is carbamazepine, as its clinical detectable effects in toxic serum levels are impressive and observation of saccadic changes has been reliable in detecting adverse effects in patients.

5.6.1 Conventional AEDs

5.6.1.1 Benzodiazepines

Most research on saccades and anti-epileptic drugs has focused on diazepam. One of the earliest studies was carried out some 40 years ago by Ashcoff (Ashcoff, 1968). Several new studies, using more accurate eye movement recording techniques (magnetic search coil) were recently published (Wang *et al.* 2005). Even though the methodologies varied and the recording of the saccadic eye movements were carried out by various techniques, at least 15 studies have reported that the peak velocity of saccades were significantly affected by benzodiazepines (Ashcoff, 1968; Ball *et al.* 1991; Bittencourt *et al.* 1981; Fafrowicz *et al.* 1995; Gentles and Thomas, 1971; Griffiths *et al.* 1984; Hofferberth *et al.* 1986; Hommer *et al.* 1986; Jurgens *et al.* 1981; Rothenberg and Selkoe, 1981; Roy-Byrne *et al.* 1990; Tedeschi *et al.* 1983; Tedeschi *et al.* 1986; van der Mayden *et al.* 1989; Wang *et al.*

2005). Some of the studies even reported that these effects could be dose-related (Ball *et al.* 1991; Bittencourt *et al.* 1981).

Padoans (Panoan *et al.* 1992) reported that besides decreased peak saccadic velocity, the latency was prolonged and the amplitude was decreased after administration of diazepam. The effects were maximal 1 hour after taking the drug. However, no significant correlation between the effects and the serum drug concentrations was reported. Jurgens and co-workers (Jurgens *et al.* 1981) reported that the peak saccadic velocity and duration of saccades were influenced by diazepam, but the accuracy and latency remained almost constant before and after taking diazepam. In contrast to these, Blau (Blau *et al.* 2005) found that saccades latency is the most sensitive parameter in measuring the effect of diazepam, but accuracy and peak velocity of saccades are less reliable. These authors concluded that this finding may reflect a dose of diazepam and a longer time interval between eye movements recording, although using electronystamogaphy in recording saccades may also contribute to the differing results.

Lorazepam, which is widely used in status epilepticus, has been reported to have a similar effect to diazepam, i.e it prolonged saccadic latency (Salonen *et al.* 1986; Tedeschi *et al.* 1983), increased the duration and decreased the peak velocity and amplitudes of saccades. Similar effects were also found in pursuit, that the latency (reaction time) prolonged and decreased gain (Masson *et al.* 2000).

Van der Mayden (van der Mayden *et al.* 1989) compared the side effects of two benzodiazepines on saccades: clonazepam and clobazam. He found that clonazepam markedly slowed peak saccade velocity, while clobazam did not impair saccadic eye movements and had much less side effects on psychological functions such as cognition.

Two studies have investigated the effect of midazolam on saccades and reported slowed saccadic velocity (Ball *et al.* 1991; Salonen *et al.* 1986). Ball and co-workers also found that the latency of saccades was not affected, but peak acceleration and deceleration and the ratio of acceleration to deceleration as well as accuracy were reduced. They also reported a correlation between the magnitude of changes of parameters measured and the drug concentration, while Solanen and co-workers found no such correlation (Salonen *et al.* 1986).

5.6.1.2 Phenytoin and Phenobarbital

Although it has been frequently observed that phenytoin can induce nystagmus (especially down beating nystagmus) with both toxic and therapeutic serum drug levels, there are surprisingly few studies of saccadic eye movements and phenytoin. In a case report of a patient with a toxic serum level of phenytoin and phenobarbital (phenytoin 27.5 mg/ml, phenobarbital 18.8 mg/ml), it was demonstrated that the patient exhibited hypometric saccades (Thurston *et al.* 1984). In a review, the slowing of saccades and saccadic pursuit by phenytoin were mentioned (Esser and Brandt, 1983). No study has focused on describing the effects of phenytoin on other parameters of saccades.

Tedeschi (Tedeschi *et al.* 1989) compared the influences of carbamazepine and phenobarbital on saccades. It was concluded that both drugs exhibited a marked influence on saccades, with a reduction of peak velocity, poor saccades accuracy and prolonged latency. However, there were no significant diurnal changes of saccades in the phenobarbital group compared to the carbamazepine group.

5.6.1.3 Carbamazepine and oxcabazepine

Although widely recognized, only a few studies have demonstrated the effects of carbamazepine on saccades. Early studies found that carbamazepine slowed the peak saccadic velocity (Mühlau *et al.* 1987; Tedeschi *et al.* 1989), while more recent work in patients on steady carbamazepine therapy did not demonstrate significant changes of saccades parameters (including peak velocity, reaction time [latency] and accuracy) compared to the age-matched control group (Pieters *et al.* 2003). The fact that serum concentrations measured across the time interval of the experiment were relative stable in these patients probably contributed to this result. Another study quantitatively compared the effects of carbamazepine and oxcarbazepine on saccades, concluded that both drugs produced a significant reduction in the peak velocity of saccades, and carbamazepine had a greater effect than oxcarbazepine (Zaccara *et al.* 1992). Noachtar and co-workers (Noachtar *et al.* 1998) confirmed this finding in a study investigating carbamazepine and gabapentin and additionally noted that the duration of saccades was prolonged and the gain (accuracy) were reduced. However, no further detail about the quantitative relationship or

any statistic analysis between the serum drug levels and the magnitude of change in saccades parameters were available from these studies.

5.6.2 New generation AEDs

For most of the new generation AEDs, their effects on eye movements have not yet been observed or established. Only Gabapentin, Tiagabine, and Lamotrigine have been studied so far.

5.6.2.1Gabapentin and Tiagabine

There is only one report about gabapentin and saccades, which showed that gabapentin mildly slowed the peak velocity and prolonged the latency of saccades (Noachtar *et al.* 1998). The effects of gabapentin were not as strong as carbamazepine on parameters of saccades. In a recent study of tiagabine in healthy volunteers, it showed that this drug had no significant effect on peak velocity and the amplitude of saccades, although a trend was found for increased latencies after tiagabine treatment (Zwanzger *et al.* 2005).

5.6.2.2 Lamotrigine

Hamilton *et al* (Hamilton *et al.* 1993) reported that lamotrigine did not have significant influences on peak velocity or duration of saccades at the dose of 150 mg and 300 mg compared to placebo group. In his study, eye movements were tested ½ hour before taking lamotrigine and then repeated every 1.5 hours after taking the drug up to 6 hours after medication. Another study comparing the psychomotor effects of phenytoin and lamotrigine and reported the same result (Cohen *et al.* 1985).

For all other novel anti-epileptic drugs, even those that have been widely prescribed for epilepsy treatment such as levetiracetam and topiramate, there are no published reports available.

5.7 Evidence that AEDs may affect saccades through ion channels and receptors

Complicate networks within the brain are required to generate saccades. Neuronal activity depends on sophisticated neuron networks across various parts of cortex, superior colliculus and brainstem with modification by the cerebellum and the basal ganglia. These activities

rely on the interactions of neurotransmitters, receptors and ion channels. The role of ion channels in the generation of saccades remain ill defined, but recent research has provided some indirect evidence that ion channels, especially calcium channels, sodium channels and GABA receptors may have their role in saccadic eye movements.

5.7.1. Ion Channels

5.7.1.1 Calcium channel

Mutations of the calcium channel gene CACN1A which encodes the $\alpha_1$2.1 subunit of the Ca_v2.1 (P/Q type) voltage gated calcium channel is known to be involved in four inherited neurological disorders: generalized epilepsy with febrile convulsion plus (GEFS+), progressive spinocerebellar ataxia, familial hemiplegic migraine, and episodic ataxia type 2 (also named spinocerebellar ataxia type 6, SCA 6). There is one study, which has demonstrated that patients with Ca_v2.1 mutation are more likely to have selective floccular atrophy leading to impairment of smooth pursuit, although saccadic velocity remain normal (Ying *et al.* 2005). In another study, Subramony and colleague (Subramony *et al.* 2006) found that SCA6 patients with a new CACN1A mutation have slow, hypometric saccades together with markedly impaired smooth pursuit. It was also reported that 9 out of 11 subjects in this study, fever was a trigger factor for ataxia, suggesting that the function of the mutated calcium channels was sensitive to high temperature. This could be a possible explanation for febrile seizures caused by calcium channel mutations. A more recent study using the scleral coil search method showed that besides hypometric saccades, impaired peak saccadic velocity of the upward saccades could be an early manifestation of SCA 6 and might help in determining the time for treatment (Christova *et al.* 2008). However, the most direct evidence demonstrating that there is an association between slow saccades and ion channels was reported by Miura and Optican (Miura and Optican, 2006). Using a conductance-based saccades generating model, they proved that lesions of the omnipause neurons (OPN) caused slow saccades, because of a reduced T- current (the transient current mediated by T –type calcium channel) and reduced NMDA currents (mediated by NMDA receptors), which were caused by loss of glycine released from OPN.

5.7.1.2 Sodium channel

There is no direct evidence that sodium channels affect saccades. However, the fact that phenytoin influences saccades and exerts its anti-epileptic effect through the sodium channel provides indirect evidence that the sodium channel may have its impact on saccade. A recent study showed that mice with β1 subunit mutation of the voltage-gated sodium channel, which is associated with generalized epilepsy with febrile seizure plus (GEFS+) had reduced sensitivity to phenytoin (Lucas *et al.* 2005). This could be the first step in exploring the relationship between saccades, susceptible genes to epilepsy and AEDs.

The other indirect evidence that sodium channel may affect saccades came from another report of a familial disorder with limb tremor and saccadic oscillation (Shaikh *et al.* 2007). In this report, a conductance based simulated model with deficits on ion channel functions (including calcium, sodium and potassium ion channels) were used to explain both the oscillation of saccades and limb tremor. Although such hypothesis is yet to be proved, they nevertheless suggest new genetic, experimental and clinical approaches to such disorders which may involve ion channels.

5.7.2 GABA Receptors

The relationship of saccades and GABA receptors has been well defined by Hikosaka and Wurtz (Hikosaka and Wurtz, 1985), who used muscimol (GABA agonist) and bicuculline (GABA antagonist) to evaluate their effect on saccades. They were injected into the monkey's superior colliculus (SC) and the effects on saccades were measured. Injection of muscimol markedly slowed peak saccadic velocities, slightly reduced amplitudes and prolonged latencies, while injection of bicuculline had the opposite effects. The effect of a GABA antagonist and agonist on saccades was demonstrated through their actions on GABA receptors in the SC. A more recent study further confirmed that in addition to the SC, GABA agonists also have an effect on the nucleus reticularis tegmenti pontis (NRTP) (Kaneko and Fuchs, 2006). Ipsiversive saccades were difficult to elicit following the injection of muscimol on NRTP, with marked slow peak saccadic velocity, prolonged latency, and moderately impaired accuracy. Further work showed that muscimol injection into the caudal fastigial nucleus produced hypermetric saccades of the ipsilesional site and hypometric saccades to the contralesional side of injection (Goffart *et al.* 2004).

Although lack of direct evidence, the fact that conventional anti-epileptic drugs act on sodium (such as phenytoin and carbamazepine) may indicate that their actions on these ion channels are crucial to saccadic generation. Some novel AEDs, such as lamotrigine, also acts on the sodium channel.

Lidocaine, a local anaesthetic drug, which has the same action on sodium channels as several AEDs has been used in various animal studies to suppress saccades (McPeek and Keller, 2004; Sommer and Tehovnik, 1997). Benzodiazepines such as diazepam and lorazepam are GABA agonists. Another novel AED tiagabine is a selective GABA blocker. Muscimol, a GABA agonist, has been used in numerous studies to suppress or alter saccadic generation to elucidate saccadic control pathways. This provides some evidence to support the hypothesis that the mechanisms underlying the effects on saccades and the anti-epileptic effects of many AEDs might exert such effect through actions on various ion channels and receptors. (Kuo *et al.* 2000; Lingamaneni and Hemmings Jr, 2003). Thus, measuring the change of saccadic eye movements in patients on AEDs therapy may be a good surrogate marker in evaluating the pharmacodynamic effect of an AED.

5.8 Studies using saccades as clinical measurements

Saccadic parameters of saccades have been in common use as a clinical measurement to monitor disease progression and to predict disease genotype. Measuring express saccades and memory guided saccades have revealed their impairment in association with cognitive defects in patients with Parkinson disease (Chan *et al.* 2005). In the comparison of the differences of saccades characteristics between Alzheimer disease, Dementia with Lewy bodies and dementia of Parkinson disease, it showed that the latter two groups were similar in that they both had impaired complex saccadic performance and impaired execution of reflexive saccades compared to the control group, and patients with Alzheimer disease were only impaired in complex saccades performance, thus impaired execution of reflex saccades allowed discrimination between dementia with Lewy bodies and Alzheimer's disease (Mosimann *et al.* 2005). The existence of abnormal saccades allows differentiation between brainstem or cerebellum involvement in patients with multiple sclerosis (Downey *et al.* 2002); dysmetric saccades suggest cerebellar lesions, while disconjugate saccades without marked dysmetria favours brainstem lesion (Serra *et al.* 2008). In Gaucher's

disease, measuring peak saccade velocity has been used as a clinical marker of the therapeutic effects of enzyme replacement and in monitoring the disease progression (Pensiero *et al.* 2005). By measuring and comparing the latencies, the peak velocity and the accuracy of saccades, Ali et al (Ali *et al.* 2006) had successfully identified patients with chorea mimicking Huntington Disease who were later proved to be genetically uninvolved. These studies have demonstrated that the measurement of saccadic parameters and characteristics of saccades may be an useful tool for monitoring and evaluating various neurological diseases.

5.9 Conclusion

Measuring parameters of saccades is very useful in diagnosing and monitoring neurological diseases. Understanding the physiology of saccades and their characteristics are crucial. The application of saccadic eye movement measurements may be extended to monitor therapeutic effects and to predict disease genotype. Current digitalized laboratory facilities such as electro-oculography, video-oculography and magnetic search scleral coil are sensitive and reliable in detecting subtle changes of saccades which are difficult to detect in bedside examination.

There have not been many studies investigating saccadic eye movements on patients taking anti-epileptic drugs as a measurement to their therapeutic effects, especially those taking new generation AEDs. The use of saccadic eye movements seems promising as a biomarker for epilepsy therapy or as a marker of certain epilepsy phenotypes. However methodological issues need to be clarified including, the effects of (novel) AEDs on saccades, and the influences of epilepsy susceptible genes on saccades (if there is any). As the advances of eye movement recording facilities, accumulating knowledge on genetics of epilepsy, pharmacokinetic and pharmacokinetic on AEDs, measuring parameters of saccades in the future can be reliable and robust in monitoring epilepsy treatment and possibly in the prediction of pharmocodynamic effects.

[This chapter is adapted and modified from *Saccadic Eye Movements and Anti-epileptic drugs* (Lo *et al,* 2008) with permission].

Material And Methods

Section 6

6.0 Overview

The Ethic Approval of the linkage analysis study in familial epilepsy syndromes was granted by the Central Office for Research Ethic Committees (COREC, later as the National Research Ethic Service) and the Reference Number was 04/Q0512/57. The methodology in this genetic linkage study was divided into three parts: the identification and recruitment of study subjects; laboratory based molecular genetics; and computational linkage analysis as well as other works involving bio-informatics in genetics.

6.1 Genetic linkage analysis

6.1.1 Recruitment of subjects

The recruitment of the subjects in the linkage analysis project was done in three different ways. For the two families with mesial temporal lobe epilepsy, subjects were recruited through probands identified in the out patient clinic in the *NHNN, Queen Square, London, and Royal London Hospital, Whitechapel, London.* Provisional drafts of the family pedigrees were obtained from the probands, and potential participants were identified from these drafts. The permission to contact these potential participants was made though the probands who contacted their relatives in advance before forwarding back their contact details and their agreement. Once the permission was obtained, visits to these subjects were arranged and visited all the subjects in person at their homes. This included travel to Great Yarmouth, Glasgow, Chingford, Romford, Kent, East London, Essex and several small villages in Swansea, South Wales, where these family members were based.

The recruitment of the subjects in the Photosensitive Epilepsy pedigree was made around 15 years ago in Birmingham in a study in collaboration with Dr. Peter M Jeavons. The DNA samples in this pedigree were already in storage in the Neurogenetic Laboratory, Department of Molecular Neuroscience, Institute of Neurology, Queen Square, London

when this study began. The access of the DNA samples and the permission to study these DNA samples was made by collaborating with Professor Nick Wood, Head of the Department.

The recruitment of the subjects in the five Kohlschütter-Tönz pedigrees was made by collaborating with Dr Henry Houlden, who wrote to each family or the researchers studying these families to obtain their permission to have their blood samples and to use DNA samples for further studies. The clinical investigations of these families and the details had been previously published in journals(Christodoulou *et al.* 1988; Donnai *et al* 2005; Guazzi *et al.* 1994; Musumeci *et al.* 1995; Petermöller *et al.* 1993).

6.1.2 Modification of the family pedigrees and taking consents

When a proband of was identified in the out patient clinic, a provisional draft of the proband's family pedigree was made according to the proband. On each visit to the subjects identified according to the pedigree, the pedigree might be adjusted if there were mistakes recognized in the provisional draft by family members. The amended draft was then shown to key members in the same family pedigree to ensure that the information was as correct as possible.

An information leaflet was given to each participating subject upon each visit, the aim of the study was explained orally if necessary and relevant questions were answered before the consent was signed.

6.1.3 Interview and blood sample collection

The subjects recruited from the families with familial mesial temporal lobe epilepsy were interviewed in person with a questionnaire. This questionnaire was modified from The New Patient Questionnaire used in the Epilepsy Out Patient Clinic of NHNN, according to the characteristics of the pedigrees identified. The questionnaire gathered back ground information of each subject, their medical histories, seizure patterns and their characteristic, states of seizure control, medical investigations which had been done in the past, and their medications in controlling epilepsy.

Upon each visit, around 10 -15 ml of peripheral blood were taken from each consenting subject and transferred into EDTA tubes. These EDTA tubes were brought back to the

Neurogenetic Laboratory in the NHNN to extract DNA. The DNA samples were stored in freezers at the temperature of -72°C until further use.

6.1.4 Molecular biology techniques

The molecular biology techniques involved in this study included DNA extraction from peripheral blood, whole genome wide scan with microsatellite markers, fine mapping with microsatellite markers, polymerase chain reaction (PCR) and allele sizing (genotyping), agarose gel electrophoresis, sequencing reaction and gene sequencing. With the exception of the 8-cM whole genome wide scan which was done in deCODE Genetics in Iceland, other molecular genetic work was carried out in the Neurogenetic Laboratory, National Hospital for Neurology and Neurosurgery, Institute of Neurology, Queen Square, London.

6.1.4.1 DNA extraction from blood

The material used in DNA extraction included blood samples collected from subjects, reagent A (320mM sucrose, 5mM$MgCl_2$, 10mMTris-HCL pH8, 1% Triton x 100), lysis buffer, proteinase K, phenol-chloroform, chloroform, 100% ethanol, and 1x TE (10mM Tris pH8, 1mM EDTA)

10 -15 ml Blood (in EDTA) was mixed with 40ml reagent A and centrifuged for 5 minutes at 2600 rpm twice. The pellet was suspended in 4mls lysis buffer with proteinase K added and incubated overnight at 55^0C. Two phenol-chloroform extraction and two chloroform extraction were performed. The genomic DNA was precipitated in 100% ethanol and was then removed into a tube containing 0.5 ml of sterile 1xTE. The extracted DNA samples were stored in freezers at -72°C until further use.

6.1.4.2 Whole genome wide scans with microsatellite markers

Microsatellite markers (sometimes referred to as a variable number of tandem repeats, VNTRs) are short segments of DNA that have a repeated sequence, such as CACACACA, and tend to occur in non-coding DNA. In some microsatellites, the repeats of the unit are highly variable and may be different from one individual to the other. In diploid organisms, each individual will have two copies of any particular microsatellite segment with one copy from the father and the other from the mother, thus the number of the repeats can be

different in each copy. Such characteristics of the microsatellite marker have been applied to genotype individuals. The most common way to detect microsatellites is to design polymerase chain reaction (PCR) primers which are unique to one locus in the genome

The microsatellite marker genome wide scan was divided into 2 steps: polymerase chain reaction (PCR) and sizing of the PCR product (genotyping). The majority of the microsatellite genotyping process was done in the deCODE Genetics in Iceland. Genotyping for fine mapping after computational linkage analysis was done by myself in the Neurogenetic Laboratory, National Hospital for Neurology and Neurosurgery, Institute of Neurology, Queen Square, London.

6.1.4.2.1 Polymerase Chain Reaction (PCR)

The material needed for PCR includes DNA primers (both forward and reverse for each microsatellite marker), DNA samples, DNA polymerase, 5x Buffer, dNTP (Deoxyribonucleotide triphosphate), sterile water, and PCR plate. The machine used for PCR in this study was the Applied Biosystems 9700 Thermocycling machine.

Prior to each PCR, gradient block might be necessary to determine the optimal temperature and reacting condition of the primers for microsatellite markers. The preparation of the reaction for gradient block was the same as ordinary PCR.

In the gradient block, there were 8 rows and each row contains 12 wells. Each well of the block was assigned a different temperature, stepwise ranging from a low temperature to a high temperature (for example, from 50° C to 64° C, with 50° C in the first well, 51.4° C for the second well, 52.8° C for the third well… and so on, with 64 ° C for the last well). The mixture for PCR prepared in advance was evenly pipette into each well, so the content for PCR in each well of the row was the same but the reaction was done in different temperatures, depending on the position of the well in the block. Such a design was necessary to figure out the most optimal temperature and reaction condition for primers used in PCR.

To make 25 ml of PCR product, mix 1ul of forward and reverse primers each with 5 ul of 5x buffer, 5ul of dNTP, 12.7ul of water, 1ul of DNA, and 0.3 ul of DNA polymerase (Taq). In order to avoid contamination, a master mix sufficient for the reactions up to one PCR plate was prepared instead of mixing the agents repeatedly for each well. The master

mix contains the same ratio of each agent needed for the reaction. Twenty-five ul of mixture was pipette to each well on a PCR plate from the master mix prepared in advance. To avoid unnecessary reactions happening before the PCR plate was placed onto the thermocycling machine, the DNA polymerase was added last to the master mix. The plates were centrifuged after the mixture was evenly pipette to the wells and put onto the Applied Biosystems 9700 Thermocycling machine. Primers which had similar reacting temperature were organized in the same plates. The optimal temperature and the numbers of cycles needed for PCR was set on thermocycling machine before starting the PCR. Such setting might be different according to the DNA polymerase used in the PCR. For gradient block, the PCR plate was put onto the gradient block machine and the desired temperature range was set before starting the reaction.

6.1.4.2.2 Allele sizing (genotyping using microsatellite markers)

Although the majority of the genotyping was done in deCODE Genetics, if fine mapping was necessary after linkage analysis such genotyping was carried out in the Neurogenetic Laboratory Queen Square.

The material needed for allele sizing included PCR products, Formamide, sizing ladder (Liz 500), PCR plate, Applied Biosystem 3730 DNA Analyzer.

For each well on a PCR plate, 1ul of PCR product, 0.3 ul of sizing ladder, and 12 ml of Formamide was added. To avoid contamination, a master mix was prepared first, which contains the same ratio of each agent needed for the reaction, and 12.3 ul of mixture was pipette into each well on a PCR plate. The prepared PCR plate was heated up to 95^0C for 5 minutes then placed on top of ice for 1- 2 minutes before centrifugation and loaded to the Applied Biosystem 3730 DNA Analyzer.

Before starting the Applied Biosystem 3730 DNA Analyzer, the information of the subject including DNA sample numbers, sizing protocol, and the file path in saving the analyzed data were keyed in and set up on the computer operating the DNA analyzer. The analysis was then started after the analyzer was set.

6.1.4.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to examine the quality and quantity of extracted DNA and also to examine whether the PCR products were the expected products in the right size. The percentage of the agarose mixture (weight of agarose/volume of solvent) was prepared according to the molecular weight of the element being analyzed. For example, 1% (w/v) Agarose gel is suitable for larger molecule product (such as DNA) while 2.5-3 % (w/v) Agarose gel is suitable for small molecular product (such as PCR product). The percentage of the agarose gel is usually between 0.8% and 3 %.

The PCR products are fragments of DNA which are negatively charged. They migrate toward the positive electrode during electrophoresis. Thus the cable connecting the positive electrode is always connected to the electrode of the mini-plates located at the far end of the mini-wells used for electrophoresis, so the negatively charged PCR products will move downward the gel during the electrophoresis which separates fragments of different molecular weights. Ideal PCR products contain only fragments with the same product size projected during primer design.

The material used in agarose gel electrophoresis included 10x TBE, agarose powder, ethidium bromide, mini-plates for electrophoresis, and transilluminator. To prepare 1 litre of 10x TBE, added 121.1 gram of Tris, 61.8 gram of boric acid (anhydrous) and 7.4 gram of EDTA to a flask, then water was added until the total volume was 1 litre. The agents were mixed well until all the components were properly dissolved.

Ten ml of 10x TBE was added to 90 ml of water to make 100 ml of 1 x TBE buffer. 1.25 mg of agarose was added to 50 ml of the 1x TBE buffer and heated up in a microwave until the agarose powder was all dissolved. 2.5ul of ethidium bromide was added to the liquid form of agarose gel while it was still hot in the fume hood and then poured into a mini plate. Comb(s) with 16 teeth was (were) placed on the mini-pate to form mini-wells on the gel. After the gel was fully set, 50-70 ml of 1 x TBE was added to the mini plate and the combs were removed. To load the PCR product to the mini-wells formed on the gel, 4 ul of 5% orange loading dye was mixed with 5 ul of each PCR product and the PCR-dye mixture was loaded to each mini-well formed on the agarose gel. The 100 bp ladder was used to assess the fragments sizes. The mini-plate was covered with a lid with cables which were connected to the electrophoresis machine. The cable connecting the positive electrode of

the electrophoresis machine was connected to the electrode of the mini-plates located at the far end of the mini-wells. The voltage for electrophoresis was set at 50-60 mV. The time needed to complete the electrophoresis was around 20-30 minutes. Once the electrophoresis had completed, the agarose gel was removed from the mini-plate and placed under the ultraviolet light in the transilluminator to read the results. The image of the gel exposed under the ultraviolet light was saved to a computer for further interpretation. Under the ultraviolet light, the DNA fragments in the PCR products were seen as bands. Once the size of the PCR products were confirmed to be correct and the reactions were ideal, these PCR products were filtered to eliminate unwanted small fragments and excessive primers left during the reaction. These purified PCR products were then used as the templates in the sequencing reaction.

6.1.4.4 Gene Sequencing

6.1.4.4.1 Purification of PCR products

The PCR products may contain small molecular fragments, such as the primers that were not used up in the PCR process. These small fragments must be eliminated before the PCR products can be used as the templates in sequencing reaction.

The material needed for filtering PCR products are manifolds, vacuum, shaker, sterile water, and PCR filtering plates, and PCR plates.

First, 60 ul of sterilized water was added to each PCR product in each well on the PCR plate, then each of the PCR-water mixture was pipetted into the wells of the PCR filter (clean – up) plate. The filter plates were placed onto a vacuum and left for 5-10 minutes, until all liquid had been sucked out. This procedure eliminated unwanted small molecular fragments in the PCR products and left the PCR products in the filter plates. The filtered plates were removed from the vacuum, 40 ul of water was added into each well, the plates were placed onto shaker and left to re-suspend for 30 minutes. The cleaned and re-suspended PCR products were then pipetted into clean, labelled standard 96 PCR plates for storage; to be used as the templates in the sequencing reaction.

6.1.4.4.2 Sequencing reaction

The material needed for sequencing includes primers for each exon, both forward and reverse, Big dye, sequencing 5x buffer, filtered PCR product, and sequencing plate.

The mixture for sequencing reaction was 1 ul of cleaned PCR products, 1 ul of Big Dye, 2 ul of 5x Sequencing Buffer, 1ul of primer (3.2 p mol), 3.5 ul of cleaned PCR product, and 2.5 ul of sterile water. To avoid contamination, master mix excluding the templates (filtered PCR products) was prepared first, which contains the same ratio of each agent needed for the reaction, and 6.5 ul of mixture was pipette to each well on a PCR plate. Then 3.5 ul of templates were added to the wells using multi-pipette. The loaded PCR plates were placed onto the Thermocycling machine and the program "Big Dye" was chosen for the sequencing reaction. The products of the sequencing reaction were filtered again to remove excessive Big Dye and unwanted fragments before loading into the 3730 DNA analyzer for sequencing.

6.1.4.4.3 Purification of Sequencing Reaction products

The material needed to eliminate the unwanted residuals and excessive Big Dye from the products of the sequencing reactions were the BigDye Terminator kit and sterile water. First the Dye Terminator kit was centrifuged at the speed 950 RCF for 3 minutes to spin off the buffer in each well. The buffer was centrifuged down to a container plate placed underneath the BigDye Terminator kit and was removed after centrifugation. Fifty ul of sterile water was added to each well which contained the sequencing reaction product from the sequencing reaction process and the mixture were loaded to the wells in BigDye Terminator kit by using multi-pipette. The loaded BigDye Terminator was centrifuged again at 950 RCF for 3 minutes with a PCR plate underneath, and the cleaned sequencing reaction products were centrifuged down to the wells of the PCR plate, ready to be loaded to the 3730 DNA analyzer.

Before starting the Applied Biosystem 3730 DNA Analyzer, the subjects ID, gene ID, and exons information file which was prepared in advance were imported to the Applied Biosystem 3730 DNA Analyzer, and sequencing process was started after the computer was set up. .

6.1.4.4.4 Analysis of Sequencing Data

The data in gene sequencing were analyzed by SeqScape, version 2.5. Before importing the data, a reference data group which contained the sequence of the exons and the sequence of approximately 50 base pair of the introns flanking each exon of was set up for each gene sequenced. A project template was build using the reference data group later and serves as the reference in the project. The sequence of the exons and introns of genes were downloaded from the website http://www.ensemble.org.

6.1.5 Computational Linkage Analysis and Genetic Bioinformatics

Software necessary for computational linkage analysis and genetic bioinformatics were downloaded from various websites, including http://linkage.rockefeller.edu/soft/, http://www.ncbi.nlm.nih.gov/sites/entrez, http://watson.hgen.pitt.edu/register/, http://www.sph.umich.edu/csg/abecasis/Merlin/index.html, http://www.ensemble.org, http://www.soe.ucsc.edu/~kent/dnaDust/dnadust.html, and http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.

Both Model-based and model-free linkage analysis were carried out in this project. The model-based (parametric) analysis was mainly done by using MLINK of the LINKAGE package, FASTLINK version. The model-free (Non-parametric) analysis was either done by MERLIN, version1.1.2 or Simwalk2, version 2.91. The file manipulating/constructing program Mega2 version 5.0 R1 (Mukhopadhyay *et al.* 2005) was used to format input files for MERLIN and SimWalk2. Software Cyrillic 2.1 was used for both pedigree drawing and haplotype analysis. Some of the Genetic Bioinformatics such as primer design, genetic maps, marker sequence, and candidate gene information were also carried out through the websites mentioned above.

6.1.5.1 MLINK of the FASTLINK package

FASTLINK package version 4.1P(Lathrop *et a.l* 1984; Cottingham *et al.* 1993; Schaffer *et al.* 1994) and the LINKAGE package version 5.2 were downloaded from the website http://linkage.rockefeller.edu/soft/. They were installed to a laptop partitioned with a UNIX operating system. The input files in the LINKAGE format necessary for the

linkage analysis via MLINK were formatted by importing the genotype data to Microsoft Excel and transformed the files into LINKAGE format.

There are two types of basic LINKAGE input files. The pedfiles contain information of the pedigree, such as information of the subjects' parents, sex, affection status, and genotype data of each individual. The information of the pedigree in the pedfiles was based on the pedigrees obtained and the genotype data from deCODE Genetics. The affection states were assigned based on the results of phenotyping according to clinical diagnosis. The dat.files are parameter files which contain parameters used in the parametric linkage analysis, such as mode of inheritance, allele/gene frequencies, penetrance rate, liability class, and the increment of recombination fraction, etc. The dat.files were formatted by the PREPLINK program in the LINKAGE package. The gene/allele frequencies were taken from data provided by deCODE Genetics.

Before entering the MLINK analysis, the UNKNOWN program of the LINKAGE package was used to check genotyping errors or incompatibility in all the input files. Genotyping errors were corrected by setting the data in question to unknown, because the original sizing figures from deCODE were not available. The LCP program of the MLINK was used to facilitate the calculation of the LOD scores, and the LRP program was used to generate tables for the output LOD scores. Multipoint analysis was performed by using LINKMAP when necessary. Detailed protocols of linkage analysis using MLINK can be found in Handbook of Human Genetic Linkage (Terwilliger and Ott, 1994) and Analysis of Human Genetic Linkage (Ott, 1999a).

6.1.5.2 MERLIN

Part of the non-parametric (model – free) linkage analysis in this project was done by MERLIN version 1.1.2. This program was downloaded from the website http://www.sph.umich.edu/csg/abecasis/Merlin/index.html and was installed to the laptop with a UNIX operating system. The formats of the input files are different from MLINK and were prepared by the file manipulating program Mega2. There are three basic types of input files in MERLIN. A map file which contains genetic map information, such as the genetic distance between each marker and the order of the markers on the chromosomes is necessary. The other two input files are pedigree files which contain the pedigree

information and genotype data, and the data files which contain parameters used in the analysis. Both types of files were constructed by Mega 2 using data stored in Linkage-format files. The map files were formatted directly by text editor in UNIX operation system.

The input files of MERLIN were checked by PEDSTATS to check genotyping errors. The genotyping errors were adjusted by setting the incompatible genotype data unknown. Because MERLIN cannot analyze large pedigrees, the pedigree files were adjusted by trimming off some individuals to fit the analysis in MERLIN when necessary.

To do NPL by MERLIN, in the command prompt page of MERLIN, the file name of the pedigree file, the corresponding map files and data files, and the option "NPL analysis" were specified in the commands. The output format of the analysis results can also be elected in the command lines specifying which format was in favour of, choices include graph, table, or plain text files. The LOD score generated by MERLIN and the corresponding pointwise *p* value is available, and the estimated maximum LOD score is also provided. The details of using Merlin can be found on the website: http://www.sph.umich.edu/csg/abecasis/Merlin/index.html.

6.1.5.3 Simwalk2

Part of the NPL analysis in this project was done by Simwalk2 version 2.91. Simwalk2 was downloaded from the website http://watson.hgen.pitt.edu/register/ and was installed to the laptop with an UNIX operating system.

The formats of the input files of SimWalk2 are different from MERLIN and MLINK. Similar to MERLIN, map files containing the information of genetic maps and the genetic distance between each marker are necessary in NPL analyses using SimWalk2. Other input files included were the locus data file, the pedigree data file, the penetrance data file, and the BATCH2.DAT control file. The penetrance files are only necessary for parametric linkage analysis. The BATCH2.DAT control files contain the user-specified instruction parameters, but usually it has already been set by default. The input files were constructed from data stored in Linkage-format files by Mega2, and was checked by Simwalk2 by the "setup" option before entering any specified linkage analysis. This option performed no likelihood-based analysis on the data but merely checks that the data files are consistent and

that the pedigrees have no incompatibilities. If genotyping errors were found, the errors were adjusted by setting the genotype data in question to unknown.

After all the input files were ready and checked, on the command prompt page of Simwalk2 the NPL analysis option was selected and the types of statistics was specified (including BLOCKS, MAX-TREE, ENTROPY, NPL_{ALL}, and NPL_{PAIR}) before starting the analyses. The data output of the analyses were all in plain text format and contained location scores at each marker under statistics selected. The corresponding *P* values were also available. Other information in the output data includes the estimated and observed recombination events between each marker, its *P* value, and the parameters set in the BATCH2. DAT file.

6.1.6 Other computing works

Besides computational linkage analysis, other works involving Genetic Bioinformatics in this study were primer designs and to look up candidate gene.

6.1.6.1 Design of primers

Most of the sequence of the primers for microsatellite markers is available online. For fine mapping, primers design might be necessary if the primers sequence available from websites failed to work in PCR. Primers design was also necessary for the PCR of each exon before go on gene sequencing. All the software needed for primer design are available online from websites, including http://frodo.wi.mit.edu/cgi-bin/primer3/primer3, www.gdb.org, http://www.ensembl.org/, http://www.soe.ucsc.edu/~kent/dnaDust/dnadust.html, and http://genome.ucsc.edu/cgi-bin/hgPcr?command=start.

The primers for exons were designed using sequence of introns flanking each exon. The sequence of the exons and up to 650 base pair of introns at the splice sites of each exon was retrieved online from http://www.ensembl.org/. A segment of intron sequence which contained several hundreds base pair and at least 200-250 base pair away from the splicing site was selected, copied and pasted into the DNA duster on the website http://www.soe.ucsc.edu/~kent/dnaDust/dnadust.html to eliminate unwanted strings or letters. This “dusted” sequence of the intron was copied and pasted to the primer picking

software on http://frodo.wi.mit.edu/cgi-bin/primer3/primer3 to pick up primers. The criteria in picking up primers for the exons in gene sequencing were 23bp for minimum primer size and 28 maximum; minimum melting temperature was 60°C and 68°C maximum, minimum CG content was 20%, optimum at 50-60 % and 80% maximum. The designed primers were then sent to PAN facility in Stanford University, California, USA to be synthesized.

The primers for microsatellite markers were designed in a similar way. The sequence of the markers was retrieved from the NCBI UniSTS website http://www.ncbi.nlm.nih.gov/sites/entrez?db=unists. A segment of the sequence which contained a few hundred base pair was selected from the sequence of the marker flanking the bi- or tri-nucleoside repeating segment. This segment was pasted to the DNA duster on the website http://www.soe.ucsc.edu/~kent/dnaDust/dnadust.html to eliminate unwanted strings or letters. This "dusted" sequence was then copied and pasted to the primer picking software on http://frodo.wi.mit.edu/cgi-bin/primer3/primer3 to pick up primers. The criteria in picking up primers for the exons in gene sequencing were 18 base pair for minimum primer size and 20 bp maximum; minimum melting temperature was 57°C and 63°C maximum, minimum CG content was 20%, optimum at 50-60 % and 80% maximum.

6.1.6.2 Candidate genes screening

When a possible disease locus was identified, the genes within the locus and their functions were searched from the websites http://www.ensembl.org/ and http://www.genecards.org/index.shtml. Both known genes and pseudo-genes within the regions of interest were first identified through the ensemble website, and the functions of the known gene were looked up in the Gene Cards website. The Gene Cards website also provides web links that provide relevant of the gene functions that are not yet summarized in their website.

6.2 Saccadic Eye Movement Recording

The study "Eye movement and anti-epileptic drugs" was approved by the Central Office for Research Ethic Committees (COREC, later as the National Research Ethic Service) and the Reference Number was 05/Q0505/119. The subjects in this project were recruited from the National Hospital for Neurology and Neurosurgery, Queen Square, London and the laboratory work were carried out in the Eye Movement Laboratory of the Institute of Child Health, Great Ormond Street Hospital, London.

6.2.1 Subjects

In this pilot study, twelve healthy individuals who were not taking any medication and who did not have any visual fields defects or eye movement disorders were recruited as the control group. Their age were recorded at the time of recording. These healthy individuals were either staff or research fellows in the Institute of Neurology, Queen Square, London.

The subjects of the case group were patients with epilepsy who were taking either phenytoin or carbamazepine as their single anti-epileptics drug treatment. They were recruited from the out patient clinic of the NHNN. Information leaflets were given to each subject and consents were obtained before any eye movements were recorder and blood samples were taken. In this preliminary pilot investigation, five patients were recruited but only three subjects completed the whole recordings.

6.2.2. Methodology

6.2.2.1. Battery of eye movement recording

The stimulus for eye movements consisted of a row of 3 red LEDs which were spaced 15 degrees apart [0°, −15° (left) and +15° (right)]. Each LED subtended 0.35° at the eye. The subject was asked to look at the illuminated LED with both eyes open. The stimulus sequence consisted of cycles in which first the LED in the primary position was illuminated (0°), followed by the LED at −15°, +15° and 0° again. The eye movements were recorded using an IRIS 6500 infrared limbal tracker (Skalar Medical, Delft, The Netherlands). The eye movements of each eye were recorded simultaneously. The analogue output was filtered by a 100 Hz low-pass filter, digitised to 12-bit resolution, and then sampled at 1 ms intervals. The system was linear over a range of ±25° (horizontal), with a resolution of

0.03°. Calibration was carried out using the 15° saccades (i.e. right and left), manually adjusting the graphs so that the foveating part of the waveform was set at approximately 0° and ±15°, respectively.

The eye movements was recoded when the head was stabilized in the primary position with a chin rest for one recording, and the whole battery of eye movement recording was repeated again without the chin rest used while the subject remain in the primary position. Such saccades recorded without chin rest is the "Eye-head saccades".

6.2.2.2. The control group

Two sets of eye movement recording using the regime described above were carried out on the control group. Their age at the time of recording were recorded. The time gap between the first and the second set of eye movement recording varied, from several hours to one week, depending on the availability of the control subjects. The purpose of having two sets of recording was to test the intra-individual reliability of the saccadic eye movement recording.

6.2.2.3. The subject group

Two sets of recording using the same regime in the control group were carried out on the subject group. The first set of recording was done before the subjects took the daily (or morning) dose of their antiepileptic drugs, and the second set recording was carried out several hours after they had taken their medication. The time gap between the first and the second set of eye movement recording varied, depending on the time needed in different AEDs to reach peak serum drug level. The serum drug levels were measured right before the beginning of their eye movement recording. If subjects also consenting for genetic analysis, another 10mls of blood samples would be taken. They could drop out the recording at any stage if they felt any discomfort or inability to concentrate. The condition of their seizure control, anti-epileptic medication in use during the eye movement recording, and detail medical history were reviewed according to their medical notes.

6.2.3. Analysis

The recorded eye movements were exported to a 100 Hz low-pass filter, digitised to 12-bit resolution, and then sampled at 1 ms intervals before imported to Mathematica version 5.0 for further analysis.

The eye movements of each eye were recorded simultaneously but analyzed separately. For each eye, the saccades generated when looking towards to right (+15°) and looking towards to left (-15°) were analyzed separately. The peak velocity of each saccade was plotted against its amplitude, and the main sequence of each eye was obtained by fitting a curve that best fitted these dots plotted. The constant OCCURRED was then calculated according to the fitted curve. The main sequence was shown as "C * $\sqrt{A}$" (the square root equation model). As shown in Figure 6.1, each curve represents the main sequence of one eye looking to one direction; the curve at the right side represents the main sequence of the saccades generated when looking to right; and the left side curve side represents the main sequence of the saccades generated when looking to the left. These data were pooled later to obtained the average value of the main sequence in each eye.

6.2.4 Statistics

The statistics used in this project were Independent T test, Paired T test, Pearson regression correlation, and Intraclass Correlation (ICC). To simplify the comparison, A (amplitude) was assigned of the same value in each subject, thus comparison of the constant OCCURRED was directly comparable to the comparison of the peak velocity of saccades in each subject. The comparisons of the constant of the main sequence were analyzed by two tail paired T test using Microsoft Excel. The result was presented by P value. P_1 represents the result of comparison between the first and the second set of eye movements recording in the same subject (Main Sequence obtained using chin rest in the first recording to main sequence obtained using chine rest in the second recording; and main sequence obtained without using chin rest in the first recording to Main Sequence obtained without using chin rest in the second recording). P_2 represents the result of comparison of the main sequence obtained with chin rest to the recording obtained without using chin rest in the same individual. Thus P_1 represented whether there were statistical differences between the 1st and the 2nd recording in each individual, while P_2 represents

whether there were differences between the main sequence recorded with and without chin rest in the same individual.

Independent T test was carried out to compare the main sequence of the control group to subject group.

The test-retest reliability calculation was carried out by Pearson regression correlation and Intraclass correlation (ICC). ICC estimates the variability arising from the same individual during different testing.

Figure 6.1

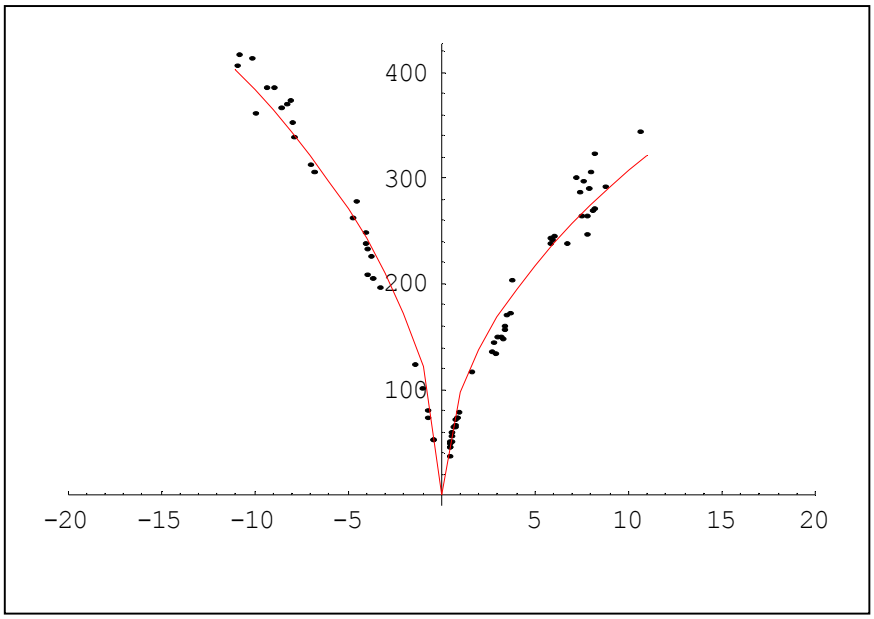


Figure 6.1, showing an example of fitting the curve to obtain the constant of the main sequence of saccades. The x axis is the amplitude and the y axis is the peak velocity calculated. Dots are plotted peak velocity against its amplitude in each saccade measured.

Results

Section 7: Linkage analysis in familial mesial temporal lobe epilepsy- family B

Two families with familial medial temporal lobe epilepsy (fMTLE) were identified from the epilepsy out patient clinic: family B and family W. The clinical presentations of both families were similar, but the modes of inheritance were different.

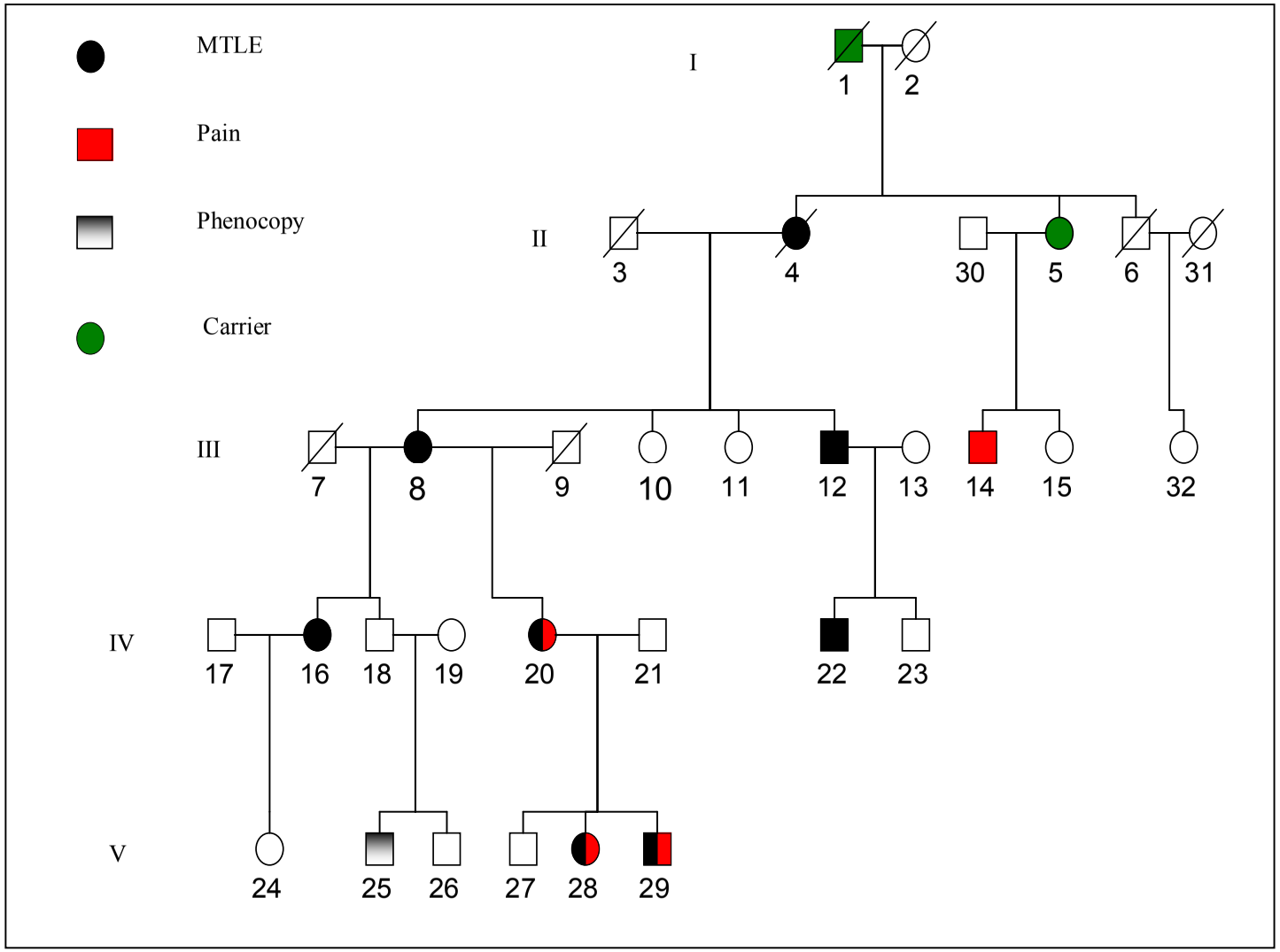
7.1 Pedigree

The pedigree of family B is shown in Figure 7.1. Twenty four consenting subjects in this family took part in this study and all their DNA samples were available. Individuals who are marked green were those who did not have clinical symptoms suggesting epilepsy but were considered carriers because either their children or parents were affected. Such phenomenon also suggested that the mode of inheritance in this pedigree could be an incomplete penetrance mode.

7.1.1 Clinical presentation and investigation

The medical history and medical investigations (if available) of the affected individuals are presented in Table 7.1. Detailed medical histories of the first and second generations were not available. I-1 was labelled as a carrier in the parametric analysis. II-4 was epileptic according to the interview of her daughter (III-8) and granddaughter (IV-20). II-5 and II-6 were not affected, but II-5 was marked as a possible carrier if the pain syndrome was considered a phenotype in this pedigree. Their spouses II-30 and II-31 were not affected. There were another three unaffected individuals in the II generation, who were brothers and one sister of II-4 and II-5 but are not shown in the pedigree.

Figure 7.1 Pedigree B



In the third generation, III-8 had several episodes of fainting, described as fits according to her daughter, we accepted her as a case. However, no definite convulsions had been witnessed. One of her brothers, III-12 had typical mesial temporal lobe epilepsy, onset at the age of 5. His clinical presentations included absence seizures, complex partial seizures and simple partial seizures without history of febrile convulsion. III-10 and III-11 were unaffected. III-14 had an atypical pain syndrome since the age of 9. The symptoms began after he had recovered from rheumatic fever and was bed ridden for 2 years. The pain was always evoked by startle response, such as sudden movement of the lift or unexpected horn sounding while walking on the street. The episodes were brief, lasting for seconds only with spontaneous recovery and the pain was intense. It could be so severe that he might fall down to the ground before the pain subsided. The pain originated in his lower back and propagated rapidly to his occipital area before it subsided. Other diagnoses suggested in this subject included Crohn's colitis and probable multiple sclerosis, both were made several years after the onset of such pain syndrome. His sister III-15 was unaffected. III-32 was unaffected.

In the fourth generation, IV-16 had late onset seizures. She had her first generalized seizure while she was in labour giving birth to her 4th child. Several episodes of simple partial seizure were noticed after this generalized seizure. The characteristics of these simple partial seizures included typical stomach rising sensation, pallor and dizziness after the aura, and a feeling of fear at the same time occasionally. She was unable to respond during the episodes but remained conscious. Post-ictally she would feel very tired and it might take up to half an hour for full recovery. She had 5 children and at the time of interview none of them had clinical syndromes suggesting seizures or epilepsy. IV-16 was also a Fragile X syndrome carrier (confirmed genetically) and she had 2 miscarriages. Her half sister IV-20 had intractable migraine, and the diagnosis of simple partial seizure was made after several EEG recordings and MRI scanning. She was also a Fragile X syndrome carrier. IV-22 was epileptic (confirmed by genetic testing). According to his mother, he had absence in his early childhood and several episodes of complex partial seizures with a similar pattern to his father's. Other symptoms during the seizures included twitching of limbs, especially in the morning, panic sensation and an inability to think. Triggering factors included sleep deprivation and rarely very bright lights. The complex partial

seizures subsided after the age of 20, but the other symptoms remained. His brother IV-23 was unaffected.

In the fifth generation, V-25 had three episodes of generalized seizures, all occurred when he was drunk. No other symptoms related to temporal lobe epilepsy were reported in this individual. He was marked as a possible phenocopy of mesial temporal lobe epilepsy. V-28 had Fragile X syndrome (confirmed by genetic testing), intractable complex partial seizures with secondary generalization, and moderate learning disabilities. MRI did not reveal hippocampal sclerosis, the EEG suggested that the seizures were temporal lobe in origin. Her brother V-29 was epileptic, with an impression of temporal lobe epilepsy and fully was investigated, his MRI showed right hippocampal sclerosis and he underwent a temporal lobectomy. He was seizure free after surgery. Both V-28 and V-29 had febrile convulsions and several episodes of status epilepticus. V-24, V-26, and V-27 were unaffected.

7.2 Linkage analysis

7.2.1 Genotyping

The DNA samples were genotyped using a set of 546 microsatellite markers for an 8 centi-Morgan(cM) Whole Genome-wide Scan.

7.2.2 Mode of inheritance and analysis program

In this pedigree, there was male to male transmission, so the mode of inheritance (MOI) was not an X linked and or a mitochondrial inheritance. It was also not autosomal recessive because there were affected individuals in more than one generation. We therefore considered the MOI to be likely autosomal dominant and so therefore this pedigree was analyzed by parametric linkage analysis using MLINK.

In this pedigree, III-8 was not epileptic, but her daughter IV-16 and IV-20 were epileptic. This suggested that there the mode of inheritance of this pedigree could be autosomal dominant (AD) with a reduced penetrance. This pedigree was analyzed both under the mode of AD with full penetrance and again under the mode of AD with reduced penetrance.

Table 7.1 Characteristics of seizure and pain of the B family

ID	Age of onset	Gender	Seizure pattern	Febrile convulsion	Status Epilepticus	Pain	MRI	EEG
8		F	fainting only	_	_	_	_	_
12	5	M	Partial seizure	_	_	_	_	_
14	9	M	_	_	_	Atypical back pain which is always evoked by startle response, usually starts at left lower back and then propagates along his spine up to head, usually lasts less then 1 minute with spontaneous recovery.	Multiple white patches in periventricular white matter, demyelination change is likely	_
16	early thirties	F	Generalized seizure and complex partial seizure	_	_	_	_	_
20	uncertain	F	Simple partial seizures	_	_	Migraine	Normal	Normal
22	childhood	M	Complex partial seizure, simple partial seizure	_	_	_	_	_
25	20	M	3 generalized seizure, all evoked by alcohol	_	_	_	_	_

(continued)

Table 7.1 Characteristics of seizure and pain of the B family (continued)

ID	Age of onset	Gender	Seizure pattern	Febrile convulsion	Status Epilepticus	Pain	MRI	EEG
28	2.5	M	Complex partial seizure with secondary generalization	+	+	Migraine/ post seizure headache	normal	Sharpened delta waves which contain mixed spikes, maybe generalized from right side, most obvious in centro-sylvian and anterior temporal area
29	2	M	Complex partial seizure with 2ndary generalization	+	+	post seizure headache	Right hippocampal sclerosis	occasional sharp, spike and slow wave elements are seen over the right hemisphere maximum over the anterior temporal region

7.2.3 Parameters in linkage analysis

The disease allele gene frequency was set as 0.0001. The gene frequencies were provided by deCODE Genetics. The recombination increment was 0.1, started with 0.1 and the finishing value was 0.45. The LOD score was also calculated at θ= 0, 0.01, and 0.05. (θ= recombination fraction). When the mode of inheritance was set as AD with a reduced penetrance, the penetrance rate was set as 0.8.

7.2.4 Results

This pedigree was analyzed in three different ways. First, this pedigree was analyzed assuming that migraine, pain and mesial temporal lobe epilepsy were caused by the same gene. The analysis result is presented in Table 7.2 showing the maximum LOD score (2.37) was obtained at θ = 0.0 at marker D6S1660, at 6p22.3 – 6p21.33. Multipoint analysis showed a much smaller LOD (0.773) which suggested that there was recombination event that was not picked up by two point parametric linkage analysis. Further fine mapping (Table 7.4) and haplotype analysis (Figure 7.2) confirmed that recombination had occurred. The haplotype analysis showed that there was homozygosity in marker D6S1660 in 4 affected individuals; this uninformative marker could lead to a false positive LOD score. When this pedigree was analyzed under AD with a reduced penetrance mode, the maximum LOD score was 2.19 at θ = 0.0 at the same marker D6S1660 (Table 7.3).

Table 7.2 LOD Scores in chromosome 6 (AD with a full penetrance)

θ / ***Marker***	***0.0***	***0.01***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***
D6S422	-infini	-4.62	-1.80	-0.66	0.22	0.43	0.30
D6S1660	***2.37***	***2.33***	***2.17***	***1.96***	***1.51***	***1.04***	***0.53***
D6S273	-infini	-2.47	-1.04	-0.45	0.03	0.18	0.15

θ: recombination fraction

Table 7.3 Fine mapping of chromosome 6

θ / Marker	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D6S422	-infini	-4.62	-1.80	-0.66	0.22	0.43	0.30
D6S1588	-infini	-2.59	-1.28	-0.76	-0.28	-0.06	0.02
D6S1660	***2.37***	***2.33***	***2.17***	***1.96***	***1.51***	***1.04***	***0.53***
D6S461	-infini	-1.92	-0.53	0.02	0.40	0.43	0.27
D6S299	-infini	-3.26	-1.30	-0.54	0.06	0.23	0.18
D6S306	-infini	-2.10	-0.69	-0.15	0.22	0.25	0.15
D6S276	-infini	-4.74	-2.12	-1.13	-0.36	-0.1	-0.02
D6S273	-infini	-2.47	-1.04	-0.45	0.03	0.18	0.15

θ: recombination fraction

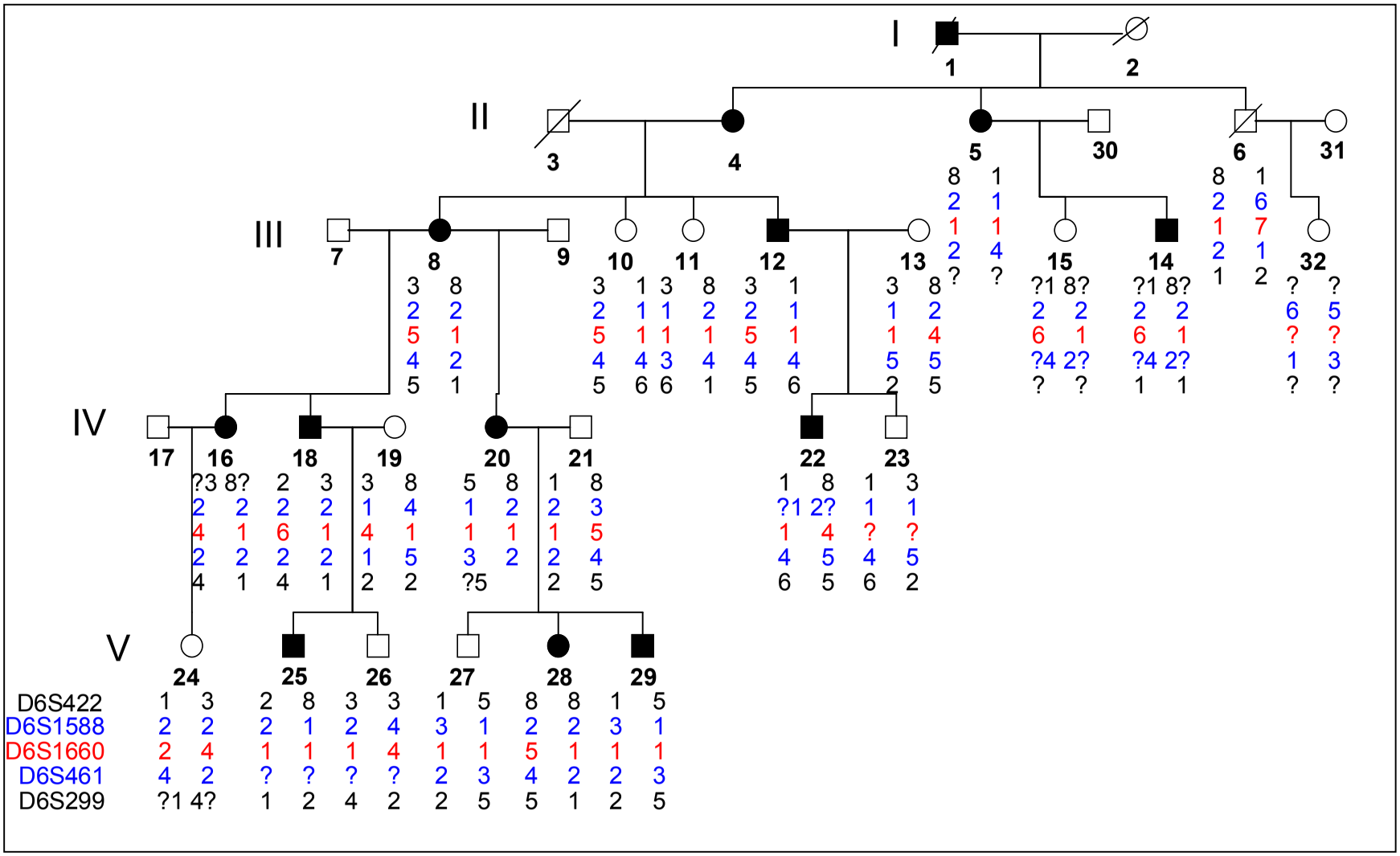
Table 7.4 LOD Scores in chromosome 6(AD with a reduced penetrance)

θ / Marker	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D6S422	-8.40	-1.97	-0.55	0.03	0.45	0.48	0.29
D6S1660	***2.19***	***2.15***	***2.00***	***1.80***	***1.39***	***0.95***	***0.48***
D6S273	**-3.66**	**-0.91**	**-0.21**	**0.08**	**0.29**	**0.29**	**0.19**

θ: recombination fraction

Fine mapping in this pedigree failed to fine a marker that showed a LOD greater than 3, but did help to narrow down the region of interest. However, there are only two genes in the region narrowed down: the prolactin gene and the HDGFL1 (hepatoma derived growth factor-like 1) gene, but neither of them seems likely to be related in epileptogenesis so far. We therefore, excluded this region identified on chromosome 6 and the finding suggest that the migraine, pain syndrome and temporal lobe seizures were unlikely to be caused by the same gene.

Figure 7.2 Haplotype analysis of chromosome 6



In the second parametric analysis; only subjects with seizures were marked as affected and individuals with the pain syndrome only were marked unaffected, i.e III-14 and II-5 were marked unaffected in this pedigree. The maximum LOD score was 1.67 at θ = 0.1 at marker D4S1586 (Table 7.5) and no LOD scores were larger than 2 in this analysis.

Table 7.5 LOD of chromosome 4 (seizures only, AD with a full penetrance)

θ ***Marker***	***0.0***	***0.01***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***
D4S1575	-infini	-1.38	-0.63	-0.31	-0.03	0.06	0.06
D4S424	-infini	-2.60	-0.52	0.26	0.76	0.75	0.47
D4S1586	-infini	1.04	1.59	1.67	1.49	1.12	0.62
D4S2962	-infini	-5.24	-1.95	-0.73	0.19	0.42	0.32
D4S3046	-infini	0.14	0.82	1.05	1.08	0.88	0.52
D4S1539	**infini**	**-7.39**	**-3.38**	**-1.81**	**-0.52**	**-0.04**	**0.07**

θ: recombination fraction

The second analysis was therefore inconclusive and did not support the hypothesis that all individuals with seizures in this pedigree shared a disease gene, V-25 and his father IV-18 were further excluded as affected individuals in the third parametric analysis, based on the clinical presentations. We did this on the assumption that V-25 who had three generalized seizures all evoked by alcohol with no other symptoms suggesting mesial temporal lobe epilepsy, was a phenocopy. His father IV-18 had no clinical symptoms suggesting epilepsy but was marked as a carrier in the first and second parametric analysis.

The third parametric analysis showed a maximum LOD score of 2.35 at θ = 0.0 at marker D3S1266, at 3p23-3p24 (Table 7.6). The LOD score of multipoint analysis was 2.72. Haplotype analysis (Figure 7.3) shows that there might be a disease locus in this region, although the haplotype analysis in the individuals of the second and third generations was hampered by the fact that their parents were not genotyped and the phase was unknown. It was also possible that II-5, III-10 and III-11 might share the same haplotype (2-2-3-3-7) with the affected individuals, thus there could be reduced penetrance. When analyzed under the mode of a reduced penetrance, the maximum LOD score was 2.17 at θ = 0.0 at marker D3S1266 (Table 7.7), and there were another 2 LOD scores which were larger than 2: 2.15 at θ=0.0 at D5S674 (Table 7.8) and 2.15 at θ=0.0 at D7S672 (Table

7.9). The haplotype analysis of chromosome 5 (Figure 7.4) and chromosome 7 (Figure 7.5) showed that the haplotypes segregated with affected individuals both in the regions of 5p13.3-5p-13.2 and 7q11.22-7q21.11, although the haplotypes of the individuals in the second and third generation could not be considered certain because their parents were not genotyped and the phase was unknown.

Because this pedigree had only 7 affected individuals, 2.35 (or 2.17 under AD with reduced penetrance mode) might be the largest LOD score this pedigree could have through parametric linkage analysis. From the haplotype analysis, all three regions identified appeared equally likely to be the candidate disease locus in this pedigree when analyzed under the mode of AD with a reduced mode. I therefore investigated the genes within these regions, and sequenced gene in the region identified on chromosome 3.

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Table 7.6 LOD scores on chromosome 3 (phenocopy excluded, AD with a full penetrance)

θ / *Marker*	*0.0*	*0.01*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*
D3S1567	-infini	-1.45	-0.81	-0.57	-0.33	-0.17	-0.07
D3S2335	0.54	0.56	0.61	0.63	0.56	0.42	0.23
D3S1266	***2.35***	***2.31***	***2.14***	***1.94***	***1.51***	***1.07***	***0.57***
D3S3547	1.18	1.17	1.12	1.05	0.87	0.63	0.34
D3S3521	-infini	-3.17	-1.03	-0.19	0.40	0.49	0.34

θ: recombination fraction

Table 7.7 LOD scores on chromosome 3 (phenocopy excluded, AD with a reduced penetrance)

θ / *Marker*	*0.0*	*0.01*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*
D3S1567	-0.66	-0.62	-0.52	-0.44	-0.31	-0.19	-0.08
D3S2335	0.43	0.44	0.48	0.49	0.46	0.36	0.20
D3S1266	***2.17***	***2.14***	***1.98***	***1.79***	***1.39***	***0.97***	***0.51***
D3S3547	1.08	1.07	1.02	0.96	0.79	0.57	0.31
D3S3521	-8.38	-2.25	-0.74	-0.12	0.33	0.39	0.26

Figure 7.3 Haplotypes analyses of markers within 3p24.1-3p24.2

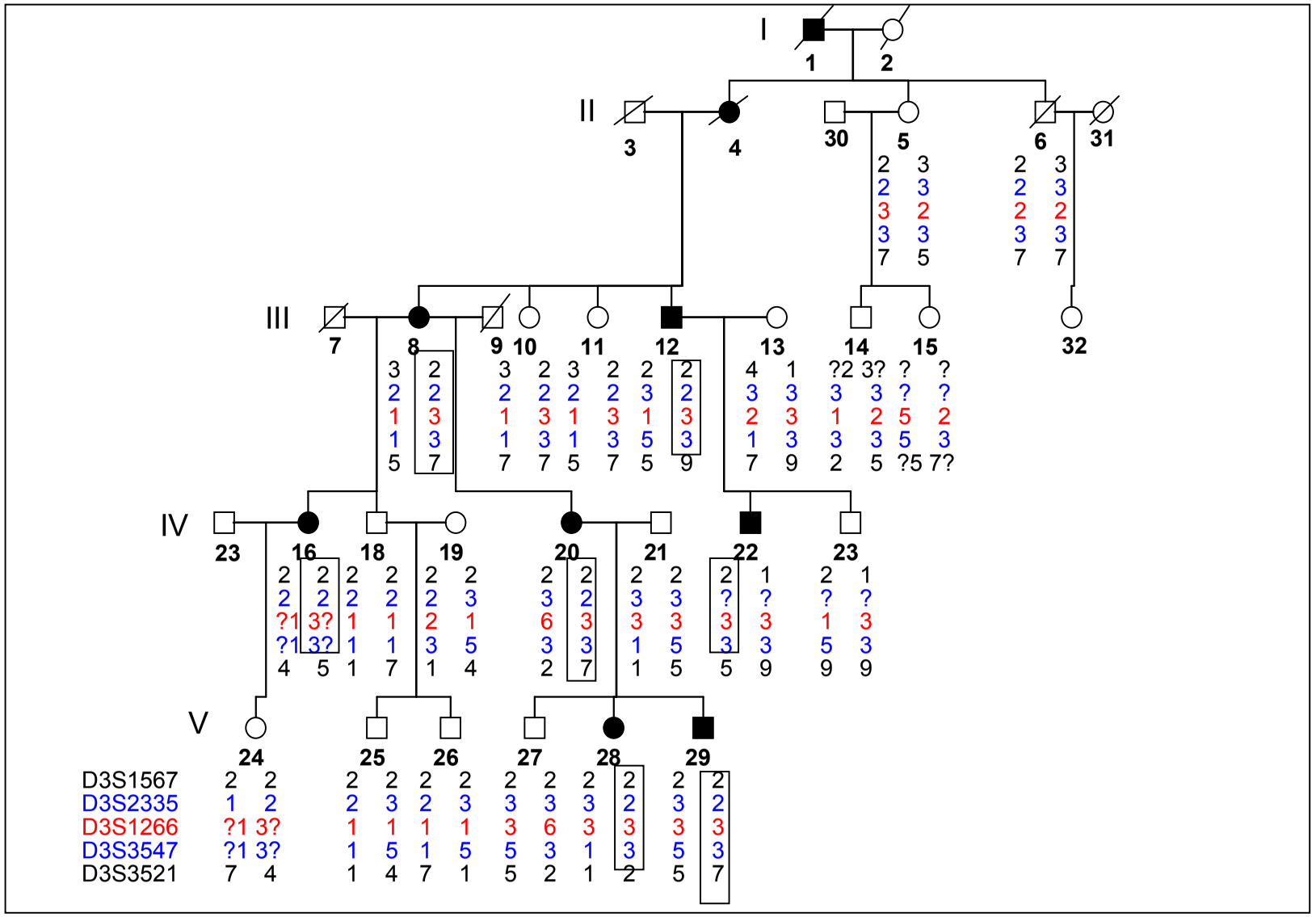


Table 7.8 LOD scores on chromosome 5 (phenocopy excluded, AD with a reduced penetrance)

θ / Marker	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D5S2081	-8.74	-2.89	-1.53	-1.01	-0.56	-0.34	-0.16
D5S2031	-2.40	0.20	0.80	0.97	0.96	0.76	0.44
D5S674	***2.15***	***2.13***	***2.03***	***1.88***	***1.51***	***1.07***	***0.57***
D5S2021	-3.61	-0.92	-0.25	0.00	0.18	0.20	0.14
D5S628	**0.09**	**0.08**	**0.07**	**0.05**	**0.03**	**0.01**	**0.00**

Table 7.9 LOD scores on chromosome 7 (phenocopy excluded, AD with a reduced penetrance)

θ / Marker	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D7S502	-4.02	-1.24	-0.47	-0.13	0.14	0.20	0.14
D7S2500	-5.37	-2.00	-1.10	-0.62	-0.13	0.07	0.11
D7S672	***2.15***	***2.13***	***2.06***	***1.92***	***1.55***	***1.07***	***0.54***
D7S2443	0.81	0.81	0.82	0.82	0.74	0.58	0.33
D7S2485	-3.21	-0.52	0.14	0.38	0.52	0.47	0.29

Figure 7.4 Haplotypes analyses of markers within 5p13.3-5p13.2

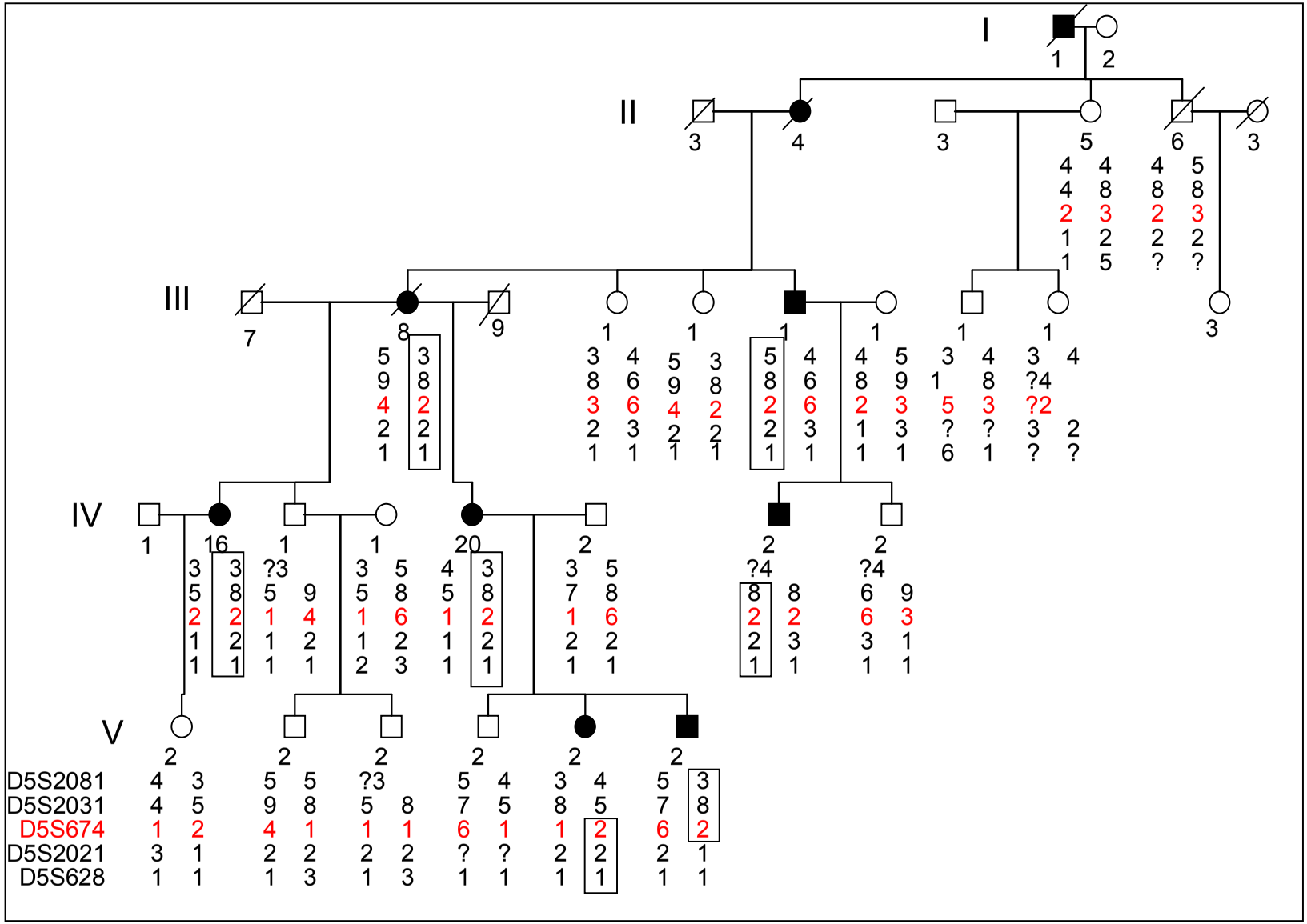
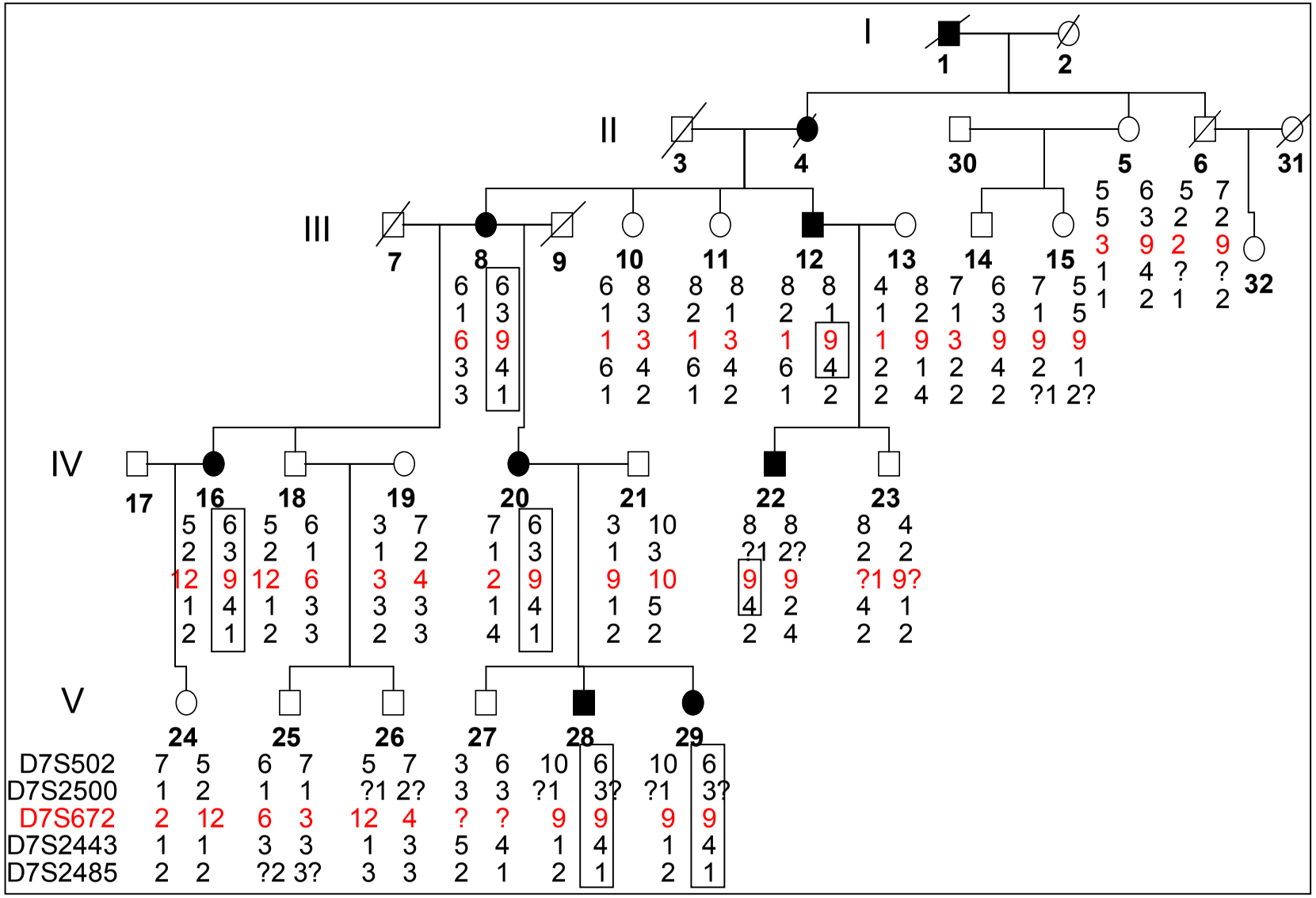


Figure 7.5 Haplotypes analyses of markers within 7q11.22-7q21.11



7.3 Candidate genes on chromosome 3 and gene sequencing

Candidate genes were screened in this region (3p24.2-3p24.1), inclusion priorities are the ion channel genes and genes that express in the brain with no other criteria set. Seven genes were identified as candidates in this region. The function of these candidate genes are presented in Table 7.12.

Five of the seven genes were sequenced on 5 individuals only (III-8, IV-20, IV-21, V-28, and V-29) in this pedigree. The five genes sequenced were SLC4A7, LRRC3B, AZI2, NEK10, and ZCWPW2. No rare mutations or disease causing polymorphisms were found in the exons of these genes. Table 7.13 summarized the polymorphisms found in gene sequencing. The polymorphisms found in the sequencing all segregated with mesial temporal lobe epilepsy in this small sample. Several polymorphisms were found in the introns near the splicing sites (within a distance of 50 bp).

7.4 Genes within 5p13.3-5p13.2

There are 32 known genes and pseudo-genes located within 5p14.3-5p 13.2. Their functions are summarized in Table 7.14. There are no ion channel genes included. None of the genes in this region were sequenced in this study.

7.5 Genes within 7q11.22-7q21.11

There are 105 known genes and pseudo-genes located within 7q11.22-7q21.11. Their functions are summarized in Table 7.15. None of the genes in this region were sequenced in this study.

7.6 Summary

The aim of this study was to identify the genetic basis of the familial epilepsy, to determine the mode of inheritance in this family, to identify linked regions, and to sequence candidate genes within the linked regions. In summary, we found that the inheritance mode of this family was likely to be AD with a reduced penetrance. We identified three regions of interest in this pedigree, sequenced 5 candidate genes on one of the region identified (3p24.2-3p24.1) on five individuals, although no pathological mutations were found. There are another 2 regions of interest await further investigation.

Table 7.12 Candidate genes in 3p24.2-3p24.1

Official Symbol	Official Full Name	Function Summary	sequencing result
EOMES	Eomesodermin homolog (Xenopus laevis)	This gene encodes a member of a conserved protein family that shares a common DNA-binding domain, the T-box. T-box genes encode transcription factors involved in the regulation of developmental processes. A occurred gene disrupted in mice is shown to be essential during trophoblast development and gastrulation.	Not successfully sequenced
LRRC3B	Leucine rich repeat containing 3B	Also named LRP15, expression is extremely high in brain kidney and lung. Several reports showed that it functions as protein interaction, signal transduction, cell adherence, development, DNA repair, gene recombination and transcription according to its structure. Some reports showed that it might have relation to colon cancer and leukemia, however the real function is unknown.(Tian *et al.* 2009).	no mutation found
NEK10	NIMA (never in mitosis gene a)-related kinase 10	Unknown. Possible function includes ATP binding, kinase activity, magnesium ion binding, nucleotide binding, protein serine/threonine kinase activity, protein tyrosine kinase activity, and transferase activity. No related human disease has been reported in relation to this gene.	No mutation found
AZI2	5-azacytidine induced 2	AZI2, or NAP1, contributes to the activation of NFKB (see MIM 164011)-dependent gene expression by activating IKK-related kinases, such as NAK	no mutation found

(continued)

Table 7.12 Candidate genes in 3p24.2-3p24.1 (continued)

Official Symbol	Official Full Name	Function Summary	sequencing result
ZCWPW2	zinc finger, CW type with PWWP domain 2	Function of this gene is not known yet. Possibly related to zinc ion binding or metal ion binding.	No mutation found
RBMS3	RNA binding motif, single stranded interacting protein	The protein encoded by this gene is a member of a small family of proteins which bind single stranded DNA/RNA. The observation that this protein localizes mostly in the cytoplasm suggests that it may be involved in a cytoplasmic function such as controlling RNA metabolism, rather than transcription. Multiple alternatively spliced transcript variants encoding different isoforms have been found for this gene. The latest study showed that the depletion of this gene might be associated with neuroblastoma (Carén *et al.* 2008)	not successfully sequenced
SLC4A7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	Functions as Na/HCO3 cotransporter, which regulates intracellular pH and may play a role in bicarbonate salvage in secretory epithelia. May also have an associated sodium channel activity.	No mutation found

Table 7.13 Sequence result summary

Gene	Position	polymorphism	Frequencies in Caucasian population	Type of polymorphism
SLC4A7	Exon 3	210A/G	A: 0.758, G:0.242	Synonymous
SLC4A7	Exon 7	846T/C	A:0.720, G: 0.280	Synonymous
SLC4A7	Exon 7	976G/A, Gly>Lys	A: 0.080, G:0.920,	Non- Synonymous
SLC4A7	Exon 16	2319C/T	C: 0.750, T: 0.250	Synonymous
SLC4A7	Exon 24	3499T/C	T: 0.092, C: 0.908	Synonymous
LRRC3B	Exon 1	330A/T	N/A	Synonymous
NEK10	Exon 20	1684A/G	A: 0.742, G: 0.259	Synonymous
NEK10	Exon 24	2071C/G	C: 0.491, G: 0.508	Synonymous
AZI2	no polymorphisms found in gene sequencing			
ZCWPW2				

Table 7.14 Functions of the genes within 5p14.3-5p13.2

GENE ID	FUNCTION
CDH12	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.
PMCHL1	Expressed in developing brain, found in fetal newborn and adult brain, function undetermined
PRDM9	May be involved in transcriptional regulation
CDH10	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.
CDH9	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.
CDH6	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types.
RNASEN	Executes the initial step of microRNA (miRNA) processing in the nucleus, that is cleavage of pri-miRNA to release pre-miRNA. Involved in pre-rRNA processing. Cleaves double-strand RNA and does not cleave single-strand RNA.
PDZD2	possibly be involved in intracellular signalling, this gene is upregulated in primary prostate tumours and may be involved in the early stages of prostate tumourigenesis.
GOLPH3	The protein encoded by this gene is a peripheral membrane protein of the Golgi stack and may have a regulatory role in Golgi trafficking.
MTMR12	Inactive phosphatase that plays a role as an adapter for the phosphatase myotubularin to regulate myotubularin intracellular location.
ZFR	Involved in postimplantation and gastrulation stages of development. Involved in the nucleocytoplasmic shuttling of STAU2. Binds to DNA and RNA.
SUB1	General coactivator that functions cooperatively with TAFs and mediates functional interactions between upstream activators and the general transcriptional machinery. May be involved in stabilizing the multiprotein transcription complex. Binds single-stranded DNA. Also binds, in vitro, non-specifically to double-stranded DNA.

(continued)

Table 7.14 Functions of the genes within 5p14.3-5p13.2 (continued)

GENE ID	FUNCTION
NPR3	Receptor for natriuretic peptide hormones. Has broad specificity and can bind several distinct natriuretic peptides, including atrial natriuretic peptide (NPPA) and brain natriuretic peptide (NPPB).
TARS	A human gene that catalyze the aminoacylation of tRNA by their cognate amino acid.
ADAMTS12	The enzyme encoded by this gene contains eight TS-1 motifs. It may play roles in pulmonary cells during fetal development or in tumor processes through its proteolytic activity or as a molecule potentially involved in regulation of cell adhesion.
RXFP3	Receptor for relaxin-3. Binding of the ligand inhibit cAMP accumulation
SLC45A2	Melanocyte differentiation antigen. May transport substances required for melanin biosynthesis (By similarity).
C1QTNF3	Function undetermined.
RAI14	Function undetermined.
TTC23L	Function undetermined.
BXDC2	Required for biogenesis of the 60S ribosomal subunit
RAD1	Component of the 9-1-1 cell-cycle checkpoint response complex that plays a major role in DNA repair. The 9-1-1 complex is recruited to DNA lesion upon damage by the RAD17-replication factor C (RFC) clamp loader complex.
DNAJA1	Co-chaperone of Hsc70. Seems to play a role in protein import into mitochondria.
AGXT2	The protein encoded by this gene is a class III pyridoxal-phosphate-dependent mitochondrial aminotransferase. It catalyzes the conversion of glyoxylate to glycine using L-alanine as the amino donor.
RPLR	This is a receptor for the anterior pituitary hormone prolactin (PRL). Isoforms 4 and 6 are unable to transduce prolactin signaling
SPEF2	Required for correct axoneme development
IL7R	The protein encoded by this gene is a receptor for interleukine 7 (IL7). The function of this receptor requires the interleukin 2 receptor, gamma chain (IL2RG), which is a common gamma chain shared by the receptors of various cytokines, including interleukine 2, 4, 7, 9, and 15. This protein has been shown to play a critical role in the V(D)J recombination during lymphocyte development. This protein is also found to control the accessibility of the TCR gamma locus by STAT5 and histone acetylation.

(continued)

Table 7.14 Functions of the genes within 5p14.3-5p13.2 (continued)

GENE ID	FUNCTION
CAPSL	Function undetermined.
UGT3A1	UDP-glucuronosyltransferases catalyze phase II biotransformation reactions in which lipophilic substrates are conjugated with glucuronic acid to increase water solubility and enhance excretion. They are of major importance in the conjugation and subsequent elimination of potentially toxic xenobiotics and endogenous compounds.
UGT3A2	UDP-glucuronosyltransferases catalyze phase II biotransformation reactions in which lipophilic substrates are conjugated with glucuronic acid to increase water solubility and enhance excretion. They are of major importance in the conjugation and subsequent elimination of potentially toxic xenobiotics and endogenous compounds.
LMBRD2	Function undetermined.
	Substrate recognition component of a SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complex which mediates the ubiquitination and subsequent proteasomal degradation of target proteins involved in cell cycle progression, signal transduction and transcription.
RANBP3L	Function undetermined.
SLC1A3	Transports L-glutamate and also L- and D-aspartate. Essential for terminating the postsynaptic action of glutamate by rapidly removing released glutamate from the synaptic cleft. Acts as a symport by cotransporting sodium.
NIPBL	Probably plays a structural role in chromatin. Involved in sister chromatid cohesion, possibly by interacting with the cohesin complex.
WDR70	Function undetermined.
GDNF	Neurotrophic factor that enhances survival and morphological differentiation of dopaminergic neurons and increases their high-affinity dopamine uptake.

Table 7.15 Functions of the genes within 7q11.22-7q21.11

GENE ID	FUNCTION
AUTS2	Autism candidate gene, involved in neurodevelopment and neurotransmission.
WBSCR17	May catalyze the initial reaction in O-linked oligosaccharide biosynthesis, the Transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on the protein receptor.
CALN1	May play a role in the physiology of neurons and is potentially important in memory and learning.
POM121	Essential component of the nuclear pore complex (NPC). The repeat-containing domain may be involved in anchoring components of the pore complex to the pore membrane. When overexpressed in cells induces the formation of cytoplasmic annulate lamellae.
NSUN5C	May have S-adenosyl-L-methionine-dependent methyl-transferase activity (Potential).
TRIM74	Function undetermined
NCF1B	May be required for activation of the latent NADPH oxidase (necessary for superoxide production) (By similarity)
TRIM50	Function undetermined
NSUN5	May have S-adenosyl-L-methionine-dependent methyl-transferase activity (Potential). Gene is deleted in Williams syndrome, a multisystem Developmental disorder caused by the deletion of contiguous genes at 7q11.23.
FZD9	The FZD9 gene is located within the Williams syndrome common deletion region of chromosome 7, and heterozygous deletion of the FZD9 gene may contribute to the Williams syndrome phenotype. FZD9 is expressed predominantly in brain, testis, eye, skeletal muscle, and kidney.
BAZ1B	Forms a chromatin eveloped g complex that mobilizes nucleosomes and reconfigures irregular chromatin to a regular nucleosomal array structure. This gene is deleted in Williams-Beuren syndrome
BCL7B	May play a role in lung tumour development or progression
TBL2	This gene encodes a member of the beta-transducin protein family. Most proteins of the beta-transducin family are involved in regulatory functions. This protein is possibly involved in some intracellular signaling pathway. This gene is deleted in Williams-Beuren syndrome.

(continued)

Table 7.15 Functions of the genes within 7q11.22-7q21.11 (continued)

GENE ID	FUNCTION
MLXIPL	This gene encodes a basic helix-loop-helix leucine zipper transcription factor of the Myc/Max/Mad superfamily. This protein forms a heterodimeric complex and binds and activates, in a glucose-dependent manner, carbohydrate response element (ChoRE) motifs in the promoters of triglyceride synthesis genes. The gene is deleted in Williams-Beuren syndrome.
VPS37D	Component of the ESCRT-I complex, a regulator of vesicular trafficking process. Required for the sorting of endocytic ubiquitinated cargos into multivesicular bodies. May be involved in cell growth and differentiation.
DNAJC30	This intronless gene encodes a member of the DNAJ molecular chaperone homology domain-containing protein family. This gene is deleted in Williams syndrome.
STX1A	Potentially involved in docking of synaptic vesicles at presynaptic active zones. May play a critical role in neurotransmitter exocytosis.
WBSCR22	Methyltransferase that may act on DNA.
ABHD11	This gene encodes a protein containing an alpha/beta hydrolase fold domain. This gene is deleted in Williams syndrome, a multisystem developmental disorder caused by the deletion of contiguous genes at 7q11.23.
CLDN3	The protein encoded by this intronless gene, a member of the claudin family, is an integral membrane protein and a component of tight junction strands.
CLDN4	This gene encodes an integral membrane protein, which belongs to the claudin family. The protein is a component of tight junction strands and may play a role in internal organ development and function during pre- and postnatal life.
WBSCR27	This gene encodes a protein belonging to ubiE/COQ5 methyltransferase family. The gene is deleted in Williams syndrome.
WBSCR28	Function undetermined
ELN	Major structural protein of tissues such as aorta and nuchal ligament, which must expand rapidly and recover completely. Molecular determinant of the late arterial morphogenesis, stabilizing arterial structure by regulating proliferation and organization of vascular smooth muscle (By similarity).
LIMK1	Protein kinase which regulates actin filament dynamics. Phosphorylates and inactivates the actin binding/ depolymerizing factor cofilin, thereby stabilizing the actin cytoskeleton. Isoform 3 has a dominant negative effect on actin cytoskeletal changes. May be involved in brain development.

(continued)

Table 7.15 Functions of the genes within 7q11.22-7q21.11 (continued)

GENE ID	FUNCTION
WBSCR1	This gene encodes one of the translation initiation factors, which functions to stimulate the initiation of protein synthesis at the level of mRNA utilization. This gene is deleted in Williams syndrome.
LAT2	Involved in FCER1 (high affinity immunoglobulin epsilon receptor)-mediated signaling in mast cells. May also be involved in BCR (B-cell antigen receptor)-mediated eveloped in B-cells and FCGR1 (high affinity immunoglobulin gamma Fc receptor I)-mediated eveloped in myeloid cells. Couples activation of these receptors and their associated kinases with distal intracellular events through the recruitment of GRB2.
CYLN2	Seems to link microtubules to dendritic lamellar body (DLB), a membranous organelle predominantly present in bulbous dendritic appendages of neurons linked by dendrodendritic gap junctions. May operates in the control of brain-specific organelle translocations (By similarity).
GTF2IRD1	May be a transcription regulator involved in cell-cycle progression and skeletal muscle differentiation. May repress GTF2I transcriptional functions, by preventing its nuclear residency, or by inhibiting its transcriptional activation. May contribute to slow-twitch fiber type specificity during myogenesis and in regenerating muscles.
NCF1	May be required for activation of the latent NADPH oxidase (necessary for superoxide production) (By similarity).
GTF2I	This gene encodes a multifunctional phosphoprotein with roles in transcription and signal transduction. It is deleted in Williams-Beuren syndrome.
GTF2IRD2	This gene is unusual in that its coding sequence is mostly derived from Charlie8 repeat elements. However, there is mRNA and EST evidence to suggest that this gene is transcribed, and the encoded protein has a homolog in mouse, with which it shares 78% sequence identity. The exact function of this gene product is not known.
GTF2IRD2B	This gene encodes a glycosylated phosphoprotein with a leucine zipper motif, two helix-loop-helix motifs (I repeats) that are similar to domains found in the TFII-I family of transcription factors, one CHARLIE8 transposable element-like sequence, and a BED zinc finger.
WBSCR16	This gene encodes an RCC1-like G-exchanging factor. It is deleted in Williams syndrome.

(continued)

Table 7.15 Functions of the genes within 7q11.22-7q21.11 (continued)

GENE ID	FUNCTION
PMS2L2	Function undetermined
TRIM73	Function undetermined
NSUN5B	This gene shares high sequence similarity with the genes WBSCR20A and WBSCR20C; these three genes are the products of gene duplication during evolution. The protein encoded by this gene is smaller than the proteins encoded by WBSCR20A and WBSCR20C. This gene is deleted in Williams syndrome.
POM121B	Putative component of the nuclear pore complex (NPC). The repeat-containing domain may be involved in anchoring components of the pore complex to the pore membrane (By similarity).
PMS2L3	Function undetermined
HIP1	Plays a role in clathrin-mediated endocytosis and trafficking. Involved in regulating AMPA receptor trafficking in the central nervous system in an NMDA-dependent manner. Enhances androgen receptor (AR)-mediated transcription. May act as a proapoptotic protein that induces cell death by acting through the intrinsic apoptosis pathway. Binds 3-phosphoinositides (via ENTH domain). May act through the ENTH domain to promote cell survival by stabilizing receptor tyrosine kinases following ligand-induced endocytosis. May play a functional role in the cell filament networks. May be required for differentiation, proliferation, and/or survival of somatic and germline progenitors.
CCL24	Chemotactic for resting T-lymphocytes, and eosinophils. Has lower chemotactic activity for neutrophils but none for monocytes and activated lymphocytes. Is a strong suppressor of colony formation by a multipotential hematopoietic progenitor cell line. Binds to CCR3.
CCL26	Chemotactic for eosinophils and basophils. Binds to CCR3.
POR	This gene encodes an endoplasmic reticulum membrane oxidoreductase with an FAD-binding domain and a flavodoxin-like domain. The protein binds two cofactors, FAD and FMN, which allow it to donate electrons directly from NADPH to all microsomal P450 enzymes. Mutations in this gene have been associated with various diseases, including apparent combined P450C17 and P450C21 deficiency, amenorrhea and disordered steroidogenesis, congenital adrenal hyperplasia and Antley-Bixler syndrome.

(continued)

Table 7.15 Functions of the genes within 7q11.22-7q21.11 (continued)

GENE ID	FUNCTION
RHBDD2	Function undetermined
TMEM120A	Function undetermined
STYXL1	Probable pseudophosphatase. Contains a Ser residue instead of a conserved Cys residue in the dsPTPase catalytic loop which probably renders it catalytically inactive. . The binding pocket may be however sufficiently preserved to bind phosphorylated substrates, and maybe protect them from phosphatases.
MDH2	Malate dehydrogenase catalyzes the reversible oxidation of malate to oxaloacetate, utilizing the NAD/NADH cofactor system in the citric acid cycle. The protein encoded by this gene is localized to the mitochondria and may play pivotal roles in the malate-aspartate shuttle that operates in the metabolic coordination between cytosol and mitochondria.
HSPB1	The protein encoded by this gene is induced by environmental stress and developmental changes. The encoded protein is involved in stress resistance and actin organization and translocates from the cytoplasm to the nucleus upon stress induction. Defects in this gene are a cause of Charcot-Marie-Tooth disease type 2F (CMT2F) and distal hereditary motor neuropathy (dHMN).
YWHAG	Adapter protein implicated in the regulation of a large spectrum of both general and specialized eveloped pathway. Binds to a large number of partners, usually by recognition of a phosphoserine or phosphothreonine motif. Binding generally results in the modulation of the activity of the binding partner.
SRCRB4D	The scavenger receptor cysteine-rich (SRCR) superfamily is an ancient and highly conserved group of cell surface and/or secreted proteins, some of which are involved in the development of the immune system and the regulation of both innate and adaptive immune responses. Group B SRCR domains usually contain 8 regularly spaced cysteines that give rise to a well-defined intradomain disulfide-bond pattern.
ZP3	The mammalian zona pellucida, which mediates species-specific sperm binding, induction of the acrosome reaction and prevents post-fertilization polyspermy, is composed of three to four glycoproteins, ZP1, ZP2, ZP3, and ZP4. ZP3 is essential for sperm binding and zona matrix formation.

(continued)

Table 7.15 Functions of the genes within 7q11.22-7q21.11 (continued)

GENE ID	FUNCTION
DTX2	Regulator of Notch signalling, a signalling pathway involved in cell-cell communications that regulates a broad spectrum of cell-fate determinations. Probably acts both as a positive and negative regulator of Notch, depending on the developmental and cell context. Mediates the antineural activity of Notch, possibly by inhibiting the transcriptional activation mediated by MATCH1. Functions as an ubiquitin ligase protein in vitro, suggesting that it may regulate the Notch pathway via some ubiquitin ligase activity.
UPK3B	Component of the asymmetric unit membrane (AUM); a highly specialized biomembrane elaborated by terminally differentiated urothelial cells. May play an important role in AUM-cytoskeleton interaction in terminally differentiated urothelial cells. It also contributes to the formation of urothelial glycocalyx which may play an important role in preventing bacterial adherence (By similarity).
POMZP3	This gene appears to have resulted from a fusion of DNA sequences derived from 2 distinct loci, specifically through the duplication of two internal exons from the POM121 gene and four 3' exons from the ZP3 gene. The 5' end of this gene is similar to the 5` coding region of the POM121 gene which encodes an integral nuclear pore membrane protein. However, the protein encoded by this gene lacks the nuclear pore localization motif. The 3' end of this gene is similar to the last 4 exons of the zona pellucida glycoprotein 3 (ZP3) gene and the encoded protein retains one zona pellucida domain. Multiple protein isoforms are encoded by transcript variants of this gene.
CCDC146	Function undetermined
FGL2	May play a role in physiologic lymphocyte functions at mucosal sites.
PION	Function undetermined
PTPN12	The protein encoded by this gene is a member of the protein tyrosine phosphotase (PTP) family. PTPs are signalling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. This PTP contains a C-terminal PEST motif, which serves as a protein-protein interaction domain, and may regulate protein intracellular half-life. This PTP was found to bind and dephosphorylate the product of the oncogene c-ABL and thus may play a role in oncogenesis. This PTP was also shown to interact with, and dephosphorylate, various products related to cytoskeletal structure and cell adhesion, such as p130 (Cas), CAKbeta/PTK2B, PSTPIP1, and paxillin. This suggests it has a regulatory role in controlling cell shape and mobility.

(continued)

Table 7.15 Functions of the genes within 7q11.22-7q21.11 (continued)

GENE ID	FUNCTION
RSBN1L	Function undetermined
PHTF2	Function undetermined
TMEM60	Function undetermined
MAGI2	Seems to act as scaffold molecule at synaptic junctions by assembling neurotransmitter receptors and cell adhesion proteins. May play a role in regulating activin-mediated signalling in neuronal cells. Enhances the ability of PTEN to suppress AKT1 activation.

Section 8: Linkage analysis in familial mesial temporal lobe epilepsy – family W

8.1. Pedigree W

Family W was a large pedigree based in Swansea, South Wales. The complete family pedigree is shown in Appendix 1. A simplified pedigree showing the second, the third, and the fourth generation is presented in Figure 7.1. Twenty seven DNA samples were collected from this pedigree, and 20 of them were sent for genotyping.

8.2 Clinical presentation and investigation

Of these 20 individuals, 9 had mesial temporal lobe epilepsy, five were considered carriers and 6 were unaffected. Of the 9 individuals who had temporal lobe epilepsy, seven of them also had migraine, and 2 of these 7 subjects also had trigeminal neuralgia. The diagnosis of migraine was based on the ICHD-II Diagnostic Criteria for Migraine (OCCURRED, 2004). The clinical presentation and the medical investigations of the affected individuals in this pedigree are summarized in Table 7.1.

Detail medical history of II-1 and II-30 were not available, both were epileptic according to family members interviewed and there were no medical records available to confirm this. Their spouses II-2 and II-29 were unaffected. In the third generation, III-3 and III-4 were both unaffected, they denied having any seizure or fainting related events but four out of their four children were epileptic. IV-15 had cerebral palsy, whether this was related to her epilepsy could not be clarified because she did not agree to participate in this study and refused to be interviewed. IV-16 died at the age of 3, with a diagnosis of intractable seizures and severe learning disability. No further description or diagnosis of his epilepsy syndrome was available. IV-17 had typical manifestations of mesial temporal lobe epilepsy. She had febrile convulsions at the age of 8 months and her first tonic-clonic generalized epilepsy at the age of 31 months. There was a seizure free period for a few years in her childhood until she was 10 years old when generalized seizures relapsed. She became seizure free when she was 12 years old under the control of valproate until she was 15. She remained seizure free and was not on medication until the age of 18 when seizures

relapsed again with complex partial seizures, generalized tonic-clonic seizures, and several episodes of status epilepticus. Her paralytic migraine started around at this time. Her complex partial seizures started with unpleasant smells or tastes, followed with talking rubbish, flushed face and altered consciousness. Other symptoms were oro-facial automatism, and jerky limbs movement which was usually confined to right side. The duration of her complex partial seizures was within 30 seconds to a few minutes. Postictally she was often exhausted with severe headache. From the age of 18 to 26, anti-epileptic drugs including lamotrigine, gabapentin and vigabatrin were described but failed to control the seizures. Her MRI scan showed left hippocampal sclerosis and the EEG showed left temporal epileptiform discharge. She had left temporal lobectomy at the age of 26 and remained seizure free since. Left side trigeminal neuralgia was found after surgery. She had one son who had intractable epilepsy and severe learning disability (not shown in the pedigree) but the father of her child was unaffected. Her brother IV-18 had febrile convulsion, complex partial seizures and generalized seizures. His first seizure was a febrile convulsion when he was 3 months old. He denied frequent seizures but his partner had witnessed at least 1 episode and was aware of the existence of 4 other episodes of seizures during approximately one year time (2007). According to his partner who witnessed one episode of seizures, he fell down suddenly with no obvious warning, with whole body shaking, jerky limbs and wet himself. Confusion with slow recovery was noticed. He claimed that his seizure frequencies were low and had not had any medical investigation. He had two daughters who had not had any seizures by the time of the interview. His partner was unaffected.

III-5 was epileptic according to his wife. He had one generalized seizure after they were married and although a brain CT was done, the report was not available. Whether he was epileptic and what epilepsy syndrome he had could not be certain. III-6 was not epileptic, but four out of her five children with III-5 were affected and three of them had migraine, only one was free of symptoms of seizures or migraine. IV-19 had simple partial seizures, symptoms including becoming pale for a few seconds, accompanied by mumbling. When it happened, although he knew what he was going to say but could not avoid mumbling. No loss of consciousness had been noticed. IV-20 had generalized seizures, déjà vu, and hemiplegic migraine. Her seizure onset was at the age of 12 years old. According to her

description, she would suddenly fall to the ground without having any warning usually with arms outstretched accompanied with unidentifiable vocal sound. These were followed with shaking of the whole body with the eyes widely open, with or without tongue biting and loss of consciousness. She noticed that she often injured her right thumb during seizures. Stiffness of one side of the body was recalled sometimes during seizures but she could not be sure which side. She had déjà vu and the frequency was 1-2 per week, often with a strong feeling that she had been to some places familiar and was doing some familiar tasks. The onset of migraine was at the age of 15, the frequency of migraine was around 1-2 per week, often started with bright light and spots followed with darkened right side visual field and her right side body would become numb, eventually becoming right hemiplegic. Other less frequent symptoms were pain, and a sensation of needles over her head after the aura, severe pulsating headache at right side, feeling nauseous and vomiting. The duration of migraine could last 6 hours. Trigger factors including stress, bright light and sleep deprivation. IV-21 had simple partial seizures with déjà vu but was not certain about the frequency. Her déjà vu usually were feelings that she was in a place she actually had not been to or was at the middle of doing something but in the reality she was not. She also had migraine without aura, starting with right pulsating periorbital pain and gradually spreading to right head with nauseous sensation which could last for hours. Trigger factors were lack of sleep and tiredness. The other medical history of IV-21 included several episodes of pre-syncope or near fainting which usually relieved after chocolate intake, and 4-5 episodes of what have been taken to be sleep paralysis. IV-22 had simple partial seizures and migraine without aura. Her seizure onset was at the age of 12 when she started to have rising sensation of a stomach followed by giddiness lasting for seconds to minutes, and then tiredness. There was no vomiting, or symptoms suggesting abdominal diseases. The frequency ranged from 2 times per week to 2 times per month. She also had frequent déjà vu, around 4 times a week, with the feeling that she was suddenly in a familiar place. Her migraine was evoked by having cheese or seeing bright light. The pain was unilateral and throbbing in character and started with mild headache which gradually worsened. There was no aura. IV-23 was the only child of III-5 and III-6 and was free from any symptoms of seizures and migraine.

III-7 and III-8 denied any symptoms of epilepsy or migraine, despite careful interviewing. Two of their three children were epileptic. IV-24 was the only unaffected child. IV-25 was epileptic and his seizures onset was at the age of 4 with no history of febrile convulsion. His seizures included complex partial seizures and generalized tonic-clonic seizures. The complex partial seizures started with a sensation of an unpleasant smell and taste which lasted for several minutes, followed with head turning to one side (could not recall which side) and convulsions. He had déjà vu which started at the age of 8 years old that he might recall a scene suddenly which he was sure he had not been to. He was on Epilim and had been seizure free from the age of 13. He also had migraine without aura, but was infrequent. His migraine was throbbing or stabbing in character, located at right frontotemperal area, with no nauseous feeling. III-26 had complex partial seizures. The seizures onset was at the age of 4 years. As far as his parents could recall his first seizures clustered and were not febrile seizures. He denied having further seizures until the age of 22, when he developed complex partial seizures started with a funny and unpleasant taste and smell, followed by stiffness. The duration was about 1 minute. He was not on medication and refused to give blood sample because of needle phobia.

III-11, III-12 and III-13 were not epileptic. III-12 had a severe head injury falling from a pile of cargo placed on a truck, and was comatose for 3 week in the hospital. Prior to the accident she did not have history of seizures or migraine. After the accident, she recovered well but had chronic daily headache, impaired visual acuity (damaged optic nerve), and a damaged lacrimal gland as the consequence of the accident. Her relatives noticed also that a marked personality change with prominent indifference. She denied having seizures or déjà vu. According to the interview of III-12, her partner III-11 and III-13 had no symptoms suggesting epilepsy. Her daughters IV-27 and IV-28 were half sisters. IV-27 had déjà vu, migraine, and trigeminal neuralgia on the right side. Her déjà vu was quite frequent with the feeling as that she was in a place she actually had not been to, or felt certain in some circumstances about what was happening as if she had witnessed or experienced them before. She had several episodes of pre-syncope, starting with blurred vision, severe dizziness and sometimes mixed with a rising sensation in the stomach. No loss of consciousness or convulsion had occurred. Her migraine started with blurred vision and a right sided shooting headache, with occasionally light spots and bright lights seen before

the headache. The light was not “zig-zag” in shape. She usually also felt nauseous. The trigeminal neuralgia was located at right side. IV-28 had infrequent déjà vu about twice a year, experienced as if she was in a familiar place, or with a strong feeling that she had witnessed something happening before. The déjà vu episodes could happen at any time without any warning.

In the third generation, III-9, III-10 and III-14 were unaffected. Their children were not interviewed and they were not affected according to the parents.

Figure 8.1 Pedigree W

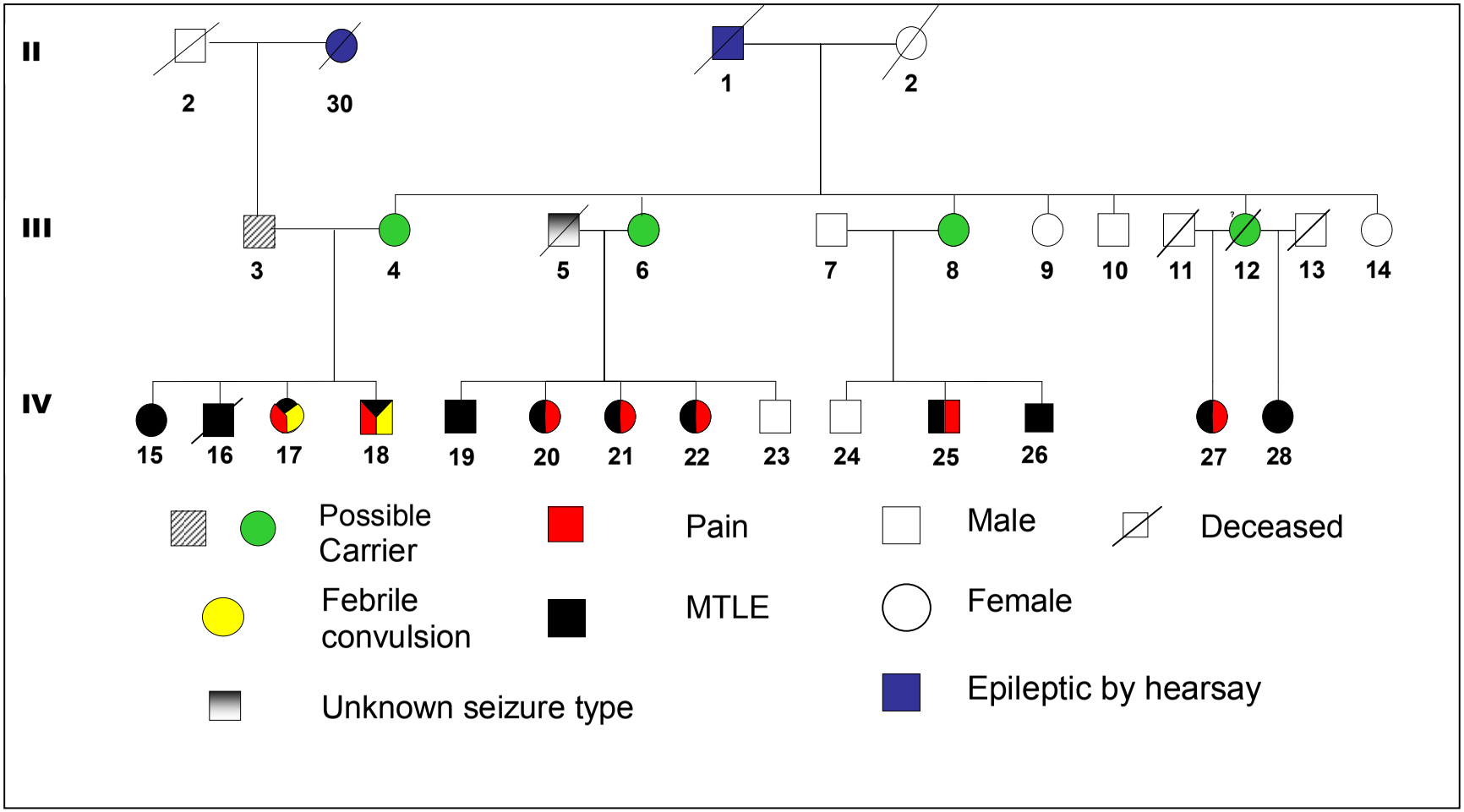


Table 8.1 Clinical presentation and investigation of affected individuals

ID	Gender	DNA samples	Age of onset	Seizures	Febrile convulsion	Migraine/ Trigeminal neuralgia	Medication
1	M	N/A	N/A	N/A	N/A	N/A	N/A
5	M	-	?	One generalized seizure, diagnosis unconfirmed	N/A	N/A	N/A
15	F	N/A	N/A	Unconfirmed type	N/A	N/A	N/A
16	M	-	?	Generalized	+	N/A	N/A
17	F	+	8 months old	Generalized and complex partial seizure. Status epilepticus(+)	+	Migraine with aura, Trigeminal neuralgia (+) after temporal lobectomy	Pregabalin
18	M	+	3 months old	Complex partial seizures, generalized seizures	+	-	nil
19	M	+	? 27 years old	Simple partial seizure(mumbling of words or pale looking for seconds)	-	-	nil
20	F	+	12 years old	Simple partial and generalized seizures	-	Migraine with aura	nil
21	F	+	uncertain	Simple partial seizures(Déjà vu)	-	Migraine without aura	nil
22	F	+	16 years old	Simple partial seizures (frequent Déjà vu, stomach rising sensation)	-	Migraine without aura	nil
25	M	+	4 years old	Complex partial seizures and secondary generalized seizures	-	Migraine without aura	seizure free after 13 y/o On Epilium

(continued)

Table 8.1 Clinical presentation and investigation of affected individuals (continued)

ID	Gender	DNA samples	Age of onset	Seizures	Febrile convulsion	Migraine/ Trigeminal neuralgia	Medication
26	M	**-**	4 years old	Simple and complex partial seizure	**-**	-	nil
27	F	**+**	Couldn't recall	Simple partial seizures (frequent Déjà vu)	**-**	Trigeminal neuralgia	nil
28	F	**+**	Couldn't recall	Simple partial seizures (Déjà vu)	**-**	**-**	nil
30	F	N/A	N/A	N/A	N/A	N/A	N/A

8.3 Phenotyping and the mode of inheritance

In this pedigree, some affected individuals did not have detailed medical investigation. In most individuals, the seizure frequencies were not high and the severity of seizures was mild. A few of them had stereotyped déjà vu only as the main clinical presentation.

My interpretation of the inheritance pattern in this pedigree was likely to be admixture effect that the susceptibility genes of epilepsy were brought in by unrelated founders: from individual II-1, II-30, and III-5. It was not possible to know whether these genes were the same or different. Assuming these genes were the same and individual III-3, III-4, III-6, III-8, and III-12 were carriers, the inheritance mode of this pedigree could be autosomal dominant (AD) with a incomplete penetrance. Male to male transmission and the occurrence of affected individuals were in more than one generation and no history of consanguity excluded X linked disease, mitochondrial disease, and autosomal recessive inheritance. This is considered to be an incomplete penetrance because in the third generation there were only carriers but no affected individuals. This hypothesis could explain why there were many affected individuals in the fourth generation.

However the probability of three unrelated individuals carrying the same susceptibility gene and brought it into the same pedigree is low. If the genes these founders carried were different, this pedigree would exhibit polygenic and this could explain the different seizure patterns, severities, and frequencies observed from the affected individuals in this pedigree. This seems to be likely that the possibility that the three founders were not carrying the same susceptible gene.

If the founders II-1 and II-30 were not considered epileptic because of the lack of evidence, and III-5 might not be epileptic because he had only one late onset seizure, III-3, III-4, III-6, III-8, and III-12 would less likely to be carriers. Under this assumption, to explain the cause of epilepsy in this pedigree entirely by genetics appeared difficult, because it would be very unlikely that the four (five) couples of the third generation (III-3 and III-4, III-5 and III-6, III-7and III-8, III-11, III-12, and III-13) were carrying the same susceptible gene to make the pedigree autosomal recessive (AR). Even though this family was based in a small valley of Swansea and inbreeding was possible, they seemed unlikely.

Another possible mode of inheritance is to consider II-1, II-30 and III-5 as unaffected individuals in which case the pedigree could be interpreted as showing a maternal

transmission pattern suggesting mitochondrial inheritance. Until now, there is no report that mesial temporal lobe epilepsy has been caused by mitochondrial disease, but mitochondrial functional impairment has been found in temporal lobe of those who have mesial temporal lobe epilepsy (Vielhaber *et al.* 2008).

Environmental factors might be part of the cause of mesial temporal lobe epilepsy in this pedigree, however no such factors shared between the affected individuals were found based on the information gathered from interviews.

In summary, the mode of inheritance of this pedigree is not certain. Several situations are considered but each had its uncertainty. Parametric analysis would not be suitable because the MOI could not be defined in the analysis, so Non-parametric linkage analysis was the choice in this pedigree. Two programs (MERLIN and SimWalk2) were chosen to do this analysis.

8.4 NPL analysis

8.4.1 MERLIN

Because this pedigree was too large for MERLIN, so II-29, II-30, IV-15, IV-16, and IV-26 (these individuals were not genotyped), III-14 (unaffected individuals, parents were not genotyped) and IV-23 (unaffected individuals, but one of their parents was not genotyped) and IV-24 (unaffected individual whose parents were fully genotyped) were trimmed off to suit the NPL analysis by using MERLIN. Trimming off unaffected individuals whose parents were fully genotyped would not affect the NPL analysis. However, in this pedigree, only IV-24 fulfilled these criteria.

8.4.1.2 Input files, parameters setting and error detection

The input files for MERLIN NPL were prepared by Mega2 using standard protocol. The allele frequencies were provided by deCODE Genetics. The affection states were assigned according to the pedigree shown in Figure8.1. Genotyping error was checked when preparing and converting the input files by Mega2. If genotyping errors were found they were adjusted by setting the incompatible genotype data to unknown. The formatted input files were checked again by PEDSTATS (part of the MERLIN package) before

starting the NPL analysis. Any file preparation errors and incompatible genotype data were corrected accordingly.

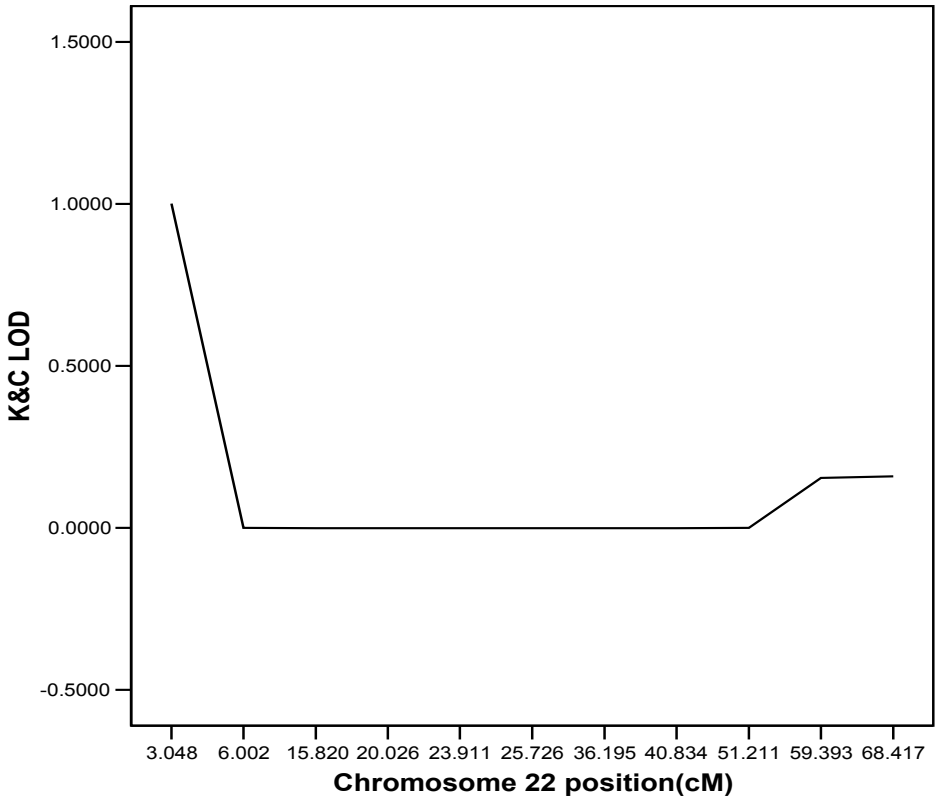
8.4.1.3 LOD scores obtained by MERLIN

The LOD score obtained by MERLIN is the *King and Cox LOD score*, (see Section 4). The maximum King and Cox LOD score obtained by MERLIN was 1.001 at marker D22S420 at chromosome 22 (*P value* = 0.01588) (Table 8.2). The expected maximum K&C LOD score was 2.006 in this pedigree. The LOD score distribution of chromosome 22 is shown in Figure 8.2. The result did indicate linkage.

Table 8.2 LOD scores obtained by MERLIN

Chr. 22	Position (Haldane, in cM)	Makers	Z score	Delta (δ)	LOD score (*King and Cox LOD*)	*P* value
22	***3.048***	***D22S420***	***1.279***	***7.602***	***1.001***	***0.01588***
22	6.002	D22S427	- 0.003	- 0.018	0	0.504
22	15.820	D22S539	- 0.091	- 0.018	- 0.001	0.5227
22	20.026	D22S1174	- 0.113	- 0.018	- 0.001	0.5253
22	23.911	D22S315	- 0.123	- 0.018	- 0.001	0.5263
22	25.726	D22S1154	- 0.125	- 0.018	- 0.001	0.5266
22	36.195	D22S531	- 0.126	- 0.018	- 0.001	0.5267
22	40.834	D22S1265	- 0.127	- 0.018	- 0.001	0.5268
22	51.211	D22S276	- 0.027	- 0.018	0	0.5125
22	59.393	D22S928	0.060	7.602	0.154	0.1996
22	68.417	D22S1170	0.062	7.602	0.159	0.1964

Figure 8.2 King &Cox LOD scores distribution obtained in MERLIN



8.4.2 Simwalk2

This pedigree was re-analyzed by SimWalk2 NPL, using the same parameter setting as used in MERLIN without trimming off any individual. The advantage of SimWalk2 is that it can analyze large pedigrees but the time needed for analysis using SimWalk2 NPL was much longer than the time needed for MERLIN NPL.

8.4.2.1 Parameters settings and error detection for Simwalk2

The input files were constructed from data stored in Linkage-format files by Mega2. The allele frequencies were provided by deCODE Genetics. Genotyping errors were checked by Simwalk2 by the "setup" option before entering any specified linkage analysis. Genotyping errors were adjusted by setting the incompatible data to unknown.

8.4.2.2 Location scores by SimWalk2

In Simwalk2, the location score is calculated from the equation:

$$-\log_{10}(p-value).$$

A p-value of 0.5 (i.e a location score of 0.3010 [$-\log_{10}(0.5)$]) indicates equal evidence for and against linkage. The maximum p-value was set as 1.0, the smaller the p value is, the higher the significance. In this SimWalk2 analysis, the default minimum p value was set as 0.0001, with a maximum location score of 4. Under Simwalk2 NPL analysis, there are 5 statistics methods available. The BLOCKS statistic is the number of founder-alleles contributing alleles to the affected individuals. This statistic was designed for traits best modelled by recessive inheritance. The MAX-TREE statistic is the largest number of affected subjects inheriting an allele from one founder-allele. This statistic was designed for traits best modelled by dominant inheritance. The ENTROPY statistic is a measure of the entropy of the alleles among the affected individuals. The NPL_{pair} statistic is roughly the sum of conditional kinship coefficients for all affected pairs. This statistic was designed for traits best modelled by ADDITIVE inheritance. The NPL_{all} statistic is a measure of whether a few founder-alleles are overly represented in affected individuals. This statistic was designed for traits best modelled by ADDITIVE inheritance.

The largest location score obtained by SimWalk2 NPL analysis was 1.733 in NPL_{pair}, at the position 90.7876 cM on chromosome 13, the nearest marker was D13S1241 (Table 8.3). Another location score larger than 1 was on chromosome 22, with a location score of 1.235 by NPL pair analysis and 1.343 by NPL_{all}. However, in none of the location scores obtained via Simwalk2 NPL did the analysis exceed the significant threshold.

The reason that SimWalk2 showed a maximum location score at marker D13S1241 but not D22S420 could be that several genotyped individuals were trimmed off in MERLIN NPL analysis, but the entire pedigree shown in Figure 8.1 was included in the SimWalk2 NPL analysis, including those who were not genotyped.

8.5 Summary

In this analysis, we aimed to identify the genetic basis of this familial epilepsy, to determine the mode of inheritance in this family, to identify linked regions, and to to

determine whether there was a similar genetic cause as in the other pedigree (pedigree B) However, the mode of inheritance of this pedigree could not be determined, and the NPL analysis by MERLIN and SimWalk2 were both inconclusive; the maximum K&C LOD was 1.001 at marker D22S420, and the maximum location score by SimWalk2 NPL_{pair} was 1.733 at marker D13S1241. In pedigree B, the mode of inheritance was AD with a reduced penetrance, and three regions of interest were identified. Thus the underlying genetics in this pedigree and pedigree B is likely to be different.

Table 8.3 Location scores on chromosome 13 (Simwalk2 NPL)

Chromosome	Position (Haldane, in cM)	Marker	Block	Max Tree	Entropy	NPL (pair)	NPL (all)
13	53.9549	D13S272	0.402	0.738	1.207	1.168	1.138
13	65.4734	D13S279	0.501	0.827	1.831	1.626	1.510
13	79.9789	D13S271	0.503	0.822	1.792	1.617	1.513
13	***90.9876***	***D13S1241***	***0.507***	***0.992***	***1.844***	***1.733***	***1.652***
13	100.3041	D13S1256	0.282	0.948	0.844	0.925	1.000
13	107.2660	D13S1809	0.280	0.945	0.858	0.942	1.014

Figure 8.3 Distribution of the location score on chromosome 13 (SimWalk2NPL)

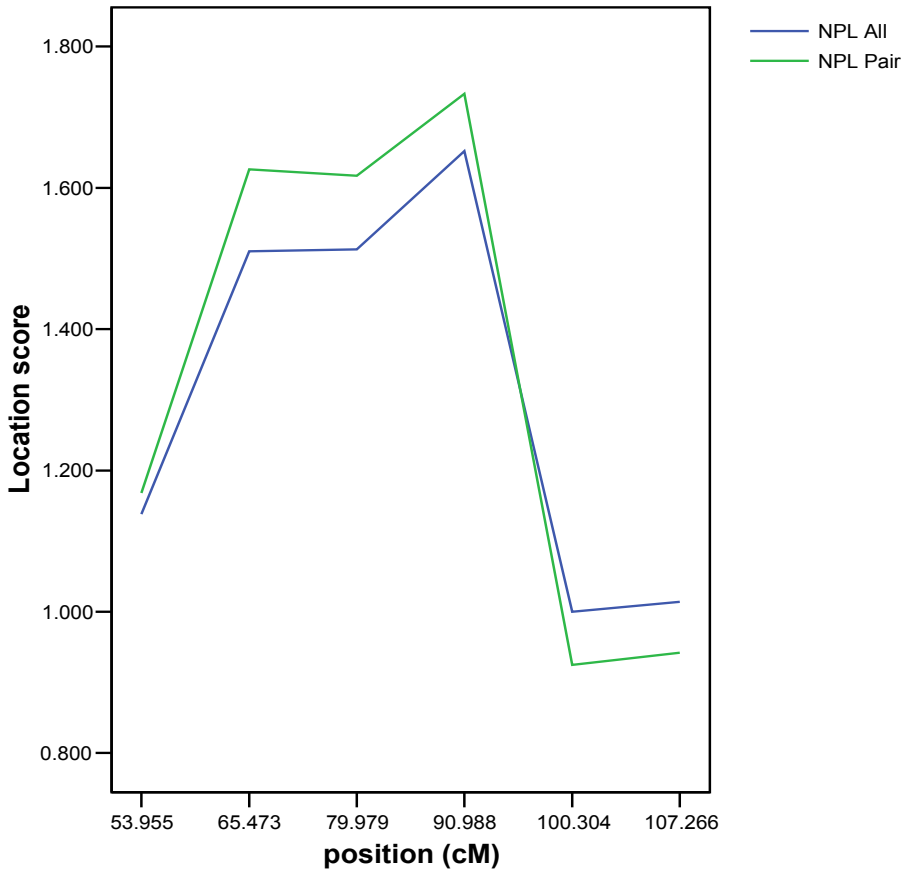
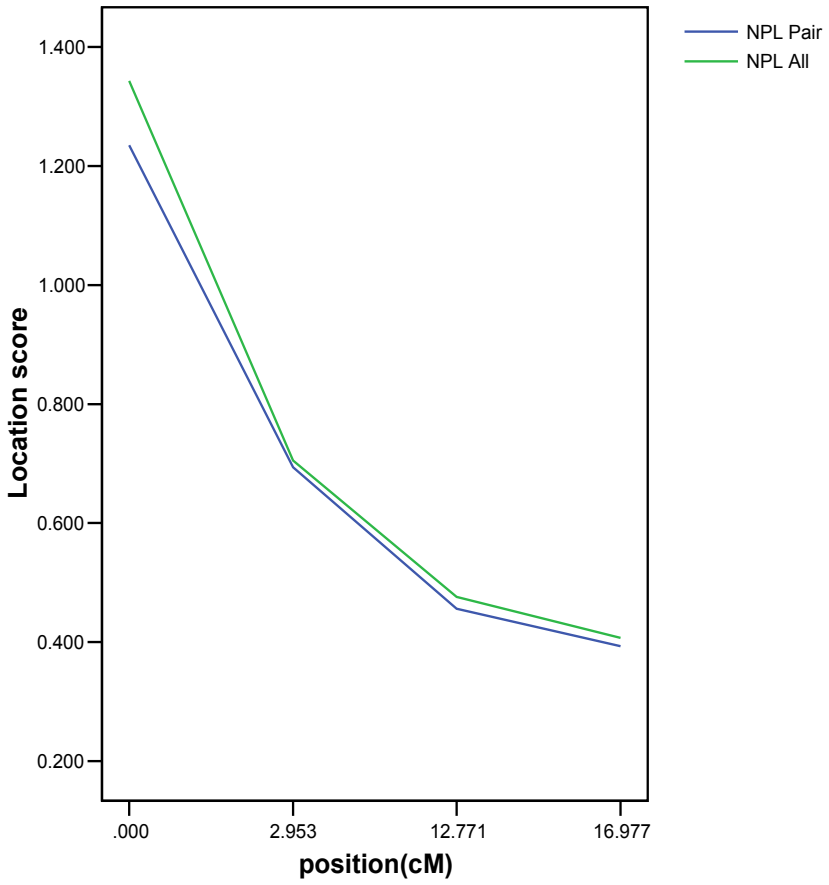


Table 8.4 Location scores on chromosome 22 (Simwalk2 NPL)

Chromosome	Position (Haldane, in cM)	Marker	Block	Max Tree	Entropy	NPL (pair)	NPL (all)
22	***0.0000***	***D22S420***	***0.250***	***1.268***	***1.046***	***1.235***	***1.343***
22	*2.9535*	D22S427	0.222	0.538	0.699	0.694	0.705
22	12.7713	*D22S539*	0.151	0.337	0.466	0.456	0.476
22	16.9774	*D22s1174*	0.130	0.279	0.406	0.393	0.407

Figure 8.4 Distribution of the location scores on chromosome 22(SimWalk2 NPL)



Section 9: Linkage Analysis in Photosensitive Epilepsy

9.0 Overview

The DNA samples of this pedigree were collected approximately 15 years ago, the access of the DNA samples was made through collaborating with Professor Nick Wood, Head of the Department of Molecular neuroscience, Institute of Neurology, Queen Square, London. Electroencephalography (EEG) was used as a phenotyping tool in this study. Some of the EEGs of the individuals were repeated 8 to 17 years after their first EEGs using the same protocol. The EEGs were reported by Professor Graham F.A. Harding and reviewed by Professor Peter M Jeavons.

9.1 Family pedigree

The pedigree of this family is shown in Figure 9.1. Nineteen DNA samples were available from this family. Of these 19 individuals, 10 had a photoparoxysmal response (PPR) based on their EEG investigation with intermittent photic stimulation (IPS) by using Grass PS-22. The intensity was set at 2, with and without grid testing.

9.2 Clinical presentation and investigation

The medical records of this pedigree are summarized in Table 9.1. In the first generation, the DNA sample of I-2 was not available. Her EEG investigation was carried out at the age of 48 and 65. The first EEG showed a clear PPR at 35 and 40Hz photic stimulation. In the second EEG, whole body myoclonic jerks were induced during the IPS at 50-60 Hz. I-1 was unaffected. His DNA sample was not available.

In the second generation, II-3 was not affected. II-4 had 2 seizures. The first one was evoked by watching TV and the last seizure was at the age of 18. He had one EEG at the age of 35 which showed marked occipital spiking with anterior involvement at 35, 38, 40, and 45 Hz photic stimulation. He was seizure free after the age of 18 and was not on medication. II-5 was not affected. II-6 had seizures, but his EEG report and medical history were not available; whether he had photosensitivity was unknown. II-7 had 2 seizures which were both induced by watching TV. She was seizure free after the age of 16. She had 2 EEG recordings, the first one was carried out at the age of 19 and an inconstant PPR were

recorded at 20, 50 Hz photic stimulation as well as occipital spikes induced at 30-40 Hz photic stimulation. The second EEG was carried out at the age of 31 which showed occipital spiking and a degraded PPR. II-8 had no seizures, but bright sun light bothered her with dizziness. She had one EEG at the age of 15, showing a definite PPR at 20 and 50 Hz photic stimulation. II-8 was unaffected. II-9 had no seizures and his EEG showed, on photic stimulation, occipital spikes at 50 Hz stimulation. II-10 and II-11 were not affected. II-12 did not have seizures, but responded to bright light with "jumping". She had no problem in watching TV and had no jerky limb movements in the morning. On her EEG record at the age of 28, when the PPR were induced at 40 Hz photic stimulation, she felt "funny". II-13 was not affected. II-14 had photosensitive seizures, with an onset at the age of 11 and was always induced by watching TV. He was on phenytoin and was seizure free after the age of 16. He had 1 EEG at the age of 15 years old and 10 months, which showed a PPR at 16, 18, 24, 25, 40, and 52 Hz photic stimulation. His follow-up EEG was at the age of 27, which did not show a typical PPR but did show some abnormality (sharp waves) at the occipital lobe with anterior spread at 40, 45 Hz stimulation, suggesting that his PPR had deteriorated or degraded. II-15 was not affected. II-16 did not have seizures, on her EEG a PPR was recorded at 14-25 Hz photic stimulation with her eye closed or at 9-25 Hz stimulation on eye closure. EEG was not tested on II-13, who did not have history of seizures.

In the third generation, III-17 was not affected. III- 18 did not have seizures. On her EEG at the age of 15, a definite PPR were recorded at 20 and 50 Hz photic stimulation. III-19 had epilepsy, but his EEG did not show any PPR. III-20 and III-21 was not affected. III-22 had no seizures, and no symptoms suggesting photosensitivity but a degraded PPR was found on his EEG, mainly confined to the occipital area. On 1 or 2 occasions, there was a slight anterior involvement. III-22 also had asthma. III-23 had seizures with mild learning disability, with an onset at the age of 7. His seizures were evoked by watching TV and flashing lights. There were also nocturnal seizures usually preceded by seeing flashing lights, particularly in the right visual field. With the onset of the sparkling light, he would say "I cannot see" and this would be followed by left side jerky limb movements and mild left side Todd's paralysis. The partial seizures could evolve to generalized tonic-clonic seizures. He was on Epilim 2000 mg per day. The imaging study of brain was normal. He

had 2 EEGs, the first EEG was carried out at the age of 12 and showed a PPR at 35 and 40 Hz photic stimulation. His second EEG was carried out at the age of 18 and showed PPR at 50 Hz photic stimulation and at the same time he reported seeing flashing light. A focal seizure was evoked on pattern testing at 200 Hz. III-24 had no seizures. On her EEG examination, PPR were recorded at 15, 17, 18, 19, 35, 36, 37, 40, 42, Hz photic stimulation. No special sensation was reported by this individual when a PPR were recorded.

The clinical presentations of the affected individuals are summarized in Table 9.1. Except for individual I-2, II-3, II-9, and II-12 in whom the first EEGs were conducted after the age of 20 years, the rest of the subjects had EEGs recorded between the age of 10 and 20 years. A degraded PPR (Doose Type 1) were found on the follow-up EEG of I-2 (68y/o), II-7 (31 y/o), and on the first EEG recording of II-4 (35 y/o), II-9 (36 y/o), and II-22 (15y/o).

Figure 9.1 Family tree of the photosensitivity epilepsy family

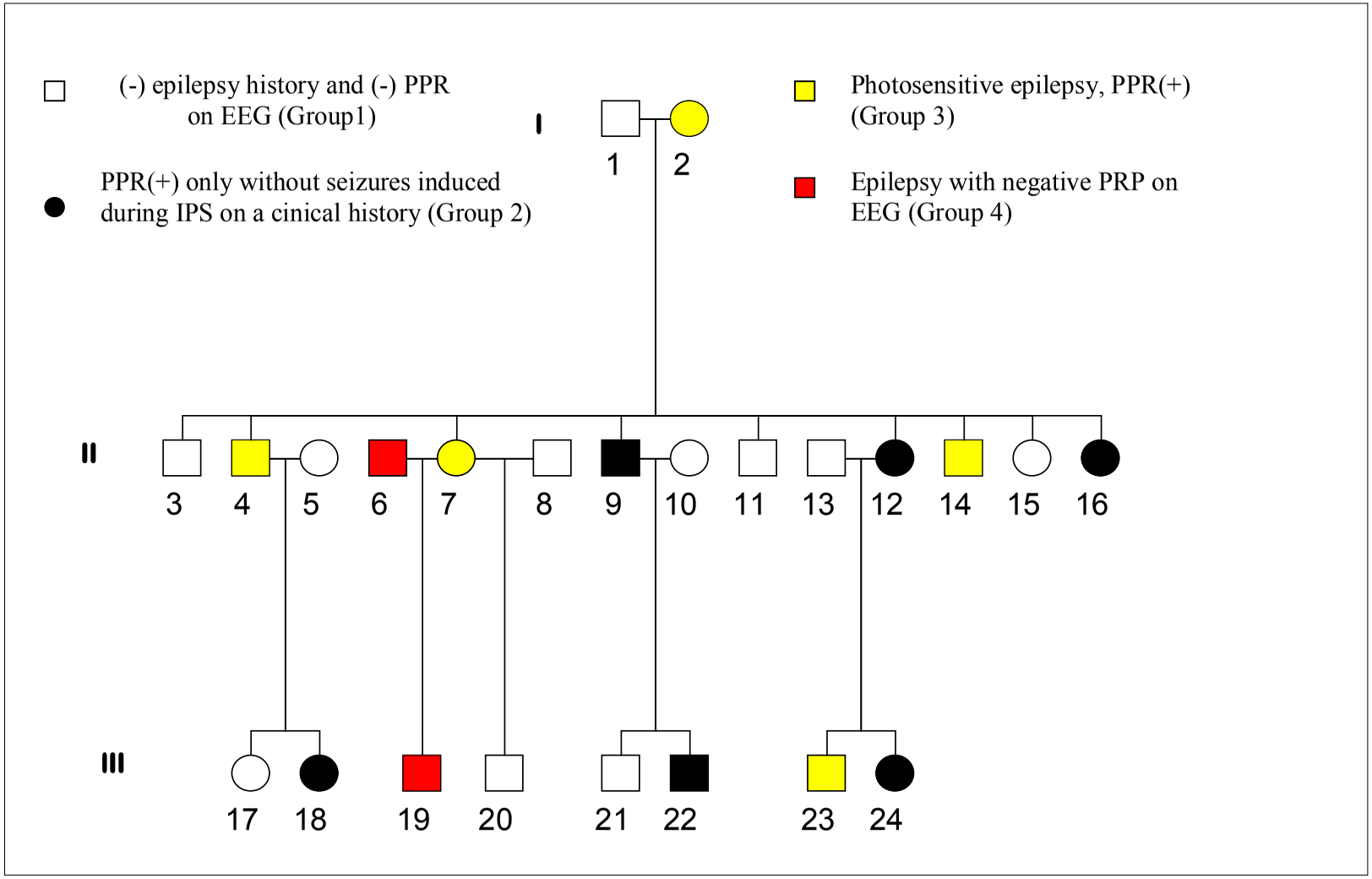


Table 9.1 Clinical presentation of affected individuals

ID	Gender	DNA samples	PPR induced by IPS	Seizures	Age of onset	Medication	Age at 1st EEG recording	Degraded PPR
2	F	-	+	+ (whole body myoclonic jerk induced during IPS at the age 65, none in daily activity)	-	-	48, 65	+(the 2nd one done at the age 65)
3	M	+	-	-	-	-	-	-
4	M	+	+	+(TV)	18	-	35	+
5	F	+	-	-	-	-	-	-
6	M	-	N/A	+	?	?	?	?
7	F	+	+	+(TV)	11	Phenobarbital	19, 31	yes (the 2nd one done at the age 31)
9	M	+	+	-	-	-	36	+
11	M	+	-	-	-	-	-	-
12	F	+	+	-	-	-	28	-
13	M	+	N/A	-	-	-	-	-
14	M	+	+	+(TV)	11	Phenobarbital (15-16 y/o)	15, 23	-
15	F	+	-	-	-	-	-	-
16	F	+	+	-			12,	
17	F	+	-	-	-	-	-	-
18	F	+	+	-		-	15	
19	M	+	-	+	?	?	?	?
20	M	+	-	-	-	-	-	-
21	M	+	N/A	-	-	-	?	-
22	M	+	+	-	-	-	15	+
23	M	+	+ (TV, sparkling light)	+ [partial simple seizures (sparkling light in right visual field, left jerky arm, Todds paralysis) , may evolve to generalized seizures]	7	phenytoin at the age of 8 and then Epilim 2000/day when17	8, 17	-
24	F	+	+	-	-	-	14	-

9.3 Linkage analysis

9.3.1 Phenotyping and mode of inheritance

This pedigree was divided into 4 groups based on both their clinical presentation and EEG reports:

Group 1: Unaffected individuals; who had no history of seizures and in whom there were no PPRs evoked on EEG during IPS. There were 11 individuals in this group and 8 DNA samples were available from this group.

Group 2: Individuals showing PPR on EEG recording during IPS; but who clinically did not have history of epilepsy and had no seizures were induced during IPS. There were 6 individuals in this group. All of their DNA samples were available.

Group 3: Individuals with photosensitive epilepsy; and in whom PPRs were induced and recorded on EEG during IPS. There were 5 individuals in this group and 4 DNA samples were available from this group.

Group 4: Individuals with epilepsy but whose seizures were not related to photosensitivity, and whose epilepsy was not visually sensitive (no PPRs and no seizures induced by photosensitivity). There were 2 individuals in this group and 1 DNA sample was available.

The different definition of phenotype in the family inducted different mode of inheritance. If PPR is the single factor considered in determining the case status (group2 and group3), there was male to male transmissions indicating this was not an X-linked or an mitochondrial inheritance, and affected individuals were seen in three generations, indicating that this was not autosomal recessive inheritance. The inheritance mode would thus likely to be autosomal dominant. In this analysis, the phenotype was defined as positive PPRs on EEG, which included both group 2 and group 3.

9.3.2 Parameters for MILNK analysis

Parametric linkage analysis was carried out by MLINK. The mode of inheritance was set as autosomal dominant with full penetrance. Other parameters such as allele frequencies were set according to the data provided by deCODE Genetics, the increment of recombination fraction was set at 0.1, starting from 0 and ended at 0.45. The LOD scores were also calculated when the recombination fraction was at 0.01 and 0.05. The program

UNKNOWN was used to check genotyping errors. Such errors were corrected by changing the wrongly genotyped data as "unknown".

Clinically, statistics has suggested that photosensitivity is an age-related phenomenon with maximum prevalence in the age group between 5-20 years old. A degraded PPR is common when patients exceed 20 years old. Most of the degraded PPRs (Doose Type 1, 2, and 3) are confined to occipital area, parietal area, or frontal lobe without generalized spreading. Female are more likely to be affected than male with the ratio of 2-2.5:1. In this pedigree, with the exception of 4 individuals, the EEG investigations were carried out between the ages of 10-20 years, in this way, the influence of age was minimized.

9.4 LOD scores by MLINK

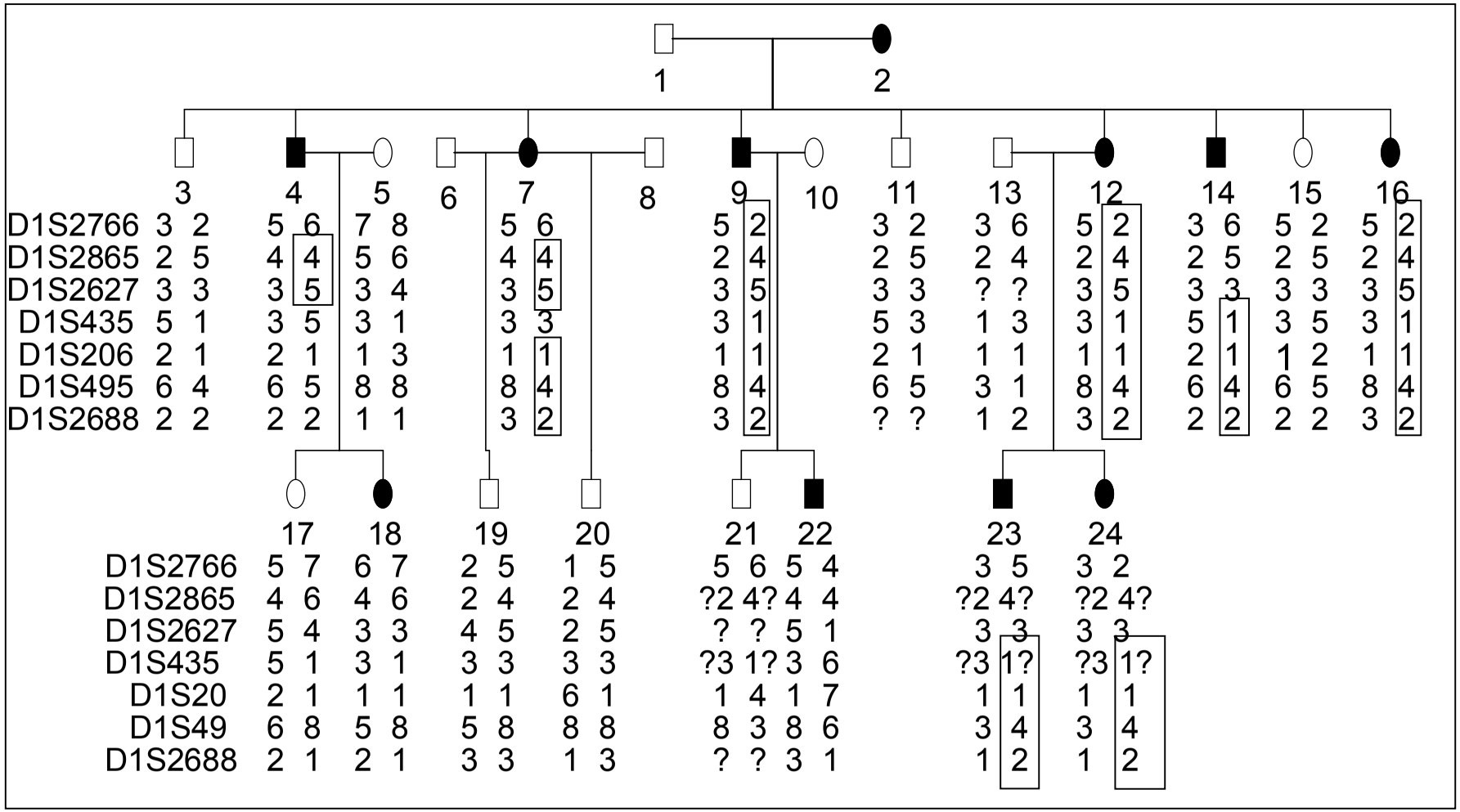
The maximum LOD score was 1.14 at marker D1S2865 at $\theta = 0.1$, with another comparable LOD score was 1.06 at marker D1S495 at $\theta = 0.2$. The LOD scores distribution is presented in Table 5.2. Multipoint linkage analysis at this locus the LOD score was 1.57.

The haplotype analysis of this region is shown in Figure 9.2. If the genotype data is missing it is shown as "?". If the haplotype is not certain because the phase of meiosis was unknown, it is marked with an "?" beside the haplotype. The haplotype analysis of chromosome 1 did not support the existence of a disease locus at this region, and demonstrated that any disease locus was not adjacent to the markers used. No haplotype segregated with the affected individuals.

Table 9.2 LOD score of chromosome 1

θ ***Marker***	***0.0***	***0.01***	***0.05***	***0.1***	***0.2***	***0.3***	***0.4***
D1S2766	-infini	-5.54	-2.26	-1.03	-0.13	0.11	0.08
D1S2865	***-infini***	***0.53***	***1.06***	***1.14***	***0.95***	***0.58***	***0.18***
D1S2627	-infini	-0.25	0.34	0.50	0.51	0.37	0.16
D1S435	-infini	-3.35	-1.37	-0.63	-0.07	0.07	0.04
D1S206	0.35	0.34	0.29	0.24	0.17	0.13	0.06
D1S495	***-infini***	***-1.55***	***0.30***	***0.87***	***1.06***	***0.79***	***0.31***
D1S2688	-infini	-0.84	-0.21	-0.00	0.11	0.08	0.03

Figure 9.2 Haplotype analysis of chromosome 1



The phenotyping of this pedigree is based as it was on the existence of a PPR, which was interpreted by 2 experts in photosensitivity, and false phenotyping was unlikely. The MOI in the pedigree suggested a single gene disease which was autosomal dominant with full penetrance. However parametric analysis by MLINK failed to find a disease linkage, because of this, the pedigree was re-analyzed using NPL by SimWalk2 to test the hypothesis that photosensitivity in this pedigree was polygenic with a dominant gene.

9.5 NPL by SimWalk2

The input files for Simwalk2 were constructed by Mega2 using Linkage format files. The input files were checked by selecting the "option" of SimWalk2. Genotyping errors (if there were any) were adjusted by setting the data as "unknown". The parameters used were the same as used in MLINK, and the map file was based on the Decode genetic map.

The NPL analysis by Simwalk2 showed that the location score [$-\text{Log}_{10}$ (p-value)] of NPL_{pair} at marker D16S2621 was 2.231, the corresponding *P* value was 0.0059, and the location score of NPL_{all} was 1.629 at the same location, the corresponding pointwise *P* value was 0.0235. Part of the NPL location score distributions by SimWalk2 on chromosome 16 is presented in Table 9.3 and Figure 9.4. The haplotype analysis showed that recombination was found on III-18 and III-24; these 2 affected individuals did not share the haplotype with the other affected individuals.

If photosensitivity is a single gene disease, the NPL analysis as well as the parametric analysis by MILNK failed to identify the disease locus. If photosensitivity is a polygenic disease with a dominant gene (susceptibility gene that is shared by the majority of affected individuals), the region identified by SimWalk2 NPL analysis showed that a single haplotype was shared by most of the affected individuals except III-18 and III-24, whose photosensitivity could be caused either by non-dominant genes or non-genetic factor.

The region identified via NPL analysis was located at 16q24.1-16q24.3, and is around 6.1 Mb in length. There are around 90 known genes and pseudo-genes in this area, but no ion channel gene is found within this region. One ion channel gene, KCNG4 (the potassium voltage-gated channel gene, subfamily G, member 4), was adjacent to this region. But, there is no evidence that this gene expresses in the brain and its function has not been determined. The functions of the known genes in this region are summarized in Table 9.4.

9.6 Summary

In this photosensitive epilepsy pedigree, we aimed to identify the genetic basis of the Photoparoxysmal response (PPR) in a large family with photosensitive epilepsy, to determine the mode of inheritance, and to identify linkage.

In summary, the NPL analysis of Simwalk2 suggested a region of interest on chromosome16 in which a haplotype was shared by the majority of affected individuals. This pedigree could be thus explained by a polygenic inheritance in which involving a dominant gene.

Figure 9.3 Haplotype analysis of Chromosome 16

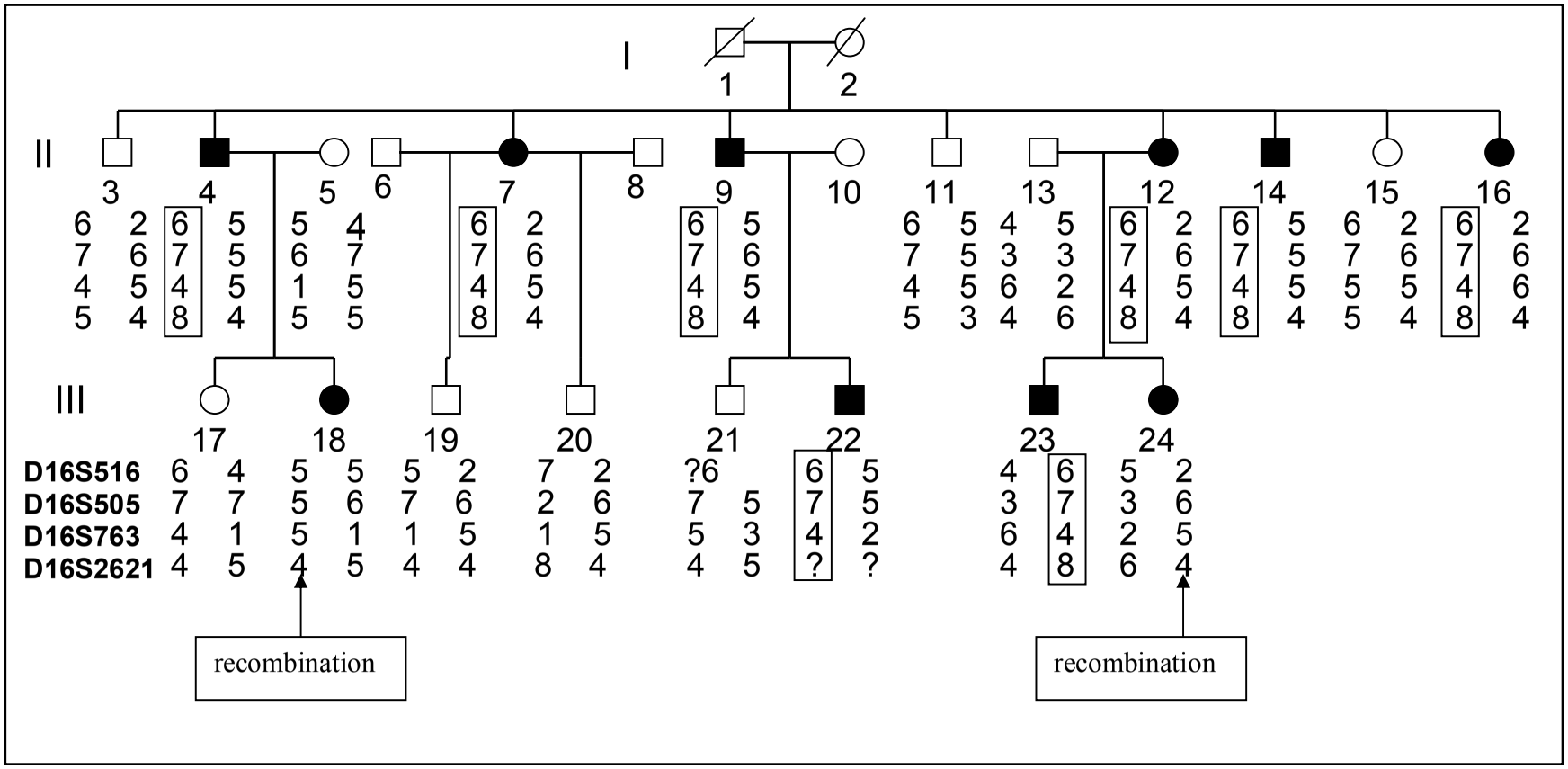


Table 9.3 Distribution of NPL statistics on chromosome 16 (SimWalk2)

Marker Names	Position (in Haldane)	-Log_{10}(p value)				
		Blocks	Max-Tree	Entropy	NPL_{pairs}	NPL_{all}
D16S3080	52.9102	0.000	0.258	0.284	0.412	0.423
D16S3034	60.0841	0.000	0.237	0.173	0.289	0.348
D16S3057	68.1689	0.006	0.291	0.157	0.293	0.363
D16S514	75.2574	0.000	0.391	0.165	0.342	0.442
D16S503	76.1188	0.000	0.390	0.164	0.343	0.443
D16S515	86.5020	0.032	0.158	0.158	0.173	0.182
D16S516	93.0485	0.047	0.283	0.286	0.324	0.320
D16S505	99.8807	0.072	0.404	0.441	0.506	0.476
D16S763	112.4373	0.117	0.786	1.672	1.893	1.609
D16S2621	***123.2668***	***0.266***	***1.066***	***2.153***	***2.231***	***1.629***

Figure 9.4 Distribution of NPL scores on Chromosome 16

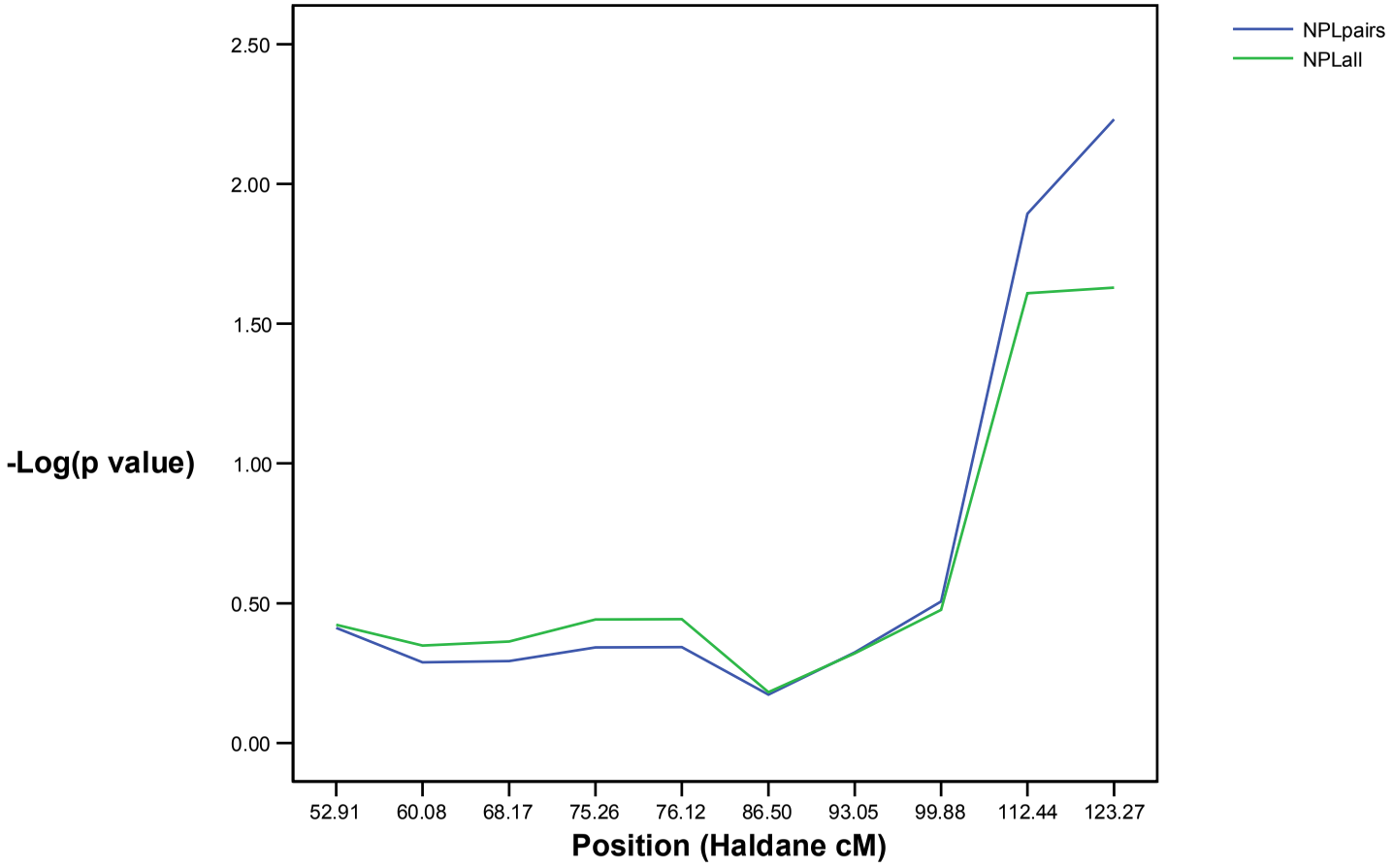


Table 9.4 Genes within 16α24.1-16α24.3

Gene ID	Function
USP10	This gene encodes a member of the ubiquitin-specific protease family of cysteine proteases, may be associated with poor survival of glioblastoma multiform.
CRISPLD2	Candidate gene for Non-syndromic cleft lip with or without cleft palate.
ZDHHC7	Palmitoyltransferase with broad specificity. Palmitoylates SNAP25 and DLG4/PSD95. May palmitoylate GABA receptors on their gamma subunit (GABRG1, GABRG2 and GABRG3) and regulate their synaptic clustering and/or cell surface stability.
KIAA0513	a novel signaling molecule that interacts with modulators of neuroplasticity, apoptosis, and the cytoskeleton.
FAM92B	candidate gene of Crohn Disease.
TMEM148	function unknown
KIAA0182	function unknown
GINS2	The GINS complex plays an essential role in the initiation of DNA replication, and progression of DNA replication forks. GINS complex seems to bind preferentially to single-stranded DNA.
C16orf74	function unknown
COX4NB	function unknown
COX4I1	This gene encodes the nuclear-encoded subunit IV soforms 1 of the human mitochondrial respiratory chain enzyme.
IRF8	Interferon regulatory factor 8 The IRF family proteins bind to the IFN-stimulated response element (ISRE) and regulate expression of genes stimulated by type I IFNs, namely IFN-alpha and IFN-beta. IRF family proteins also control expression of IFN-alpha and IFN-beta-regulated genes that are induced by viral infection.
MTHFSD	function unknown
FOXF1	This gene belongs to the forkhead family of transcription factors which is characterized by a distinct forkhead domain. The specific function of this gene has not yet been determined; however, it may play a role in the regulation of pulmonary genes as well as embryonic development.
FOXL1	Candidate gene of primary lymphedema.
FOXC2	This gene belongs to the forkhead family of transcription factors which is characterized by a distinct DNA-binding forkhead domain. The specific function of this gene has not yet been determined; however, it may play a role in the development of mesenchymal tissues.

(continued)

Table 9.4 Genes within 16q24.1-16q24.3 (continue)

Gene ID	Function
FBXO31	Probably recognizes and binds to some phosphorylated proteins and promotes their ubiquitination and degradation. May act as a breast tumour suppressor.
MAP1LC3B	Probably involved in formation of autophagosomal vacuoles (autophagosomes).
ZCCHC14	function unknown
JPH3	Contributes to the stabilization of the junctional membrane complexes, which are common to excitable cells and mediate cross-talk between cell surface and intracellular ion channels. Probably acts by anchoring the plasma membrane and endoplasmic reticulum. May play an active role in certain neurons involved in motor coordination. Had been reported as a candidate gene for Huntington disease.
KLHDC4	function unknown
SLC7A5	Sodium-independent, high-affinity transport of large neutral amino acids such as phenylalanine, tyrosine, leucine, arginine and tryptophan, when associated with SLC3A2/4F2hc. Involved in cellular amino acid uptake. Acts as an amino acid exchanger. Involved in the transport of L-DOPA across the blood-brain barrier, and that of thyroid hormones triiodothyronine (T3) and thyroxine (T4) across the cell membrane in tissues such as placenta. Plays a role in neuronal cell proliferation (neurogenesis) in brain. Involved in the uptake of methylmercury (MeHg) when administered as the L-cysteine or D,L-homocysteine complexes, and hence plays a role in metal ion homeostasis and toxicity. Involved in the cellular activity of small molecular weight nitrosothiols, via the stereoselective transport of L-nitrosocysteine (L-CNSO) across the transmembrane. May play an important role in high-grade gliomas. Mediates blood-to-retina L-leucine transport across the inner blood-retinal barrier which in turn may play a key role in maintaining large neutral amino acids as well as neurotransmitters in the neural retina. Acts as the major transporter of tyrosine in fibroblasts
CA5A	Reversible hydration of carbon dioxide
BANP	Controls V(D)J recombination during T-cell development by repressing T-cell receptor (TCR) beta enhancer function. Binds to scaffold/matrix attachment region beta (S/MARbeta), an ATC-rich DNA sequence located upstream of the TCR beta enhancer. Represses cyclin D1 transcription by recruiting HDAC1 to its promoter, thereby diminishing H3K9ac, H3S10ph and H4K8ac levels. Promotes TP53 'Ser-15' phosphorylation and nuclear accumulation, which causes cell cycle arrest.

(continue)

Table 9.4 Genes within 16q24.1-16q24.3 (continue)

Gene ID	Function
ZFPM1	Transcription regulator that plays an essential role in erythroid and megakaryocytic cell differentiation. Essential cofactor that acts via the formation of a heterodimer with transcription factors of the GATA family GATA1, GATA2 and GATA3. Such heterodimer can both activate or repress transcriptional activity, depending on the cell and promoter context. The heterodimer formed with GATA proteins is essential to activate expression of genes such as NFE2, ITGA2B, alpha- and beta-globin, while it represses expression of KLF1. May be involved in regulation of some genes in gonads. May also be involved in cardiac development, in a non-redundant way with ZFPM2/FOG2.
ZC3H18	function unknown
IL17C	Stimulates the release of tumour necrosis factor alpha and IL-1-beta from the monocytic cell line THP-1.
CYBA	Critical component of the membrane-bound oxidase of phagocytes that generates superoxide. Associates with NOX3 to form a functional NADPH oxidase constitutively generating superoxide.
MVD	Performs the first committed step in the biosynthesis of isoprenes
SNAI3	Seems to inhibit myoblast differentiation. Transcriptional repressor of E-box-dependent transactivation of downstream myogenic bHLHs genes. Binds preferentially to the canonical E-box sequences 5'-CAGGTG-3' and 5'-CACCTG-3'.
FAM38A	Expressed in numerous tissues. In normal brain, expressed exclusively in neurons, not in astrocytes. In Alzheimer disease brains, expressed in about half of the activated astrocytes located around classical senile plaques. In Parkinson disease substantia nigra, not detected in melanin-containing neurons nor in activated astrocytes.
RNF166	Function unknown
APRT	Catalyzes a salvage reaction resulting in the formation of AMP, that is energically less costly than de novo synthesis.
GALNS	This gene encodes N-acetylgalactosamine-6-sulfatase which is a lysosomal exohydrolase required for the degradation of the glycosaminoglycans, keratan sulfate, and chondroitin 6-sulfate. Sequence alterations including point, missense and nonsense mutations, as well as those that affect splicing, result in a deficiency of this enzyme. Deficiencies of this enzyme lead to Morquio A syndrome, a lysosomal storage disorder.

(continue)

Table 9.4 Genes within 16q24.1-16q24.3 (continue)

Gene ID	Function
CBFA2T3	Functions as a transcriptional repressor. Regulates the proliferation and the differentiation of erythroid progenitors by repressing the expression of TAL1 target genes. Plays a role in granulocyte differentiation. Isoform 2 functions as an A-kinase-anchoring protein.
TRAPPC2L	Function undetermined
ACSF3	Acyl-CoA synthases catalyze the initial reaction in fatty acid metabolism, by forming a thioester with CoA. May have some preference toward very-long-chain substrates.
ZNF778	May be involved in transcriptional regulation
CDH15	Cadherins are calcium dependent cell adhesion proteins. They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. M-cadherin is part of the myogenic program and may provide a trigger for terminal muscle differentiation.
ANKRD11	May recruit HDACs to the p160 coactivators/nuclear receptor complex to inhibit ligand-dependent transactivation.
SPG7	This gene encodes a nuclear-encoded mitochondrial metalloprotease protein that is a member of the AAA (ATPases associated with a variety of cellular activities) protein family. Members of this protein family share an ATPase domain and have roles in diverse cellular processes including membrane trafficking, intracellular motility, organelle biogenesis, protein folding, and proteolysis. Two transcript variants encoding distinct isoforms have been identified for this gene. Mutations associated with this gene cause Autosomal recessive spastic paraplegia 7.
RPL13	This gene encodes a ribosomal protein that is a component of the 60S subunit of ribosome. This gene is expressed at significantly higher levels in benign breast lesions than in breast carcinomas.
CPNE7	May function in membrane trafficking. Exhibits calcium-dependent phospholipid binding properties.
DPEP1	Hydrolyzes a wide range of dipeptides. Implicated in the renal metabolism of glutathione and its conjugates. Converts leukotriene D4 to leukotriene E4; it may play an important role in the regulation of leukotriene activity.
CHMP1A	This gene encodes a member of the CHMP/Chmp family of proteins which are involved in multivesicular body sorting of proteins to the interiors of lysosomes.
ZNF276	May be involved in transcriptional regulation

(continued)

Table 9.4 Genes within 16q24.1-16q24.3 (continue)

Gene ID	Function
CDK10	The protein encoded by this gene belongs to the CDK subfamily of the Ser/Thr protein kinase family.The CDK subfamily members are highly similar to the gene products of S. cerevisiae cdc28, and S. pombe cdc2, and are known to be essential for cell cycle progression.
FANCA	Mutations in this gene are the most common cause of Fanconi anemia.
SPIRE2	Acts as a actin nucleation factor, remains associated with the slow-growing pointed end of the new filament. Involved in vesicle transport processes providing a novel link between actin organization and intracellular transport.
TCF25	TCF25 is a member of the basic helix-loop-helix (bHLH) family of transcription factors that are important in embryonic development.
AFG3L1	Possible candidate gene of human spastic paraplegia
TUBB3	Tubulin is the major constituent of microtubules. It binds two moles of GTP, one at an exchangeable site on the beta chain and one at a non-exchangeable site on the alpha-chain. Over expression of this gene may be a maker of poor clinical outcome of advanced ovarian cancer patients.
DEF8	Function unknown
PRDM7	The protein encoded by this gene is a transcription factor of the PR-domain protein family. It contains a PR-domain and multiple zinc finger motifs. Transcription factors of the PR-domain family are known to be involved in cell differentiation and tumorigenesis.
DBNDD1	Function undetermined
GAS8	This gene includes 11 exons spanning 25 kb and maps to a region of chromosome 16 that is sometimes deleted in breast and prostrate cancer. The second intron contains an apparently intronless gene, C16orf3, which is transcribed in the opposite orientation. This gene is a putative tumour suppressor gene.

Section 10: Linkage Analysis in Kohlschütter-Tönz Syndrome

10.0 Overview

Five small families were identified with Kohlschütter-Tönz Syndrome. These families were identified and diagnosed in several different countries, including Italy (2 families), Germany (1 family), United Kingdom (1 family) and Australia (1 family). All of the DNA samples were obtained from consenting individuals of these families. The detail clinical presentations and medical history, pedigrees of these families have been published (Christodoulou *et al.* 1988; Guazzi *et al.* 1994; Donnai *et al* 2005; Musumeci *et al.* 1995; Petermöller *et al.* 1993).

10.1 The Kohlschütter-Tönz Pedigrees

Pedigree 1, 3, and 5 are shown in Figure 10.1, pedigree 2 is shown in Figure 10.2, and pedigree 4 in Figure 10.3. All the families except pedigree 4 showed a typical autosomal recessive inheritance. There was consanguinity in pedigree 2 and in pedigree 4; the parents were first cousins. In pedigree 4, there were four phenotypes and the mode of inheritance was complicated, depending on the phenotypes defined.

Figure 10 .1 Pedigrees 1, 3, and 5

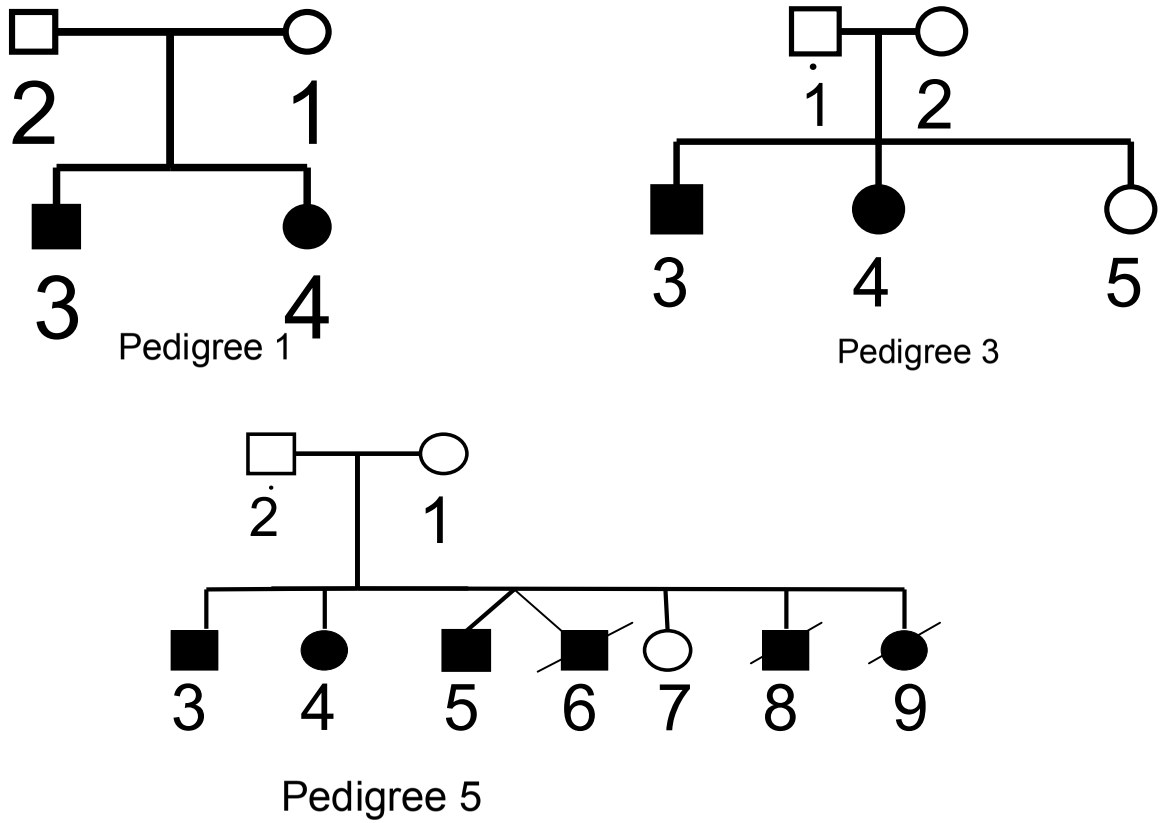
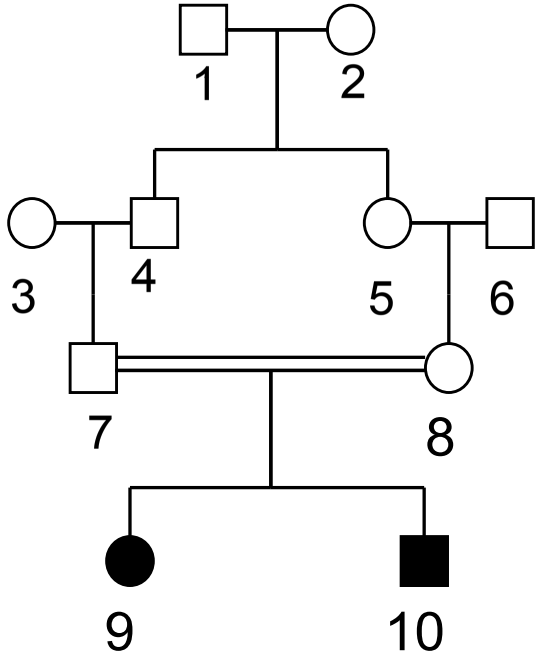
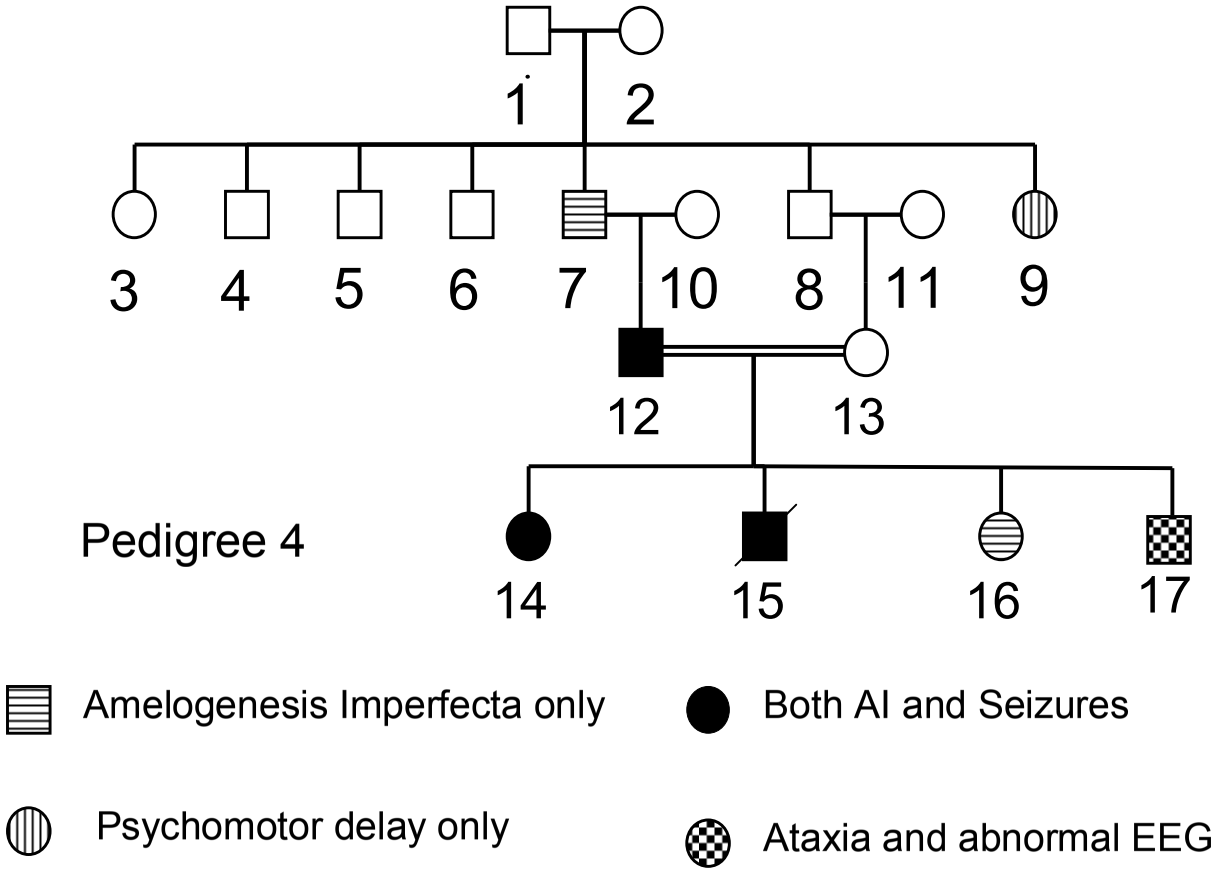


Figure 10.2 pedigree 2



Pedigree 2

Figure 10.3 Pedigree 4



10.1.1 Clinical presentation

The clinical presentation and some of their clinical investigations of these 5 pedigrees are summarized in Table 10.1.

Pedigree 1 was a UK family reported by Donnai (Donnai *et al.* 2005). The parents were healthy unrelated individuals who had no family history of dental abnormality or neurological disorders. II-3 had a seizure onset at the age of 5 weeks, and the EEG at four months showed hypsarrhythmia. His CT scan at the same time showed enlargement of the lateral and third ventricles as well as abnormality of the cortical sulci particularly over the surface of the left hemisphere. His teeth appeared to have essentially normal dentine structure and morphology but the enamel was poorly formed with an irregular rod structure and prominent hypoplasia, which was consistent with the diagnosis of amelogenesis imperfecta (AI). His psychomotor development was severely impaired and he could not talk or walk at the age of 13 and only smiled to a lot of attention. II-4 was sister of II-3. At the age of nine weeks she appeared hypotonic and had feeding problems with lactose intolerance. At the age of 11 weeks she had the first episode of partial seizures accompanied by myoclonic jerks of the left arm which lasted 45 minutes. Despite combined anticonvulsant therapy she continues to have 5 to 10 seizures per day and subtle signs of seizure activity such as twitches and unusual eye and lip movements. The EEG at this time showed an abnormal pattern with loss of normal rhythmic activities and frequent multi-focal discharges. The MRI scan showed incomplete myelination and enlargement of the lateral ventricles, possibly consistent with cerebral atrophy. She demonstrated more awareness of her surroundings than her brother and was able to communicate with her mother by eye movements. Other symptoms shared by the siblings included facial flushing and the passage of sediment in the urine every few weeks. The crystals have been analyzed and were 75% calcium phosphate and 25% magnesium ammonium phosphate. The mode of inheritance was autosomal recessive in this pedigree.

Pedigree 2 was an Italian family with consanguity reported by Musumeci (Musumeci *et al.* 1995). The parents (III-7 and III-8) were first cousins. There were family members reported to have epilepsy or mental retardation in this pedigree, but were not specified which side these family members were from (paternal or maternal) and the detailed symptoms and signs were not available. History and DNA of I-1, I-2, II-3, II-4, II-5 and II-

6 were not available. IV-10 was found to have psychomotor development delay at the age of 2 months when his first seizure occurred. They seizures were characterized by cyanosis, staring spell and loss of muscular tone which lasting a few seconds. During the following months more seizures were observed, sometimes with head turning to the left and intense cyanosis of the lips. Other types of seizures included left unilateral tonic-clonic seizures with a prolonged Todd's paralysis and generalized tonic-clonic seizures, mostly during sleep. On physical examination, spastic hypertonia of the 4 limbs was found together with bilateral Babinski sign, severe mental retardation with no verbal language, yellow teeth, and broad thumbs and toes. The interictal EEG during awake and sleep showed high-voltage diffuse continuous theta and delta waves with multifocal spikes over the frontal regions, asynchronously occurring over the two hemispheres and over the right temporo-occipital areas. Brain CT scans and MRI showed hypoplasia of the cerebellar vermis, asymmetric dilation of the lateral ventricles, the subarachnoid spaces and cisternae. IV-9 was sister of IV-10, she had delayed neuromotor development noticed at the age of 4 months. Her seizures onset was at the age of 10 months and was a febrile convulsion with loss of consciousness. Most of her seizures were correlated with sleep onset and were characterized by rigidity of the 4 limbs, rotation of the head to the right and occasionally the left, eyelid and facial myoclonus, and oro-facial automatisms. These episodes were short and consciousness was promptly recovered. These episodes were rare in the beginning but showed a progressive increase in frequency which was not modified by valproate or carbamazepine. The interictal EEG showed high-voltage diffuse slow wave activity at 3-4 c/s. Intermittent photic stimulation did not show any abnormal effects. Brain MRI showed only an enlargement of the lateral ventricles. In both patients, seizure frequencies were greatly reduced by clobazam. In both case, biochemistry or metabolic screening tests were normal.

Pedigree 3 was a German family reported by Petermöller (Petermöller *et al.* 1993). The parents were healthy unrelated individuals who had no remarkable family history of abnormal enamel or neurological disorders. II-3 had delayed psychomotor development noticed as early as 5 months old. For the first five months he was very restless but became very quiet thereafter, showed no interests to toys, became extremely reluctant to being touched and made very few movements. The seizures (febrile seizure) started at the age of

8.5 months and were very difficult to control. The seizure pattern changed to generalized seizures with left preponderance later, and was resistance to most of anti-epileptic drugs. The teeth were noticed to be yellow and crumble from the start of dentition. All biochemistry tests were normal and the MRI showed only slight ventricular enlargement. II-4 had normal development milestones for the first year. At the age of 8 months, however, she had her first febrile convulsion which was a generalized seizure with a left sided preponderance. Frequent seizures started at the age of 13 months and were resistant to phenytoin and carbamazepine. Psychomotor development regressed from the age of two. Her teeth were yellowish and prone to crumble at the start of dentition. The EEG showed generalized slow activity with discrete focal sharp waves. The mode of inheritance of this pedigree appeared to be autosomal recessive. III-5 was unaffected.

Pedigree 4 was an Italian family, which was investigated by Guazzi (Guazzi *et al.* 1994). The first generation I-1 and I-2 were both unaffected. In the second generation, there were 7 siblings, II-7 had amelogenesis imperfecta but not neurological disorders. His sister II-9, on the contrary, had psychomotor delay only but not amelogenesis imperfecta. In the third generation, III-12 was affected with both amelogenesis imperfecta and seizures. He had absence seizures during childhood and later tonic-clonic generalized epilepsy, but was well controlled by anti-epileptic drugs. He had no learning disability. He married his first cousin III-13, who was unaffected. In the fourth generation, IV-14 had a normal birth but early psychomotor development was delayed. Other later neurological symptoms included ataxia, dysarthria, hyperactive lower limb deep tendon reflexes, and bilateral positive Babinski sign. Neuropsychologic examination showed difficulty in maintaining attention, slowness, slight reduction of recent memory, and infantilism. EEG showed normal alpha activity in the posterior regions with bursts of theta waves, but clinically there was no record of seizures. IV-15 had psychomotor deterioration after the age of 2, and amelogenesis imperfecta was diagnosed at the age of 9. Other abnormalities included kyphoscoliosis of the dorsal spine. From the age of 10, episodes of partial loss of consciousness appeared. At the same age the child once became comatose for 36 hours and during hospitalization, spasticity of all limbs was described. The EEG was characterized by the presence of widespread theta and delta waves in all records. He died suddenly at the age of 12 years in 1979. III-16 had amelogenesis imperfecta only and no other abnormality

such as psychomotor delay or neurological disorders. III-17 was cyanotic at birth, but did not need any therapy or intensive care and his psychomotor development was slightly delayed. From the age of 10, he developed a ataxic gait and complained of cramps in the legs. Neurological examination showed only brisk tendon reflexes in the lower limbs. The EEG showed alpha activity (8 c/sec) in the posterior regions interrupted by 2 brief generalized bursts of sharp waves. MRI of the brain showed slight periventricular gliosis and hypoplasia of the corpus callosum. The patient did not have enamel hypoplasia of the teeth. Whether these symptoms were related to the hypoxia during birth was not determined.

The mode of inheritance of pedigree 4 was more complicated than the other 4 pedigrees as there were four different phenotypes: amelogenesis imperfecta only; delayed psychomotor developments; ataxia and abnormal EEG; neurological disorders or seizures and amelogenesis imperfecta. Guazzi in his report concluded that this was an autosomal dominant pedigree with amelogenesis imperfecta as the main phenotype but with additional variable manifestations, including seizures, ataxia, and psychomotor retardation. However, this pedigree could be autosomal recessive if amelogenesis imperfecta and neurological disorders was the defined phenotype. Under such a phenotype definition, II-7 was unaffected, only III-12, IV-14 and IV-15 were affected. Because of consanguity, III-13 could be a carrier, with a 50% chance that offspring would be affected.

Pedigree 5 was an Australian family. This family was investigated by Christodoulou (Christodoulou *et al.* 1988). There were 6 affected individuals in this pedigree, three of them had expired and DNA samples were only available from the other three affected individuals, one unaffected individual, and one of the parents (mother). Both parents were unaffected with no remarkable dental or neurological disorders. There were four maternal relatives which had mental retardation of unknown cause. The affected individuals in the second generation all had the same phenotype, including amelogenesis imperfecta, seizures, and delayed neuromotor development. The seizure onset of II-3 was at 7 months and the diagnosis of Kolhschütter-Tönz syndromes was confirmed at the age 1.5 years. No investigation details of this individual were available. II-4 was sister of II-3, her seizure onset was at 22 months old and the clinical presentation included delayed neuomotor development and amelogenesis imperfecta. II-5 was one of the twins survived. His seizure

onset was at the age of 18 months, with regression of neuromotor development as well as amelogenesis imperfecta. His biochemistry tests were all normal, which included normal pyruvate dehydrogenase, cytochrome c oxidase, succinatecytochrome c reductase, NADH-cytochrome c reductase, El ATPase, serum uric acid, blood porphyrin screen, blood ammonia, CSF lactate, pyruvate, and amino acids. His G banded karyotype was also normal. II-6 had seizures began at the age of 22 months, with delayed neuromotor development. II-7 was the only unaffected individual in this generation. II-8 was normal until the age of 11 months when he was brought to hospital in status epilepticus. He had severe learning disability and intractable epilepsy, died at the age of 10 and ½ years old of pneumonia and emaciation. II-9 had seizures began at the age of 11 months with amelogenesis imperfecta and delayed neuromotor development. She died at the age of 1.5 years old. The mode of inheritance in pedigree 5 was autosomal recessive.

Table 10.1 Clinical presentations and investigations

Pedigree	ID	Country origin	Amelogenesis Imperfecta	Seizures	Other neurological disorder	EEG	Image study	Biochemistry tests
1	II-3	UK	+	epileptic convulsion	Psychomotor development delay and learning disabilities, hypotonia	Hypsarrythmia	Enlargement of cortical sulci, lateral and third ventricles	normal
1	II-4	UK	+	Myoclonic seizures	Psychomotor development delay and learning disabilities, hypotonia	Lack of normal background activities with multifocal discharges	Lack of myelination and enlarged lateral ventricles	normal
2	IV-9	Italy	+	Orofacial automatism, rigid 4 limbs and head turning, either to right or left	Severe psychomotor development delay,	High voltage slow activities, 3-4 Hz	Enlarged lateral ventricles	normal
2	IV-10	Italy	+	Partial seizures with secondary generalization	Psychomotor development delay and learning disabilities, spasticity	High voltage diffuse and continuous theta and delta waves	Hypoplasia of vermis, enlarged ventricles	normal
3	II-3	German	+	Generalized seizure with left preponderance	Severe psychomotor development delay	Focal or multifocal sharp wave discharges	Slight ventricular enlargements	normal
3	II-4	German	+	Generalized seizure with left preponderance	Severe psychomotor development regression after the age of 2	Generalized slow activity with discrete focal sharp waves.	Normal	normal

Table 10.1 Clinical presentations and investigations (continued)

Pedigree	ID	Country origin	Amelogenesis Imperfecta	Seizures	Other neurological disorder	EEG	Image study	Biochemistry tests
4	II-7*	Italy	+	-	-	N/A	N/A	N/A
4	II-9*	Italy	+	-	Psychomotor development delay only	N/A		N/A
4	III-12	Italy	+	Absence, generalized tonic-clonic seizures	-	N/A	N/A	N/A
4	IV-14	Italy	+	-	Pain, cramps in the leg, deterioration of psychomotor performance,	Normal alpha background activity interrupted by burst of sharp waves	Peri-ventricular gliosis and hypoplasia of the corpus callosum	increase in glutamate oxalacetate and glutamate pyruvate transaminases, otherwise normal.
4	IV-15	Italy	+	Partial lost of consciousness, with spasticity of 4 limbs, abnormal EEG,	Severe psychomotor development delay and learning disability	Presents of theta and delta waves on all records	normal	Mucopolysaccharides were absent in the urine
4	IV-16*	Italy	+	-	normal	N/A	N/A	N/A
4	IV-17*	Italy	-	-	Ataxia, stiffness in the legs, mild psychomotor development delay, with abnormal EEG	Occipital sharp waves, alpha back ground activity	Peri-ventricular gliosis and hypoplasia of the corpus callosum	N/A

* Individuals that had either amelogenesis imperfecta only or neurological disorders only in pedigree 4 (continued)

Table 10.1 Clinical presentations and investigations (continued)

Pedigree	ID	Country origin	Amelogenesis Imperfecta	Seizures	Other neurological disorder	EEG	Image study	Biochemistry tests
5	II-3	Australian	+	+	N/A	N/A	N/A	N/A
5	II-4	Australian	+	+	N/A	N/A	N/A	N/A
5	II-5	Australian	+	+	N/A	N/A	N/A	normal
5	II-6	Australian	+	+	N/A	N/A	N/A	N/A
5	II-7	Australian	-	-	-	-	-	-
5	II-8	Australian	+	+	N/A	N/A	N/A	N/A
5	II-9	Australian	+	+	N/A	N/A	N/A	N/A

10.2 Linkage Analysis

10.2.1 Mode of inheritance and choosing linkage analysis program

The mode of inheritance of these 5 pedigrees was considered as autosomal recessive. The analysis was done by parametric linkage analysis with MLINK.

10.2.2 Setting parameter files

The parameter files were prepared by PREPLINK. The mode of inheritance was set as autosomal recessive with full penetrance. Liability class was set at 1, the gene frequencies were provided by deCODE Genetics. Because there were consanguity in two of the pedigrees, a loop data file indicating where to break the loop was made when converting the pedigree files to pedfiles to facilitate the speed in analysis.

10.2.3 Genotyping errors

The genotype data and compatibility were checked by UNKNOWN. Genotyping errors (if there is any) were adjusted by setting the genotypes in question as "unknown".

10.2.4 LOD scores

The maximum LOD score obtained from MLINK was 3.05, at θ =0 at D16S423. The LOD scores is presented in Table 10.2.The haplotype analysis is shown in Figure 10.4 to Figure 10.8. 3.05 is considred significant linkage. The haplotype analysis further suggested that there was a disease locus between 16p13.3-13.2. There are approximately 30 known genes and pseudo genes within this region, and the functions of the known genes are summarized in Table 10.3 There are no known ion channel genes in this area, nor are there any ion channel regulating genes. Possible candidate genes are marked red in this table.

Table 10.2 LOD score on chromosome 16

θ / *Marker*	*0.0*	*0.01*	*0.05*	*0.1*	*0.2*	*0.3*	*0.4*
D16S521	-infini	-5.59	-2.35	-1.17	-0.31	-0.04	0.02
D16S3065	-infini	-0.57	0.56	0.82	0.75	0.47	0.18
D16S423	***3.05***	***2.97***	***2.64***	***2.23***	***1.44***	***0.74***	***0.22***
D16S418	-infini	-0.58	0.57	0.85	0.81	0.53	0.23
D16S3062	-infini	-3.92	-1.40	-0.54	0.00	0.09	0.06

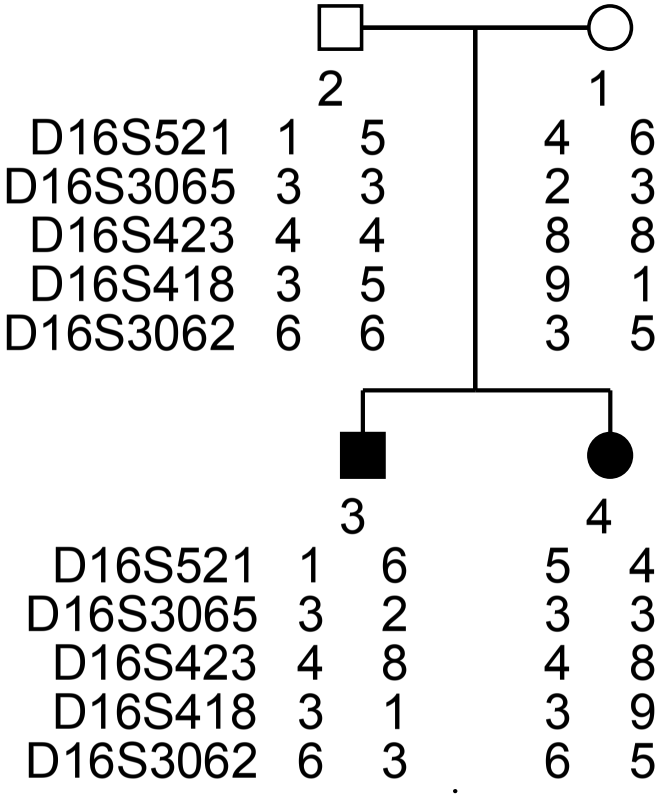
10.3 Microarray Analysis

In order to seek further evidence to support our result, 4 DNA samples were sent for Microarray analysis for SNPs detection. Two of the DNA samples (one affected and one control) failed on Microarray analysis. The other 2 DNA samples successfully analyzed showed a region of homozygosity from the location 37354 bp of chromosome 16 (snp rs8466) to the location 6005359 bp (snp rs955905) on the affected individual while the same locus showed heterozygosity in the unaffected individuals. This locus shown on the microarray chip analysis was larger than the disease locus identified through linkage (which started approximately from 3762270 bp to 7579883 bp on chromosome 16) but there was an obvious overlap. The flanking SNP that showed homozygosity in the chip analysis were rs8466 and rs955905, located at 37354 bp and 6005359bp on chromosome 16, respectively. This finding provided supporting evidence that there could be a disease locus on chromosome 16p13.3-16p13.2. If only the region overlap in two analyses was taken into consideration, the microarray analysis results was able to narrow down the region in interest by 1574524 bp.

10.4 Summary

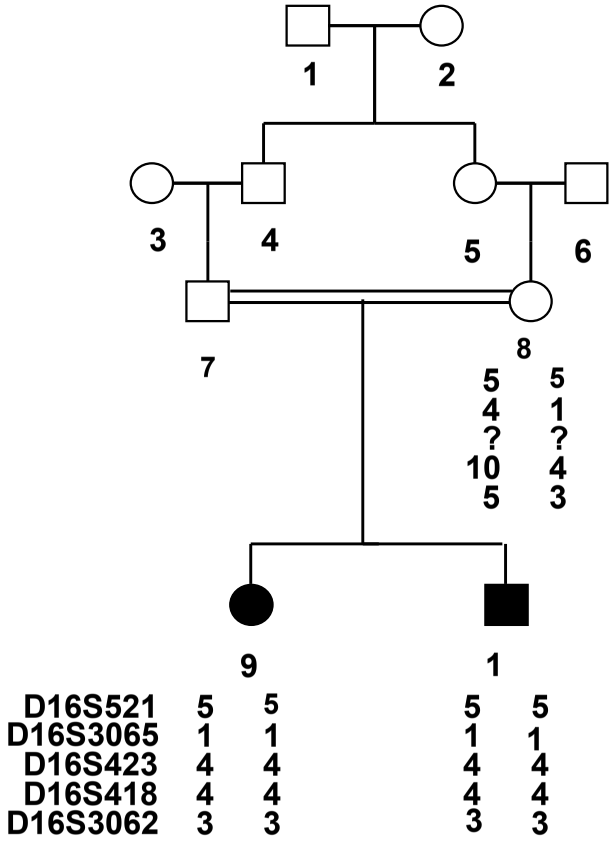
The aim of this analysis was to identify the genetic basis (linkage) of this rare syndrome (in five families), to determine the mode of inheritance, to identify linkage, to define the clinical phenotype associated with the linked area, and to if possible identify the causative gene. We confirmed a disease linkage on chromosome 16 through both linkage analysis and microarray chip analysis. There are 30 known genes within this area however there are no ion channel genes. More investigation is necessary both in identifying candidate genes as well as in establishing the pathogenesis once the gene is identified.

Figure 10.4 Haplotype analysis



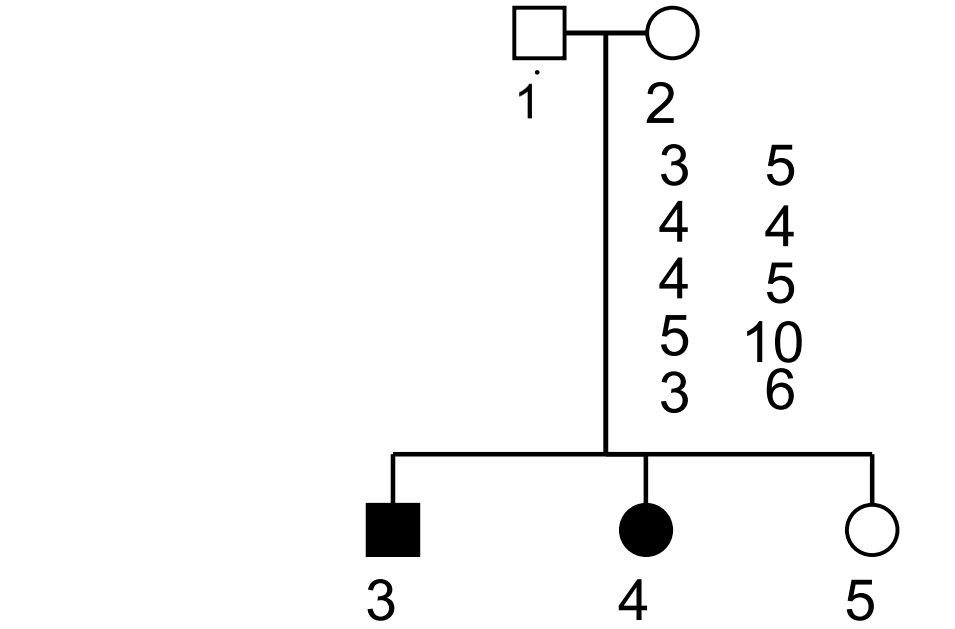
Pedigree 1

Figure 10.5 Haplotype analysis



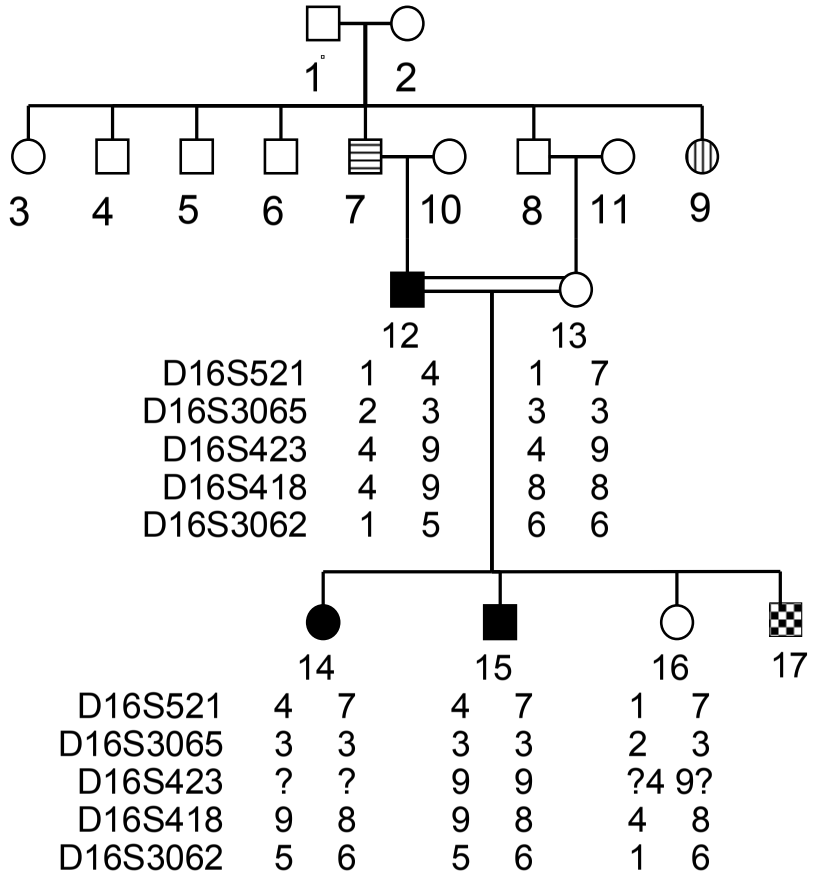
Pedigree 2

Figure 10.6 Haplotype analysis



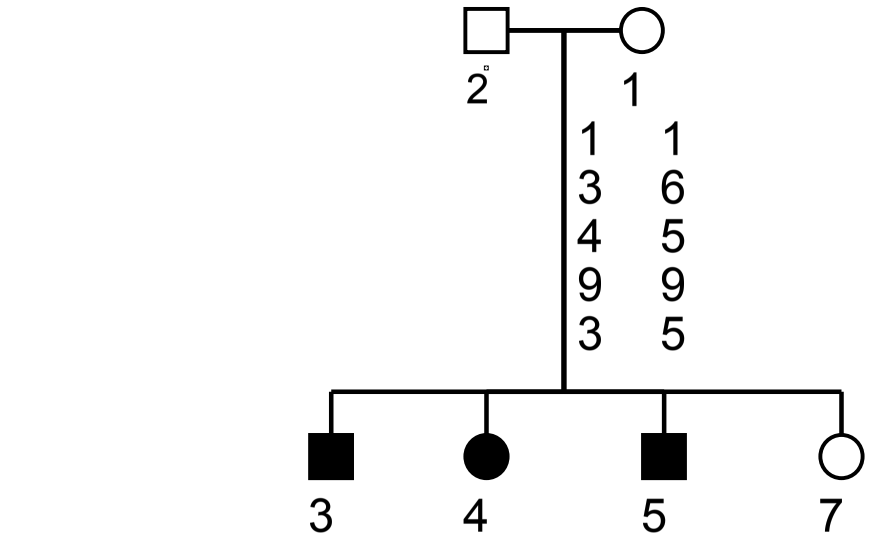
Pedigree 3

Figure 10.7 Haplotype analysis



Pedigree 4

Figure 10.8 Haplotype analysis



Pedigree 5

Table 10.3 Summary of the genes within 16p13.3-16p13.2

Gene ID	Function
CREBBP	Acetylates histones, giving a specific tag for transcriptional activation. Also acetylates non-histone proteins, like NCOA3 coactivator. Binds specifically to phosphorylated CREB and enhances its transcriptional activity toward cAMP-responsive genes.
ADCY9	May play a fundamental role in situations where fine interplay between intracellular calcium and cAMP determines the cellular function. May be a physiologically relevant docking site for calcineurin.
SRL	Function undetermined
TFAP4	Transcription factor that activates both viral and cellular genes by binding to the symmetrical DNA sequence 5'-CAGCTG-3'.
GLIS2	Members of the Kruppel-like zinc finger protein family, such as GLIS2, function as activators and/or repressors of gene transcription.
CORO7	May play a role in the maintenance of the Golgi apparatus morphology and in the protein export from the Golgi.
VASN	May act as an inhibitor of TGF-beta signaling
DNAJA3	Modulates apoptotic signal transduction or effector structures within the mitochondrial matrix. Affect cytochrome C release from the mitochondria and caspase 3 activation, but not caspase 8 activation. Isoform 1 increases apoptosis triggered by both TNF and the DNA-damaging agent mytomycin C; in sharp contrast, isoform 2 suppresses apoptosis. Can modulate IFN-gamma-mediated transcriptional activity.
NMRAL1	Function undetermined
HMOX2	Heme-oxygenase cleaves the heme ring at the alpha methene bridge to form biliverdin. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Under physiological conditions, the activity of heme oxygenase is highest in the spleen, where senescent erythrocytes are sequestrated and destroyed. Heme oxygenase 2 could be implicated in the production of carbon monoxide in brain where it could act as a neurotransmitter.
MGRN1	Probable E3 ubiquitin-protein ligase.
ZNF500	May be involved in transcriptional regulation
FAM100A	Function undetermined
NUDT16L1	Probable adapter protein, which may link syndecan-4 (SDC4) and paxilin (TGFB1I1 and PXN) receptors.

(continued)

Table 10.3 Summary of the genes within 16p13.3-16p13.2 (continued)

Gene ID	Function
ANKS3	Function unknown
SEPT12	Septins, such as SEPT12, are conserved GTP-binding proteins that function as dynamic, regulatable scaffolds for the recruitment of other proteins. They are involved in membrane dynamics, vesicle trafficking, apoptosis, and cytoskeleton remodeling, as well as infection, neurodegeneration, and neoplasia.
ROGDI	May act as a positive regulator of cell proliferation.
UBN1	May be required for replication-independent chromatin assembly.
PPL	The protein encoded by this gene is a component of desmosomes and of the epidermal cornified envelope in keratinocytes. Component of the cornified envelope of keratinocytes. May link the cornified envelope to desmosomes and intermediate filaments. May act as a localization signal in PKB/AKT-mediated signaling.
SEC14L5	Function undetermined
NAGPA	Catalyzes the second step in the formation of the mannose 6-phosphate targeting signal on lysosomal enzyme oligosaccharides by removing GlcNAc residues from GlcNAc-alpha-P-mannose moieties, which are formed in the first step.
ALG1	Participates in the formation of the lipid-linked precursor oligosaccharide for N-glycosylation. Involved in assembling the dolichol-pyrophosphate-GlcNAc(2)-Man(5) intermediate on the cytoplasmic surface of the ER.
FAM86A	Function unknown
A2BP1	RNA-binding protein that regulates alternative splicing events by binding to 5'-UGCAUGU-3' elements. Regulates alternative splicing of tissue-specific exons and of differentially spliced exons during erythropoiesis.

Section 11: Saccadic eye movement experiments

11.1 Control Group (n=12)

The main sequence of saccades recorded from each subject in the control group is presented in Table 12.1 in the form of square root model (Main Sequence = C * $\sqrt{A}$). "A" represents the amplitude the eye ball had travelled in each saccadic eye movement, "C" represents the constant and was calculated after fitting the line to the dots which plotted the peak velocity of each saccade against the amplitude after data conversion and analysis. Paired T test was carried out to compare the main sequence recorded between the first and second recording (data recorded with chin rest in the first recording compared to the second recording with chin rest, and without chin rest to without chin rest) as well as main sequence recorded by chin rest and without chin rest within 1 individual in the same set of recording. *P_1 values* represented the results of the comparison in each individual between the 1st and the 2nd recording, and *P_2 values* represented the results of comparison in each individual with and without chin rest.

Twelve healthy subjects were recruited in the control group. The recording of one of the healthy subjects was not available because of technical error during recording. Case 7 was able to repeat the eye movement battery both with and without chin rest during the second set of recording twice, and Case 8 was able to repeat the battery three times in both sets of recording with and without chin rest. The chin-rest-free saccadic eye movement recording was not done in case 1, 2, 3, and 4. In the recordings of case 8, sufficient data were available to calculate the mean and 95% CI of the main sequence in this subject.

From Table 11.1, it shows that most of the 1st set and 2nd set recording from the same individual did not show any significant statistical difference when analyzed with paired T test, except case 3, case 4 and case 9 ((*P1=0.036, 0.0013,and 0.041, respectively)*. In other words, 81% (9/11) of cases showed no significant difference in their main sequence recorded at different time when using chin rest, and 85.7% (6/7) of cases showed no significant difference in their main sequence recorded at different times while chin rest was not used.

In the comparison between main sequence obtained using chin rest and without chin rest, only case 10 showed that there was a significant difference of the main sequences between using chin rest and without using chin rest ($P_2=0.005$).

The test-retest reliability in the control group was calculated with Pearson regression correlation, and Intraclass Correlation Coefficient (ICC). The 95% confidence interval of ICC value and typical error is presented in Table 12.2. The results showed that both Pearson Correlations Coefficient (r) and ICC was 0.433, (95% CI 0.014-0.723) when chin rest was used. Pearson Correlations Coefficient (r) was 0.615 and ICC was 0.588 (95% CI 0.083-0.852) when the chin rest was not used. In general *r values* are within 0.7- 0.9 for adequate reliability and correlation, and > 0.9 if of very good reliability. The test-retest reliability in the control group was below these values and thus the reliability has to be considered poor.

Figure A and B are graphs with data of the second recording plotted against the first recording, with data form the left eye plotted to left eye and right eye to right eye. Figure C and D are graphs with the subtraction of recording 2 to recording 1 plotted against recording 1, with data from the left eye plotted against to left eye and right eye against to right eye. The graphs showed that the correlation was slightly better in the main sequence recorded when chin rest was not used.

To sum up, the analysis of the results of the main sequence measurements in the control group did not show good test-retest correlation, although paired T test did not suggest that the measurement of the first recording are statistically different from the second recording except in three cases. The reliability was thought to be such that the identification of small differences due to drug actions in the subject group would not be able to be detected.

Table 11.1 Main sequence of the control group

Case ID (age)		main sequence (C* $\sqrt{A}$)(with chin rest)		main sequence (C* $\sqrt{A}$)(without chin rest)
Case 1(37) Recording 1	left eye	113.967 $\sqrt{A}$	*P_1=0.70*	-
	right eye	98.2409 $\sqrt{A}$		-
Case 1 Recording 2	left eye	103.757 $\sqrt{A}$		-
	right eye	101.416 $\sqrt{A}$		-
Case 2 (24) Recording 1	left eye	94.812 $\sqrt{A}$	*P_1=0.44*	-
	right eye	94.4832 $\sqrt{A}$		-
Case 2 Recording 2	left eye	127.007 $\sqrt{A}$		-
	right eye	113.352 $\sqrt{A}$		-
Case 3 (32) Recording 1	left eye	101.249 $\sqrt{A}$	*P_1=0.036 <0.05*	-
	right eye	98.7713 $\sqrt{A}$		-
Case 3 Recording 2	left eye	123.814 $\sqrt{A}$		-
	right eye	118.9 $\sqrt{A}$		-
Case 4 (35) Recording 1	left eye	116.123 $\sqrt{A}$	*P_1=0.0013 <0.5*	-
	right eye	121.983 $\sqrt{A}$		-
Case 4 Recording 2	left eye	134.846 $\sqrt{A}$		-
	right eye	140.785 $\sqrt{A}$		-

Table 11.1 Main sequence of the control group (continue)

Case ID (age)		main sequence (C* $\sqrt{A}$)(with chin rest)		main sequence (C* $\sqrt{A}$)(without chin rest)	
Case 5 (41) Recording 1	left eye	$117.276\sqrt{A}$	P_1=0.36	$126.171\sqrt{A}$	P_1=0.5 P_2=0.22
	right eye	$118.187\sqrt{A}$		$133.829\sqrt{A}$	
Case 5 Recording 2	left eye	$112.936\sqrt{A}$		$126.083\sqrt{A}$	
	right eye	$98.428\sqrt{A}$		$112.563\sqrt{A}$	
Case 6(64) Recording 1	left eye	$90.6071\sqrt{A}$	P_1=0.43	$96.5234\sqrt{A}$	P_1=0.40 P_2=0.08
	right eye	$72.7373\sqrt{A}$		$104.527\sqrt{A}$	
Case 6 Recording 2	left eye	$92.3701\sqrt{A}$		$99.4043\sqrt{A}$	
	right eye	$88.9638\sqrt{A}$		$105.003\sqrt{A}$	
Case 7 (35) Recording 1	left eye	$103.729\sqrt{A}$	P1=0.19	$124.501\sqrt{A}$	P_1=0.15 P_2=0.44
	right eye	$124.455\sqrt{A}$		$98.452\sqrt{A}$	
Case 7 Recording2.1	left eye	$98.8823\sqrt{A}$		$121.795\sqrt{A}$	
	right eye	$106.664\sqrt{A}$		$106.564\sqrt{A}$	
Case 7 Recording2.2	left eye	$107.748\sqrt{A}$		$131.088\sqrt{A}$	
	right eye	$106.627\sqrt{A}$		$105.815\sqrt{A}$	
Case 8 (30) Recording1.1	left eye	$118.11\sqrt{A}$	Mean: 114.43	$126.759\sqrt{A}$	Mean: 114.10
	right eye	$113.766\sqrt{A}$		$113.651\sqrt{A}$	
Case 8 Recording1.2	left eye	$113.01\sqrt{A}$	95% C.I: 114.43± 5.09	$103.22\sqrt{A}$	95% C.I: 114.10±8.9
	right eye	$124.978\sqrt{A}$		$104.853\sqrt{A}$	
Case 8 Recording1.3	left eye	$109.168\sqrt{A}$		$118.409\sqrt{A}$	
	right eye	$107.538\sqrt{A}$		$117.736\sqrt{A}$	

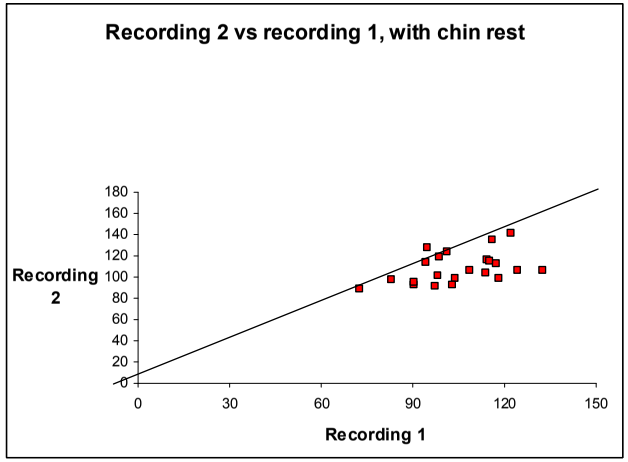
Table 11.1 Main sequence of the control group (continue)

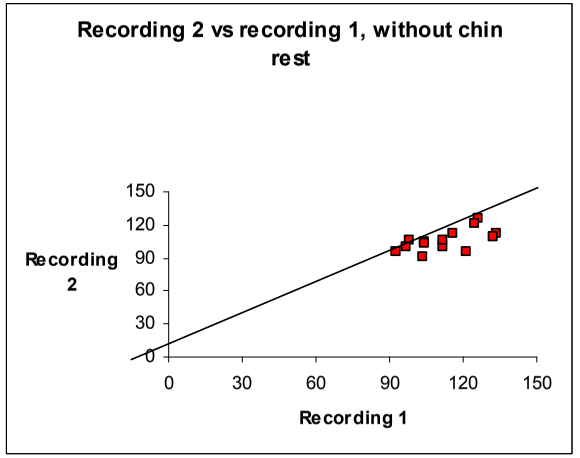
Case ID (age)		main sequence (C* $\sqrt{A}$)(with chin rest)		main sequence (C* $\sqrt{A}$)(without chin rest)	
Case 8 Recording2.1	left eye	125.901 $\sqrt{A}$	Mean: 116.58	125.503 $\sqrt{A}$	Mean: 107.79
	right eye	110.991 $\sqrt{A}$		100.74 $\sqrt{A}$	
Case 8 Recording2.2	left eye	102.063 $\sqrt{A}$		101.765 $\sqrt{A}$	95% C.I: 107.79± 9.47
	right eye	116.531 $\sqrt{A}$	95% C.I: 116.58± 7.05	94.4703 $\sqrt{A}$	P_1=0.052
Case 8 Recording2.3	left eye	122.683 $\sqrt{A}$		118.697 $\sqrt{A}$	P_2=0.19
	right eye	121.304 $\sqrt{A}$	P_1= 0.65	105.573 $\sqrt{A}$	
Case 9 (23) Recording 1	left eye	132.393 $\sqrt{A}$	P_1=0.43	132.091 $\sqrt{A}$	P_1=0.041<0.05
	right eye	108.573 $\sqrt{A}$		121.07 $\sqrt{A}$	P_2=0.82
Case 9 Recording 2	left eye	106.151 $\sqrt{A}$		109.239 $\sqrt{A}$	
	right eye	105.64 $\sqrt{A}$		95.0452 $\sqrt{A}$	
Case 10 (31) Recording 1	left eye	97.5044 $\sqrt{A}$	P_1=0.91	112.029 $\sqrt{A}$	P1=0.35
	right eye	90.622 $\sqrt{A}$		104.629 $\sqrt{A}$	P_2=0.005<0.05
Case 10 Recording 2	left eye	90.9332 $\sqrt{A}$		106.527 $\sqrt{A}$	
	right eye	95.5524 $\sqrt{A}$		103.313 $\sqrt{A}$	
Case 11(25) Recording 1	left eye	82.9552 $\sqrt{A}$	P_1=0.89	92.5056 $\sqrt{A}$	P_1=0.68
	right eye	102.966 $\sqrt{A}$		103.757 $\sqrt{A}$	P_2=0.52
Case 11 Recording 2	left eye	97.5104 $\sqrt{A}$		96.1572 $\sqrt{A}$	
	right eye	92.7528 $\sqrt{A}$		91.3723 $\sqrt{A}$	

Table 11.2 Reliability calculation in the control group

	With chin rest	Without chin rest
	Recording 2-1	Recording 2-1
Change in mean	**3.22**	**-6.63**
Lower conf limit	-3.62	-12.78
Upper conf limit	10.06	-0.49
Typical error	**10.91**	**7.52**
Lower conf limit	8.39	5.45
Upper conf limit	15.59	12.12
Degrees of freedom	21	13
TE^2	119.01	56.61
Pearson r	**0.433**	**0.615**
Intraclass r	**0.433**	**0.588**
Lower conf limit	0.014	0.083
Upper conf limit	0.723	0.852

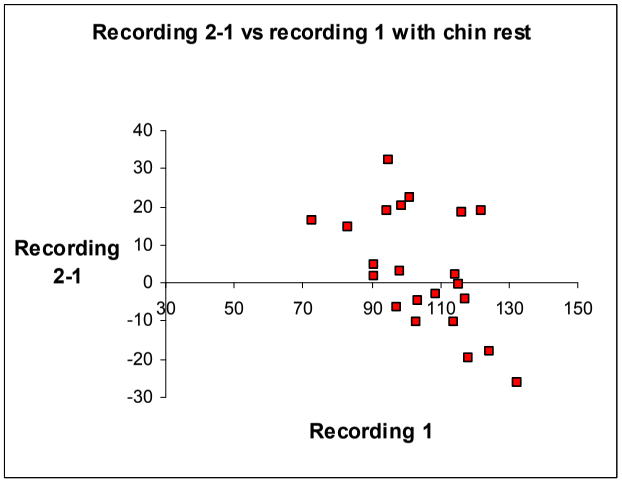
A

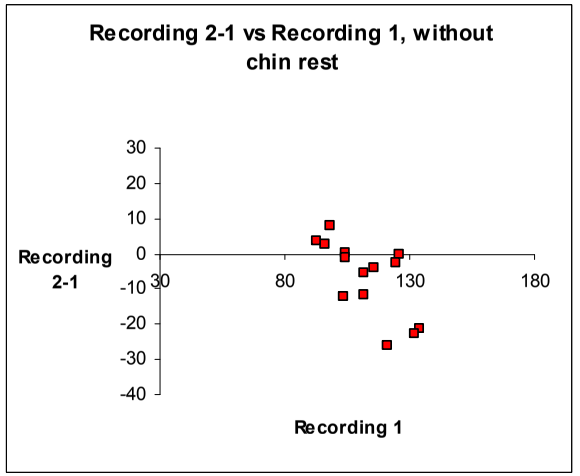




B

C





D

Figure 11.1 Reliability analyses. A and B are graphs of recording 2 plotted against recording 1, left eye to left eye and right eye to right eye. Figure C and D presents the graph of the data (recording 2-recording 1) plotted against recording 1.

11.2 Subject group (n=3)

The main sequence measurements changes in the subject group and the results of the analysis are presented in Table 12.3. None of the case showed significant change in main sequence measurements before and after medication (all *P values* > 0.05). The serum drug levels at the time of eye movement recording are also listed.

The eye movement of Case 1 and Case 3 were recorded before and once again after they took their daily carbamazepine (CBZ) medication, and the two set of testing were completed in the same day with a time interval of three hours and 4 hours respectively. The first recording of case 2 was done when the case was experiencing phenytoin (PHT) intoxication. Her serum drug level was 84 umol/l and was higher than standard therapeutic level and symptoms and signs of intoxication were also found at the time of the first recording. The second set of recordings was done 1 week after the first recording while the serum drug level had returned to the normal range 62 umol/l.. Case 3 declined to have serum drug level tested again for the second set of recording. All cases had their eye movements recorded with and without chin rest.

Case 1 had intractable seizures and did not show any optimal drug response to either old or new generation anti-epileptic drugs, carbamazepine was the only drug that she had showed partial responses and she was considered a non-respondent. Case 2 had reasonable seizure control, with a seizure frequency around 1-2 times annually, usually with apparent triggering factors such as sleep deprivation. Case 3 had optimal seizure control and at the time of recording she had been seizure free for approximately one year. We expected that there would be saccadic eye movement parameter changes seen in these 2 cases.

We hypothesized that the main sequence of case 1 would not show mark changes between the 2 sets of eye movements recordings of the paramters we used. In case 2, however, the subject did not show the expected significant change of main sequence parameter between the 2 recordings, despite the fact that she had fairly good seizure control and a considerable change of the phenytoin serum drug level was observed between the two sets of eye movements recordings. In case 3, again we expected there to be changes of the main sequence parameter, but no significant changes were observed between the 2 sets of recordings.

11.3 The comparison between the control group and the subject group

The comparison of the main sequence between the control group and the subject group is presented in Table 12.4. The comparison was made case by case to the control group, because in each case their serum drug levels and the medication they took were different.

The *P values* presented in Table 12.4 show that only the main sequence of case 1 was statistically different from the control group. The main sequence of case 2 and case 3 did not differ from the control group, even though they were either on carbamazepine or phenytoin therapy. The main sequence of case 1 obtained without using chin rest before and after taking carbamazepine, as well as the main sequence obtained with chin rest after taking medication (carbamazepine) showed significant difference from the control group.

Table 11.3 Main sequence of the subject group

Case ID		main sequence (C* $\sqrt{A}$) with chine rest		main sequence (C* $\sqrt{A}$) without chin rest		Serum drug level (umol/l)
Case 1 Recording1	left eye	97.2428 $\sqrt{A}$	*P_1=0.25*	93.0793 $\sqrt{A}$	*P_1=0.25* *P_2=0.12*	24.6 (CBZ)
	right eye	82.9895 $\sqrt{A}$		82.9825 $\sqrt{A}$		
Case 1 Recording2.1	left eye	80.0339 $\sqrt{A}$		79.1122 $\sqrt{A}$		26.3(CBZ)
	right eye	81.3006 $\sqrt{A}$		77.2754 $\sqrt{A}$		
Case 1 Recording2.2	left eye	76.3089 $\sqrt{A}$		-		
	right eye	87.8169 $\sqrt{A}$		-		
Case 2 Recording1	left eye	116.977 $\sqrt{A}$	*P_1=0.67*	136.119 $\sqrt{A}$	*P_1=0.39* *P_2=0.66*	84(PHT)
	right eye	119.768 $\sqrt{A}$		107.583 $\sqrt{A}$		
Case 2 Recording 2	left eye	121.412 $\sqrt{A}$		99.4612 $\sqrt{A}$		62(PHT)
	right eye	103.325 $\sqrt{A}$		101.391 $\sqrt{A}$		
Case 3 Recording1	left eye	113.45 $\sqrt{A}$	*P_1=0.99*	127.229 $\sqrt{A}$	*P_1=0.75* *P_2=0.56*	23.8(CBZ)
	right eye	121.672 $\sqrt{A}$		104.043 $\sqrt{A}$		
Case 3 Recording2	left eye	110.991 $\sqrt{A}$		109.258 $\sqrt{A}$		N/A
	right eye	124.04 $\sqrt{A}$		111.379 $\sqrt{A}$		

Table 11.4 comparison between control group and subject group

	with chin rest, 1st	with chin rest 2nd	without chin rest 1st	without chin rest 2nd
Case 1	*P*=0.09	***P=0.00039<0.05***	***P=0.0147<0.05***	***0.000678<0.05***
Case 2	*P*=0.18	*P*=0.49	*P*=0.21	*P*=0.26
Case 3	*P*=0.21	*P*=0.22	*P*=0.56	*P*=0.99

11.4 Summary

In this pilot (or scoping) phase we aimed to:

1. to devise a clinically-applicable method for measuring saccadic main sequence and peak saccadic velocity.
2. to carry out measurements in a suitable control group, to identify mean values and the range of normality.
3. to carry out a pilot study in patients with epilepsy, to determine whether changes in blood levels were reflected by measurable changes in saccadic velocity in a reliable fashion, and to determine the extent of this effect
4. In a preliminary manner, to whether the there is an association between the magnitude of the effect of saccadic variation and clinical effects (side-effects/efficacy).

In summary of the results, the Pearson regression and correlation coefficient and Intraclass coefficient did not suggest that there was a high correlation and a good reliability of main sequence (peak saccadic velocity). Thus it was not possible to obtaine a reliable normative data of the main sequence. The analysis results from the three cases in the subject group were inconclusive. None of the cases in this group showed significant changes of their main sequence recorded before and after taking their AEDs. When comparing their main sequence to the control group, only case 1 had a peak velocity of saccades which were slower than the mean of the control group.

Discussion

Section 12: The Linkage Studies

12.0 Hypothesis

The hypothesis underpinning the genetic linkage analyses in this thesis is that the cause of these familial epilepsy syndromes (mesial temporal lobe epilepsy, photosensitive epilepsy, and Kohlschütter-Tönz syndromes) is genetic in origin, and genetic linkage analysis could detect a disease locus for each epilepsy syndrome.

In this body of work, I studied 8 families within epilepsy syndromes (familial temporal lobe epilepsy, photosensitive epilepsy, Kohlschütter-Tonz syndrome) and collected appropriate clinical phenotypic data (from clinical examination, note review and hospital inquiry) to carry out the laboratory genetic analysis of the DNA from individuals in this families (preparation, genotyping and sequencing). I devised and conducted the computerised linkage analysis using various linkage programmes.

The aims of the study were:

i. Familial temporal lobe epilepsy (two families). To identify the genetic basis of the familial epilepsy, to determine whether there was a similar genetic cause in the two families, to determine the mode of inheritance in the families, to identify linked regions, to sequence candidate genes within the linked regions and to determine the clinical phenotype associated with any linkage discovered.

ii. Photosensitive epilepsy (one large family): To identify the genetic basis of the Photoparoxysmal response (PPR) in a large family with photosensitive epilepsy, to determine the mode of inheritance, and to identify linkage.

iii. Kohlschütter-Tonz Syndrome: To identify the genetic basis (linkage) of this rare syndrome (in five families), to determine the mode of inheritance, to identify linkage, to define the clinical phenotype associated with the linked area, and to if possible identify the causative gene.

The linkage analyses confirmed a disease linkage in the five small families with the Kohlschütter-Tönz syndrome, and a less confirmed linkage with three regions of interest in

pedigree B with mesial temporal lobe epilepsy. The genetics underlying the photosensitive family was likely to be polygenic, and a region of interest was identified through NPL. In the pedigree W with mesial temporal lobe epilepsy, the mode of inheritance could not be determined, and the analysis was negative. In this discussion, I will speculate on these results and the reasons for these positive and negative findings.

12.1 The fMTLE pedigrees

12.1.1 Pedigree B

The B pedigree was a middle size pedigree with 8 affected individuals, including a person who was a possible phenocopy and who was excluded as an affected individual in the third parametric analysis. The first parametric analysis in which both pain and seizures were the defined phenotypes showed a maximum LOD score of 2.37 at chromosome 6, but multipoint analysis, fine mapping, and haplotype analysis excluded this region. The second parametric analysis in which seizures only was the defined phenotype did not show any LOD scores larger than 2. In the third parametric analysis, a possible phenocopy was further excluded and the result showed that there might be a disease locus on chromosome 3 with a LOD score 2.35, however the haplotype analysis showed that there could be a reduced penetrance. The same pedigree was re-analyzed assuming an AD with a reduced penetrance inheritance and showed that were three regions of interest on chromosome 3 (3p24.1-3p24.2, LOD 2.17), chromosome 5 (5p13.3-5p13.2, LOD 2.15) and chromosome 7 (7q11.22-7q21.11, LOD 2.15). Five genes within 3p24.1-3p24.2 were sequenced, including SLC4A7, LRRC3B, AZI2, NEK10, and ZCWPW2 genes.

The SLC4A7 gene functions as a Na^{+}/HCO^{-} co-transporter and has been found in the dendrite and soma of neurons in the hippocampus especially. Recent research showed that this gene might increase the cytotoxicity in magnesium depletion, as found in the primary cultures of hippocampal neurons in rat (Cooper *et al.* 2009). However it is not known whether this upregulation effect on cytotoxicity might contribute to hippocampal sclerosis or epileptogenesis. I did not find any rare mutations on this gene but did discover a few polymorphisms that are considered to be normal variations in Caucasian population (Table 12.1). I therefore do not consider these polymorphisms to be pathogenic. Most of the polymorphisms are synonymous, which means they do not alter the amino acid coded to

that region and they do not change the peptide products. There is only one non-Synonymous polymorphism, which is considered non-pathogenic.

Table 12.1 Polymorphisms on SLC4A7 gene and LRRC3B gene

Gene	Position	polymorphism	Frequencies in Caucasian population	Type of polymorphism
SLC4A7	Exon 3	210A/G	A: 0.758, G:0.242	Synonymous
SLC4A7	Exon 7	846T/C	A:0.720, G: 0.280	Synonymous
SLC4A7	Exon 7	976G/A, Gly>Lys	A: 0.080, G:0.920	Non-Synonymous
SLC4A7	Exon 16	2319C/T	C: 0.750, T: 0.250	Synonymous
SLC4A7	Exon 24	3499T/C	T: 0.092, C: 0.908	Synonymous
LRRC3B	Exon 1	330A/T	N/A	Synonymous
NEK10	Exon 20	1684A/G	A: 0.742, G: 0.259	Synonymous
NEK10	Exon 24	2071C/G	C: 0.491, G: 0.508	Synonymous
AZI2	no polymorphisms found in gene sequencing			
ZCWPW2				

The LRRC3B gene belongs to the Leucine-rich gene family, as does the LGI1 gene. This gene is usually considered to be a tumour suppressor gene, and some researches suggested that it might be related to leukaemia and colon cancer. Its expression is extremely high in brain, but its function in brain is unknown, for instance, whether it is involved in the regulation of ion channel in the primate brain or what its function is likely to be in the CNS system (Kim *et al.* 2008; Tian *et al.* 2009).

The AZI2 gene, also known as NAP1, was identified as an activator of IκB kinase [(IKK)-related kinases] in a recent study (Fujita *et al.* 2003). IKK contributes to the activation of NF-κB-dependent gene expression, and the transcription factor NF-κB plays a central role in inducing the expression of many genes that contribute to diverse biological functions, including cell proliferation, cell survival, oncogenesis, inflammatory and immune responses (Baeuerle. & Baltimore**.** 1996; Ghosh *et al.* 1998; Silverman and Maniatis, 2001). It is not known whether such biological functions of the AZI2 gene might

indirectly contribute to epileptogenesis via activation of other genes. No mutations or rare polymorphism were found in the exons of this gene.

The functions of the other two genes, NEK10 and ZCWPW2, are even less clear. Most of the reported functions of these two genes have been predicted by the structure of their protein transcription. Although the expression of these genes is also found in the brain, there are yet no reports available as to what influences or functions they have in the CNS system. No rare mutation or polymorphisms were found on the exons of these two genes, either. There are 7 transcripts (variation) of NEK10 but only one of them was sequenced. The first two exons of ZCWPW2 gene were not sequenced, because they appeared to be non-expression exons.

There are several possible reasons which might explain why no disease gene has been identified through linkage analysis in the B pedigree. First, the sample size of this pedigree was not large enough. There were only 7 affected individuals in this pedigree and 2.1 was as large a LOD score can be with a sample size of 7 (Table 12.2) It is not however appropriate to interpret this result as a genuinely strong linkage. Second, there were three regions of interest identified, but only 5 genes within 3p24.1-3p24.2 were successfully sequenced on 5 individuals in this pedigree, and the other 2 regions have not been investigated. Third there were several polymorphisms identified in the introns adjacent to the spicing site of exons which did segregate with the affected individuals sequenced. Although unlikely, the polymorphisms in the introns adjacent to exons might cause splicing errors and contribute to epileptogenesis in this pedigree; however this could not be proved in this study. In general, polymorphisms or mutations in the intron rarely are pathological, however, in recent genetic association studies, some of the hotspot (or hitSNPs) identified were either in the non-coding areas or “gene desert”, where there were no adjacent genes and is an example in the study of Crohn disease (Libioulle *et al.* 2007; The Wellcome Trust Case Control Consortium, 2007). Such phenomenon suggested that regulatory regions or element of a gene can be located far from the gene itself and yet still be able to affect expression and function of the gene, as found in the ENCODE Project (The ENCODE Project Consortium, 2007). The exploration of such phenomenon remains a challenge, as biological studies such as functional determination and the transcript

regulation of the protein transcript are difficult, and to explore the functional role of SNPs located in non-coding areas or gene deserts is going to be challenging.

Finally, it is also possible in this family that Fragile X syndrome is partially involved in the epileptogenesis in this pedigree, even though the mode of inheritance did not suggest an X-linked disease pattern. Fragile X syndrome is the most common inherited form of mental retardation, with a prevalence of about 1 in 4000 males and 1 in 8000 females (Turner *et al.* 1996). The mental retardation results from a triplet repeat (CGG) expansion mutation (Verkerk *et al.* 1991) which inactivates the FMR1 gene (fragile X mental retardation 1 gene), resulting in loss of expression of the FMR1 gene product, known as fragile X mental retardation protein FMRP(Devys *et al.* 1993; Pieretti *et al.* 1991). Another milder form of Fragile X syndrome has been identified as Fragile XE syndrome, FRAXE (Orr and Zoghbi, 2007). FRAXE is caused by the expansion of a CCG repeat in the 5′ UTR of the fragile X mental retardation 2 gene (*FMR2*). When the CCG repeat expands beyond 200, the CpG island upstream of the *FMR2* gene is hypermethylated causing transcriptional silencing of *FMR2* and leads to loss of protein function. Patients with Fragile XE syndrome (FRAXE) present with mild mental retardation, learning deficits, and developmental delay (Mulley *et al.* 1995). Epilepsy can also be present.

Berry-Kravis (Berry-Kravis, 2002) investigated the seizure patterns in 16 Fragile X syndromes patients, twelve of them had complex partial seizure and four of them had generalized tonic–clonic seizures. In the abnormal EEG recording of these patients, 6 showed centro-temporal spikes. Most of the EEG patterns were similar to the EEG in Benign Focal Epilepsy of Childhood, however, clinically it might be difficult to differentiate the seizures of Fragile X syndrome from temporal lobe epilepsy.

No study has reported a structural anomaly of either the temporal lobe or the hippocampus in the patients with Fragile X syndrome or the carriers. One study using high resolution fMRI did not find any volume change of the temporal lobe or the hippocampus in mice with Fragile X syndrome (Kooy *et al.* 1999). Another study which investigated the hippocampal volume, the neuropsychological performances, and the CGG repeat number in full mutation Fragile X syndrome subjects (those which the CGG repeat was larger than 200 with hypermethylation of the CpG island and who were mentally retarded) and premutation Fragile X syndrome subjects (those which the CGG repeat was within 60 to

200, no hypermethylation of the CpG island, with normal or nearly normal phenotype without severe learning disability) suggested that there were only minor structural anomalies of the temporal lobes in patients with full mutation Fragile X Syndrome (Jäkälä *et al.* 1997). Whether such a subtle anomaly could contribute to temporal lobe epilepsy has not been further clarified.

Both FMRP and FMR2 protein have been trafficked in hippocampus (Antar *et al.* 2005; Miller *et al.* 2000). It was believed that lack of expression of these proteins may contribute to the memory impairment, learning disability or mental retardation in Fragile X syndromes. Whether the lack of these protein expressions in the hippocampus also contributes to mesial temporal lobe epilepsy or hippocampal sclerosis has not been investigated.

In this pedigree, there were 2 Fragile X carriers (IV-16, and IV-20) who had a normal phenotype without memory impairment or severe learning disability and one Fragile X syndrome victims with moderate mental retardation, learning disability and poorly controlled epilepsy (V-28). It was possible that Fragile X syndrome might have influenced the epileptogenesis at least in some of the members in this pedigree, and this may have complicated the analysis of inheritance in this pedigree.

12.1.2 Pedigree W

The linkage analysis of pedigree W was negative. No single K&C LOD scores using MERLIN or single location score using SimWalk2 was larger than 2. There are several possible explanations.

First, it was possible that there were false phenotyping in this pedigree. Accurate phenotyping was particularly important in parametric linkage analysis. A phenocopy not carrying the disease locus shared by other *genuine* affected individual (is considered as having recombination in the linkage analysis) will significantly reduce the LOD score, especially when the sample sizes are small (Table 12.2). Some of the subjects who were classified as affected in this pedigree did not have detailed medical investigations (for instance MRI), particularly those who had very mild symptoms and infrequent seizures. Under such circumstances, a phenotyping error is possible. Even after detailed medical investigation, a phenotyping error cannot be totally avoided as epilepsy is essentially a

clinical diagnosis and a negative EEG or MRI cannot definitively exclude a diagnosis of MTLE.

Table 12.2 Influences of sample size and number of recombinants on LOD score

Number of recombinants→

(Sample Size)	0	1	2	3	4	5	6	7
2	0.6021	0	0	0	0	0	0	0
3	0.9031	0.0738	0	0	0	0	0	0
4	1.2041	0.2272	0	0	0	0	0	0
5	1.5051	0.4185	0.0437	0	0	0	0	0
6	1.8062	0.6321	0.1476	0	0	0	0	0
7	2.1072	0.8604	0.2884	0.0311	0	0	0	0
8	2.4082	1.0992	0.4545	0.1097	0	0	0	0
9	2.7093	1.3458	0.6388	0.2213	0.0242	0	0	0
10	3.0102	1.5985	0.8371	0.3574	0.0874	0	0	0
11	3.3113	1.856	1.0463	0.5121	0.1799	0.02	0	0
12	3.6124	2.1175	1.2642	0.6817	0.2951	0.073	0	0
13	3.9134	2.3823	1.4895	0.8635	0.4285	0.152	0.0167	0
14	4.2144	2.6499	1.7209	1.0553	0.5769	0.252	0.0623	0
15	4.5154	2.9199	1.9574	1.2556	0.7376	0.369	0.1312	0.0145
20	6.0206	4.2963	3.197	2.349	1.6742	1.136	0.7147	0.3969
25	7.5257	5.7023	4.4991	3.5419	2.7521	2.093	1.5425	1.0878

Second, the inheritance pattern in this pedigree could be a quantitative trait. The severity of the clinical presentation of the affected individuals in this pedigree varied from one to another, which is a characteristic of quantitative trait or polygenic diseases. In pedigrees with polygenic disease, a dominant gene could be shared by the majority of the affected individuals and it would be possible to detect a linkage if the pedigree was large enough. However, this was not the situation in this pedigree. It appeared that no dominant gene was shared by the majority of the affected individuals; therefore linkage analysis would not be able to map the disease locus successfully.

Third, is the possibility of a founders' effect. Some of the un-related founders (II-30 and II-1) in this pedigree were said to be epileptic although no medical records are available to prove the diagnosis. However, this may well be the case, as those founders' unaffected and unrelated offspring were married to each other (III-3 and III-4) and all of their offspring appeared to have epilepsy (IV-15, IV-16, IV-17, and IV-18). The possibility of an

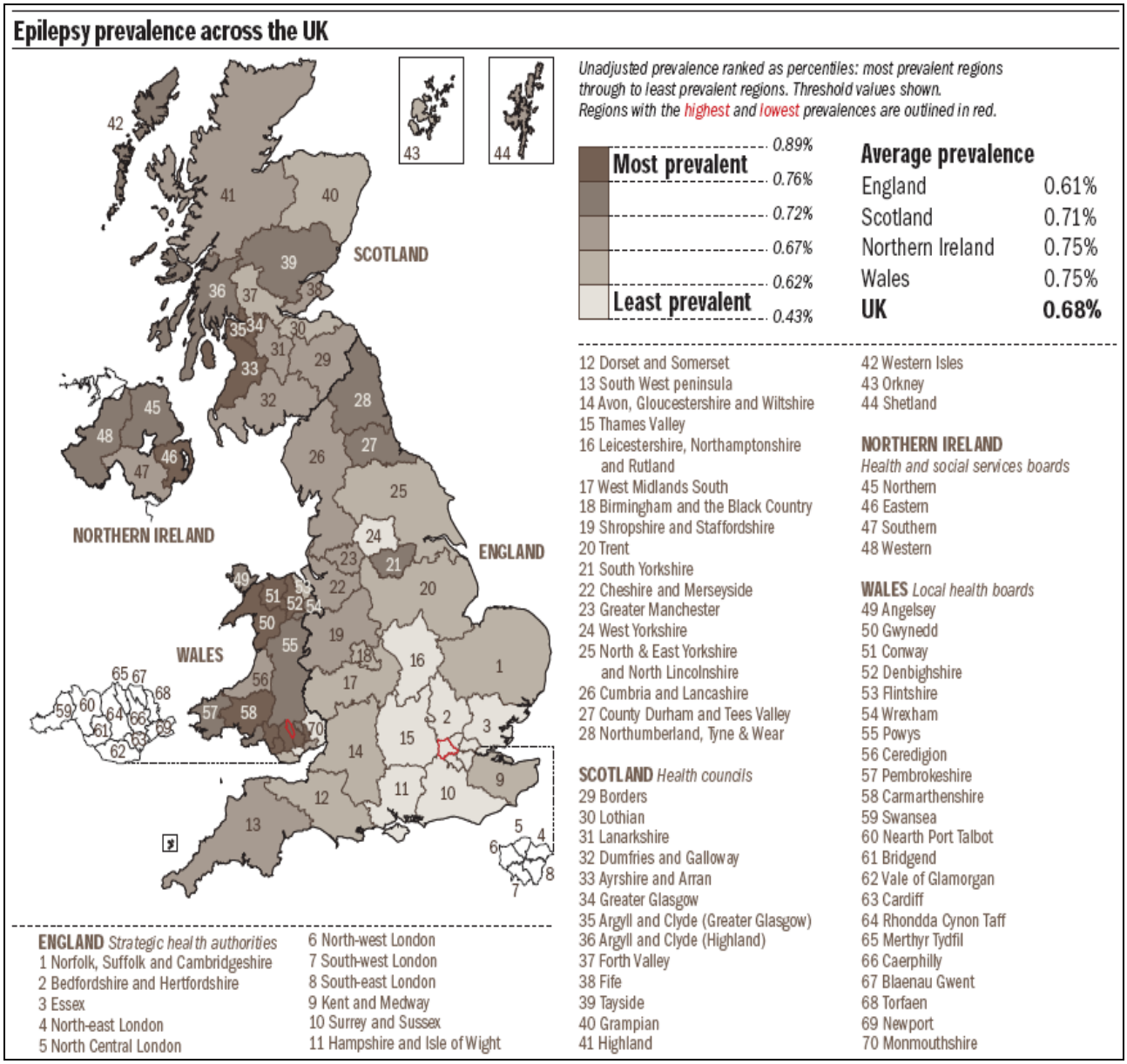
autosomal recessive inheritance pattern can not be excluded, but it seems more likely that two susceptibility genes, coming from two different founders, influenced each other and contributed to the epileptogenesis in this situation. Hence, the possibility and the effect of epistasis (gene to gene interaction) cannot be overlooked in this pedigree. Neither parametric nor non-parametric linkage analysis can easily identify interactions between genes. Moreover, the phenomenon that one whole generation (the III generation) was free from symptom of epilepsy while their next generation (the IV generation) was greatly affected by epilepsy cannot be easily explained by genetics alone.

Finally, it has long been realised that a disease gene does not always cause the expected disease and environmental factors may be necessary to induce its expression. Although we failed to identify any possible environmental factors from the interviews and investigations, Swansea is one of the areas in the UK that has a high incidence and prevalence of epilepsy and this might suggest that some environmental influences cannot be overlooked in this pedigree (Figure 12.1).

In summary, non-parametric linkage analysis did not successfully identify the disease locus in this pedigree. Uncertainties in the founder states, phenocopies, polygenic inheritance, epistasis, and the gene-environment interactions might have contributed to the negative findings.

Figure 12.1 Epilepsy prevalence across the UK

(Information from General Medical Service, UK)



12.2 The linkage analysis in fMTLE

12.2.1 A comparison

In this study, genetic linkage analysis was carried out in two families diagnosed with familial mesial temporal lobe epilepsy to map the disease locus. Both families had more than two members that were diagnosed to have mesial temporal lobe epilepsy and both

fully fulfilled the criteria of familial mesial temporal epilepsy. There was one individual in each family who had hippocampal sclerosis and both were seizure free after surgery. There were two individuals in each family who had history of febrile convulsion, and status epilepticus were found in severely affected individuals in both families. A considerable variation of disease severities and seizure frequencies between affected individuals were observed in both families. There were several individuals in family W who had stereotyped déjà vu as the only symptom of MTLE but such clinical presentation was not found in family B. In family B, the mode of inheritance (MOI) was likely to be autosomal dominant, or a single gene inheritance, but the MOI could not be clearly defined in family W. Such phenomenon suggested that the underlying genetics of MTLE in these two families might well be different.

In comparison to other linkage analyses of familial MTLE, Claes *et al* (Claes *et al.* 2004) mapped the disease locus to 12q22-q23.3 in their linkage analysis of a family with FTLE associated with FS based on a 2 point LOD score of 6.9 and a multipoint LOD score of 7.8 at the same marker. The sample size of this study was larger than ours (5 generation, 53 participants and 22 affected individuals). 10 cM whole genome scan was done in this pedigree and MLINK was used for the linkage analysis. Hedera (Hedera *et al.* 2007) identified a disease locus at 4q13.2-q21.3 with a LOD score of 4. The samples size in Hereda's was smaller than Claes but larger than ours, with 12 affected individuals distributed in 3 generations. 8 cM whole genome scan was carried out and the linkage analysis was done by MLINK using the 'affected only' method. In both studies, the mode of inheritance was autosomal dominant. No disease gene has been mapped in these two loci, and the results have not yet been replicated. In contrast to these studies, the sample size in B family was much smaller, the mode of inheritance appeared to be AD with a reduced penetrance, and three regions of interest were identified. As was also the result in the two studies mentioned, no susceptibility genes have been mapped despite the fact that I sequenced five candidate genes in one of the regions identified. The mode of inheritance of W family could not be defined, non-parametric linkage analysis failed to identify any disease locus, and this pedigree was unlikely to have a single gene disease inheritance pattern.

Current meta-analysis of the association studies in mesial temporal lobe epilepsy showed that Interleukin –β gene might be one of the susceptibility genes of MTLE (Kauffman *et al.* 2008b). The linkage analysis in the two families did not show that this gene was related to either pedigree. Other candidate genes that had been reported in association study of MTLE include APOE4, PRNP (M129V), GABBR1, PDYN residue 171, and 5-HTTVNTR (Briellmann *et al.* 2000; Gambardella *et al* 2003; Labate *et al.* 2007; Manna *et al.* 2007; Walz *et al.* 2003). Except the Interleukin –β gene, none of the results of these candidate gene association studies have been replicated, and these genes are not in the loci identified in the B family.

In summary, we did not replicate any of the studies results either in association or in genetic linkage studies of mesial temporal lobe epilepsy. We provided evidences that familial mesial temporal lobe epilepsy can be polygenic, and complicated epistasis or gene-environmental interaction may exist and both involved in the development of mesial temporal lobe epilepsy. Conventional genetic linkage analysis may not be suitable in these circumstances to map the disease locus in familial mesial temporal lobe epilepsy, without enhancement in analytical methods on larger samples.

12.2.2 The future of fMTLE gene mapping

Despite the fact that we did not successfully map the disease gene from the two pedigrees, they form potentially good material for future genetic studies of fMTLE, and future detailed within-in family investigation.

In the B family, the pedigree size was not large enough to have a LOD score larger than 3 (2.1 might be the largest LOD score that could be obtained through MLINK in this pedigree). It will be worthwhile sequencing more candidate genes on the other 2 regions identified on chromosome 5 (5p13.3-5p13.2) and chromosome 7 (7q11.22-7q21.11). It would also be worthwhile attempting to collect more subjects from the 6^{th} generation of this pedigree to enlarge the sample size ; however there is no guaranteed that there will be more cases available. It may also be worthwhile sequencing the FRM1 gene and/or FRM2 gene in all subjects that had seizures in this pedigree. A study of the numbers of CGG repeat in relation to the clinical presentation of epilepsy might be worthwhile in understanding the impact of these genes on epileptogenesis, which is a polygenic setting.

In family W, conventional linkage analysis did not prove adequate to map the disease locus. More advanced mapping methods might be more successful, for instance using quantitative trait mapping techniques. Such mapping methods became available in 1972 (Haseman and Elston, 1972), but their implementation remain challenging even if advanced computing facilities are available nowadays (Forabosco *et al.* 2005). The current pedigrees we have alone was not large enough to carry out such analysis. To do quantitative trait mapping in mesial temporal lobe epilepsy, more information would be needed to define the model of quantitative trait, to evaluate the magnitude of the gene-gene interaction and the gene-environmental interaction, or to define different endophenotypes within mesial temporal lobe epilepsy. In quantitative trait mapping, a good disease model is as important as an accurate MOI in parametric linkage analysis. Whole genome assessment using dense tagging SNPs across the whole genome for linkage analysis is another choice in the future. Such technology is available and the cost has been largely reduced recently (Maresso and Broeckel, 2008). I will seek to further this work in the future.

The susceptibility genes underlying both pedigrees could be different, because the MOI were clearly different and the LOD scores which were larger than 1.5 in each pedigree did not show any regional overlap. We therefore considered that pooling the data of the two pedigrees for linkage analysis was not likely to be helpful, but if more pedigrees with similar clinical presentations could be collected in the future, quantitative trait locus mapping or family based whole genome linkage/association study might be possible on the combined material.

Finally, I would recommend that further refinement is carried out in relation to the criteria for phenotyping mesial temporal lobe epilepsy. If such phenotyping methods lead to a more homogeneous study sample population, the power of the linkage analysis would be higher. A good example is the association studies in patients with hippocampal sclerosis which led to the association of Interleukin –β gene with mesial temporal lobe epilepsy. In our study, the phenotype in fMTLE was decided mainly according to clinical symptoms and we did not restrict our analysis to those with hippocampal sclerosis on MRI or those who had epileptiform discharge on EEG suggesting MTLE. It is possible that subcategories of MTLE exist, with specific inheritance patterns and a specific analysis for each would be helpful.

To sum up, the future work for mapping disease locus in fMTLE includes developing more specific phenotyping, and the development of advanced analysis programmes for linkage analysis, especially when whole genome assessment using SNPs has become available for linkage analysis. In this situation, more powerful statistic tools will be needed to accommodate the massive amount of data accumulated.

12.3 The photosensitive epilepsy pedigree

The linkage analysis of the photosensitive epilepsy pedigree turned out to be inconclusive in this study. The pedigree size was large enough for linkage analysis and genetics seem to play a major role in the pathogenesis of photosensitivity in this pedigree, but the analysis did not successfully identify the disease locus. In the MLINK parametric analysis, the maximum LOD score was 1.14 on chromosome 1 and the haplotype analysis did not support a disease linkage. The largest location score was 2.231 on chromosome 16 in NPL_{pair} using SimWalk2. In some circumstances, such location score may have its significance, however the haplotype analysis at this region on chromosome 16 showed recombination in 2 affected individuals, which suggested that the locus identified was unlikely a disease locus if photosensitivity is a single gene disease. There are several possible explanations.

First, photosensitivity itself could be a quantitative trait or a polygenic disease. The phenotype defined in this pedigree was based on photoparoxysmal response (PPR) proved by EEG, and the subjects with seizures only were excluded. The phenotype appeared to be well defined and the study population appeared homogeneous. However, it could be argued that the affected individuals were not as homogeneous as they appeared to be, and that some of the affected individuals did not have seizures evoked by photosensitivity. Whether these two groups (photosensitivity with or without seizures) were of the same genotype has been widely discussed without definite conclusion (Stephani *et al.* 2004). Our results suggested either situation was possible. This was supported by the haplotype analysis on chromosome 16 showing that 2 individuals (III-18 and III-24) who had photosensitivity without seizures did not have the haplotype shared by those who had both photosensitivity and seizures. Instead, they inherited the other haplotype that was not shared by all the other affected individuals. Such a phenomenon could be explained if more than one gene could

cause photosensitivity. Another simple explanation of this analysis was that these two groups were of the same genotype and these two subjects who had different haplotype were phenocopies and that the photosensitivity was caused by other nongenetic factors. However it is generally rare to find 2 phenocopies in one pedigree. In summary, the analytic result of this study did not provide a definite answer as to whether the two phenotypes (PPR with seizure and PPR without seizure) have the same genotype.

Second, in polygenic gene disease, two or more genes could have equal potential in causing photosensitivity and possessing any of them is sufficient for a subject to express photosensitivity. It was possible that most of the affected individuals in this pedigree might share a dominant gene but a few of them did not. If the pedigree was large enough, linkage analysis might be able to identify the locus where the dominant gene was linked to. However, this would prove difficult in small pedigrees.

Third, it might not be entirely appropriate to phenotype this pedigree based on the existence of PPR on EEG only. Doose and Waltz (Waltz *et al.* 1992) categorized the PPRs on EEG into 4 different types: Spike within the occipital rhythm (type 1), Parietal-occipital spikes with biphasic slow waves (type 2), Parieto-occipital spikes with biphasic slow waves and spread to the frontal region (type 3), and Generalized spike and waves or polyspikes (type 4). In our analysis, PPR were not subcategorized. The age-dependency of the patterns is a particular issue. Deterioration of the generalized spike and wave (type 4) into occipital spike (type 1) was observed on the same subject in the follow up EEG years after the first recording in this pedigree, indicating that different types of PPR might arise from the same genotype but regulated by different factors. Similar findings were made in the previous investigation by Waltz and Dose (Waltz *et al.* 1992). In their study, it was concluded that "the phenotypical expression of the PPR is age-related and modified by other factors predisposing to generalized epilepsy, and the varied patterns of the PPR only represents different levels of expression of the same genetically determined trait". Furthermore, PPR often totally disappears after the third decade and this renders the use of EEG in adults of doubtful validity. To assign all individuals who failed to show a PPR on EEG recording as unaffected individuals risks inaccuracy. Age is not the only influence and gender is also important as females are more likely to be photosensitive than males. Drug treatment can greatly affect the PPR. Moreover, multiple, successive examinations with varying time

intervals in EEG recordings tend to show considerable variation in the PPR, which further indicates that a number of other unknown factors are in play and many of them have not been fully identified (Stephani *et al.* 2004).

Fourth, a PPR are seen in other epilepsy syndromes, such as idiopathic generalized, idiopathic partial, cryptogenic, and symptomatic epilepsies, not just in idiopathic photosensitive occipital lobe epilepsy (IPOLE). The susceptibility genes of some of these epilepsy syndromes have been identified, such as GABRA1 gene in Juvenile Myoclonic Epilepsy (JME) (Cossette *et al.* 2002), but these susceptibility genes have not been shown to be responsible in causing photosensitivity (Stephani *et al.* 2004). These might suggest that complicated gene-to-gene interactions could be a part of the mechanisms leading to photosensitive epilepsies, and may further complicate gene mapping in photosensitive epilepsies.

In conclusion, photosensitivity is likely to be a quantitative trait or a polygenetic disease. EEG although accurate and sensitive in detecting the PPR might not be reliable in detecting individuals whose photosensitivity has been fully suppressed because of various regulating factors. The locus identified on chromosome 16 near marker D16S2621 could be a region of interest, but more supportive evidence should be gathered before going on the next step of investigation.

12.3.1 Comparison with other research

As reviewed in the introduction, no susceptibility genes for the PPR or photosensitivity have been conclusively mapped. Pinto (Pinto *et al.* 2005) in a family based linkage analysis mapped two susceptibility loci for epilepsy-related photosensitivity (PPR) at regions 7q32 (PPR type 1, HLOD=3.47 with $\alpha = 1$, $P\ value_{(NPL)} = 3.39\ 3\ 10^{-5}$ at whole genome wide level) and 16p13 (PPR type 2, HLOD = 2.44 with $\alpha = 1$, $P\ value_{(NPL)} = 7.91\ 3\ 10^{-5}$ at whole genome wide level). The subjects were families with a PPR and prominent myoclonic seizures (MS-related PPR). In a later study, the same research group reanalyzed the data using two locus linkage analysis to evaluate whether certain degree of interaction (epistasis) exist between the two loci identified. They suggested that the two loci may have similar functions or act in the same biochemical pathway. No definite gene has been mapped from these two loci so far.

Our study population differed from theirs. We did not subclassify the PPR into different liability classes in the analysis. The analysis program used in their study was Allegro, whereas we used Simwalk2. The largest NPL_{all} location score was 2.231 at 16q24 (D16S2621) with a pointwise *P value* 0.0059 in our study. Pinto suggested that the two loci they identified could have similar biological functions; while our parametric linkage analysis suggested that photosensitivity is unlikely to be a single gene disease, and polygenic inheritance is likely. Another familial linkage study mapped a disease locus on chromosome 6 (6p21.2) and chromosome13 (13q31.3) (Tauer *et al.* 2005). No significant location score was obtained either on chromosome 6 or on chromosome 13 in our pedigree.

Compared with the results of the association studies in photosensitivity and JME which suggested an association with BRD2 gene (Cavalleri *et al.* 2007; Lorenz *et al.* 2006b), there was no evidence that the disease gene in this pedigree was linked to the BRD2 gene.

In summary, we did not replicate any of the results reported in other genetic studies of photosensitive epilepsies. No definite susceptibility locus or gene was mapped in our study, but we provide some evidence that photosensitivity (PPR) is likely to have polygenic inheritance.

12.3.2 *Future work*

To map the susceptibility genes of photosensitive epilepsies is one of the major challenges of epilepsy genetic research. Little is known about the role of genetics in its pathogenesis. Several reported pedigrees had shown that the mode of inheritance (MOI) was likely to be AD with an age-dependent incomplete penetrance. The inheritance pattern in our pedigree resembled an AD pattern however the parametric analysis using MLINK did not demonstrate linkage. This study did not successfully map any disease locus, but provided evidence that photosensitivity is unlikely to be a single gene disease. The non parametric analysis further suggested that there might be a locus of interest on chromosome 16 but more investigation is necessary.

Accurate phenotyping is mandatory in any kind of genetic analysis. It may be worthwhile, in my opinion, combining EEG with other diagnostic tools such as MEG, VEP or SPECT which have shown potential in refining the diagnosis of the PPR to detect photosensitivity. As discussed above, EEG alone cannot be reliable in detecting affected

individuals. It would be very helpful if an alternative biomarker for photosensitivity was available, and the identification of such a biomarker would potentially rapidly move this field forward.

More attention to the clinical epidemiology of photosensitive epilepsy syndromes might also be profitable, and contribute to genetic studies. For example, current studies showed that photosensitivity is both gender and age dependent, and it is also associated with many epilepsy syndromes. More epidemiological figures will help in the study design of genetic studies. For example, different disease traits might be further stratified to provide a homogeneous study population, such as PPR + JME, or PPR + IGE, and pure PPR, etc. Such information will also be useful in quantitative trait locus mapping by providing a better defined quantitative trait models.

Whole genome association studies are another approach to mapping the genes causing photosensitivity. However, it will be very difficult to recruit a large enough sample size, since pure photosensitive epilepsy is rare. Even though broader-defined photosensitive epilepsies may be helpful in obtaining a large sample size, as photosensitivity co-exists with disparate epilepsy syndromes, it is quite possible that the underlying genotype of each photosensitive epilepsy syndrome will differ, and in such situation a large sample size will not improve the power of study (in fact, the reverse). An association study will still face the same phenotyping issues which confound the linkage analysis such as age, gender, and the difficulties in finding affected individuals whose photosensitivity has been fully delineated. Furthermore, assessing the complexity of epistasis remains difficult and challenging.

In summary, the gene mapping and genetics in photosensitivity remain challenging. Existing gene mapping methods and phenotyping tools have not yet satisfactorily overcome the difficulties in mapping genes arising from the complexity of photosensitivity.

12.4 The Kohlschütter-Tönz pedigree

The Kohlschütter-Tönz syndrome is comprised of early onset seizures, mental retardation or deterioration, spasticity, ataxia, and amelogenesis imperfecta (abnormal enamel formation). Patients often die before the third decade of their life because of complications arising from severe handicap. Currently there is no cure. The syndrome is quite rare but mapping the gene and understanding its expression may provide a tool for

understanding the pathogenesis of the condition and of its epileptogenesis, which in turn will contribute to the development of treatment. An example of the potential value of gene-identification is that of the identification of the ion channel genes such as SCN1A and 2A in Idiopathic Generalized Epilepsies (IGEs) which have had an enormous impact on the understanding of these forms of epilepsy and in facilitating ongoing development of new AEDs.

The linkage analysis of the 5 small families with Kohlschütter-Tönz syndrome suggested significant linkage of a disease locus on chromosome 16. The maximum LOD score was 3.05 in parametric linkage analysis at 16p13. The haplotype analysis and the preliminary microarray chip analysis provided convincing confirmatory evidence. There were two main reasons for the successfully mapping of the disease locus by MLINK, one was because it clearly showed that the inheritance mode was autosomal recessive, and second because there was consanguity reported in two of the families. Both factors contributed to the power of this study to detect linkage.

To date, the genetic basis of Kohlschütter-Tönz syndrome is still unknown. There are around 24 known genes located in the region identified in this study. Clearly, the next stage is to identify candidate genes in this region to find the disease mutations, to explore the gene expression through functional studies, and to establish the pathogenesis of this syndrome. However, in this case the identification of candidate genes is not straightforward. There are no known ion channel genes in this location, and little is known about the genes that play a role in regulating ion channels or ion channel genes. Furthermore, very little is known about the genetics of abnormal enamel formation. To sequence all known genes within this locus is possible, but is probably not considered cost-effective. Fine mapping would be an ideal way to narrow the region of interest before choosing candidate genes for sequencing. I attempted this in this study but no ideal microsatellite marker was available. Preliminary microarray chip analysis provided was somewhat helpful in narrowing the region in interest. As the cost of microarray chip analysis has been reduced rapidly, whole genome assessment with SNPs in linkage analysis is now available (John *et al.* 2004; Middleton *et al.* 2004), and could be considered in the near future. An alternative approach might be a whole genome association study, but these are not generally recommended for such "rare disease, rare variant" traits, as a large

sample, mandatory in these studies, is not possible and such studies will inevitably underpowered and fail to map rare disease variants.

12.5 Discussion of the Linkage analysis:

12.5.1 Overview

In this study, 8 families with different epilepsy syndromes were genotyped by an 8 cM whole genome scan using a set of microsatellite markers. Three potential disease loci were identified in one of the families with fMTLE, but no disease mutations were found on five of the candidate genes that were sequenced. No disease locus was identified in the other fMTLE pedigree which the MOI was more complicated. The parametric linkage analysis by MLINK did not identify any disease locus under the inheritance mode AD in the photosensitive pedigree, but the NPL analysis showed that there might be a region of interest, if photosensitivity is a polygenic disease with a dominant gene. In the five small pedigrees with Kohlschütter-Tönz syndrome, a disease locus was identified with approximately 24 known genes in this region. No disease mutation genes have yet been identified.

According to the linkage analysis of the fMTLE pedigrees, it seemed that although the clinical presentation of the two pedigrees was similar, the MOI and the susceptibility genes underlying each pedigree might be different. This suggests that fMTLE is likely to be polygenic, that family based studies in gene mapping of fMTLE will be difficult, unless large samples can be collected.

The parametric linkage analysis on the pedigree with photosensitive epilepsy showed that photosensitivity was unlikely to be a single gene disease. The NPL analysis helped to locate a region of interest on chromosome 16, however, more supporting evidence is needed. Another problem in this pedigree is that we could not be certain whether the unaffected individuals defined by negative PPR on EEG were truly unaffected, because EEG can not identified subjects whose PPR were completely suppressed or has resolved .

The linkage analysis in the Kohlschütter-Tönz families was promising, with the convincing evidence gathered from microarray chip analysis and haplotype analysis. Future work would be to identify the mutation genes and the work on gene expression to establish the pathogenesis of this syndrome.

12.5.2 Gene mapping methods

More and more emerging evidence suggests that most epilepsy syndromes are unlikely to be single gene diseases but to have polygenic causation. Conventional linkage analysis is therefore unlikely to be the only method of value in identifying causal genes. In the past, candidate gene association studies had been the main method of mapping disease genes but little has been achieved. As the advances in technology and the completion of Human Genome Project, whole genome assessment association studies using different microarray platforms have become the major tool in genetic mapping. However, although such studies were able to demonstrate rigorously the association between certain common diseases and disease variants, so far they have only explained a small fraction of the underlying genetics of most of the common diseases, and have not been able to detect rare disease variants but with considerable effect size (effect size is a function of what a variant dose) (Goldstein, 2009). In contrast to association studies, many rare disease variants have been identified through linkage analysis but such studies often fail to map common disease variants.

Various modifications to linkage analysis programs have been made. Quantitative trait mapping has been introduced in 1972, and at least three types of quantitative trait mapping are available now (Table 12.3). Their reliability and validity have not been well established yet, and it has been a main challenge to implement the algorithm to computation, despite the advanced computing facilities are available. Besides using microsatellite markers for genotyping in the linkage analysis, genotyping using Single Nucleotide Polymorphisms (SNPs) are now available and will provide more information (John *et al.* 2004; Maresso and Broeckel, 2008) (Table 12.4). The major challenge in applying SNPs in linkage analysis is that powerful statistical tools are mandatory to accommodate huge amount of data.

Genetic association studies had been considered to be more powerful than linkage analyses in gene mapping, and were originally designed to solve the disadvantages of linkage analysis in mapping complex traits. However, to date not much progress has been made by association studies in the gene mapping of epilepsy syndromes. Most such studies were candidate-gene based, and many were underpowered by utilising too small a sample size. In contrast, genetic linkage studies have mapped many disease genes, and indeed are

still responsible for most of the successful gene-finding investigations in epilepsy as well as other diseases. There has been a fierce debate about whether association study or linkage studies are the most suitable choice for gene mapping, and a comparison of the linkage and association study is appropriate here to clarify some of the issues.

12.5.3 Comparison between different gene mapping methods

The comparison of quantitative trait mapping, genetic association study and genetic linkage study is presented in Table12.3, which provides an outline of the advantages and disadvantages in each type of gene mapping method.

Parametric linkage analysis has been very successful in mapping Mendelian traits in the past two decades. However its value is greatly reduced in mapping genes in complex disease trait or quantitative traits. Most linkage analysis has taken the form of non-parametric linkage analysis because most pedigrees of interest are not single gene diseases. However, non-parametric linkage analysis is less powerful in detecting linkage in a Mendelian trait disorder and often ignores available genotype data from unaffected individuals, lessening its power in detecting linkage. Quantitative trait mapping was another method developed to solve this disadvantage. However, the quantitative trait mapping required several steps and can be laborious and complicated in comparison to conventional linkage analysis. To perform quantitative trait mapping, segregation analysis, establishing model definitions, and relevant epidemiological information are often necessary.

The design of association study is essentially different from linkage analysis. It aims at finding common disease variations with moderate gene strength shared by a large population, based on the hypothesis “common disease, common variation”. In other words, it seeks genes that express moderately by majority rather than genes that expressed strongly but only by minority, such as in a Mendelian trait pedigree. There are several different forms of association study. Based on the study design, these can be roughly classified into three types: candidate gene based, linkage disequilibrium (LD), family based, or population based. Each has its advantages and disadvantages in mapping disease genes (Table 12.3).

Despite the fact that there have been many debates and disagreements about whether linkage or association is the better way to find causation genes, it might be reasonable to

conclude that these two methods complement rather than compete with each other. Both have a place. For example, although whole genome assessment has been applied to association studies using different platforms of tagging SNPs, most of the new associations do not explain previous genetic linkage results, suggesting that even whole genome association studies cannot replace linkage analysis in mapping rare disease variants (Mullen *et al.* 2009). On the other hand, genetic linkage analysis is much less powerful in identifying common variants than the association studies. Ideal study design varies according to the characteristic of the study population, and carefully selecting suitable mapping methods remains a vital ingredient in gene mapping.

Recently, Goldstein (Goldstein, 2009) estimated that to explain 80% of a common variation, such as human height, as much as 93,000 SNPs are needed based on the data reported by Manolio (Manolio *et al* 2008) and the formula by Risch and Merikangas (Risch *et al* 1996). He also suggested that the associations with several common diseases (such as type II diabetes) that have been reported through genome wide association studies might be those that have the strongest effect size, however the majority are the SNPs of weak effect size which might not be able to be further identified through GWAS with a mass number of study population. Thus, it is unlikely that such variations identified through genome wide association studies can be ideally applied to clinical practice, either in gene screening or in developing therapy, because these variants only account for a small fraction of these common diseases. Therefore, it seems reasonable that in clinical practice, seeking genes with larger effect size would be more clinically applicable and cost-effective. This further point out that linkage analysis has its place and cannot be replaced by GWAS.

Table 12.3 Comparison of different gene mapping methods
(Almasy and Blangero, 2008; Forabosco *et al.* 2005; Hirschhorn and Daly, 2005; Maresso and Broeckel, 2008; Rice *et al.* 2008)

Gene mapping method		Study design/assumptions	Strength	Weakness
Linkage	**Parametric**	1. Hardy Weinberg Equilibrium 2. Linkage equilibrium. 3. Random mating. 4. No epistasis. 5. No chiasma interference.	1. Powerful in detecting disease locus in rare Mendelian traits. 2. Unaffected by allelic heterogeneity. 3. P value and LOD score can be accurately given.	1. The locus identified is usually larger than 10 cM with hundreds of genes in between. 2. Not suitable for non-Mendelian traits. 3. Assess within pedigree, not across pedigrees
	Non-parametric	1. Model-free, to compare the observed and expected numbers of genes shared IBD between a selected pairs in the pedigree.	1. Suitable for complex trait or quantitative trait.	1. Ignores genotyped data in unaffected individuals. 2. Conservative in detecting linkage.

(continued)

Table 12.3 Comparison of different gene mapping methods (continue)
(Almasy and Blangero, 2008; Forabosco *et al.* 2005; Hirschhorn and Daly, 2005; Maresso and Broeckel, 2008; Rice *et al.* 2008)

Gene mapping method		Study design/assumptions	Strength	Weakness
Quantitative trait	**ANOVA**	For each marker, each of the genotypes is considered a class, and all of the members of the population with that genotype are considered an observation for that class. The measured means values of each quantitative trait are compared by ANOVA.	1. Suitable for quantitative trait, or diseases with polygenic inheritance. 2. Reduces expected genetic heterogeneity and environmental voice by stratification of the population.	1. An approximate accurate quantitative trait model is necessary, which may be difficult to establish. 2. Might be complicated and laborious. 3. Difficulties in obtaining adequate parameter estimates. 4. The power and effects depend on the density of the markers used. 5. Recombination effects between markers can reduce the real quantitative trait locus effect on the markers
	Regression method	1. Using regression model to estimate the degree of IBD sharing among different relatives in correlation to trait similarity.	1. Less demanding in computation, compared to VC method.	1. Might be less powerful than the VC method if the distributions of the trait are nearly normal.
	Variance component method (VC)	1. Using maximum likelihood to estimate the components that model the phenotypic covariance among relatives.	1. Can be easily extended to complex genetic models, allowing additional sources of genetic and nongenetic variance, such as environmental factors.	1. Might be complicated and laborious in defining the quantitative traits and genotypes. Accurate estimation of the model is difficult if relevant epidemiological information is not available. Segregation analysis is needed before the analysis. 2. Many pedigrees are needed as large sample size is necessary.

Table 12.3 Comparison of different gene mapping methods (continued)
(Almasy and Blangero, 2008; Forabosco *et al.* 2005; Hirschhorn and Daly, 2005; Maresso and Broeckel, 2008; Rice *et al.* 2008)

Gene mapping method		Study design/assumptions	Strength	Weakness
Association	**Candidate gene**	1. Compares the candidate genes allele frequencies between control and affected subjects.	1. might be more powerful than linkage analysis if the sample size is large enough	1. Large sample number of control and subjects are needed. 2. Low disease gene detect rate so far. 3. Selection of candidate genes is not easy and often questionable.
	Linkage disequilibrium (LD)	1. Calculates the correlation coefficient γ of two loci to evaluate whether one locus can be substituted for the other without loss of information. If γ^2 =0, there is independence between the 2 loci, γ^2 =1 if they are of complete disequilibrium.	1. Non-independence between alleles of tightly linked loci, robust to find disease mutation near the linked marker.	1. Admixture (the union of two or more genetically distinct populations) and migration (the introduction of new genes from one previously distinct population) may have significant impact on LD
	Population stratification	1. Compares the marker frequencies between unrelated individuals and controls.	1. Easy in recruiting subjects.	1. Potential of spurious associations due to population stratifications, when disease prevalence and marker allele frequencies differ among the subpopulation.
	Family based [transmission disequilibrium test (TDT)]	1. Compares the frequency of transmission versus non-transmission of marker alleles to affected offspring by means of a simple X^2.	1. Eliminates population stratification effects completely	Less powerful than population based approaches when there is no population stratification.

12.5.4 SNPs vs microsatellite markers in linkage analysis

Single Nucleotide Polymorphisms (SNPs) has been replacing microsatellite markers in genotyping after the completion of Human Genome Project and now the ongoing HapMap project. The application of dense SNPs sets for whole genome linkage scans has been reported (John *et al.* 2004; Middleton *et al.* 2004) and showed that using the Affymetrix Mapping 10 K array offers significantly higher information content (IC) across the whole genome when compared to standard 300-400 10 cM spaced microsatellite markers scan. Further more, the LOD scores obtained using SNPs were more precisely defined and the loci identified were narrower compared with microsatellite markers scans (John *et al.* 2004). Although SNPs are less polymorphic than microsatellite markers, local clusters or haplotypes of SNPs can result in more genetic information being extracted from a particular genomic region than microsatellite markers (Schaid *et al.* 2004).

However, using SNPs as a whole genome scan in linkage analysis is not without disadvantages. For example, while linkage disequilibrium (LD) is a substantial benefit in association study, its impact on linkage analysis can be negative leading, for instance, to an upward bias in identical by descent (IBD) estimates though underestimation of haplotype frequencies. However, the overall impact of LD on linkage analysis requires further study as not enough data have been accumulated. Table 12.4 is a brief comparison of the advantages and disadvantages using SNPs and microsatellite markers for genotyping in linkage analysis.

Table 12.4 Comparison of microsatellite markers and SNPs (Maresso and Broeckel, 2008; Schaid *et al.* 2004)

	Structure characteristics	Information content (IC)	In linkage analysis
Microsatellite marker	contains of various di-or tri-nucleotide repeats, varies in each individual	High IC but less powerful as their densities in human genome are not as dense as SNPs	1. Relatively easy in computation then SNPs. Less precise. 2. The loci detected are usually quite large.
SNPs	A single base of DNA is substituted with another or is either inserted or deleted.	1. Low IC but compensated by their abundance and uniform density, making it more informative in mapping genes. 2. All markers can be used to construct haplotypes, increase the amount of information extracted from a given region DNA 3. Lower IC of SNPs is offset by the ease with which they can be typed.	1. More precisely defined than microsatellite marker with higher LOD scores and narrower LOD unit interval. 2. Able to detect loci that are undetectable with microsatellite, with relatively ease and speed in genotyping. 3. A better resolution of quantitative traits. 4. LD may lead to upward bias in IBD estimates through underestimation of haplotype frequencies. 5. Upgrade of both algorithms and software are needed to handle genome wide data efficiently.

12.5.5 Whole genome wide assessment and genome wide association studies (GWAS)

Dense SNP maps are now widely available through the human genome mapping and the HapMap studies (International HapMap Consortium, 2005), which cataloged SNPs that could be used to capture the majority of human variability. There are approximately 500,000 SNPs whose genetic analysis can be used to assess about 95% of genetic variability (Hardy and Singleton, 2008). With the advances in microarray chip analysis technology along with dense informative tagging SNPs, whole genome assessment to map disease gene has become a feasible option in non-Mendelian trait diseases, and has facilitated the genome wide association studies (GWAS) of many common diseases, especially after the cost in microarray chip analysis has been rapidly reduced. Genome wide association studies (GWAS) have been successful in mapping genes of several complex disease, such as breast cancer and prostate cancer (Easton *et al.* 2007; Yeager *et al.* 2007).

Two types of microarray chips are now available, one is the Affymetrix Genechip platform, which randomly selects SNPs available across the whole genomes for microarray analysis. The other is the Illumina BeadChip platform, which employs an LD-based selection strategy, that different specific tagging SNPs can be selected according to the population in interest. The Affymetrix Genechip seeks event spacing of the SNPs distribution across the whole genome, while the density of the SNPs platform on Illumina varies according to LD, so different SNPs in strong LD (genetically identical SNPs, giSNPs, or redundant markers) are not selected as they tend to give the same result in association study. The coverage of the Snips across the genome directly affects the information content (IC) and the results of GWAS. Newer platforms also include the ability to detect copy number variation, as this may play a major role in contributing to the complex relationship between phenotype and genotype (Mares so and Broeckel, 2008).

Several factors affect the results of genome wide association study. First, a large sample size is necessary to provide adequate statistical powers. An underpowered association study is likely to fail. It was estimated that for an adequate association study, at least 2500 subjects are necessary (Altshuler and Daly, 2007), although these figure may be more or less adjusted according to disease prevalence and incidence (Mullen *et al.* 2009). Such large sample sizes often make the replication of association studies difficult. Careful selection of tagging SNPs for a good coverage across the genome is another variable that

contributes to success of an association study. The magnitude (potency) of the alleles in causing disease and the allele frequency are also determining factors, however neither is likely to be known prior to study (Maresso and Broeckel, 2008). Finally, because a large number of SNPs are genotyped in a GWAS, *P values* of 10^{-6} -10^{-7} after Bonferroni correction are currently accepted as the significance threshold to confirm an association in GWAS. Such stringent *P values* threshold may have excluded many true association results, and such a threshold is only reasonable when the sample size in genome wide scan is large enough and the statistical power is adequate enough. Carefully choosing the significance threshold is therefore another determining factor of successful GWAS.

The strengths of GWAS are that it is hypothesis free and is able to comprehensively interrogate the entire human genome, which enables investigators to identify novel loci or genes for various diseases. It is especially good in complex diseases or quantitative trait, powerful in detecting alleles that are common but with minor or moderate effects on disease phenotypes. These are great advantages over conventional linkage analysis. However, there are also limitations in GWAS (Goldstein, 2009). The major limitation is that a large sample size is mandatory. Genotyping more SNPs does not compensate for a lack of power to detect real effects if the sample size is not large enough. Hence, collaboration is almost mandatory and a universal approach is important in pooling data. Second, GWAS is largely based on the principals of LD, therefore the markers identified through association studies are unlikely to be the disease variants. Extensive re-sequencing and fine mapping are required to discern the disease variants. Re-sequencing will enable investigators to uncover novel and uncommon variants, and biological studies of the genes are required. This has been a great challenge since LD does not further help in fine mapping once a SNP or marker is identified, because the genetically identical SNPs (giSNPs) in LD with the identified SNP nearby offer the same information as the identified SNP, and the functional studies are limited in regions that are not well characterized (Kruglyak, 2008; Seng and Seng, 2008). Currently, it remains difficult to establish the functional role of the disease variants as well as the transcription regulation. Moreover, since GWAS offers massive genetic data output, it is hypothesis-generating rather than hypothesis-testing, therefore replication is paramount to confirm the results. Unbiased and rigorous replication is essential for all kinds of association discoveries as this validates the

study results, and offers a wider evidence base with which to try to make inferences about the validity and biases (Ioannidis, 2008). Replication is not always possible in such large scale studies, especially with disease that have low prevalence rate.

12.6 GWAS in epilepsy syndromes

GWAS has not generally been a tremendous success in elucidating the cause of neurological disorders. Associations have been reported in Restless Leg Syndrome, Multiple Sclerosis, and Periodic Limb Movements in Sleep and these have been considered the most successful GWAS in neurological disorders (Simón-Sánchez and Singleton, 2008; Stefansson *et al.* 2007; Winkelmann *et al.* 2007). Most of GWAS in neurological disorders have been small in scope and designed to detect moderate to large effect sizes (examples are the GWAS in Parkinson disease, Amyotrophic lateral Sclerosis and Ischemic Stroke)(Cronin *et al.* 2008; Fung *et al.* 2006; Matarin *et al.* 2007; Schymick *et al.* 2007). In Amytrophic Lateral Sclerosis, although several disease loci identified through GWAS have been published, none has been replicated, suggesting that the genetics of ALS is more heterogeneous that previous thought (Chio *et al.* 2009; Valdmanis *et al.* 2009) In the GWAS of Parkinson disease, none of the disease loci have been replicated and one of the studies did not identify any associations in the SNPs genotyped in 276 patients and 276 controls (Fung *et al.* 2006). In the GWAS of ischemic stroke, a recent study reported that a genetic locus on chromosome 12p13 is associated with an increased risk of stroke, in a sample size of 1544 incidence of stroke among 19602 persons. This result has not been replicated (Ikram *et al.* 2009). In the Alzheimer Disease GWAS, several disease associated loci (including APO-E gene) have been reported, but with limited replication to support the findings except the APOE gene (Waring and Rosenberg, 2008).

The GWAS in epilepsy syndromes are on going in several research centres, and preliminary reports are yet available. In comparison to other common disease, there are additional challenges in GWAS in epilepsy syndromes. The difficulties of performing GWAS in Idiopathic Generalized epilepsy can be taken as an example.

Idiopathic Generalized Epilepsy syndromes (IGEs) have a cumulative incidence around 0.25% with several sub-syndromes, which are usually diagnosed using clinical and electrographic criteria. These syndromes have been proved to have a strong genetic

predisposition and several ion channel gene mutations have been identified in rare families with single gene disorders (Kjeldsen *et al.* 2003; Meisler and Kearney, 2005). Such findings have facilitated many candidate-gene based association studies in the last 20 years but all have failed to provide convincing reproducible results. Although GWAS may offer a new hope in mapping genes for IGE, there are several challenges. The first challenge is the difficulties in phenotyping. Symptoms may overlap in some of the IGE syndromes. For example, Juvenile Myoclonic Syndrome is characterized with myoclonic seizures, with or without generalized tonic clonic seizures; and Juvenile Absence Epilepsy is characterized with absence with or without myoclonic seizures. The overlap of seizure types in these IGEs has made the phenotyping and population stratification difficult. Secondly, to include all types of IGEs in GWAS might fail as distinct genetic determinants may underlie different sub-syndromes. Phenotyping based on different IGE syndromes faces the difficulty of setting clear boundaries for population stratification. However, the small sample size in sub-syndrome carries the risk of rendering a GWAS underpowered (Mullen *et al.* 2009). Such a situation may also apply if seizure types are used as the basis of phenotyping in GWAS. The phenotyping difficulties and the dilemma in population stratification also apply to studies of familial temporal lobe epilepsy, which consists of a wide variety of seizure types and symptoms. Furthermore, complicated epistasis and environmental factors may have their roles in epileptogenesis, and a GWAS is less likely to be able to take these factors into account (Kruglyak, 2008).

12.7 Biomarker and phenotype

In both GWAS and in genetic linkage analysis, phenotyping is a substantial factor in determining successful gene mapping. In linkage analysis, there are two common situations that hamper the power of gene mapping. First, when an allele has only a minor or moderate effect on a phenotype, some relatives might be affected because of other causes and may not carry the risk allele. Second, when an allele is common, it can enter the family through multiple founders, erasing a clear inheritance pattern. An association study is influenced by the first phenomenon as much as in the linkage analysis. Such an effect might not be easily compensated for even if a large number of cases are involved. Moreover, as discussed above, a homogenous population will enhance the success rate in either types of study. It is

therefore necessary to develop sensitive and unique biomarkers for common diseases, especially in those in which clinical phenotyping is complicated and a major impediment to gene mapping. Many studies to identify markers are on going and many hypotheses are being developed and tested, but in epilepsy syndromes, no reliable biomarkers have yet been identified.

The advantages of developing sensitive and unique biomarkers will assist in other ways. Biomarkers can also be used to predict the therapy outcome and in turn contribute to further adjustment of therapies. In comparison to expensive gene screening, biomarkers can be cost-effective in the practice of Public Health.

12.8 Conclusion

The gene mapping in familial epilepsy syndromes remain challenging. We concluded that familial mesial temporal lobe epilepsy might be either a single gene disease or polygenic disease, while photosensitive epilepsy is probably polygenic. Whole genome association study may provide new hopes in mapping the disease genes of mesial temporal lobe epilepsy. However, the susceptibility genes of the two epilepsy syndromes (familial mesial temporal lobe epilepsy and photosensitive epilepsy) in the pedigrees collected in this study are likely to be rare disease variants with moderate to strong effects, and it remains questionable whether GWAS is suitable to map the susceptibility genes in such a situation. Gene mapping in familial epilepsy syndromes can be improved in several ways: adequate epidemiological information, precise phenotyping via unique biomarkers, advanced linkage analysis software, and genotyping with dense SNPs across the whole genomes.

Section 13: Saccadic eye movement Studies

13.0 Hypothesis

Saccadic eye movements are influenced by anti-epileptic drugs, especially those act on ion channels or receptors to exert the anti-epileptic effects, such as benzodiazepine, phenytoin, and carbamazepine. The most common effects of these AEDs on saccades are slowing of the peak velocities, and prolongation of the latencies.

In this body of work, we aimed to conduct a pilot study as part of a programme to investigate the potential of saccadic eye movement parameters (main sequence, peak saccadic velocity) to act as a biomarker of value in pharmacogenomic studies of epilepsy.

The long-term aim of this work is to determine whether saccadic eye-movements were useful as a measure of the pharmacological effects of antiepileptic drugs – in other words to determine whether they would prove a useful *pharmacogenomic biomarker*. The specific objective is to determine whether large effects on saccadic velocity due to drugs correlated with large drug effects on epilepsy in terms of side-effects or efficacy. If the AED effects on saccades did indeed correlate with clinical AED effects, a second phase of the programme was planned to identify whether polymorphic variation in SCN1A (or other candidate genes) could be identified as underpinning the saccadic variation – thus potentially leading eventually to the use of saccadic eye-movements as a biomarker, and identification of genotypes which correlate to clinical response. The first phase of the project reported in this thesis, the pilot (or scoping) phase, which had the following narrower aims:

1. to devise a clinically-applicable method for measuring saccadic main sequence and peak saccadic velocity.
2. to carry out measurements in a suitable control group, to identify mean values and the range of normality.
3. to carry out a pilot study in patients with epilepsy, to determine whether changes in blood levels were reflected by measurable changes in saccadic velocity in a reliable fashion, and to determine the extent of this effect
4. In a preliminary manner, to whether the there is an association between the magnitude of the effect of saccadic variation and clinical effects (side-effects/efficacy).

13.1 Findings of the study

There were significant variations on test-retest comparisons in the control group. Although many differences were not significant, due to small sample numbers, we concluded that small differences due to drug action will be lost within the noise. In the test-retest reliability calculation, the Pearson regression correlation and the Intraclass correlation coefficient (ICC), we showed that the peak saccadic velocity did not have adequate reliability, with $R_{Pearson} = 0.433$ (same as in R_{ICC}) when chin rest was used and $R_{ICC} = 0.588$ ($R_{Pearson} = 0.615$) when chin rest was not used. The analysis of the subject group showed that the main sequence measured before and after the subjects took their medication, did not show any significant changes. The comparison of the main sequence of the subject group to the control group showed that only the peak saccadic velocity in case 1 was significantly lower than the control group. The other 2 subjects did not differ from the control group even when one of them was experiencing phenytoin intoxication. Although only three cases were tested in the subject group, the results would seem such that the peak saccadic velocity was neither reliable nor sensitive enough in reflecting the changes of serum drug level on saccadic eye movements.

13.2 Possible explanations

There are several reasons for the failure to achieve good test-retest reliability, which was the main reason for our decision not to proceed beyond the pilot stage of this study.

13.2.1 The natural variability of saccades:

The variability of saccades was noticed as early as 1978 by Boghen (Boghen *et al.* 1974) using electro-oculogram to record and analyze the characteristics of the velocity of saccadic eye movements. He found that there was a considerable intra-subject variability in the peak velocity of saccades, and in one of his subject tested, the two sets of recording which was carried out in a time interval of several months showed significant statistic difference. This finding was similar to the results in our control group. A subsequent study done by Bollen (Bollen *et al.* 1993) that used an infrared tracking device to analyze the main sequence and peak velocity in a sample size of 58 showed that the R_{ICC} of the peak velocity was 0.34 calculated at 10°, and 0.10 when calculating the peak velocity at the

amplitude of 5°. This also suggests poor intra-individual reliability of the saccadic peak velocity.

A few studies managed to publish the normative data base of human saccadic eye movement, despite the variability noticed. Bahill (Bahill *et al.* 1981) recorded over 900 saccadic eye movements from 13 normal subjects ranging from 20 to 35 years of age. Table 14.1 shows the suggested normative data of human saccadic eye movements from his study. Raab (Raab, 1985) reported that the peak velocity of saccades distributed normally and ranged from 281 to 541 (mean and SD = 393 +/- 50) deg/sec. Wilson (Wilson *et al.* 1993) provided another set of normative data (Table 14.2). The normative data suggested by Bahill and Wilson are difficult to compared, because they were obtained at different amplitude and were presented in different parameters. The exception was the peak saccadic velocity acquired at 15°, which showed partial overlap in figures and may be comparable to each other. Recently, there have not been studies working on the normative parameters of saccades, but comparisons of main sequence using different methods (Träisk *et al.* 2006).

Table 13.1 Normative data of human saccadic eye movement [Adapted from (Bahill *et al.* 1981) with permission]

Saccadic magnitude (deg)	Peak velocity and SD (deg/sec)	Duration and S.D (msec)
5	261 ± 42	42 ± 8
10	410 ± 67	51 ± 8
15	499 ± 43	54 ± 7
20	657 ± 78	64 ± 6

Table 13.2 Normative data of human saccadic eye movement, mean ± SD [Adapted from(Wilson *et al.* 1993) with permission]

Saccadic magnitude (deg)	peak velocity (degree/sec)	peak acceleration (deg/sec^2)	peak deceleration (deg/sec^2)	saccades accuracy (degree)	saccades latency (ms)
15	332 ± 20	51 ± 6	-48 ± 7	-0.6 ± 1.7	134 ± 46
25	445 ± 41	59 ± 8	-51 ± 9	-0.8 ± 0.8	153 ± 41
35	507 ± 55	63 ± 9	-48 ± 9	-0.8 ± 1.0	160 ± 38
male 35	510 ± 59	64 ± 9	-49 ± 9	-0.9 ± 0.8	161 ± 38
female 35	504 ± 49	62 ± 8	-48 ± 10	-0.7 ± 1.1	157 ± 37

More recently, Smeets (Smeets and Hooge, 2003) investigated the nature of such variability by using 2 different recording facilities to record the saccadic eye movements: the video tracking method and the scleral coil search method. They found that the variability of main sequence was larger when the saccades were recorded by scleral coil search method than the less accurate video tracking method, and suggested that part of the variability could be explained by the method used. They also examined the variability on three hypotheses: the pulse-height noise hypothesis (such motor variability is caused by noise in the firing intensity of motoneurons), the localization noise hypothesis (inaccuracy of target determination by the experimental subjects), and the independent noise hypothesis (measurement inaccuracy). They suggested that the variability in the scleral coil search method might come from the pulse-height noise, while a combination of localization noise and independent noise best described the data obtained by the video system. Another study done by van Beers (van Beers, 2007) by analyzing saccades trajectories suggested that the uncertainty in the sensory signals and the noise in movement planning as well as execution all contributed to the variability in saccade trajectories, and this could be the main resource of the natural variability of the main sequence of saccades. However, these two studies used hypothetical models to analyze the possible origins of the variability rather than directly measuring the signals intensities and relevant noises from neurons, and these theories await further validation.

13.2.2. The reliability of quantitative saccadic recording

Because of the considerable variability seen in the peak velocity of saccades, some researchers have questioned whether the quantitative measurement of the main sequence would be reliable, either as neurophysiologic tool in the longitudinal evaluation of disease progression, or in the evaluation of therapeutic effects of pharmacologic treatments. Version and colleagues (Versino *et al.* 1993) recorded saccadic eye movements from 20 healthy subjects in a time interval of 1 week. They took into account the normal range of variability of saccades in a normal population and modified the ICC accordingly. In contrast to our study, they concluded that the quantitative measurement of saccades and pursuits were reliable, but our interpretation of their data is that the considerable variability demonstrates unreliability.

Another study (Roy-Byrne *et al.* 1995) has investigated the diurnal variation in saccades. Parameters for comparison included peak velocity of saccades and mean latency of saccades. Types of saccades used for testing included visually guided saccades, memory guided saccades, anti-saccades and saccades to moving target. Infrared reflecting device was used for recording, and standard ICC was used for all reliability calculation. The recordings were done twice in the morning and again twice in the afternoon in a random time interval of one week to two months. Roy-Byrne and colleagues found good AM to AM and PM to PM reliability (R_{ICC} within 0.67 to o.78) in the mean latency of all saccades types, and R_{ICC} was within 0.62 to 0.82 in the comparison of AM to PM. All comparisons of peak saccadic velocity showed moderate to excellent reliability, that the R_{ICC} was within 0.70 to 0.95. Our R_{ICC} was smaller than theirs, and we did not test the different types of saccades in our investigation.

More recently, Klein et al (Klein and Fischer, 2005) investigated the reliability of saccadic measurements over time. He tested 446 patients in an average time interval of 19 months, aged from 6 to 88 years old. The saccades types used for recording were anti-saccades and pro-saccades. Parameters used for comparison however, was not typical parameters of saccades, but reaction time for pro-saccades; error reaction time, and direction error for anti-saccades mainly. Both instrumental reliability and test- retest reliability were calculated using Pearson correlation coefficient or Intraclass correlation coefficient. He found that in his sample population there was excellent instrumental reliability (using infrared reflecting device, IRIS, Skalar; Delft, Netherlands, r was within 0.67 – 0.97). The R_{ICC} was within 0.69 – 0.77 in the test-retest reliability test in all saccades parameter tested. While calculating the reliability, he also took into account the normal range of variability. Klein's study might suggest that some unique parameters in specific types of saccades might have better reliability than the dynamic parameters such as peak velocity of voluntary saccades which we used in our study (we chose our measurements on the advice of Professor Linda Luxon, who is in charge of the Neuro-otology Unit of the National Hospital for Neurology and Neurosurgery). The variability of the measurements is in fact striking, and this may also be the reason, for the fact that reliable normative data is absent from the literature.

In summary, the reported reliability of saccades has varied in different research studies. The major differences between our study and some of the studies described above were types of saccades tested and parameters measured. Only visual guided voluntary saccades was tested in our study, while anti-saccades, memory guided saccades or saccades to moving target which involve more sophisticated psychomotor function were tested in the studies and showed better reliability. In these studies, different parameters were chosen for comparison according to different types of saccades tested, which might also be another factor leading to different results of the reliability. It could be concluded that the unique parameters of these saccades which involve more complicated psychomotor performance had higher reliability for intra-individual comparison.

13.2.3. Peak saccadic velocity alone might not be sensitive enough to detect changes of serum drug level.

Our study design aimed to compare main sequence (the peak velocity of saccades) when subjects were in different serum drug levels, and to correlate the results to their treatment outcome. If the magnitude of change in the peak velocity of saccades caused by the change of serum drug level was not great enough to show above the natural variability of saccades, such difference will not be detected. Despite of negative results, it may be true that the majority of patients taking carbamazepine or phenytoin have a slightly slower peak velocity of saccades compared to the control group.

From our study, we can conclude, that the peak velocity of saccades was not sensitive enough to be reliably used to detect the changes and the effect of serum drug level. Even in one subject whose phenytoin serum drug level decreased significantly from toxic level to normal therapeutic level, the Paired T test did not suggest of a significant change in the peak velocity measured. It is clear from our own findings and also our review of the literature, that the detail of the fact of changing blood levels on saccadic velocity, including the effect of intoxication, are not known – although it is clinical experience that eye movements are indeed affected by anti-epileptic drugs (for instance, drug induced nystagmus).

13.2.4. Factors which affect saccadic measurements

Nicotine, Caffein, Alcohol

Besides drugs, other substances in common daily use such as nicotine, alcohol and caffeine also influence saccades. Some studies found that nicotine improve anti-saccades performance without affecting pro-saccades and might improve the psychomotor function in schizophrenic patients (Larrison *et al.* 2004; Larrison-Faucher *et al.* 2004). Alcohol impaired the velocity and initiation of saccadic eye movement, and increases the latencies in anti-saccades (Khan *et al.* 2003; Nyberg *et al.* 2004). Caffeine increases the peak velocity of saccades and may reduce the reaction time in finding the targets (Smith *et al.* 2003). In our study, all cases in the control group and the subject group were tested when they had not had cigarette, coffee or any alcoholic drinks for at least 12 hours before their recording. Thus effects from these substances should have been much minimized in our recording.

Fatigue

Fatigue has marked effects on saccades (Crevits *et al.* 2003). The latency of saccades was prolonged in Crevits' study when fatigue was induced by sleep deprivation, but this prolongation did not reach a statistical significant level. Schleicher (Schleicher *et al.* 2008) used the duration of saccades as the parameter for comparison, and showed that the duration was only moderately increased, but the blink rate after sleep deprivation were significantly increased and appeared to be a good index of fatigue. However, De Gennaro (De Gennaro *et al.* 2000) found that both latency and velocity of saccades were significantly affected in addition to blink rates. Porcu (Porcu *et al.* 1998) found that in the saccadic eye movement, the accuracy (gain) was sensitive to the severity of sleep deprivation and decreased significantly in sleep deprivation. In our studies, although we made all reasonable attempts to minimize the effects of fatigue, it was possible, that many of the subjects were experiencing fatigue during the second recording and this may have contributed to the variability of main sequence, especially those who were tested in the same day.

Leaning effect (adaptation)

Adaptation or learning effect has been shown in several studies (Kojima *et al.* 2005; Watanabe *et al.* 2003). Most of these studies examined modification in continuous saccades, or saccades occurred consecutively within a very short time interval. It has been shown, for instance, that when saccades consistently overshoot their targets, saccade amplitudes gradually decrease to maintain the accuracy. (Herman *et al.* 2009a). In another study, it was found that the amplitude and peak deceleration of saccades were significantly decreased during the process of adaptation, while the other dynamic parameters such as duration, latency and average velocity did not show significant change (Collins *et al.* 2008). In our eye movements recording, most of the cases repeat the whole battery at least twice in a time interval which ranged from half an hour to 1 week apart. It is possible that a degree of adaptation occurred in some individuals,

Age

Finally, the performance of saccades varies with age. A negative linear correlation was found between the saccadic eye movement parameters and age (peak saccade velocity, latency and amplitude) in a study population aged from 15 to 75 (Tedeschi *et al.* 1989). Irving's investigation and survey (Irving *et al.* 2006) showed that the latency of saccades gradually decreased from the age of around/before 5 until before the third decades of life, remain more or less the same until the fifth decades, and then gradually increased in older age. The peak velocity of saccades gradually increases from the age of 3 until 14, and gradually deceases thereafter. The accuracy of saccades (gain) also differs with a larger variation according to the magnitude of saccades made. In general, the gain increases after the age of 10 until 19, slightly decreases (or increased, depending on the amplitude) during the 20s until the age of 30, remains relatively stable until late forties, then gradually decreases thereafter. A similar tendency for the parameters in saccades to change with age was also reported in Klein study on pro- and anti- saccades (Klein *et al.* 2005). In our control group, the age range was wide (from 24 to 64 years). Averaging their main sequence measurements did not account for this when making comparisons between the subject group and the control group. An age matched comparison might have been more appropriate.

13.2.5. Recording facility and analytic programs

Although generally minor in its influence on the results of this study, there were several issues related in the recording process and the programs designed for analysis:

Head gear

The saccadic eye recording was conducted by using an infrared reflection device, with head gear used to place and fix the infrared reflection device in front of the eyes during the recording. It was noticed that because of sweating sometimes the position of the head gear changed slightly during recording. This would change the angle of infrared reflection and might bias the data collected. Such a bias could be observed when converting the data by the program designed with Mathematica. Visually and manually adjustment of such data might have further biased the analysis.

Eye lashes and blinking

Long eye lashes often cause unnecessary noise during recording, especially when the infrared reflector could not be moved further away from the cornea. Blinking was another source of noise during recording. Such effects could be partially overcome by the noise filter but not all. The noise caused by eye lushes and blinking could be further eradicated by manually eliminating them after the data was converted graphically. However, such trimming of data trades off the amount of data collected against the accuracy of recording.

13.3 Limitations

Small sample size was the major limitation of this study. The sample number in both the control group and subject group were less than 15. Although statistics were carried out, it was obvious that the power of this study was not good enough to reach any definite conclusion. We did not increase the number, because it was our opinion that because of the wide intra-subject variation in the control group, it is unlikely that small but genuine changes in the subject group would be detectable. Furthermore, our experience from case 2, the patient intoxicated with phenytoin, who showed no real differences at intoxicating and normal levels of phenytoin, raised doubt about the chances of detecting drug-induced

pharmacodynamic changes. Similarly there was no significant change in eye movement measurements in case 3 in spite of changing drug levels.

Another limitation of this study was that we did not test different types of saccades other than voluntary visual guided saccades. Other types of saccades or saccades task such as anti-saccades, memory guided saccades, or reflexive saccades which involve more sophisticated psychomotor performance and the parameters unique to these saccades might be more reliable and sensitive in terms of the intra-individual comparison. Anti-saccades is a good choice as error rate can be easily calculated and this parameter has been used in the analysis of psychomotor performance under many circumstances (Khan *et al.* 2003; Larrison-Faucher *et al.* 2004). The parameters used in reflex saccades and memory guided saccades are often not traditionally dynamic saccadic parameters such as latency or peak velocity, but reaction time or error rates. Other parameters, such as duration, acceleration, and deceleration of saccades are also worth measuring at the same time, as combinations of these measurements often help in interpretation when a single parameter measurement fail to provide conclusive results. Alternatively, the mathematical relationship between parameters can also be applied for comparison. The Q value is the ratio between peak velocity and average velocity and is often used to evaluate the skewness of saccades waves. The Q value is quite consistent (typically about 1.6), even for slow saccades made by tired subjects and some patients with neurological disorders (Leigh and Zee, 2006; Liao *et al.* 2005). If fatigue or neurological disorders are thought to be confounding factors, the use of the Q value might be a good choice.

13.4 Suggestions for future work

This preliminary study results suggested that modifications should be made on the study design, also that new hypothesis could be addressed.

13.4.1 Modifications to the study design

Several modifications of the study design can be considered.

1. In the speculation that parameters of saccades which involve more sophisticated psychomotor performance are likely to be more reliable and sensitive, and should be tested on the recording battery adjusted. A careful designed regime to include different types of

saccades, such as anti-saccades, memory guided saccades is likely to be more suitable. The length of the time needed for testing should also be considered to avoid fatigue. The effects of learning and adaptation should be minimized as much as possible. To eliminate adaptation, it would be worthwhile having the subjects make a saccade to a 10° target that remained on and not to step back before the second recording. This procedure was used to complete the extinction of any residual adaptation from previous recordings (Herman *et al.* 2009). It would be better if three recordings were carried out on the subject group, so that the peak and trough are less likely to be missed.

2. An age matched control-subject group is necessary, and a larger sample size is mandatory to improve the power of this study.

3. Well defined criteria for alcohol and caffeine intake as well as cigarette use before eye movement recording are necessary, both in the control and subject group.

4. The analysis program must be modified so that simultaneous measurement of different parameters from the output data is possible.

13.4.2 New hypotheses

The findings of our preliminary studies pose other questions worth pursuing:

(1) It would be valuable to test patients who are experiencing carbamazepine or phenytoin intoxication. The saccadic eye movement can be recorded at the time of intoxication and then again when the intoxication has subsided.

(2) The psychomotor performance of these patients should be correlated to saccades parameters, and this might further provide valuable information for epilepsy phenotyping. It is often noticed that patients taking AEDs have relatively worse psychomotor performance, even if they have achieved adequate seizure control or have been seizure free for a period of time, and withdrawing AEDs often improves the performance. It is therefore worth investigating whether there are unanticipated or unknown confounding factors affecting saccadic parameters that are related to psychomotor performance.

(3) The effects of novel AEDs on saccades should be studied. Many new generation AEDs have been introduced into clinical treatment, such as levetiracetam, zonisamide, topiramate, and recently lacosamide. The effects of these novel AEDs on saccades are not yet available. These AEDs often have excellent effects on some epilepsy syndromes that respond poorly

to conventional AEDs in some individuals, which may suggesting that such individuals have specific phenotypes. It may be worth seeing whether saccadic eye movements would be a useful biomarker of treatment outcome of these novel AEDs.

13.5 Conclusion

Our pilot study looking at the effects of AEDs on saccadic eye movements did not enable us to draw any definite conclusions. However, the preliminary analysis results permit us to speculate on the possible problems arising from original study design, and to modify the study design. Our findings also allow us to refine the hypothesis and to tender suggestions for further work.

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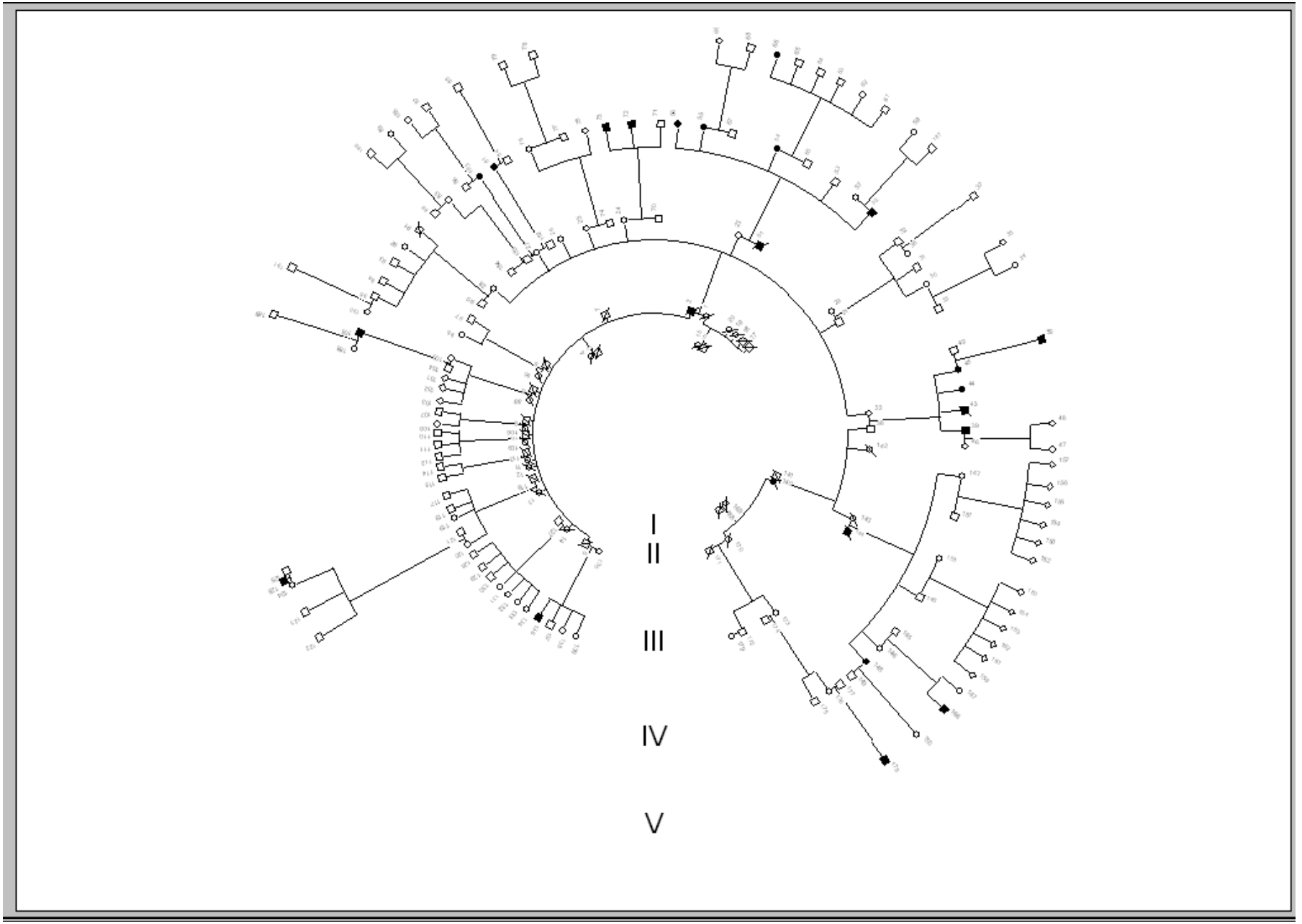
Appendix 1 Pedigree W



Appendix 2 Questionnaire for interview

UCL

PATIENT INFORMATION:
NAME:
ADDRESS:
DATE OF BIRTH: ----------
PHONE NUMBERS:

Home:_____
Work:_____
Mobile:_____
EMAIL ADDRESS:-_____

1. **BIRTH HISTORY:**
   Do you know if there were any problems during your mother's pregnancy with you, during your birth, or during the period immediately following your birth?

2. **PAST MEDICAL PROBLEMS:**
   As a child, did you suffer from febrile convulsions, meningitis or a head injury, and have you had any other serious illness requiring medical, surgical, psychological or psychiatric treatment?

3. **EDUCATION LEVEL:**

4. **Déjà vu**
   Please describe if there has been any repeated, similar, nice and dreamy-like feeling in your life, or other similar states.

5. **PAIN SYNDROME:**
   Please describe if you have any unusual intermittent, periodic pain, such as migraine, facial pain, etc.

   a. Pain: (character, location, duration, triggering factors, response to medicine)

   b. Migraine: (character, location, duration, triggering factors, response to medicine)

   c. Others, please describe (low back pain, neuralgia, etc)

6. **HISTORY OF BLACKOUTS OR SEIZURES:**

At what age did your attacks start? What were they like at the beginning? How often did they occur at the beginning and how often do you get them now? Do you know what caused them?

7. **DESCRIPTION OF YOUR BLACKOUTS OR SEIZURES:**

It is common to have one type of attack. However, two or three different types of attacks may occur. Please try and describe one of the most typical episodes and mention other types if they different from the typical one. In particular, remember your feelings when you very first realise an attack is coming on. It is also very useful to have the account of a witness who has seen your attacks.

a. Body and limb position

b. Usually in what circumstances does it happen?

c. Pattern: Diurnal? in the morning, at night, during sleep, on awakening, or during menstruation period? Any cluster seizure?

d. Prodromal symptoms: symptoms that happens before seizure (not aura)

e. Onset: any aura? Focal motor features? Affective symptoms, such as fear, panic? please describe them as detail as you can if there is any.

f. Features: lost of consciousness and the duration,

g. Motor characteristics: spasm? Jerky? Stiffness? Tongue biting? Incontinence? Fall or injuries?

h. Somatosensory or special sensory manifestations: tingling, numbness, electrical shock-like feeling, burning, pain or heat, stomach rising sensation?

i. Autonomic manifestation: change in skin colour, elevated blood pressure, heart rate, stomach raising sensation, pale looking, sweating…

j. Psychiatric manifestation: dysphasic, amnestic, cognitive, speech arrest or disturbance?

k. Automatism: (orofacial, laughter, cry, meaningless sound, fiddeling movements, complex action, walking, circling,violent behaviour.)

l: visual or auditory hallucination? Circular or ball shape, multiple colours, location?

m. Post-ictal symptoms: fatigue, sore tongue or lip, limbs? Affective symptoms? Confusions? Giddiness?

8. **TRIGGER FACTORS:**
   Do your episodes occur by day, by night or both? Do they occur during any particular activity, such as watching television? Do they occur in relation to any other event such as your menstrual period?

Tiredness? Alcohol? Sleep deprivation? Flickering or bright light? TV?

9. **MEDICATION:**
   Please write down what medications you have taken, roughly when you have been on them, and if possible, the does and effect of the medications:

   a. In the past:

   b. At present:

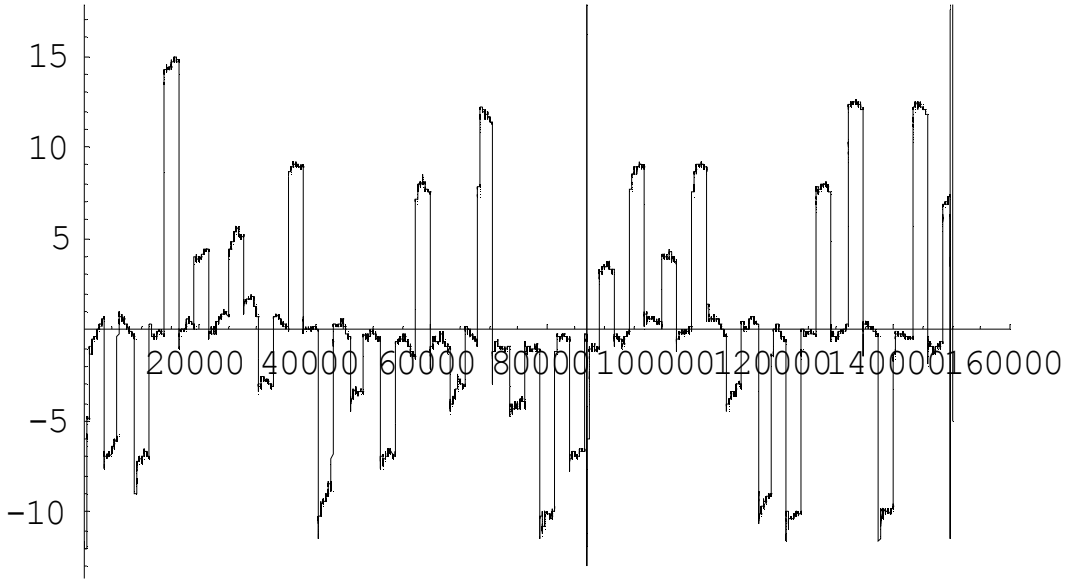
10. **WHICH HOSPITALS AND SPECIALISTS HAVE YOU PREVIOUSLY CONSULTED AND WHEN?**

11. **IS THERE ANY OTHER INFORMATION YOU WOULD LIKE US TO KNOW?**

Appendix 3

Burst cell function recovery

```
Read in data
 data = ReadList["C:\\Documents and Settings\\chiening LO\\My
Documents\\Saccades\\control\\Luis\\text\\Luis left.txt"];
data = 15( data)+4;
data= Take[data,{1,150000}];
Plot data
 ListPlot[( data), PlotJoined -> True, PlotRange->
{{1,160000},Automatic}];
```



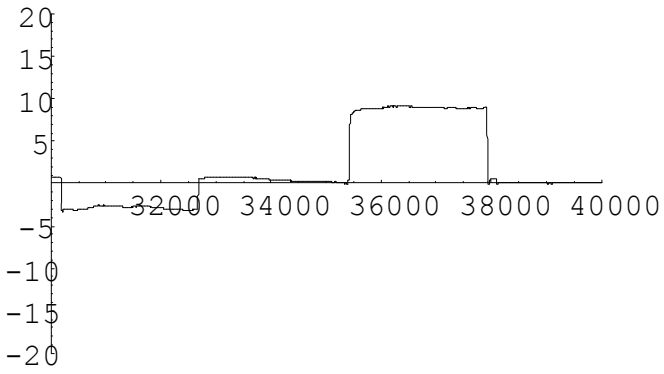
Reconstruct trajectory with discrete Legendre polynomials

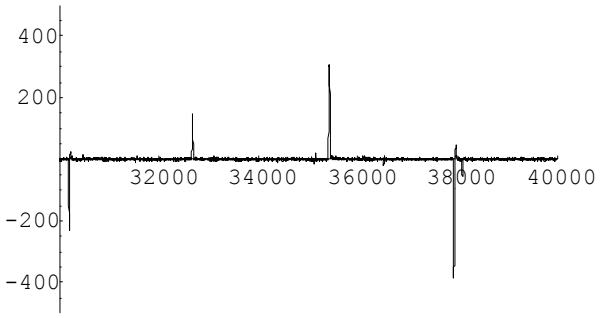
```
p = 5;
n = 2 * p + 1;
one = Table[ 1/Sqrt[2 * p + 1], {n, -p, p}];
two = Table[ n/(p * Sqrt[((2*p+1)*(p+1))/(3*p)]), {n, -p, p}];
three = Table[ (n^2 - (p*(p+1))/3)/((p^2)/3 * Sqrt[((4*p^2-1)*(2*p+3)*(p+1))/(5*p^3)]), {n, -p, p}];

delay = Transpose[Table[Drop[RotateLeft[data, i - 1], -n],
    {i, n}]];
onefac = Tr[one];
one = one / onefac;
twofac = two.Table[i * 0.001, {i, n}];
two = two / twofac;

x = delay.one;
y = delay.two;
xy = delay.Transpose[{one, two}];

axpic = ListPlot[x, PlotJoined → True,
   PlotRange → {{30000, 40000}, {-20, 20}}];
bxpic = ListPlot[y, PlotJoined → True,
   PlotRange → {{30000, 40000}, {-500, 500}}];
```



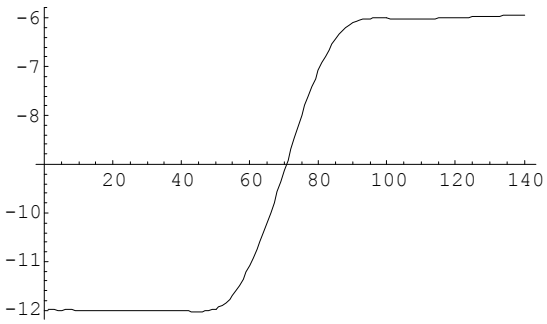


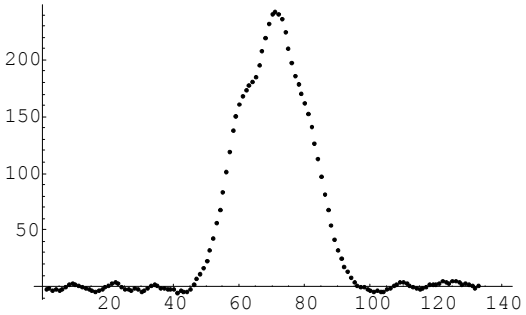
Find starting points of saccades

```
 (*Identify the start of each saccade, using a velocity criterion of 30
degrees per second*)
temp = Table[{Abs[y[[i]]],i},{i,Length[y]}];
temp = Select[temp,#[[1]]>30 &];
(*Form a separate list for the data points of each saccade*)
temp = Split[temp, #2[[2]]==#1[[2]] + 1  &];
(*Remove any saccades lasting less than 30 milliseconds which might be
noise*)
temp = Select[temp,Length[#]> 10 &];
(*Form arrays of the data point index of the start and finish of each
saccade*)
starts = Table[temp[[i,1,2]],{i,Length[temp]}];
ends = Table[temp[[i,Length[temp[[i]]],2]],{i,Length[temp]}];
 mainseq= Table[{x[[ends[[i]]]]-
x[[starts[[i]]]],Max[Abs[Take[y,{starts[[i]],ends[[i]]}]]]},{i,Length[sta
rts]}];
```

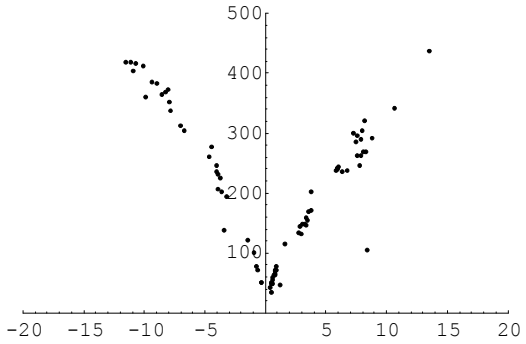
List all figures

```
 Do[
    Print[i];
    ListPlot[Take[x,{starts[[i]]-50,ends[[i]]+50}], PlotJoined → True,
PlotRange → All];ListPlot[Take[y,{starts[[i]]-50,ends[[i]]+50}],
PlotRange → All],{i,Length[mainseq]}];
```



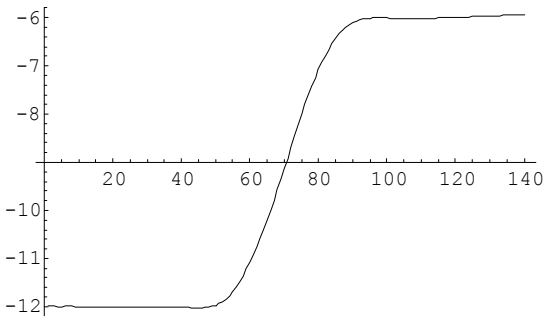


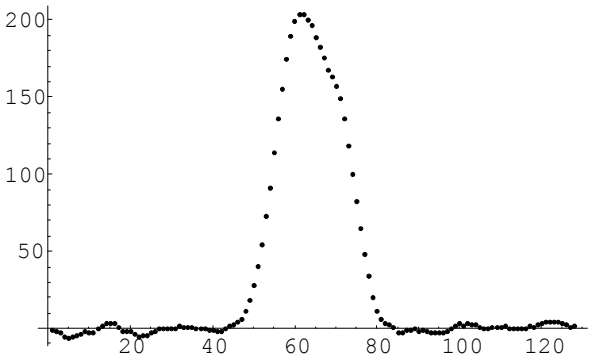

```
ListPlot[mainseq,PlotRange ->{{-20,20},{0,500}}];
```

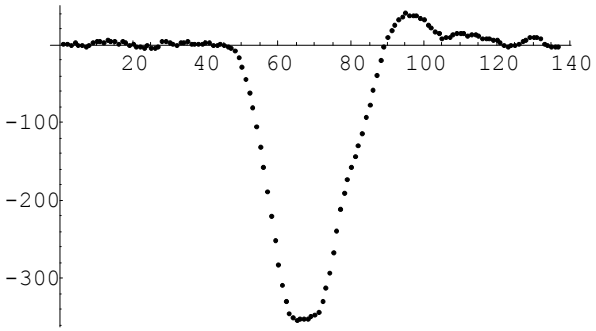


Selection of desirable data

```
goodones={};
goodones=Drop[goodones,{}];
const=Length[goodones];
goodones=Drop[goodones,{const}];
Show[temp1 = Table[ListPlot[Take[x,{starts[[goodones[[i]]]]-
50,ends[[goodones[[i]]]]+50}], PlotJoined -> True, PlotRange ->
All],{i,Length[goodones]}]];
Show[temp1 = Table[ListPlot[Take[y,{starts[[goodones[[i]]]]-
50,ends[[goodones[[i]]]]+50}],  PlotRange -> All],{i,Length[goodones]}]];
(*Do[
      Print[i];
      ListPlot[Take[x,{starts[[goodones[[i]]]]-
50,ends[[goodones[[i]]]]+50}], PlotJoined -> True, PlotRange ->
All];ListPlot[Take[y,{starts[[goodones[[i]]]]-
50,ends[[goodones[[i]]]]+50}], PlotRange -> All],{i,Length[goodones]}];*)
```

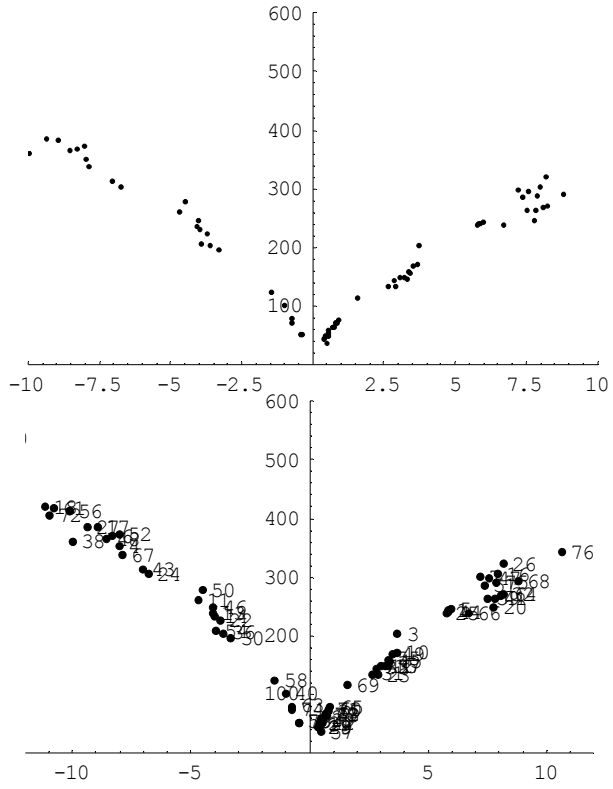






Further modification

```
<<Graphics`Graphics`;
goodones={};
goodones=Drop[goodones,{}];
const = Length[goodones];
goodones = Drop[goodones,{const}];
mainseq= Table[{x[[ends[[goodones[[i]]]]]]-
x[[starts[[goodones[[i]]]]]],Max[Abs[Take[y,{starts[[goodones[[i]]]],ends
[[goodones[[i]]]]}]]]},{i,Length[goodones]}];ListPlot[mainseq,PlotRange -
>{{-10,10},{0,600}}];
LabeledListPlot[mainseq,PlotRange ->{{-12,12},{0,600}}]
```



Examination

```
 i = 49;
ListPlot[Take[data,{starts[[i]]-50,ends[[i]]+50}]];
i = 96;
ListPlot[Take[data,{starts[[i]]-50,ends[[i]]+50}]];
i = 105;
ListPlot[Take[data,{starts[[i]]-50,ends[[i]]+50}]];
i = 103;
ListPlot[Take[data,{starts[[i]]-50,ends[[i]]+50}]];
i = 152;
ListPlot[Take[data,{starts[[i]]-50,ends[[i]]+50}]];
```

Find data points on nullcline

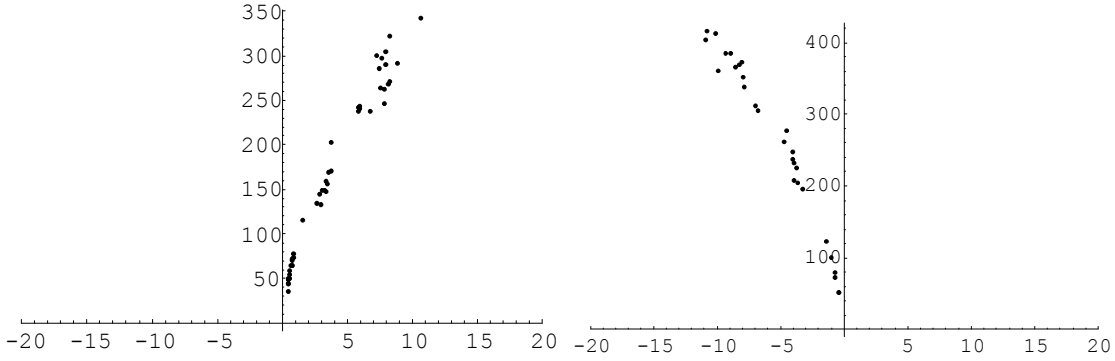
```
<< Statistics`NonlinearFit`;
uppersize = 7.5;
mainseq1 = Select[mainseq, ((#[[1]] > 0) && (#[[1]] < 11)) &];
mainseq2 = Select[mainseq, ((#[[1]] < 0) && (#[[1]] > -11)) &];
mainseq2 = Table[{-mainseq2[[i, 1]], mainseq2[[i, 2]]},
   {i, Length[mainseq2]}];
mainseq3 = Select[mainseq, (Abs[#[[1]]] < uppersize) &];
mainseq3 =
  Table[If[(mainseq3[[i, 1]] < 0), {-mainseq3[[i, 1]], mainseq3[[i, 2]]},
    mainseq3[[i]]], {i, Length[mainseq3]}];
curve1 = NonlinearFit[mainseq1, α Sqrt[q], q, α]
curve2 = NonlinearFit[mainseq2, α Sqrt[q], q, α]
curve3 = NonlinearFit[mainseq3, α Sqrt[q], q, α]
mainseq1 = Select[mainseq, ((#[[1]] > 0) && (#[[1]] < 11)) &];
mainseq2 = Select[mainseq, ((#[[1]] < 0) && (#[[1]] > -11)) &];
mplot1 = Table[{q, curve1}, {q, 0, 11}];
mplot2 = Table[{-q, curve2}, {q, 0, 11}];
mplot3 = Table[{q, curve3}, {q, 0, uppersize}];
```

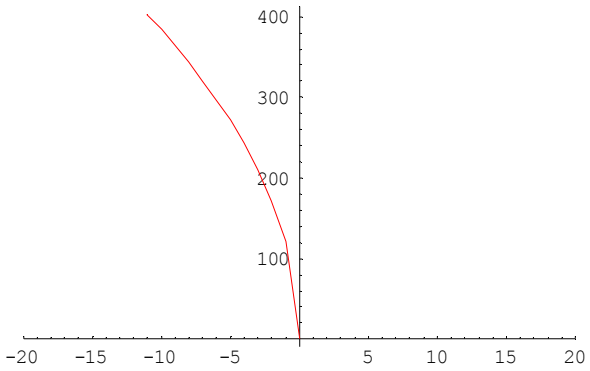
$97.0284\sqrt{q}$

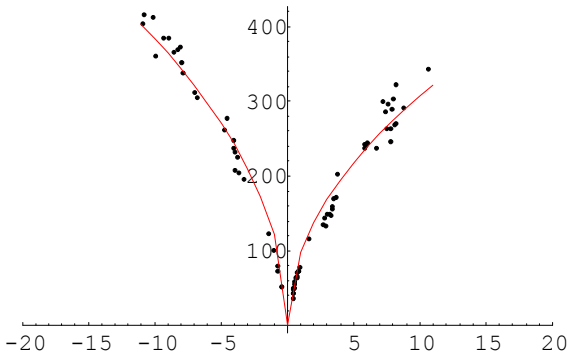
$121.503\sqrt{q}$

$101.249\sqrt{q}$

```
Show[
   ListPlot[mainseq1, PlotRange -> {{-20,20},Automatic}],
   ListPlot[mplot1, PlotStyle -> RGBColor[1,0,0],PlotJoined ->
True,PlotRange -> {{-20,20},Automatic}],
   ListPlot[mainseq2, PlotRange -> {{-20,20},Automatic}],
   ListPlot[mplot2,PlotStyle -> RGBColor[1,0,0],PlotJoined -> True,
PlotRange -> {{-20,20},Automatic}]];
```




400
300
200
100
-20
-15
-10
-5
5
10
15
20


400
300
200
100
-20
-15
-10
-5
5
10
15
20