CLINICAL AND MOLECULAR GENETIC STUDIES ON JUVENILE MYOCLONIC EPILEPSY

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Ph.D. THESIS (2003)

SREE CHITRA TIRUNAL INSTITUTE
FOR
MEDICAL SCIENCES AND TECHNOLOGY
THIRUVANANTHAPURAM - 695 011, INDIA

The thesis entitled

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Submitted by

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for
Doctor of Philosophy
of

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CERTIFICATE

I J.VIJAI hereby certify that I had personally carried out the work depicted in the thesis entitled **"CLINICAL AND MOLECULAR GENETIC STUDIES ON JUVENILE MYOCLONIC EPILEPSY"** except where external help sought and acknowledged.

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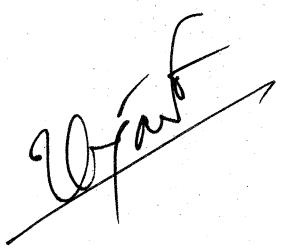

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DECLARATION

This is to certify that Sri J. VIJAI in the department of Neurology of this Institute, has fulfilled the requirements of the regulations relating to the nature and prescribed period of research for the Ph.D. degree of the Sree Chitra Tirunal Institute For Medical Sciences And Technology, Thiruvananthapuram. The work relating to his thesis entitled **"CLINICAL AND MOLECULAR GENETIC STUDIES ON JUVENILE MYOCLONIC EPILEPSY"** was carried out under my direct supervision.

Signature :.....

K. Radhakrishnan
(Guide)

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- J. VIJAI

We shall not cease from exploration

And the end of all our exploring

Will be to arrive where we started

And know the place for the first time.

-T.S. Eliot, "Little Gidding".

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LIST OF ABBREVIATIONS

ACE	:	Angiotensin converting enzyme
ADNFLE	:	Autosomal dominant nocturnal frontal lobe epilepsy
AD	:	Autosomal dominant
AED	:	Antiepileptic drug
AR	:	Autosomal recessive
BAM	:	Broad affection mode
BFNC	:	Benign familial neonatal convulsions
CACN1A	:	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
CAE	:	Childhood absence epilepsy
CEPH	:	Center de Etude Polymorphism Humaine
CHRNA7	:	Cholinergic receptor, nicotinic, alpha polypeptide 7
CLIC5	:	chloride intracellular channel 5
cM	:	Centimorgan
CNS	:	Central nervous system
CT	:	Computerized tomography
$\hat{d}$	:	$\hat{d}$, MLS function of δ (where δ = allele sharing)
DZ	:	Dizygotic
EEG	:	Electroencephalogram
GABA	:	γ-amino-butyric acid
GABRA	:	γ-amino-butyric acid receptor subunit α
GADPH	:	Glyceraldehyde 3 Phosphate Dehydrogenase
GCLC	:	Glutamate-cysteine ligase, catalytic subunit
GEFS+	:	Generalized epilepsy with febrile seizure plus
GTCS	:	Generalized tonic-clonic seizures
HIV	:	Human immunodeficiency virus
HLA	:	Human leukocyte antigen
hSKCa3	:	Small conductance calcium activated potassium channel subunit 3 gene
HWE	:	Hardy-Weinberg equilibrium
IAE	:	Idiopathic absence epilepsy
IBD	:	Identity-by-descent
IED	:	Interictal epileptiform discharges
IGE	:	Idiopathic generalized epilepsy
ILAE	:	International League Against Epilepsy
JAE	:	Juvenile absence epilepsy
JME	:	Juvenile myoclonic epilepsy

JNCASR	:	Jawaharlal Nehru Center for Advanced Scientific Research
KCNAB2	:	Potassium voltage-gated channel, shaker-related subfamily, beta member 2
KCNJ	:	Potassium inwardly-rectifying channel, subfamily J
KCNQ2	:	Potassium voltage-gated channel, KQT-like subfamily, member 2
KCNQ3	:	Potassium voltage-gated channel, KQT-like subfamily, member 3
K_v	:	Voltage gated potassium
LOD	:	Logarithm of odds
MLS	:	Maximum likelihood estimator score
MPT	:	Multipoint
MRI	:	Magnetic resonance imaging
mRNA	:	Messenger ribonucleic acid
mtDNA	:	Mitochondrial deoxyribonucleic acid
MZ	:	Monozygotic
nAChR	:	Nicotinic acetylcholine receptor
NAM	:	Narrow affected model
NKM Buffer	:	Sodium-Potassium-Magnesium buffer
NPL	:	Non-parametric linkage
O_E	:	Occurrence expectation
OMIM	:	Online Mendelian Inheritance in Man
PCR	:	Polymerase chain reaction
REM	:	Rapid eye movement
RFLP	:	Restriction fragment length polymorphism
RR	:	Relative risk
S_{all}	:	All sib-pairs selected for analysis
SCA	:	Spinocerebellar ataxia
SCN1B	:	Sodium channel, voltage-gated, type I, beta
SCTIMST	:	Sree Chitra Tirunal Institute for Medical Sciences and Technology
SNP	:	Single nucleotide polymorphism
S_{pairs}	:	Sib-pair scoring function
$S_{pairs\text{-}p(0.5)}$	:	Sib-pair scoring function with equal power of 0.5 to each sib-pair
SPT	:	Single point
Taq	:	*Thermus aquaticus*
TDT	:	Transmission disequilibrium test
TEN Sol.	:	Tris-EDTA-NaCl solution
θ	:	Recombination fraction
θ_f	:	Recombination fraction (female)
$\theta_{m=f}$	:	Recombination fraction (male = female)
θ_m	:	Recombination fraction (male)
VNTR	:	Variable number of tandem repeats
Z_{all}	:	Maximum LOD score with all affected members
Z_{max}	:	Maximum LOD score

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SYNOPSIS

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INTRODUCTION

Juvenile myoclonic epilepsy (JME) is a well-defined age-related idiopathic generalized epilepsy (IGE) syndrome with an estimated prevalence of 0.5 to 1 per 1000 population. Myoclonic jerks, frequently involving the upper extremities and occurring shortly after awakening, often precipitated by sleep deprivation and alcohol ingestion, are the characteristic features of the syndrome. The majority of patients seen in epilepsy clinics also have generalized tonic-clonic seizures (GTCS), and about one-third of them report antecedent history of absence seizures. The age at onset is usually around puberty and a positive family history of epilepsy is obtained in nearly half of the patients. Individuals with JME have normal intellect and neurological status. The characteristic interictal electroencephalographic (EEG) finding is paroxysmal, generalized, symmetric, fast multiple spike and slow wave discharges over a normal background activity, and photoparoxysmal response in approximately one-third of patients. Antiepileptic drug (AED) treatment with valproate results in seizure freedom in the majority, but relapses are inevitable on AED discontinuation.

Problems in the diagnosis of JME and phenotypic variability

Despite these distinctive clinical and EEG features, JME is frequently unrecognized and misdiagnosed in both developed and developing countries. Lack of familiarity with the syndrome, failure to elicit a history of myoclonic jerks, and misinterpretation of åbsences, asymmetric myoclonic jerks and focal EEG findings in favor of partial seizures have been found to be the factors responsible for the misdiagnosis of JME. However, with the documentation of a sizeable number of patients with JME in recent years from different parts of the world, variability in the phenotypic expression of this epilepsy syndrome is becoming increasingly apparent. JME with myoclonic jerks alone, with onset very early in life as well as in

old age, with focal EEG abnormalities, and with resistance to valproate therapy have been recognized.

Genetics of JME

JME is one of the most genetically explored epileptic syndrome. However, till today, no single gene or mutation has been consistently implicated in its pathogenesis. While the presence of susceptibility locus for JME on chromosome 6p was observed in the majority of the United States (Greenberg *et al.,* 1988) and German (Sander *et al.,* 1997) families, linkage studies in families from the United Kingdom, Denmark, Sweden, Spain and Greece failed to find evidence for such a locus (Elmslie *et al.,* 1996). Linkage of JME to a locus on chromosome 15q has been suggested and negated by different investigators (Elmslie *et al.,* 1997; Durner *et al.,* 2000). A JME gene was identified on chromosome five in a large French Canadian family and a *GABRA1* mutation was uncovered (Cossette *et al.,* 2002). Quite recently, another gene myoclonin has been reported on chromosome six (Delgado-Escueta *et al.,* 2002).

Channelopathies and epilepsy

Studies done on animal models by pharmacological inhibition of potassium channels and with induced deletions of potassium channel genes have revealed that alterations in these channels can result in seizures. Two distinct mutations involving voltage-gated potassium channel genes (*KCNQ2* and *KCNQ3*) have been identified in the rare human idiopathic epilepsy syndrome, benign familial neonatal convulsions (Charlier *et al.,,* 1998). The neuronal small conductance calcium activated potassium channel gene 3 (*hSKCa3*) plays a critical role in determining the firing pattern of neurons through the generation of slow after-hyperpolarization and regulation of intracellular calcium signals. These physiological attributes make *KCNQ3* and *hSKCa3* intriguing candidate genes for investigation in JME.

OBJECTIVES OF THE STUDY

This study was conceived with the following objectives:

1) To describe the problems and pitfalls in the diagnosis, phenotypic peculiarities, and clinical genetics of JME among the Kerala population.
2) To explore linkage of EJM1 locus on chromosome 6 to JME using parametric and non-parametric methods in a large family from Kerala.
3) To test EJM1 locus in South Indian multiplex JME families using non-parametric linkage analysis.
4) To investigate genetic association of JME to potassium channel candidate genes *KCNQ3* and *hSKCa3* using transmission disequilibrium test (TDT) and case control strategies.

MATERIALS AND METHODS

Selection of patients and controls

For the clinical study, patients fulfilling the standard diagnostic criteria of JME were selected through the epilepsy clinic of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvanathapuram. For molecular genetic studies, JME probands were recruited through neurology centers situated in the South Indian cities of Thiruvanathapuram, Bangalore, Chennai, Hyderabad, Kochi and Kozhikode. Each individual institution's internal review board approved the study, and all subjects provided informed consent to participate.

The subjects utilized for genetic analysis were: 1) One large family from Kerala with 18 members, five of whom were diagnosed with JME and five members with febrile seizures or a generalized EEG epileptiform abnormality, which seemed amenable to both parametric and non-parametric linkage analysis; 2) One hundred and nineteen probands sampled along with the parents forming a "trio"; a family unit ideal for TDT; 3) Fifty multiplex families with atleast one affected sib-pair,

which fits for non-parametric linkage study design; 4) Two hundred and twenty two JME probands from mutually unrelated families used for case control association study for *hSKCa3*; and 5) Two hundred and forty eight control subjects of South Indian origin without family history of epilepsy, unexplained blackouts or other chronic neurological disorders.

Molecular genetic studies

Genotyping

The molecular genetic studies were undertaken at the Jawaharlal Nehru Center for Advanced Scientific Research, Bangalore. Genomic DNA was extracted using phenol-chloroform method from 10ml venous blood. Twenty-two polymorphic DNA markers spanning 49.4cM of chromosome six (6p22.3 to 6q12) of EJM1 locus from the Gènèthon reference map were selected with an average intermarker distance of 2.35cM. Polymerase chain reaction (PCR) was standardized according to published guidelines for the Gènèthon reference map set. Emphasis on finding intragenic polymorphic markers for association studies led to final selection of two such potassium channel genes namely *KCNQ3* and *hSKCa3*. In *KCNQ3*, the polymorphic (CA_n) marker D8S558 in intron 1 was amplified by PCR.

Statistical analysis

Transmission disequilibrium test for KCNQ3

Allelic association between JME and the marker D8S558 was tested by using TRANSMIT. The program produces asymptotic χ^2 tests for each allele, a test on 1-df for excess transmission of that allele and calculates significance based on a large bootstrap. TRANSMIT analysis using the 10,000 bootstrap samples was performed excluding rare haplotypes, so that the approximate chi-squared distribution would hold valid.

***Chi-square and test of proportions for** hSKCa3*

Test of proportions was carried out to determine the significance of individual alleles of *hSKCa3*. The CLUMP software was for calculation of χ^2 values and a Monte-Carlo procedure employed for computing p values.

Parametric analysis for the large family

Two point LOD score analysis was performed using the computer programs MLINK and ILINK through Linkage Control Program (LCP) version 5.1. LOD scores were calculated at equal male: female recombination fractions $\theta_{m=f}$ of 0.1, 0.2, 0.3, 0.4 and 0.5. Analysis was performed with both autosomal dominant (AD) and autosomal recessive mode of inheritance. Analysis was done under two affection models. In the narrow affection model (NAM), only members with JME were used. In the broad affection model (BAM), in addition to JME members, individuals with fast spike and wave EEG abnormalities were also included. Multipoint and two-point linkage analysis was carried out using Génehunter.

Non-parametric analysis for small multiplex families

Genotypes were checked for Mendelian consistencies and founder allele frequencies were inferred using TRANSMIT. Statistical analysis was carried out using ALLEGRO computational package (deCode Genetics, Iceland). ALLEGRO can perform both classical parametric linkage analysis and analysis based on allele sharing models. In the allele-sharing model, posterior pair-wise identity-by-descent (IBD) sharing probabilities are calculated based on the pedigree and genotype data. Prior probabilities are given by the pedigree within the constraints of Mendelian rules of inheritance

In two-point analysis, the IBD distribution was estimated based on the marker genotypes for each marker taken individually. In multipoint analysis, a 0.06cM scanning increment was used to infer IBD distribution along the map used. ALLEGRO was

used to estimate the NPL scores and the exact P values associated with the NPL and Z_{lr} scores, where Z_{lr} = sign ($\hat{d}$) $\sqrt{}$(2ln10.LOD) where $\hat{d}$ is the extent of allele sharing. Empirical significance testing of the observed scores was done by simulation using 100 randomly generated datasets based on the original dataset.

RESULTS

Clinical study

At referral, only six (3.3%) out of the 183 JME probands carried the diagnostic label of JME. Default in diagnosis resulted from failure to elicit the history of myoclonic jerks by the referring physicians. During the mean delay of 8.6 ± 7.0 years in diagnosing JME, seizure control in the majority was poor due to inappropriate AED therapy with carbamazepine (38%). There was relatively increased occurrence of absence seizures (40%) and low frequency of photoparoxysmal response (10%). A history of epileptic seizures was obtained in 6.2% of the first-degree and 2.2% of the second-degree relatives of the probands; 37.7% and 11.1% of them, respectively, were diagnosed as JME.

Molecular genetic studies

Association between KCNQ3 and JME

Sixteen alleles were observed at the marker locus D8S558 ranging in length from 157bp to 193bp [$(CA)_n$; n=15 - 32]. The modal allele had 20 CA repeats. $(CA)_{19}$ to $(CA)_{23}$ accounted for >68% of all alleles. The p value obtained for $(CA)_{20}$ was 0.0076 (χ^2 5.6685). Empirical power estimated with TDT Power Calculator was 99.96%, at simulated type I error of 0.0001. Suggestive evidence of transmission disequilibrium was noted (P 0.008).

Protective influence of an hSKCa3 allelic variant on JME

Among 222 JME cases and 248 ethnically matched controls, 16 alleles were observed, 13 in controls and 16 in cases. No dynamic repeats were detected in any sample. Modal allele was CAG_{18}. Z-test of proportions showed significance for three alleles: CAG_{16} (P 0.009), CAG_{18} (P 0.004) and CAG_{19} (P 0.0000011). While allele CAG_{16} and CAG_{18} were present at higher frequencies in JME patients, allele CAG_{19} was quite rare in JME cases but present at a high frequency in the control group. CAG_{18} and CAG_{19} were significant after Bonferroni correction for 11 alleles at α=0.0045. Relative risk (RR) of the common alleles (f >0.01) was found to be maximum for allele CAG_{18} (1.178) and minimum for allele CAG_{19} (0.514). Regression analysis of relative risk versus allele length variation of the most common alleles did not yield a statistically fit model.

Linkage exclusion of EJM1 locus in a large Kerala family

Two point LOD score analysis using MLINK shows no evidence of linkage to the chromosome 6p markers used in the study. Except marker D6S1548, all other markers showed exclusion LODs (< -2) at θ=0 with 90% AD under the narrow affection model. The Z_{max} obtained from ILINK did not cross LOD of 0.5 at any $\theta_{m=f}$. Multipoint LOD scores again were exclusionary in most regions of the map. When the BAM was used, multipoint LOD scores were clearly exclusionary along the entire map. Analysis was also done with the unaffected members reclassified as unknown. This also failed to show any positive trend towards linkage.

Non-parametric linkage analysis of putative locus EJM1 in South Indian JME sib-pairs

Highest two-point LOD score was observed at D6S452 (LOD 2.31, P 0.00055). Another locus with a LOD approaching and crossing 1 was also found; the maximum

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LOD was 1.54. There were three consecutive markers with a promising score in this HLA locus spanning 3.36cM. When analysis was done with all families weighted equally in the exponential mode and all sibs selected (S_{all}); the maximum LOD of 1.34 was observed at map location 11.54cM (P 0.006383) between markers D6S265 and D6S1568. However, the maximum Z_{lr} score of 2.36 was obtained at 10.22cM (P 0.0106) at marker locus D6S265. A region starting near marker D6S1691 (8.82cM) showed a LOD>0.9 and extended upto a region (14.4cM) between markers D6S1568 and D6S291. The total sub-chromosomal region that shows a trend of Z_{lr}>2 is 5.9cM long. The P values are consistently below the nominal significance level of 0.05 in this region (spanning eight markers) except for two points near the second marker D6S1663 at 4.8 and 6.0cM. The breadth of this significance region is 16.3cM.

DISCUSSION

Problems and pitfalls in the diagnosis

The diagnosis of JME should be straightforward in a person of the adolescent age group with normal intellect, neurological status, and an antecedent history of early morning myoclonic jerks during wakefulness, who presents with a GTCS following sleep deprivation. However, several studies have observed delay in diagnosis, ranging from a mean of 6.8 to 15.0 years, both from developed and developing countries. Only episodes of myoclonic jerks during wakefulness clearly support the diagnosis of JME, and delay in the diagnosis usually results from failure to elicit this history. In the present 183 patients, only six (3.3%) were diagnosed with JME when first referred; the average duration from onset of seizures to diagnosis of JME was 8.6 years. Effective AED therapy was delayed and seizure control was poor at the time of referral in nearly all the patients.

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Phenotypic peculiarities

The two notable differences in the clinical features of JME observed in the present study compared to those reported in the literature were the relatively increased occurrence of absence seizures and low frequency of photoparoxysmal response among Kerala JME probands.

The prevalence of absence seizures in the present series was 40% when compared to 10 to 35% reported among patients belonging to different ethnic groups. Two other series from India found absences in 8% of JME probands from Delhi and in 21% from Hyderabad. Although the low frequency of absence seizures among JME patients reported from certain geographical regions may most likely be due to incomplete ascertainment, it may well be an expression of the clinical heterogeneity of the syndrome in different ethnic groups.

The prevalence of photoparoxysmal response in JME patients has been observed to vary from 10% to 40%. Among the JME patients, photoparoxysmal response was encountered in 10%. Other studies involving JME patients from South India, North India, and Japan have also noted a relatively low prevalence of photoparoxysmal response. The variability in the prevalence of photoparoxysmal response among JME patients from different ethnic groups may be yet another reflection of the genetic variability of this syndrome.

Clinical genetics

Forty six percent of the present patients had a positive family history, which is comparable to the reported rates of 24% to 44% from India and 25% to 65% from other nations. In all series, including the present one, generalized epilepsies and syndromes were more common than localization related epilepsies among affected relatives. First-degree relatives were three fold or more often affected as compared to second-degree relatives.

Molecular genetic studies

Association between KCNQ3 and JME

Analysis of 119 JME nuclear families from the present series showed linkage disequilibrium between marker D8S558 and JME indicating *KCNQ3* is associated to JME. A study from Berlin using 38 JME and 33 childhood absence epilepsy patients failed to find a significant association (Haug *et al.,* 2000). However, the authors admit that the small sample size and low statistical power in their experiment cannot exclude small existing gene effects. Apart from genetic heterogeneity known to be present in ethnic populations, the heterogeneity of their sample population consisting of different epilepsy syndromes could also have confounded the results. The subtle effect of linkage disequilibrium noted in the present study may or may not be applicable to other populations. Replication studies need to be carried out to ascertain the validity of the results in different ethnic populations.

Association of hSKCa3 and JME

The present study tested association of an expressed polymorphic CAG repeat tract in a functionally important calcium activated potassium channel gene *hSKCa3* that has been previously implicated in schizophrenia (Chandy *et al.,* 1998). A significant difference in the allelic distribution among South Indian JME probands compared to ethnically matched control subjects was observed. A plausible biological model could involve the pathogenic polymorphisms to be associated with CAG_{18} in cases, while CAG_{19} provides a protective mechanism in controls and hence was extremely rare in JME probands. We did not observe any dynamic CAG repeat expansions outside the normal range either in the patients or controls. However, in Kennedy's disease and spinocerebellar ataxia type 6, CAG repeats within the normal range are known to result in a disease state.

Contrary to above results, lack of evidence for an association between idiopathic generalized epilepsies and *hSKCa3* gene has been reported earlier (Sander *et al.,* 1999). Genetic heterogeneity, mixture of different syndromes within

the samples used, small sample size and low statistical power could perhaps explain the apparently conflicting results in this report.

EJM1 and JME

At present, there are only two reports of large families of JME that have been used for linkage analysis: 1) LA-Belize family (Liu *et al.,* 1996) 2) French Canadian family (Cossette *et al.,* 2002). The analysis of the third such family is reported here. It is interesting to note that both the above families were mapped to different chromosomes; LA-Belize family to chromosome six and French Canadian family to chromosome five. The Kerala family was found to be negative for the *GABRA1* mutation reported on chromosome five. The results from two-point and multipoint mapping from the Kerala family suggest that there is no linkage to EJM1 in this family. Most of the map showed LOD scores that were clearly exclusionary, except the region between markers D6S1548 and D6S282 which showed LOD > -2. The marker informativeness in this region was only 50% and hence the LOD dropping down further upon usage of additional markers cannot be ruled out.

When the investigation at EJM1 was expanded with more markers for 50 multiplex families only, we found positive evidence of locus heterogeneity. In the initial two-point analysis, the marker D6S452 gave a LOD of 2.31. However, in multipoint analysis, this marker did not show robust allele sharing. On the other hand, three markers at the HLA locus gave Z_{lr} >2. The results obtained from allele-sharing, non-parametric linkage analysis in the 50 South Indian multiplex families suggest a weak linkage to markers D6S265 and D6S1568.

The important observation from the multiplex families study is a trend towards positive allele sharing in both the locations previously described as the EJM1 locus, namely at the HLA region (6p21.33 to 6p21.31) and at the centromeric region implicated in the classic JME variety (6p11.2 to 6p12.1).

SIGNIFICANCE OF THE STUDY

Genetic analysis of complex disorders is a scientifically challenging venture and has seldom been attempted in developing countries. Most genetic studies on common epilepsies have either lumped different syndromes in order to find common gene effects governing all the syndromes there in, or have attempted to find linkage of a well-characterized epilepsy syndrome to a single locus. In this study, the role of the causative gene and the influence of minor susceptibility or protective genes were investigated in a well-characterized patient population utilizing a systematic approach.

The pitfalls and problems associated with the diagnosis of JME highlights the difficulty in clinically characterizing the syndrome, which could have influenced results of previous genetic studies on JME. The protective allele effect of a calcium activated potassium channel gene *hSKCa3* in JME is revealed for the first time through a case control strategy. The role of EJM1 locus in JME has been a matter of severe debate in the epilepsy research community. Although this study using an independent dataset from the South Indian population suggests a weak linkage to EJM1, it also suggests that EJM1 is not the sole locus involved in JME predisposition. For the first time, proof for the emerging concept that JME has a complex mode of inheritance that is dependent on one or more disease genes and interplay of modifier genes is provided through this genetic research.

CONCLUSIONS

Based on the results of this study utilizing a well-characterized group of JME patients and normal subjects from South India, I wish to conclude: 1) A majority of the JME patients in the community remain inadequately diagnosed and treated; 2) Minor phenotypic variability in the syndrome is common; 3) JME in South Indian population shows a trend towards linkage to EJM1, however, locus and genetic heterogeneity exist; and 4) Potassium channel genes *KCNQ3* and *hSKCa3* may be involved in modifying the genetic expression of JME.

REFERENCES

Chandy KG, Fantino E, Wittekindt O, *et al.,* (1998). Mol Psychiatry 3:32-7.

Charlier C, Singh NA, Ryan SG, *et al.,* (1998). Nat Genet 18:53-5.

Cossette P, Liu L, Brisebois K, *et al.,* (2002). Nat Genet 31:184-9.

Delgado-Escueta AV, Bai D, Bailey J, *et al.,* (2002). Rev Neurol 35: 82-6.

Durner M, Shinnar S, Resor SR, *et al.,* (2000). Am J Med Genet 96:49-52.

Elmslie FV, Rees M, Williamson MP, *et al.,* (1997). Hum Mol Genet 6:1329-34.

Elmslie FV, Williamson MP, Rees M, *et al.,* (1996). Am J Hum Genet 59:653-63.

Greenberg DA, Delgado-Escueta AV, Widelitz H, *et al.,* (1988). Am J Med Genet 31:185-92.

Haug K, Hallmann K, Horvath S, *et al.,* (2000). Epilepsy Res 42:57-62.

Liu AW, Delgado-Escueta AV, Gee MN, *et al.,* (1996). Am J Med Genet 63:438-46.

Sander, T., Bockenkamp, B., Hildmann, T. *et al.,* (1997) Neurology; 49: 842-7.

Sander T, Scholz L, Janz D, Epplen JT, Riess O (1999). Epilepsy Res 33:227-33.

I. INTRODUCTION

Juvenile myoclonic epilepsy (JME) is a well-defined age-related idiopathic generalized epilepsy (IGE) syndrome with an estimated prevalence of 0.5 to 1 per 1000 population (Durner and Janz, 1997). Myoclonic jerks, frequently involving the upper extremities and occurring shortly after awakening, often precipitated by sleep deprivation and alcohol ingestion, are the characteristic features of the syndrome (Grünewald and Panayiotopoulos, 1993; Durner and Janz, 1997). The majority of patients seen in epilepsy clinics also have generalized tonic-clonic seizures (GTCS), and about one-third of them report antecedent history of absence seizures. The age at onset is usually around puberty and a positive family history of epilepsy is obtained in nearly half of the patients. Individuals with JME have normal intellect and neurological examination. The characteristic interictal electroencephalographic (EEG) finding is paroxysmal, generalized, symmetric, fast multiple spike and slow wave discharges over a normal background activity, and photoparoxysmal response in approximately one-third of patients (Grünewald and Panayiotopoulos, 1993; Durner and Janz, 1997). Antiepileptic drug (AED) treatment with valproate results in seizure freedom in the majority, but relapses are inevitable on AED discontinuation (Penry *et al.,* 1989; Kleveland and Engelsen, 1998).

Despite these distinctive clinical and EEG features initially described nearly five decades ago (Janz and Christian, 1957) and recognized by the International league against Epilepsy (ILAE) more than a decade ago (ILAE, 1989), even today, JME is frequently unrecognized and misdiagnosed in both developed (Kleveland and Engelsen, 1998; Montalenti *et al.,* 2001) and developing countries (Atakli *et al.,* 1998; Murthy *et al.,* 1998; Gunatilake *et al.,* 2000). Lack of familiarity with the syndrome, failure in elicit a history of myoclonic jerks, and misinterpretation of absences, asymmetric myoclonic jerks and focal EEG findings in favour of partial seizures have been found to be the factors responsible for the misdiagnosis of JME (Panayiotopoulos *et al.,*1991; Vazquez *et al.,* 1993; Lancman *et al.,* 1994; Murthy, 1999). However, with the documentation of a sizeable number of patients with JME in recent years from different parts of the world, variability in the phenotypic expression of this epilepsy syndrome is becoming increasingly apparent. JME with myoclonic jerks alone (Jain *et al.,* 1997), with onset very early in life as well as in old age (Gram

et al., 1988), with focal EEG abnormalities (Aliberti *et al.,* 1994), and with resistance to valproate therapy (Fernando-Dongas et al. 2000; Gelisse *et al.,* 2001) have been recognized.

JME is one of the most genetically explored epileptic syndromes (McNamara, 1999). However, till today, no single gene or mutation has been consistently implicated in its pathogenesis. While the presence of susceptibility locus for JME on chromosome 6p was observed in the majority of the United States (Greenberg *et al.,* 1988) and German (Sander *et al.,* 1997) families, linkage studies in families from the United Kingdom (Whitehouse *et al.,* 1993), Denmark (Elmslie *et al.,* 1996), Sweden (Elmslie *et al.,* 1996), Spain (Obach *et al.,* 2000) and Greece (Elmslie *et al.,* 1996) failed to find evidence for such a locus. Linkage of JME to a locus on chromosome 15q has been suggested (Elmslie *et al.,* 1997) and negated (Durner *et al.,* 2000) by different investigators. A JME gene was identified on chromosome five in a large French Canadian family and a *GABRA1* mutation was uncovered (Cossette *et al.,* 2002). Quite recently, another gene myoclonin has been reported on chromosome six (Delgado-Escueta *et al.,* 2002).

Genetic heterogeneity could perhaps explain dissimilarities in the phenotypic expression of JME, as well as the conflicting linkage results from different populations. Ethnic background of the population, by contributing to heterogeneity in susceptibility alleles and polygenetic effects, may contribute to genotypic and phenotypic heterogeneity.

The genes encoding for ion channels have become the favorite candidates in the investigation about the pathogenesis of several paroxysmal neurological disorders, including epilepsies (McNamara, 1999; Prasad *et al.,* 1999). Studies done on animal models by pharmacological inhibition of potassium channels (Juhng *et al.,* 1999) and with induced deletions of potassium channel genes (Smart *et al.,* 1998) have revealed that alterations in these channels can result in seizures. Two distinct mutations involving potassium channel genes (*KCNQ2* and *KCNQ3*) have been identified in the rare human idiopathic epilepsy syndrome benign familial neonatal

convulsions (BFNC) (McNamara, 1999; Prasad *et al.,* 1999).

The calcium-activated potassium channels are gated by intracellular calcium ions and their activity is responsible in part for the afterpolarization that follows a single action potential or a train of action potentials in the neurons (Coetzee *et al.,* 1999). According to their single-channel conductance in symmetrical potassium solutions, these channels are classified as big (BK), intermediate (IK), or small (SK) (Coetzee *et al.,* 1999). The neuronal small conductance calcium-activated potassium channel gene (*hSKCa3*) plays a critical role in determining the firing pattern of neurons through the generation of slow after-hyperpolarization and regulation of intracellular calcium signals (Nicoll, 1988). *In situ* hybridization demonstrates abundant *hSKCa3* transcripts within the substantia nigra and ventral tegmental area, and along the dopaminergic neurons from these regions into the nigrostriatal and mesolimbic pathways (Dror *et al.,* 1999). These physiological and anatomical attributes make *hSKCa3* an intriguing candidate gene for investigation in an IGE syndrome such as JME. *hSKCa3*, located on chromosome 1q21, encodes a 371 amino acid protein comprising two polyglutamine arrays in its N-terminus of which the second polyglutamine repeat is highly polymorphic (Chandy *et al.,* 1998).

It is evident from the above discussion that the phenotypic and genotypic features of JME are complex. The available evidence on JME genetics points to involvement of atleast three major genes (EJM1, EJM2 and *GABRA1*) and perhaps several modifying genes. The following pertinent questions arise:

1) Is JME phenotypically homogeneous syndrome or are there distinct subtypes such as JME with myclonus alone, EEG trait alone, with or without absences, with or without photosensitivity, or with valproate resistance?
2) Is JME a genetically heterogeneous syndrome, as the molecular genetic data indicates from the available association and linkage studies?
3) If there is phenotypic and genotypic heterogeneity, can there be distinct subtypes, which can be characterized by genotype-phenotypic correlations?
4) Does genetic dysfunction involving the potassium channels modify the susceptibility to JME?

OBJECTIVES OF THE STUDY

This study was conceived with the following objectives:

1) To describe the problems and pitfalls in the diagnosis, phenotypic peculiarities, and clinical genetics of JME among the Kerala population.

2) To explore linkage of EJM1 locus on chromosome 6 to JME using parametric and non-parametric methods in a large family from Kerala.

3) To test EJM1 locus in South Indian multiplex JME families using nonparametric linkage analysis.

4) To investigate genetic association of JME to potassium channel candidate genes *KCNQ3* and *hSKCa3* using transmission disequilibrium test (TDT) and case control strategies.

II. REVIEW OF LITERATURE

1.The Epilepsies

A seizure is defined as a paroxysmal disorder of the central nervous system (CNS) characterized by abnormal cerebral neuronal discharge with or without a loss of consciousness (International League Against Epilepsy [ILAE], 1993, 1997). Epilepsy is defined as a condition characterized by a tendency for recurrent seizures (two or more), unprovoked by any known proximate cause (ILAE, 1993, 1997). This definition excludes seizures that occur due to acute systemic metabolic derangement or to an acute CNS insult.

The ILAE introduced a classification of seizures in 1981 based on clinical semiology, interracial EEG findings and ictal patterns (ILAE, 1981), which divided seizures into two major groups: first characterized by generalized seizures and the latter with partial, focal or localization-related seizures. Among the generalized seizures, are included, absence seizures myoclonic seizures and GTCS. Partial seizures are classified into simple and complex depending upon whether consciousness is impaired or not during a seizure episode.

In 1989, ILAE evolved a system for classifying epilepsies and epileptic syndromes (ILAE, 1989). A syndrome is defined as a group of signs and symptoms that occur together and which collectively characterize a particular disease. The syndromic classification takes into account factors such as the age at onset of the seizures, clinical characterizations and grouping of seizure symptoms, and the presence or absence of evidence of organic brain disease. The epilepsies with known etiologies were called symptomatic or secondary, those with uncertain etiologies were called as idiopathic or primary, and those with presumed etiologies were called cryptogenic. The term cryptogenic was subsequently omitted and the current classification recognizes only idiopathic and symptomatic epilepsies. More recently, the ILAE Task Force on Classification and Terminology recommended an elaborate five-axis diagnostic scheme for epilepsy consisting of 1) descriptive ictal phenomenology, 2) diagnostic seizure types, 3) epileptic syndromes, 4) etiology, and 5) impairment (Engel, 2001).

1.1 Idiopathic generalized epilepsies

Idiopathic generalized epilepsies are forms of generalized epilepsies in which all seizures are initially generalized and associated with EEG changes that are generalized and bilaterally synchronous and symmetrical (ILAE, 1989). The patient usually has a normal interictal state, without neurological or neuroradiological abnormalities. In general, the EEG show normal background activity and generalized interictal epileptiform discharges (IEDs) such as spikes, polyspikes, spike-wave and polyspike-waves of 3 Hz. The various syndromes in idiopathic generalized epilepsies differ mainly in the age of onset and are listed in Table 2.1. Juvenile myoclonic epilepsy (JME) is the commonest idiopathic generalized epilepsy syndrome (ILAE 1989).

Table 2.1. Idiopathic generalized epilepsy syndromes

1.	Benign neonatal familial convulsions
2.	Benign neonatal convulsions
3.	Benign myoclonic epilepsy in infancy
4.	Childhood absence epilepsy (pyknolepsy)
5.	Juvenile absence epilepsy
6.	Juvenile myoclonic epilepsy
7.	Epilepsy with generalized tonic-clonic seizures on awakening

1.2 The JME syndrome

1.2.1 A historical perspective

Herpin (1867) first gave a detailed description of a patient with JME. He called the myoclonic jerks as "secousses" or compulsions that shake the body as an electric shock.

Rabot (1899) emphasized the intermittency and mildness of the jerks and involvement of isolated muscles of the neck and shoulders, distinguishing this pattern from the global and massive trunk and limb spasms that can propel a patient to the floor (Lundborg, 1903). Lennox (1945) distinguished and separated the progressive myoclonus epilepsy described by Unverricht (1895) from the intermittent myoclonus epilepsy of Rabot. In 1955, Janz introduced the term *impulsive pètit mal* and by 1957 had accumulated 47 patients, describing the characteristic symptoms known today. The following year, Castells and Mendilaharsu (1958) at the Montevideo School of Medicine reported 70 patients with "bilateral and conscious myoclonic epilepsy". However, both these reports in German and Spanish, respectively, went unnoticed in the English literature. The term "juvenile myoclonic epilepsy" was coined by Lund (1975). Delgado-Escueta and Enrile-Bacsal (1984) introduced the syndrome to the American neurology community under the name of "juvenile myoclonic epilepsy of Janz". Another report involving 12 patients with JME followed in the American neurology literature in the same year (Asconape and Penry, 1984).

1.2.2 Prevalence

JME is estimated to account for about 10% of all epilepsies. Studies from Germany (Janz, 1989; Janz, 1990) and United Kingdom (Grünewald *et al.,* 1992) reported a frequency varying from 7% to 11.4%. The prevalence rates reported from other regions include 10.2% from Saudi Arabia (Panayiotopoulos *et al.,* 1994), 5.6% from among the Singapore men (Kun *et al.,* 1999) and 2% from Malaysia (Manonmani and Tan, 1993).

The prevalence of epilepsies has been studied from different parts of India. In a series of 131 JME patients from Hyderabad, Andhra Pradesh, a prevalence of

7.7% was noted (Murthy *et al.,* 1998). In a hospital based study from North India, JME accounted for 350 of 3,442 (10%) of all cases with epilepsy (Jain *et al.,* 1999). From Bangalore, Karnataka, (Mani and Rangan, 1995) diagnosed JME in 5.9% of 2,548 patients of various epilepsies. In an elaborate epidemiological survey on epilepsy conducted in the central part of Kerala, JME accounted for 13% of all epilepsies (Radhakrishnan, unpublished data).

1.2.3 Clinical features of JME

1.2.3.1 Demographic characteristics

JME affects both sexes equally, however, female preponderance has been noted in some series (Panagariya *et al.,* 2001). Age of onset varies from eight to 20 years (mean 14 years) (Janz and Durner, 1997). The seizures that occur in JME are absences, myoclonus and GTCS (Janz and Durner, 1997). The mean age at onset of absences reported by Delgado-Escueta is 11.5 years (range, 8 to 16), myoclonic seizures 15.4 years (range, 8-30) and GTCS 15.5 years (range, 8 to 29). Absences began at a mean age of 9.5 years (range 7 to 13 years), myoclonic jerks at 14.3 years (range 10 to 19 years) and GTCS at 16.1 years (range 9 to 14 years) in a series from United Kingdom (Grünewald *et al.,* 1992). Diagnosis of JME should be considered for all age groups as the syndrome has been reported to develop in unexpected age groups (Gram *et al.,* 1988). The chronological order of seizure types is fairly consistent in the JME syndrome. Absence seizures predated myoclonic jerks by 3.4 ± 2.5 years and myoclonic jerks predated GTCS by 0.8 ± 3.4 years (Panayiotopoulos *et al.,* 1994). In some patients myoclonus may be the only clinical symptom, but GTCS follows usually within two years of the onset of myoclonic jerks (Janz, 1989).

1.2.3.2 Precipitating factors

The major seizure types in JME, namely myoclonus and GTCS occur usually within one hour after awakening in the morning (Janz and Durner, 1997). These may be precipitated by lack of sleep, fatigue, emotional stress or alcohol. Janz (1985) mentioned sleep deprivation as the most important precipitating factor, an observation that has been confirmed by other researchers (Asconape and Penry, 1984; Delgado-Escueta and Enrile-Bacsal, 1984; Obeid and Panayiotopoulos, 1988; Grünewald *et al.,* 1992; Pedersen and Petersen, 1998). Fatigue, sleep deprivation and alcohol were the most important precipitating factors identified in western series (Grünewald *et al.,* 1992). Absence seizures may be precipitated by acts involving prolonged intense concentration, however, absences are not as sensitive to factors that provoke myoclonic jerks or GTCS. Emotional stress frequently precipitates seizures in JME (Janz, 1985). Seizures often exacerbate during menstrual periods (Delgado-Escueta and Enrile-Bacsal, 1984; Janz, 1985).

1.2.3.3 Physical and neurological examination

Neurological examinations do not show any abnormalities and intellect is normal (Janz and Durner, 1997). Neuroimaging with computed tomographic (CT) or magnetic resonance imaging (MRI) shows normal results. Positron emission tomography (PET) studies using ^{18}F-2 deoxyglucose have shown normal findings in the majority (Swartz *et al.,* 1996a; Swartz *et al.,* 1996b; Swartz *et al.,* 1996c). However, voxel-based statistical parametric mapping comparison between a group of JME patients and control subjects have shown an increase in cortical grey matter in the mesial frontal lobes among patients (Woermann *et al.,* 1999a; Woermann *et al.,* 1999b). Further analysis revealed significant abnormalities of cortical grey matter in five out of 20 JME patients (Woermann *et al.,* 1999b). Limited autopsy data have not provided any substantial structural abnormalities except microdysgenesis in few cases, characterized by an increase of partially dystopic neurons in the stratum moleculare (Meencke and Janz, 1984; Meencke and Janz, 1985).

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1.2.3.4 Seizure semiology

1.2.3.4.1 Myoclonic seizures

Myoclonus in JME consists of mild to moderate jerks of extensor muscles in neck, shoulders, arms or legs occurring during the alert state. Myoclonias are more frequent in the upper than the lower extremities and are usually bilaterally symmetric and synchronous (Janz and Durner, 1997). Myoclonic jerks in the upper extremities can often cause the patient to drop objects and can interfere with daily activities. The jerks can be repetitive or single (Janz, 1985). Myoclonic jerks become massive preceding a GTCS. Sometimes the myoclonias of JME are perceived only as an electric shock inside the body with no exterior movement.

1.2.3.4.2 GTCS

GTCS are often preceded by mild to moderate myoclonic jerks of increasing frequency and intensity. Because consciousness is preserved during myoclonic jerks, the patient often adapts a safe position to avoid injuries during the GTCS, which might follow. Consciousness is abruptly lost when the head, face, neck and trunk extend with a tonic contraction. This phase lasts 10 to 20 seconds and lead to a final phase of clonic trunk and limb jerks. A history of GTCS is elicited in 90% to 95% of JME patients attending the epilepsy clinic.

1.2.3.4.3 Absence seizures

The absences in JME are generally mild, relatively short, and are often unnoticed by the patients and their caregivers (Janz and Durner, 1997). The reported frequency of myoclonic jerks therefore show wide variation in different series. Absences were present in 33% of patients described from USA (Delgado-Escueta and Enrile-Bacsal, 1984), in 10% of cases as reported from Germany (Janz, 1969), 33% of patients from Saudi Arabia (Panayiotopoulos *et al.,* 1994), 40.8% from Turkey (Atakli *et al.,* 1998), 23% from Balkans (Ercegovac *et al.,* 1998), 12% from Japan (Matsuoka, 1989) and 19.2% from Brazil (Figueredo *et al.,* 1999).

Two series from India found absences in 8% of JME probands from Delhi (Jain *et al.,* 1998b) and in 21% from Hyderabad (Murthy *et al.,* 1998).

1.2.3.5 Electroencephalographic features

1.2.3.5.1 Interictal EEG

The resting awake EEG background activity in JME is uniformly normal (Janz and Durner, 1997). The characteristic IEDs consists of bilateral symmetric and synchronous polyspike and wave complexes of 4-6 Hz (Fig. 2.1). These discharges are often prominent over the fronto-central regions. The polyspike-wave discharges are more frequent during the time of awakening. The IEDs are less frequent during drowsiness and during stage I of non-REM sleep. The IEDs seldom occur during REM sleep, hardly ever during stage 2 non-REM sleep, and never during stages 3 and 4 of non-REM sleep (Touchon, 1982).

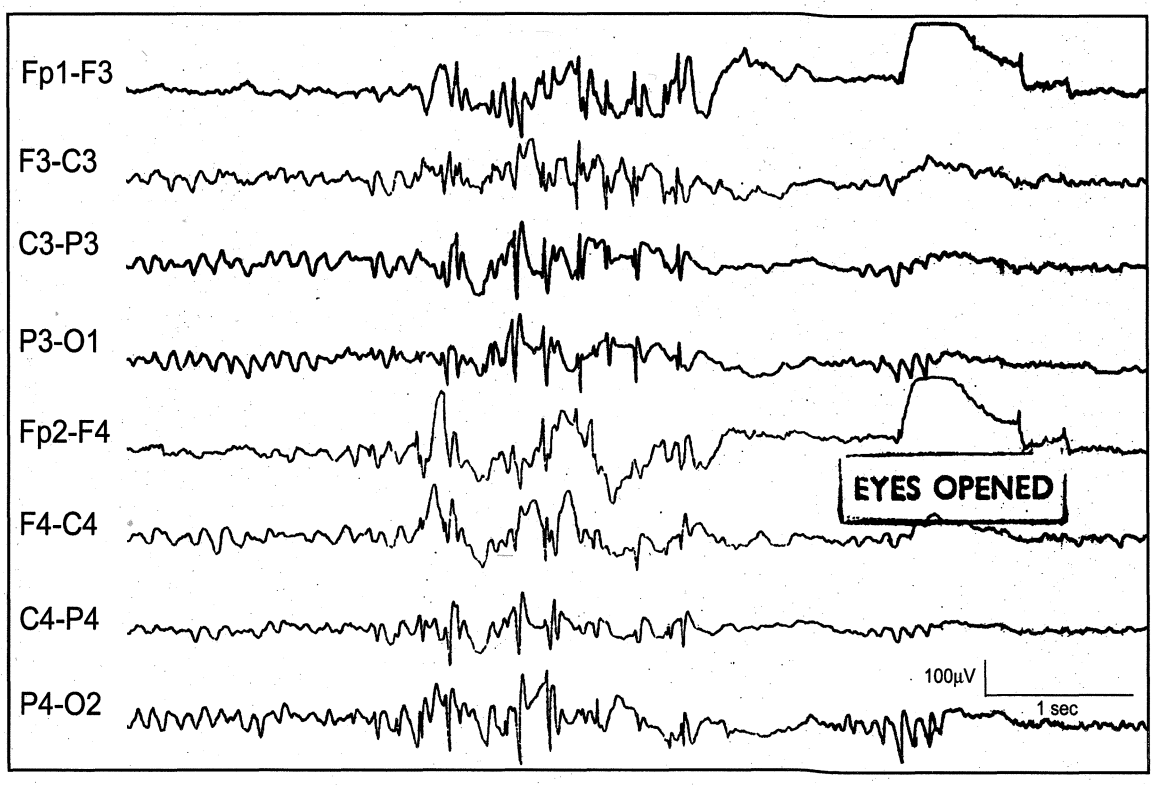


Fig 2.1 Interictal EEG of a 17-year old female showing bilateral symmetric and synchronous spike- and multiple spikes-wave complexes over a normal background activity

1.2.3.5.2 Ictal EEG

The EEG correlate of the myoclonic jerks is a burst of medium to high amplitude 10-16Hz polyspikes followed by irregular 1-3Hz slow waves of different amplitude. A diffuse discharge of irregular 2-5 Hz spike and wave complexes may precede the multispike discharges (Janz and Durner, 1997). During absences, fast diffuse polyspike and wave complexes of 4-6 Hz change to 3 Hz frequencies. During GTCS, diffuse high voltage rapid spikes are interrupted by quiescence on the EEG.

1.2.3.5.3 Photosensitivity

Photosensitivity or the precipitation of bilaterally synchronous spike and wave patterns by intermittent photic stimulation in the EEG laboratory is frequent in JME (Fig. 2.2). Photosensitivity has been reported in 30% of 121 JME patients in one series, which is the highest rate in all epileptic syndromes (Wolf and Goosses, 1986). The prevalence of photosensitivity in other studies are as follows: 33% from the United States (Asconape and Penry, 1984), 27.3% from Saudi Arabia (Panayiotopoulos *et al.,* 1994) and 17.5% from Japan (Shiraishi *et al.,* 2001). Television sensitivity has been reported in 17.4% of JME patients (Petrukhin *et al.,* 1997). The prevalence of photosensitivity reported from India are 13% from South India (Murthy *et al.,* 1998), 9% (Jain *et al.,* 1998b) and 11.6% (Mehndiratta and Aggarwal, 2002) from North India.

Photosensitivity is strongly age and gender related (Jeavons *et al.,* 1986; Kasteleijn-Nolst Trenite, 1989). Of the 61 children with a diagnosis of JME with a median age of 13 (range 7 to 16) years, 55 (90%) were photosensitive (Appleton *et al.,* 2000). Twice as many females as male patients exhibit photosensitivity (Jeavons *et al.,* 1986; Kasteleijn-Nolst Trenite, 1989).

Eye closure can facilitate EEG discharges and myoclonic jerks in JME patients. Eye closure sensitivity was found in 26 out of 115 (20.6%) JME patients (Wolf and Goosses, 1986)

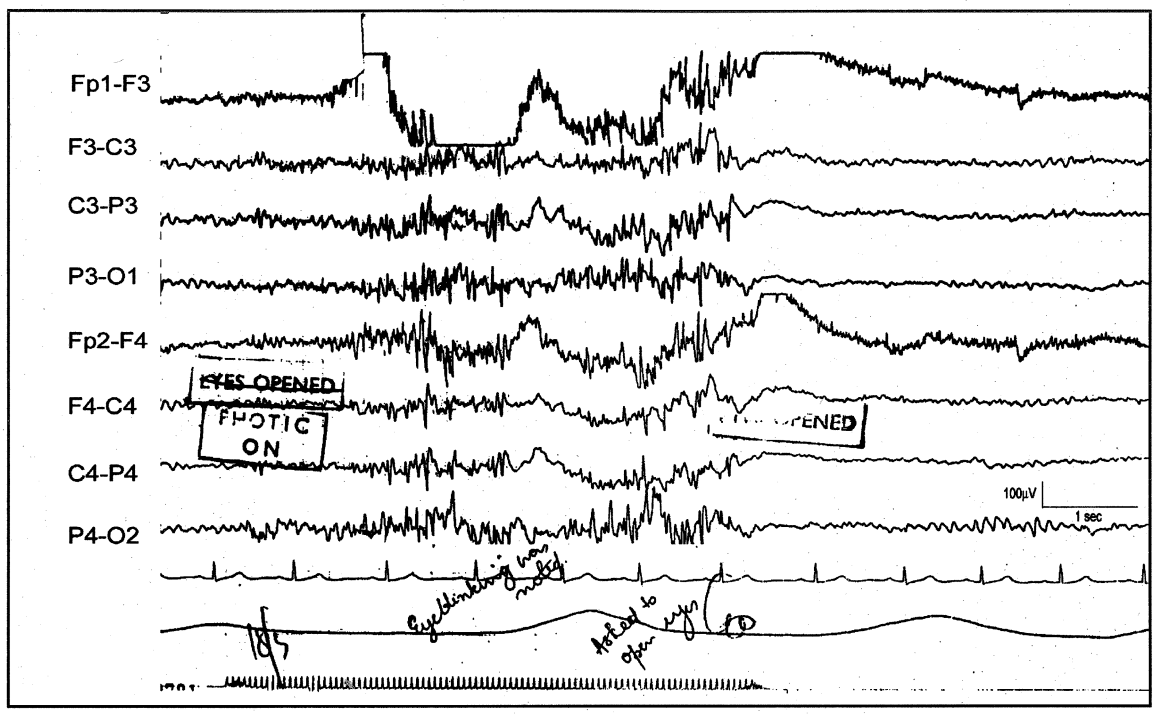


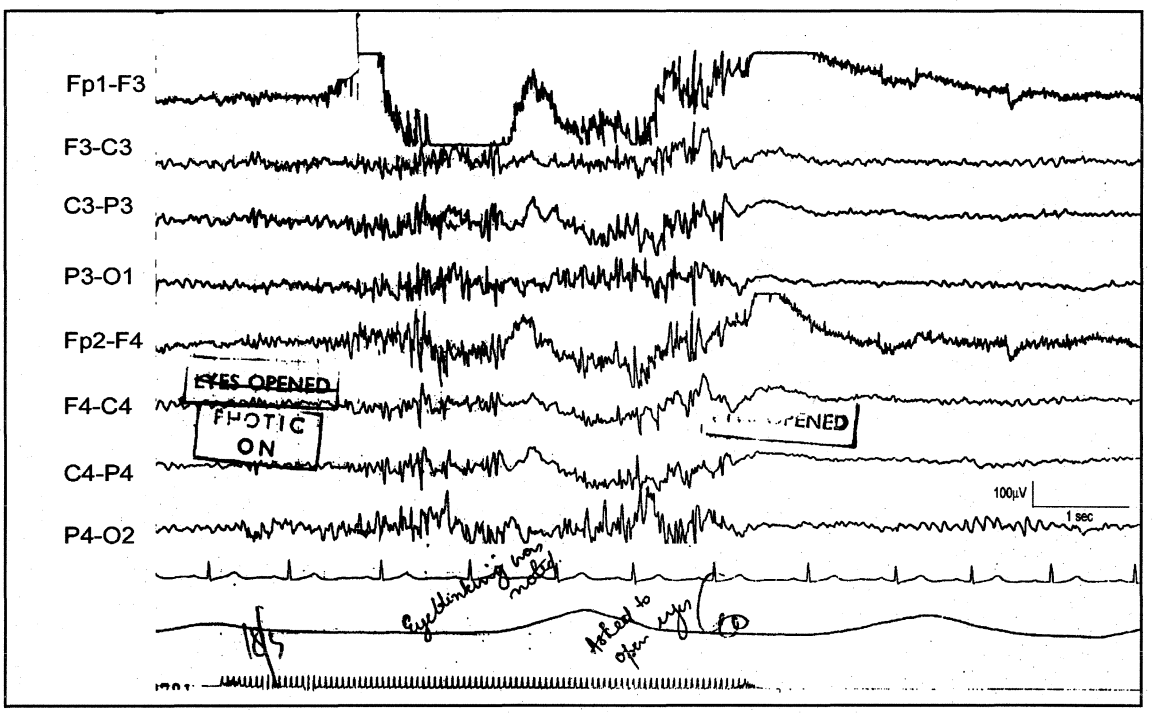
Fig 2.2 EEG of the same patient in Figure 2.1 during intemittent photic stimulation at 18 Hz showing generalized multiple-spike discharges with eye lid myoclonus.

1.2.3.6 Management

Management of the patient with JME should, not only include pharmacologic treatment of epileptic seizures, but also life style modifications to alleviate the precipitating factors. Patients with JME should be made aware of the precipitating factors such as alcohol intake, sleep deprivation and excessive fatigue. Those with known photosensitivity should avoid flickering lights or such stimuli. A regular sleep cycle, providing 6 to 8 hours of night sleep should be maintained. Staying late at night and waking up in the middle of the night should be avoided.

1.2.3.6.1 Diagnosis

The involvement of minor components of seizure like absences and myoclonus are not easily elicited from the patients. Unilateral myoclonus, misinterpretation of



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myoclonic jerks as simple partial seizures, focal EEG asymmetries and failure to elicit history of myoclonus makes JME a highly underdiagnosed disease in spite of its well differentiating characteristics. The rate of misdiagnosis may vary from 25% to 90% (Grünewald *et al.,* 1992; Vazquez *et al.,* 1993; Kleveland and Engelsen, 1998; Murthy, 1999; Gunatilake and Seneviratne, 2000).

1.2.3.6.2 Differential diagnosis

JME should be differentially diagnosed from the progressive myoclonus epilepsies, other primary generalized epilepsies such as myoclonic absences, epilepsy with grand mal seizures on awakening, and partial epilepsies with focal motor seizures. JME, childhood absence epilepsy (CAE), and epilepsy with grand mal on awakening may occur rarely in the same patient at different times in life. A non-progressive epilepsy syndrome, JME should be easily distinguished from the rare progressive myoclonus epilepsies because of the lack of progressive neurologic deterioration, dementia, and ataxia (ILAE, 1989). However, at disease onset, when intelligence and neurologic status are still normal, the clinical picture of progressive myoclonic epilepsy may resemble that of JME. Conversely, patients with JME who are inappropriately treated and intoxicated with antiepileptic drugs may mimic the symptoms and signs of the progressive myoclonus epilepsies (Berkovic *et al.,* 1986). JME should also be distinguished from myoclonic absences, a very rare form of epilepsy that develops in early or middle childhood (Tassinari *et al.,* 1969). Lasting from 10 to 60 seconds, the attacks are frequent and involve rhythmic, bilateral, prominent myoclonias of shoulders, arms, and legs on a background of arrested movement and staring. A majority of the patients have an altered intellectual function and respond poorly to antiepileptic medication (Tassinari *et al.,* 1969). Epilepsy with

grand mal seizures on awakening is another related IGE syndrome (ILAE, 1989). GTCS most often occur shortly after awakening from sleep. Myoclonic seizures are not noted, even though the seizures start in the young age groups or in the second decade of life.

1.2.3.6.3 Pharmacologic treatment

The antiepileptic drug (AED) of choice in JME is sodium valproate (Janz and Durner, 1997). Given as monotherapy, 80 to 95% of patients become seizure free (Janz and Durner, 1997). Among the newer AEDs, lamotrigine is equally beneficial (Janz and Durner, 1997). In uncontrolled studies, clonazepam as a co-medication has been reported to be effective against myoclonic jerks, but showed no beneficial effect on GTCS (Janz and Durner, 1997). Seizures invariably return after valproate is withdrawn and a lifetime treatment is usually required.

There is also a small group of AED resistant JME patients (Gelisse *et al.,* 2001). In a recent study from North India, while JME patients with photoparoxysmal response were uniformly responsive to sodium valproate, those with a combination of all the three seizure types (myoclonus, absences and GTCS) were poor responders (Jain *et al.,* 2003).

Some of the commonly used AEDs can exacerbate the seizures in JME. Carbamazepine appears to have the strongest aggravating potential, whereas the aggravating effect of phenytoin is less prominent (Genton *et al.,* 2000). Aggravation was mostly in the form of increased myoclonic jerks (Genton *et al.,* 2000).

1.2.3.6.4 Follow-up

Patients with JME require a careful long-term follow-up in order to ensure compliance to medication and to exclude progressive myoclonic epilepsy syndromes. One

of the common causes of relapse of seizures in a well-controlled JME patient is inadvertent reduction or stoppage of AED. Females in the reproductive age group on treatment with sodium valproate also require counseling regarding marriage and pregnancy in view of the potential teratogenic effects of valproate. Patients also require advice regarding the need to maintain an optimum sleep pattern and abstinence from excessive use of alcohol.

1.2.3.6.5 Prognosis

JME carries an excellent prognosis for seizure control in the majority of patients. However, remission even in the later age groups appears unlikely necessitating life long AED treatment (Janz and Durner, 1997). Experience to date has shown that relapse occurs in 75% to 100% of patients if AEDs are discontinued, even after many years of seizure control (Janz, 1985; Shinnar and Berg, 1994).

2. Genetics of epilepsies

2.1 Clinical genetics

Genetic causes contribute to a diversity of human epilepsies. There are over 200 inherited disorders listed in the Online Mendelian Inheritance in Man catalogue (McKusick, 1998), where seizures occur in association with other neurological symptoms and signs. A few of those with simple inheritance, where mutations are described are listed in Table 2.1. While rare forms of idiopathic epilepsy follow a Mendelian inheritance pattern, genetics of a majority of human epilepsies are complex. Considerable advances in the understanding of genetics of human epilepsies have occurred in the past 10 years through clinical and molecular genetic studies.

Table 2.2. Symptomatic epilepsies with Mendelian inheritance

Gene	Chromosome	MIM	Mode	Type of mutant alleles	Clinical syndrome
GABRG2 $GABA_A$ receptor	5q31	604233	AD	Missense	GEFSC3
SCN2A sodiumchannel alphasubunit	2q24	604233	AD	Missense	GEFSC
SCN1A sodiumchannel alphasubunit	2q24	604233	AD AD	Missense null,missense	GEFSC2 SMEI
CHRNB2 acetylcholine recepter beta subunit	1p21	605375	AD	Missense	ADNFLE3
SCN1B sodiumchannel beta1subunit	19q13	604233	AD	Missense	GEFSC1
KCNQ2 potassium channel	20q13	602235	AD	Missense,null	BFNC1(EBN1)
KCNQ3 potassium channel	8q24	121201	AD	Missense	BFNC2(EBN2)
CHRNA4 acetylcholine receptor α	20q13	600513	AD	Missense	ADNFLE1

AD, autosomal dominant; GEFSC, generalized epilepsy with febrile seizures plus; SMEI, severe myoclonic epilepsy of infancy; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; BFNC, benign familial neonatal convulsions

2.1.1 Family history of epilepsy

In both clinical practice and genetic research, information about seizures in family members is usually obtained indirectly. Most early studies of the genetics of epilepsy were devoted to demonstrating familial aggregation and an increased risk for epilepsy in relatives of the affected persons compared with the general population. One of the best works on the familial incidence of seizures has been from the Rochester-Olmsted County Record Linkage Project (Annegers *et al.,* 1982; Ottman *et al.,* 1988; Ottman *et al.,* 1991; Ottman *et al.,* 1996). In these studies, the standardized morbidity ratios (SMRs) for unprovoked seizures in relatives of individuals with idiopathic, childhood-onset epilepsy were 2.5 in siblings and 6.7 in offspring. There was no evidence for unprovoked seizures in more distant relatives. Evidence of familial aggregation has only limited utility in evaluating genetic hypotheses. Clustering can also occur by environmental exposures and shared behavioural practices.

Variability in the clinical presentation of seizures among family members has been reported from many geographical locations and ethnicities. Family studies of 68 JME probands from California revealed 50% of all families reported seizures in first- or second-degree relatives (Delgado-Escueta *et al.,* 1989). Twelve percent of all family members other than the proband had epileptic seizures. Eighty percent of symptomatic siblings and 6% of asymptomatic siblings had diffuse 4- to 6-Hz multispike-wave complexes in their EEG. Twelve percent of asymptomatic parents

had diffuse, nonspecific slow waves mixed with spikes or sharp waves (Delgado-Escueta *et al.,* 1989). From Saudi Arabia (Obeid and Panayiotopoulos, 1988), there was a high positive family history of epilepsy (48.7%) and a high prevalence (10.7%) of other forms of epilepsy in family members of JME probands. Of the 60 JME patients studied from Italy, 33% had a positive family history of epilepsy (Canevini *et al.,* 1992). Fifty-six percent of the cases showed family history of epilepsy and/or febrile convulsions in a 68 patient series from Spain (Salas Puig *et al.,* 1994). Family history of seizures was positive in 23.6% of JME patients in a series from South India (Murthy *et al.,* 1998). Thirty-five percent of first degree relatives (Jain *et al.,* 1998b) and 24.2% of first and second degree relatives (Mehndiratta and Aggarwal, 2002) were afflicted with seizures in studies from North India.

2.1.2 Twin studies

The classical twin study, which uses monozygotic (MZ) and dizygotic (DZ) twins reared together from birth to adulthood, has been a popular research design utilized in the search for genetic influences on many traits and disorders. Within families, two individuals are more likely to have the same syndrome if they have a closer genetic relationship than if they are more distantly related (Harvald and Hauge, 1965; Italian League Against Epilepsy Genetic Collaborative Group, 1993). The studies of twins in idiopathic epilepsies have shown that affected MZ twins share more of the same sub-syndrome than DZ twins (Rosanoff *et al.,* 1934; Lennox, 1953; Oradovskaia, 1970; de Morsier, 1971). Epileptologists were quick to spot that there was a greater

risk of idiopathic forms in the patients than the general population. Risk for epilepsy was not increased among relatives of probands with postnatal symptomatic epilepsy (Ottman *et al.,* 1996; Callenbach *et al.,* 1998). Studies of families with multiple affected individuals show that most affected individuals have IGEs or febrile seizures. Children with febrile seizures were more likely to have a first-degree or a second-higher-degree relative with febrile seizures and less likely to have CAE and absence seizures compared with children without febrile seizures. This was especially true for simple febrile seizures (Berg *et al.,* 1999).

A study involving 358 twins showed an overwhelming concordance in MZ twins for epilepsy syndromes, suggesting that there may be syndrome specific genetic determinants (Berkovic *et al.,* 1998). However twin birth by itself is not a risk factor for epilepsy (Berkovic *et al.,* 1993; Jain *et al.,* 1998a) suggesting that results from genetic analysis using twins are applicable to the non-twin population. The Finnish Twin Cohort study suggests that 8% to 27% of the incidence of epileptic seizures is related to genetic variability (Sillanpaa *et al.,* 1991). A similar Danish twin study (Kjeldsen *et al.,* 2001) has dissected the probable genetic and environmental determinants; 70 and 88% of the liability to develop epilepsy being accounted for by genetic factors in the younger and older cohorts, respectively. Individual specific environmental factors explained the remaining 30 and 12%, respectively (Kjeldsen *et al.,* 2001). There are wide fluctuations in the concordance rates described in most of the twin studies cited above. Possible reasons for this disparity may be ascertainment bias in referral setup versus community based twin registries, and the

lack of well defined epilepsy classifications in those early studies which were conducted before the ILAE guidelines on classification of seizures and epilepsies were published.

2.1.3 Segregation analysis

The benefit of approaching an epileptic disorder within the background of its genetic risk is to elucidate which epilepsies have a high relative risk and which do not. This can be a marker for differential diagnosis. A better scrutiny of the mode of inheritance can aid in the clinical differentiation of various rare epileptic syndromes (Greenberg *et al.,* 1988a; Berkovic *et al.,* 1996; Jimenez *et al.,* 1996). This is done by segregation analysis.

Various modes of inheritance have been proposed for JME using data from different populations. Earlier reports were favoring AD inheritance (Weissbecker *et al.,* 1991; Minassian *et al.,* 1995; Sander *et al.,* 1997). Segregation analysis of 48 families ascertained through JME probands favored autosomal dominant transmission with incomplete penetrance and sporadic cases of heterogeneity in JME (Greenberg *et al.,* 1988a). Three definitions for the affected-ness status were used. In the first, all family members with a history of clinical generalized seizures or an abnormal EEG were classified as affected. In the second analysis, individuals with non-specific EEGs were classified as not affected and in the final analysis, only individuals with a history of seizures were considered affected. Febrile seizures were not included in this analysis. An AR mode of inheritance was

proposed by the examination of consanguineous marriages in the Saudi Arabian JME families (Panayiotopoulos and Obeid, 1989; Panayiotopoulos *et al.,* 1994).

Recent studies have emphasised a more complex mode of inheritance for JME involving perhaps an oligogenic or multifactorial inheritance model (Leppert *et al.,* 1993; Greenberg 1992; Haug *et al.,* 2000b).

2.2 Molecular genetics

The last one-decade has seen an explosion of molecular genetic advances aided by the rapid progress in mapping the human genome and finally culminating in the Human Genome Project, which sought to sequence the entire human genome. The project was finished on June 26, 2000. This has influenced every field of medicine, including epilepsy. At present there is a much better understanding of the molecular mechanisms of a few types of epilepsy. A number of epilepsy genes have been characterized, mutations uncovered and in many of the more complex ones, loci linked to the phenotypes have been discovered (Table. 2.3, page 26).

Table 2.3 Idiopathic human epilepsies with genetic determinants

Disorder/Syndrome	OMIM	Mode of inheritance	Chromosomal loci	Putative mechanism
Generalized epilepsies (idiopathic and symptomatic)				
JME	254770	Complex	6p21.1-p11	Genetic and locus heterogeneity
			15q14	CHRNA7?
BFNC (EBN1)	121200	AD	20q13.3	*KCNQ2*
BFNC (EBN2)	121201		8q24	*KCNQ3*
Northern epilepsy syndrome	600143	AR	8pter-p22	Cathepsin B? gene 3, human GKAP homolog
CAE	600131	Complex, age dependent penetrance	8q24	GABA(A) receptor gamma2-subunit? jerky gene? kainate sensitive Glu-receptor (GRIK1)
IGE	600669	Multilocus	Unknown	
Generalized 3 Hz spike and waves			8q24	Syntenic with *stargazer* mouse chromosome 15 (stg locus)
			? 15q11.2-q12	? linkage to GABA(A) α_5, β_3, γ_5 subunit cluster on chromosome 15
GEFS+	121210	AD		
FEB1	602476		8q	
FEB2	602477		19p 2q 19q13.1	SCN1B
Localization related (partial) epilepsies	600512	AD	10q22-q24	Glutamate dehydrogenase (GLUD-1)
	600513	AD	20q13.2-13.3	CHRNA4
	6032304		15q24	?
BECTS	117100	Complex	15q14	CHRNA7
BIFC	601764	AD	9q11-13	?
			16q	
PME of Unvericcht Lundborg (EJM1)	254800	AR	21q22.3	Unstable microsattelite expansion, Cystatin B
Dentatorubro pallidoluysian atropy (DRPLA)	125370	AD	12p13.31	(>49) of $(CAG)_n$ leads to an altered gene product "atrophin"; may alter glycolytic enzyme GAPDH
PMR of lafora epilepsy	254780	AR	6q24	Tyrosine phosphatase (laforin)
Myoclonic epilepsy with ragged red fibers MERRF)	545000	Mitochondrial tRNA mutations, tRNA(lys)		Respiratory chain enzyme deficiencies
Gaucher's disease type III	231000	AR	1q21	Glucocerebroside deficiency
Sialidosis I & II	256550	AR	6p21.3	α-Neuraminidase deficiency
Neuronal ceroid lipofuscinoses (CLN1)	256730	AR	1p32	Lysosymal enzyme palmitoyl protein thioesterase
CLN2, CLN5	204500	AR	11p15	Pepstatin-insensitive lysosomal
Batten disease (CLN3)	204200	AR	9q34	Hamartin, ? tumor suppressor?

For Abbreviations, see page - xii

2.2.1 Molecular markers

The application of molecular tools in the study of inherited human diseases has brought about a remarkable transformation in the way these diseases are now understood. The spectacular progress that has happened in hereditary human diseases is largely due to the availability of polymorphisms in the human genome (Botstein and Donis-Keller, 1984). DNA polymorphisms are variations in base sequence that have no apparent functional significance, but can be used as genetic markers for linkage analysis (Botstein and Donis-Keller, 1984). Human geneticists follow the transmission patterns of various diseases through generations using neutral DNA segments called 'markers'. These markers have many different forms called 'alleles' whose inheritance can be traced across generations. The first markers used for human genetic mapping were blood group antigens. These were far few and most of them were not informative. The next generation of markers were the HLA antigenic markers, which were again few and localized only to chromosome six (Race and Sanger, 1975). Restriction fragment length polymorphisms (RFLP) (Kan and Dozy, 1978; Botstein *et al.*, 1980; Donis-Keller *et al.*, 1987) which are enzyme based restriction sites on DNA were used as the next generation of markers. Their numbers were again only in a few hundreds. Polymorphic DNA markers amenable to amplification by polymerase chain reaction (PCR) were used in the late 1980s and they heralded a new era in human genome mapping (Weber and May, 1989). A comprehensive Gènèthon human linkage map was published in 1996 (Dib *et al.*, 1996). Polymorphic DNA

markers had a high heterozygosity and wider distribution along the genome when compared to the HLA markers. The usefulness of DNA markers is thus directly related to the informativeness of human DNA polymorphisms. This remarkable paradigm has allowed successful localization of aberrant genes responsible for a number of neurological disorders like Duchenne muscular dystrophy (Burmeister and Lehrach, 1986), Huntington's disease (1993b), Alzheimer's disease (Goate *et al.*, 1991; Sherrington *et al.*, 1995), Fragile X syndrome (Oberle *et al.*, 1991), and hyperkalemic periodic paralysis (Bulman, 1997) to name a few. The newer generation of single nucleotide polymorphisms (SNP) has provided an even denser reference map for dissecting complex human diseases such as diabetes, asthma, stroke, migraines and epilepsies (Nowotny *et al.*, 2001).

2.3 Channelopathies

Epilepsy is a disorder of altered neuronal excitability. Since ion channels underlie the fundamental physiological process of the excitable membrane, mutations in ion channels have been sought diligently in paroxysmal neurological disorders including epilepsies (Steinlein, 2002; Mulley *et al.*, 2003). Many of the neurological diseases with channelopathy as the etiological basis manifest with paroxysmal symptoms such as periodic paralysis, hemiplegic migraine (Terwindt *et al.*, 1998) and episodic ataxias (Gordon, 1998). Epileptic conditions that have been established as channelopathies include, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (Steinlein *et al.*, 1995b; Hirose *et al.*, 1999), BFNC (Biervert *et al.*, 1998; Charlier *et al.*, 1998; Singh *et al.*, 1998), generalized epilepsy

with febrile seizures plus (GEFS+) (Wallace *et al.*, 1998; Sugawara *et al.*, 2001; Wallace *et al.*, 2001).

2.3.1 Voltage-gated potassium channels

Voltage-gated potassium (K_v) channels control action-potential waveforms and neuronal firing patterns by opening and closing in response to membrane-potential changes (Yi *et al.*, 2001). They are involved in the regulation of fast repolarizing phase of action potentials (Connor and Stevens, 1971) and delayed spiking. They are prominent in nervous system, acting in delicate and accurate ways to control or modify many physiological and pathological functions including membrane excitability, neurotransmitter release, cell proliferation or degeneration, signal transduction in neuronal network (Jin and Wang, 2002). In excitable cells they serve the crucial function of repolarizing the membrane to its resting voltage after an action potential and maintaining resting membrane potential near equilibrium potential for K^+ ions (Choe, 2002). Reducing the activity of potassium channels in the nerve fiber delays action potential repolarization and lowers the amount of excitation needed to produce action potentials (Cooper and Jan, 1999). K^+ channel mutations with these effects underlie hereditary forms of myokymia. Several human diseases like cardiac arrhythmias, deafness, diabetes, and epilepsy are known to be due to disruption of K^+ channels (Cooper and Jan, 1999). Two such genes *KCNQ2* (Singh *et al.*, 1998) and *KCNQ3* (Charlier *et al.*, 1998) are mutated in rare idiopathic epilepsy called BFNC. The channels resulting from *KCNQ2* and *KCNQ3* co-expression exhibit gating properties

and linopirdine sensitivity similar to those of neuronal M-channels (Wang *et al.*, 1998). The M-channel is a slowly activating and deactivating K^+ conductance that plays a critical role in determining the sub threshold electroexcitability of neurons as well as the responsiveness to synaptic inputs (Wang *et al.*, 1998). *In situ* hybridization and Northern blotting has shown that *KCNQ2* and *KCNQ3* mRNA are expressed in overlapping patterns in brain and sympathetic ganglia (Cooper *et al.*, 2000). These data suggest that *KCNQ2* and *KCNQ3* co-assemble *in vivo* to form the M-channel (Wang *et al.*, 1998). Disease-causing missense mutations in *KCNQ2* and *KCNQ3* are responsible for only modest reductions (20-30%) in current magnitude (Schroeder *et al.*, 1998).

2.3.2 Calcium-activated potassium channels

Calcium-activated potassium channels perform fundamentally important functions for excitable cells in the CNS and peripheral tissues (Schumacher and Adelman, 2002). Activated by elevated levels of intracellular Ca^{++} such as that occur, following an action potential, hyperpolarizing mechanism of K^+ channels modulates the time between action potential spikes within a burst (Vergara *et al.*, 1998). This gives rise to spike-frequency signatures where their accumulated activity results in a hyperpolarization that is deep and long lasting, prohibiting a subsequent action potential (Bond *et al.*, 1999). Thus, the regulation of interspike interval and spike-frequency adaptation by these channels shape the basic pattern of neuronal

communication (Schumacher and Adelman, 2002). The phenomenon of anticipation in genetic diseases involving trinucleotide repeat expansions (CAG/CTG) is of great concern to geneticists and clinicians (Timchenko and Caskey, 1999; Parniewski and Staczek, 2002). Unstable dynamic DNA repeats expansions have been reported in the etiology of Huntington's disease, spinal and bulbar muscular atrophy, spinocerebellar ataxia 1 (SCA1), Machado-Joseph disease and many others (Robitaille *et al.*, 1997; Yamada *et al.*, 2000). Based on its physiological and expression features, such a potassium channel gene *hSKCa3*, has generated considerable interest for its possible involvement in neurological and neuropsychiatric disorders (Chandy *et al.*, 1998; Dror *et al.*, 1999; Saleem *et al.*, 2000).

The calcium-activated potassium channels are gated by intracellular Ca^{++} ions and their activity is responsible in part for the afterpolarization that follows a single action potential or a train of action potentials in the neurons (Bond *et al.*, 1999). According to their single-channel conductance in symmetrical potassium solutions, these channels are classified as big (BK), intermediate (IK), or small (SK) (Latorre *et al.*, 1989). The neuronal small conductance calcium-activated potassium channel gene (*hSKCa3*, chromosomal location 1q21*)* plays a critical role in determining the firing pattern of neurons through the generation of slow after-hyperpolarization and regulation of intracellular Ca^{++} signals by binding with calmodulin (Fanger *et al.*, 1999). *In situ* hybridization demonstrates abundant *hSKCa3* transcripts within the human hippocampus, amygdala, substantia nigra and ventral tegmental area and along the

dopaminergic neurons from these regions into the nigrostriatal and mesolimbic pathways (Dror *et al.*, 1999). Lower levels are also seen in the cortex and cerebellum. *In situ* hybridization revealed that mRNAs encoding the SK family subunits are widely expressed in the brain and show distinct but overlapping patterns (Dror *et al.*, 1999). *hSKCa3* encodes a 731 amino acid protein comprising two polyglutamine arrays in its N-terminus of which the second polyglutamine repeat is highly polymorphic (Chandy *et al.*, 1998) These physiological and anatomical attributes make *hSKCa3* an interesting candidate gene for investigation in IGE syndrome such as JME..

2.4 Genetic analysis

For most single gene disorders very little is known about the biochemical events underlying the disorders that there are no obvious candidate genes. This is true for heritable disorders such as Huntington's disease and idiopathic epilepsies.

DNA polymorphisms, which are present throughout the human genome, can be found nearer the genetic loci that may be predisposing towards the disorder (Weber and May, 1989). If the polymorphisms are very close to the gene responsible for the disease of interest, then crossing over or recombination between the two is less likely to occur than if the two were unlinked. Consequently, within families, in which different forms of the polymorphism exist, individuals with the disease will be more likely than the unaffected individuals to inherit a particular form of polymorphism (Weiss, 1993). Thus, one form of a linked polymorphism and the disease causing

allele will be transmitted together during meiosis. In Mendelian terms, they will "co-segregate". This co-segregation forms the basis of identifying a chromosomal location of a disease-causing gene, when the location of the linked polymorphism is known (Weiss, 1993).

2.4.1 Genetic linkage

The basic purpose of a linkage study is to determine whether a marker and locus responsible for a genetic disease segregate as if they are physically linked, i.e., if the probability of recombination between them is $\theta < ½$, or whether, on the contrary, they segregate independently with $\theta = ½$. Linkage mapping is thus dependent on the offspring haplotypes produced by various parental mating types via recombination (Weiss, 1993). In linkage analysis the probability that the observed associations are caused by linkage is calculated and then, the probability that the observations arose by chance (no linkage) is calculated. The ratio of these two probabilities is expressed as logarithm (base 10) of odds for linkage (LOD) (Ott, 1999). Usually, LOD in each family is calculated and the LOD scores are added together. If the mode of inheritance is known for a particular disease, a combined LOD score of 3 (log of base 10), for which the odds are 1000 to 1 in favor of linkage, is generally accepted as proof of linkage (Ott, 1999). A LOD score of -2 is considered proof that two loci are not linked.

2.4.1.1 Parametric linkage analysis

Parametric linkage analysis is performed when the genetic parameters of the disease such as mode of inheritance and penetrance are known. For most rare Mendelian disorders, parametric linkage can be used (Chakravarthi and Lynn, 1999). Parametric linkage analysis is powerful than linkage methodologies that do not use a genetic model. Traditionally, in human genetics, linkage analysis has been carried out as a sequence of pairwise (two point or also called as singlepoint mapping [SPT) comparisons between a trait locus and each of a number of marker loci (Ott, 1999). For each comparison, trait versus the *i*th marker, LOD scores are computed and combined over families and investigators. For a single-gene two allele model, the full parametric model would include the frequency *a*, of the trait causing allele, as well as pentrances f_0, f_1, f_2 for non carriers, heterozygote carriers, and homozygote cariers. This model can be extrapolated to include many alleles. In such a case, for a single gene model with *h* alleles with frequencies a_1, a_2, ...a_h, respectively and penetrance f_{ij} for an individual whose genotype consists of the *i*th and *j*th alleles or multigene versions of the above model where the loci are unlinked and effects are additive across loci. A likelihood approach is then used to evaluate the strength of evidence of linkage (H_1) relative to the evidence for no linkage (H_0) by calculating the following likelihood ratio (LR) and corresponding Log Odds (LOD) score (Ott 1999):

LR = Proability (Data | H_1))/ Probability (Data| H_0)

LOD score = $\log_{10}$ (LR)

The power of linkage tests is greatly enhanced if several markers are tried simultaneously in the same family data (Ott, 1999). In cases when the parameters of the model are not known, they are sometimes impossible to estimate, and the maximum likelihood analysis had been found to be very sensitive to model misspecifications (Clerget-Darpoux *et al.,* 1986). Another problem arises when marker data are missing for some family members. In this case, linkage analysis also depends on marker allele frequencies; misspecification of these frequencies can affect both the power and robustness of the method (Ott, 1999). Computation of LOD scores is often mathematically intensive and thus a variety of computer programs have been developed for calculating linkage statistics (Ott, 1999). A LOD score less than –2.0 may be viewed as evidence that the region under consideration does not contain a disease susceptibility gene (exclusion). If the LOD falls between –2 and 3.0, additional families must be integrated into the data set before a determination can be made. Linkage is declared if the LOD score exceeds the critical threshold of 3.0 (Lander and Kruglyak, 1995)

2.4.1.2 Non-parametric linkage analysis

In some complex traits, an appropriate mode of inheritance is unknown. Allele sharing methods have been proposed as a way to avoid the above-mentioned difficulties. For this reason, NPL analysis methods have been introduced, as they do not require specification of an inheritance model (Fishman *et al.*, 1978). This class of methods includes the sib pair method, originated by Penrose (1935) and developed by many

others Suarez (1978); Hodge (1984) Lange (1986); Fimmers *et al.,* (1989). The objective is to evaluate departures from the null hypothesis that the markers are not near a disease gene. Under the null hypothesis, Mendel's laws give the probabilities that a set of relatives exhibits a particular allele-sharing pattern, and the null hypothesis is rejected if the extent of allele sharing among affected relatives exceeds Mendelian expectation (Morton, 1996). These methods are clearly not model free, but they are believed to be more robust than full parametric likelihood analysis in those cases where the model is not known. Because the rationale for allele-sharing methods is intuitively plausible and easily grasped, geneticists have used these methods for more than 30 years, well before the advent of the large sets of polymorphic markers that have made linkage analysis so fruitful today (Shih and Whittemore, 2001). Here, a test statistic for allele sharing is calculated and the linkage results are better understandable in P values (Chakravarthi and Lynn, 1999). NPL analyses study the distribution of marker alleles inherited from a same ancestor, i.e., alleles identical by descent (IBD) (Campbell and Elston, 1971; Elston and Stewart, 1971), in persons from the same family (e.g., siblings), whereas association studies examine the distribution of a given marker allele (Hirschhorn *et al.*, 2002). Allele sharing models commonly used are S_{pairs} and S_{all}. S_{pairs} (Week and Lange 1988; Flimmers *et al.*, 1989; Whittemore and Halpern 1994; Kruglyak *et al.* 1996) counts for each pair of affected relatives, the number of alleles they share, and then sums that over all pairs of affected relatives. For a pair of relatives with respective IBD genotypes (*I, j*) and

(*k, l*) the number of alleles they share is calculated as $\delta(l, k) + \delta(i, l), + \delta(j,l)$, where $\delta(x, y) = 1$ if $x=y$, 0 otherwise. S_{all} scoring function (Whittemore and Halpern 1994) is described as follows:

Consider a vector of length *m*, where *m* is the number of affecteds, whose *i*th component is one of the two alleles of the *i*th person at the given location. There are 2^m such possible vectors ω. For each ω, let $h(\omega) = \prod_{j=1}^{\#\text{alleles}} gj.!$, where *gj* is the number of times allele *j* occurs in ω, i.e $h(\omega)$ is the number of permutation that preserve ω. Define $S_{all} = 1/2^m \times \Sigma\omega\varepsilon\Omega\, h(\omega)$. The value assigned to a configuration by S_{all} increases with the number of people sharing the same allele.

Multiple marker testing and misspecification of marker allele frequencies are also common problems to the NPL methods (Ott, 1999). Each method has advantages and disadvantages; however, the two methods complement each other. The choice of a design for a particular study depends on several factors related to the phenotype (e.g., nature, frequency), population, accurate measurement of environmental factors, and known genetic background (Ott, 1999). Both methods have led to successful gene localizations and identifications in the analysis of several disease phenotypes.

2.4.1.2.1 Sib-pair analysis

The most commonly used NPL analysis is the affected sib-pair method (Penrose, 1953; Payami *et al.*, 1984; Morton, 1996). Two siblings can share 0, 1, or 2 parental IBD alleles of any locus, and the respective proportions of this sharing under random

segregation are simply 0.25, 0.5, and 0.25. For sibling pairs, the probability of sharing 2, 1, and 0 alleles (IBD), assuming that there is only a single locus are, $z_0=1/4\lambda_s$, $z_1=\lambda_0/2\lambda_s$ and $z_2=\lambda_M/4\lambda_s$ (Risch, 1990a), where λ_s, λ_0 and λ_M are the risk ratios for siblings, offspring and MZ twins of proband. When the phenotype under study is a clinical disease (affected/unaffected), the method tests whether affected sib-pairs share more parental alleles than expected under random segregation. This excess allele sharing can be tested by a simple chi-square, in particular when all parental marker data are known. Maximum likelihood methods have also been developed to analyze data from affected sib-pairs data, such as the maximum likelihood score (MLS) (Risch, 1990b) and a maximum likelihood binomial approach (Abel *et al.*, 1998), and can lead to more powerful tests. Haseman and Elston algorithm, which regresses the squared difference of the sib-pair phenotypic values on the expected proportion of alleles shared IBD by the sib-pair is one of the classic statistical approach to the sib-pair problem (Haseman and Elston, 1972). It must however be noted that under the null hypothesis (H_0); that a locus is not linked to a disease susceptibility gene, the statistical behaviour of the number of alleles IBD among individuals depend only on their relationships to each other, as determined by the pedigree structure and not on their disease status. For a locus that is linked to a disease susceptibility gene, there is expected to be an increase in the number of alleles IBD among the affected, relative to null expectation. So testing for linkage becomes a test for excess allele sharing. Many recent studies have used other methods not detailed here

(Holmans, 1998). Some of these methods are implemented in popular packages, such as MAPMAKER/SIBS (Kruglyak and Lander, 1995) and ALLEGRO (Gudbjartsson *et al.*, 2000) which also allow SPT and MPT analysis of sib-pair data.

2.4.2 Genetic association

As with other epidemiological studies, the design and analysis of a study including genetic polymorphisms generally involve relating a particular disease or health outcome to a particular exposure or genetic trait, while assessing the presence of systematic error, controlling random error and assessing effect modification (interaction) with other exposures or traits traits (Boffetta and Pearce, 1999; Schulze and McMahon, 2002). In studies of determinants and mechanisms of disease, markers of genetic polymorphism are generally treated either as exposure variables or as effect modifiers.

2.4.2.1 Case control association

The study may use cases (patients) of the disease (or condition) under investigation and a control group sampled from the study base that generated the cases (Boffetta and Pearce, 1999). Their major methodological problem is the selection of controls, which may be an important source of bias (Daly and Day, 2001). Another important limitation of many available studies is the small sample size, which may be inadequate for assessing statistical interaction (Daly and Day, 2001).

2.4.2.2 Family based association study

Spielman, McGinnis, and Ewens (1993) developed a family-based statistical test of linkage for the situation where a population association is already known to exist. The test is called transmission disequilibrium test (TDT). Family based association study designs circumvent the problem of population stratification by using the parents or siblings (Curtis, 1997) as controls, eliminating underlying population stratification (Zhao *et al.*, 1997). Multiple testing is involved when the marker used has many alleles, and corrections for multiple testing is essential to guard against false positives (Lazzeroni and Lange, 1998). The TDT differs from IBD methods or parametric linkage methods because the TDT evaluates departures from random assortment of alleles across families, whereas the other methods evaluate departures from random assortment of alleles within families. In other words, the TDT focuses on linkage between a specific marker allele and the disease allele; the other linkage tests focus on linkage between a specific marker locus and the disease locus (McGinnis, 1998).

2.5 Genetic linkage analysis of JME

JME is genetically the maximally explored human epilepsy syndrome. Intensive research in different parts of the world for the last 15 years have given us information regarding underlying complexities in the genetics of JME. Nevertheless, no single gene or mutation has been consistently established as responsible for this common epilepsy syndrome.

2.5.1 The chromosome 6p locus (EJM1)

Due to the use of HLA markers in early linkage and association studies (Race and Sanger, 1975), the chromosome 6 was one of the earliest loci studied for most human genetic disorders.

2.5.1.1 Evidence for linkage to EJM1

Using serological markers available in the HLA locus, a Los Angeles based group (Greenberg *et al.*, 1988b) first identified a locus linked to Bf and human leukocyte antigen (HLA) designated EJM1. Linkage analysis with HLA-DQ RFLPs on 21 extended JME families (Durner *et al.*, 1991) obtained a highest LOD score of 3.9 (recombination fraction [θ]; $\theta_m = 0.01$, $\theta_f = 0.01$) assuming AD and 70% penetrance when family members with JME, absence epilepsy, or epilepsy with GTCS were considered as affected. The LOD increased to 4.1 when clinically normal family members with generalized spike-wave discharges in the EEG were classified as "affected" under AD, 90% penetrance (Durner *et al.*, 1991).

A subsequent study (Weissbecker *et al.*, 1991) confirmed this linkage assignment in 23 nuclear families, although at a larger recombination fraction. The affected status of relatives of the probands was assigned by four different clinical criteria, and separate analyses were done assuming an AD with 90% penetrance and AR with full penetrance. The maximum LOD score obtained was 3.11 at $\theta_m = 0.001$, $\theta_f = 0.20$, assuming AD. There was also support for linkage of a JME locus to

this region under the AR model, although the results varied depending upon the definition of the disease phenotype. These authors (Weissbecker *et al.*, 1991) did not find significant evidence for linkage heterogeneity.

The HLA-linked locus was investigated to test the robustness of the linkage results when the assumptions of mode of inheritance and penetrance were changed; and whether absence and clonic-tonic-clonic in JME families are influenced by the same gene locus as JME (Greenberg and Delgado-Escueta, 1993). It was found that linkage is stable within a wide range of assumptions of penetrance and mode of inheritance, and that the EEG traits seen in unaffected family members reflect the actions of the same gene that is involved in the expression of JME (Greenberg and Delgado-Escueta, 1993). The data also suggested that the same locus is responsible for non-JME forms of epilepsy seen in JME families, and that either different doses of the disease allele at the JME locus may lead to different epilepsy phenotypes or that another locus influences the final disease phenotype.

In a large family study (Liu *et al.*, 1995) involving 38 members of a four-generation LA-Belize family with classical JME but with no pyknoleptic absences; pairwise analysis tightly linked markers centromeric to HLA, namely D6S272 (peak LOD score [Z_{max}] = 3.564-3.560 at $\theta_{m=f}$ = 0-.001) and D6S257 (Z_{max} = 3.672-3.6667 at $\theta_{m=f}$ = 0-.001), spanning 7 cM, to convulsive seizures and EEG multispike wave complexes. Five living members had JME; four clinically asymptomatic members had EEG multispike wave complexes (Liu *et al.*, 1995). A recombination between

D6S276 and D6S273 in one affected member placed the JME locus within or below HLA. Pairwise, multipoint, and recombination analyses in this large family independently showed that a JME gene is located on chromosome 6p, centromeric to HLA (Liu *et al.*, 1995). Seven additional multiplex pedigrees with classic JME were screened with the same chromosome 6p21.2-p11 polymorphic markers. When LOD scores for small multiplex families were added to LOD scores of the LA-Belize pedigree, Z_{max} values for D6S294 and D6S257 are > 7 ($\theta_{m=f}$ = 0.000) indicating that chromosome 6p21.2-p11 locus is linked to a phenotype consisting of classic JME with convulsions and/or EEG rapid multispike wave complexes.

2.5.1.2 Evidence for locus heterogeneity at EJM1

Subsequently evidence for locus heterogeneity at the 6p locus was revealed for JME mixed with pyknoleptic absences (Liu *et al.*, 1996). The JME locus was further refined to be outside the HLA loci between markers D6S272 and D6S257 (Z_{max} = 4.442). A single recombination event between D6S276 and D6S273 helped place the JME locus within or below (centromeric to) HLA. Admixture test in the small multiplex families suggested linkage with heterogeneity where α=0.5 for D6S294 and D6S272 (Liu *et al.*, 1995; Liu *et al.*, 1996; Serratosa *et al.*, 1996).

Analysis of small multiplex families from Germany (Sander *et al.*, 1997) and Austria showed that JME locus is located 10cM below HLA. Haplotype analyses revealed key recombinations in five families, which locate EJM1 to the centromeric side of the HLA-DQ locus. This study refined a candidate region of 10.1 cM in the

chromosomal region 6p21 between the flanking loci HLA-DQ and D6S1019 (Sander *et al.*, 1997).

A subsequent study (Greenberg *et al.*, 2000) sought to partition the linkage phenomenon on independent θ_m and θ_f. Linkage analysis $\theta_m = \theta_f$ showed evidence for linkage (LOD score 2.5), but at a higher θ, suggesting heterogeneity. When linkage analysis was redone to allow independent θ_m and θ_f, the LOD score was significantly higher (4.2) at a θ_m, θ_f =.5, 0.01 suggesting that a maternal inheritance component may predominate (Greenberg *et al.*, 2000).

Thus there are two loci separated by 10 cM on chromosome 6p that are linked to JME and EEG spike and wave trait.

2.5.1.3 Evidence against linkage to EJM1

Linkage analysis in a third set of 25 families including a patient with JME and at least one first-degree relative with IGE showed no significant evidence in favour of linkage (Whitehouse *et al.*, 1993). Pairwise and MPT linkage analysis was carried out assuming AD and AR inheritance and age-dependent high or low penetrance. MPT analysis generated significant linkage exclusion (LOD score < -2.0) at HLA and for a region 10-30 cM telomeric to HLA, the extent of which varied with the level of penetrance assumed (Whitehouse *et al.*, 1993). These observations indicate that genetic heterogeneity exists within this epilepsy phenotype.

Nineteen families recruited from United Kingdom, Greece, Spain and Denmark when tested for seven polymorphic DNA markers on chromosome 6p did

not give any evidence for linkage (Elmslie *et al.*, 1996). The families were selected using a JME proband and atleast one first degree and two second degree relatives with JME. The region was excluded (LOD score < -2) by using multipoint analysis.

A study conducted to check the EPM1 locus of progressive myoclonic epilepsy (Unverricht-Lundborg disease) on those JME families which showed linkage exclusion to EJM1 showed clear linkage exclusion at EPM1 showing that JME and Unverricht-Lundborg disease were not allelic variants (Rees *et al.*, 1994).

Another study (Obach *et al.*, 2000) of seven Spanish families did not find evidence of linkage to 6p locus. In three of the families, linkage exclusion for different markers was seen (Obach *et al.*, 2000).

2.5.2 The chromosome 15q locus (EJM2)

2.5.2.1 Evidence for 15q locus (EJM2)

Neuronal nicotinic acetylcholine receptor (nAChR) gene *CHRNA4* was implicated in a rare idiopathic epilepsy ADFNLE (Steinlein *et al.*, 1995b). Chromosomal regions harboring genes for nAChR subunits were tested for linkage to the JME trait in 34 pedigrees (Elmslie *et al.*, 1997). Significant evidence for linkage with heterogeneity was found to polymorphic loci encompassing the region in which the gene encoding the α7 subunit of nAChR (*CHRNA7*) maps on chromosome 15q14 (HLOD = 4.4 at α = 0.65; Z_{all} = 2.94, P = 0.0005). This major locus EJM2 contributed to genetic susceptibility to JME in a majority of the families studied (Elmslie *et al.*, 1997).

2.5.2.2 Evidence against the 15q locus (EJM2)

An independent German sample set involving JME and CAE samples failed to replicate the linkage on 15q14 (Sander *et al.*, 1999c). They used MPT parametric and NPL analyses with seven microsatellite polymorphisms encompassing the region of the *CHRNA7*.

2.5.3 Linkage to chromosome 5q (*GABRA1*)

In a genome scan using 383 evenly distributed microsatellite markers in a large French Canadian (Cossette *et al.*, 2002) family, there was evidence of linkage to chromosome 5q34, with a maximum LOD score of 3.1 at θ=0 for marker D5S414. Fine mapping showed that the candidate region includes a cluster of $GABA_A$ receptor subunits-β2 (*GABRB2*), α1 (*GABRA1*) and γ2 (*GABRG2*) that are flanked by the markers D5S1955 and D5S422. Mutation in these GABA receptor genes was investigated using both single-stranded conformation polymorphism analysis and denaturing high-performance liquid chromatography for *GABRG2* (Cossette *et al.*, 2002). A variant in exon nine of *GABRA1* in all of the affected (n = 8) was detected, but none of the unaffected (n = 6), members of the family. Sequence analysis indicated that the affected individuals are heterozygous with respect to a C $\rightarrow$ A substitution, which is predicted to change a GCC (alanine) to a GAC (aspartic acid) codon at position 322 of the *GABRA1* cDNA (Cossette *et al.*, 2002). This alanine residue is located in the third transmembrane domain of the predicted protein and is conserved

in all α-subunits of $GABA_A$ receptors of different species. This Ala322Asp variation was not found in 400 control chromosomes of individuals of French Canadian origin, nor in an individual, who showed only an abnormal EEG or in people with sporadic IGE, which included individuals with JME (n = 31) and CAE (n = 52) (Cossette *et al.*, 2002).

2.5.4 Whole genome scans for IGE

In a European collaborative study (Sander *et al.*, 2000) that included 130 IGE-multiplex families ascertained through a proband with either an idiopathic absence epilepsy (IAE) or JME, and one or more siblings affected by an IGE trait, 413 microsatellite polymorphisms were genotyped in 617 family members (Sander *et al.*, 2000). NPT MPT linkage analysis, using the Genehunter program (Kruglyak *et al.*, 1996), provided significant evidence for a novel IGE susceptibility locus on chromosome 3q26 (Z_{NPL} = 4.19 at D3S3725; P = 0.000017) and suggestive evidence for two IGE loci on chromosome 14q23 (Z_{NPL} = 3.28 at D14S63; P = 0.000566), and chromosome 2q36 (Z_{NPL} = 2.98 at D2S1371; P = 0.000535) (Sander *et al.*, 2000). The linkage findings provide suggestive evidence that at least three genetic factors confer susceptibility to generalized seizures in a broad spectrum of IGE syndromes (Sander *et al.*, 2000). The chromosomal segments identified harbor several genes involved in the regulation of neuronal ion influx, which are plausible candidates for mutation screening.

Varying lines of evidence point to the involvement of several interacting genes in the etiology of IGE. A genome scan in 91 families (Durner *et al.*, 2001) ascertained through a proband with adolescent-onset IGE support an oligogenic model for IGE. The IGEs included JME, juvenile absence epilepsy (JAE), and epilepsy with generalized tonic clonic seizures (EGTCS). The linkage results show strong evidence for a locus common to most IGEs on chromosome 18 (LOD score 4.4/5.2 MPT/ SPT) and other loci that may influence specific seizure phenotypes for different IGEs (Durner *et al.*, 2001). Loci showing positive evidence included a previously identified locus on chromosome six (Greenberg *et al.*, 1988b) for JME (LOD score 2.5/4.2 MPT/SPT); a reported locus on chromosome eight (Zara *et al.*, 1995) influencing non-JME forms of IGE (LOD score 3.8/2.5 MPT/SPT), and, more tentatively, two newly discovered loci for absence seizures on chromosome five (LOD scores 3.8/ 2.8 and 3.4/1.9 MPT/SPT) (Durner *et al.*, 2001). It also suggests that the genetic classification of different forms of IGE is likely to cut across the clinical classification of these sub forms of IGE.

2.5.5 Genetic association studies and mutation screens for JME

The discovery of mutant genes coding for aberrant proteins in affected JME patients is the final proof of the genetic etiology in JME. In the absence of conclusive linkage evidence and locus and genetic heterogeneity, many investigators pursued promising candidate genes based on their physiological role, pharmacological importance, available animal models of epilepsy and sometimes on their genomic localizations on the putative JME loci.

The gene encoding the human GABABR1 receptor (*GABABR1*) maps to human chromosome 6p21.3 (Kaupmann *et al.*, 1998). Mutation screening of the entire coding region of *GABABR1* in 18 German patients with JME, who were linked to the EJM1 locus revealed several DNA sequence polymorphisms (Peters *et al.*, 1998), two of which result in amino acid changes occurring in all IGE-affected members of two families. However, clinically unaffected relatives carried the same variations, excluding these amino acid substitutions as the cause for IGE in these families (Peters *et al.*, 1998). Genetic association analysis too did not find any allelic association of the *GABABR1* sequence variants with either JME or IAE, ($P > 0.18$) (Sander *et al.*, 1999a).

2.6 Current status of molecular genetics data on JME

Mutations in the *CACN1A* play a causative role in the epileptogenesis of absence seizures in *tottering* mutant mice (Barclay and Rees, 1999). An expressed polymorphic CAG trinucleotide repeat in the 3' end of *CACN1A4* was assessed in 70 German (Sander *et al.*, 1998) families ascertained through members with CAE, JAE, or JME. There was no evidence of genetic variants of *CACN1A4* in the causative role in the pathogenesis of common subtypes of IGE in humans (Sander *et al.*, 1998).

The *weaver* mice phenotype, characterized by ataxia, tremor, male infertility, and tonic-clonic seizures, is caused by a point mutation in the inwardly rectifier K^+ channel gene *KCNJ6* (*GIRK2*) (Liao *et al.*, 1996). Mutation screening of *KCNJ6/*

KCNJ3 heteromeric receptors did not find any mutations that may be involved in the etiology of JME (Hallmann *et al.*, 2000).

Mutational analyses of *CLIC5, KIAA0057* and *GCLC* genes near to the EJM1 region did not provide any mutations co-segregating with JME phenotype, and suggested that they may not be the EJM1 gene (Suzuki *et al.*, 2002). Mutation screens for *CHRNA7* did not yield a genotypic correlate for EJM2 (Taske *et al.*, 2002).

2.7 Problems and pitfalls in the molecular genetic analysis of JME

In the absence of clear etiologies, the science of dissection of complex phenotypes present unique challenges, which are at times overwhelming with the limited tools available to human geneticists.

2.7.1 Inadequate phenotypic characterization of JME syndrome

JME is a benign epilepsy syndrome with its most important characteristic being the presence of myoclonic jerks without loss of consciousness. However, as described previously, eliciting the history of myoclonus in a clinical examination is difficult. This results in an invariable delay in diagnosis. JME may also coexist with other epilepsy syndromes and then a clear phenotypic characterization becomes even more difficult.

2.7.2 Lumpers and splitters

Traditionally, linkage and association analysis have depended on robust sample sizes. This meant collection of patient biological samples over an extended period of time

for a statistically powerful analysis. There are two schools of thought on the characterization of epileptic syndromes. The first school advocates lumping together idiopathic epilepsies of different clinical presentations together to find a common trait-defining gene. The seizure components (absence, myoclonus, GTCS) of all the major IGE syndromes are common with considerable overlap (Reutens and Berkovic, 1995). The second school of thought advocates, splitting the different epilepsies on syndromic classification (ILAE, 1989) and thus reduces clinical heterogeneity that may interfere with robust analysis scores. In fact, much of the controversy on gene mapping of complex disorders like JME has focused on the definition of the "affectedness" status.

2.7.3 Phenotypic heterogeneity in JME

With the increasing familiarity of JME among clinicians, clinical characterization of JME has been well documented. Recent studies from various centres however add a caveat to the usual description of a homogeneous entity. Occurrence of JME with other generalized seizure patterns in siblings and offsprings and occurrence of EEG spike and waves in asymptomatic members of large families and twin pairs shows that there is considerable phenotypic variability in this syndrome (Reutens and Berkovic, 1995). Several reports point to the presence of a sub group of patients who exhibit only myoclonic jerks as the seizure type (Jain *et al.*, 1997; Jain *et al.*, 2003). Perhaps, JME can again be re-classified into 'sub-syndromes' based on

seizure types as patients with or without pyknoleptic absences or GTCS; based on EEG profile as those with and without photic sensitivity and photoparoxysmal responses; on the basis of AED response as monotherapy-responsive and non-responsive. This could be a reflection of the complex genetic inheritance pattern and phenotypic and genotypic variability that underlies this common epilepsy. Recognition of clinical 'subtypes' among JME could have therapeutic implications and help improve JME phenotypic characterization for molecular studies (Jain *et al.*, 2003).

2.7.4 Inclusion and exclusion of subjects with EEG trait alone

Investigators have used different yardsticks to define the "affectedness" status. The use of 3.5-6.0 Hz spike-wave or spike-wave complex EEG pattern as a clinical marker of affection has attracted much debate (Greenberg *et al.*, 1988b; Durner *et al.*, 1991; Weissbecker *et al.*, 1991; Whitehouse *et al.*, 1993; Serratosa *et al.*, 1996). Some authors argue that, since EEG 3.5-6.0 Hz polyspike-wave complexes are associated with myoclonic and grand mal seizures; their presence in asymptomatic family members may be significant (Serratosa *et al.*, 1996). 3.5-6.0 Hz polyspike-wave complexes occur in normal children and adolescents only at a frequency of 0.2% (Okubo *et al.*, 1994). Nevertheless, what is of contention is if this EEG trait can be grouped with the JME phenotype (Serratosa *et al.*, 1996). Similar irregularly formed "diffuse, bilateral synchronous bursts of 2.0-5.0-Hz slow waves with random, poorly

developed spikes between the slow waves" were found in 2.3% of awake EEGs (during rest, hyperventilation, or photic stimulation) and 7.9% of drowsy and sleep EEGs of normal children and adolescents (Eeg-Oloffson *et al.*, 1971).

2.7.5 Genetic heterogeneity

Results from different centres have not reached a consensus on the putative loci governing the JME gene. While, clinical heterogeneity, differences in ascertainment of samples and criteria for defining the affectedness status may be playing a large role in these discrepancies, one cannot rule out the genetic heterogeneity that may be prevalent in divergent and geographically distinct human populations. Genetic heterogeneity has been well documented even in rare monogenic epilepsies like BFNC (Leppert *et al.*, 1993; Lewis *et al.*, 1993; Steinlein *et al.*, 1995a) and ADFNLE (Mochi *et al.*, 1997; De Fusco *et al.*, 2000). It is only logical to presume that common complex variants of epilepsies like CAE and JME may show a greater level of heterogeneity (Durner *et al.*, 1999). GEFS+ is one of the best example of such heterogeneity (Scheffer and Berkovic, 1997; Baulac *et al.*, 1999; Moulard *et al.*, 1999; Lopes-Cendes *et al.*, 2000; Escayg *et al.*, 2001).

Both linkage analysis and case-control studies suffer in the case of locus heterogeneity; however, allelic heterogeneity affects case-control studies but has no affect on linkage analysis (Gulcher *et al.*, 2001). Population stratification also affects case-control studies much more than linkage studies (Gulcher *et al.*, 2001).

III. MATERIALS AND METHODS

3.1 Geographical area of the study and the organizations

3.1.1 Kerala population

The state of Kerala, from where a majority of the JME probands included in this study was ascertained, is situated in the southwest coast of the Indian peninsula. It is inhabited by over 30 million people, is distinguished from the rest of India by the high level of literacy and health awareness of its population. The intimate fusion of the Aryan and Dravidian cultures, as well as the welcome accorded to foreigners, who came to spread entirely different religious ideas, have helped to transform Kerala to a multi-religious state. Its present population consists of three major religious groups, namely Hindus (57%), Muslims (23%), and Christians (20%). Kerala, unlike other Indian states, enjoys an extensive health infrastructure and that is well distributed all over the State and thereby ensuring easy accessibility to urban as well as rural population.

3.1.2 South Indian population

Various anthropological analyses have documented extensive regional variation among populations on the subcontinent of India using morphological, protein, blood group, and nuclear DNA polymorphisms (Easteal *et al.*, 1989; Bamshad *et al.*, 1996; Das *et al.*, 2002). These patterns are the product of complex population structure (genetic drift, gene flow) and a population history noted for numerous branching events. Several genetic studies have been conducted on the South Indian populations and it has been found that the genetic distance between even the tribal populations is small, signifying a closer genetic relationship (Watkins *et al.*, 1999; Saha *et al.*, 2000). Populations belonging to the state of Maharashtra in western India (Konkanastha

Brahmins and Marathas) and from the state of Kerala (Nairs, Ezhavas and Muslims) in South India have shown a low level of gene differentiation (GST) for two loci, indicating a close relationship among the population groups (Das *et al.*, 2002). Genetic distance estimates using the gene frequency data indicate that the closest groups are the Nair and Izhava and the Brahmin and Nair (Saha *et al.*, 1976). Gene frequencies for the systems showing variation are within the range for other South Indian populations too (Rao *et al.*, 1978). In a new study, genetic variation at six tetranucleotide microsatellites (HUMTHO1, HUMVWA, F13A01, D3S1359, D12S66, and D12S67) has been determined in five endogamous ethnic population groups of India belonging to two major linguistic families (Ghosh *et al.*, 2003). The populations analyzed were Konkanastha Brahmins and Marathas (Maharashtra state) from the Indo-Aryan linguistic family and Nairs, Ezhavas, and Muslims (Kerala state) from the Dravidian family. All six loci show high gene diversity, ranging from 0.63 ± 0.04 to 0.84 ± 0.02 (Ghosh *et al.*, 2003). The average GST value observed was 1.7%, indicating that the differences between the populations account for less than 2% of the diversity, while the genetic variation is high within the five population groups studied (>98%). The phylogenetic tree fails to show any clear cluster. The absence of any cluster along with low average GST is suggestive of substantial genetic similarity among the studied populations, in spite of clear geographical, linguistic, and cultural barriers (Ghosh *et al.*, 2003). This similarity indicates either a greater gene flow between these groups or, alternatively, may reflect a recent evolution for them, considering that the Indian caste system evolved only about 3000 years ago (Ghosh *et al.*, 2003). Another study has also pointed out that the non-tribal South Indian population clusters closely with the main caste populations in North India (Kamboh, 1984).

3.1.3 Centres in South India

For molecular genetic studies, JME probands were recruited through neurology centers situated in the South Indian cities of Thiruvananthapuram, Bangalore, Chennai, Hyderabad, Kochi and Kozhikode. Each individual institution's internal review board approved the study, and all subjects provided informed consent to participate.

3.1.3.1 Sree Chitra Tirunal Institute for Medical Sciences and Technology

This study was undertaken at the R. Madhavan Nayar Center for Comprehensive Epilepsy Care attached to the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), a tertiary referral center situated at Trivandrum, the capital city of Kerala. The comprehensive epilepsy program receives patients referred from primary and secondary care facilities all over Kerala and neighboring states (Radhakrishnan *et al.*, 1998)

3.1.3.2 Jawaharlal Nehru Centre for Advanced Scientific Research

Molecular genetic analysis was done at the Human Genetics Laboratory, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore. The laboratory is equipped to handle medium scale genomic DNA extraction and PCRs. The laboratory has one Applied Biosystems ABI Prism 3100 genetic analyzer for fluorescent marker based genotyping and DNA sequencing. It has access to state of the art computational facilities of JNCASR.

3.1.4 Subjects for study

3.1.4.1 Patient selection

The patients were selected from the epilepsy clinic by the consultant neurologists with special interest in epilepsy; at least two of who independently examined the patients to verify the diagnosis. They were then interviewed and a detailed proforma was filled up, which included demographic features, family pedigree, and seizure

characteristics. Routine 16-channel scalp EEG was performed during wakefulness and sleep after the patients were partially deprived of sleep during the previous night. Intermittent photic stimulation was performed during EEG recording on all patients. Neuroimaging and prolonged video-EEG monitoring were undertaken only if specially indicated. Venous blood samples were collected from patients and family members after written informed consent.

For the clinical study, only JME patients who are residents of Kerala were included. One hundred eighty three JME patients were recruited based on the inclusion and exclusion criteria described in Table 3.1.

Table 3.1 Diagnostic criteria for JME

Inclusion criteria
(a) Characteristic repetitive myoclonic jerks involving the upper extremities, occurring after awakening, without loss of consciousness;
(b) Age at onset of myoclonic seizures between 8 and 25 years;
(c) Normal background activity and paroxysmal generalized spike and wave discharges in the EEG;
(d) Otherwise normal neurological status and intelligence.
Exclusion criteria
(a) Evidence of structural, metabolic or degenerative diseases of the brain
(b) Atonic/astatic or tonic seizures
(c) Stimulus-induced myoclonic jerks alone
(d) Partial seizures of any form
(e) Family history of progressive myoclonus epilepsy.

EEG -Electroencephalogram

The subjects utilized for genetic analysis were:

1. One large family from Kerala with 18 alive members, 5 of whom were diagnosed with JME and 5 members with febrile seizures or a generalized EEG epileptiform abnormality, which seemed amenable to both parametric and non-parametric linkage analysis
2. One hundred and nineteen probands sampled along with the parents forming a "trio"; a family unit ideal for TDT
3. Fifty multiplex families with atleast one affected sib pair, which fits for NPL study design
4. Two hundred and twenty two JME probands from mutually unrelated families used for case control association study for *hSKCa3*
5. Two hundred and forty eight control subjects of South Indian origin without family history of epilepsy, unexplained blackouts or other chronic neurological disorders.

3.2 Molecular genetic studies

The reagents used for DNA isolation and their preparation are provided in Appendix.1.

3.2.1 Isolation of genomic DNA from venous blood

1) 10 ml of whole blood was mixed with 30ml of cold NKM buffer. Vortexed and centrifuged for 30 min at 6000rpm, 4°C.
2) Removed all but ~10ml supernatant. Added 30ml of cold re-suspension buffer and re-suspended the pellet by vortexing to break up any clumps. Centrifuged for 30 min at 6000rpm, 4°C.
3) Removed all but ~4ml supernatant. Added 0.5ml 10x TEN solution, 0.025ml of 2mg/ml Proteinase K, 0.5ml of 10%SDS and mixed gently and digested overnight at 37°C or 2 hrs at 65°C.
4) Added 5ml of buffered Phenol, vortexed. centrifuged for 20 min at 5000rpm at room temperature. Separated the aqueous phase and discarded the organic phase.

5) To the aqueous phase, added equal volume of chloroform/ isoamyl alcohol (24:1); (centrifuged for 20 min at 3000rpm at room temperature, this step was repeated twice).
6) To the purified aqueous phase, added 5M NaCl such that final concentration became 0.4M. Added twice the volume of 100% Ethanol or equal volume of Isopropyl alcohol by the sides of the tube. Mildly mixed the contents so that the DNA is precipitated.
7) DNA is recovered directly by pippeting with a cut micropipette tip or by spooling out into the microfuge tube using a glass rod.
8) Washed the DNA pellet with 70% ethanol (500µl), centrifuged at 8000 rpm for 10 min at 4°C.
9) Air-dred and resuspended DNA in 200µl TE buffer. Kept the tube at 65°C for 10 min (to inactivate DNase) and left overnight at room temperature for DNA to dissolve completely.
10) Checked the purity (260/280nm) and yield (260nm) of DNA.
11) Stored DNA at –20°C

3.2.2 Genetic association analysis of candidate genes *KCNQ3* and *hSKCa3*

Emphasis on finding intragenic polymorphic markers for association studies led to final selection of two such potassium channel genes namely *KCNQ3* and *hSKCa3*.

3.2.2.1 Transmission disequilibrium test for *KCNQ3*

3.2.2.1.1 PCR and genotyping

A hypothesis was tested that *KCNQ3* predisposes to common forms of IGEs such as JME using TDT (Spielman *et al.*, 1993) in 119 JME probands using the parents as family based controls. The intragenic polymorphic (CA_n) marker D8S558 in intron 1 of *KCNQ3* was amplified by PCR using the primers AFM351zh1 (GDB:200088) 5'- TET-GGAACCACGCTTCGTTT; and AFM351zh1m 5'-GGGGCTTTAAGACCCAT.

PCR was done in 10μl consisting of 50ng of DNA, 20pmol of primers, 200mM of each dNTP, and PCR buffer with 1.5mM of $MgCl_2$ and 0.5 U of recombinant *Taq* DNA polymerase. Amplification was performed for 35 cycles in a Perkin-Elmer 9700 thermocycler (Applied Biosystems, CA, USA) with the following conditions: denaturation at 95°C for 30 sec, annealing at 57°C for 30 sec, and extension at 72°C for 40 sec. Initial denaturation and final extension times given were 5 minutes each. Genotyping was done on a ABI Prism 377 genetic analyzer (Applied Biosystems, CA, USA) using TAMRA dye set as reference standard (which was loaded in each lane for accurate sizing); allele sizes were counted using Genotyper™ version 1.1r8 (Applied Biosystems, CA, USA). Homozygous samples were sequenced on ABI Prism 3100 genetic analyzer (Applied Biosystems, CA, USA) using sequencing software version 3.7 to confirm identity and sequence of the DNA and also to assign correct numeric designation to the CA repeat alleles.

3.2.2.1.2 Transmission disequilibrium test for *KCNQ3* using TRANSMIT

Allelic association between trait and the marker was tested by using TRANSMIT version 2.5.2 (Clayton, 1999) software package that implements TDT. TRANSMIT tests for association between genetic marker and disease by examining the transmission of markers from parents to affected offspring. The main features that differ from other similar programs are that it can deal with transmission of multi-locus haplotypes, even if phase is unknown and parental genotypes may be unknown (Clayton, 1999). The tests are based on a score vector, which is averaged over all possible configurations of parental haplotypes and transmissions consistent with the observed data. The program produces the following asymptotic chi-squared tests (Clayton, 1999): It performs for each haplotype or allele, a test on 1-df for excess transmission of that haplotype and a global test for association on H-1 df, where H is the number of alleles for which transmission data are available. In the version used,

a bootstrap test procedure is implemented, which is more accurate than the chi-squared approximations (Clayton, 1999). This also avoids the need for Bonferroni correction for multiple testing. Power of TDT was calculated using a freely available program TDT Power Calculator (Chen and Deng, 2001). The parameters provided were disease allele frequency (0.0001), genotype penetrance (AA=0.8, Aa=0.2, aa=0.0001) and parameters of family structures, number of families (119), significance level (0.0001) and the number of simulations (10000).

3.2.3 Case control genetic association for *hSKCa3*

A case control study design was envisaged for studying the role of *hSKCa3* in etiology of JME. Two hundred and twenty two JME cases and adequately matched 248 South Indian controls formed the two sample populations. PCR mediated amplification of the second polyglutamine CAG tract of *hSKCa3* was carried out in all subjects. Each reaction comprised of 50 ng of genomic DNA, 20 pmol primers (*hSKCa3*-F: 5'-CAC CGT CAG TGT CAC CAG TAG TCC CC-3' and *hSKCa3*-R: 5'-Hex-GAA GGG GTT GCT GTC CCG CCG GT-3'), 200μM of each dNTP, 50 mM KCl, 10mM Tris-HCl (pH 8.3) 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase in 10μl final volume. 40 cycles of PCR were carried out; each cycle with denaturation at 94°C (40 sec), annealing at 52°C (40 sec) and extension at 72°C (45 sec) (Saleem *et al.*, 2000). Initial denaturation and final extension time were of 5 minutes each. Genotyping was performed using ABI 3100 genetic analyzer (Applied Biosystems, LA, USA) using GeneScan HD-400 (Applied Biosystems, LA, USA) as size standard mixed with each sample. Genotyping was done without the knowledge of affection status of individuals. Alleles were visualized using the Genescan version 3.7 (Applied

Biosystems, LA, USA). PCR product sizes between 374-419 bp were detected conforming to the CAG repeat size variation (10-25 copies). To ensure genotyping accuracy of these triplet repeats, the gene sequence and number of repeats present were confirmed in several homozygous samples by sequencing of the PCR amplified products.

3.2.3.1 Statistical analysis for case control association of *hSKCa3* using test of proportions and CLUMP

Test of proportions in controls and cases was used to check significance of each individual allele. The software CLUMP (Sham and Curtis, 1995) was also used to assess the overall significance of departure of the observed values from the expected values conditional on the marginal totals in a contingency table. CLUMP produces four χ^2 values termed T1, T2, T3 and T4 statistic and associated P values (Sham and Curtis, 1995). T1 is the chi-square associated with the original data input table. T2 is the χ^2 value of clumped table produced by lumping small-expected columns. Analyzing one column of the table against total of all other columns give T3. T4 is obtained by clumping the columns of the original table to maximize the chi-squared value. Monte Carlo simulation is used to assess significance and hence avoids multiple correction (Sham and Curtis, 1995). CLUMP is particularly useful in situations where the contingency table has many columns with sparse values, which is a common situation in genetic studies involving multiallelic markers (Sham and Curtis, 1995). Both the cases and control population were tested for Hardy-Weinberg equilibrium (HWE) to get a crude idea about genotyping errors or population stratification (Schaid and Jacobsen, 1999). The relative risks (RR) of the significant alleles were calculated by computing the ratio of presence and absence of each allele in the cases and controls. In order to find if there is any linear or nonlinear mathematical relation between the RR of each allele and the number of CAG repeats at the marker locus, regression analysis of the allele length versus relative risk was performed.

3.2.4 Parametric linkage analysis for the large Kerala family

3.2.4.1 Genetic analysis

High molecular weight genomic DNA was extracted by phenol/chloroform extraction followed by isopropanol precipitation (Sambrook *et al.*, 1989b). PCR amplification was done in 10μl reaction in a ABI 9700 thermal cycler as per the protocol for polymorphic DNA markers (Hudson *et al.*, 1995; Weissenbach *et al.*, 1992). The reverse primer was fluorescent labelled for tracking on an ABI 3100 genetic analyzer (Applied Biosystems, LA, USA). The PCR products of different size ranges and flourescent labels were pooled and loaded into the ABI 3100 genetic analyzer for genotyping. Size standard Genescan HD-400 was loaded with each sample for accurate sizing of the products.

3.2.4.2 Loci studied

Twelve polymorphic microsatellite markers covering chromosome six from 6p24 to 6q11 were typed. All markers and intermarker distances were from the Gènèthon collection (Dib *et al.*, 1996). The loci studied were D6S1678 -8.5cM - D6S1691-1.72 cM - D6S265-3.3 cM - D6S1568-5.0 cM - D6S1548-13.8 cM - D6S282-2.3 cM - D6S1650-3.56 cM - D6S452-1.16 cM - D6S465-4.9 cM - D6S294-2.0 cM - D6S1628-2.8 cM - D6S1619.

3.2.4.3 Linkage analysis

Power calculation using SLINK (Weeks *et al.*, 1990) was carried out on the assumption of AD model. Allele frequencies for all the 12 markers were determined by a special routine in ILINK as described by (Terwilliger and Ott, 1994). This was done because two founders were not genotyped (B4 and C20, Fig 3.1).

Assigning equal allele frequencies for the markers can results in inflated LOD scores and increased false positives. Frequency of the disease allele was estimated to be 0.0001. AD mode of inheritance with penetrance of 90% was used.

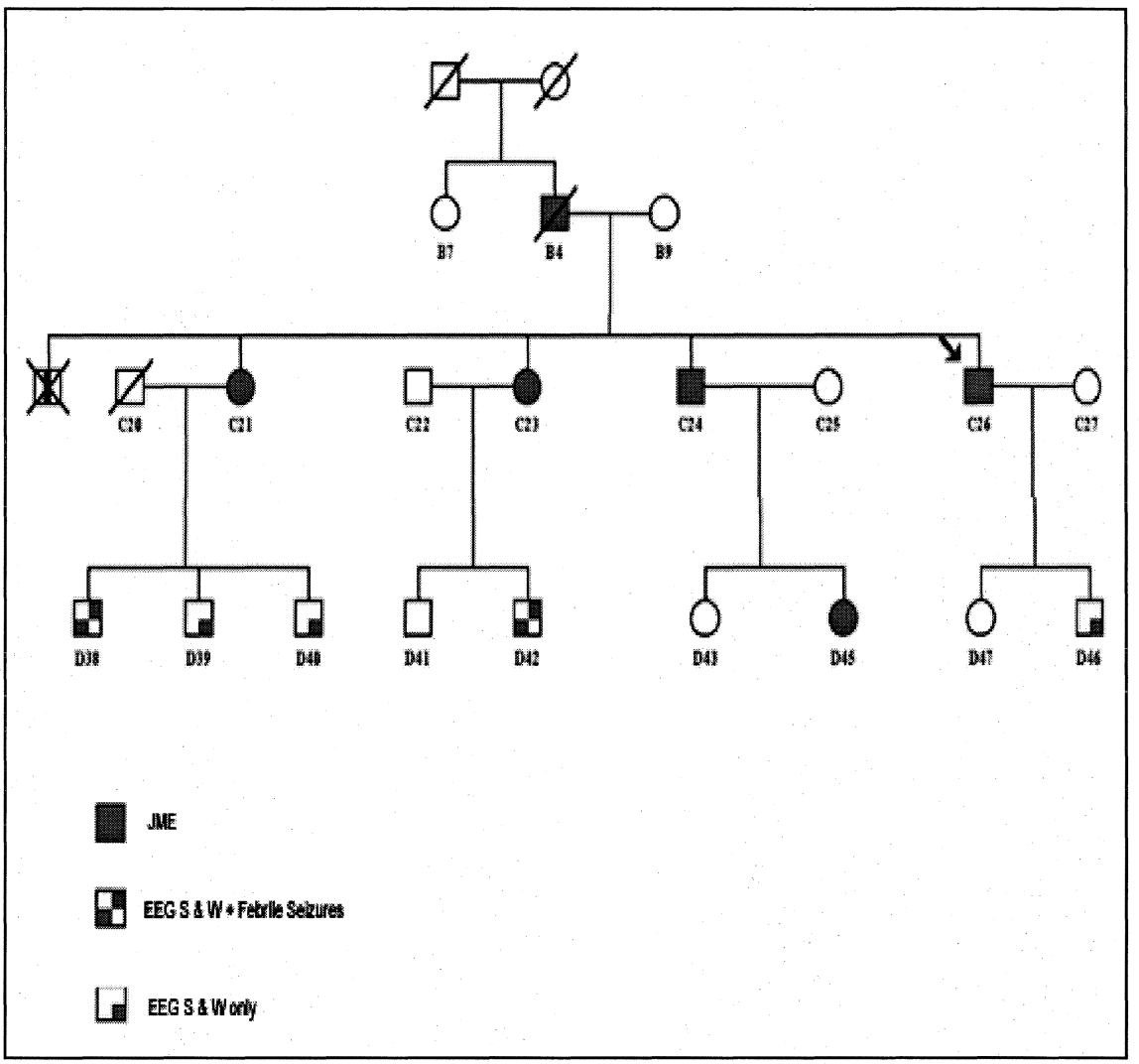

Fig 3.1 Pedigree chart of the large Kerala family with JME, febrile seizures and EEG spike and wave trait

Two point LOD score analyses was performed using the computer programs MLINK and ILINK through Linkage Control Program (LCP) version 5.1 (Lathrop and Ott, 1990). LOD scores were calculated at equal male: female recombination fractions ($\theta_{m=f}$) of 0.1, 0.2, 0.3, 0.4 and 0.5. Analysis was performed with both AD and AR mode of inheritance at various recombination fractions θ. Analysis was done under two affection models. In the narrow affection model (NAM), only members with JME were used. In the broad affection model (BAM), in addition to JME members,

individuals with fast spike and wave EEG abnormalities were also included. SPT and MPT linkage analysis were carried out using Genehunter version 2.1_r3 beta (Kruglyak *et al.*, 1996). Analysis was performed with both affection models (NAM and BAM). Genehunter counted the observed recombinations and the marker informativeness.

3.2.4.4 Haplotype analysis

Genehunter was also used to compute the probable haplotype of the deceased founder members B4 and C20 (Fig.4.4).

3.2.5 Non-parametric linkage analysis of sibpairs

3.2.5.1 Genotyping

Genomic DNA was isolated from 10ml of venous blood using standard phenol chloroform method (Sambrook *et al.*, 1989a). PCR amplification of the fluorescent labeled primers were done in 10μl reaction in a ABI 9700 thermal cycler (Applied Biosystems, LA) as per published guidelines (Hudson *et al.*, 1995; Weissenbach *et al.*, 1992). PCR products were pooled and genotyping was carried out on an ABI 3100 genetic analyzer (Applied Biosystems, LA). Allele sizes were detected using Genotyper software version 3.7 NT (Applied Biosystems, LA). Genotyping was done blinded to affection status, checked independently by two people.

3.2.5.2 Loci studied

Polymorphic CA repeat markers used were selected from the CEPH-Généthon reference marker map (Dib *et al.*, 1996). Twenty-two markers were selected to include region 6p22.3 (D6S1678, AFM329te9) to 6q12 (D6S1557, AFMa191zh5) at an average inter-marker distance of 2.35. Loci investigated were D6S1678 - 6.0 - D6S1663 -2.5 - D6S1691 -1.6 - D6S1621 - 0.06 - D6S464 - 0.06 - D6S265 -3.3 - D6S1568 -2.2 - D6S291 -2.8 - D6S1548 -1.3 - D6S1610 -12.5 - D6S282 - 2.3 - D6S1650 -3.5 - D6S1638 -0.06 - D6S452 -0.06 - D6S269 -1.1 - D6S465 - 4.9 -

D6S294 - 2.0 - D6S1628 - 0.9 - D6S402 -0.7 - D6S430 -1.2 - D6S1619 - 0.06 - D6S1557 encompassing a 49.2cM region on chromosome six.

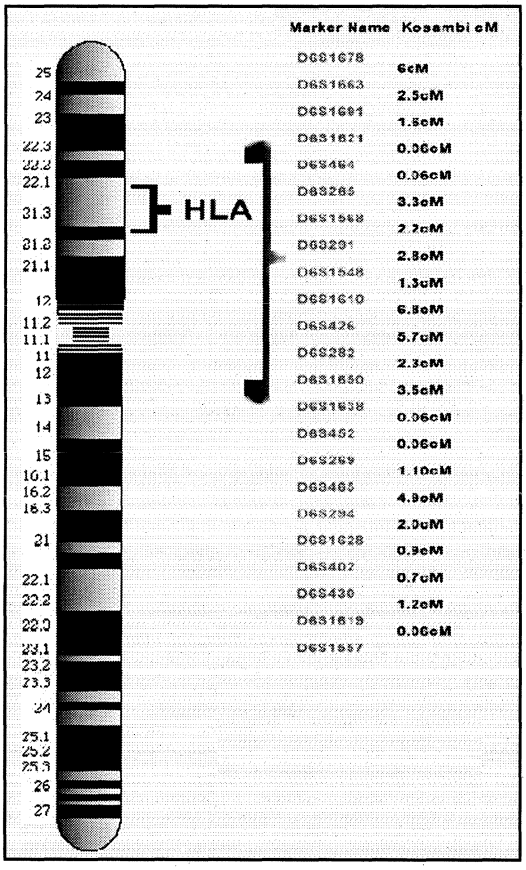

Figure 3.2 DNA markers used on the EJM1 region scan with the relative marker positions and intermarker distances in kosambi cM

3.2.5.3 Statistical analysis

Genotypes were checked for Mendelian consistencies and founder allele frequencies were inferred using TRANSMIT (Clayton, 1999). Statistical analysis was carried out using ALLEGRO (deCode Genetics, Iceland) computational package (Gudbjartsson *et al.*, 2000). ALLEGRO can perform both classical parametric linkage analysis and analysis based on allele sharing models. ALLEGRO offers the investigator the same scoring functions S_{pairs} and S_{all} (Whittemore and Halpern, 1994; Kruglyak *et al.*, 1996) as Genehunter, and adds additional scoring functions described by McPeek (1999).

S_{pairs} is simply the number of pairs of alleles from distinct affected pedigree members that are IBD. In comparison, S_{all} puts extra weight on three or more affected sharing the same allele IBD. Two basic types of analysis can be run namely single-point (SPT, also called two-point mapping) and multi-point (MPT). There are three allele-sharing models; parametric, exponential and linear to fit the data (Kong and Cox, 1997) and this is its biggest advantage compared to other multipoint analysis programs. The exponential model is quite useful if there are small numbers of pedigrees with excess allele sharing, while linear model is advantageous in case of missing data. In the allele-sharing model, posterior pair-wise IBD sharing probabilities are calculated based on the pedigree and genotype data. Prior probabilities are given by the pedigree within the constraints of Mendelian rules of inheritance. Two major features that ALLEGRO provide are the calculation of NPLEXACTP and LODEXACTP values for parity with Genehunter.

In SPT, the IBD distribution was estimated based on the marker genotypes for each marker taken individually. In MPT, a 0.06cM scanning increment was used to infer IBD distribution along the map used. The genetic distances used were from the Généthon map. Wherever the genetic distances on the Généthon reference map were given as zero, these distances were given as 0.06cM (map function = kosambi) to account for possible recombinants in the samples. A single liability class was provided which was utilized by ALLEGRO only in the parametric analysis mode and heterogeneity analysis. In parametric analysis, the liability class was varied to reflect

an AD or AR mode with penetrance varied from 50% to 90%. For extracting maximum information from families with more than two affected siblings, analysis was performed using S_{all} and S_{pairs} scoring functions. ALLEGRO was used to estimate the non-parametric linkage scores (Z_{NPL}) (Kruglyak *et al.*, 1996) and the exact P values associated with the NPL and Zlr scores, where Z_{lr} = sign $(\hat{d})\sqrt{(2\ln 10.LOD)}$. When the descent information is incomplete, which nearly is always the case, (due to presence of untyped members, low heterozygosity of markers and wide unequal spacing of markers) the NPL statistic has shown to be overtly and sometimes unacceptably conservative, and hence Z_{lr} score is chosen as a better alternative (Kong and Cox, 1997). As with most common complex diseases, the correct genetic model of JME is not known; model misspecification can lead to considerable loss of power and hence Z_{lr} scores are used in this analysis. Empirical significance testing of the observed scores was done by simulation in ALLEGRO (Liu *et al.*, 2001).

IV. RESULTS

4.1 Clinical characteristics of JME patients from Kerala

The demographic, clinical and EEG features observed in these patients are summarized in Table 4.1. To define the dispersion, mean ± standard deviation was used.

Table 4.1 Characteristics of 183 probands with JME from Kerala, South India

Attribute		
Gender		
Male	:	95 (51.9%)
Female	:	88 (48.1%)
Mean age ± standard deviation (year)		
At presentation	:	24.4 ± 7.18
Onset of absences	:	6.4 ± 7.93
Onset of myoclonic jerks	:	14.6 ± 3.6
Onset of GTCS	:	13.7 ± 3.93
Seizure type		
Absences	:	74 (40.4%)
Myoclonic seizures	:	183 (100%)
GTCS	:	170 (92.9%)
Precipitating factors		
Sleep deprivation	:	144 (78.7%)
Menstruation	:	7 (7.9% of women)
None	:	21 (11.5%)
EEG findings		
Generalized spike-wave discharges	:	114 (62.3%)
Photoparoxysmal response	:	18 (9.9%)
Lateralized/focal spikes only	:	34 (18.6%)
Normal EEG	:	21 (11.5%)

EEG – electroencephalogram; GTCS – generalized tonic clonic seizure

4.1.1 Demographic data

The mean age of 183 JME probands (95 males, 88 females) at presentation was 24.4 ± 7.2 years. The distribution of patients according to the age at presentation is shown in Fig. 4.1. At the time of recruitment, 54% of the patients were between the ages 20 and 30 years.

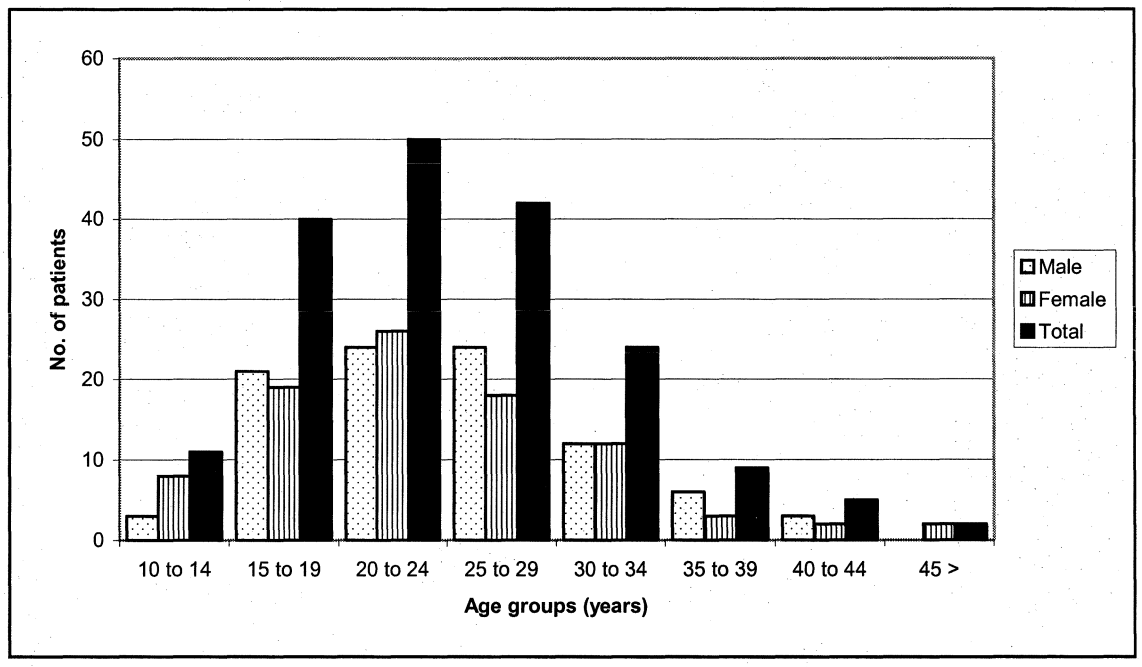

Fig 4.1 Distribution of patients according to the age at presentation

4.1.2 Seizure data

The seizure characteristics are provided in Table 4.1. All patients had myoclonic jerks, however, only in six (3.3%) of them, the referring doctors had elicited this history. One hundred and seventy (92.9%) patients had at least two episodes of GTCS at the time of recruitment. In 156 (85.3%) of them GTCS occurred in the early morning. An antecedent history of febrile seizures was present in 18 (9.8%) of the JME probands. Fig. 4.2 shows different seizure types and their combinations observed among these patients. Ten (5.5%) patients had myoclonic seizures alone. The mean age at onset of myoclonus in these patients was 15.4 ± 5.9 years and the mean delay between the onset of myoclonic jerks and diagnosis of JME was 9.9 ± 4.8 (median 10) years.

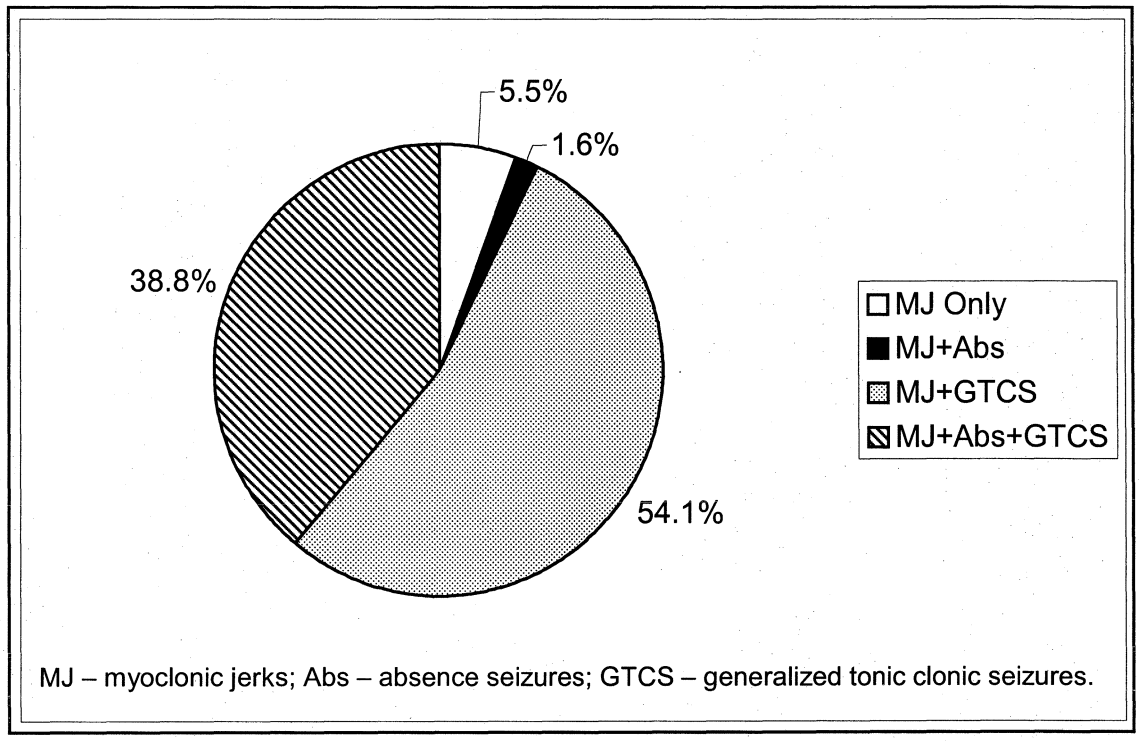

Fig 4.2 Distribution of patients according to seizure type(s) and their combinations

4.1.3 Precipitating factors

A majority of patients had their GTCS episodes related to sleep deprivation (Table 1). Poor AED compliance was responsible for GTCS in 38 (20.8%), watching television in 6 (3.3%) and alcohol in 3 (1.6%) patients.

4.1.4 EEG data

The EEG findings noted in these patients are summarised in Table 4.1. Interictal epileptiform discharges in the form of spikes, multiple spikes and wave discharges occurred in the scalp EEG recordings in 148 (80.9%) patients, which were generalized in 62.3% of patients, and lateralized or focal in 18.6%. Photoparoxysmal response to intermittent photic stimulation was elicited in 18 (9.9%) patients. One hundred and forty-six (79.8%) patients were receiving valproate at the time of the EEG study.

4.1.5 Pitfalls in the diagnosis

The diagnosis, when these patients were initially seen, is cited in Table 4.2. Although a majority of patients were diagnosed as having an epileptic seizure disorder, only six (3.3%) patients carried the diagnostic label of JME. The mean delay in diagnosing JME was 8.6 ± 7.0 years. The maximum delay from onset to diagnosis was 32 years. One hundred and thirteen (61.7%) patients had consulted at least one qualified neurologist prior to enrolment at the epilepsy clinic.

Table 4.2 Distribution of patients according to referral diagnoses

Diagnosis	n	%
Seizure disorder	154	84.1
Juvenile myoclonic epilepsy	6	3.3
Primary generalized seizures	2	1.1
Generalized seizures	5	2.7
Grand mal epilepsy	7	3.8
Absence seizures	1	0.6
Partial seizures	8	4.4
Total	183	100.0

4.1.6 Treatment and follow-up

At the time of referral, 61% of patients were on a combination of AEDs (Table 4.3), subsequently they were weaned off other AEDs, and optimised on sodium valproate alone (85%) or a combination of valproate with clonazepam. At referral, all the patients had experienced at least one GTCS in the preceding three months. At the time of

last follow-up, 98 (53.6%) patients were completely seizure-free for two years or more and 152 (83.1%) did not have more than two GTCS in the preceding year. Common causes for seizure recurrence were sleep deprivation and noncompliance to AED.

Table 4.3. Distribution of patients according to antiepileptic drug (AED) use at presentation

	n	%
Number of AED(s)		
None	2	1.1
1 AED	72	39.3
2 AEDs	69	37.7
3 AEDs	30	16.4
4 AEDs	10	5.5
AED		
Carbamazepine	69	37.7
Phenobarbitone	63	34.4
Sodium valproate	53	28.9
Phenytoin	46	25.1
Other	27	14.8

4.1.7 Family history

The distribution of seizures and epileptic syndromes among the 850 first-degree and 2013 second-degree relatives of the 183 JME probands are summarized in Table 4.4. There was a positive family history of seizures in 84 families (46%). First

and second-degree family members were affected in 53 (6.2%) and 45 (2.2%) of total members respectively. JME was found in 25.5% and other IGEs in 30.6% of the total affected first and second-degree relatives. The diagnosis was verified among affected relatives in 21 of the 25 with JME and 23 of the 30 with other IGEs.

Table 4.4 Prevalence of epilepsy syndromes among the relatives of 183 Kerala JME probands

Epilepsy syndrome	First degree affected		Second degree affected		Total	
	N	%	n	%	n	%
JME	20	37.7	5	11.1	25	25.5
Other idiopathic generalized epilepsies	18	34.0	12	26.7	30	30.6
Idiopathic localization related	0	0.0	2	4.4	2	2.0
Symptomatic localization related	0	0.0	1	2.2	1	1.0
Febrile seizures plus	5	9.4	6	13.3	11	11.2
Affected relatives not examined/ dead/ single seizures or neonatal seizures	10	18.9	19	42.2	29	29.6
Total affected relatives	53	100.0	45	100.0	98	100.0
Total relatives	850	6.2	2013	2.2	2863	3.4

4.2 Transmission disequilibrium between allele 20 of D8S558 and *KCNQ3*

4.2.1 Clinical characteristics of the patients

Clinical characteristics of the 119 JME patients selected for the TDT study are given in Table 4.5.

Table 4.5 Clinical characteristics of probands with JME used for *KCNQ3* TDT

Attribute		n (%)
Gender		
Males	:	48 (40.3)
Females	:	71 (59.7)
Mean age ± S.D (years)		
At presentation	:	23.33 ± 8.13
Average age of onset of absences	:	10.9 ± 5.9 (24.3%)
Average age of onset of myoclonus	:	14.5 ± 3.5 (100%)
Average age of onset of GTCS	:	14.7 ± 4.7 (86%)
EEG findings		
Generalized fast spike and wave	:	87 (73.1%)
Lateralized focal spikes	:	2 (1.68)
Normal	:	30 (25.22%)
Photoparoxysmal response	:	40 (33.6%)

EEG, Electroencephalogram; GTCS, generalized tonic-clonic seizure.
All probands were unrelated to each other. There was a positive family history of seizures in 28 cases (23.5%). Only one affected member was taken from each family.

4.2.2 Genotypic characteristics of the patients

Sixteen alleles were observed at the marker locus D8S558 ranging in length from 157bp to 193bp [$(CA)_n$; n=15 - 32]. CA_{19-23} accounted for more than 68% of all alleles. $(CA)_{16}$ and $(CA)_{31}$ were absent in the sampled individuals. The modal allele had $(CA)_{20}$ repeats. TRANSMIT analysis for 10,000 bootstrap samples were used to test for association, the P value was calculated for each of the allele (Table 4.6). The P-value obtained for $(CA)_{20}$ was 0.0076 (χ^2 =5.6685). Empirical power estimated with TDT Power Calculator was 99.96%, at simulated type I error of 0.0001.

Table 4.6. Results of TDT analysis for individual alleles of marker D8S558 using TRANSMIT

Alleles	Observed	Expected	Var(O-E)	ChiSq (1df)	P value
$(CA)_{17}$	7	10.16	5.019	1.986	0.168
$(CA)_{18}$	16	14.02	6.500	0.601	0.497
$(CA)_{19}$	18	26.05	11.502	5.625	0.014
$(CA)_{20}$	71	59.05	25.21	5.668	0.008
$(CA)_{21}$	51	50.08	21.007	0.039	0.837
$(CA)_{22}$	21	26.06	12.403	2.062	0.142
$(CA)_{23}$	17	16.53	8.251	0.027	0.906
$(CA)_{24}$	14	12.52	6.250	0.349	0.562
$(CA)_{25}$	5	4.51	2.250	0.108	0.826
$(CA)_{26}$	7	6.511	3.250	0.074	0.852
$(CA)_{27}$	7	6.01	3.000	0.326	0.602
$(CA)_{28}$	3	2.50	1.25	0.196	0.767
Agg*	1	4.01	1.5	6.0268	0.139

Alleles *shows alleles at the locus in increasing order of the number of (CA) repeats,* ***Observed*** *and* ***Expected*** *are the observed and expected transmissions,* ***Var (O-E)*** *is the variance of difference between observed and expected;* ***Chi-Sq (1df) is*** *the test statistic expressed as asymptotic chi-square with 1 degree of freedom and* ***P value*** *is computed using 10,000 bootstraps.* ***Agg**** *is the aggregate of rare alleles* $(CA)_{15}$, $(CA)_{29}$, $(CA)_{30}$ *and* $(CA)_{32}$ *which were present with frequency less than 0.01. This flag was used as a method to exclude rare alleles from the analysis.*

4.3 Statistical significance in transmission of *hSKCa3* alleles in JME

4.3.1 Clinical characteristics of the patients

The mean age-at-onset of the JME probands was 14.9±1.4 (average ±S.D) years. The triad of myoclonus, absences and GTCS was observed in 27.9%, the combination of myoclonus and GTCS in 64.7% and myoclonus alone in 7.4% of patients. Eighty-four percent of the patients were receiving treatment with sodium valproate. Scalp EEG during wakefulness and sleep exhibited generalized spikes, spike and wave or polyspike epileptiform abnormalities in about 70% of the patients. 30% were seizure free for two or more years and perhaps therefore, exhibited normal EEG. Clinical details of a major subset of these patients were recently described.

4.3.2 Genotypic characteristics of the patients

Sixteen distinct *hSKCa3* alleles were observed. Thirteen alleles were seen in the controls and 16 in the patients. Repeat size ranged from 10 to 25 (Figure 4.3). The modal repeat size was CAG_{18}. No unstable dynamic CAG triplet repeat expansions either in the controls or patients was observed. For the 10 common alleles, with frequency greater than 0.01, statistical tests were carried out to find out if one or more of these allelic variants show significant frequency differences in either the control (protective effect) or patient group (susceptibility effect). Z-test of proportions showed significance for three alleles: CAG_{16}, P=0.009; CAG_{18}, P= 0.004 and CAG_{19}, P=0.0000011. Allele CAG_{16} and CAG_{18} were present at higher frequencies in JME patients. Allele CAG_{19} was quite rare in JME cases but present at a high frequency

in the control group (Table 4.7). Bonferroni correction for 11 alleles was carried out to tackle the problem of multiple comparisons. CAG_{18} and CAG_{19} were significant after Bonferroni correction for 11 alleles at α=0.0045. While evidence for CAG_{18} is highly suggestive in nature; CAG_{16} was found not to be significant after employing Bonferroni correction. CAG_{19} was found to be significant.

Software CLUMP was used to investigate the overall significance of the allele frequencies of cases and controls in *hSKCa3* gene using CLUMP. Significance was obtained in T1 with 14 df, (P=0.000750), T2 with 9 df, (P=0.000051), T3 with 1 df, (P=0.000011) and T4 with 1 df, (P=0.000100) using Monte Carlo simulation for 10,000 times. T4 is the largest of the Pearson chi-square statistics of all possible 2 x 2 tables comparing any combination of alleles and is considered quite robust.

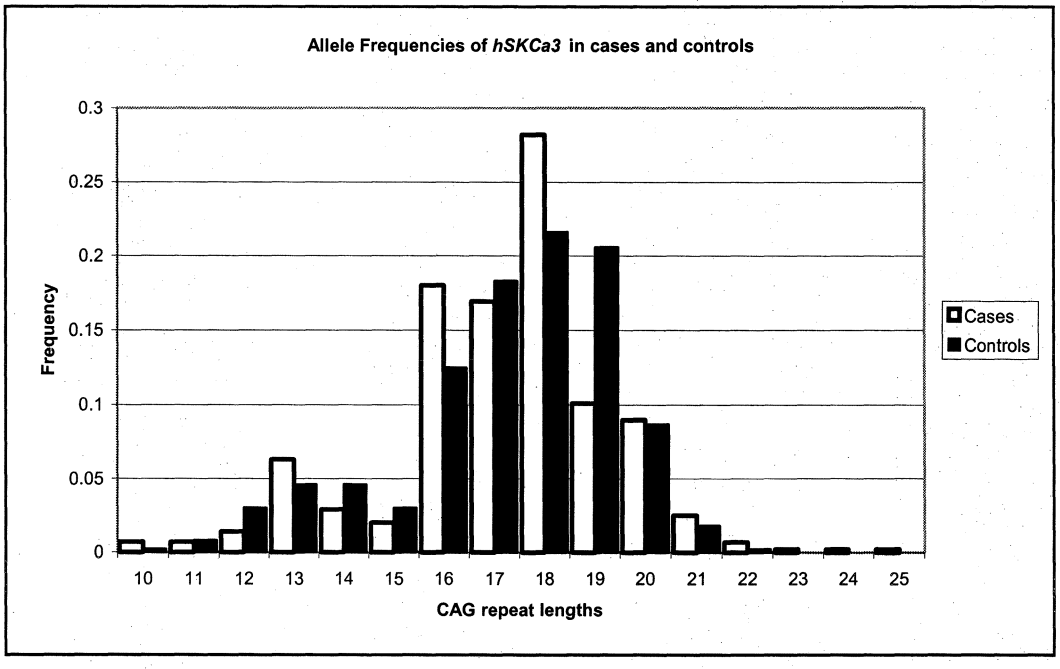

Fig 4.3 Allele sizes and frequencies of *hSKCa3* in cases and controls

Table 4.7: Allele-frequencies of *hSKCa3* and pair-wise Z tests of JME cases and controls

Alleles	Cases	Freq	Ctrls	freq	Z value	P value
CAG_{10}	3	0.007	1	0.002	1.117	0.264
CAG_{11}	3	0.007	4	0.008	-0.234	0.815
CAG_{12}	6	0.014	15	0.030	-1.753	0.080
CAG_{13}	28	0.063	23	0.046	1.162	0.245
CAG_{14}	13	0.029	23	0.046	-1.391	0.164
CAG_{15}	9	0.020	15	0.030	-0.981	0.327
CAG_{16}	80	0.180	62	0.125	2.601	0.009
CAG_{17}	75	0.169	91	0.183	-0.659	0.510
CAG_{18}	125	0.282	107	0.216	2.849	0.004
CAG_{19}	45	0.101	102	0.206	-4.869	0.0000011
CAG_{20}	40	0.090	43	0.087	0.193	0.847
CAG_{21}	11	0.025	9	0.018	0.711	0.477
CAG_{22}	3	0.007	1	0.002	1.117	0.264
CAG_{23}	1	0.002	0	0.000	1.058	0.290
CAG_{24}	1	0.002	0	0.000	1.058	0.290
CAG_{25}	1	0.002	0	0.000	1.058	0.290

CAG_n denotes the number of CAG repeats in the marker allele. Z value is the Z-test of proportions result for each allele.

As mentioned earlier, the alleles that were not included in analysis had very low frequencies in both populations and were unlikely to provide additional information in this test. RR of the common alleles (*f>0.01*) was found to be high (Table 4.8) for allele CAG_{18} (1.178) and minimum for allele CAG_{19} (0.514). Curve fitting using regression analysis of relative risk versus allele length variation of the most common alleles did not yield a statistically fit model.

Table 4.8 Relative risks (RR) for *hSKCa3* alleles computed using allele present vs. absent in cases and controls

Allele No:	Relative Risk#
$(CAG)_{12}$	0.560
$(CAG)_{13}$	1.047
$(CAG)_{14}$	0.705
$(CAG)_{15}$	0.739
$(CAG)_{16}$	1.198
$(CAG)_{17}$	0.854
$(CAG)_{18}$	1.178
$(CAG)_{19}$	0.514
$(CAG)_{20}$	0.956
$(CAG)_{21}$	1.105

If R1 = A/(A+B), R2 = C/(C+D), then R1/R2 is the relative risk of the allele where A = alleles present in cases, B is alleles present in controls, C=alleles not present in cases, D=alleles not present in controls.

4.4 Parametric analysis of a large family from Kerala

4.4.1 Clinical examination

C26: The proband was a 38-year-old male who developed myoclonus and GTCS at the 20 years. He has a history of febrile seizures at the age of seven years. The seizures were precipitated by sleep deprivation, alcohol consumption or by flashing lights. At referral, the patient was taking phenobarbitone 100mg and phenytoin 50mg daily. The neurological examination was unremarkable. An awake EEG recording showed a background activity of 9Hz over the posterior head region, which was symmetric and synchronous. Infrequent bursts of generalized frontally dominant IEDs

were seen at a frequency of 4Hz lasting around 1 sec. During photic stimulation at 6Hz, 11Hz, 15Hz and 25Hz there was jerking of the whole body and frontally dominant spike, polyspikes and waves discharges obscured by movement artifacts.

The family pedigree is given in Fig 4.4 and a detailed description of the affected members is given below. There was a strong family history of seizures in all the siblings alive (Fig 3.1). Both the patient's offsprings **D46** and **D47** were clinically normal, however, his son showed generalized activation of spike and wave disharges during EEG. The proband's father **B4** was reliably learned to have had myoclonic seizures until death. Mother **B9** was asymptomatic and normal.

The proband's eldest sister **C21** (46 years) had similar seizures starting at the age of 15 years. She also complained about absence attacks. The frequency of GTCS was once in six months precipitated by emotional stress or sleep deprivation. She was on similar medication as the proband at referral. EEG showed generalized atypical fast spike-wave discharges, which were maximum over both frontal regions. Generalized isolated spike and wave discharges were also seen in sleep. Photic stimulation did not produce any abnormality. Three of her sons (all older than 20 years) **D38**, **D39** and **D40** who were clinically asymptomatic, but exhibited generalized spike-wave discharges on EEG. **D38** also had a history of febrile seizures.

The proband's younger sister **C23** aged 42 years also had absences, myoclonus and GTCS from the age of 15 years. Her EEG showed abnormalities as described above, which were consistent with the diagnosis of JME. One of her two sons **D42** had a history of febrile seizures, but was seizure free during adolescence. His EEG showed frontally dominant generalized spike-wave discharges associated with myoclonus during sleep.

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The elder brother of the proband (40 years) **C24** had early morning myoclonus and GTCS from the age of 17 years. He was, however, seizure free from the age of 35 years. No precipitating factors were involved. His EEG shows characteristic generalized spike and wave discharges. His younger daughter **D45** was affected and had myoclonus and GTCS from the age of 12 years. In addition, antecedent history of febrile seizures was present at age five years. She was seizure free and off medications for four years, however, seizures recurred; and was currently taking 60mg phenobarbitone daily. Her EEG shows frontally dominant generalized atypical spike and wave discharges and photoparoxysmal response at 8Hz and 16Hz.

Phenotypically in this family with 18 live members, 5 had JME, 2 had a history of febrile seizures with generalized EEG abnormalities and 3 had generalized EEG abnormalities alone.

Two point LOD score analyses using MLINK shows no evidence of linkage to the chromosome 6p markers used in the study. Minor differences were noted when the penetrance was varied to 70 or 90%. A penetrance of 90% is presented here as a realistic scenario involving possible phenocopies. However except markers D6S1548 and D6S1619, all other markers showed exclusion LODs (<-2) at θ=0 with 90% AD under the NAM (Table. 4.9). When AR was used, marker D6S1548 gave a non-negative score (0.1627) at θ=0. The Z_{max} obtained from ILINK did not cross LOD of 0.5 at any $\theta_{m=f}$. LOD decreased further, when the BAM was used, however marker D6S265 showed a LOD of -1.92 and D6S1548 had LOD of 0.430 (Table. 4.10).

Table 4.9. Two point parametric linkage analysis: narrow affection model with only clinically verified JME

JME Only Kerala family linkage analysis using 12 markers by LCP							
Loci	**0**	**0.1**	**0.2**	**0.3**	**0.4**	**Z_{max} from ILINK**	**θ**
D6S1678	-2.577	-0.447	-0.040	0.094	0.096	0.108	0.35
D6S1691	-2.902	0.002	0.197	0.208	0.132	0.217	0.26
D6S265	-2.975	0.002	0.197	0.208	0.132	0.217	0.26
D6S1568	-2.975	-1.331	-0.595	-0.226	-0.045	0.001	0.49
D6S1548	0.430	0.307	0.191	0.092	0.024	0.430	0.00
D6S282	-3.716	-0.677	-0.269	-0.086	-0.010	0.002	0.47
D6S1650	-8.585	-1.170	-0.577	-0.282	-0.109	0.086	0.68
D6S452	-4.511	-0.752	-0.397	-0.209	-0.089	0.182	0.85
D6S465	-4.276	-1.170	-0.577	-0.282	-0.109	0.086	0.68
D6S294	-3.276	-0.872	-0.302	-0.054	0.031	0.034	0.42
D6S1628	-5.003	-1.146	-0.438	-0.127	-0.000	0.013	0.45
D6S1619	-0.433	-0.096	0.015	0.035	0.019	0.035	0.29

(LOD scores reported by MLINK at incremented recombination fractions (θ). Results reported by ILINK; the maximum LOD Z_{max} and the associated θ.) Disease allele frequency (d=0.001), AD=autosomal dominant, penetrance=AA=0, Aa=0.9,aa=0.9)

Table. 4.11 Maximum LOD scores (Z_{max}) reported by Genehunter in two-point analysis with broad affection model

JME + spike and wave trait KERALA family linkage analysis using 12 markers by Genehunter				
Loci	**Z_{max}**	**NPL Score**	**P value**	**Information**
D6S1678	-2.5780	-0.7665	0.9063	0.8000
D6S1691	-2.9025	0.2986	0.2422	0.8500
D6S265	-2.9759	0.1075	0.3906	0.9066
D6S1568	-2.9759	-0.5697	0.7109	0.9510
D6S1548	0.4304	1.4458	0.0703	0.4108
D6S282	-3.7163	0.1075	0.3906	0.9500
D6S1650	-8.5851	-0.7162	0.8828	0.7500
D6S452	-4.5115	-0.5104	0.6875	0.4410
D6S465	-4.2769	-0.2403	0.4688	0.7162
D6S294	-3.2769	-0.5057	0.6875	0.6959
D6S1628	-5.0036	-0.5503	0.6875	0.6226
D6S1619	-0.4340	1.1333	0.0703	0.8154

Z_{max} =maximum LOD scores; NPL score=non-parametric linkage score; Information=marker informativeness; model; disease allele frequency d=0.001)

Multipoint LOD scores again were exclusionary in most regions of the map (Fig. 4.5). Using heterogeneity option, in NAM, Genehunter gave consistently negative LODs along the entire map in two-point mapping. Computed haplotypes of the pedigree members including those (B4 and C20) who were not genotyped is given in Fig. 4.4. When the BAM was used, multipoint parametric LOD scores were clearly exclusionary along the entire map (Fig. 4.6). NPL did not show any region with a LOD > 0.2 (Fig. 4.7). Average information content was 0.79 (Fig. 4.8). A region spanning 8.2cM between markers D6S1548 and D6S282 showed LOD between -1 and -2. Bounding markers showed LOD approaching negative-infinity. Analysis was also done with the unaffected members reclassified as unknown. This also failed to show any positive trend towards linkage

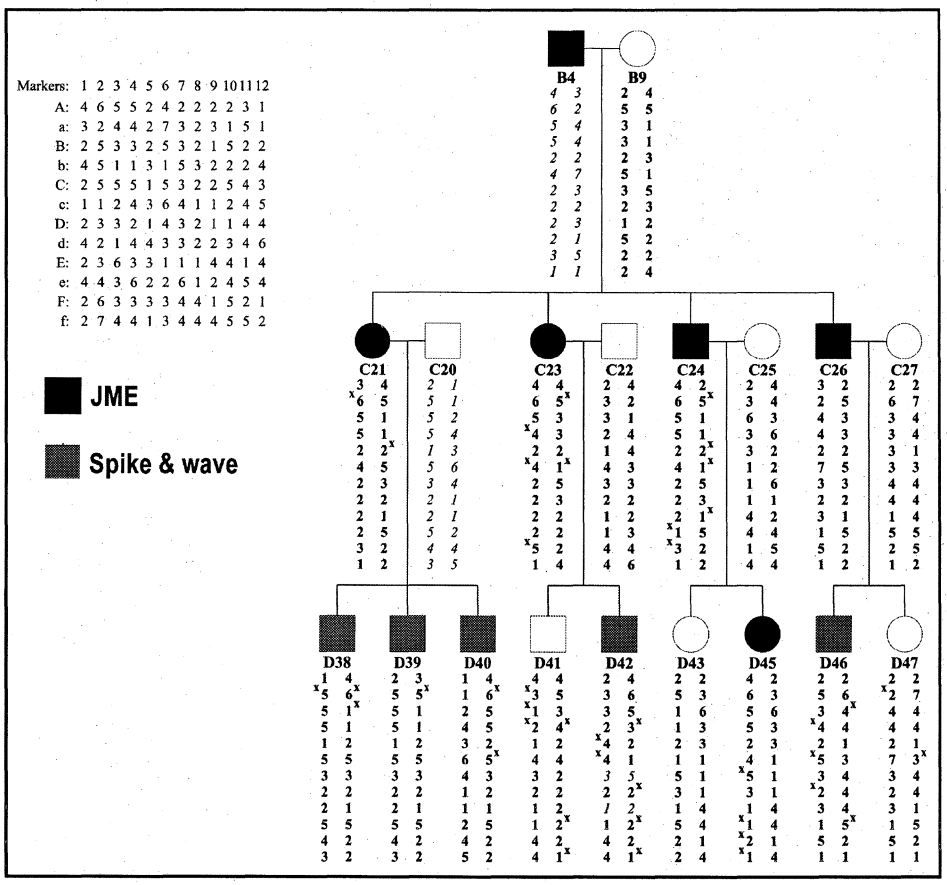


Fig 4.4 Haplotypes of the alleles of chromosome 6p markers in the large Kerala family constructed using Genehunter

Dark circles and squares represent JME and lighter shaded ones EEG spike and wave. X denoted observed recombinations in pedigree members

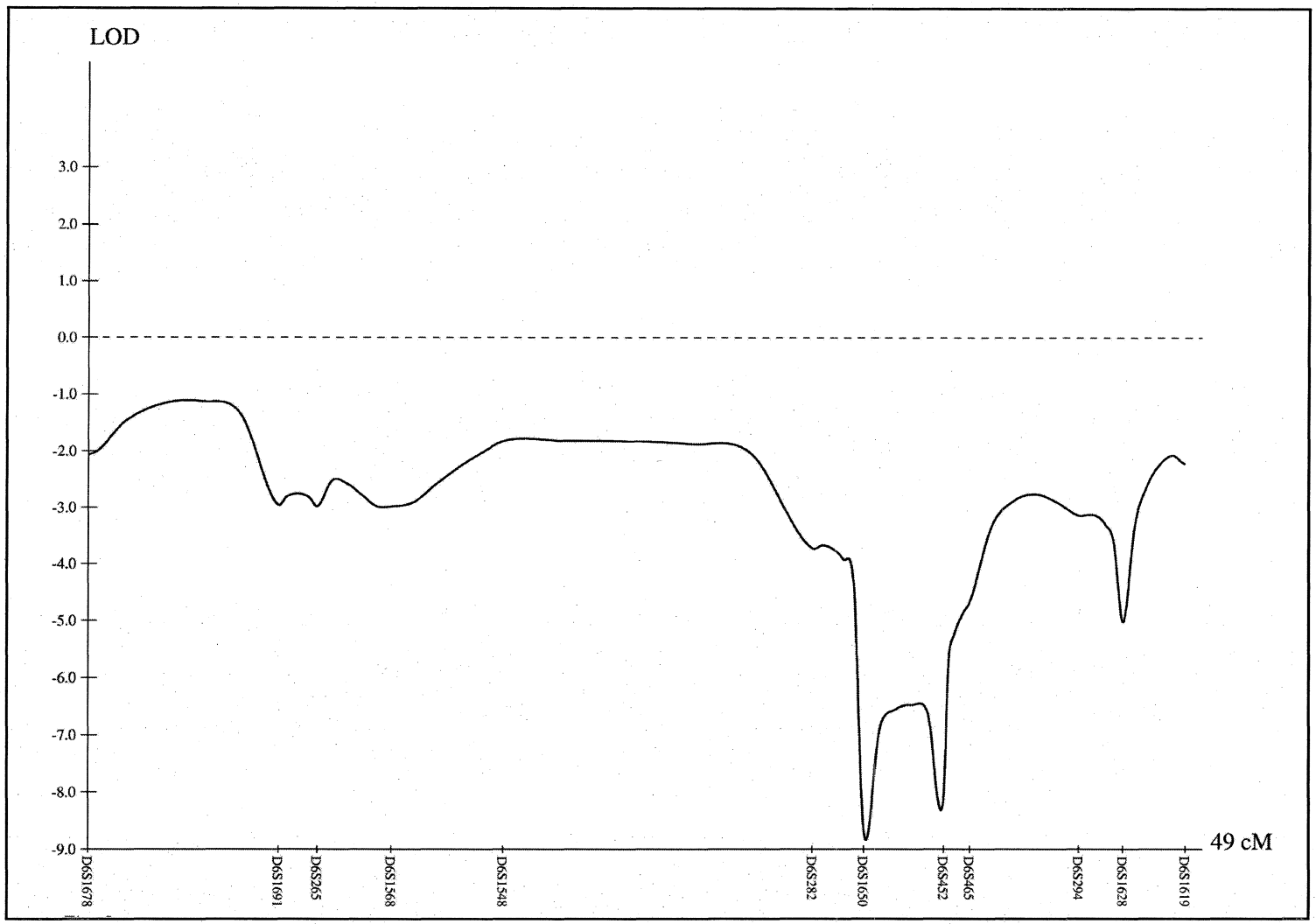


Fig. 4.5 Multipoint parametric analysis using Genehunter. Narrow affection model, penetrance 90% AD.

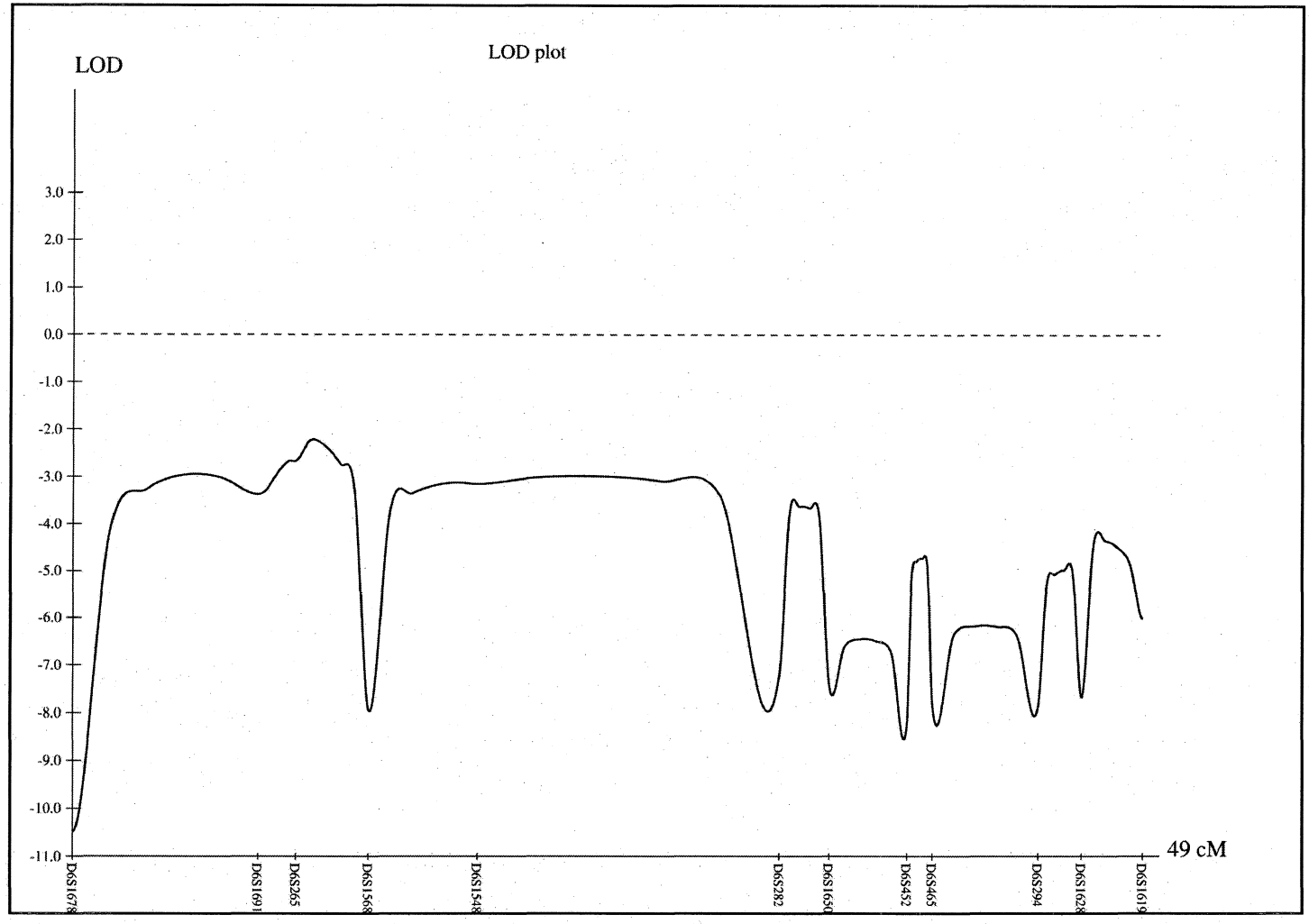


Fig. 4.6 Multipoint analysis using Genehunter. Broad affection model, penetrance 90% AD.

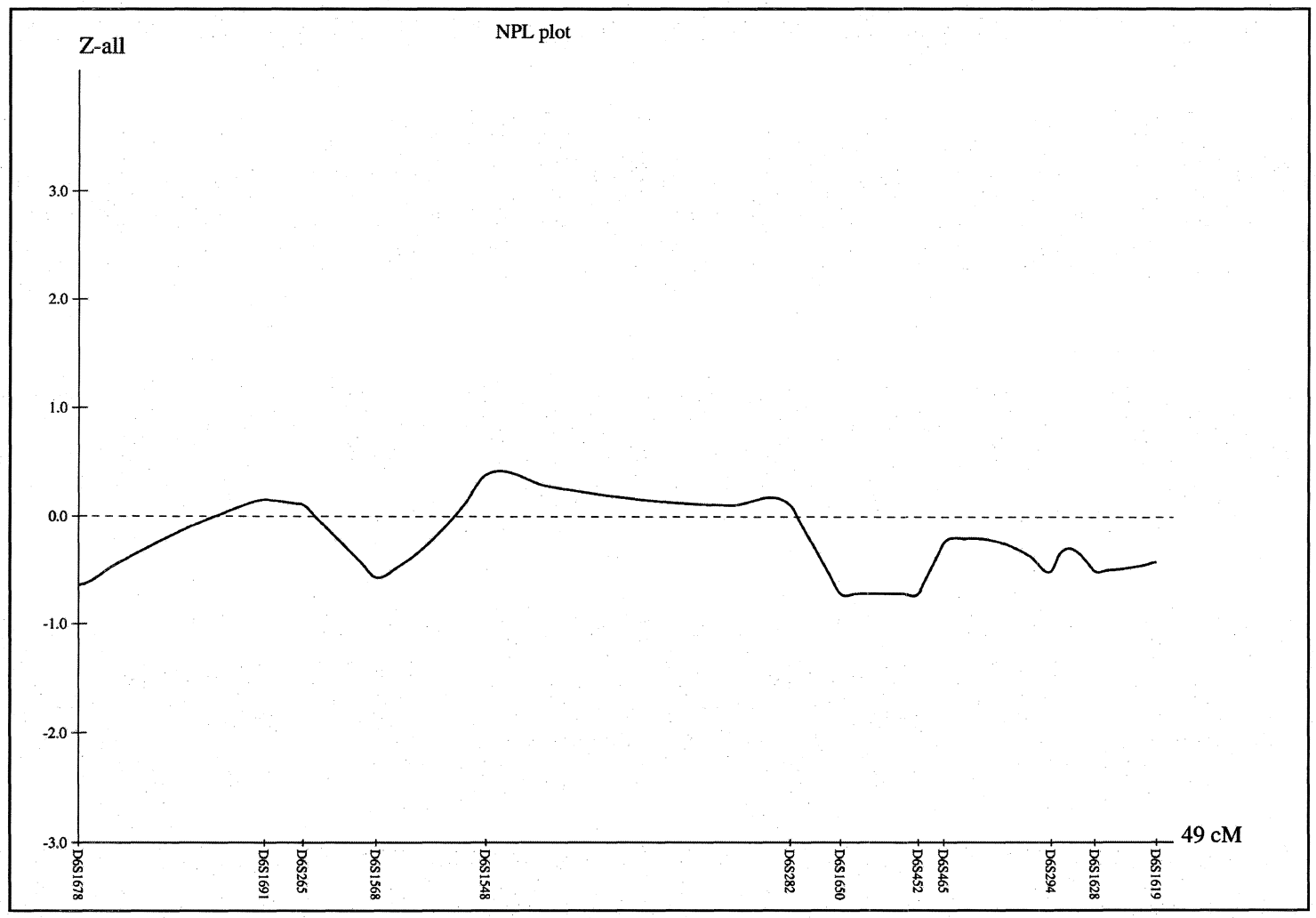


Fig. 4.7 Multipoint non-parametric linkage analysis using Genehunter. Narrow affection model, penetrance 90% AD.

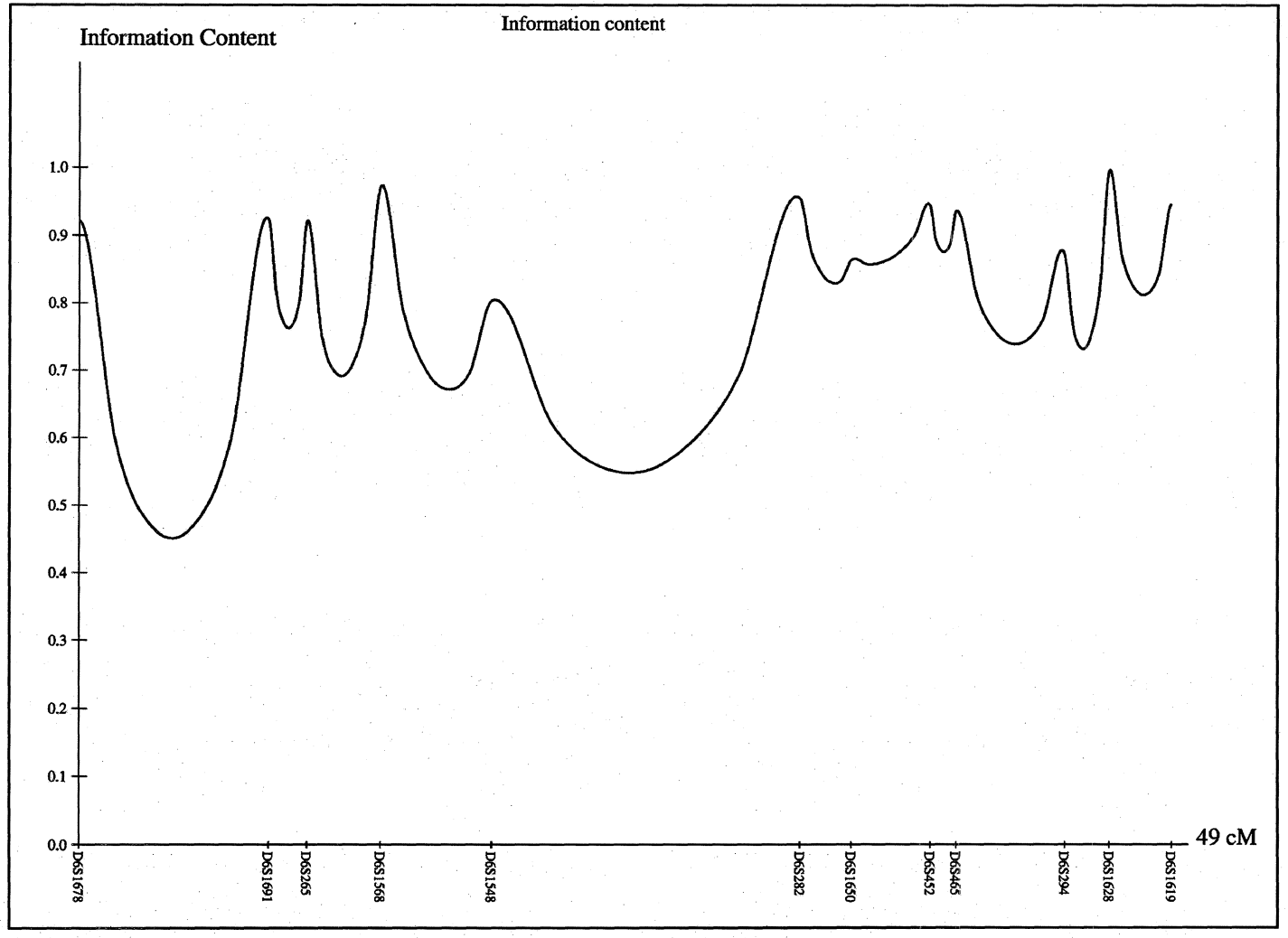


Fig. 4.8 Marker informativeness in multipoint linkage analysis using Genehunter. Narrow affection model, penetrance 90% AD.

4.5 Non-parametric analysis of 50 sibpairs

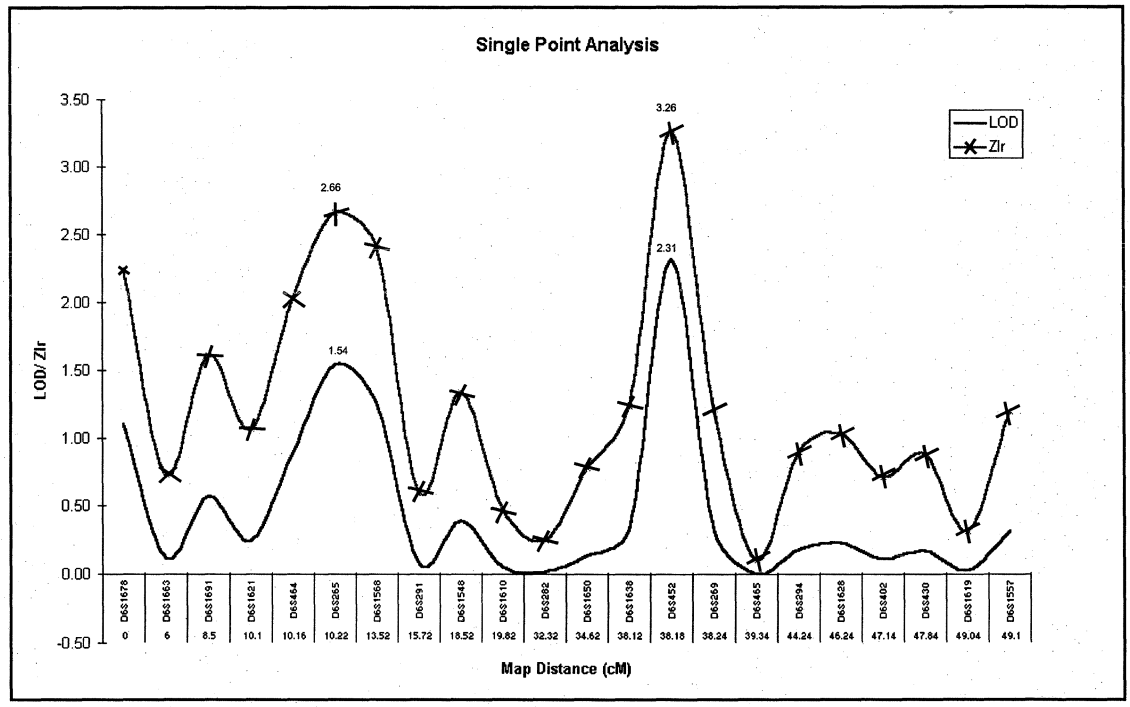
4.5.1 Clinical examination

Among the 50 JME families, there were 62 affected females as well as males. Total sample size from 50 multiplex families was 244. At entry, the average age of affected member was 28 years. Six patients exhibited absences (Avg. age of onset =9.3 years). The average age of myoclonus was 14.3 years and that of GTCS was 15.3 years. Myoclonus occurred predominantly in early morning hours with sleep deprivation being the primary precipitating factor. Sodium valproate was the drug of choice and seizure control was good upon medication. EEG recordings were taken on all patients and wherever possible on other members of the parents. Eleven patients exhibited photoparoxysmal responses in EEG. Family members either had a history of IGE, a history of tonic clonic seizures during childhood or a spike and wave epileptiform abnormality on routine scalp EEG recording. Affectedness status was confirmed before genotyping.

4.5.2 Single marker analysis

Highest SPT LOD score was observed at D6S452 (LOD=2.31, Z_{lr}=3.26 P=0.00055) (Fig 4.9). Another locus with a LOD approaching and crossing one was also found using S_{all} scoring function in ALLEGRO. The maximum LOD here was 1.54 for marker D6S265 (Table 4.12). There were three consecutive markers with a promising score spanning 3.36cM in this HLA locus. The Z_{lr} scores for the markers were higher than 2.0 (D6S464, Z_{lr}=2.034, P=0.043; D6S265 Z_{lr} =2.664, P=0.026 and D6S1568 Z_{lr} =2.405, P=0.034). The information content at D6S452 was poor (30%), while markers D6S464, D6S265 and D6S1568 showed healthy (average 61%). When S_{pairs} scoring

was employed, the LOD dropped slightly at both loci (Table 4.13) [D6S464, LOD 0.85, P=0.023; D6S265, LOD1.45, P=0.005; P=0.007; D6S452 LOD 2.27 P=0.00062].



Highest LOD score of 2.31 at marker D6S452 (P=0.00055), Z_{lr}=3.26; markers at HLA locus showing another peak (Z_{lr}=2.66, P=0.00384)

Fig 4.9 Single point analysis (S_{all}) equal, exponential model

Table 4.12. Two point analysis: exponential model in ALLEGRO using $S_{all-equal}$ statistic

$S_{all-equal}$ scoring function : SPT analysis							
location	Marker	LOD	$\hat{d}$	Zlr	NPLexact-P	LODexact-P	Info
0	**D6S1678**	1.10	0.522	2.250	0.090	0.01218	36%
6	**D6S1663**	0.12	0.146	0.733	0.301	0.23060	50%
8.5	**D6S1691**	0.57	0.259	1.614	0.080	0.05296	76%
10.1	**D6S1621**	0.25	0.211	1.071	0.222	0.14132	50%
10.16	**D6S464**	0.90	0.336	2.034	0.043	0.02089	71%
10.22	**D6S265**	1.54	0.514	2.664	0.026	0.00384	52%
13.52	**D6S1568**	1.26	0.430	2.405	0.034	0.00805	60%
15.72	**D6S291**	0.08	0.116	0.597	0.331	0.27391	52%
18.52	**D6S1548**	0.39	0.258	1.339	0.164	0.08977	52%
19.82	**D6S1610**	0.05	0.092	0.470	0.366	0.31756	52%
32.32	**D6S282**	0.01	0.053	0.243	0.436	0.40237	42%
34.62	**D6S1650**	0.13	0.170	0.782	0.306	0.21593	41%
38.12	**D6S1638**	0.33	0.237	1.235	0.184	0.10783	53%
38.18	**D6S452**	2.31	0.842	3.263	0.039	0.00055	29%
38.24	**D6S269**	0.32	0.229	1.216	0.183	0.11141	55%
39.34	**D6S465**	0.00	0.019	0.102	0.468	0.45752	56%
44.24	**D6S294**	0.18	0.165	0.902	0.245	0.18269	58%
46.24	**D6S1628**	0.23	0.171	1.031	0.192	0.15047	72%
47.14	**D6S402**	0.11	0.118	0.709	0.273	0.23809	71%
47.84	**D6S430**	0.17	0.162	0.877	0.252	0.18922	57%
49.04	**D6S1619**	0.02	0.062	0.318	0.408	0.37382	52%
49.1	**D6S1557**	0.31	0.223	1.197	0.184	0.11497	56%

Location: Location of dinucleotide marker on the genetic map; LOD: allele sharing LOD; $\hat{d}$=extent of allele sharing; NPLexactP= P value for Z_{lr} score; LODexactP= P value for LOD score; Info= marker informativeness

Table 4.13 Two point analysis using ALLEGRO using S_{pairs} scoring function

S_{pairs} scoring function : SPT analysis							
location	Marker	LOD	$\hat{d}$	Zlr	NPLexact-P	LODexact-P	Info
0	**D6S1678**	0.96	0.486	2.107	0.103	0.01756	37%
6	**D6S1663**	0.10	0.135	0.670	0.318	0.25048	49%
8.5	**D6S1691**	0.70	0.290	1.796	0.060	0.03625	76%
10.1	**D6S1621**	0.28	0.223	1.140	0.206	0.12653	51%
10.16	**D6S464**	0.85	0.334	1.980	0.049	0.02386	69%
10.22	**D6S265**	1.45	0.497	2.587	0.029	0.00486	54%
13.52	**D6S1568**	1.32	0.455	2.464	0.033	0.00690	58%
15.72	**D6S291**	0.10	0.132	0.674	0.312	0.24969	52%
18.52	**D6S1548**	0.35	0.242	1.277	0.172	0.10056	55%
19.82	**D6S1610**	0.03	0.075	0.384	0.389	0.34999	52%
32.32	**D6S282**	0.01	0.048	0.215	0.444	0.41397	40%
34.62	**D6S1650**	0.18	0.201	0.903	0.283	0.18251	40%
38.12	**D6S1638**	0.43	0.276	1.406	0.158	0.07959	51%
38.18	**D6S452**	2.27	0.797	3.230	0.034	0.00062	34%
38.24	**D6S269**	0.51	0.289	1.527	0.128	0.06338	55%
39.34	**D6S465**	0.02	0.054	0.287	0.412	0.38560	57%
44.24	**D6S294**	0.10	0.127	0.686	0.301	0.24564	57%
46.24	**D6S1628**	0.29	0.193	1.155	0.167	0.12375	71%
47.14	**D6S402**	0.17	0.145	0.879	0.226	0.18900	73%
47.84	**D6S430**	0.21	0.184	0.993	0.224	0.15981	58%
49.04	**D6S1619**	0.02	0.052	0.269	0.421	0.39264	53%
49.1	**D6S1557**	0.39	0.254	1.340	0.161	0.08972	55%

Location: Location of dinucleotide marker on the genetic map; LOD: allele sharing LOD; $\hat{d}$=extent of allele sharing; NPLexactP= P value for Z_{lr} score; LODexactP= P value for LOD score; Info= marker informativeness

4.5.3 Multi point analysis

A consolidated multipoint analysis of the twenty-two markers on chromosome 6p was carried out in ALLEGRO using exponential and linear modes with S_{pairs} and S_{all} options of sib-pair selection. Both, allele sharing LODs and Z_{lr} scores were estimated along with their exact P values. When analysis was done with all families weighted equally in the exponential mode and all sibs selected (S_{all}) (Fig. 4.10); the maximum LOD of 1.34 was observed at map location 11.54cM (P=0.006383, MapInfo=0.78) between markers D6S265 and D6S1568. However, the maximum Z_{lr} score of 2.36 was obtained at 10.22cM (P= 0.0106 MapInfo=0.95) at marker locus D6S265 (Fig. 4.10). A region starting near marker D6S1691 (8.82cM) showed a LOD>0.9 and extends upto a region (14.4cM) between markers D6S1568 and D6S291. The total sub-chromosomal region that shows a trend of Z_{lr}>2 is 5.9cM long. The breadth of this significance region is 16.3cM. A second region at D6S430 (47.8cM) revealed LOD of 0.6 (P=0.04, MapInfo=0.97) and another at D6S269 flanking D6S452. Flanking markers did not seem to show a trend towards greater allele sharing, having very little support for the breadth of the allele-sharing peak. P values are consistently below the nominal significance level of 0.05 in this region spanning eight markers except for two points near the second marker D6S1663 at 4.8 and 6.0cM (Fig 4.11). LOD at the locus near D6S265 increased to 1.45 (P=0.0049) at 11.54cM when S_{pairs} scoring function was used (Fig. 4.12). The total connected sub-chromosomal region that shows a trend of Z_{lr}>2 was now 6.2cM long and that showing Z_{lr}>1 was 24.8cM

spanning 10 markers (Fig 4.12) from D6S1678 and D6S1610. It is of importance to point out that nowhere on the entire map, did '$\hat{d}$' assume a negative value showing the existence of positive allele sharing. The positive trend near marker locus D6S430 continued as mentioned above in the S_{all} analysis too; with little or no support from the flanking markers. An additional region that showed marginal significance (<0.05) similar to that observed at locus D6S430 was identified in the S_{pairs} analysis. This region was between markers D6S269 and D6S465. Here again, the breadth of the region was small. In genome scans, true peaks tend to be wider than false peaks; this must be true and applicable for region scans too. It may also be noted that here, the intermarker distance is only 1.1cM. The results were not very different when s= ½ was used as family weights. Average marker informativeness was 89.85% (Fig. 4.13).

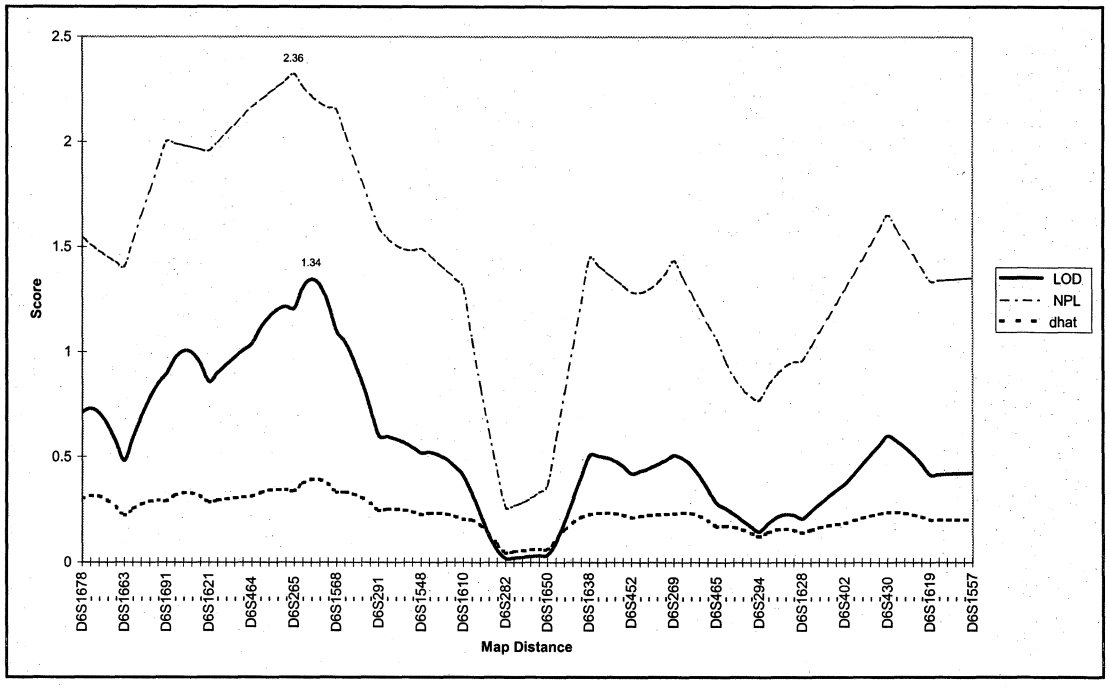
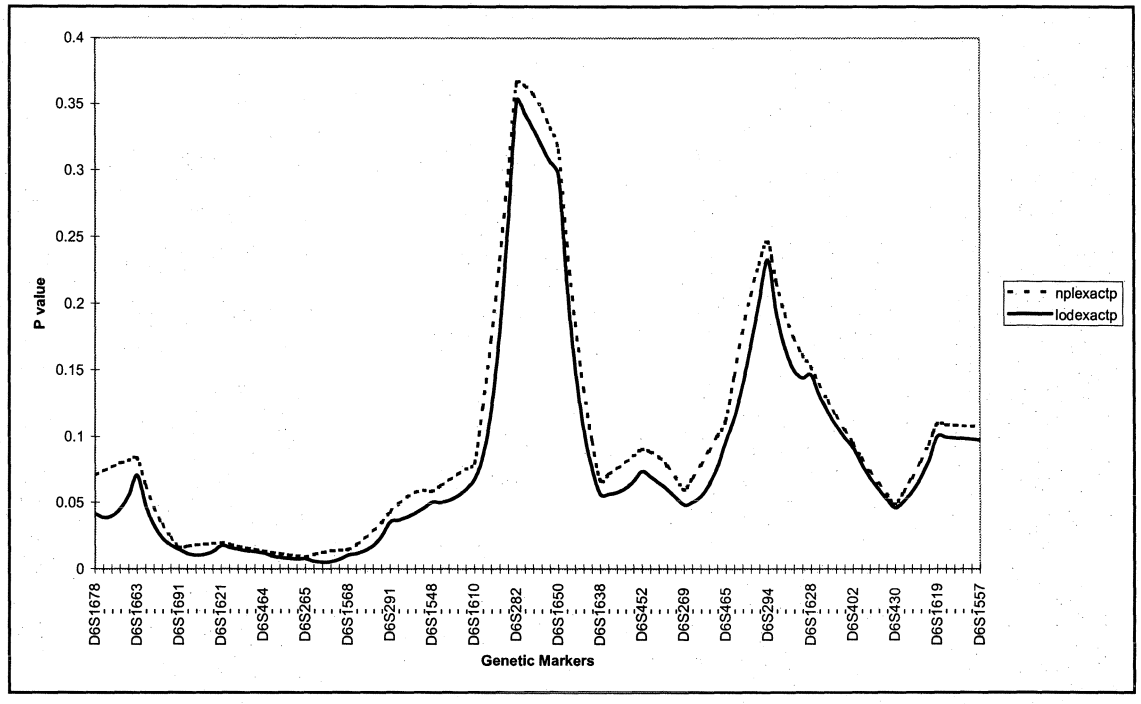
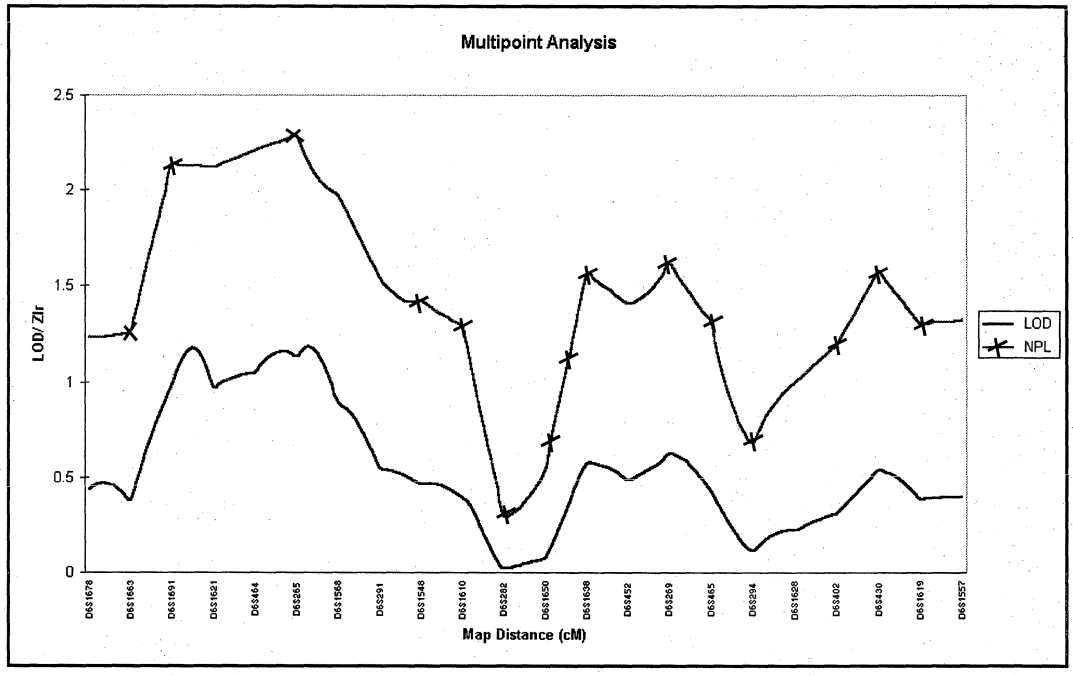


Fig 4.10 Non-parametric linkage analysis: exponential mode, S_{all} with equal weight



Regions showing very low P values in multipoint mapping; X-axis shows the marker locations, Y-axis shows the associated P values.

Fig 4.11 Significance levels during $S_{pairs-eq}$ multipoint mapping using ALLEGRO



Regions showing positive LOD and NPL scores (Z_{lr}) during multipoint mapping; X-axis shows the marker locations, Y-axis shows the associated LOD/Z_{lr} scores.

Fig 4.12 Multipoint Analysis $S_{pairs-0.5}$ showing LOD scores and Z_{lr} scores

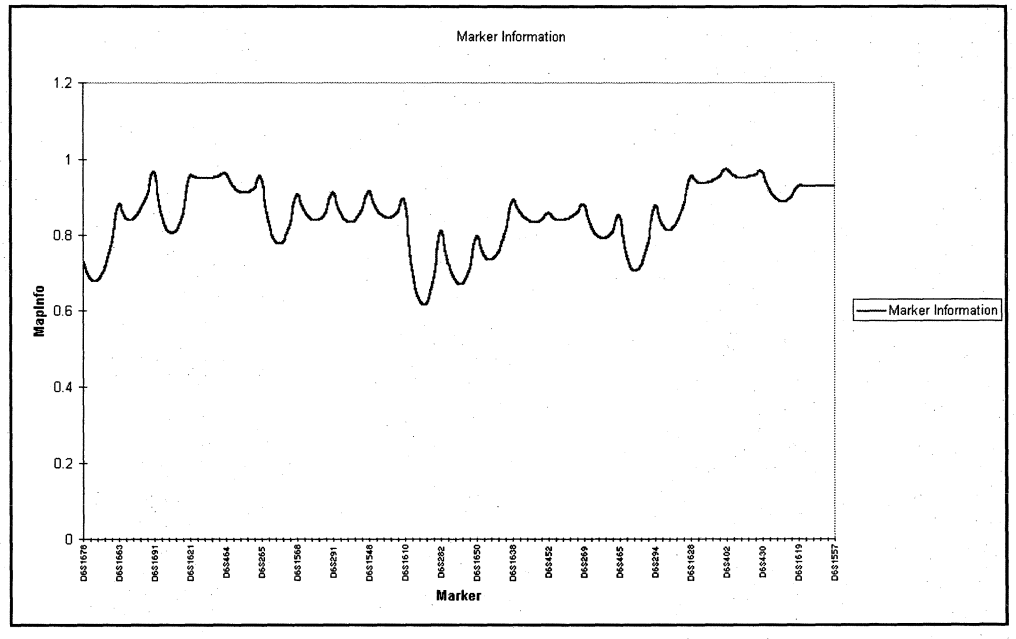


Fig 4.13 Map information in multipoint analysis using ALLEGRO

4.5.4 Simulation and estimation of occurrence expectation (significance testing)

Lander and Kruglyak presented the guidelines for interpretation of linkage results in genome scans in their seminal paper published in 1995 (Lander and Kruglyak, 1995). A LOD score of 3.6 (P=0.000022) is accepted as 'evidence' of linkage, LOD 2.2 (0.00074) as 'suggestive evidence' of linkage and replication studies of an initial significant result 'confirmed' with a P<0.01. However, these values are obtained only in an ideal scenario of 100% marker informativeness and an infinitely dense map. To aid in the interpretation of data for this region scan, 5000 replicates were used (100 pre-files with 50 sets of each family) of the data using the same family structure, simulated marker information (under Mendelian inheritance constraints) and simulated against the null hypothesis of unlinked loci. The disease locus was simulated 50cM away from the marker loci.

4.5.5 Single marker analysis

A $S_{pairs\text{-}P(0.5)}$ LOD of 2.74 (P=0.00019) or Z_{lr} of 3.55 (P=0.021) obtained at D6S452 during analysis of the actual data set was not exceeded once in the 100 analyzed data during simulation resulting in the occurrence expectation (O_E) <0.01. While the LOD 1.44 (P=0.0048), Z_{lr}=2.58 (P=0.035) obtained at D6S265 was exceeded in the simulation five times out of 100 resulting in the O_E =0.05. This means that such a value may be randomly obtained only once in every 20 such region scans.

4.5.6 Multi point analysis

$S_{pairs\text{-}P(0.5)}$ LOD 1.178 (P=0.0097) was equaled or exceeded in four instances out of 100 files analyzed O_E =0.04, each with 50 families replicated 50 times, implying similar scores can be obtained only once in 12 such region scans. In fact, a LOD>1 or Z_{lr}>2.15 was obtained in simulations only nine times out of 100 anywhere on the map. This suggests that the region spanning the 10 markers, which has shown a LOD or Z_{lr} greater than the above value has a very remote chance of being a random event. The marker D6S430 (LOD=0.6, Z_{lr}=1.69) did not assume significance in the simulations. This score was repeatedly attained in the simulations.

V. DISCUSSION

5.1 Problems and pitfalls in the diagnosis of JME in South India

Based on this prospective study, undertaken through a tertiary care epilepsy centre, I wish to describe the problems and pitfalls in the diagnosis, phenotypic peculiarities, and clinical genetics of JME as seen in the population of Kerala, South India.

JME is a well-defined, relatively common, epileptic syndrome that when correctly diagnosed can be easily treated with a guaranteed long-term seizure-free outcome in the majority. The diagnosis of JME should be straightforward in a person of the adolescent age group with normal intellect, neurological status, and an antecedent history of early morning myoclonic jerks during wakefulness, who presents with a GTCS following sleep deprivation. However, significant delays in diagnosis of JME continue to be the rule rather than the exception. Several studies have observed delay in diagnosis of JME, ranging from a mean of 6.8 (Murthy et al., 1999) to 15.0 years (Vazquez *et al.*, 1993), both from developed (Grünewald *et al.*, 1992; Vazquez *et al.*, 1993; Kleveland and Engelsen, 1998) and developing countries (Murthy, 1999; Gunatilake and Seneviratne, 2000). In a patient, who presents with a GTCS following sleep deprivation, only antecedent history of myoclonic jerks during wakefulness clearly supports the diagnosis of JME, and delay in the diagnosis usually results from failure to elicit this history (Panayiotopoulos *et al.*, 1991; Vazquez *et al.*, 1993; Lancman *et al.*, 1994; Murthy, 1999). The state of Kerala is distinguished from the rest of India by the high level of literacy and health awareness of its population, and a comprehensive health care system easily accessible to the population (Thankappan and Valiathan, 1998; Zachariah, 1998). While there is one neurologist for 13,500 persons with

epilepsy for the whole of India, this ratio for Kerala is one for 3000. Despite these demographic and medical attributes favouring an early diagnosis of JME in the Kerala population, little difference was found in pitfalls in diagnosis compared to the data from less developed regions. In this group of 183 patients, only six (3.3%) were diagnosed with JME when first referred to us; the average duration from onset of seizures to diagnosis of JME was 8.6 years. Over 60% of the patients had consulted at least one qualified neurologist. Effective AED therapy was delayed and seizure control was poor at the time of referral in nearly all the patients.

Carbamazepine, which is known to exacerbate or precipitate absence and myoclonic seizures, and GTCS in JME patients (Genton *et al.*, 2000), was the commonest AED in use, more often in combination with other AEDs. After JME diagnosis was made and optimal treatment with valproate started, seizure control was achieved in over 80% of the patients.

A majority of patients with epilepsy in developing countries are treated and followed up by primary and secondary care physicians without specific training or expertise in this disorder. The need for educating the primary and secondary care physicians about the recent trends in the diagnosis and treatment of epilepsy cannot be overemphasized. In developing regions, even among the neurologists, the awareness about JME is poor. In a recent report from Sri Lanka, two third of the JME patients, like in this study, had remained undiagnosed despite already being seen by at least one neurologist (Gunatilake and Seneviratne, 2000).

A majority of the clinical and EEG features observed in this JME cohort were in accordance with literature (Janz, 1985; Penry *et al.*, 1989; Janz, 1990;

Panayiotopoulos *et al.*, 1994; Murthy *et al.*, 1998). Nearly 10% of patients had antecedent history of febrile seizures, consistent with the rate of 12% reported for JME patients from Turkey (Baykan *et al.*, 1991). Myoclonic jerks as the only seizure type noted in 5.5% of patients have been reported to occur in 7-17% of JME probands (Asconape and Penry, 1984; Janz, 1985; Jain *et al.*, 1997). Abnormal EEGs, defined as those with spike and/or multiple spike-wave discharges were found in 81% of patients, consistent with 60-90% reported in the literature (Janz, 1969; Janz, 1985; Grünewald and Panayiotopoulos, 1993). Nearly 20% of patients exhibited focal or lateralized epileptiform discharges, which has been reported to occur in about one third of patients with JME (Aliberti *et al.*, 1994; Lancman *et al.*, 1994). The two notable differences observed were the relatively increased occurrence of absence seizures and low frequency of photoparoxysmal response among Kerala JME probands.

In this series the prevalence of absence was 40% when compared to 10-35% reported among patients belonging to different ethnic groups (Delgado-Escueta and Enrile-Bacsal, 1984; Janz, 1985; Grünewald and Panayiotopoulos, 1993; Panayiotopoulos *et al.*, 1994). Two other series from India found absences in 8% of JME probands from Delhi (Jain *et al.*, 1998b) and in 21% from Hyderabad (Murthy *et al.*, 1998). The absences in JME are generally mild, relatively short and are often unnoticed by the patients and their relatives (Panayiotopoulos *et al.*, 1989). Although the low frequency of absence seizures among JME patients reported from certain geographical regions may most likely be due to incomplete ascertainment, it may well be an expression of the clinical heterogeneity of the syndrome in different ethnic groups.

The prevalence of photoparoxysmal response in JME patients has been observed to vary from 10 to 40% (Janz, 1969; Janz, 1985; Grünewald and Panayiotopoulos, 1993). The frequency of photoparoxysmal response can be influenced by several factors, such as age, gender, race, environment, sleep deprivation, technique of intermittent photic stimulation, definition of photoparoxysmal response, and AED regimen (Kasteleijn-Nolst Trenite, 1989). Photosensitivity appears at the age of 12-14 years and disappears between the ages of 20 and 30 years in a proportion of patients (Jeavons *et al.*, 1986). In a recent study, out of 61 children with a diagnosis of JME with a median age of 13 (range 7-16) years, 55 (90%) were photosensitive (Appleton *et al.*, 2000). Two thirds of the patients with photosensitivity are females (Jeavons *et al.*, 1986; Kasteleijn-Nolst Trenite, 1989). A single dose of valproate can suppress the photoparoxysmal response (Myslobodsky *et al.*, 1980). Photoparoxysmal response was encountered in 10% of the JME patients in the present study. A low prevalence of photoparoxysmal response among epilepsy patients has been observed in African and Asian populations (de Graaf, 1992; Saleem *et al.*, 1994; de Graaf *et al.*, 1995), compared to whites living in western countries (Jeavons *et al.*, 1986; Kasteleijn-Nolst Trenite, 1989). However, reported prevalence of photoparoxysmal response of 3.5% in epilepsy patients from Kerala (Radhakrishnan *et al.*, 1998), is comparable to that reported from the western countries. The mean age of JME patients in the study was 24.4 years, and nearly 80% were receiving valproate at the time of EEG study. These two factors may be responsible for the relatively low prevalence of photoparoxysmal response among JME patients. However, studies involving JME patients from South India (Murthy *et*

al., 1998), North India (Jain *et al.*, 1998b) and Japan (Shiraishi *et al.*, 2001) have noted a relatively low prevalence of photoparoxysmal response. The variability in the prevalence of photoparoxysmal response among JME patients from different ethnic groups may be yet another reflection of the genetic variability of this syndrome.

Forty-six percent of our patients had a positive family history, which is comparable to the reported rates of 24–44% from India (Jain *et al.,* 1998b; Murthy *et al.*, 1998), and 25–65% from other nations (Janz and Durner, 1997; Janz, 1985; Grünewald and Panayiotopaulos, 1993). In all series, including ours, generalized epilepsies and syndromes were more common than localisation-related epilepsies among affected relatives. First-degree relatives were threefold or more often affected as compared to second-degree relatives (Panayiotopaulos *et al.*, 1994; Jain *et al.*, 1998b). We diagnosed JME in 25.5% of affected relatives, similar to the 19% noted in a North Indian series (Jain *et al.*, 1998b) comprising larger number of patients and affected relatives. In a study from Saudi Arabia (Panayiotopaulos *et al.*, 1994), a family history of epilepsy was detected in 66% of the probands, and 36% of the families had at least two members affected by JME; these higher figures compared to studies from other regions may be attributable to large family size and high degree of consanguinity in this Arab population. A potential methodological limitation of these studies, including ours, is the absence of controlled family data. There are conflicting reports regarding the mode of inheritance of JME. Based on the study of a three-generation pedigree of 33 members, 10 of whom were diagnosed as JME, Serratosa *et al.*, (1996) suggested an AD inheritance. AD inheritance was also noticed in a recently reported large French Canadian family (Cossette *et al.*, 2002). In the Saudi

Arabian population, Panayiotopoulos *et al.* (Panayiotopoulos *et al.*, 1994) postulated an AR inheritance for JME. Although two different loci on chromosomes 6p (Greenberg *et al.*, 1988b; Durner *et al.*, 1991; Weissbecker *et al.*, 1991) and 15q (Elmslie *et al.*, 1997) have been proposed to be carrying JME predisposing genes, no single gene or mutation has so far been consistently implicated in the pathogenesis of JME. Latest linkage studies point to a complex mode of inheritance that is dependent on two or more disease genes and interplay of modifier genes (McNamara, 1999; Prasad *et al.*, 1999). It is likely that multiple genetic factors that influence the neuronal excitatory and inhibitory pathways may play a collective role in predisposing to JME and in expressing the differential phenotypic components of the syndrome (Avoli *et al.*, 2001).

5.2 Suggestive evidence of allelic association between KCNQ3 and JME

The moderate intrafamilial recurrence rates observed for most idiopathic epilepsies indicate that several different genes contribute to the increased neuronal excitability in common forms of idiopathic epilepsy. Potassium channels play a vital role in modulating neuronal excitability. Loss of the potassium channel β subunit gene, *KCNAB2*, is associated with epilepsy in patients with 1p36 deletion syndrome (Heilstedt *et al.*, 2001). *KCNQ2* and *KCNQ3* show an overlapping expression pattern in nearly all brain regions. Interestingly *KCNQ3* is expressed later than *KCNQ2* (Tinel *et al.*, 1998). *In situ* hybridization shows that *KCNQ2* is mainly present in the cerebellar cortex, the neocortex and the hippocampal formation including the dentate gyrus. This is interesting since these three structures present distinct epileptic seizure susceptibility (Madeja *et al.*, 1997). *KCNQ3* is localized in the same areas in mouse

brain. However, *KCNQ2* expression appears earlier than that of *KCNQ3* and rapidly increases during the first week of life. *KCNQ2* is expressed three days after birth (P3) in the mouse, and its expression is increased by a factor of 2.5 at one week after birth. On the other hand, *KCNQ3* is expressed in very low amounts during birth and its expression increases continuously until the adult stage. This indicates that different profiles of association of *KCNQ2* and *KCNQ3* will probably occur during development. Recently, it has been reported that mice lacking the voltage-gated *Shaker*-like potassium channel $K_v1.1$ α-subunit develop recurrent spontaneous seizures early in postnatal development and throughout adult life (Smart *et al.*, 1998). BFNC usually occurs between day three after birth and the fourth month and seizures disappear spontaneously. Interestingly, 11-15% of the BFNC patients will have epileptic seizures again in life, which are generalized tonic clonic seizures with variable age of onset and frequency. This provides a model for comprehending the mechanism underlying common IGEs such as JME.

Analysis of 119 JME nuclear families shows linkage disequilibrium between marker D8S558 and JME. The results indicate the involvement of *KCNQ3* in predisposition of JME. A group from Berlin had performed genetic association study of *KCNQ3* (Haug *et al.*, 2000a) using a small sample size of 38 JME and 33 CAE cases, but failed to find a significant association. However the authors admit (Haug *et al.*, 2000a) that the small sample size and low statistical power in their experiment cannot exclude small existing gene effects. Apart from genetic heterogeneity shown to be existent in ethnic populations, the heterogeneity of their sample population involving two different syndromes could also have confounded the results. The subtle

effect of linkage disequilibrium noted in the study samples may or may not be widespread in other populations. Replication studies have to be carried out to ascertain the validity (Roberts *et al.,* 1999) of these results in different ethnic populations and are very important in genetics of complex disorders.

Recent reports on modulation of *KCNQ2/Q3* channels by novel anticonvulsant retigabine suggests a shift in the activation threshold and voltage for half-activation in the hyperpolarizing direction (Main *et al*., 2000)., leading to an increase in current amplitude. It is likely that M-current modulation can explain the anticonvulsant actions of retigabine in animal models of epilepsy (Main *et al*., 2000). The present results assume importance when viewed in light of above observations. These would present *KCNQ3* as an important target for drugs supporting the hypothesis that M-channel agonism is a new mode of action for anticonvulsant drugs. In the future, the identification of ion channel defects as predisposing factors in the common epilepsies could herald a new era of genotype-specific therapies.

5.3 Evidence of protective allele of hSKCa3 in controls

Genetic association of JME was tested against an expressed polymorphic CAG repeat tract in a functionally important calcium activated potassium channel gene *hSKCa3* that has been previously implicated in schizophrenia (Chandy *et al*., 1998; Saleem *et al*., 2000). While lack of evidence for association between idiopathic generalized epilepsies and *hSKCa3* has been reported earlier in 126 German IGE cases, which included 78 JME and 48 CAE or JAE patients (Sander *et al*., 1999b), Results in this sample population is however different. The sample size used in the present study is moderately large and consisted only cases of JME. A significant

difference in the allelic distribution among South Indian JME probands compared to ethnically matched control subjects was found. CAG_{18} was significantly common while CAG_{19} was found to be very rare in JME patients. A plausible biological model could involve the pathogenic polymorphisms to be associated with CAG_{18} in cases, while CAG_{19} provides a protective mechanism in controls and hence is extremely rare in JME probands. The high RR of CAG_{18} (1.178) and minimum RR of CAG_{19} (0.514) suggests it possible that CAG_{18} harbors a loss-of-function effect and CAG_{19} a gain-of-function.

The presence of CAG_{19} seemed to reduce the effect of CAG_{18}. The RR of genotype $CAG_{(18,18)}$ was found as 0.79, while that of $CAG_{(18,19)}$ dropped to 0.18. While these observations could also be just a coincidence in the generated data, when viewed in the light of the allelic association, they assume importance. Regression analysis of relative risk (RR) versus allele length variation $(CAG)_n$ of the most common alleles did not yield a statistically relevant model. However, JME is widely thought to be a complex genetic disorder and hence genetic heterogeneity could play a major role in causation. Even as controls were selected from the same ethnic population (South Indians) that the JME samples were drawn from, the possible and unmeasured population stratification that may exist in the population is not accounted for. The South Indian population has been described as homogeneous by mtDNA analysis (Edwin *et al.*, 2002).

Protective alleles are important modifiers of the phenotype. Unlike alleles of susceptibility genes that are over-represented in affected individuals (cases) versus unaffected individuals (controls), protective alleles occur preferentially in healthy

individuals, implying that their presence prevents disease despite presence of other disease-promoting (susceptibility) alleles at genes elsewhere in the genome. Several recent reports have highlighted importance of protective alleles in various disease conditions. Deletion of the CCR5 gene protects from HIV infection (Huang *et al.*, 1996). HLA-DRB11 and HLA-DRQ03032 alleles are both over-represented in controls versus breast cancer cases (Chaudhuri *et al.*, 2000). Protective association of genetic variation in alcohol dehydrogenase (ADH2) with alcohol dependence in Native American Mission Indians (Wall *et al.*, 2003) and a protective effect of RET proto-oncogene in pathogenesis of Hirschsprung's disease (Griseri *et al.*, 2002) were reported. Short polyglutamine tracts in the androgen receptor are protective against breast cancer in the general population (Giguere *et al.*, 2001). Protective effects of aggrecan VNTR alleles for osteoarthritis have been reported in an Australian twin sample (Kirk *et al.*, 2003). *Alu* DNA polymorphism in *ACE* is protective for age-related macular degeneration (Hamdi *et al.*, 2002).

CAG repeat expansions have been implicated in many neurological diseases. There are atleast two reported scenarios in which CAG repeat length polymorphisms within the normal reported range for most polyglutamine proteins can result in a disease state. They are Kennedy's disease (Chamberlain *et al.*, 1994) and spinocerebellar ataxia type 6 (SCA-6)(Zhuchenko *et al.*, 1997). This offers insights into the plausible role of such CAG repeats in the *hSKCa3* in modulating the K^+ channel function and neuronal excitability. The results obtained could mean an allelic association and could for instance implicate a gene quite near *hSKCa3*. This however would not explain the relative risks, because the marker used is a polyglutamine-

coding region. The next closest marker available within the gene sequence is the first CAG tract of *hSKCa3,* but shows only rare polymorphism and is non-informative. Further studies on the structural and functional role of CAG repeat polymorphisms of *hSKCa3* as well as SNP marker based studies are needed to validate the results obtained here.

5.4 Linkage exclusion of a large family with JME from Kerala

Studies on the genetic mapping of JME have given mixed results so far. A part of the complexity in dissection of this disease has been the intricate clinical variability seen in the general population as described in the introduction. Analyses of single large families are considered to reduce the phenotypic variability. However, different yardsticks are used by investigators to define the 'affectedness' status. The use of 3.5-6.0-Hz spike-wave or spike-wave complex EEG pattern as a clinical marker of affection has attracted much debate (Greenberg *et al.*, 1988b; Durner *et al.*, 1991; Weissbecker *et al.*, 1991; Whitehouse *et al.*, 1993; Serratosa *et al.*, 1996). Some authors argue that, since EEG 3.5-6.0 Hz polyspike-wave complexes are associated with myoclonic and grand mal seizures, their presence in asymptomatic family members may be significant (Serratosa *et al.*, 1996). 3.5-6.0-Hz polyspike-wave complexes occur in normal children and adolescents only at a frequency of 0.2% (Okubo *et al.*, 1994). Nevertheless, what is of contention is: can this EEG trait be grouped with the JME phenotype? Similar irregularly formed "diffuse, bilateral synchronous bursts of 2.0-5.0-Hz slow waves with random, poorly developed spikes between the slow waves" was found in 2.3% of awake EEGs (during rest, hyperventilation, or photic stimulation) and 7.9% of drowsy and sleep EEGs of normal

children and adolescents (Eeg-Oloffson *et al.*, 1971). However, this report only analyzes whether JME phenotype or the phenotype of JME and fast EEG spike and wave, complexes are linked to the EJM1 locus in this family from Kerala. The results from two-point and multipoint mapping suggest that there is no linkage to EJM1 in this family. Most of the map showed LOD scores that were clearly exclusionary. The region only between markers D6S1548 and D6S282 showed LOD > -2. The marker informativeness in this region was only 50% and hence the LOD dropping down further upon usage of additional markers is not ruled out. In the present map, this is the only region that was not conclusively ruled out.

The results obtained from this study shows that there are several genes that predisposes to the familial variety of JME. This is the second large family after the French Canadian family that has been excluded from linkage to EJM1. The trait of fast spike and wave in EEG of unaffected individuals was also not found to be linked, when taken together with JME patients.

5.5 Positive evidence of weak linkage to 6p locus EJM1

This is the first allele sharing linkage study of JME from Asia. A positive evidence of locus heterogeneity is found in the results. In the initial SPT analysis, the marker D6S452 gave a LOD of 2.31. However, in MPT mapping, this marker did not show robust allele sharing. On the other hand, three markers at the HLA locus gave $Z_{lr} > 2$. Here, the average marker informativeness (Mapinfo) was greater than 60%. A single family with six affected siblings and with unavailable paternal genotype contributed a LOD 0.95 at locus D6S452. When this family was excluded from the analysis, LOD at D6S452 was still greater (LOD 1.78, Z_{lr} 2.86, LODEXACTP=0.002) than the LOD

at D6S265 (LOD 1.32, Z_{lr} 2.46, LODEXACTP=0.007). What is remarkable was that the flanking markers to D6S452 did not contribute a robust allele sharing LOD even though there is another peak at D6S269. Hence, the allele-sharing peak was narrow and support interval small. Seven families (14%) showed a positive LOD only at D6S265 locus, while nine families (18%) showed a positive LOD at D6S452. Fourteen families (28%) contributed positive LOD to both these loci. Twenty families (40%) did not contribute a positive LOD to either locus. The contributions from each of the nuclear family are quite small, and hence these observations should be treated with caution.

In MPT analysis, the locus D6S452 did not give positive evidence of linkage. On the other hand, D6S265 and flanking markers at HLA locus gave LOD in excess of one. The breadth of the linkage peak here was broad and the total sub-chromosomal region that shows a trend of Z_{lr}>2 was 5.9cM long. The allele sharing LOD does not cross the suggested threshold of 2.2. Nevertheless, a positive trend of linkage cannot be ruled out. The region showing, a LOD > 1 also shows positive allele sharing defined by parameter '$\hat{d}$'(which remained positive in the entire region). Simulation data suggests that the SPT LOD at D6S452 was significant. However in view of the low marker information during SPT analysis and the absence of a reasonable LOD during MPT, the SPT score reported may not be a reliable IBD measure. Simulation also showed MPT LOD score of 1.178 was marginally significant. It is quite clear that there is substantial genetic heterogeneity in the samples used.

The results obtained from allele sharing, non-parametric linkage analysis in the 50 South Indian families suggest a weak linkage to markers D6S265 and

D6S1568. Only families with classic JME, JME with pyknoleptic absences and JME with myoclonus alone have been included in the analysis. Further splitting of these samples using more stringent clinical criteria may yield benefits for linkage analysis. However, the present sample size seems inadequate to do so. The important observation from this study is the positive signs towards linkage in both the locations previously described as the EJM1 locus; namely, at the HLA region (6p21.33 to 6p21.31), and at the centromeric region implicated in the classic JME variety, (6p11.2 to 6p12.1); even though the latter did not show a decent support interval and was non-significant in simulation performed to assess O_E.

VI. SIGNIFICANCE OF THE STUDY

Genetic analysis of complex disorders is a scientifically challenging venture and has seldom been attempted in developing countries. Most genetic studies on common epilepsies have either lumped different syndromes in order to find common gene effects governing all the syndromes therein, or have attempted to find linkage of a well-characterized epilepsy syndrome to a single locus. In this study, the role of the causative gene and the influence of minor susceptibility or protective genes were investigated in a well-characterized patient population utilizing a systematic approach.

The pitfalls and problems associated with the diagnosis of JME highlights the difficulty in clinically characterizing the syndrome, which could have influenced results of previous genetic studies on JME. The protective allele effect of a calcium activated potassium channel gene *hSKCa3* in JME is revealed for the first time through a case control strategy. The role of EJM1 locus in JME has been a matter of severe debate in the epilepsy research community. Although this study using an independent dataset from the South Indian population suggests a weak linkage to EJM1, it also suggests that EJM1 is not the sole locus involved in JME predisposition. For the first time, proof for the emerging concept that JME has a complex mode of inheritance that is dependent on one or more disease genes and interplay of modifier genes is provided through this genetic research.

VII. CONCLUSIONS

Based on the results of this study utilizing a well-characterized group of JME patients and normal subjects from South India, I wish to conclude: 1) A majority of the JME patients in the community remain inadequately diagnosed and treated; 2) Minor phenotypic variability in the syndrome is common; 3) JME in South Indian population shows a trend towards linkage to EJM1, however, locus and genetic heterogeneity exist; and 4) Potassium channel genes *KCNQ3* and *hSKCa3* may be involved in modifying the genetic expression of JME.

VIII. RECOMMENDATIONS FOR FURTHER INVESTIGATIONS

The discovery of very large number of novel SNP polymorphisms recently in the human genome can aid in the gene mapping process in a number of ways. Application of microarrays for the analysis of point mutations and SNPs in genomic DNA is currently under intensive investigation. The availability of the complete sequence of the human genome calls for large-scale investigation of its diversity. In humans and other organisms, SNPs are the most frequent (about one per kilobase in the human genome) DNA markers and are evenly spread. Large-scale association studies by genotyping many SNPs, in individuals with well-characterized phenotypes, are considered as promising methods to identify the cause of many complex diseases, or for improved understanding of the basis of variable response to drugs (pharmacogenetics). Ideally, such studies should be free of biological hypotheses and be done at the whole genome level, to maximize the likelihood of success. In complex disorders like JME, the thrust should be to screen a family of candidate genes like the potassium channels, calcium channels or GABA receptor subunit genes using many SNPs evenly distributed. In the apparently disheartening scenario of large family linkage studies unearthing distinct localizations/mutations that are not reflected in the general population of JME patients, candidate gene associations seems to be the way to go foreward.

IX. BIBLIOGRAPHY

Abel L, Alcais A, Mallet A (1998). Comparison of four sib-pair linkage methods for analyzing sibships with more than two affecteds: interest of the binomial maximum likelihood approach. Genet Epidemiol 15:371-90.

Aliberti V, Grünewald RA, Panayiotopoulos CP, Chroni E (1994). Focal electroencephalographic abnormalities in juvenile myoclonic epilepsy. Epilepsia 35:297-301.

Annegers JF, Hauser WA, Anderson VE, Kurland LT (1982). The risks of seizure disorders among relatives of patients with childhood onset epilepsy. Neurology 32:174-9.

Appleton R, Beirne M, Acomb B (2000). Photosensitivity in juvenile myoclonic epilepsy. Seizure 9:108-11.

Asconape J, Penry JK (1984). Some clinical and EEG aspects of benign juvenile myoclonic epilepsy. Epilepsia 25:108-14.

Atakli D, Sozuer D, Atay T, Baybas S, Arpaci B (1998). Misdiagnosis and treatment in juvenile myoclonic epilepsy. Seizure 7:63-6.

Avoli M, Rogawski MA, Avanzini G (2001). Generalized epileptic disorders: an update. Epilepsia 42:445-57.

Bamshad M, Fraley AE, Crawford MH, Cann RL, Busi BR, Naidu JM, Jorde LB (1996). mtDNA variation in caste populations of Andhra Pradesh, India. Hum Biol 68:1-28.

Barclay J, Rees M (1999). Mouse models of spike-wave epilepsy. Epilepsia 40 Suppl 3:17-22.

Baulac S, Gourfinkel-An I, Picard F, Rosenberg-Bourgin M, Prudhomme JF, Baulac M, Brice A, LeGuern E (1999). A second locus for familial generalized epilepsy with febrile seizures plus maps to chromosome 2q21-q33. Am J Hum Genet 65:1078-85.

Baykan B, Gokyigit A, Calskan A (1991). Juvenile myoclonic epilepsy. Arch. Neuropsych (Turkey) 28:41-7.

Berg AT, Shinnar S, Levy SR, Testa FM (1999). Childhood-onset epilepsy with and without preceding febrile seizures. Neurology 53:1742-8.

Berkovic SF, Andermann F, Carpenter S, Wolfe LS (1986). Progressive myoclonus epilepsies: specific causes and diagnosis. N Engl J Med 315:296-305.

Berkovic SF, Howell RA, Hay DA, Hopper JL (1993). Twin birth is not a risk factor for seizures. Neurology 43:2515-9.

Berkovic SF, Howell RA, Hay DA, Hopper JL (1998). Epilepsies in twins: genetics of the major epilepsy syndromes. Ann Neurol 43:435-45.

Berkovic SF, McIntosh A, Howell RA, Mitchell A, Sheffield LJ, Hopper JL (1996). Familial temporal lobe epilepsy: a common disorder identified in twins. Ann Neurol 40:227-35.

Biervert C, Schroeder BC, Kubisch C, Berkovic SF, Propping P, Jentsch TJ, Steinlein OK (1998). A potassium channel mutation in neonatal human epilepsy. Science 279:403-6.

Boffetta P, Pearce N (1999). Epidemiological studies on genetic polymorphism: study design issues and measures of occurrence and association. IARC Sci Publ:97-108.

Bond CT, Maylie J, Adelman JP (1999). Small-conductance calcium-activated potassium channels. Ann N Y Acad Sci 868:370-8.

Botstein D, Donis-Keller H (1984). A molecular approach to defining the inherited components in epilepsy and other diseases of uncertain etiology. Epilepsia 25 (Suppl 2):S150-5.

Botstein D, White RL, Skolnick M, Davis RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314-31.

Bulman DE (1997). Phenotype variation and newcomers in ion channel disorders. Hum Mol Genet 6:1679-85.

Burmeister M, Lehrach H (1986). Long-range restriction map around the Duchenne muscular dystrophy gene. Nature 324:582-5.

Callenbach PM, Geerts AT, Arts WF, van Donselaar CA, Peters AC, Stroink H, Brouwer OF (1998). Familial occurrence of epilepsy in children with newly diagnosed multiple seizures: Dutch Study of Epilepsy in Childhood. Epilepsia 39:331-6.

Campbell MA, Elston RC (1971). Relatives of probands: models for preliminary genetic analysis. Ann Hum Genet 35:225-36.

Canevini MP, Mai R, Di Marco C, Bertin C, Minotti L, Pontrelli V, Saltarelli A, Canger R (1992). Juvenile myoclonic epilepsy of Janz: clinical observations in 60 patients. Seizure 1:291-8.

Castells C (1958). *La epilepsia mioclonica bilateral y consciente*. *Acta Neurol Latinoam* 4:23-48.

Chakravarthi A, Lynn A (1999). Meiotic mapping in humans. In: Birren B, Green D, Hieter P, Klapholz S, Myers RM, Riethman H, Roskams J (eds) Genome Analysis. Vol 4. New York, Cold Spring Harbor Laboratory Press, pp 1-64.

Chamberlain NL, Driver ED, Miesfeld RL (1994). The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. Nucleic Acids Res 22:3181-6

Chandy KG, Fantino E, Wittekindt O, Kalman K, Tong LL, Ho TH, Gutman GA, Crocq MA, Ganguli R, Nimgaonkar V, Morris-Rosendahl DJ, Gargus JJ (1998). Isolation of a novel potassium channel gene *hSKCa3* containing a polymorphic CAG repeat: a candidate for schizophrenia and bipolar disorder? Mol Psychiatry 3:32-7.

Charlier C, Singh NA, Ryan SG, Lewis TB, Reus BE, Leach RJ, Leppert M (1998). A pore mutation in a novel KQT-like potassium channel gene in an idiopathic epilepsy family. Nat Genet 18:53-5.

Chaudhuri S, Cariappa A, Tang M, Bell D, Haber DA, Isselbacher KJ, Finkelstein D, Forcione D, Pillai S (2000). Genetic susceptibility to breast cancer: HLA DQB*03032 and HLA DRB1*11 may represent protective alleles. Proc Natl Acad Sci U S A 97:11451-4.

Chen WM, Deng HW (2001). A general and accurate approach for computing the statistical power of the transmission disequilibrium test for complex disease genes. Genet Epidemiol 21:53-67.

Choe S (2002). Potassium channel structures. Nat Rev Neurosci 3:115-21.

Clayton D (1999). A generalization of the transmission/disequilibrium test for uncertain-haplotype transmission. Am J Hum Genet 65:1170-7.

Clerget-Darpoux F, Bonaiti-Pellie C, Hochez J (1986). Effects of misspecifying genetic arametres in LOD score analysis. Biometrics 42: 393-399.

Connor JA, Stevens CF (1971). Prediction of repetitive firing behaviour from voltage clamp data on an isolated neurone soma. J Physiol 213:31-53.

Cooper EC, Aldape KD, Abosch A, Barbaro NM, Berger MS, Peacock WS, Jan YN, Jan LY (2000). Colocalization and coassembly of two human brain M-type potassium channel subunits that are mutated in epilepsy. Proc Natl Acad Sci U S A 97:4914-9.

Cooper EC, Jan LY (1999). Ion channel genes and human neurological disease: recent progress, prospects, and challenges. Proc Natl Acad Sci U S A 96:4759-66.

Cossette P, Liu L, Brisebois K, Dong H, Lortie A, Vanasse M, Saint-Hilaire JM, Carmant L, Verner A, Lu WY, Wang YT, Rouleau GA (2002). Mutation of GABRA1 in an autosomal dominant form of juvenile myoclonic epilepsy. Nat Genet 31:184-9.

Curtis D (1997). Use of siblings as controls in case-control association studies. Ann Hum Genet 61 (Pt 4):319-33.

Daly AK, Day CP (2001). Candidate gene case-control association studies: advantages and potential pitfalls. Br J Clin Pharmacol 52:489-99.

Das B, Chauhan PS, Seshadri M (2002). Genetic variation observed at two tetrameric short tandem repeat loci on chromosome 12 (D12S66 and D12S67) among five distinct ethnic groups of India: detection of two new alleles. Ann Hum Biol 29:513-25.

De Fusco M, Becchetti A, Patrignani A, Annesi G, Gambardella A, Quattrone A, Ballabio A, Wanke E, Casari G (2000). The nicotinic receptor beta 2 subunit is mutant in nocturnal frontal lobe epilepsy. Nat Genet 26:275-6.

de Graaf AS (1992). Prevalence of a photoparoxysmal response in the electroencephalograms of epileptics in the three main ethnic groups of Namibia. Clin Neurol Neurosurg 94 (Suppl):S67-9.

de Graaf AS, Lombard CJ, Claassen DA (1995). Influence of ethnic and geographic factors on the classic photoparoxysmal response in the electroencephalogram of epilepsy patients. Epilepsia 36:219-23.

de Morsier G (1971). [Case study. Concordance and discordance in two monozygotic twins with epilepsy]. J Genet Hum 19:355-9.

Delgado-Escueta AV, Enrile-Bacsal F (1984). Juvenile myoclonic epilepsy of Janz. Neurology 34:285-94.

Delgado-Escueta AV, Greenberg DA, Treiman L, Liu A, Sparkes RS, Barbetti A, Park MS, Terasaki PI (1989). Mapping the gene for juvenile myoclonic epilepsy. Epilepsia 30 Suppl 4:S8-18; discussion S24-7.

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996). A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152-4.

Donis-Keller H, Green P, Helms C, Cartinhour S, Weiffenbach B, Stephens K, Keith TP, Bowden DW, Smith DR, Lander ES, *et al.* (1987). A genetic linkage map of the human genome. Cell 51:319-37.

Dror V, Shamir E, Ghanshani S, Kimhi R, Swartz M, Barak Y, Weizman R, Avivi L, Litmanovitch T, Fantino E, Kalman K, Jones EG, Chandy KG, Gargus JJ, Gutman GA, Navon R (1999). hKCa3/KCNN3 potassium channel gene: association of longer CAG repeats with schizophrenia in Israeli Ashkenazi Jews, expression in human tissues and localization to chromosome 1q21. Mol Psychiatry 4:254-60.

Durner M, Keddache MA, Tomasini L, Shinnar S, Resor SR, Cohen J, Harden C, Moshe SL, Rosenbaum D, Kang H, Ballaban-Gil K, Hertz S, Labar DR, Luciano D, Wallace S, Yohai D, Klotz I, Dicker E, Greenberg DA (2001). Genome scan of idiopathic generalized epilepsy: evidence for major susceptibility gene and modifying genes influencing the seizure type. Ann Neurol 49:328-35.

Durner M, Sander T, Greenberg DA, Johnson K, Beck-Mannagetta G, Janz D (1991). Localization of idiopathic generalized epilepsy on chromosome 6p in families of juvenile myoclonic epilepsy patients. Neurology 41:1651-5.

Durner M, Zhou G, Fu D, Abreu P, Shinnar S, Resor SR, Moshe SL, Rosenbaum D, Cohen J, Harden C, Kang H, Wallace S, Luciano D, Ballaban-Gil K, Klotz I, Dicker E, Greenberg DA (1999). Evidence for linkage of adolescent-onset idiopathic generalized epilepsies to chromosome 8-and genetic heterogeneity. Am J Hum Genet 64:1411-9.

Easteal S, Voorhoeve AH, Zimmet P, Serjeantson SW (1989). RFLP-defined HLA-DP polymorphism in four ethnic groups. Hum Immunol 25:169-79.

Edwin D, Vishwanathan H, Roy S, Usha Rani MV, Majumdar PP (2002). Mitochondrial DNA diversity among five tribal populations of Southern India. Paper presented at Recent Trends in Genomics, XXVII Annual conference of Indian Society of Human Genetics. P3: 49

Eeg-Oloffson O, Petersen I, Sellden U (1971). The development of electroencephalogram in normal children from age 1 through 15: paroxysmal activity. Neuropadiatrie 2:375-404.

Elmslie FV, Rees M, Williamson MP, Kerr M, Kjeldsen MJ, Pang KA, Sundqvist A, Friis ML, Chadwick D, Richens A, Covanis A, Santos M, Arzimanoglou A, Panayiotopoulos CP, Curtis D, Whitehouse WP, Gardiner RM (1997). Genetic mapping of a major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q. Hum Mol Genet 6:1329-34.

Elmslie FV, Williamson MP, Rees M, Kerr M, Kjeldsen MJ, Pang KA, Sundqvist A, Friis ML, Richens A, Chadwick D, Whitehouse WP, Gardiner RM (1996). Linkage analysis of juvenile myoclonic epilepsy and microsatellite loci spanning 61 cM of human chromosome 6p in 19 nuclear pedigrees provides no evidence for a susceptibility locus in this region. Am J Hum Genet 59:653-63.

Elston RC, Stewart J (1971). A general model for the genetic analysis of pedigree data. Hum Hered 21:523-42.

Engel J, Jr. (2001). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 42:796-803.

Ercegovac MD, Vojvodic N, SokicDv, Jankovic SM, Drulovic J, Stojsavljevic N, Levic Z (1998). [Juvenile myoclonic epilepsy]. Srp Arh Celok Lek 126:335-44.

Escayg A, Heils A, MacDonald BT, Haug K, Sander T, Meisler MH (2001). A novel SCN1A mutation associated with generalized epilepsy with febrile seizures plus—and prevalence of variants in patients with epilepsy. Am J Hum Genet 68:866-73.

Fanger CM, Ghanshani S, Logsdon NJ, Rauer H, Kalman K, Zhou J, Beckingham K, Chandy KG, Cahalan MD, Aiyar J (1999). Calmodulin mediates calcium-dependent activation of the intermediate conductance KCa channel, IKCa1. J Biol Chem 274:5746-54.

Figueredo R, Trevisol-Bittencourt PC, Ferro JB (1999). [Clinical-epidemiological study of patients with juvenile myoclonic epilepsy in Santa Catarina State, Brazil]. Arq Neuropsiquiatr 57:401-4.

Fimmers R, Seuchter SA, Neugebauer M, Knapp M, Baur MP (1989). Identity-by-descent analysis using all genotype solutions. In Elston RC, Spence MA, Hodge SE, MacCluer JW (eds) Multipoint mapping and linkage based on affected pedigree members: Genetic Analysis Workshop 6, New York, Liss, pp 123-128.

Fishman PM, Suarez B, Hodge SE, Reich T (1978). A robust method for the detection of linkage in familial disease. Am J Hum Genet 30:308-21.

Gelisse P, Genton P, Thomas P, Rey M, Samuelian JC, Dravet C (2001). Clinical factors of drug resistance in juvenile myoclonic epilepsy. J Neurol Neurosurg Psychiatry 70:240-3.

Genton P, Gelisse P, Thomas P, Dravet C (2000). Do carbamazepine and phenytoin aggravate juvenile myoclonic epilepsy? Neurology 55:1106-9.

Ghosh A, Das B, Seshadri M (2003). Population genetic analysis among five Indian population groups using six microsatellite markers. Hum Biol 75:189-203.

Giguere Y, Dewailly E, Brisson J, Ayotte P, Laflamme N, Demers A, Forest VI, Dodin S, Robert J, Rousseau F (2001). Short polyglutamine tracts in the androgen receptor are protective against breast cancer in the general population. Cancer Res 61:5869-74.

Goate A, Chartier-Harlin MC, Mullan M, Brown J, Crawford F, Fidani L, Giuffra L, Haynes A, Irving N, James L, *et al.* (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704-6.

Gordon N (1998). Episodic ataxia and channelopathies. Brain Dev 20:9-13.

Gram L, Alving J, Sagild JC, Dam M (1988). Juvenile myoclonic epilepsy in unexpected age groups. Epilepsy Res 2:137-40.

Greenberg DA, Delgado-Escueta AV (1993). The chromosome 6p epilepsy locus: exploring mode of inheritance and heterogeneity through linkage analysis. Epilepsia 34 (Suppl) 3:S12-8.

Greenberg DA, Delgado-Escueta AV, Maldonado HM, Widelitz H (1988a). Segregation analysis of juvenile myoclonic epilepsy. Genet Epidemiol 5:81-94.

Greenberg DA, Delgado-Escueta AV, Widelitz H, Sparkes RS, Treiman L, Maldonado HM, Park MS, Terasaki PI (1988b). Juvenile myoclonic epilepsy (JME) may be linked to the BF and HLA loci on human chromosome 6. Am J Med Genet 31:185-92.

Greenberg DA, Durner M, Delgado-Escueta AV (1992). Evidence of multiple gene loci in the expression of the common generalized epilepsies. Neurology 37:993-1000.

Greenberg DA, Durner M, Keddache M, Shinnar S, Resor SR, Moshe SL, Rosenbaum D, Cohen J, Harden C, Kang H, Wallace S, Luciano D, Ballaban-Gil K, Tomasini L, Zhou G, Klotz I, Dicker E (2000). Reproducibility and complications in gene searches: linkage on chromosome 6, heterogeneity, association, and maternal inheritance in juvenile myoclonic epilepsy. Am J Hum Genet 66:508-16.

Griseri P, Pesce B, Patrone G, Osinga J, Puppo F, Sancandi M, Hofstra R, Romeo G, Ravazzolo R, Devoto M, Ceccherini I (2002). A rare haplotype of the RET proto-oncogene is a risk-modifying allele in hirschsprung disease. Am J Hum Genet 71:969-74.

Grünewald RA, Chroni E, Panayiotopoulos CP (1992). Delayed diagnosis of juvenile myoclonic epilepsy. J Neurol Neurosurg Psychiatry 55:497-9.

Grünewald RA, Panayiotopoulos CP (1993). Juvenile myoclonic epilepsy. A review. Arch Neurol 50:594-8.

Gudbjartsson DF, Jonasson K, Frigge ML, Kong A (2000). Allegro, a new computer program for multipoint linkage analysis. Nat Genet 25:12-3.

Gulcher JR, Kong A, Stefansson K (2001). The role of linkage studies for common diseases. Curr Opin Genet Dev 11:264-7.

Gunatilake SB, Seneviratne SL (2000). Juvenile myoclonic epilepsy: a study in Sri Lanka. Seizure 9:221-3.

Hallmann K, Durner M, Sander T, Steinlein OK (2000). Mutation analysis of the inwardly rectifying K(+) channels KCNJ6 (GIRK2) and KCNJ3 (GIRK1) in juvenile myoclonic epilepsy. Am J Med Genet 96:8-11.

Hamdi HK, Reznik J, Castellon R, Atilano SR, Ong JM, Udar N, Tavis JH, Aoki AM, Nesburn AB, Boyer DS, Small KW, Brown DJ, Kenney MC (2002). Alu DNA polymorphism in ACE gene is protective for age-related macular degeneration. Biochem Biophys Res Commun 295:668-72.

Harvald B, Hauge M (1965). Hereditary factors elucidated by twin studies. In: Neel JV, Shaw MW, Schull WJ (eds) Genetics and the epidemiology of chronic diseases. US Department of Health and Human Services, Washington, D.C., Public Health Service, pp 61-96.

Haseman JK, Elston RC (1972). The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2:3-19.

Haug K, Hallmann K, Horvath S, Sander T, Kubisch C, Rau B, Dullinger J, Beyenburg S, Elger CE, Propping P, Heils A (2000a). No evidence for association between the *KCNQ3* gene and susceptibility to idiopathic generalized epilepsy. Epilepsy Res 42:57-62.

Haug K, Kremerskothen J, Hallmann K, Sander T, Dullinger J, Rau B, Beyenburg S, Lentze MJ, Barnekow A, Elger CE, Propping P, Heils A (2000b). Mutation screening of the chromosome 8q24.3-human activity-regulated cytoskeleton-associated gene (ARC) in idiopathic generalized epilepsy. Mol Cell Probes 14:255-60.

Heilstedt HA, Burgess DL, Anderson AE, Chedrawi A, Tharp B, Lee O, Kashork CD, Starkey DE, Wu YQ, Noebels JL, Shaffer LG, Shapira SK (2001). Loss of the potassium channel beta-subunit gene, KCNAB2, is associated with epilepsy in patients with 1p36 deletion syndrome. Epilepsia 42:1103-11.

Herpin TH (1867). *Des Accès Incomplets de L'èpilepsie. J Balliere et Fils.*

Hirose S, Iwata H, Akiyoshi H, Kobayashi K, Ito M, Wada K, Kaneko S, Mitsudome A (1999). A novel mutation of CHRNA4 responsible for autosomal dominant nocturnal frontal lobe epilepsy. Neurology 53:1749-53.

Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K (2002). A comprehensive review of genetic association studies. Genet Med 4:45-61.

Hodge SE (1984). The information contained in multiple sibling pairs. Genet Epidemiol 1:109-122.

Holmans P (1998). Affected sib-pair methods for detecting linkage to dichotomous traits: review of the methodology. Hum Biol 70:1025-40.

Huang Y, Paxton WA, Wolinsky SM, Neumann AU, Zhang L, He T, Kang S, Ceradini D, Jin Z, Yazdanbakhsh K, Kunstman K, Erickson D, Dragon E, Landau NR, Phair J, Ho DD, Koup RA (1996). The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. Nat Med 2:1240-3.

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, Baptista R, Krugylak L. Xu SH, et al. (1995). An STS based map of the human genome. Science 270: 1919-1920.

Huntington's Disease Collaborative Research Group (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971-83.

ILAE (1981). Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 22:489-501.

ILAE (1989). Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 30:389-99.

ILAE. (1993). Commission on Epidemiology and Prognosis. Guidelines for epidemiologic studies on epilepsy. Epilepsia 34:592-6.

Italian-League-Against-Epilepsy-Genetic-Collaborative-Group (1993). Concordance of clinical forms of epilepsy in families with several affected members. Italian League Against Epilepsy Genetic Collaborative Group. Epilepsia 34:819-26.

Jain S, Jain MS, Padma MV, Puri A, Sen P, Maheshwari MC (1998a). Epilepsies among twins born in families of Indian probands with epilepsy. Seizure 7:139-43.

Jain S, Padma MV, Maheshwari MC (1997). Occurrence of only myoclonic jerks in juvenile myoclonic epilepsy. Acta Neurol Scand 95:263-7.

Jain S, Padma MV, Maheswari MC (1999). Juvenile myoclonic epilepsy: The Indian Perspective. In: Murthy JMK (ed) Epilepsy: The Indian Perspective. CARE Foundation, Hyderabad, pp 107-116.

Jain S, Padma MV, Puri A, Maheshwari MC (1998b). Juvenile myoclonic epilepsy: disease expression among Indian families. Acta Neurol Scand 97:1-7.

Jain S, Tripathi M, Srivastava AK, Narula A (2003). Phenotypic analysis of juvenile myoclonic epilepsy in Indian families. Acta Neurol Scand 107:356-62.

Janz D (1969). *Die Epilepsien*. Stuttgart, Germany, Georg Thieme.

Janz D (1985). Epilepsy with impulsive petit mal (juvenile myoclonic epilepsy). Acta Neurol Scand 72:449-59.

Janz D (1989). Juvenile myoclonic epilepsy. Epilepsy with impulsive petit mal. Cleve Clin J Med 56 (Suppl) Pt 1:S23-33; discussion S40-2.

Janz D (1990). Juvenile myoclonic epilepsy. In: Dam M, Gram L (eds) Comprehensive epileptology. New York, NY, Raven Press, pp 171-185.

Janz D, Durner M (1997). Juvenile myoclonic epilepsy. In: Engel J, Jr., Pedley TA (eds) Epilepsy: A Comprehensive Textbook. Vol 2. Philadelphia, Lippincott-Raven Publishers, pp 2389-400.

Jeavons PM, Bishop A, Harding GF (1986). The prognosis of photosensitivity. Epilepsia 27:569-75.

Jimenez I, Mora O, Jimenez M, Zuluaga L, Isaza R, Sanchez JL, Uribe CS, Blanco R, Arcos-Burgos M (1996). Complex segregation analysis of non-myoclonic idiopathic generalized epilepsy in families ascertained from probands affected with idiopathic epilepsy with tonic-clonic seizures in Antioquia, Colombia. Hum Genet 98:214-8.

Jin HW, Wang XL (2002). [Voltage-gated potassium channels and human neurological diseases]. Sheng Li Ke Xue Jin Zhan 33:21-5.

Kamboh MI (1984). Population genetic studies of PI, Tf, Gc and PGM1 subtypes among various caste groups in North India. Acta Anthropogenet 8:159-79.

Kan YW, Dozy AM (1978). Polymorphism of DNA sequence adjacent to human beta-globin structural gene: relationship to sickle mutation. Proc Natl Acad Sci U S A 75:5631-5.

Kasteleijn-Nolst Trenite DG (1989). Photosensitivity in epilepsy. Electrophysiological and clinical correlates. Acta Neurol Scand Suppl 125:3-149.

Kaupmann K, Schuler V, Mosbacher J, Bischoff S, Bittiger H, Heid J, Froestl W, Leonhard S, Pfaff T, Karschin A, Bettler B (1998). Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K^+ channels. Proc Natl Acad Sci U S A 95:14991-6.

Kirk KM, Doege KJ, Hecht J, Bellamy N, Martin NG (2003). Osteoarthritis of the hands, hips and knees in an Australian twin sample—evidence of association with the aggrecan VNTR polymorphism. Twin Res 6:62-6.

Kjeldsen MJ, Kyvik KO, Christensen K, Friis ML (2001). Genetic and environmental factors in epilepsy: a population-based study of 11900 Danish twin pairs. Epilepsy Res 44:167-78.

Kleveland G, Engelsen BA (1998). Juvenile myoclonic epilepsy: clinical characteristics, treatment and prognosis in a Norwegian population of patients. Seizure 7:31-8.

Kong A, Cox NJ (1997). Allele-sharing models: LOD scores and accurate linkage tests. Am J Hum Genet 61:1179-88.

Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES (1996). Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347-63.

Kruglyak L, Lander ES (1995). Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 57:439-54.

Kun LN, Ling LW, Wah YW, Lian TT (1999). Epidemiologic study of epilepsy in young Singaporean men. Epilepsia 40:1384-7.

Lancman ME, Asconape JJ, Penry JK (1994). Clinical and EEG asymmetries in juvenile myoclonic epilepsy. Epilepsia 35:302-6.

Lander ES, Kruglyak L (1995). Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 11:241-7.

Lange K (1986). The affected sib-pair method using identity by state relations. Am. J. Hum Genet 39: 148-150.

Lathrop M, Ott J (1990). Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443-3446.

Latorre R, Oberhauser A, Labarca P, Alvarez O (1989). Varieties of calcium-activated potassium channels. Annu Rev Physiol 51:385-99.

Lazzeroni LC, Lange K (1998). A conditional inference framework for extending the transmission/disequilibrium test. Hum Hered 48:67-81.

Lennox WG (1945). The petit mal epilepsies. JAMA 129:1069-73.

Lennox WG (1953). [Heredity of epilepsy, studies in families of patients and in twins.]. Pediatr Am 10:251-2.

Leppert M, McMahon WM, Quattlebaum TG, Bjerre I, Zonana J, Shevell MI, Andermann E, Rosales TO, Ronen GM, Connolly M, *et al.* (1993). Searching for human epilepsy genes: a progress report. Brain Pathol 3:357-69.

Lewis TB, Leach RJ, Ward K, O'Connell P, Ryan SG (1993). Genetic heterogeneity in benign familial neonatal convulsions: identification of a new locus on chromosome 8q. Am J Hum Genet 53:670-5.

Liao YJ, Jan YN, Jan LY (1996). Heteromultimerization of G-protein-gated inwardly rectifying K^+ channel proteins GIRK1 and GIRK2 and their altered expression in weaver brain. J Neurosci 16:7137-50.

Liu AW, Delgado-Escueta AV, Gee MN, Serratosa JM, Zhang QW, Alonso ME, Medina MT, Cordova S, Zhao HZ, Spellman JM, Donnadieu FR, Peek JR, Treiman LJ, Sparkes RS (1996). Juvenile myoclonic epilepsy in chromosome 6p12-p11: locus heterogeneity and recombinations. Am J Med Genet 63:438-46.

Liu AW, Delgado-Escueta AV, Serratosa JM, Alonso ME, Medina MT, Gee MN, Cordova S, Zhao HZ, Spellman JM, Peek JR, *et al.* (1995). Juvenile myoclonic epilepsy locus in chromosome 6p21.2-p11: linkage to convulsions and electroencephalography trait. Am J Hum Genet 57:368-81.

Liu J, Nyholt DR, Magnussen P, Parano E, Pavone P, Geschwind D, Lord C, Iversen P, Hoh J, Ott J, Gilliam TC (2001). A genomewide screen for autism susceptibility loci. Am J Hum Genet 69:327-40.

Lopes-Cendes I, Scheffer IE, Berkovic SF, Rousseau M, Andermann E, Rouleau GA (2000). A new locus for generalized epilepsy with febrile seizures plus maps to chromosome 2. Am J Hum Genet 66:698-701.

Lund M, Reintoft H, Simonsen N (1975). [A controlled social and psychological investigation of patients with juvenile myoclonic epilepsy]. Ugeskr Laeger 137:2415-8.

Lundborg H (1903). *Die Progressive Myoklonus-Epilepsie (Unverricht's Myoklonie)*. Uppsala, Sweden, Almquist and Wiksell.

Madeja M, Musshoff U, Speckmann EJ (1997). Diversity of potassium channels contributing to differences in brain area-specific seizure susceptibility: sensitivity of different potassium channels to the epileptogenic agent pentylenetetrazol. Eur J Neurosci 9:390-5.

Main MJ, Cryan JE, Dupere JR, Cox B, Clare JJ, Burbidge SA (2000). Modulation of *KCNQ2*/3 potassium channels by the novel anticonvulsant retigabine. Mol Pharmacol 58:253-62.

Mani KS, Rangan G (1995). Primary generalized epilepsies-Absence seizures and juvenile myoclonic epilepsy: An Indian experience. Paper presented at Proceedings of first Indo-US workshop on genetics in epilepsies. AIIMS, New Delhi.

Manonmani V, Tan CT (1993). Juvenile myoclonic epilepsy—a report of 11 patients at the University Hospital, Kuala Lumpur. Singapore Med J 34:378-80.

Matsuoka H (1989). [A clinical and electroencephalographic study of juvenile myoclonic epilepsy: its pathophysiological considerations based on the findings obtained from neuropsychological EEG activation]. Seishin Shinkeigaku Zasshi 91:318-46.

McGinnis RE (1998.) Hidden linkage: a comparison of the affected sib pair (ASP) test and transmission/disequilibrium test (TDT). Ann Hum Genet 62 (Pt 2):159-79.

McKusick, V.A (1998). Mendelian Inheritance in Man. A Catalog of Human Genes and Genetic Disorders, 12th edn. Baltimore, MD, Johns Hopkins University Press.

McNamara JO (1999). Emerging insights into the genesis of epilepsy. Nature 399:A15-22.

McPeek MS (1999). Optimal allele-sharing statistics for genetic mapping using affected relatives. Genet Epidemiol 16:225-49.

Meencke HJ, Janz D (1984). Neuropathological findings in primary generalized epilepsy: a study of eight cases. Epilepsia 25:8-21.

Meencke HJ, Janz D (1985). The significance of microdysgenesia in primary generalized epilepsy: an answer to the considerations of Lyon and Gastaut. Epilepsia 26:368-71.

Mehndiratta MM, Aggarwal P (2002). Clinical expression and EEG features of patients with juvenile myoclonic epilepsy (JME) from North India. Seizure 11:431-6.

Minassian BA, Sainz J, Delgado-Escueta AV (1995). Genetics of myoclonic and myoclonus epilepsies. Clin Neurosci 3:223-35.

Mochi M, Provini F, Plazzi G, Corsini R, Farnedi F, Tinuper P, Valentino ML, Lugaresi E, Montagna P (1997). Genetic heterogeneity in autosomal dominant nocturnal frontal lobe epilepsy. Ital J Neurol Sci 18:183.

Morton NE (1996). Logarithm of odds (lods) for linkage in complex inheritance. Proc Natl Acad Sci U S A 93:3471-6.

Moulard B, Guipponi M, Chaigne D, Mouthon D, Buresi C, Malafosse A (1999). Identification of a new locus for generalized epilepsy with febrile seizures plus (GEFS+) on chromosome 2q24-q33. Am J Hum Genet 65:1396-400.

Mulley JC, Scheffer IE, Petrou S, Berkovic SF (2003). Channelopathies as a genetic cause of epilepsy. Curr Opin Neurol 16:171-6.

Murthy JM (1999). Factors of error involved in the diagnosis of juvenile myoclonic epilepsy: A study from South India. Neurol India 47:210-3.

Murthy JM, Rao CM, Meena AK (1998). Clinical observations of juvenile myoclonic epilepsy in 131 patients: a study in South India. Seizure 7:43-7.

Myslobodsky MS, Mintz M, Douglas R (1980). Electroencephalographic and behavioural effects of sodium valproate in patients with photosensitive epilepsy. A single dose trial. J Neurol 224:111-23.

Nowotny P, Kwon JM, Goate AM (2001). SNP analysis to dissect human traits. Curr Opin Neurobiol 11:637-41.

Obach V, Arroyo S, Santamaria J, Grinberg D, Oliva R (2000). No evidence of linkage to 6p markers in Spanish families with juvenile myoclonic epilepsy. Neurosci Lett 286:213-7.

Obeid T, Panayiotopoulos CP (1988). Juvenile myoclonic epilepsy: a study in Saudi Arabia. Epilepsia 29:280-2.

Oberle I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, Bertheas MF, Mandel JL (1991). Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 252:1097-102.

Okubo Y, Matsuura M, Asai T, Asai K, Kato M, Kojima T, Toru M (1994). Epileptiform EEG discharges in healthy children: prevalence, emotional and behavioural correlates, and genetic influences. Epilepsia 35:832-841.

Oradovskaia IV (1970). [The use of twins in the study of epilepsy in children]. Vopr Okhr Materin Det 15:41-6.

Ott J (1999). Analysis of Human Genetic Linkage. Vol 3rd Edition. Baltimore, Johns Hopkins University Press.

Ottman R, Annegers JF, Hauser WA, Kurland LT (1988). Higher risk of seizures in offspring of mothers than of fathers with epilepsy. Am J Hum Genet 43:257-64.

Ottman R, Annegers JF, Kurland LT (1991). Familial aggregation and severity of epilepsy. Epilepsia 32:523-9.

Ottman R, Annegers JF, Risch N, Hauser WA, Susser M (1996). Relations of genetic and environmental factors in the etiology of epilepsy. Ann Neurol 39:442-9.

Panagariya A, Sureka RK, Sardana V (2001). Juvenile myoclonic epilepsy—an experience from north western India. Acta Neurol Scand 104:12-6.

Panayiotopoulos CP, Obeid T (1989). Juvenile myoclonic epilepsy: an autosomal recessive disease. Ann Neurol 25:440-3.

Panayiotopoulos CP, Obeid T, Tahan AR (1994). Juvenile myoclonic epilepsy: a 5-year prospective study. Epilepsia 35:285-96.

Panayiotopoulos CP, Obeid T, Waheed G (1989). Differentiation of typical absence seizures in epileptic syndromes. A video EEG study of 224 seizures in 20 patients. Brain 112 (Pt 4):1039-56.

Panayiotopoulos CP, Tahan R, Obeid T (1991). Juvenile myoclonic epilepsy: factors of error involved in the diagnosis and treatment. Epilepsia 32:672-6.

Parniewski P, Staczek P (2002). Molecular mechanisms of TRS instability. Adv Exp Med Biol 516:1-25.

Payami H, Thomson G, Louis EJ (1984). The affected sib method. III. Selection and recombination. Am J Hum Genet 36:352-62.

Pedersen SB, Petersen KA (1998). Juvenile myoclonic epilepsy: clinical and EEG features. Acta Neurol Scand 97:160-3.

Penrose LS (1953). The general purpose sibpair linkage test. Ann Eugen 18:120-4.

Penry JK, Dean JC, Riela AR (1989). Juvenile myoclonic epilepsy: long-term response to therapy. Epilepsia 30 (Suppl) 4:S19-23; discussion S24-7.

Peters HC, Kammer G, Volz A, Kaupmann K, Ziegler A, Bettler B, Epplen JT, Sander T, Riess O (1998). Mapping, genomic structure, and polymorphisms of the human GABABR1 receptor gene: evaluation of its involvement in idiopathic generalized epilepsy. Neurogenetics 2:47-54.

Petrukhin AS, Mukhin KI, Kolpachki LM, Glyzova KI, Volchenkova MM (1997). [Television epilepsy]. Zh Nevrol Psikhiatr Im S S Korsakova 97:13-6.

Prasad AN, Prasad C, Stafstrom CE (1999). Recent advances in the genetics of epilepsy: insights from human and animal studies. Epilepsia 40:1329-52.

Rabot L (1899). *De la Myoclonie Épileptique*. Thesis, Paris, *Faculté de Médecine de Paris*.

Race RR, Sanger R (1975). Blood groups in man. Oxford, Blackwell Scientific Publications.

Radhakrishnan K, Nayak SD, Nandini VS, Venugopal A (1998). Prevalence of photoparoxysmal response among South Indian epilepsy patients. Seizure 7:397-401.

Rao PM, Blake NM, Veerraju P (1978). Genetic studies on the Savara and Jatapu Tribes of Andhra Pradesh, India. Hum Hered 28:122-31.

Rees M, Curtis D, Parker K, Sundqvist A, Baralle D, Bespalova IN, Burmeister M, Chung E, Gardiner RM, Whitehouse WP (1994). Linkage analysis of idiopathic generalised epilepsy in families of probands with Juvenile Myoclonic Epilepsy and marker loci in the region of EPM 1 on chromosome 21 q: Unverricht-Lundborg disease and JME are not allelic variants. Neuropediatrics 25:20-5.

Reutens DC, Berkovic SF (1995). Idiopathic generalized epilepsy of adolescence: are the syndromes clinically distinct? Neurology 45:1469-76.

Risch N (1990a). Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 46:222-8.

Risch N (1990b). Linkage strategies for genetically complex traits. III. The effect of marker polymorphism on analysis of affected relative pairs. Am J Hum Genet 46:242-53.

Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS (1999). Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 65:876-84.

Robitaille Y, Lopes-Cendes I, Becher M, Rouleau G, Clark AW (1997). The neuropathology of CAG repeat diseases: review and update of genetic and molecular features. Brain Pathol 7:901-26.

Rosanoff AJ, Handy LM, Rosanoff IA (1934). Etiology of epilepsy with special reference to its occurence in twins. Arch Neurol Psychiatry 31:1165-93.

Saha A, Husain S, Bamezai R (2000). Characterization of a subcloned fragment (pBA0.6) of pCMM86 located on 17q21 and its potential use in generating an individual-specific DNA profile. DNA Cell Biol 19:219-26.

Saha N, Kirk RL, Shanbhag S, Joshi SR, Bhatia HM (1976). Population genetic studies in kerala and the nilgiris (South West India). Hum Hered 26:175-97.

Salas Puig J, Tunon A, Vidal JA, Mateos V, Guisasola LM, Lahoz CH (1994). [Janz's juvenile myoclonic epilepsy: a little-known frequent syndrome. A study of 85 patients]. Med Clin (Barc) 103:684-9.

Saleem Q, Sreevidya VS, Sudhir J, Savithri JV, Gowda Y, C BR, Benegal V, Majumder PP, Anand A, Brahmachari SK, Jain S (2000). Association analysis of CAG repeats at the KCNN3 locus in Indian patients with bipolar disorder and schizophrenia. Am J Med Genet 96:744-8.

Saleem SM, Thomas M, Jain S, Maheshwari MC (1994). Incidence of photosensitive epilepsy in unselected Indian epileptic population. Acta Neurol Scand 89:5-8.

Sambrook J, Fritsch E, Maniatis T (1989a). Molecular cloning: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sambrook J, Fritsch EF, Maniatis T (1989b). Molecular Cloning: a laboratory Manual. Vol 2. Cold Spring harbor: Cold Spring Harbor Laboratory Press.

Sander T, Bockenkamp B, Hildmann T, Blasczyk R, Kretz R, Wienker TF, Volz A, Schmitz B, Beck-Mannagetta G, Riess O, Epplen JT, Janz D, Ziegler A (1997). Refined mapping of the epilepsy susceptibility locus EJM1 on chromosome 6. Neurology 49:842-7.

Sander T, Peters C, Janz D, Bianchi A, Bauer G, Wienker TF, Hildmann T, Epplen JT, Riess O (1998). The gene encoding the alpha1A-voltage-dependent calcium channel (CACN1A4) is not a candidate for causing common subtypes of idiopathic generalized epilepsy. Epilepsy Res 29:115-22.

Sander T, Peters C, Kammer G, Samochowiec J, Zirra M, Mischke D, Ziegler A, Kaupmann K, Bettler B, Epplen JT, Riess O (1999a). Association analysis of exonic variants of the gene encoding the GABAB receptor and idiopathic generalized epilepsy. Am J Med Genet 88:305-10.

Sander T, Scholz L, Janz D, Epplen JT, Riess O (1999b). Length variation of a polyglutamine array in the gene encoding a small-conductance, calcium-activated potassium channel (hKCa3) and susceptibility to idiopathic generalized epilepsy. Epilepsy Res 33:227-33.

Sander T, Schulz H, Saar K, Gennaro E, Riggio MC, Bianchi A, Zara F, *et al.* (2000). Genome search for susceptibility loci of common idiopathic generalised epilepsies. Hum Mol Genet 9:1465-72.

Sander T, Schulz H, Vieira-Saeker AM, Bianchi A, Sailer U, Bauer G, Scaramelli A, Wienker TF, Saar K, Reis A, Janz D, Epplen JT, Riess O (1999c). Evaluation of a putative major susceptibility locus for juvenile myoclonic epilepsy on chromosome 15q14. Am J Med Genet 88:182-7.

Schaid DJ, Jacobsen SJ (1999). Biased tests of association: comparisons of allele frequencies when departing from Hardy-Weinberg proportions. Am J Epidemiol 149:706-11.

Scheffer IE, Berkovic SF (1997). Generalized epilepsy with febrile seizures plus. A genetic disorder with heterogeneous clinical phenotypes. Brain 120 (Pt 3):479-90.

Schroeder BC, Kubisch C, Stein V, Jentsch TJ (1998). Moderate loss of function of cyclic-AMP-modulated *KCNQ2/KCNQ3* K^+ channels causes epilepsy. Nature 396:687-90.

Schulze TG, McMahon FJ (2002). Genetic association mapping at the crossroads: Which test and why? Overview and practical guidelines. Am J Med Genet 114:1-11.

Schumacher M, Adelman JP (2002). Ion channels: an open and shut case. Nature 417:501-2.

Serratosa JM, Delgado-Escueta AV, Medina MT, Zhang Q, Iranmanesh R, Sparkes RS (1996). Clinical and genetic analysis of a large pedigree with juvenile myoclonic epilepsy. Ann Neurol 39:187-95.

Sham PC, Curtis D (1995). Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. Ann Hum Genet 59 (Pt 1):97-105.

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, *et al.* (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375:754-60.

Shih MC, Whittemore AS (2001). Allele-sharing among affected relatives: non-parametric methods for identifying genes. Stat Methods Med Res 10:27-55.

Shinnar S, Berg AT (1994). Does antiepileptic drug therapy alter the prognosis of childhood seizures and prevent the development of chronic epilepsy? Semin Pediatr Neurol 1:111-7.

Shiraishi H, Fujiwara T, Inoue Y, Yagi K (2001). Photosensitivity in relation to epileptic syndromes: asurvey from an epilepsy center in Japan. Epilepsia 42:393-7.

Sillanpaa M, Koskenvuo M, Romanov K, Kaprio J (1991). Genetic factors in epileptic seizures: evidence from a large twin population. Acta Neurol Scand 84:523-6.

Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M (1998). A novel potassium channel gene, *KCNQ2*, is mutated in an inherited epilepsy of newborns. Nat Genet 18:25-9.

Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, Schwartzkroin PA, Messing A, Tempel BL (1998). Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. Neuron 20:809-19.

Spielman RS, McGinnis RE, Ewens WJ (1993). Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet 52:506-16.

Steinlein O, Schuster V, Fischer C, Haussler M (1995a). Benign familial neonatal convulsions: confirmation of genetic heterogeneity and further evidence for a second locus on chromosome 8q. Hum Genet 95:411-5.

Steinlein OK (2002). Channelopathies can cause epilepsy in man. Eur J Pain 6 Suppl A:27-34.

Steinlein OK, Mulley JC, Propping P, Wallace RH, Phillips HA, Sutherland GR, Scheffer IE, Berkovic SF (1995b). A missense mutation in the neuronal nicotinic acetylcholine receptor alpha 4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet 11:201-3.

Suarez BK (1978). The affected sib pair IBD distribution for HLA-linked susceptibility genes Tisue Antigens 12: 87-93.

Sugawara T, Tsurubuchi Y, Agarwala KL, Ito M, Fukuma G, Mazaki-Miyazaki E, Nagafuji H, Noda M, Imoto K, Wada K, Mitsudome A, Kaneko S, Montal M, Nagata K, Hirose S, Yamakawa K (2001). A missense mutation of the Na+ channel alpha II subunit gene Na(v)1.2 in a patient with febrile and afebrile seizures causes channel dysfunction. Proc Natl Acad Sci U S A 98:6384-9.

Suzuki T, Morita R, Sugimoto Y, Sugawara T, Bai DS, Alonso ME, Medina MT, Bailey JN, Rasmussen A, Ramos-Peek J, Cordova S, Rubio-Donnadieu F, Ochoa A, Jara-Prado A, Inazawa J, Delgado-Escueta AV, Yamakawa K (2002). Identification and mutational analysis of candidate genes for juvenile myoclonic epilepsy on 6p11-p12: LRRC1, GCLC, KIAA0057 and CLIC5. Epilepsy Res 50:265-75.

Swartz BE, Halgren E, Simpkins F, Mandelkern M (1996a). Studies of working memory using 18FDG-positron emission tomography in normal controls and subjects with epilepsy. Life Sci 58:2057-64.

Swartz BE, Rich JR, Dwan PS, DeSalles A, Kaufman MH, Walsh GO, Delgado-Escueta AV (1996b). The safety and efficacy of chronically implanted subdural electrodes: a prospective study. Surg Neurol 46:87-93.

Swartz BE, Simpkins F, Halgren E, Mandelkern M, Brown C, Krisdakumtorn T, Gee M (1996c). Visual working memory in primary generalized epilepsy: an 18FDG-PET study. Neurology 47:1203-12.

Taske NL, Williamson MP, Makoff A, Bate L, Curtis D, Kerr M, Kjeldsen MJ, Pang KA, Sundqvist A, Friis ML, Chadwick D, Richens A, Covanis A, Santos M, Arzimanoglou A, Panayiotopoulos CP, Whitehouse WP, Rees M, Gardiner RM (2002). Evaluation of the positional candidate gene CHRNA7 at the juvenile myoclonic epilepsy locus (EJM2) on chromosome 15q13-14. Epilepsy Res 49:157-72.

Tassinari CA, Lyagoubi S, Santos V, Gambarelli G, Roger J, Dravet C, Gastaut H (1969). [Study on spike and wave discharges in man. II. Clinical and electroencephalographic aspects of myoclonic absences]. Rev Neurol (Paris) 121:379-83.

Terwilliger J, Ott J (1994). Handbook of human genetic linkage. Johns Hopkins University Press.

Terwindt GM, Ophoff RA, Haan J, Sandkuijl LA, Frants RR, Ferrari MD (1998). Migraine, ataxia and epilepsy: a challenging spectrum of genetically determined calcium channelopathies. Dutch Migraine Genetics Research Group. Eur J Hum Genet 6:297-307.

Thankappan KR, Valiathan MS (1998). Health at low cost: the Kerala model. Lancet 351:1274-75.

Timchenko LT, Caskey CT (1999). Triplet repeat disorders: discussion of molecular mechanisms. Cell Mol Life Sci 55:1432-47.

Tinel N, Lauritzen I, Chouabe C, Lazdunski M, Borsotto M (1998). The *KCNQ2* potassium channel: splice variants, functional and developmental expression. Brain localization and comparison with *KCNQ3*. FEBS Lett 438:171-6.

Touchon J (1982). Effect of awakening on epileptic activity in primary generalized myoclonic epilepsy. In: Sterman MB, Shouse MN, Passouant P (eds) Sleep and Epilepsy. New York, NY: Academic Press, pp 239-48.

Unverricht H (1895). Über familiare Myoklonie. *Dtsch Z Nervenheilk* 7:32-67.

Vazquez B, Devinsky O, Luciano D, Alper K, Perrine K (1993). Juvenile myoclonic epilepsy: clinical features and factors related to misdiagnosis. J Epilepsy 6:233-8.

Vergara C, Latorre R, Marrion NV, Adelman JP (1998). Calcium-activated potassium channels. Curr Opin Neurobiol 8:321-9.

Wall TL, Carr LG, Ehlers CL (2003). Protective association of genetic variation in alcohol dehydrogenase with alcohol dependence in Native American Mission Indians. Am J Psychiatry 160:41-6.

Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF (2001). Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28:49-52.

Wallace RH, Wang DW, Singh R, Scheffer IE, George AL, Jr., Phillips HA, Saar K, Reis A, Johnson EW, Sutherland GR, Berkovic SF, Mulley JC (1998). Febrile seizures and generalized epilepsy associated with a mutation in the Na+-channel beta1 subunit gene SCN1B. Nat Genet 19:366-70.

Wang HS, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, Dixon JE, McKinnon D (1998). *KCNQ2* and *KCNQ3* potassium channel subunits: molecular correlates of the M-channel. Science 282:1890-3.

Watkins WS, Bamshad M, Dixon ME, Bhaskara Rao B, Naidu JM, Reddy PG, Prasad BV, Das PK, Reddy PC, Gai PB, Bhanu A, Kusuma YS, Lum JK, Fischer P, Jorde LB (1999). Multiple origins of the mtDNA 9-bp deletion in populations of South India. Am J Phys Anthropol 109:147-58.

Weber JL, May PE (1989). Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388-96.

Weeks DE, Lange K (1988). The affected-pedigree-member method of linkage analysis. Am J Hum Genet 42:315-26.

Weeks DE, Ott J, Lathrop M (1990). SLINK: a general simulation program for linkage analysis. Am J Hum Genet (Suppl) 47:A204.

Weiss KM (1993). Genetic variation and human disease: principles and evolutionary approaches. Cambridge University Press, Cambridge.

Weissbecker KA, Durner M, Janz D, Scaramelli A, Sparkes RS, Spence MA (1991). Confirmation of linkage between juvenile myoclonic epilepsy locus and the HLA region of chromosome 6. Am J Med Genet 38:32-6.

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M (1992). A second-generation linkage map of the human genome. Nature 359:794-801.

Whitehouse WP, Rees M, Curtis D, Sundqvist A, Parker K, Chung E, Baralle D, Gardiner RM (1993). Linkage analysis of idiopathic generalized epilepsy (IGE) and marker loci on chromosome 6p in families of patients with juvenile myoclonic epilepsy: no evidence for an epilepsy locus in the HLA region. Am J Hum Genet 53:652-62.

Whittemore AS, Halpern J (1994). A class of tests for linkage using affected pedigree members. Biometrics 50:118-27.

Woermann FG, Free SL, Koepp MJ, Ashburner J, Duncan JS (1999a). Voxel-by-voxel comparison of automatically segmented cerebral gray matter—A rater-independent comparison of structural MRI in patients with epilepsy. Neuroimage 10:373-84.

Woermann FG, Free SL, Koepp MJ, Sisodiya SM, Duncan JS (1999b). Abnormal cerebral structure in juvenile myoclonic epilepsy demonstrated with voxel-based analysis of MRI. Brain 122: 2101-8.

Wolf P, Goosses R (1986). Relation of photosensitivity to epileptic syndromes. J Neurol Neurosurg Psychiatry 49:1386-91.

Yamada M, Tsuji S, Takahashi H (2000). Pathology of CAG repeat diseases. Neuropathology 20:319-25.

Yi BA, Minor DL, Jr., Lin YF, Jan YN, Jan LY (2001). Controlling potassium channel activities: Interplay between the membrane and intracellular factors. Proc Natl Acad Sci U S A 98:11016-23.

Zachariah KC (1998). Models of development and demographic changes: a case study of Kerala. Demography India 27:71-89.

Zara F, Bianchi A, Avanzini G, Di Donato S, Castellotti B, Patel PI, Pandolfo M (1995). Mapping of genes predisposing to idiopathic generalized epilepsy. Hum Mol Genet 4:1201-7.

Zhao LP, Hsu L, Davidov O, Potter J, Elston RC, Prentice RL (1997). Population-based family study designs: an interdisciplinary research framework for genetic epidemiology. Genet Epidemiol 14:365-88.

Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, Amos C, Dobyns WB, Subramony SH, Zoghbi HY, Lee CC (1997). Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the alpha 1A-voltage-dependent calcium channel. Nat.Genet. 15: 62-9

X. LIST OF PUBLICATIONS

Vijai. J, Cherian P.J., Sylaja P.N., Anand A., Radhakrishnan.K (2003). Clinical characterization of a South Indian cohort of juvenile myoclonic epilepsy probands Seizure 12: 490–6.

Vijai J., Kapoor A., Ravishankar H. M., Cherian P. J., Girija A. S., Rajendran B., Rangan G., Jayalakshmi S., Mohandas S., Radhakrishnan K., Anand A. (2003) Genetic association analysis of *KCNQ3* and juvenile myoclonic epilepsy in a South Indian population Hum Genet 113: 461–463

Kapoor A., Vijai J., Ravishankar H. M., Radhakrishnan, Anand A (2003). No evidence of GABRA1 mutation (Ala322Asp) in autosomal dominant juvenile myoclonic epilepsy families from South India. Journal of Genetics (In Press).

APPENDIX 1

Preparation of NKM buffer

Mixed 28ml 5M NaCl, 1.5 ml 1M $MgCl_2$, 30 ml 1M KCl, added distilled H_2O to 1 liter and stored at 4°C

Preparation of Resuspension buffer

Mixed 20 ml 5M NaCl, 10ml of 1M Tris.Cl, pH 7.5, 1.5 ml 1M $MgCl_2$, added distilled H_2O to 1 liter and stored at 4°C

Preparation of TEN solution,10X

Mixed 9ml 1 M Tris.Cl,pH 7.5, 24 ml 0.5 M EDTA, pH 8.0, 9 ml 5M NaCl and stored at room temperature

Preparation of buffered Phenol

1) Thawed frozen distilled phenol at 65°C in a water bath
2) Added 1% hydroxyquinoline (Antioxidant), Added an equal volume of 0.5 M Tris.Cl (pH 7.5) to melted phenol, mixed thoroughly for 20 min, the process was repeated till the required pH is attained. Buffered phenol was stored in 0.1 M TrisHCl at 4°C.

Proteinase K

Proteinase K, 2mg/ml, 49 ml H_2O, 0.5 ml 1 M $CaCl_2$, 0.5 ml 1M Tris.Cl, pH 7.5, 100mg proteinase-K, stored at –20°