CHAPTER I
INTRODUCTION

Background and Rationale

Benign Adult Familial Myoclonic Epilepsy (BAFME) is characterized by these criterias:

1) Infrequent epileptic seizure
2) Autosomal dominant manner, vibration movement of the limbs
3) Adult-onset
4) Abnormal polyspikes and waves detected by electroencephalogram (EEG) and partially photosensitivity
5) Giant somatosensory-evoked potential (SEP)
6) Enlarged long-loop reflex (C-reflex)
7) Positive spikes preceding myoclonus detected by jerk-locked averaging method
8) Rather benign outcome without dementia and cerebellar ataxia(1, 2)

BAFME diagnosis is based on clinical and electrophysiological criteria. An electrophysiological study is essential to confirm the cortical origin of myoclonus.BAFME was first reported in 1990 in the Japanese population. Until now about 50 Japanese, 10 European families and a French family were reported(1, 3, 4).

Recently the gene(s) responsible for this disease has not been identified but previous studies revealed that BAFME was linked to at least 3 loci. The first locus was linked to chromosome 8q23.3-q24.1 (BAFME1) by linkage analysis(1, 5), the second locus was linked to chromosome 2p11.1-q12.2 (BAFME2)(3, 6) and chromosome 5p15.31-p15 (BAFME3)(4).

In this study we reported the first BAFME family in Thailand. This family consists of 13 affected members. We aim to identify a disease-causing gene which will provide accurate information and genetic counseling for the affected families. Moreover, it would be useful for understanding the mechanism of the disease.

บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository(CUIR)
are the thesis authors' files submitted through the Graduate School.

Research questions

1. Are our Thai patients with tremors affected with BAFME?
2. Is the gene underlying BAFME in our Thai family linked with two known BAFME loci?
3. Which chromosomal region is linked to the BAFME in our Thai family?
4. What gene underlies BAFME in our Thai family?

Objectives

To identify the fourth genetic locus responsible for Benign Adult Familial Myoclonic Epilepsy.

Hypotheses

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Research design

Descriptive and *in vitro* studies

Key words

Benign Adult Familial Myoclonic Epilepsy, autosomal dominant inheritance, absence of linkage, electrophysiological studies, Genome-wide linkage study, microsatellite markers, genotyping.

Ethical consideration

The Chulalongkorn Ethics Committee has approved this study. All patients or their parents who participated in this experiment informed consent form.

Expected benefit

1. To provide further understanding into the molecular basis of epilepsy and better insight into the disease mechanism leading to more effective treatment of this disorder.

2. To provide accurate information and appropriate counseling for families with the disease.

Research methodology

To identify the BAFME family, the diagnosis of BAFME used clinical and electrophysiological criteria. Neurologists performed physical examination, family history, C-reflex, SEP, EEG, Jerk-locked averaging method.

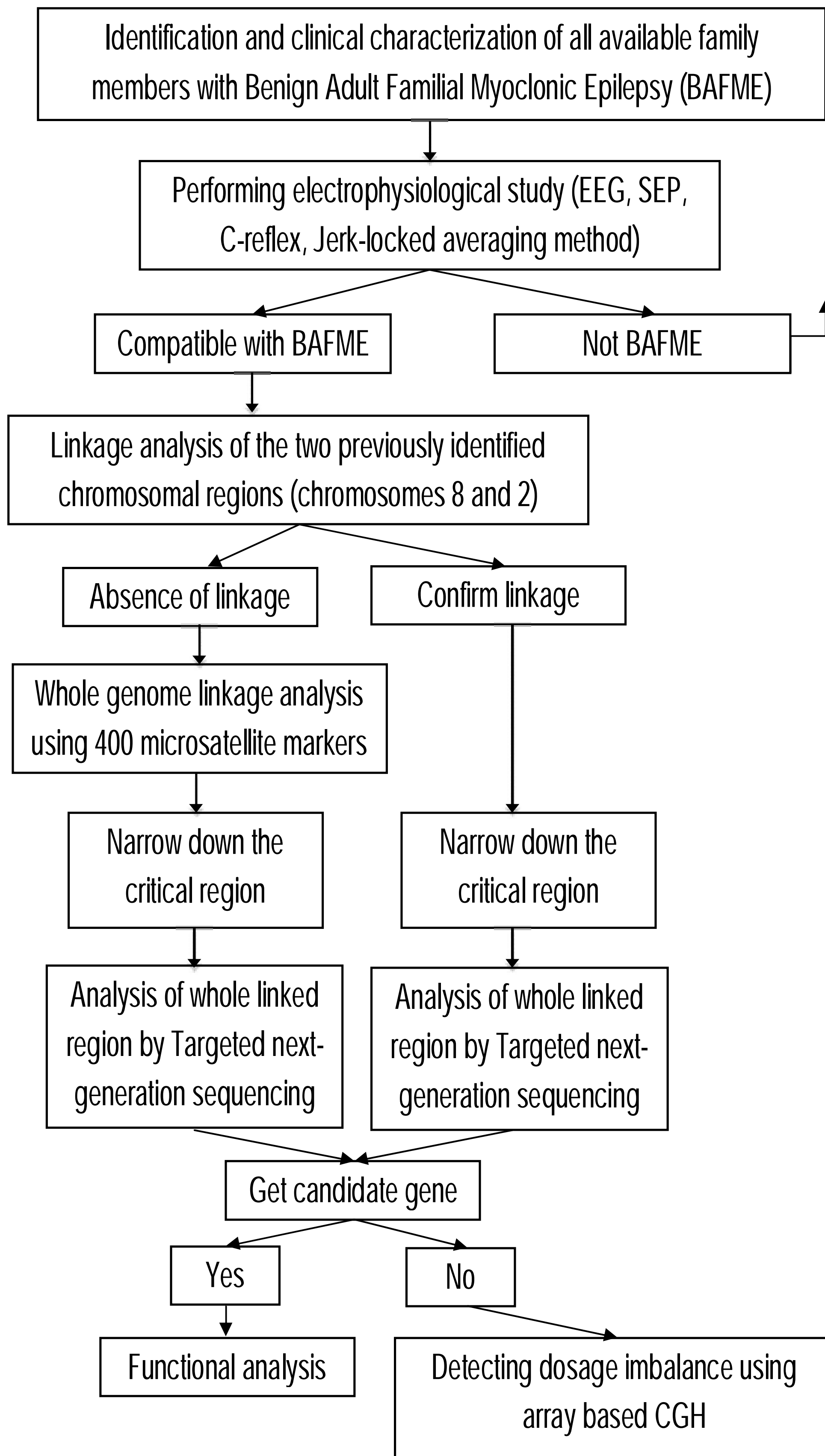
To exclude linkage with two known loci (chromosome 8 and chromosome 2) using the microsatellite markers that distributed in the previous link region.

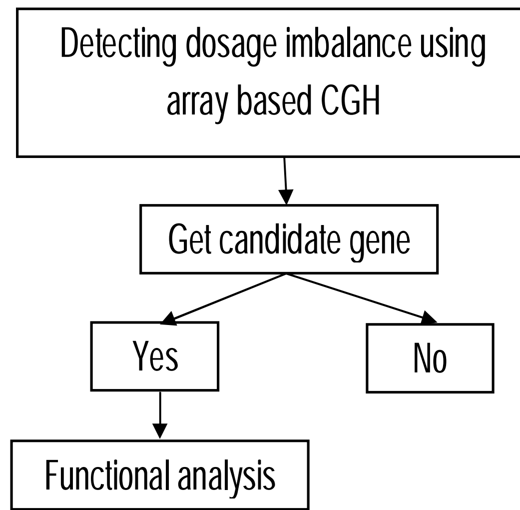
To perform whole genome linkage analysis, we use ABI linkage mapping set version 2.5. It consists of 400 microsatellite markers which distributed in the genome.

To narrow down the critical region, we selected the microsatellite marker in the critical region

We attempted to find the pathogenic mutation. We performed Targeted Next-generation sequencing which captured specific region of interest and sequence only the critical region. Then we did 385K Array CGH for detected dosage imbalance. Whole exome sequencing was also performed.

Conceptual framework




Detecting dosage imbalance using array based CGH
Get candidate gene
Yes
No
Functional analysis

CHAPTER II
REVIEW OF RELATED LITERATURE

Epilepsy

Epilepsy is a usual chronic neurological disorders characterized by seizures(7). Many people in the world have epilepsy(8). The seizures may occur in recovering patients. Epilepsy can control, but not cured, with medication. Epilepsy may be single disorder or syndromic with other symptoms, depending on abnormal electrical activity in the brain and seizures.

Some type of epilepsy occurs from mutation in several genes. The most of genes encode protein subunits of voltage-gated and ligand-gated ion channels are associated with forms of infantile seizure syndromes and generalized epilepsy(9).

Benign Adult Familial Myoclonic Epilepsy

Benign Adult Familial Myoclonic Epilepsy was first reported in 1990 Ikeda et al. reported two Japanese patients with action tremor presumably originated in the cerebral cortex(10). Surface Electromyography (EMG) showed abnormal discharge, which resembled tremor. Electrophysiological findings showed giant somatosensory evoked potentials (SEPs), enhanced long-loop reflex and jerk-locked averaging method (JLA) revealed premovement cortical spike. Treatment with beta-blocker showed no effect, but antiepileptic drug for example valproate, primidone and clonazepam were effective by suppressing the tremor and the amplitude of SEPs. Ikeda et al. have called this involuntary movement "cortical tremor," which is in fact a variant of cortical myoclonus.

In 1996 Kuwano et al. reported the 5 Japanese pedigrees with this disease dominantly inherited disorder(11). The affected individuals presented with myoclonus seizures and abnormal EEG findings with particularly photosensitivity. The age of onset was between 18 and 50 years. Performing the CAG expansion in the dentatorubral-pallidoluysian atrophy (*DRPLA*) gene showed normal results. *DRPLA* gene was excluded by linkage analysis.

In 1997 Terada et al. reported three families with six patients with tremor that showed late onset(12). It is an autosomal dominant manner. The affected individuals had tremor in the distal limbs. No cerebellar ataxia or dementia were reported. Electrophysiological studies revealed the spikes on EEG, enlarged somatosensory evoked potential, enhanced C-reflex, and polyspikes preceding the rhythmic jerk detected by the JLBA. They confirmed the cortical origin of the myoclonus.

In the same year Okino et al. described 3 adult-onset myoclonic epilepsy pedigrees (13). The age of onset of the myoclonus was between 30 and 40, with rare generalized tonic-clonic seizures (GTCS). The pedigree showed autosomal dominant pattern. Electrophysiologic studies revealed giant SEPs, polyspikes on EEG, enhanced C-reflexes, and a preceding wave on JLA. They supported the cortical origin of the myoclonus.

In 1998 Elia et al. reported a autosomal dominant European family with epilepsy, cortical tremor and mental retardation (14). All patients showed photoparoxysmal response and abnormal EEG. Long loop reflex, premyoclonus spike on JLBA and giant SEPs showed in all patients.

In 1999 Mikami et al. described a large Japanese pedigree with benign adult familial Myoclonic epilepsy. It was mapped on chromosome 8 by linkage analysis(1). At the microsatellite marker D8S555 gave the maximum 2-point lod score of 4.31 was obtained at a recombination fraction of 0.0; between D8S555 and D8S1779 gave the maximum multipoint lod score was 5.42. The locus was mapped on 8q23.3-q24.11.

In 1999 Plaster et al. inquired 4 previously reported familial adult myoclonic epilepsy Japanese pedigrees(5). The FAME locus was on chromosome 8q24 with a maximum lod score of 4.86 that flanked by microsatellite markers D8S514 and D8S1804. Researcher claimed that FAME had been identified in Japan only.

In 2001 Guerrini et al. reported a large Italian kindred in which 11 individuals (8 living) over 5 generations were affected with an autosomal dominant disorder characterized by distal myoclonus and seizures(6). All affected members had an onset in adulthood (a mean of 25 years old) with distal, rhythmic involuntary movements resembling tremors, and infrequent generalized tonic-clonic seizures (GTCS). Three patients also had intractable

complex partial seizures, which were often followed by secondary generalization. EEG showed focal frontotemporal as well as generalized interictal abnormalities. Detailed neurophysiologic studies showed enhanced long-loop reflexes, giant SEPs, and premovement cortical spikes by JLBA. Genome-wide linkage analysis of the affected family reported by Guerrini et al. yielded a maximum multipoint lod score of 3.74 with the marker D2S2175. Linkage to the locus for familial adult myoclonic epilepsy on 8q24 was excluded.

In 2002 Labauge et al. reported a 4-generation European family with clinical findings of FAME segregating in an autosomal dominant pattern(15). Ten living and three deceased members had symptoms after age 30 and a history of myoclonic movements of the extremities, and 8 of 13 also had generalized tonic-clonic seizures. Five patients underwent electrophysiologic examination with findings consistent with the diagnosis of FAME. Dementia and cerebellar ataxia were absent. Linkage to FAME1 locus on chromosome 8 was excluded.

In 2003 De Falco et al. reported 2 Italian families with non-progressive autosomal dominant BAFME(3). Cortical tremor was the presenting symptom in all affected patients, appearing at the age of 11 to 40 years (mean in the twenties). Most patients had infrequent seizures, and electrophysiologic studies suggested a cortical origin. Linkage analysis indicated linkage to chromosome 2p11.1-q12.2 (maximum cumulative lod score of 3.32). The authors noted that their patients did not have complex partial seizures or mental retardation, as was described by Guerrini et al. (2001), and suggested that the disorders might be allelic.

In 2005 Deng et al. reported a large BAFME pedigree in China(16). Genotyping using 11 microsatellite markers covering the two previously identified chromosomal regions was performed. However, evidence of negative linkage was found (LOD score
<-3.0 at no recombination). They concluded that the causative gene responsible for BAFME in the Chinese pedigree might be located on a new region other than 8q23.3–q24.1 and 2p11.1–q12.2, indicating the presence of a third locus for BAFME.

In 2007 Carr et al. reported 2 large Western Cape province of South Africa families with GTCS and myoclonus(17). Age of onset was between 13 and 31 years old. Myoclonus

was observed in upper and lower limbs. The additional features included nystagmus, dysarthria, abnormal pursuit, cerebellar, ataxia hyperreflexia and cerebellar atrophy. The families were of mixed ancestry. Carr et al. described that the symptom was more severe than the BAFME1. Exlclusion of linkage (BAFME1 and BAFME2) was found.

In 2008 Striano et al. commentated that the publication reported by Carr et al. (2007) was more severe than BAFME, and proposed that the disorder should be changed within the group of progressive myoclonic epilepsies (18). Striano and co-workers suggested that the designation 'FAME' be reserved for familial nonprogressive cortical tremor and epilepsy.

In 2008 Saint-Martin et al. studied a family which was previously reported by Labauge et al. by linkage analysis and found significant linkage to chromosome 2 (multipoint lod scores greater than 3.0 between markers D2S2114 and D2S2187)(19). Haplotype analysis identified a 40.27-Mb region segregating in all 10 affected. The region overlapped with FAME2, refining the locus to a 16.65-Mb region between D2S2161 and D2S2264. Sequence analysis of several candidate genes in that region did not identify pathogenic mutations.

In the same year (2008) Madia et al. reported 5 families with FAME2 from southern Italy, including the family reported by de Falco et al. (2003) and found significant linkage to chromosome 2p11.1-q12.2 (maximum cumulative lod score of 18.5) (20). A common 15-Mb haplotype that segregated with the disorder was identified in all the families, indicating a founder effect.

In 2010 Depienne et al. reported a large French family in which 16 individuals had a form of myoclonic epilepsy. Most patients had onset as adults in their twenties or thirties, although 1 boy had onset of cortical myoclonus at age 10. Five patients presented with cortical myoclonus, 5 with seizures, and 6 with both at the same time. Two had only cortical myoclonus without seizures. Of the 14 with seizures, 11 had GTCS and 3 had only focal seizures, characterized by visual hallucinations or transient loss of consciousness. Five patients met the electrophysiologic criteria for cortical myoclonus: paroxysmal polyspike and wave activity on EEG, photosensitivity, and giant somatosensory evoked potential,

enhanced long-loop reflex (C-reflex), and cortical transients preceding the myoclonic jerks. Exercise, uneven ground, light, and low blood sugar precipitated the episodes. All patients responded to treatment. Seven patients were older than 60 years, and all had severe myoclonus affecting both upper and lower limbs, leading to walking impairment in 6. None had mental retardation or cognitive impairment. By genome-wide linkage analysis of a large French family with cortical myoclonic epilepsy, found linkage to a 9.31-Mb region on chromosome 5p15.31-p15.1 between D5S580 and D5S2096 (multipoint lod score of 3.66). Two asymptomatic family members also shared this region. The highest 2-point lod scores were 6.3 and 6.2 for D5S486 and D5S1380, respectively. Sequencing excluded mutations in the coding regions of the SEMA5A and CTNND2 genes (4).

In 2011 Mori et al. reanalyzed the Japanese family reported by Yasuda (1991) and Mikami et al. (1999) using 10K SNP arrays and additional microsatellite markers in a genomewide linkage analysis. The FAME1 locus was mapped on chromosome 8q23.3-q24.13 (maximum 2-point lod score of 6.0 for marker rs1021897). Analysis of sequence and CNV analysis of all 38 genes located in the candidate region were completed, but no pathogenic mutation was found(21).

In 2012 Hitomi et al. reported the clinical anticipation in Japanese benign adult familial myoclonus epilepsy (BAFME), defined as earlier onset age of either cortical tremor or generalized seizures or new appearance of those symptoms in the next generation, remains unknown. The onset age and the degree of both cortical tremor and generalized seizures were investigated in nine patients of four BAFME families. Clinical anticipation in the onset age of cortical tremor or generalized seizures was observed in three families, and generalized seizures newly appeared in the next generation in those two families and in another family. Clinical anticipation was observed in four families, which suggests the clinical progression over generation in Japanese BAFME families (22).

Table 1. Different name of Benign Adult Familial Myoclonic Epilepsy (23).

Abbreviation	Full name
ADCME	Autosomal dominant cortical myoclonus and epilepsy
BAFME	Benign adult familial Myoclonic epilepsy
CrtTr	Cortical tremor
FAME	Familial adult myoclonic epilepsy
FCMT	Familial cortical myoclonic tremor
FCTE	Familial cortical tremor with epilepsy
FEME	Familial essential myoclonus and epilepsy
FMEA	Familial benign myoclonus epilepsy of adult onset
HTE	Heredofamilial tremor and epilepsy

Linkage analysis

In linkage analysis, recombination fraction is the proportion of recombinations out of all opportunities for recombination (recombinations and non-recombinations). Linked mean two genes do not independent assortment, they are close each other on the same chromosome. On the contrary genes that located on different chromosomes independent assortment and have are combination frequency of 50%, linked genes have are combination frequency that is less than 50% (Figure 1).

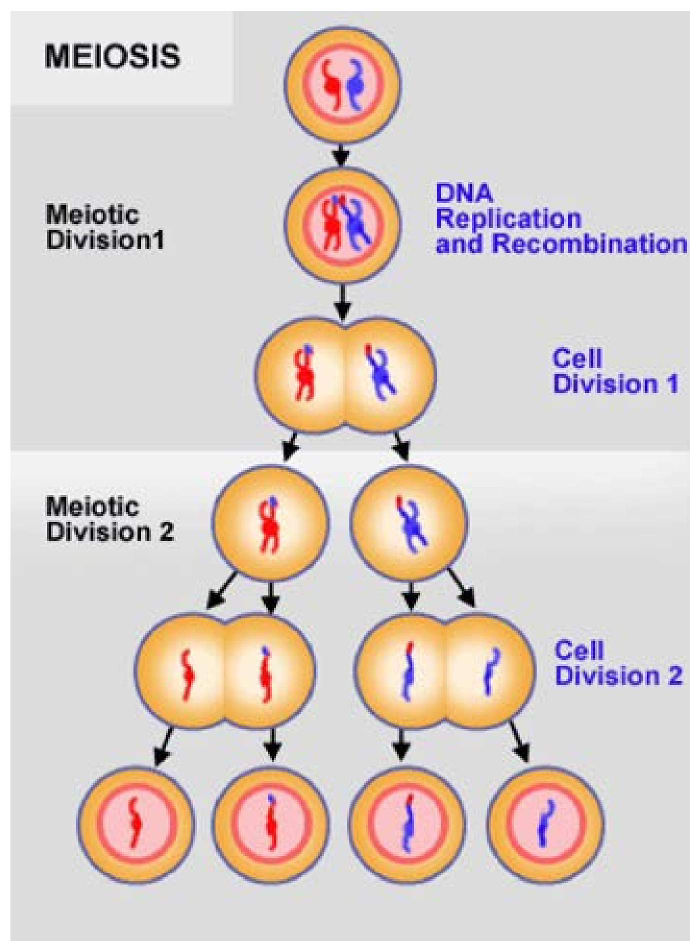


Figure 1. Process of meiosis.

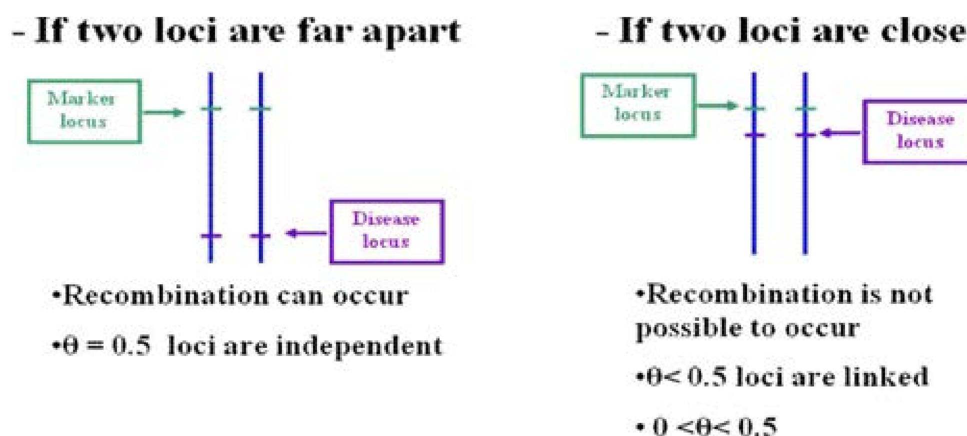


Figure 2. Recombination fraction is a measure of genetic distance.

LOD score method

Lod score is the log of odds or log of the likelihood ratio. It is developed in 1955 by Newton E. Morton. The LOD score is calculated as follows: log of the probability of birth sequence with given theta divided by probability of birth sequence with theta equal to zero point five.

$$
\begin{aligned}
LOD = Z &= \log 10 \frac{\text{probability of birth sequence with a given linkage value}}{\text{probability of birth sequence with no linkage}} \\
&= \log 10 \frac{(1-\theta)^{NR} \times \theta^{R}}{0.5^{(NR+R)}}
\end{aligned}
$$

A LOD score more than 3.0 is significance for linkage. On the other side, exclude linkage when a LOD score values less than -2.0. The positive LOD score calculation should do from the single pedigree.

Microsatellite marker

Microsatellites are short tandem repeats. The repeat units are normally di-, tri- tetra- or pentanucleotides. They distributed in the genome in non-coding part(24).

Microsatellites are highly polymorphic, so they are useful for genetic markers. The microsatellites have high mutation rate when we compared to the normal DNA regions. It can be described the frequency by slippage during DNA replication.

Microsatellites are the most significant tool for mapping genomes. They are useful in biomedical diagnosis. They are the primary marker for DNA testing in forensics(25, 26).

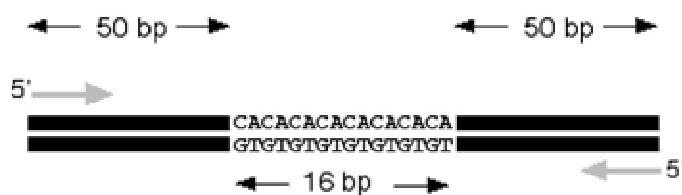


Figure 3. Designing microsatellites from genomic DNA. Forward and reverse primers are created to flank the region of microsatellite.

Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) are the newer sequencing methods. These new technologies combine the various strategies together. They are four step. First, template preparation. Second, sequencing and imaging, Third, genome alignment and finally, assembly methods. The major advantage of is the ability to produce a gigantic volume of data cheaply(27).

Targeted resequencing

The interested regions were captured and sequenced only those regions. It includes human whole exome sequencing, custom capture sequencing and ChIP-seq.

Table 2. In detail of NGS technologies (28).

Technology	Amplification	Read length (bp)	Throughput	Method of Sequencing
Roche/GS-FLX Titanium	Emulsion PCR	400-600	500 Mbp/run	Pyrosequencing
Illumina/Hiseq2000 , HiScan	Bridge PCR	2*100	200Gbp/run	Reversible terminator
ABI/SOLiD 5500*1	Emulsion PCR	50-100	>100 Gbp/run	Sequencing-by-ligation (octamer)
Polonator/G.007	Emulsion PCR	26	8-10 Gbp/run	Sequencing-by-ligation (monomer)
Helicos/Heliscope	No	35 (25-55)	21-37 Gbp/run	True single-molecule sequencing (tSMS)

Whole Exome Sequencing

The exome sequencing is the targeted sequencing of the protein coding. It is a recently and powerful tool for discovering the genetic basis of diseases(29).

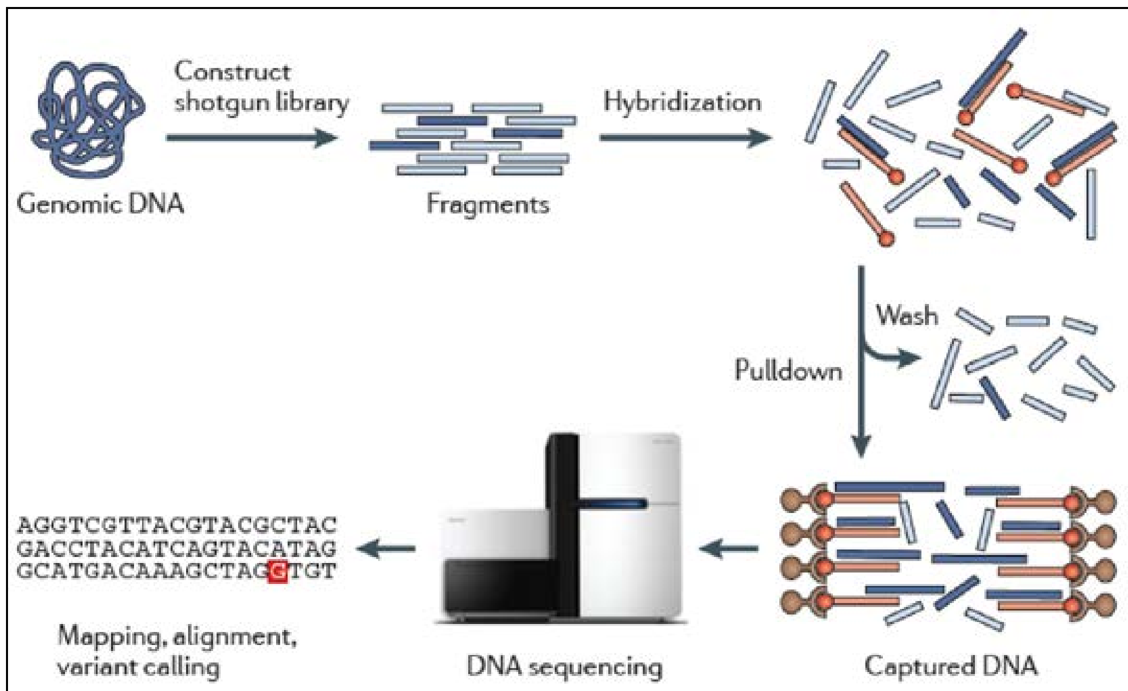


Figure 4. Workflow for exome sequencing (29). Randomly sheared the genomic DNA, and an *in vitro* shotgun library are constructed. Then, the library enrichment are performed for sequences corresponding to coding region (dark blue fragments): hybridization of fragments are performed. Washing and the targeted DNA are eluted. Targeted DNA are sequenced by high throughput DNA sequencer. Mapping, alignment and variant calling are performed.

About three companies (Illumina Agilent, and Nimblegen) offer the reagents for capturing exome. There are some difference concept between them.

CHAPTER III
MATERIALS AND METHODS

Subjects and clinical descriptions

We identified a large Thai family including 13 affected individuals in whom BAFME segregates as an autosomal dominant inheritance (figure 4). Family members underwent a complete historical interview, neurologic examination. Most electrophysiologic examinations, including electroencephalograms (EEG), somatosensory evoked potentials (SSEP), C-reflex, and jerk-locked back averaging method were performed. Blood samples were collected from all 24 family members, and genomic DNA were isolated.

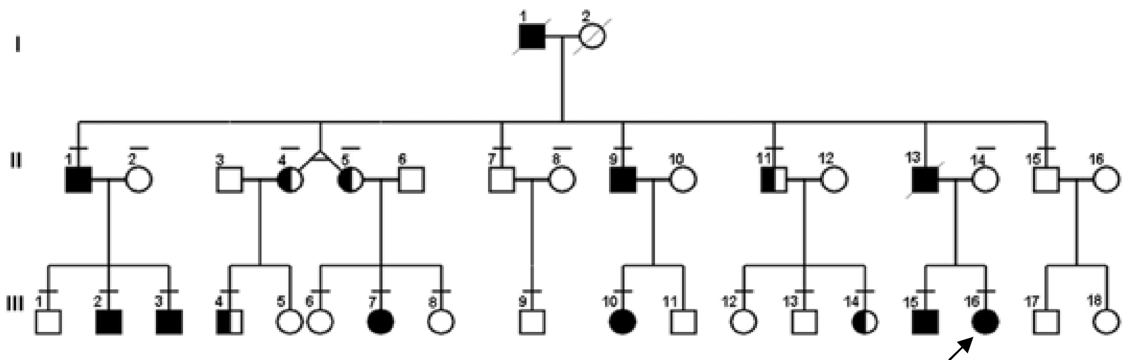


Figure 5. Pedigree of a Thai family with Benign Adult Familial Myoclonic Epilepsy (BAFME). Circles indicate female subjects and squares indicate male subjects. Affected members with cortical tremor and generalized seizures are represented by filled symbols and affected with cortical tremor only are represented by1/2-filled symbols. Bar denotes patients clinically examined by the neurologists. A slash through the symbol indicates that the subject is deceased.

After informed consent was received, peripheral blood (3 ml) was obtained from the patient and genomic DNA was extracted by standard methods. Controls were healthy volunteers unaffected with epilepsy and had no family history of epilepsy.

Electrophysiological findings

EEG-EMG polygraphy

Analyzing the relationship between cortical events and Myoclonic were performed by the recording of EEG and EMG. Surface EMG used for recording the myoclonus. Recording the cortical activities are performed by classical EEG.

Generally cortical myoclonus show a spike or a polyspike. If there is no abnormal EEG, it possible that the cortical signal is too low when compare with the EEG background(30).

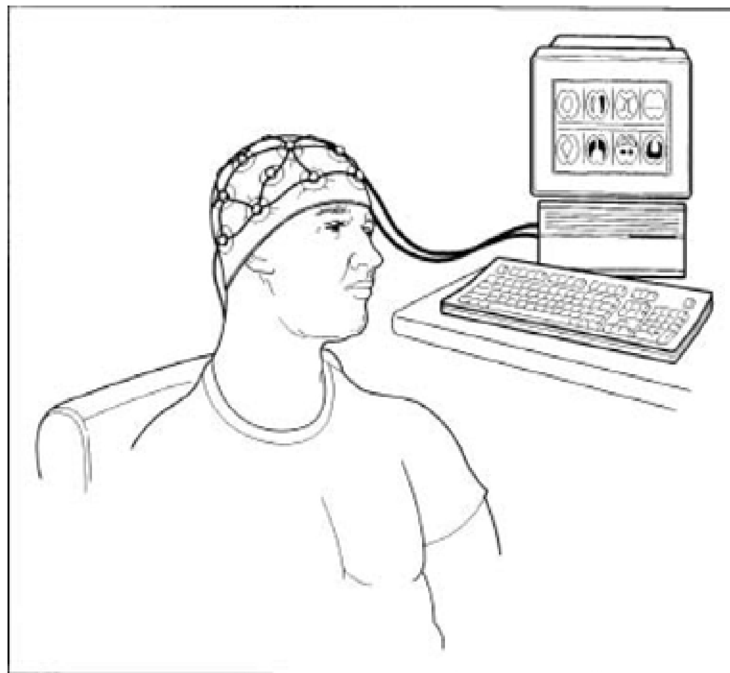


Figure 6. Method for EEG measurement.

Somatosensory Evoked Potentials (SEPs)

Myoclonic patients were performed SEPs for searching the increased cortical responses. The giant SEPs is a classical characteristic of cortical myoclonus. SEPs were performed by averaging EEG over several hundreds of peripheral-nerve stimuli. After stimulation at median-nerve, the parietal N20 is the first cortical response. Between N20 and the following componentsP27, and between P27 and N33 the peak-to-peak amplitude is measured. It is normally stimulated that 10 μV shows the enlarged cortical responses, namely giant SEPs. The components P27–N33 and N20–P27 can be found giant while N20 may be normal, implying that normal of the sensory input into the cortex and the primary cortical response.

Nevertheless giant SEPs are not found in all patients with cortical myoclonus. It is possible that the abnormality is not strong to be detected by SEPs studies or these patients have no abnormality in the cortical processing of sensory inputs(30).

Long-latency reflex or C reflex

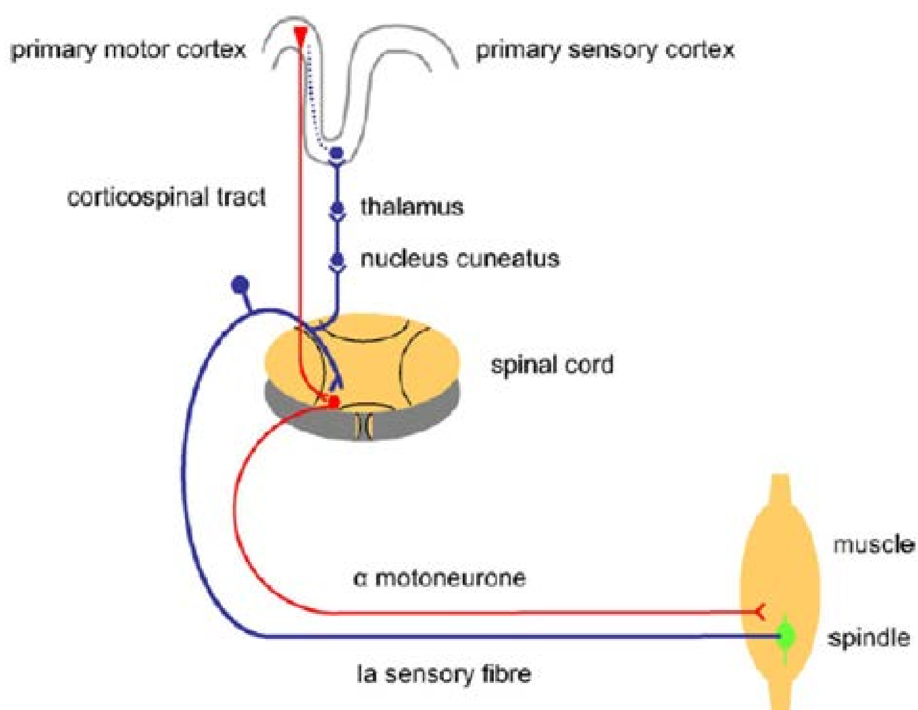


Figure 7. Two reflex loops may be associated. The short one is a spinal loop owing to an excitatory effect of Ia fibers onto homologous motoneurones called the H reflex. The long one is a transcortical loop involving the lemniscal system and the sensorimotor cortex. The connection between sensory inputs and the motor system showed at the somatosensory cortex (30).

Jerk-locked back averaging EEG

128 channels of EEG was performed for JLA, the averaging of 500 jerks were performed. The myoclonus onset is at time 0. At the left central region A premyoclonic spike is present.

DNA Extraction

Total DNA extraction was performed according to Qiagen kit protocol. Buffy coat from 3-5 ml of EDTA blood was separated by centrifugation at 1,000 x g for 10 minutes. Remove plasma and transfer buffy coat to a new 15-ml tube. Add 10 ml cold lysis buffer I and mix thoroughly. Centrifuge at 1,000 x g for 5 min, discard supernatant and then repeat this step once. Add 200 μl Buffer AL to the sample and 20 μl proteinase K. Mix by pulse-vortexing for 15 s. Incubate at 56°C for 10 min. Add 200 μl ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge to remove drops from the inside of the lid. Carefully apply the mixture to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube and discard the tube containing the filtrate. Add 500 μl Buffer AW1 without wetting the rim. Centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate. Add 500 μl Buffer AW2 without wetting the rim. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate. Add 200 μl Buffer AE or distilled water.

RNA Extraction

Total RNA extraction was performed according to Qiagen kit protocol. For blood samples, buffy coat from 3-5 ml of EDTA blood was separated by centrifugation at 1,000 x g for 10 minutes. Remove plasma and transfer buffy coat to a new 15-ml tube. Add 10 ml Buffer EL and mix thoroughly. Incubate for 10–15 min on ice. Mix by vortexing briefly 2 times during incubation. Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant. Add 10 ml Buffer EL to the cell pellet. Resuspend cells by vortexing briefly. Centrifuge at 400 x g for 10 min at 4°C, and completely remove and discard supernatant. Add 600 µl Buffer RLT to pelleted leukocytes. For lymphoblastoid cell line, harvest 10 ml of lymphoblastoid cell line with cell density of 1×10^6 cells /ml. Pellet the cells by centrifuging for 5 min at 300 x g in a centrifuge tube. Carefully remove all supernatant by aspiration. Disrupt cells by adding 600 µl Buffer RLT. Pipet lysate directly into a QIAshredder spin column in a 2 ml collection tube (provided) and centrifuge for 2 min at maximum speed to homogenize. Discard QIAshredder spin column and save homogenized lysate. Transfer lysate to new 1.5-ml tube. Disrupt cells by adding 600 µl Buffer RLT. Transfer each cell lysate after adding buffer ALT into a QIAshredder spin column in a 2 ml collection tube and centrifuge for 2 min at maximum speed to homogenize. Discard QIAshredder spin column and save homogenized lysate. Add 1 volume 600 µl of 70% ethanol to the homogenized lysate and mix by pipetting. Do not centrifuge. Carefully pipet sample, including any precipitate which may be formed, into anew QIAamp spin column in a 2 ml collection tube without moistening the rim. Centrifuge for 15 s at 8000 x g (10,000 rpm). Transfer the QIAamp spin column into a new 2 ml collection tube. Apply700 µl Buffer RW1 to the QIAamp spin column and centrifuge for 15 s at 8000 x g (10,000 rpm) to wash. Place QIAamp spin column in a new 2 ml collection tube. Pipet 500 µl of Buffer RPE into the QIAamp spin column and centrifuge for 15 s at 8000 x g(10,000 rpm).Carefully open the QIAamp spin column and add 500 µl of Buffer RPE. Close the cap and centrifuge at full speed (20,000 x g, 14,000 rpm) for 3 min. Place the QIAamp spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at fullspeed for 1 min. Transfer QIAamp spin column into a 1.5 ml microcentrifuge tube and pipet 30–50

µl of RNase-free water directly onto the QIAamp membrane. Centrifuge for 1 min at 8000 x g (10,000 rpm) to elute. Repeat this step once.

Genotyping genetic markers and linkage analysis

After informed consent, genomic DNA was extracted from peripheral blood leukocytes of 24 family members (figure 1) using ArchivePure DNA Blood Kit (5 Prime Inc., Gaithersburg, MD). We first performed linkage analysis with two known loci on chromosome 8q23.3-q24.1 and 2p11.1-q12.2. Using seven microsatellite markers (D8S1830, D8S555, D8S588, D8S1112, D8S1826, D8S572-18, D8S1799) on chromosome 8 and three markers (D2S388, D2S2175, D2S2264) on chromosome 2, we were able to exclude linkage to these loci. The details of primers were obtained from Marshfield map**. We typed all fluorescently labeled primers on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA) with GeneMapper software (Applied Biosystems). MLINK program was used to calculate the two-point linkage analysis with the following model: autosomal dominant inheritance with high penetrance, set at 0.01 and 0.99.

Remark: **(http://research.marshfieldclinic.org/genetics/GeneticResearch/compMaps.asp).

Table 3. List of microsatellite marker for exclusion of linkage on chromosome 8 and chromosome 2.

Marker	Dye	ASR		Forward sequence	Reverse sequence
D8S1830	FAM	149	187	TGCACCTTGTGGATGG	ACCTCAAATCAGATTAGAGAGCC
D8S555	FAM	165	177	GGCAAAGTTCAGAGGC	GGAGGGTTCCATATTTCAA
D8S588	FAM	177	197	AGCTCTCAAAATATGATTCTATTTC	CCATTCAAGAAACCATGCTT
D8S1112	FAM	206	234	GGATGATTGTAAGTTATAGGGAGG	CTGCAGGTGATCGAAGACTT
D8S1826	VIC	137	173	TTTCTACACTTCGCTTTTTG	GTGGTAGGAGATGCCC
D8S572-18	FAM	250	290	TGGTAATTTCAGAGGTTCCG	GATACATTACTTTTGCTTTT
D8S1799	VIC	224	270	TCACAGCAACTCCACCCCG	GGACATTCTGCCCCCTGAAGAT
D2S388	FAM	254	266	CTAAAAAAATGTGTTAAGCAAAAA	TTGGCCCTGCATTACT
D2S2175	FAM	105	135	ATGAGCCTGAGATATTGGA	CTGCTTAGAGTTATTTTGGGT
D2S2264	FAM	241	256	CATCTCAAAGGGCATGTC	TCGAATGAACAGTGCCTC

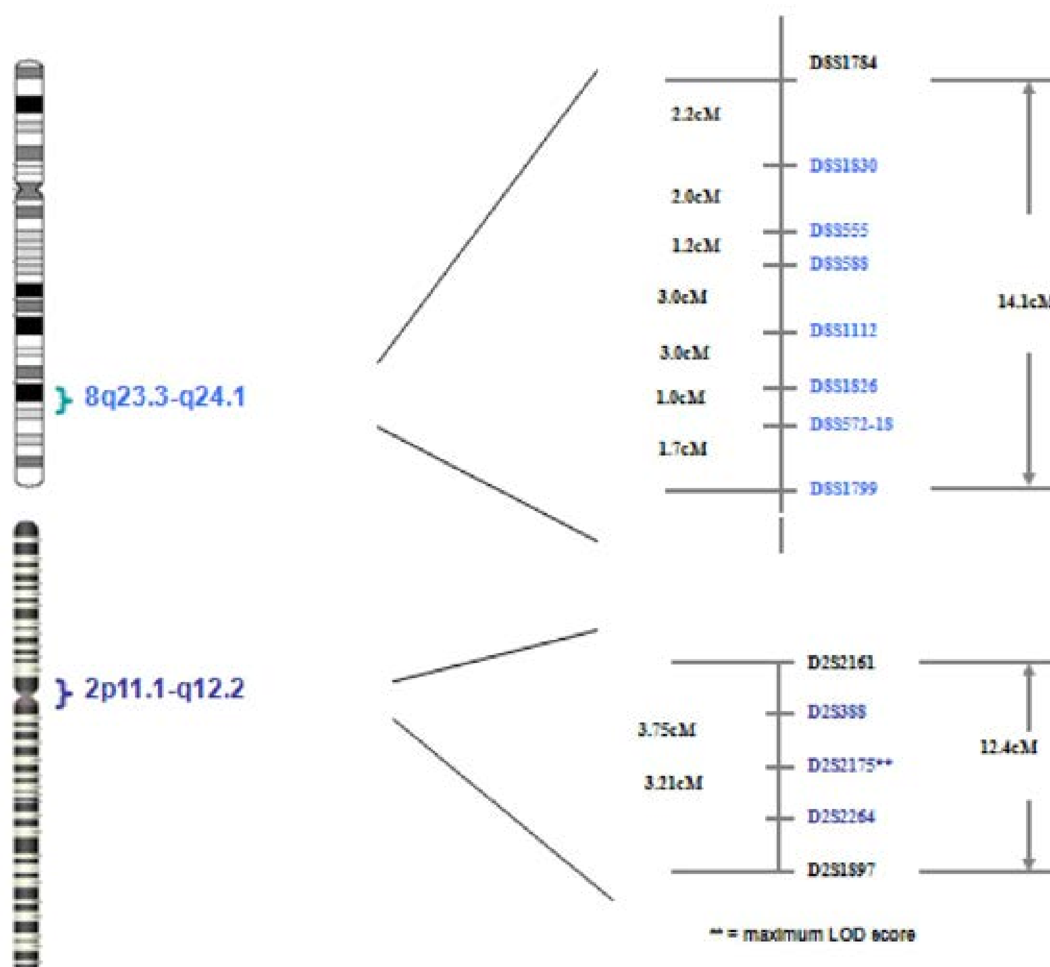


Figure 8. Genetic map of the genotyped markers flanking the previously identified regions.

Genome-wide linkage study (GWLS)

GWLS was performed using ABI Prism Linkage Mapping Sets-MD10 Version 2.5 (Applied Biosystems). This set consists of400fluorescentlylabeledprimer pairs selected to amplify dinucleotide repeats thatdefineanaverage10centimorgan (cM) resolution human index map. Forward and reverse primers are combined and supplied in a tube at 10mM concentration (5mM of each primer) in 10mM Tris-HCl, 1mM EDTA, pH 8.0.

Narrowing down the critical region.

After the linked locus was identified, we selected 11 additional markers (D3S2421, D3S3676, D3S2427, D3S3037, D3S3730, D3S1571, D3S3609, D3S3592, D3S1602, D3S3686, D3S3651) in the region on chromosome 3 for fine mapping.

Table 4. List of microsatellite markers for narrowing down the critical region.

Marker	Dye	ASR		Forward sequence	Reverse sequence
D3S2421	VIC	292	313	AGCCATGATCACACCACTCT	GGTCTTCATCATGCATCCTC
D3S3676	VIC	167	181	CCATTGAAGTAAAACTGCC	AGTGAAACACAATAGACCAAGAT
D3S2427	VIC	203	245	CTCCTCGTCACTGCAGTCTT	CTGCCTCATCTGTTCAGGAT
D3S3037	FAM	189	221	GGATTACATTTCTAATCTGGAACG	TTGAGACATGTAACTTTTAATACGC
D3S3730	VIC	138	156	GACTGGAAAATTCAGCCTCTA	AAGATGAGTCCTGAGCATGT
D3S1571	NED	160	184	ACAGTGGCTGATGCCTT	CACAGGTGGGCACTACAT
D3S3609	PET	163	185	AGCTGGGGACCAGTCT	CGAGAGTAACTTGTACGGTG
D3S3592	FAM	159	173	GCAGTTCTGAGTGATTTACCA	TCATCTGAGGTGTCTGATTG
D3S1602	FAM	275	297	AGAGCCTTCTATGGGTCTACAT	AGCTCAACCTTCAAACATACATT
D3S3686	FAM	108	134	AGGGTATTTCATTCCCATTG	CCAGGTTACGCCAAGTG
D3S3651	FAM	248	256	AGTGTGCTCTGGTTTTCTC	TTCGATATGAACTTGCTTATTG

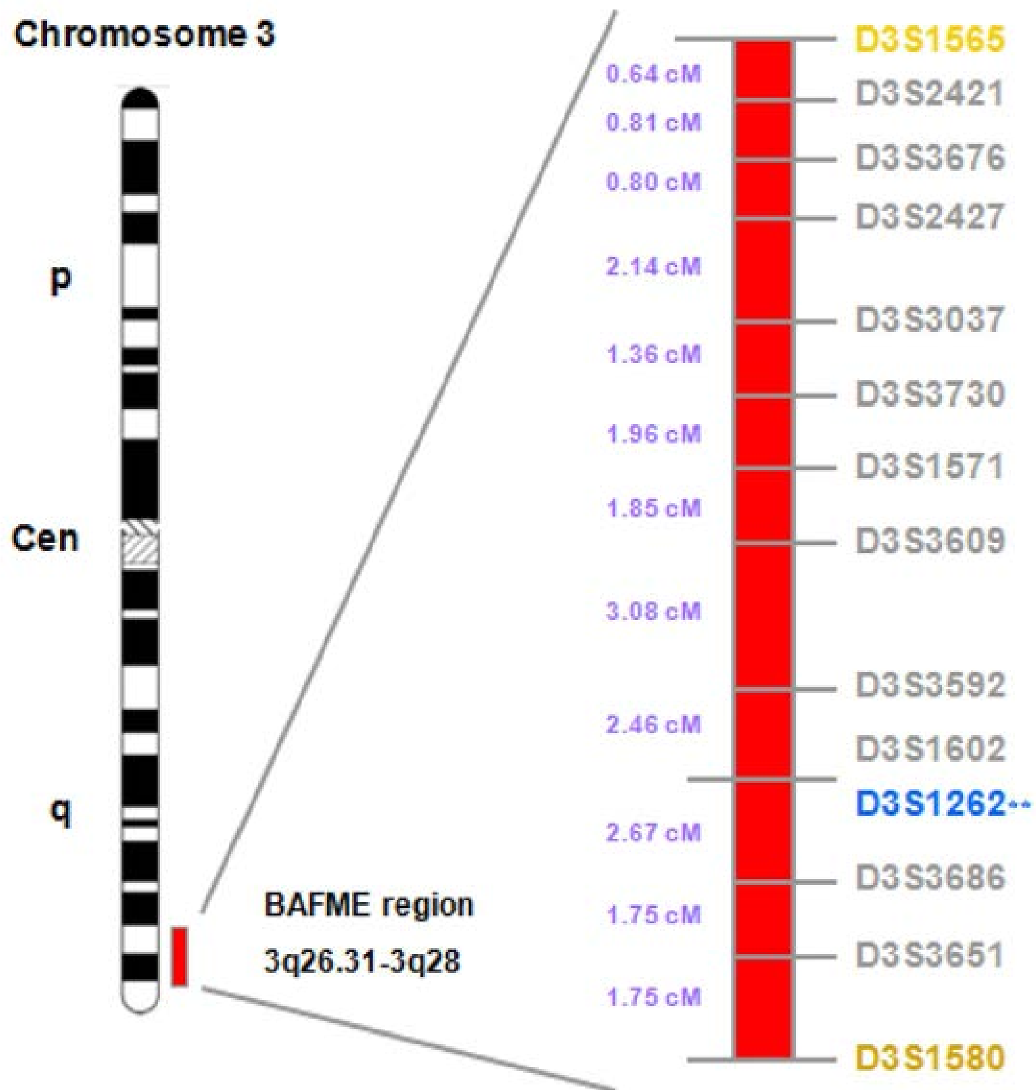


Figure 9. The diagram showed genetics distance of each marker that use for refining the critical region.

Targeted resequencing of 10 Mb linkage region on chromosome 3

The III-7 genomic DNA was used to perform targeted of entire 10 Mb linkage region between D3S2747 and D3S3663 interval. With the Next-generation sequencing (NGS) service of Macrogen Inc. (Seoul, Korea), DNA was captured on customized Nimblegen 2.1 array (Roche NimbleGen, Madison, WI, USA) with capturing capacity of 33 Mb. The targeted region was corresponded to position 178,100,000 bp and end at 188,700,000 bp on Chromosome 3 according to UCSC hg19 Assembly. Captured library was subsequently sequenced using Illumina platform Genome Analyzer II X (GAIIX) in a single-end 76 bp configuration. Sequence reads were mapped against UCSC hg19 using BWA software (http://bio-bwa.sourceforge.net/) The SNPs and Indels are detected by SAMTOOLS (http://samtools.sourceforge.net/) and annotated by SIFT (http://sift.jcvi.org/).

Mutation confirmation and restriction enzyme digestion

PCR amplification and Sanger sequencing were performed to confirm the mutation in patient III-7. Primer for the amplification of the coding exons of *HTR3D* and *MASP1* are listed in Table 5. Restriction enzyme digestion with *BsmAI* was used for normal control screening.

Table 5. List of Primer pair and restriction enzyme digestion that used for mutation confirmation.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)	Enzyme
HTR3D	GTAAAACGACGGCCAGTCAGTATCCAAGGAGCATGTC	TCTCCGTGACGCTGTAATTG	*BsmAI*
MASP1	CTTCATCACCCACCTGCTGC	GAAGGAGGCAGGAGCAGAGA	*BsmAI*

Array-bases Comparative Genomic Hybridization for detecting the dosage imbalance

Test DNA (affected male (III7)) and reference DNA (unaffected male (III6)) were sent to Macrogen, Inc, (Korea) to determine for a copy number variations. DNAs were independently labeled with fluorescent dyes, co-hybridized to a NimbleGen Human CGH 385K chromosome X Tiling array (Figure8), and scanned using a 2 μm scanner. Log2-ratio values of the probe signal intensities (Cy3/Cy5) were calculated and plotted versus genomic position using Roche NimbleGen NimbleScan software. Data are displayed in Roche NimbleGen Signal Map software.

Figure 10. 385K Array format.Human CGH 385K Chromosome 3 Tiling Array, Probe Length 50-75 mer, Median Probe Spacing 475 bp. Source: UCSC, Build: HG18, NCBI36.

Whole Exome Sequencing

The genomic DNA of two affected (III-2, III-16) and an unaffected (II-15) were sent to BGI (Shenzhen, China)

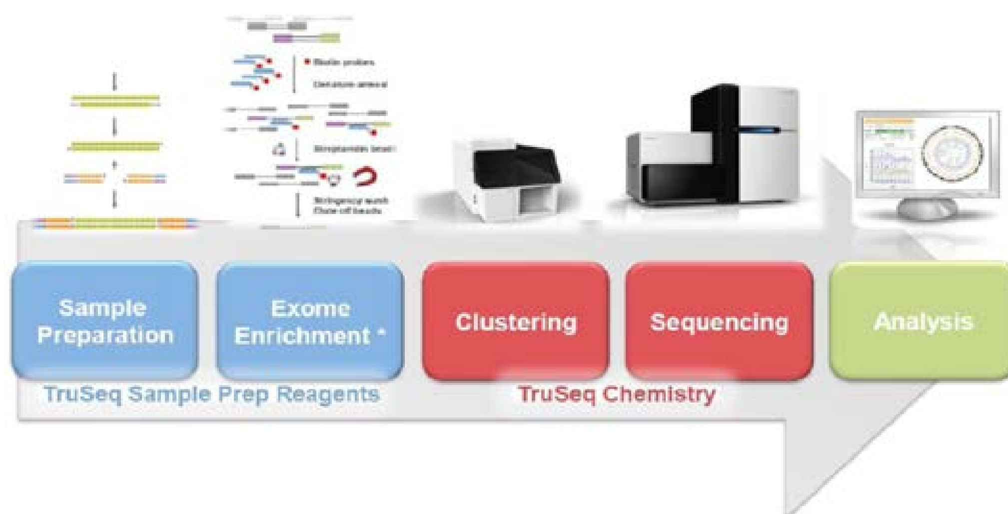


Figure 11. Experiment overview. Sample were prepared as an Illumina sequencing library, and in the second step, the sequencing libraries were enriched for the desired target using the Illumina Exome Enrichment protocol. The captured libraries were sequenced using IlluminaHiSeq 2000 Sequencer.

Captured library construction

TruSeq DNA Sample Prep

Each sequenced sample is prepared according to the Illumina protocols. Summary, one microgram of genomic DNA was fragmented by nebulization, the fragmented DNA is repaired, an 'A' is ligated to the 3'end, Illumina adapters are then ligated to the fragments, and the sample is size selected aiming for a 350–400 base pair product. The size selected product is PCR amplified, and the final product is validated using the Agilent Bioanalyzer.

First Hybridization

Before the fist hybridization, the multiple libraries are combined with different indices into a single pool prior to enrichment. The pooled DNA libraries are mixed with capture probes of targeted regions. The recommended hybridization time ensures targeted regions bind to the capture probes thoroughly.

First Wash

The streptavidin beads are used to capture probes containing the targeted regions of interest. Three wash steps remove non-specific binding from the beads. The enriched library is then eluted from the beads and prepared for a second hybridization.

Second Hybridization

The first elution of the DNA library is mixed with the capture probes of target regions. The second hybridization ensures the targeted regions are further enriched.

Second Wash

The streptavidin beads are used in order to capture probes containing the targeted regions of interest. Three wash steps remove non-specific binding from the beads. The enriched library is then eluted from the beads and prepared for sequencing. It is similar to the First Wash procedure.

PCR Amplification

PCR is used in order to amplify the enriched DNA library for sequencing. PCR is performed with the same PCR primer cocktail used in TruSeq DNA Sample Preparation.

Enriched Library Validation

Axeq Technologies performs procedures for quality control analysis on the sample library and quantification of the DNA library templates.

Clustering & Sequencing

Illumina utilizes a unique "bridged" amplification reaction that occurs on the surface of the flow cell. A flow cell containing millions of unique clusters is loaded into the HiSeq 2000 for automated cycles of extension and imaging.

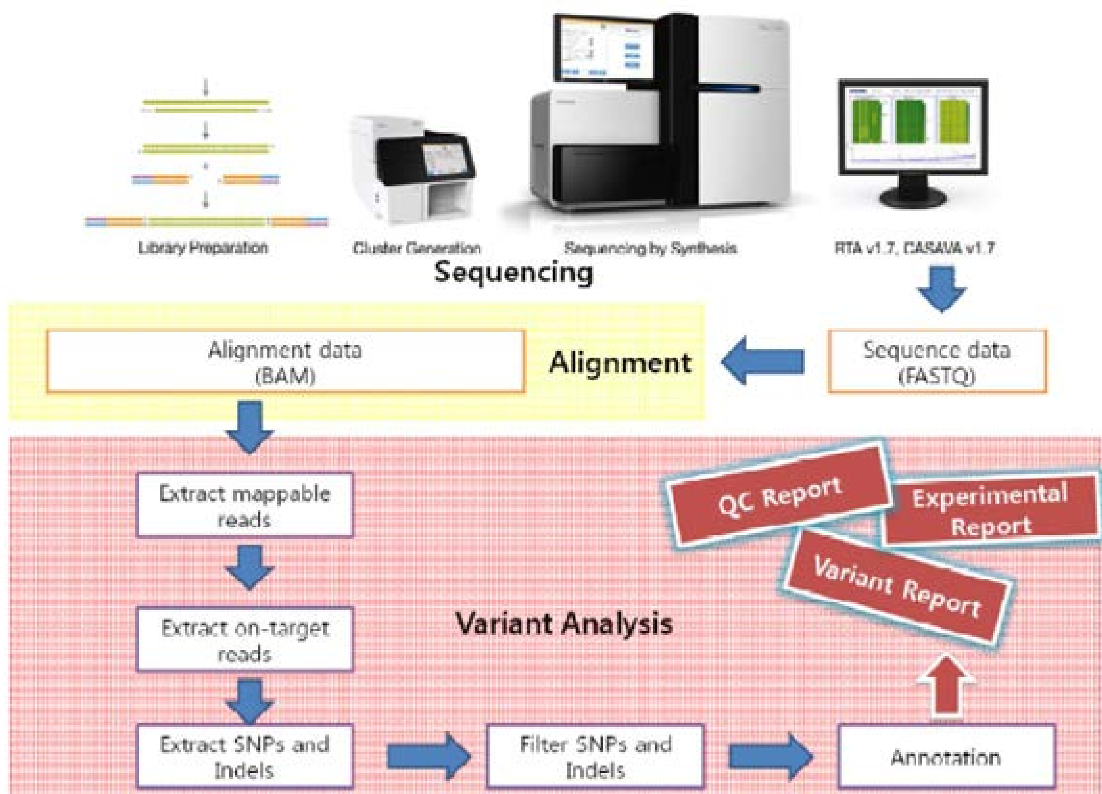


Figure 12. Data analysis pipeline.

DNA sequence analysis

Sequence data were analyzed using Sequencher (version 4.2; Gene Codes Corporation, Ann Arbor, MI) and were aligned with nucleotide BLAST program from www.ncbi.nlm.nih.gov/BLAST.

Statistical Analysis

Statistical significance was determined according to an independent sample t-test using the SPSS program version 11.5 as specified.

CHAPTER IV
RESULTS

Clinical findings

We identified a BAFME family in Thailand (Figure 11). General information including age, gender, age of onset of cortical tremor and generalized seizures, frequency of seizures, severity of tremors, family history and medical history was collected (Table 1). We investigated 24 family members. Six had cortical tremor only while the other seven had generalized seizures along with cortical tremor. Of these 13 affected, 12 had hand tremor before the onset of epileptic seizures. Only one patient, III-16 (Table 8) had seizures two years before the onset of tremor. The average age of onset was 19.5 (range 10-33 years) for tremor and 25 (range 19-33 years) for seizures. None had cognitive impairment.

EEG

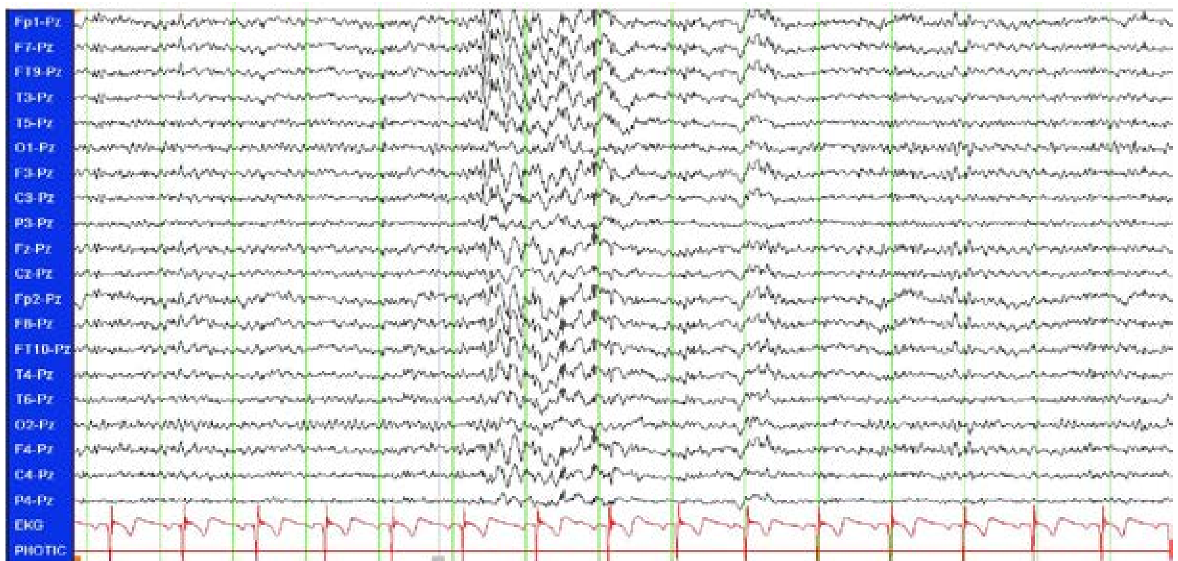


Figure 13. EEG finding showed generalized paroxysmal sharp and slow complex wave (mainly anterior head regions).

SEPs

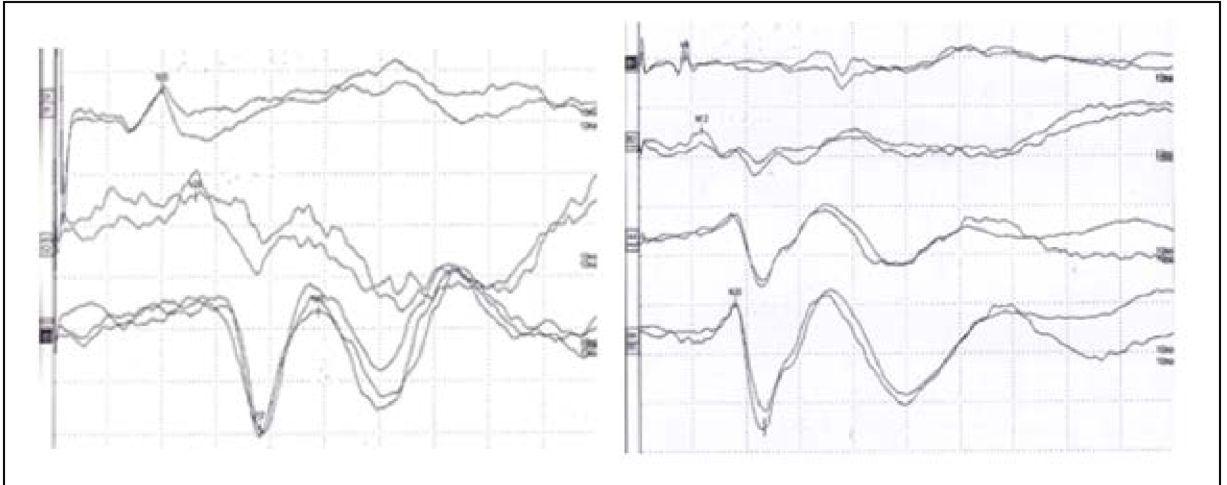


Figure 14. SEP of right tibial (left panel) and left median nerve (right panel) showed giant cortical potential in patient III-14.

Table 6. Result of SSEP

Family ID	Median SSEP (N20 amplitude)		Tibial SSEP (P37 amplitude)	
	Rt.	Lt.	Rt.	Lt.
II-7	6.30 N	6.40 N	0.99 N	1.80 N
II-11	48.00á á	68.00 á á	4.94 N	20.45á á
III-3	7.80 N	1.50 N	6.00 N	3.40 N
III-14	8.51 á	26.30 á á	5.44 N	1.77 N
III-9	5.70 N	3.30 N	2.10 N	1.30 N
II-15	3.10 N	2.80 N	0.40 N	0.40 N
III-18	1.70 N	1.20 N	1.00 N	1.80 N
III-13	5.50 N	6.60 N	2.90 N	5.30 N
III-12	2.60 N	2.80 N	0.86 N	1.30 N
III-1	5.10 N	7.20 N	2.20 N	5.30 N
III-2	5.70 N	4.50 N	3.20 N	3.50 N
III-15	2.27 N	17.61á á	6.38 N	3.48 N
III-16	13.50á	11.20 á	2.20 N	9.10á
III-6	6.80 N	6.00 N	2.40 N	3.70 N
II-5	13.60á	23.50á á	4.60 N	9.70á
III-7	14.20á	14.00á	5.00 N	5.70 N
III-8	5.30 N	7.00 N	1.00 N	2.20 N

C-reflex

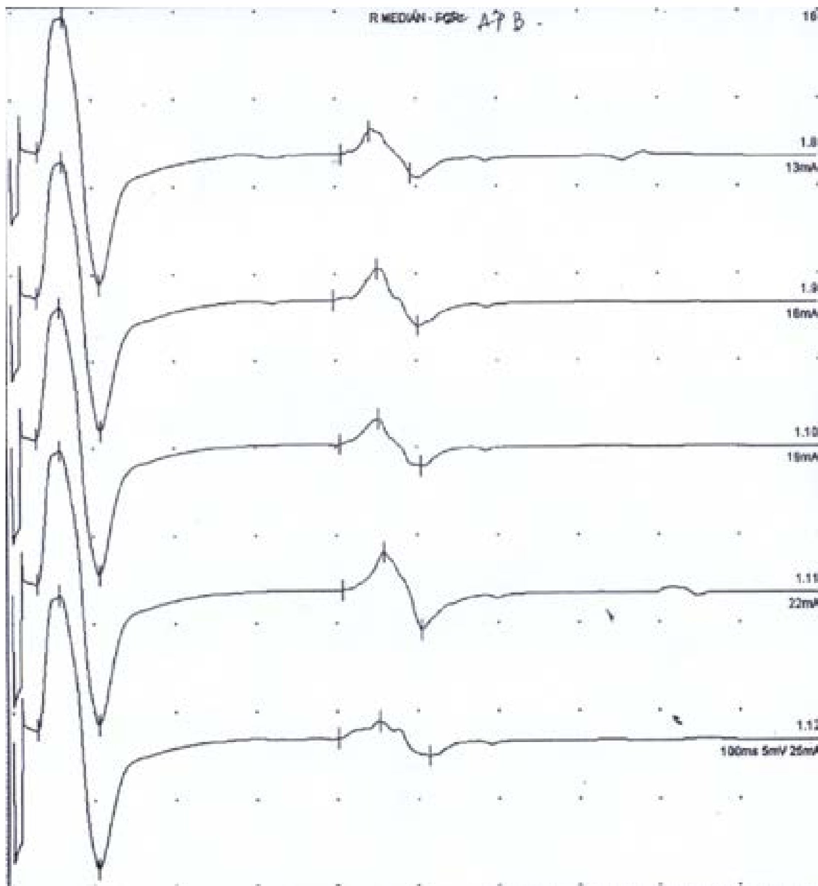


Figure 15. C-reflex was found during submaximal stimulation of the right median nerve of Patient III-15.

Table 7. Result of C-reflex

Family ID	Sex	Age	C reflex	
			Rt.	Lt.
II-7	M	58	NR	NR
II-11	M	55	41.55	39.70
III-3	M	27	40.75	40.15
III-14	F	20	36.10	37.35
III-9	M	67	NR	NR
II-15	M	51	NR	NR
III-18	F	14	NR	NR
III-13	M	28	NR	NR
III-12	F	25	NR	NR
III-1	M	32	NR	NR
III-2	M	30	39.05	40.75
III-15	M	26	40.15	40.90
III-16	F	24	37.60	38.60
III-6	F	34	NR	NR
II-5	F	62	40.85	42.65
III-7	F	22	36.95	36.00
III-8	F	28	NR	NR

Jerk-locked back averaging EEG

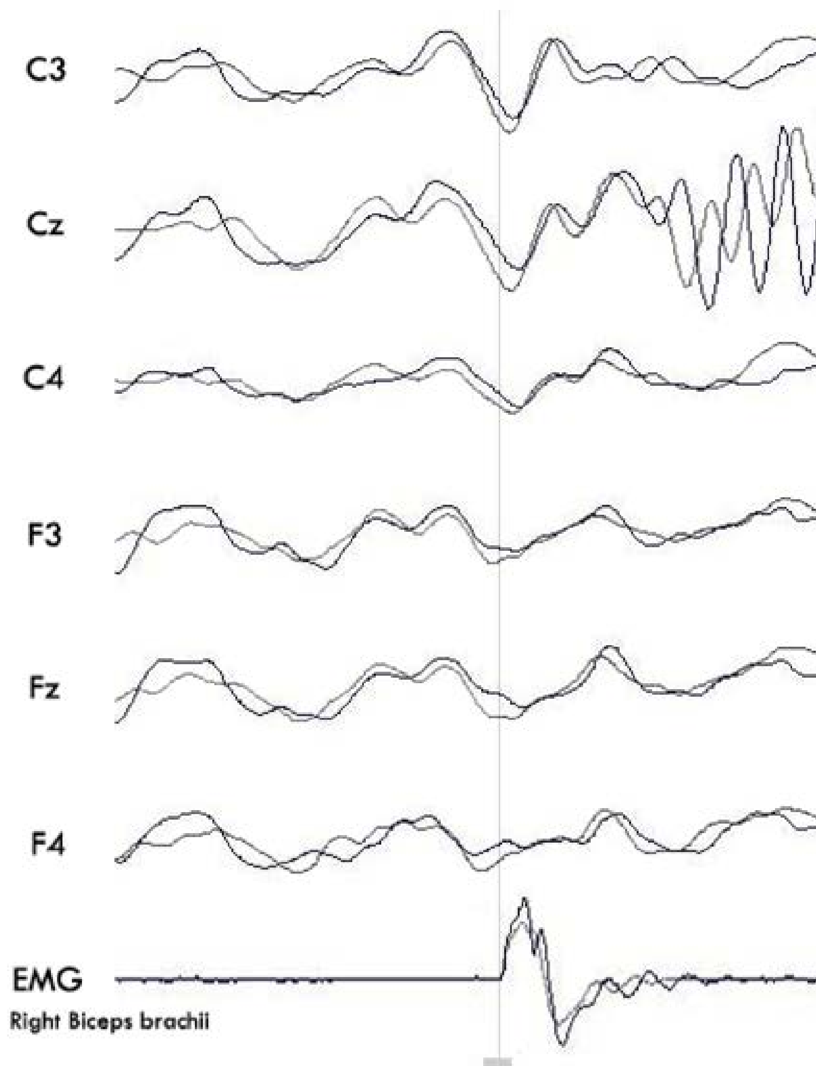


Figure 16. Jerk-locked averaging analysis showing a positive-negative potential over the contralateralcentroparietal electrodes, preceding myoclonus about 22 ms (right Biceps brachii; 2 averaging, 200 each).

Table 8. Clinical and neurophysiological findings in 13 patients with benign adult familial myoclonic epilepsy

Patient ID	Gender	Age at follow-up (years)	Age of onset (years)		Electrophysiological study					Medication	Seizure frequency in the past year
			Cortical tremor	Generalized seizures	EEG	Photic	Giant SEPs	C-reflex	JLA		
II-1	M	63	33	33	Multifocal PSW	-	N/A	N/A	N/A	Rivotril, PB	GTC once a year, Myoclonus once a month
II-4	F	62	20	None	Multifocal PSW	+	N/A	N/A	N/A	None	None
II-5	F	62	28	None	Generalized sharp wave	-	+	+	N/A	None	None
II-9	M	59	12	26	Multifocal PSW	+	-	+	N/A	VPA 400, CZP 0.5	None
II-11	M	57	10	None	N/A	N/A	+	+	N/A	None	None
III-2	M	31	21	25	N/A	-	-	+	N/A	CZP 0.5, PPN 20	None
III-3	M	30	17	20	Multifocal PSW	-	-	+	N/A	VPA 500, CZP 2	None
III-4	M	34	20	None	Normal	-	-	+	N/A	PPN 80	None

III-7	F	31	25	30	N/A	N/A	+	+	N/A	None	None
III-10	F	32	10	None	Multifocal PSW	+	+	+	N/A	None	None
III-14	F	22	17	None	Multifocal PSW	+	+	+	N/A	None	None
III-15	M	28	19	24	Multifocal PSW	-	+	+	+	LVT 4000, CZP 4	None
III-16	F	F, 27	21	19	Generalized sharp wave	+	+	+	+	CZP 2, LVT 1500	None

Abbreviations: SEPs, somatosensory evoked potentials; N/A, not available; PSW, polyspikes and wave; PB, phenobarbital; VPA, valproic acid; LVT, leviteracetam; CZP, clonazepam; PPN, propanolol; GTC, generalized tonic-clonic convulsion; -, absence; +, presence.

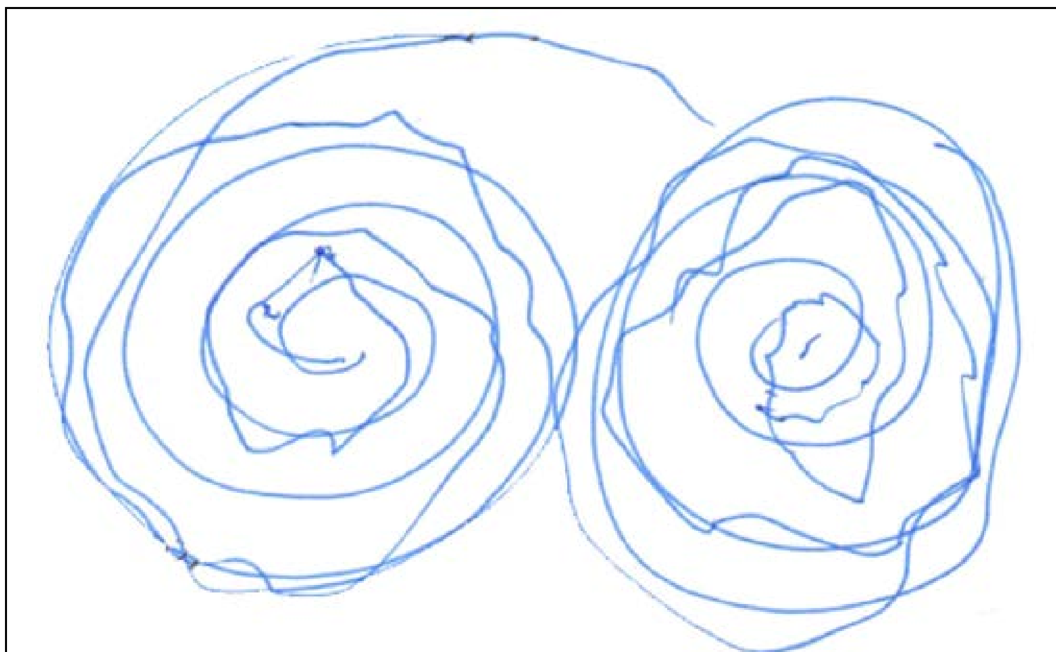


Figure 17. Archimedes spiral free-hand drawings of a patient with cortical tremor elicited irregular tremor with occasional sudden, brisk jerk.

Absence of linkage to 8q23.3-q24.1 and 2p11.1-q12.2.

Two-point linkage analysis generated negative LOD scores in every microsatellite marker at all the recombination values from 0.00 to 0.50. The LOD scores were equal to minus infinity at recombination rate of 0.00, indicating no linkage to any of the selected markers. As a result, linkage to the two chromosome regions 8q23.3–q24.1 and 2p11.1–q12.2 was excluded.

Table 9. LOD scores results of Thai family with BAFME.

No.	Marker	Recombination Fraction ( )							
		0.000	0.010	0.050	0.100	0.200	0.300	0.400	0.500
1	D8S1830	-	-1.60	-0.88	-0.56	-0.26	-0.10	-0.02	0.00
2	D8S555	-	-1.14	-0.49	-0.25	-0.08	-0.02	0.00	0.00
3	D8S588	-	-6.42	-3.05	-1.72	-0.61	-0.17	-0.02	0.00
4	D8S1112	-	-4.63	-2.51	-1.58	-0.69	-0.26	-0.06	0.00
5	D8S1826	-	-5.64	-2.89	-1.75	-0.74	-0.28	-0.06	0.00
6	D8S572-18	-	-6.73	-3.27	-1.86	-0.66	-0.19	-0.03	0.00
7	D8S1799	-	-8.12	-4.06	-2.43	-1.00	-0.36	-0.08	0.00
8	D2S388	-	-2.09	-1.20	-0.77	-0.35	-0.14	-0.03	0.00
9	D2S2175	-	-1.18	-0.53	-0.28	-0.10	-0.03	-0.01	0.00
10	D2S2264	-	-2.80	-1.44	-0.89	-0.39	-0.15	-0.04	0.00

GWLS and narrowing down the critical region

We detected preliminary evidence for linkage at D3S1262 that gave the maximum two-point LOD score of 5.419 at $\Theta = 0.00$. The critical region was 15 Mb in size and located on chromosome 3q26.31-3q28. This prompted us to select eleven additional markers on chromosome 3q (Figure 16) to refine the critical region to 10 Mb between D3S3730 and D3S1580 on 3q26.32-3q28. The results of haplotype analysis for these markers and two-point LOD scores between disease phenotype and each marker locus are shown in Figure 17, respectively.

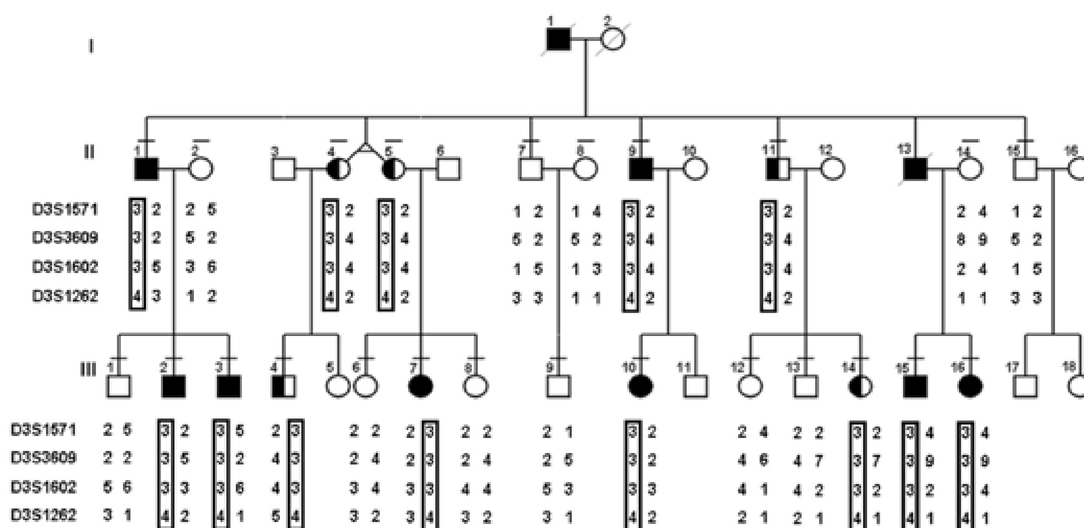
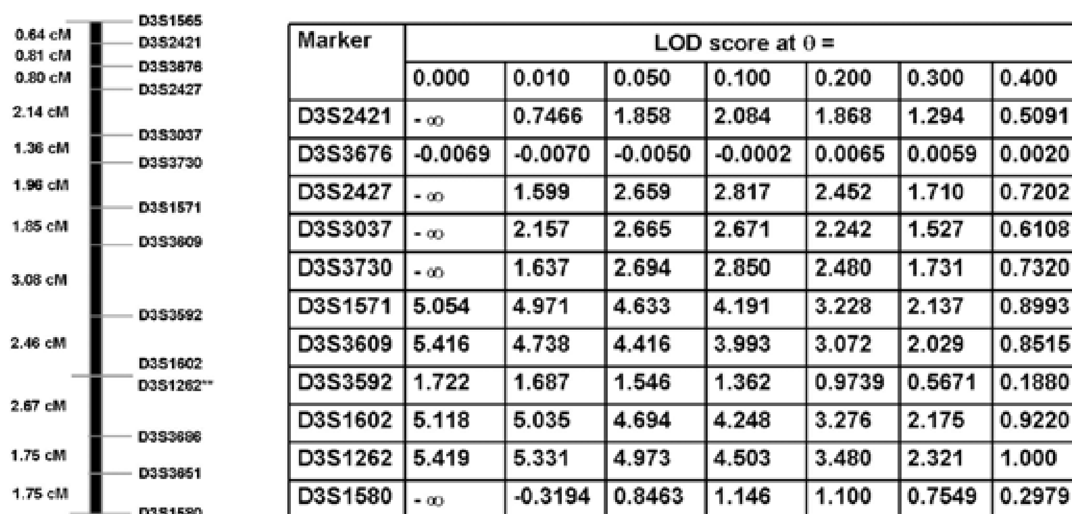


Figure 18. Pedigree of a Thai family with BAFME. Genotypes for the four informative markers of the linked loci are shown. The fourth BAFME locus is indicated in the rectangle.



Marker	LOD score at θ =						
	0.000	0.010	0.050	0.100	0.200	0.300	0.400
D3S2421	- ∞	0.7466	1.858	2.084	1.868	1.294	0.5091
D3S3676	-0.0069	-0.0070	-0.0050	-0.0002	0.0065	0.0059	0.0020
D3S2427	- ∞	1.599	2.659	2.817	2.452	1.710	0.7202
D3S3037	- ∞	2.157	2.665	2.671	2.242	1.527	0.6108
D3S3730	- ∞	1.637	2.694	2.850	2.480	1.731	0.7320
D3S1571	5.054	4.971	4.633	4.191	3.228	2.137	0.8993
D3S3609	5.416	4.738	4.416	3.993	3.072	2.029	0.8515
D3S3592	1.722	1.687	1.546	1.362	0.9739	0.5671	0.1880
D3S1602	5.118	5.035	4.694	4.248	3.276	2.175	0.9220
D3S1262	5.419	5.331	4.973	4.503	3.480	2.321	1.000
D3S1580	- ∞	-0.3194	0.8463	1.146	1.100	0.7549	0.2979

Figure 19. Genetic map of the eleven markers for fine mapping (left) and their two-point LOD scores (right).

Targeted resequencing of 10Mb linkage region on chromosome 3

Total yield of 37,446,268 reads or 2,808,470,100 bp of total sequence was achieved by using one lane of a Illumina sequencing run. Of these sequence, 97.4% were mapped back to unique regions of the human genome (hg19). The capture efficiency varied across the target with 94.7% more than 1X , 92.4% more than 10X and the mean read depth of target regions is 227.8X. A total of 8522 variants were found in this region, of these 89 variants were in the coding region. We excluded known SNPs and have not been reported in dbSNP Build 130, 1000 Genomes and HAPMAP. We filtered only the novel coding or splice site variants on the basis of heterozygosity because of autosomal dominant trait. The remaining two candidates were c.589C>G in *HTR3D* resulted in L197V and D527E in *MASP1*.

Table 10. Result of the targeted next-generation sequencing

No.	Position	Gene	Mutation	Codon	Prediction
1	183756391	*HTR3D*	Missense	CTC-gTC	Tolerated
2	186954078	*MASP1*	Missense	GAC-GAg	Tolerated

Mutation analysis and restriction enzyme digestion in *HTR3D* and *MASP1*

For confirming the mutation in *HTR3D* and *MASP1* gene, PCR amplification and Sanger sequencing were performed to confirm the mutation in III-7.Although these two variants were conserved during evolution, both of them have been detected in our internal variant database in Thai-controls with frequency of 5 out of.38 alleles and 2 out of 226 alleles, respectively using restriction enzyme digestion.

Array-bases Comparative Genomic Hybridization for detecting the dosage imbalance

From the array CGH result of chromosome3 (Figure 18)there are seven regions that gave Log2-ratio values more than 0.3 while six were found to have Log2-ratio values less than 0.3. Considering only linkage region we found Log2 ratio value at position 185459416-185463265 less than 0.3 indicating loss of DNA region. The red box (Figure 18) mark loss on chromosome 3. After searching in Database of Genomic Variants (DGV - http://projects.tcag.ca/variation/) this gain region was resided in the variation_4364 (31). Eventually, array CGH did not provide possible causative copy number variation.

UCSC Genome Browser on Human Mar. 2006 (NCBI36/hg18) Assembly

18	CHROMOSOME	START	STOP	SIZE	DATAPOINTS	LOG2_RATIO_MEAN	
19	chr3	35181	5509161	5473980	10946	0.00161	
20	chr3	5509801	5514081	4280	11	0.42983	gain
21	chr3	5514861	9494947	3980086	8067	-0.00034	
22	chr3	9495282	9505440	10158	21	0.1625	
23	chr3	9506029	15324440	5818411	11728	-0.02014	
24	chr3	15324854	15325489	535	3	0.31917	gain
25	chr3	15326199	42028399	26702200	52834	-0.00015	
26	chr3	42028569	42033913	5344	12	-0.37332	loss
27	chr3	42034018	49473100	7439082	14370	-0.01058	
28	chr3	49473435	49480058	6623	12	-0.27351	
29	chr3	49480638	53002627	3521989	6746	-0.02259	
30	chr3	53003376	53013959	10583	23	-0.44926	loss
31	chr3	53014294	54739455	1725161	3504	-0.02306	
32	chr3	54739905	54741353	1448	4	0.23397	
33	chr3	54741858	56582249	1840391	3795	0.00414	
34	chr3	56583046	56584989	1943	6	0.55008	gain
35	chr3	56587538	68823481	12235943	23919	-0.00299	
36	chr3	68824001	68830195	6194	15	0.74928	gain
37	chr3	68830745	74742632	5911887	12131	-0.00266	
38	chr3	74743230	74750541	7311	17	-0.27461	
39	chr3	74750796	100381767	25630971	41355	0.00899	
40	chr3	100382222	100385487	3265	8	-0.4912	loss
41	chr3	100385697	127558620	27172923	53427	0.00535	
42	chr3	127559491	127563292	3801	9	-0.34332	loss
43	chr3	127563782	132838133	5274351	10427	-0.0148	
44	chr3	132838678	132842553	3875	6	0.42454	gain
45	chr3	132842733	135448464	2604731	5130	-0.00203	
46	chr3	135448689	135455264	5575	13	-0.35374	loss
47	chr3	135455779	147867791	12412012	24383	-0.0019	
48	[illegible]	[illegible]	[illegible]	[illegible]	10	[illegible]	gain
49	chr3	147873222	185459206	37585984	74746	0.0038	
50	chr3	185459416	185463265	3849	9	-0.59415	loss
51	chr3	185463680	186254069	790389	1624	-0.03343	
52	chr3	186254154	186266918	12764	19	0.15948	
53	chr3	186267408	196896208	10628800	21415	-0.00018	
54	chr3	196896783	196927063	30280	44	0.50986	gain
55	chr3	196927268	199372458	2445190	4540	-0.00847	

The 4th BAFME locus:
180029295-190025487

Figure 20. Result of Array CGH.

Whole Exome Sequencing

The result of exome sequencing analysed from BGI showed that there were 212 variants and 21 indels. The results were summarized into two categories below. Number of changes represented the number of variants left after using indicated criteria.

Table 11. Summarized data for SNP_3q26.32-3q28.

Criteria	Number of changes
Total changes in 3q26.32-3q28	212
0000' in 2 patients and 1 control	24
dbSNP135	13
Found in 2 patient and didn't find in control	3
Check from Alamut program	0

Table 12. Summarized data for Indel_3q26.32-3q28.

Criteria	Number of changes
All indels in 3q26.32-3q28	21
Found in 2 patient and didn't find in control	2
Check from public database	0

0000: Information whether the SNP could be found in dbSNP, 1000 genomes data(pilot1, 2, 3), hapmap, YH project ('0' indicates could not be found in corresponding data or the MAF of the genotype less than 0.5% except YH project, '1' indicates could).

In summary, there are no interesting candidate mutations in the linked locus. It is possible that the criteria used were restricted which may lead to unidentifiable mutation.

We then attempted to reanalyze the variants using less strict criteria. The result was shown in table 13.

Table 13. Reanaysis of whole exome sequencing.

Criteria	Number of variants
Variants_3q26.32-3q28	212
Variants_shared by 2 cases not by control	74
Variants_non-synonymous	68
Variants_coding region	11

Table 14. List of variants which located in coding region after reanalysis.

Position	Reference	Gene	Control II-15	Case III-16	Case III-2
183508714	G	*YEATS2*	G44G59A2,0000,ref	A81A60G1,0101,missense	R99A22G22,0101,missense
183442219	A	*YEATS2*	A99A91G0,0000,ref	G95G87A0,1101,missense	R99A46G34,1101,missense
183508590	A	*YEATS2*	A99A44G0,0000,ref	G81G41A0,0101,missense	R99G22A17,0101,missense
186917751	C	*RTP1*	C81C29G0,0000,ref	S99C14G10,1110,missense	S99C10G5,1110,missense
191179193	A	*PYDC2*	A47A14G0,0000,ref	R99A6G6,1111,missense	R94A8G4,1111,missense
184428903	T	*MAGEF1*	T88T56G1,0000,ref	K99T30G19,1111,missense	K99G26T27,1111,missense
184429414	C	*MAGEF1*	C60C21G0,0000,ref	M99C12A10,1111,missense	M99A16C8,1111,missense
188426077	G	*LPP*	G99G41T0,0000,ref	R99G24A12,0000,missense	R99A21G21,0000,missense
183818416	G	*HTR3E*	G67G25T0,0000,ref	A96A38G0,1111,missense	A81A58G1,1111,missense
183756391	C	*HTR3D*	C99C61G0,0000,ref	G83G47A1,0100,missense	S99G41C29,0100,missense
184922294	C	*EHHADH*	C99C45G0,0000,ref	Y99C21T16,1100,missense	Y99T22C18,1100,missense

Of eleven additional variants, two variants have been reported in less than two databases. First variant is and L197V in *HTR3D*. The second variant is G379E in *LPP*. In addition, these two variants were chosen because they were not present in unaffected while it present in our two.. *The* L197V in *HTR3D* was found in5 out of 38 alleles of Thai healthy control. Another G379E in *LPP* was in progressed. This gene is unlikely to be a causative gene for BAFME

CHAPTER V
DISCUSSION

Benign Adult Familial Myoclonic Epilepsy (BAFME) was characterized by adult-onset cortical tremor and generalized seizure. This disorder is transmitted as an autosomal dominant trait with high penetrance. BAFME diagnosis is based on clinical and electrophysiological criteria. An electrophysiological study is essential to confirm the cortical origin of myoclonus. BAFME was first reported in 1990in the Japanese family which the affected patients had fine finger tremulous movement, myoclonic jerks, and occasional tonic-clonic seizures (TCS)(10).

We reported a large Thai pedigree which consists of 13 affected family members. Clinical and electrophysiological features of our patients (Table 8) confirmed the diagnosis of BAFME which showed a tremor with adult onset, similar to essential tremor but associated with generalized epilepsy. The cortical origin was confirmed by electrophysiological study showing cortical hyperexcitability (enhanced long loop reflex, giant somatosensory evoked potential (SEPs)), premyoclonus cortical spikes detected by the jerk-locked back averaging method. Of the 13 affected members, one (7.7%) developed epileptic seizure prior to tremor, originally observed in 16% of cases(32).

The patients' phenotypes were similar to those previously reported families in other populations particularly the Japanese patients. Electrophysiologic studies revealed the cortical hyperexcitability with cortical origin of tremor. Our patients had a non-progressive clinical course. Myoclonic tremor and seizure responded well to valproic acid, clonazepam, or levetiracetam. No cognitive deficit was found in our cases.

For more than twenty years since the first reported BAFME, no underlying genes have been reported but only been mapped to three chromosomal regions. Linkage analysis was used to identify chromosomal region since then. The first locus was on chromosome 8q (1), the second on chromosome 2p (3) and the third was recently reported on chromosome 5p (4). We performed exclusion of linkage only chromosome 8q and chromosome 2p and whole genome linkage analysis before 5p locus was reported in 2010. The latest article published on 2012 used Human Linkage 12 SNP

array in combination with microsatellite marker for refinement and reported a third locus on 5p region. The anticipation was suspected in two previous studies from the same Japanese research group. The latter study added three additional families out of four to the data. Clinical anticipation was divided into two types including cortical tremor and generalized seizures. There was a clear in the onset age of the cortical tremor in three families while similarly in generalized seizures Gender has no relationship with both phenotypes. However, our Thai family did not show any clinical anticipation among three generations.

We first performed genome-wide linkage analysis with 400 microsatellite markers after exclusion of chromosome 8 and chromosome 2. The D3S1262 on chromosome 3q26.32-3q28 was the only marker that gave LOD score greater than 3 (5.419). Using 11 additional subsequent markers, we successfully narrowed down the critical region from 15 Mb to 10 Mb, between the markers D3S3730 and D3S1580. This locus represents the fourth chromosomal region for BAFME. The critical region consists of 136 genes and had several candidate genes for example Chloride Channel 2 (*CLCN2*), Potassium large conductance calcium-activated channel subfamily M beta member 2 (*KCNMB2*) and 5-hydroxytryptamine (serotonin) receptor 3 family member D (*HTR3D*) etc.

Genetic heterogeneity is not uncommon in human diseases including neurological disorders. A striking example is spinocerebellar ataxia, which has at least 33 underlying genes (33). We attempted to identify the causative gene within the fourth chromosomal region using several techniques. First, we use targeted resequencing of whole 10 Mb. By that time, NGS technology is a recent research tool. The cost of this service is very high though less than traditional Sanger sequencing if all genes need to be sequenced. We therefore sent only one affected member as a trial of this technology. The result showed very good data quality with more than 100x coverage. We could obtain sequences from coding exon, intron, 5′UTR, 3′UTR, promoter and intergenic region. Unfortunately, after filtering process excluding variants present in healthy controls available in public databases, no variants in coding regions remained. the result did not reveal any candidate variants (Table 15).

Table 15. Variant from Targeted Next-Generation Sequencing

Categories	Number
All variants	8,522
Coding variants	89
Novel (not in dbSNP)	2
RFLP in healthy control	0

Secondly, we used array-based comparative genomic hybridization for detecting the dosage imbalance, It is possible that this disease may occur from copy number variation.

Our aCGH results showed that there was a gain position between chr3:196896783-196927063 in the critical region. However, it has already been reported in Database Genome Variants (DGV).

Finally, we performed Whole Exome Sequencing as some data might be missed. In collaboration with Beijing Genome Research Institute, we sent two affected and one unaffected DNA to performed whole exome sequencing.

As shown in the results, our primary filtering criterions were too strict that we could not find a candidate variant. Due to the massive information and many databases to date, some rare variants might be reported as SNPs in the public genetics database with low frequency of 1-2 alleles. This is our case, one additional variant was found with low frequency from db135. The G379E in *LPP* is now our candidate. Further study is needed to verify its pathogenicity in BAFME.

Combination of next-generation sequencing and array comparative genomic hybridization technology should cover almost all possible mutations such as nucleotide substitution, small or large insertions/deletion and dosage imbalance.. Although thousands of new variants were found in 5-UTR, 3-UTR, intergenic or intron region, our first priority is in the coding regions. Further studies are needed to verify if variants outside coding region are involved in the disorder.

Discovery of the fourth BAFME chromosomal region will facilitate the identification of the responsible gene. This will provide further understanding into the

molecular basis of epilepsy and better insight into the disease mechanism leading to more effective treatment of this disorder.

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APPENDICES

APPENDIX A
ABI Prism Linkage Mapping Set 2.5

Chromosome 1

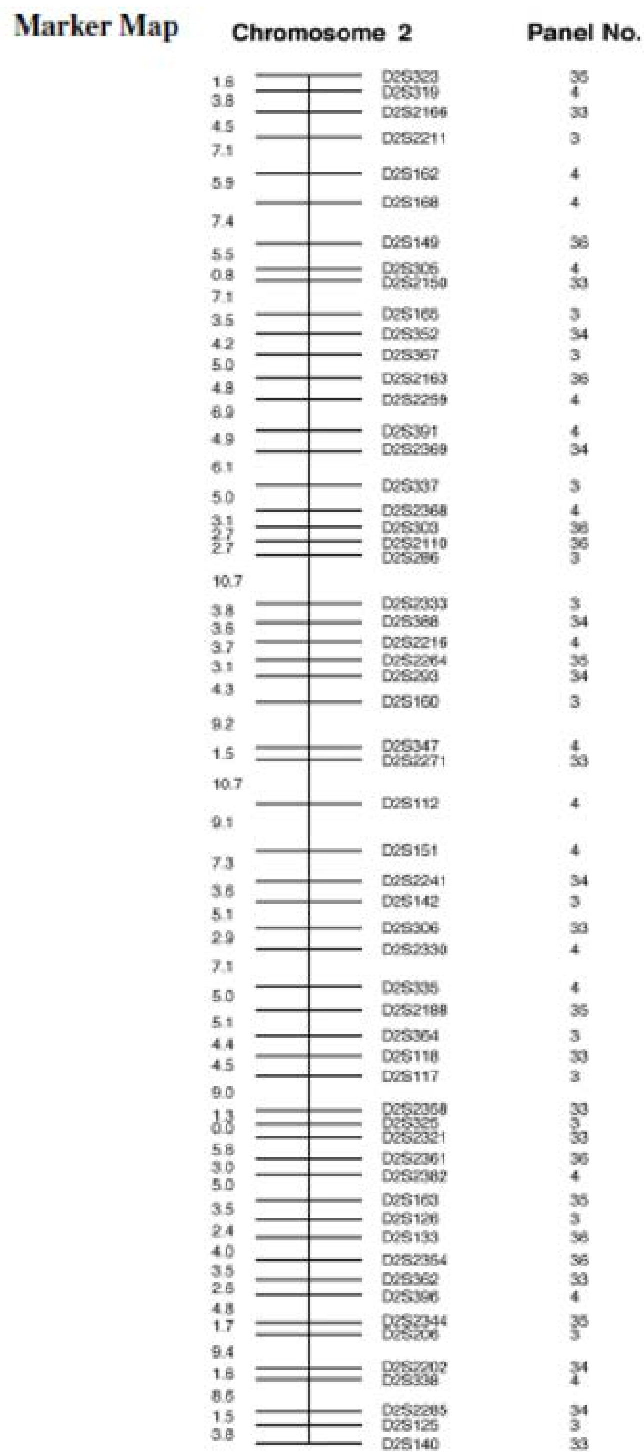
Marker Map

	Chromosome 1	Panel No.
	D1S468	2
5.0	D1S2660	31
3.9	D1S214	2
6.6	D1S450	1
3.8	D1S2667	1
2.8	D1S434	32
6.9	D1S507	31
4.7	D1S2697	2
5.4	D1S2644	29
2.0	D1S199	2
4.1	D1S2864	31
4.5	D1S234	1
6.2	D1S233	29
5.5	D1S255	1
3.5	D1S2892	30
4.3	D1S2713	30
1.9	D1S2797	1
3.7	D1S2832	29
5.5	D1S2890	1
3.9	D1S2873	30
1.3	D1S2737	32
0.1	D1S2846	32
3.9	D1S230	2
4.5	D1S198	32
7.0	D1S2841	2
0.7	D1S500	29
6.8	D1S207	2
3.6	D1S2766	31
8.3	D1S435	29
0.8	D1S2868	2
2.9	D1S2793	32
4.8	D1S206	1
2.9	D1S495	31
9.4	D1S2726	1
5.0	D1S252	2
5.4	D1S498	2
9.4	D1S2635	29
3.2	D1S484	1
9.2	D1S2878	1
4.2	D1S196	1
4.4	D1S452	29
4.2	D1S218	2
8.1	D1S2818	32
3.2	D1S238	2
2.4	D1S2877	30
4.2	D1S412	32
2.3	D1S413	2
8.8	D1S249	1
2.1	D1S2692	29
5.7	D1S245	32
3.7	D1S425	2
7.2	D1S227	31
3.3	D1S213	1
2.5	D1S2833	31
1.7	D1S2709	32
3.8	D1S2800	1
5.1	D1S2850	30
8.8	D1S2670	29
2.0	D1S2785	1
3.8	D1S304	30
4.6	D1S2842	1
4.9	D1S423	32
7.3	D1S2836	1

Associated Panels. The following panels cover chromosome 1.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
1	D1S2797	FAM	0.74	97	135	117	129
	D1S249	FAM	0.87	160	190	166	176
	D1S2800	FAM	0.77	205	221	207	207
	D1S234	FAM	0.81	262	284	270	274
	D1S450	FAM	0.81	315	341	331	339
	D1S255	VIC	0.75	85	107	89	99
	D1S2667	VIC	0.82	122	152	138	142
	D1S2785	VIC	0.76	171	185	179	183
	D1S2890	VIC	0.81	211	235	211	215
	D1S484	VIC	0.64	272	286	274	276
	D1S196	VIC	0.74	321	337	327	327
	D1S213	NED	0.86	103	129	105	115
	D1S2878	NED	0.84	148	176	154	168
	D1S206	NED	0.82	205	223	215	221
	D1S2836	NED	0.79	242	256	244	248
	D1S2726	NED	0.75	280	294	282	282
	D1S2842	NED	0.76	336	358	342	344
2	D1S199	FAM	0.83	94	120	96	104
	D1S207	FAM	0.84	146	176	158	164
	D1S2868	FAM	0.76	206	220	208	210
	D1S413	FAM	0.76	249	265	251	255
	D1S238	FAM	0.86	292	326	294	304
	D1S252	VIC	0.81	86	112	88	88
	D1S230	VIC	0.78	150	164	156	160
	D1S468	VIC	0.76	191	211	193	203
	D1S2841	VIC	0.78	230	250	236	240
	D1S2697	VIC	0.7	286	302	290	298
	D1S214	NED	0.78	117	147	121	143
	D1S498	NED	0.82	187	209	187	201
	D1S218	NED	0.83	265	291	275	277
	D1S425	NED	0.81	332	358	350	356
29	D1S2644	FAM	0.8	116	132	125	125
	D1S435	FAM	0.73	162	182	164	164
	D1S500	VIC	0.62	116	134	125	129
	D1S2670	VIC	0.83	156	182	156	166
	D1S2652	NED	0.62	93	111	95	99
	D1S452	NED	0.75	119	131	124	126
	D1S2635	NED	0.87	146	164	150	159
	D1S233	NED	0.85	196	212	206	212
	D1S2692	NED	0.86	276	316	304	310
30	D1S2892	FAM	0.88	97	133	107	111
	D1S2850	FAM	0.64	150	158	151	153
	D1S304	FAM	0.6	170	180	172	176
	D1S2713	FAM	0.76	257	283	269	269
	D1S2877	NED	0.71	143	157	150	150
	D1S2873	NED	0.69	170	186	172	172
31	D1S2833	FAM	0.82	91	113	93	103
	D1S2864	FAM	0.81	144	172	144	144
	D1S507	FAM	0.78	187	207	187	197
	D1S2660	VIC	0.78	116	124	122	122
	D1S227	NED	0.69	61	75	71	73
	D1S495	NED	0.87	143	169	152	156
	D1S2766	NED	0.74	187	199	187	197
32	D1S2793	FAM	0.77	96	132	107	115
	D1S198	FAM	0.79	313	327	313	317
	D1S412	VIC	0.7	129	147	135	135
	D1S2709	VIC	0.72	197	203	199	201
	D1S245	VIC	0.81	239	257	242	242
	D1S423	NED	0.6	89	95	91	93
	D1S434	NED	0.61	130	144	136	138
	D1S2846	NED	0.54	167	181	173	175
	D1S2737	NED	0.75	194	224	218	222
	D1S2818	NED	0.7	258	268	257	267

Chromosome 2



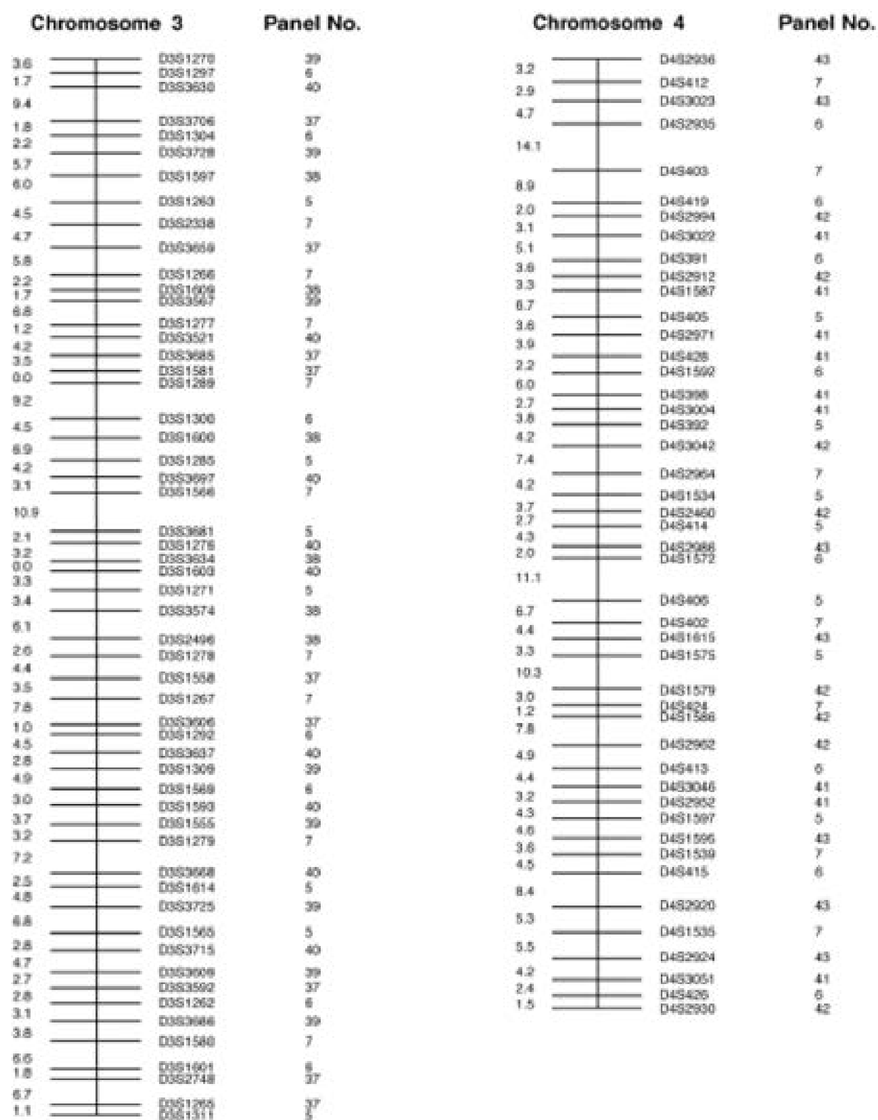
Associated Panels. The following panels cover chromosome 2.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
3	D2S286	FAM	0.66	80	102	82	92
	D2S165	FAM	0.85	141	175	153	159
	D2S160	FAM	0.78	206	224	212	214
	D2S2211	FAM	0.74	236	258	244	246
	D2S367	FAM	0.86	306	340	312	318
	D2S125	VIC	0.82	87	109	95	97
	D2S206	VIC	0.8	125	163	149	151
	D2S117	VIC	0.82	190	220	192	210
	D2S142	VIC	0.76	235	255	239	243
	D2S2333	NED	0.82	79	101	93	99
	D2S126	NED	0.82	113	145	133	137
	D2S325	NED	0.82	154	184	158	168
	D2S364	NED	0.8	230	256	234	238
	D2S337	NED	0.88	291	315	305	307
4	D2S112	FAM	0.71	73	89	75	81
	D2S162	FAM	0.75	119	148	120	132
	D2S2330	FAM	0.81	166	186	168	176
	D2S2216	FAM	0.76	208	224	210	220
	D2S347	FAM	0.8	267	299	289	289
	D2S2259	FAM	0.79	321	341	321	339
	D2S319	VIC	0.73	128	140	130	132
	D2S168	VIC	0.82	156	180	162	174
	D2S151	VIC	0.82	224	252	242	246
	D2S2382	VIC	0.81	296	336	308	316
	D2S2368	NED	0.83	93	117	103	105
	D2S391	NED	0.79	143	159	151	153
	D2S335	NED	0.79	183	205	193	197
	D2S396	NED	0.83	232	250	237	241
	D2S338	NED	0.81	264	288	274	274
	D2S305	NED	0.72	314	336	322	336
33	D2S2321	FAM	0.75	85	107	96	96
	D2S140	FAM	0.76	156	172	157	161
	D2S118	FAM	0.78	176	220	182	182
	D2S2166	FAM	0.84	236	254	246	250
	D2S362	VIC	0.77	106	121	106	106
	D2S2150	VIC	0.77	169	201	169	187
	D2S2271	NED	0.81	132	164	142	142
	D2S2358	NED	0.80	183	205	196	200
	D2S306	NED	0.70	223	250	227	249

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
34	D2S2241	FAM	0.77	86	102	91	96
	D2S293	FAM	0.83	170	196	170	178
	D2S388	FAM	0.64	218	236	227	231
	D2S2285	VIC	0.64	130	151	141	150
	D2S2202	VIC	0.67	240	250	246	250
	D2S2369	NED	0.68	107	161	151	151
	D2S352	NED	0.78	267	295	275	285
35	D2S2188	FAM	0.66	128	150	136	142
	D2S323	FAM	0.57	181	197	193	193
	D2S163	FAM	0.78	217	235	220	226
	D2S2264	VIC	0.77	245	260	248	250
	D2S2344	NED	0.78	278	298	286	288
36	D2S2110	FAM	0.78	132	146	136	140
	D2S2354	FAM	0.8	260	280	264	270
	D2S2163	VIC	0.84	119	129	123	123
	D2S133	VIC	0.66	223	249	230	238
	D2S2361	NED	0.76	128	156	135	143
	D2S303	NED	0.68	169	197	184	192
	D2S149	NED	0.81	214	232	219	221

Chromosome 3, 4

Marker Maps



Associated Panels. The following panels cover chromosome 3, 4.

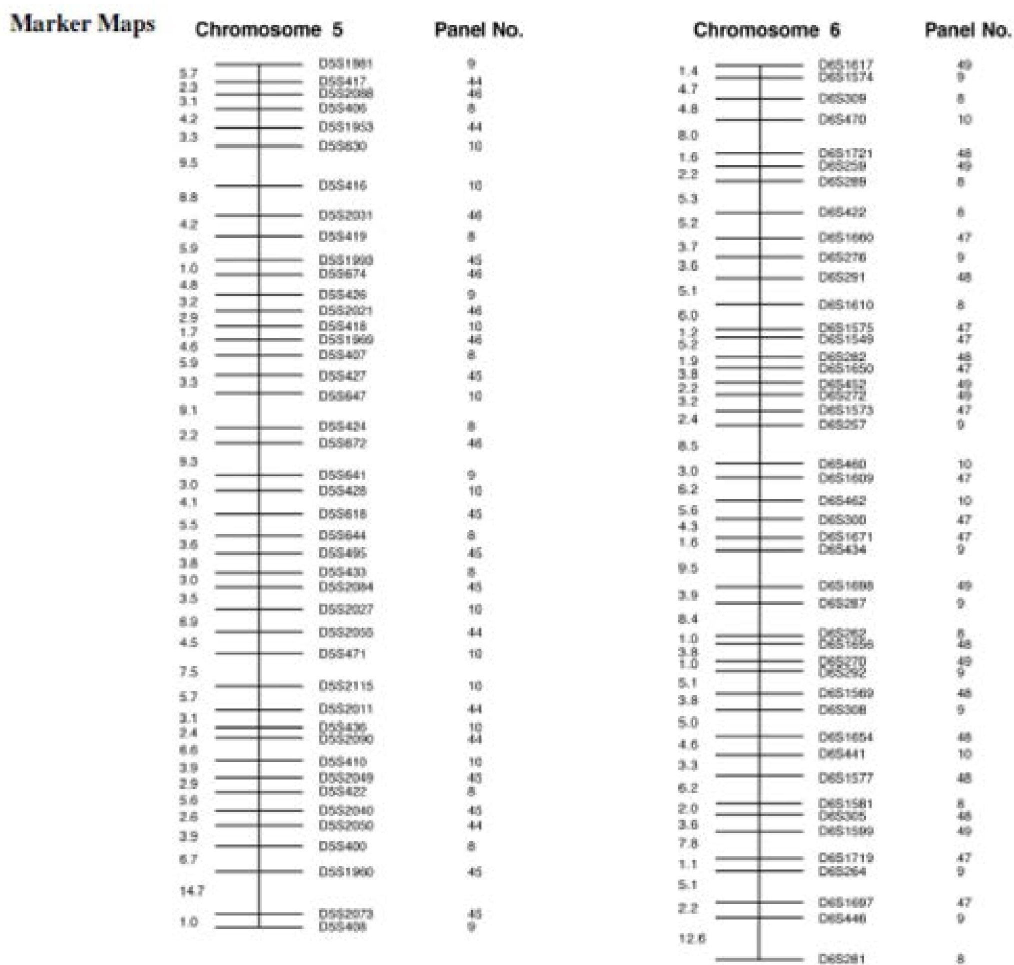
Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
5	D4S392	FAM	0.82	79	109	87	91
	D3S1311	FAM	0.83	133	159	145	147
	D3S1565	FAM	0.64	178	194	180	182
	D4S406	FAM	0.87	242	268	250	258
	D4S1575	FAM	0.65	287	305	295	297
	D3S1271	VIC	0.73	84	104	92	94
	D3S3681	VIC	0.83	121	161	151	151
	D4S414	VIC	0.89	231	249	237	237
	D4S405	VIC	0.86	281	309	293	297
	D3S1614	NED	0.83	101	125	115	115
	D4S1534	NED	0.77	147	169	149	159
	D3S1263	NED	0.86	191	211	199	205
	D3S1285	NED	0.73	233	251	237	243
	D4S1597	NED	0.76	274	300	278	278
6	D3S1262	FAM	0.80	110	132	116	122
	D3S1569	FAM	0.80	150	174	156	168
	D4S1572	FAM	0.84	195	213	199	205
	D3S1300	FAM	0.82	230	262	232	252
	D4S413	FAM	0.85	282	334	284	298
	D4S2935	VIC	0.62	85	105	87	101
	D4S1592	VIC	0.72	113	141	127	135
	D4S391	VIC	0.85	150	170	156	158
	D3S1304	VIC	0.80	254	276	264	264
	D3S1601	VIC	0.85	298	330	314	316
	D3S1297	VIC	0.82	351	369	353	359
	D3S1292	NED	0.85	111	145	119	133
	D4S426	NED	0.76	160	180	160	172
	D4S419	NED	0.77	225	245	229	235
	D4S415	NED	0.80	264	300	266	290
7	D4S402	FAM	0.91	106	146	118	128
	D4S403	FAM	0.77	170	186	172	182
	D3S1580	FAM	0.84	215	235	219	227
	D3S1279	FAM	0.85	268	286	268	270
	D4S1539	FAM	0.68	316	326	318	324
	D3S2338	VIC	0.86	89	109	93	105
	D4S2964	VIC	0.76	119	143	121	133
	D4S412	VIC	0.77	158	176	160	166
	D4S424	VIC	0.83	194	212	196	196
	D3S1278	VIC	0.87	232	260	236	240
	D3S1266	VIC	0.73	289	305	295	295
	D3S1267	NED	0.88	93	131	105	109
	D3S1566	NED	0.84	155	177	159	169
	D3S1289	NED	0.81	202	224	212	216
	D4S1535	NED	0.77	248	262	252	252
	D3S1277	NED	0.82	289	311	295	295

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
37	D3S1581	FAM	0.87	75	105	77	91
	D3S3606	FAM	0.82	160	186	175	175
	D3S1265	FAM	0.84	216	240	237	237
	D3S3706	VIC	0.83	103	119	112	112
	D3S1558	VIC	0.77	158	172	166	170
	D3S2748	NED	0.73	83	115	112	112
	D3S3592	NED	0.79	164	178	171	171
	D3S3685	NED	0.89	199	225	211	217
	D3S3659	NED	0.65	251	263	252	256
38	D3S3634	FAM	0.78	140	164	144	150
	D3S3574	VIC	0.84	92	112	97	101
	D3S1600	VIC	0.72	186	202	186	188
	D3S1597	NED	0.79	166	184	174	180
	D3S2406	NED	0.76	201	215	202	204
	D3S1609	NED	0.64	246	262	250	250
39	D3S3686	FAM	0.80	111	137	120	124
	D3S3609	FAM	0.87	168	190	171	173
	D3S1555	FAM	0.79	221	241	222	224
	D3S1309	VIC	0.75	134	152	142	144
	D3S3725	NED	0.84	75	103	76	82
	D3S1270	NED	0.75	168	190	168	168
	D3S3728	NED	0.67	226	234	226	226
	D3S3567	NED	0.70	263	285	282	284
40	D3S1276	FAM	0.71	98	114	104	108
	D3S1593	FAM	0.76	137	157	139	155
	D3S3630	FAM	0.81	177	193	184	186
	D3S3521	FAM	0.82	265	299	285	299
	D3S1603	VIC	0.70	166	184	170	170
	D3S3607	VIC	0.88	200	225	205	205
	D3S3715	NED	0.78	138	150	142	142
	D3S3637	NED	0.89	180	210	181	203
	D3S3668	NED	0.82	239	261	244	252
41	D4S398	FAM	0.82	128	150	128	140
	D4S2952	FAM	0.58	187	209	189	193
	D4S3004	FAM	0.80	266	280	268	274
	D4S3046	VIC	0.76	95	107	95	101
	D4S2971	VIC	0.80	139	161	151	157
	D4S1587	VIC	0.74	222	234	229	231
	D4S3022	NED	0.88	124	152	137	149
	D4S428	NED	0.76	191	207	193	197
	D4S3051	NED	0.51	230	244	231	231

Associated Panels. The following panels cover chromosomes 3, 4 (continued).

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
42	D4S2994	FAM	0.82	94	126	95	95
	D4S2912	FAM	0.67	171	197	181	192
	D4S3042	FAM	0.84	211	236	211	213
	D4S2962	VIC	0.82	103	129	113	115
	D4S2460	VIC	0.72	184	196	190	190
	D4S1586	NED	0.77	103	121	104	116
	D4S1579	NED	0.65	146	164	149	151
	D4S2930	NED	0.80	220	238	221	223
43	D4S2920	FAM	0.68	109	119	111	113
	D4S1596	FAM	0.72	202	212	206	208
	D4S2924	FAM	0.72	258	274	262	264
	D4S2936	VIC	0.83	172	190	174	182
	D4S1615	NED	0.74	118	128	120	124
	D4S3023	NED	0.69	147	159	148	158
	D4S2986	NED	0.80	217	233	227	229

Chromosome 5, 6

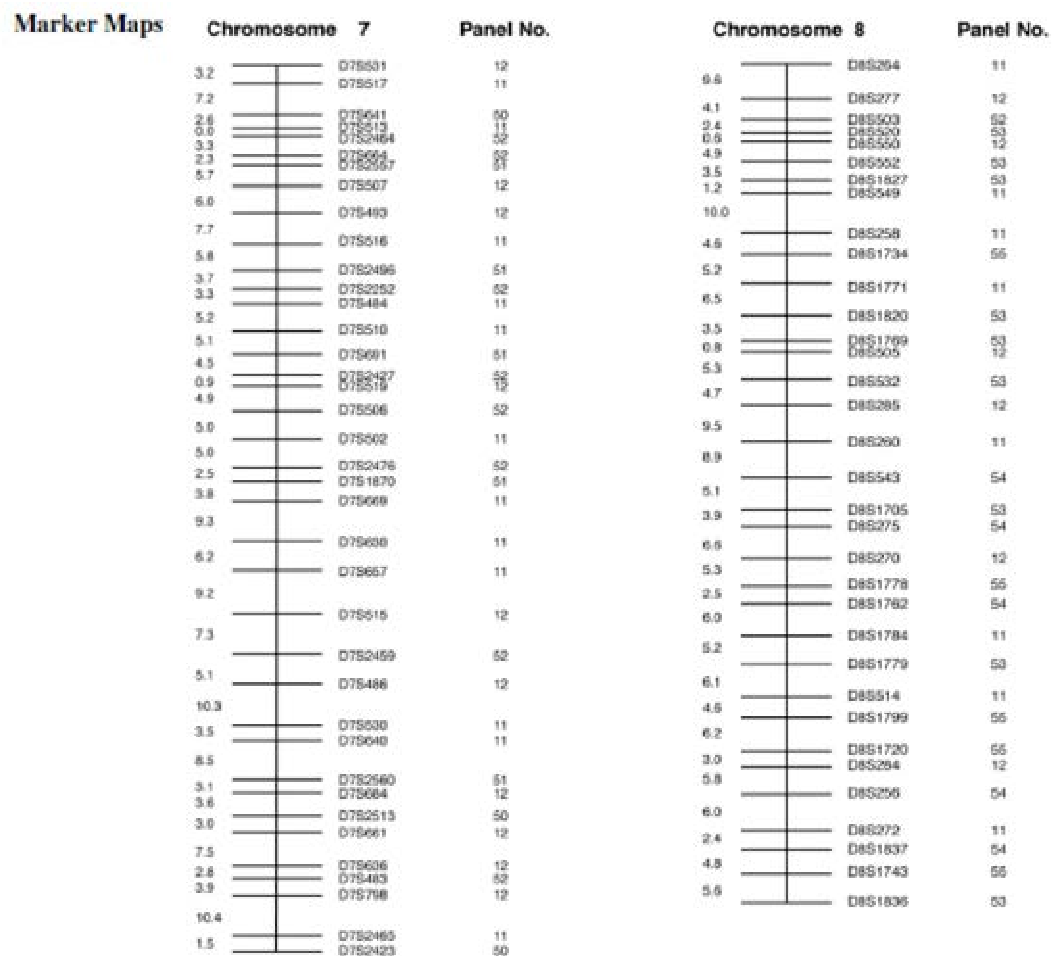


Associated Panels. The following panels cover chromosomes 5, 6 .

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
8	D6S407	FAM	0.86	82	110	100	102
	D6S289	FAM	0.70	160	182	170	172
	D6S1610	FAM	0.84	200	214	204	208
	D6S1581	FAM	0.72	257	271	261	261
	D6S422	FAM	0.77	298	320	304	306
	D5S644	VIC	0.85	82	112	84	96
	D6S281	VIC	0.68	131	151	136	136
	D6S262	VIC	0.82	169	189	171	173
	D5S424	VIC	0.76	212	234	216	218
	D5S419	VIC	0.81	256	287	271	277
	D5S433	NED	0.86	63	93	77	85
	D6S422	NED	0.84	113	135	115	131
	D5S406	NED	0.79	164	192	176	188
	D5S400	NED	0.82	217	239	223	227
	D6S309	NED	0.83	304	330	310	318
9	D6S264	FAM	0.70	108	130	112	114
	D6S1574	FAM	0.84	146	172	152	154
	D6S276	FAM	0.83	201	233	207	223
	D5S408	FAM	0.73	249	285	251	257
	D6S308	FAM	0.75	326	354	336	342
	D6S287	VIC	0.85	106	139	131	136
	D6S292	VIC	0.83	156	177	159	159
	D6S434	VIC	0.86	202	246	206	208
	D5S426	VIC	0.80	275	299	289	293
	D5S1981	NED	0.73	115	125	119	123
	D6S257	NED	0.87	167	195	181	183
	D6S446	NED	0.62	217	229	217	223
	D5S641	NED	0.77	299	339	313	315
10	D5S2027	FAM	0.78	180	202	192	194
	D5S436	FAM	0.83	238	258	240	246
	D6S460	FAM	0.81	279	303	289	299
	D5S410	FAM	0.79	329	351	331	341
	D6S462	VIC	0.68	104	121	110	112
	D5S2115	VIC	0.76	142	170	168	168
	D5S418	VIC	0.80	208	228	210	212
	D5S428	VIC	0.76	241	259	245	245
	D5S630	VIC	0.89	283	393	285	318
	D6S470	NED	0.80	120	140	128	132
	D6S441	NED	0.86	162	196	176	182
	D5S471	NED	0.76	236	255	245	249
	D5S416	NED	0.77	286	297	289	291
	D5S647	NED	0.82	326	365	340	345
44	D5S417	FAM	0.71	89	107	91	97
	D5S2011	FAM	0.86	140	158	142	148

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
44 cont'd	D5S2090	FAM	0.83	194	210	192	192
	D5S2055	FAM	0.83	272	300	280	288
	D5S2050	NED	0.79	192	210	191	195
	D5S1953	NED	0.77	251	271	253	253
45	D5S2049	FAM	0.77	82	100	84	92
	D5S1960	FAM	0.80	121	159	128	138
	D5S1993	FAM	0.77	175	195	177	193
	D5S2073	FAM	0.78	240	256	246	246
	D5S427	FAM	0.83	285	307	293	303
	D6S618	VIC	0.79	157	177	161	161
	D5S495	VIC	0.81	223	245	224	224
	D5S2084	NED	0.79	116	142	120	132
	D5S2040	NED	0.77	224	242	226	238
46	D5S2088	FAM	0.82	134	162	144	148
	D5S2031	FAM	0.75	191	215	201	209
	D5S1969	FAM	0.86	251	273	259	261
	D5S672	VIC	0.63	177	183	175	179
	D5S2021	NED	0.67	111	125	113	117
	D5S674	NED	0.77	270	280	270	276
47	D6S1575	FAM	0.82	108	130	110	122
	D6S300	FAM	0.75	187	213	192	206
	D6S1573	FAM	0.78	275	295	285	295
	D6S1609	VIC	0.81	78	104	83	97
	D6S1719	VIC	0.74	168	182	180	180
	D6S1660	VIC	0.77	204	222	206	214
	D6S1697	VIC	0.55	253	259	253	253
	D6S1650	NED	0.79	111	131	113	121
	D6S1549	NED	0.61	193	209	199	201
	D6S1671	NED	0.88	258	284	260	271
48	D6S282	FAM	0.87	102	128	114	122
	D6S1577	FAM	0.86	151	173	164	168
	D6S291	FAM	0.7	202	214	202	204
	D6S1721	FAM	0.78	257	277	257	267
	D6S1656	VIC	0.73	198	216	211	211
	D6S1569	NED	0.78	128	146	139	139
	D6S305	NED	0.83	208	234	221	229
	D6S1654	NED	0.82	225	247	235	239
49	D6S270	FAM	0.75	146	162	147	147
	D6S250	FAM	0.72	271	289	282	282
	D6S1698	VIC	0.81	167	193	167	175
	D6S1617	NED	0.86	82	104	88	88
	D6S1599	NED	0.69	134	158	131	133
	D6S272	NED	0.71	182	200	182	192
	D6S452	NED	0.84	265	284	267	277

Chromosome 7, 8

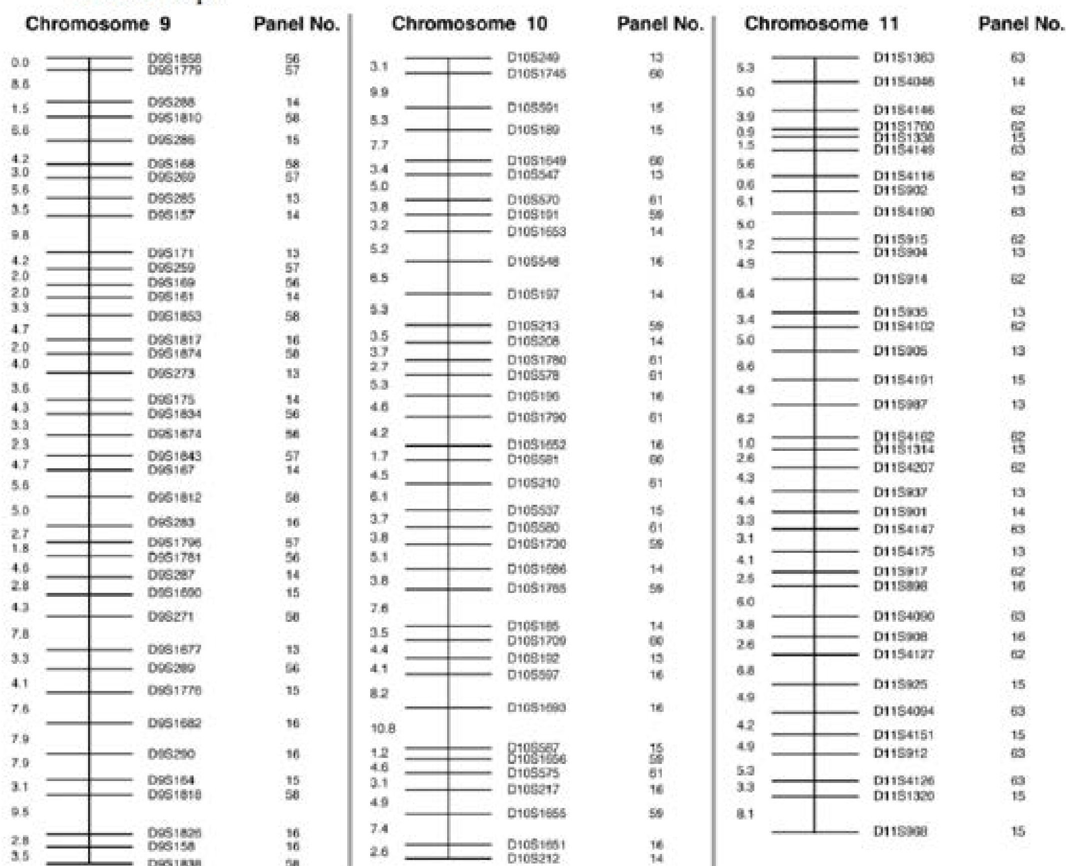


Associated Panels. The following panels cover chromosomes 7, 8.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
11	D7S484	FAM	0.74	97	115	105	105
	D8S264	FAM	0.83	136	160	144	150
	D8S260	FAM	0.81	191	217	201	211
	D7S517	FAM	0.83	243	261	257	257
	D8S1784	FAM	0.67	276	292	280	280
	D7S2465	FAM	0.83	319	343	327	333
	D8S549	VIC	0.63	73	83	77	77
	D7S530	VIC	0.78	106	123	113	115
	D8S258	VIC	0.70	142	156	150	152
	D7S669	VIC	0.80	172	194	182	184
	D8S272	VIC	0.81	211	261	245	255
	D7S502	VIC	0.84	289	309	297	301
	D7S630	VIC	0.73	327	365	349	361
	D7S510	NED	0.77	79	95	89	91
	D7S640	NED	0.85	110	150	114	142
	D7S513	NED	0.83	168	198	190	192
	D8S514	NED	0.77	212	232	220	224
	D7S657	NED	0.81	244	270	248	260
	D7S516	NED	0.76	306	326	320	320
	D8S1771	NED	0.75	343	367	351	351
12	D7S507	FAM	0.89	81.69	109.76	88	90
	D7S515	FAM	0.82	130.865	201.865	198	198
	D7S486	FAM	0.81	221.895	235.925	232	234
	D7S519	FAM	0.81	257	284.785	259	271
	D7S661	FAM	0.75	305.24	337.415	317	321
	D7S798	VIC	0.84	71.26	93.36	75	77
	D8S505	VIC	0.79	110.83	124.88	113	115
	D8S277	VIC	0.73	151.67	186.12	162	179
	D7S493	VIC	0.88	203.885	236.28	212	223
	D8S284	VIC	0.83	272.7	306.7	297	297
	D7S684	VIC	0.81	341.285	363.4	355	357
	D8S270	NED	0.79	101.506	117.506	108	110
	D7S636	NED	0.90	136.715	172.815	151	153
	D8S550	NED	0.87	167.035	217	195	211
	D7S531	NED	0.77	276	294	280	286
	D8S285	NED	0.78	314.01	330.135	322	324
50	D7S641	FAM	0.71	87	103	89	93
	D7S2513	VIC	0.74	162	186	166	176
	D7S2423	NED	0.71	229	247	233	233
51	D7S2557	FAM	0.74	152	166	160	160
	D7S691	VIC	0.74	133	151	143	149
	D7S1870	NED	0.75	111	135	122	126
	D7S2560	NED	0.86	153	199	161	161
	D7S2496	NED	0.71	212	237	212	223

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
52	D7S2459	FAM	0.76	124	138	128	130
	D7S2464	FAM	0.66	192	210	194	202
	D7S2252	FAM	0.78	251	263	251	253
	D7S2476	VIC	0.63	138	156	143	145
	D7S2427	VIC	0.80	220	252	222	224
	D7S506	NED	0.87	120	146	135	135
	D7S483	NED	0.81	170	192	183	185
	D7S664	NED	0.70	207	219	209	211
53	D8S1820	FAM	0.73	107	121	113	113
	D8S532	FAM	0.83	141	157	150	150
	D8S1706	FAM	0.83	192	212	194	204
	D8S1769	FAM	0.83	244	260	247	259
	D8S1836	VIC	0.84	124	159	138	151
	D8S1779	VIC	0.75	195	209	201	201
	D8S552	NED	0.79	110	124	124	124
	D8S1827	NED	0.66	158	168	160	164
	D8S520	NED	0.77	184	203	192	195
54	D8S543	FAM	0.75	112	136	126	126
	D8S1837	FAM	0.80	193	210	193	210
	D8S256	VIC	0.81	105	133	125	133
	D8S1762	VIC	0.76	224	242	226	236
	D8S275	NED	0.75	143	161	155	157
55	D8S1734	FAM	0.67	107	117	109	113
	D8S1720	FAM	0.81	136	150	139	139
	D8S1743	VIC	0.82	88	118	94	112
	D8S503	VIC	0.73	134	150	144	146
	D8S1778	NED	0.87	129	155	143	143
	D8S1799	NED	0.83	201	231	228	228

Chromosome 9, 10, 11



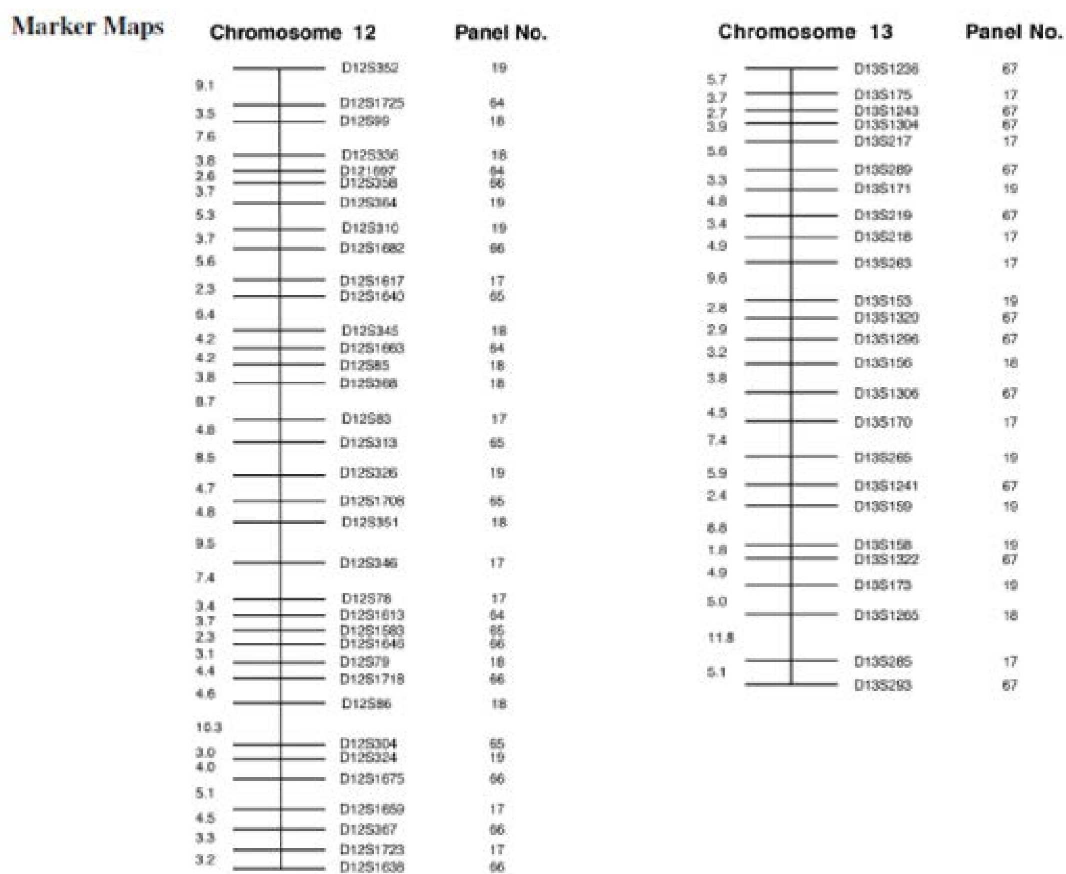
Associated Panels. The following panels cover chromosomes 9, 10, 11.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
13	D11S987	FAM	0.82	94	134	116	120
	D11S937	FAM	0.88	144	180	160	162
	D11S935	FAM	0.73	196	218	200	210
	D9S1677	FAM	0.81	229	255	231	253
	D11S1314	VIC	0.78	93	121	95	101
	D11S902	VIC	0.80	148	170	152	158
	D11S904	VIC	0.83	183	213	185	197
	D10S547	VIC	0.74	236	257	240	249
	D11S905	VIC	0.75	269	297	275	275
	D9S285	NED	0.78	80	110	104	108
	D10S249	NED	0.74	117	139	131	131
	D9S171	NED	0.79	160	186	162	162
	D9S273	NED	0.74	202	222	206	208
	D10S192	NED	0.77	238	264	240	248
	D11S4175	NED	0.89	288	340	322	332
14	D9S161	FAM	0.78	122	139	122	133
	D10S197	FAM	0.75	166	180	170	174
	D10S185	FAM	0.77	201	219	207	209
	D9S175	FAM	0.85	255	289	261	267
	D11S901	FAM	0.82	311	327	315	319
	D10S1653	VIC	0.77	118	132	124	124
	D10S212	VIC	0.71	189	207	199	199
	D10S1686	VIC	0.86	243	281	255	259
	D9S287	VIC	0.67	295	315	299	301
	D11S4046	NED	0.86	101	125	109	119
	D9S288	NED	0.84	132	154	136	144
	D10S208	NED	0.79	173	193	185	187
	D9S157	NED	0.84	225	249	225	231
	D9S167	NED	0.87	304	338	316	332
15	D9S164	FAM	0.80	84	102	92	94
	D9S286	FAM	0.88	136	170	148	162
	D9S1690	FAM	0.78	226	240	236	238
	D11S1320	FAM	0.68	259	277	263	269
	D11S4151	FAM	0.79	331	345	333	335
	D11S4191	VIC	0.87	89	119	91	95
	D11S968	VIC	0.81	140	162	150	150
	D9S1776	VIC	0.84	172	210	176	178
	D11S1338	VIC	0.74	253	271	265	265
	D10S591	VIC	0.71	309	339	317	331
	D10S587	NED	0.80	92	114	96	100
	D10S537	NED	0.83	139	165	149	153
	D10S189	NED	0.72	179	197	185	191
	D11S925	NED	0.84	260	290	262	262
16	D10S217	FAM	0.81	96	120	102	106
	D11S898	FAM	0.85	141	165	149	156
	D10S548	FAM	0.70	182	198	186	190
	D9S1826	FAM	0.69	215	231	219	219
	D9S290	FAM	0.83	240	262	246	248
	D9S1817	FAM	0.88	279	315	297	303
	D9S158	FAM	0.69	330	356	338	340
	D10S196	VIC	0.77	103	115	109	109
	D9S1682	VIC	0.68	147	159	149	151
	D11S908	VIC	0.76	172	190	180	182
	D10S1693	VIC	0.80	213	227	217	219
	D10S597	VIC	0.64	273	297	289	289
	D9S283	NED	0.80	89	115	89	91
	D10S1651	NED	0.80	206	230	208	224
	D10S1652	NED	0.78	269	295	287	289
56	D9S289	FAM	0.74	72	90	74	80
	D9S1834	FAM	0.69	186	201	187	189
	D9S1674	FAM	0.73	218	238	220	228
	D9S169	FAM	0.82	262	278	262	272
	D9S1868	VIC	0.58	241	253	251	251
	D9S1781	NED	0.79	237	257	241	251
57	D9S259	FAM	0.67	133	147	138	138
	D9S1796	VIC	0.79	155	183	163	163
	D9S1779	NED	0.63	125	149	127	143
	D9S269	NED	0.74	176	194	177	183
	D9S1843	NED	0.80	236	252	248	252
58	D9S271	FAM	0.64	138	162	154	156
	D9S1810	FAM	0.77	202	218	204	214
	D9S1853	FAM	0.63	251	269	253	255
	D9S1818	VIC	0.71	199	207	201	205
	D9S1812	VIC	0.69	274	284	280	282
	D9S1838	NED	0.83	165	181	167	171
	D9S1874	NED	0.83	194	206	195	199
	D9S168	NED	0.75	240	259	234	243
59	D10S1656	FAM	0.75	140	158	143	149
	D10S213	FAM	0.82	178	195	186	189
	D10S1730	FAM	0.83	232	266	242	244
	D10S191	VIC	0.81	121	149	122	130
	D10S1765	VIC	0.83	169	191	171	185
	D10S1655	NED	0.67	307	323	253	253

Associated Panels. The following panels cover chromosomes 9, 10, 11 (continued).

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
60	D10S1745	FAM	0.84	154	180	164	166
	D10S581	VIC	0.79	127	153	136	142
	D10S1649	NED	0.84	123	147	135	137
	D10S1709	NED	0.74	160	178	163	165
61	D10S580	FAM	0.72	133	143	136	140
	D10S578	FAM	0.65	165	189	171	173
	D10S570	FAM	0.81	292	310	298	308
	D10S1790	VIC	0.83	185	207	189	195
	D10S575	VIC	0.63	255	273	253	253
	D10S210	NED	0.78	123	137	125	125
	D10S1780	NED	0.65	226	242	228	234
62	D11S1760	FAM	0.75	84	104	90	90
	D11S4102	FAM	0.77	140	172	144	162
	D11S4116	FAM	0.80	202	221	206	206
	D11S4207	FAM	0.89	254	292	264	266
	D11S4162	VIC	0.64	159	165	163	163
	D11S914	VIC	0.71	279	291	281	289
	D11S4127	NED	0.71	91	107	99	99
	D11S917	NED	0.80	146	162	151	155
	D11S4146	NED	0.70	193	209	195	195
	D11S915	NED	0.81	221	277	269	273
63	D11S912	FAM	0.80	105	127	110	112
	D11S4090	FAM	0.83	161	189	163	175
	D11S4149	FAM	0.77	212	224	213	219
	D11S1363	FAM	0.59	239	249	244	244
	D11S4190	VIC	0.82	228	248	237	237
	D11S4126	NED	0.59	133	147	143	143
	D11S4094	NED	0.80	184	198	188	192
	D11S4147	NED	0.81	224	246	235	239

Chromosome 12, 13

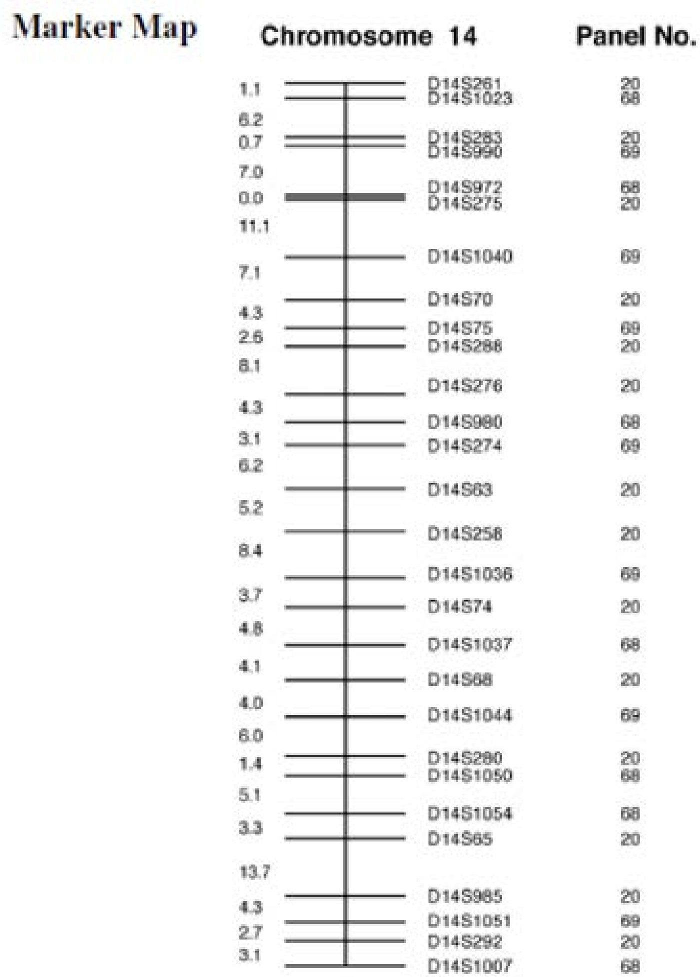


Associated Panels. The following panels cover chromosomes 12, 13.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
17	D12S83	FAM	0.81	102	122	104	110
	D13S218	FAM	0.66	141	153	143	145
	D12S78	FAM	0.91	174	212	188	190
	D13S217	FAM	0.68	242	262	252	256
	D12S1659	FAM	0.78	290	316	302	302
	D13S285	VIC	0.81	89	115	101	103
	D13S170	VIC	0.90	143	173	151	167
	D12S1723	VIC	0.67	198	216	202	208
	D13S175	NED	0.76	101	119	105	107
	D13S263	NED	0.84	146	174	152	166
	D12S346	NED	0.84	188	214	196	198
	D12S1617	NED	0.80	245	265	257	257
18	D12S85	FAM	0.67	99	131	117	125
	D12S351	FAM	0.75	147	169	155	159
	D12S368	FAM	0.81	202	222	206	214
	D13S1265	FAM	0.80	275	305	281	293
	D12S86	VIC	0.89	129	169	141	143
	D13S156	VIC	0.80	277	297	285	287
	D12S336	NED	0.82	111	129	113	123
	D12S79	NED	0.87	160	186	162	174
	D12S345	NED	0.87	211	247	213	215
	D12S99	NED	0.83	264	296	274	282
19	D13S158	FAM	0.82	116	133	122	129
	D13S159	FAM	0.90	154	196	158	180
	D13S173	FAM	0.82	232	252	238	246
	D12S364	FAM	0.87	298	326	308	308
	D13S265	VIC	0.70	89	127	109	115
	D12S352	VIC	0.73	154	174	164	166
	D12S326	VIC	0.80	207	233	223	229
	D12S310	VIC	0.69	244	252	246	250
	D13S153	NED	0.81	89	121	93	97
	D13S171	NED	0.73	177	205	187	187
	D12S324	NED	0.69	233	255	243	245
64	D12S1613	FAM	0.62	254	270	262	266
	D12S1725	VIC	0.79	221	244	221	236
	D12S1663	NED	0.77	154	181	168	176
	D12S1697	NED	0.83	222	238	230	230
65	D12S1708	FAM	0.72	172	180	172	172
	D12S1583	FAM	0.87	224	251	227	240
	D12S304	FAM	0.70	311	325	313	317
	D12S313	NED	0.79	141	157	145	153
	D12S1640	NED	0.65	266	278	268	268

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
66	D12S1718	FAM	0.43	160	172	160	160
	D12S1675	FAM	0.74	214	228	219	227
	D12S358	FAM	0.76	242	274	259	261
	D12S367	VIC	0.76	139	153	139	145
	D12S1638	VIC	0.68	204	220	209	215
	D12S1646	VIC	0.71	252	264	259	261
	D12S1682	NED	0.77	136	154	142	146
67	D13S1322	FAM	0.63	86	96	91	95
	D13S1236	FAM	0.67	122	136	124	126
	D13S1320	FAM	0.76	260	270	266	266
	D13S1241	FAM	0.82	327	347	332	336
	D13S1296	VIC	0.86	90	118	104	110
	D13S289	VIC	0.67	148	168	152	156
	D13S293	NED	0.50	93	101	95	95
	D13S219	NED	0.64	120	130	121	125
	D13S1304	NED	0.73	154	170	154	156
	D13S1306	NED	0.85	199	215	208	210
	D13S1243	NED	0.78	247	261	248	248

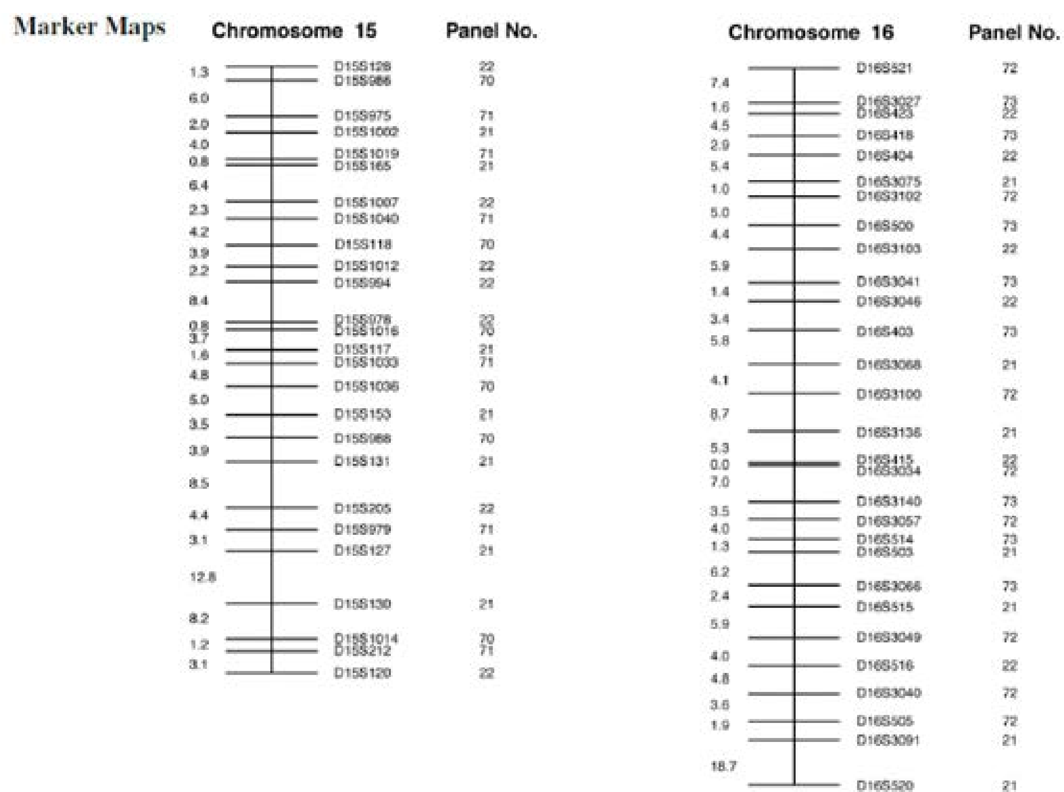
Chromosome 14



Associated Panels. The following panels cover chromosome 14.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
20	D14S292	FAM	0.73	83.54	99.59	88	90
	D14S275	FAM	0.70	145.49	159	147	147
	D14S258	FAM	0.79	192.945	213.3	195	205
	D14S280	FAM	0.68	237.9	257.91	242	244
	D14S70	VIC	0.75	98.27	114	104	104
	D14S283	VIC	0.81	127.185	158	135	148
	D14S63	VIC	0.76	177.275	195.32	187	193
	D14S985	VIC	0.76	240.935	255.085	249	253
	D14S74	VIC	0.79	297.285	321.355	301	303
	D14S65	NED	0.79	124.4	156	148	148
	D14S288	NED	0.83	192.105	215.215	200	204
	D14S276	NED	0.76	236.65	248.845	241	247
	D14S261	NED	0.75	273	305.31	273	297
	D14S68	NED	0.91	318	346.415	318	332
68	D14S1023	FAM	0.81	97	113	101	103
	D14S980	FAM	0.86	159	189	161	163
	D14S972	FAM	0.75	202	215	204	208
	D14S1007	VIC	0.77	90	108	98	100
	D14S1054	VIC	0.76	158	172	160	162
	D14S1037	NED	0.82	93	143	121	125
	D14S1050	NED	0.80	215	237	217	223
69	D14S274	FAM	0.71	118	138	120	122
	D14S1051	FAM	0.31	170	190	170	172
	D14S1044	FAM	0.65	224	238	232	234
	D14S1036	VIC	0.79	128	146	131	135
	D14S1040	NED	0.73	86	116	106	108
	D14S990	NED	0.84	148	166	151	151
	D14S75	NED	0.76	196	222	204	212

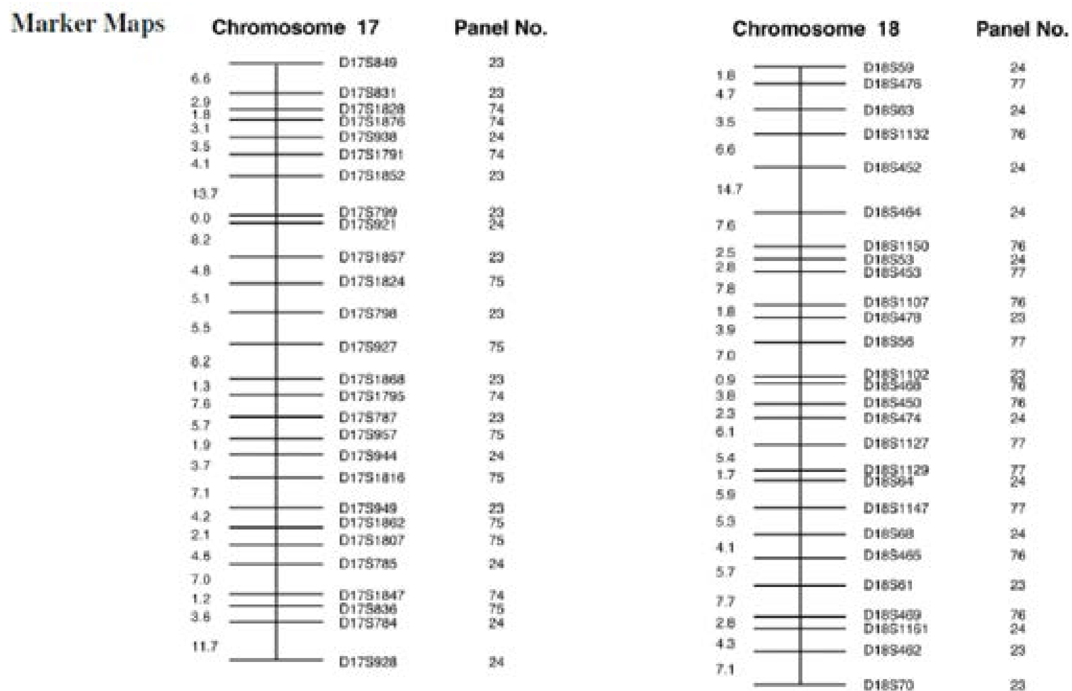
Chromosome 15, 16



Associated Panels. The following panels cover chromosomes 15, 16.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
21	D16S3075	FAM	0.79	76	94	78	78
	D16S3136	FAM	0.69	173	185	177	179
	D16S3068	FAM	0.77	219	235	221	223
	D15S130	FAM	0.66	285	299	293	295
	D16S515	FAM	0.8	325	357	331	337
	D15S1002	VIC	0.78	104	134	106	108
	D16S520	VIC	0.84	149	165	153	159
	D15S165	VIC	0.79	182	213	202	211
	D15S131	VIC	0.83	240	281	254	264
	D16S503	VIC	0.81	299	319	309	309
	D15S127	NED	0.86	118	154	138	138
	D16S3091	NED	0.73	166	182	176	180
	D15S153	NED	0.87	240	274	258	260
	D15S117	NED	0.78	321	339	335	337
22	D16S3046	FAM	0.74	83	109	99	99
	D15S205	FAM	0.88	126	166	144	162
	D16S415	FAM	0.72	213	241	227	229
	D15S1012	VIC	0.72	94	112	96	100
	D16S423	VIC	0.73	137	161	139	139
	D15S978	VIC	0.83	184	212	184	188
	D16S404	VIC	0.80	261	281	275	275
	D16S3103	VIC	0.81	315	343	327	331
	D15S1007	NED	0.86	84	108	96	100
	D15S120	NED	0.73	155	183	169	171
	D15S128	NED	0.78	197	215	209	211
	D16S516	NED	0.73	245	267	251	255
	D15S994	NED	0.73	303	315	305	305
70	D15S988	FAM	0.79	96	116	96	96
	D15S986	FAM	0.71	183	201	185	191
	D15S118	FAM	0.74	218	234	220	228
	D15S1036	NED	0.80	118	144	120	122
	D15S1014	NED	0.73	188	200	193	195
	D15S1016	NED	0.87	277	305	287	291
71	D15S979	FAM	0.85	139	171	143	155
	D15S1040	FAM	0.76	202	216	211	213
	D15S975	FAM	0.44	250	258	253	253
	D15S1033	NED	0.69	87	99	90	90
	D15S1019	NED	0.59	206	222	208	208
	D15S212	NED	0.71	343	357	345	353
72	D16S521	FAM	0.71	158	178	160	174
	D16S3102	FAM	0.69	190	208	206	206
	D16S3100	FAM	0.66	270	284	273	279
	D16S3040	VIC	0.74	88	107	95	105
	D16S3034	VIC	0.65	259	267	262	264
	D16S505	NED	0.76	137	159	154	154
	D16S3057	NED	0.72	192	210	197	205
	D16S3049	NED	0.76	232	258	234	250
73	D16S514	FAM	0.82	121	137	124	128
	D16S418	FAM	0.82	170	192	176	182
	D16S3027	FAM	0.87	210	234	210	220
	D16S403	VIC	0.85	138	157	141	152
	D16S3066	VIC	0.79	187	199	194	196
	D16S3140	VIC	0.82	283	321	288	288
	D16S500	NED	0.80	185	201	189	197
	D16S3041	NED	0.82	247	277	268	270

Chromosome 17, 18

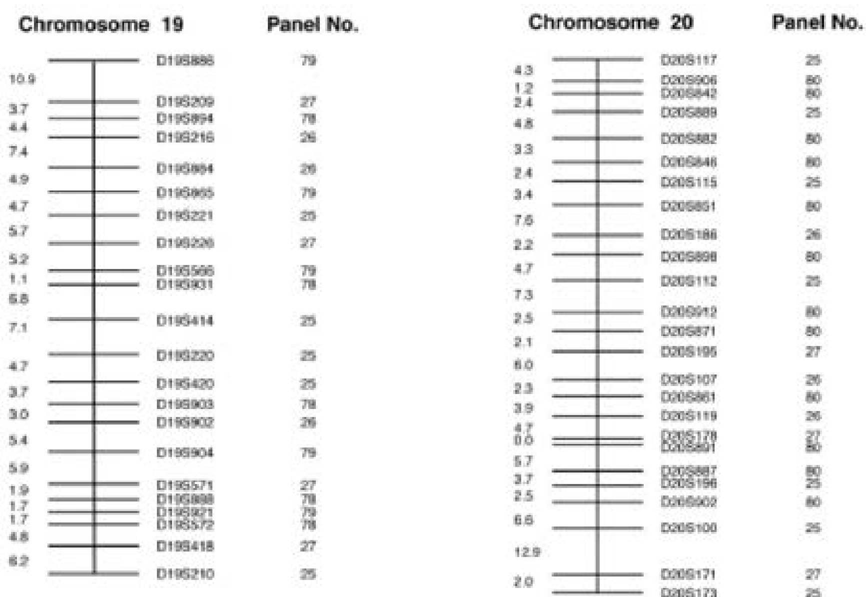


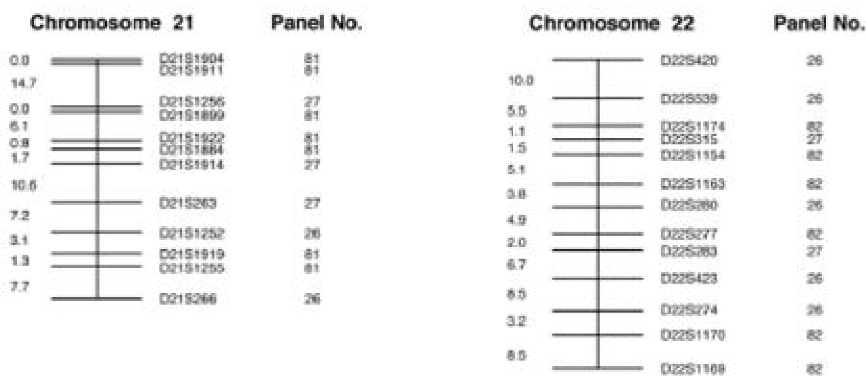
Associated Panels. The following panels cover chromosomes 17, 18.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
23	D18S70	FAM	0.83	111	131	113	113
	D17S949	FAM	0.80	210	228	216	216
	D18S478	FAM	0.64	242	256	246	252
	D17S1852	FAM	0.87	279	310	295	308
	D17S831	VIC	0.82	107	129	111	115
	D17S1857	VIC	0.64	164	174	166	168
	D17S799	VIC	0.68	186	208	190	196
	D17S1868	VIC	0.73	254	268	260	260
	D17S798	VIC	0.80	298	322	308	314
	D18S1102	NED	0.79	90	102	94	94
	D17S787	NED	0.81	138	174	144	154
	D18S61	NED	0.87	209	239	225	227
	D17S949	NED	0.67	253	267	257	261
	D18S462	NED	0.70	296	318	302	302
24	D18S474	FAM	0.82	121	143	125	137
	D18S53	FAM	0.70	152	182	164	166
	D17S938	FAM	0.76	228	258	248	250
	D18S464	FAM	0.65	298	314	308	308
	D18S63	VIC	0.79	74	100	92	96
	D18S59	VIC	0.81	152	174	164	168
	D17S921	VIC	0.72	193	211	199	207
	D17S784	VIC	0.77	230	244	238	238
	D18S64	VIC	0.74	319	345	323	327
	D17S928	NED	0.76	68	102	82	86
	D18S452	NED	0.83	126	144	128	138
	D17S785	NED	0.83	165	193	171	173
	D18S1161	NED	0.82	211	237	227	229
	D18S68	NED	0.68	269	295	287	289
	D17S944	NED	0.75	318	334	320	330
74	D17S1876	FAM	0.82	99	131	114	126
	D17S1791	FAM	0.85	270	293	272	274
	D17S1795	VIC	0.71	136	146	136	144
	D17S1828	NED	0.79	95	115	105	109
	D17S1847	NED	0.65	161	167	167	167
75	D17S1807	FAM	0.86	256	284	271	275
	D17S927	VIC	0.72	113	129	114	116
	D17S836	VIC	0.63	204	214	208	210
	D17S1824	NED	0.83	91	111	99	105
	D17S957	NED	0.44	153	185	155	157
	D17S1862	NED	0.83	202	232	212	218
	D17S1816	NED	0.82	269	300	281	285
76	D18S1132	FAM	0.67	107	131	119	121
	D18S465	FAM	0.78	234	252	236	239
	D18S468	FAM	0.79	275	287	276	280
	D18S1150	VIC	0.71	96	104	96	96
	D18S450	VIC	0.79	217	233	218	222
	D18S1107	NED	0.72	81	95	89	91
	D18S469	NED	0.65	236	256	238	244
77	D18S1147	FAM	0.85	120	148	134	142
	D18S1127	FAM	0.86	181	207	195	197
	D18S1129	FAM	0.84	236	260	248	256
	D18S56	NED	0.73	104	118	105	107
	D18S453	NED	0.82	137	167	155	155
	D18S476	NED	0.76	266	278	270	274

Chromosome 19, 20, 21, 22

Marker Maps





Associated Panels. The following panels cover chromosomes 19, 20, 21, 22.

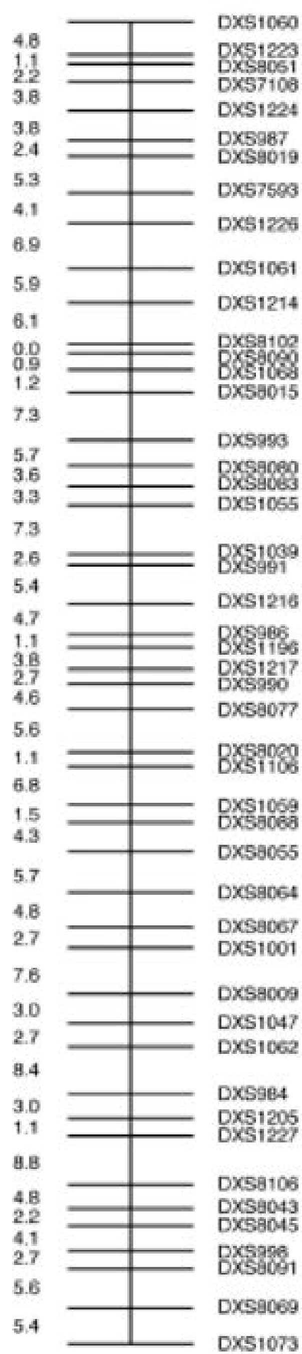
Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
25	D20S889	FAM	0.83	87	123	101	111
	D20S117	FAM	0.84	151	187	157	175
	D20S112	FAM	0.81	213	237	223	227
	D19S220	FAM	0.84	267	291	277	279
	D19S221	VIC	0.86	87	110	97	104
	D20S171	VIC	0.78	127	155	137	141
	D19S210	VIC	0.74	172	192	180	186
	D20S100	VIC	0.76	209	235	221	223
	D19S420	NED	0.79	95	117	105	107
	D19S414	NED	0.78	164	194	166	184
	D20S115	NED	0.66	234	246	238	238
	D20S196	NED	0.81	259	295	261	283
26	D20S119	FAM	0.82	103	123	111	117
	D21S266	FAM	0.59	156	178	160	172
	D20S107	FAM	0.80	197	221	205	213
	D19S902	FAM	0.79	237	273	241	253
	D20S186	VIC	0.86	113	139	115	135
	D22S420	VIC	0.77	153	169	157	157
	D22S280	VIC	0.82	213	225	219	219
	D19S216	VIC	0.76	256	274	264	264
	D22S423	VIC	0.82	287	309	305	305
	D19S884	NED	0.86	93	113	97	103
	D21S1252	NED	0.80	144	176	146	162
	D22S539	NED	0.58	199	217	201	201
	D22S274	NED	0.77	276	298	284	288
27	D20S195	FAM	0.81	128	154	140	140
	D22S315	FAM	0.78	180	210	194	196
	D19S209	FAM	0.77	238	254	240	250
	D19S418	VIC	0.66	87	107	91	91
	D20S173	VIC	0.67	128	182	174	174
	D21S263	VIC	0.75	194	229	196	201
	D21S1914	VIC	0.86	258	280	262	272
	D21S1256	NED	0.65	96	116	110	110
	D22S283	NED	0.89	127	155	131	151
	D20S178	NED	0.83	179	195	187	187
	D19S226	NED	0.85	238	270	242	248
	D19S571	NED	0.81	287	319	307	313
78	D19S572	FAM	0.80	120	138	133	133
	D19S903	FAM	0.78	210	232	222	232
	D19S931	VIC	0.77	151	179	168	174
	D19S894	NED	0.77	127	151	143	143
	D19S888	NED	0.81	174	194	176	178

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
79	D19S566	FAM	0.86	145	167	155	157
	D19S904	FAM	0.64	213	227	217	219
	D19S921	VIC	0.78	186	212	188	196
	D19S886	NED	0.63	143	159	155	157
	D19S865	NED	0.88	195	231	206	208
80	D20S906	FAM	0.71	86	104	96	98
	D20S891	FAM	0.85	193	219	211	213
	D20S902	FAM	0.81	300	316	308	310
	D20S851	VIC	0.76	66	87	70	83
	D20S871	VIC	0.74	130	162	146	152
	D20S898	VIC	0.88	235	269	247	253
	D20S912	VIC	0.80	284	302	296	300
	D20S882	NED	0.72	68	80	76	76
	D20S861	NED	0.66	123	135	125	125
	D20S842	NED	0.86	159	183	170	172
	D20S846	NED	0.77	212	230	214	220
	D20S887	NED	0.82	246	270	248	258
81	D21S1904	FAM	0.52	131	149	137	139
	D21S1899	FAM	0.86	170	190	179	185
	D21S1884	FAM	0.59	242	252	244	244
	D21S1911	VIC	0.69	133	157	137	143
	D21S1919	NED	0.81	168	198	170	178
	D21S1922	NED	0.65	244	256	248	248
	D21S1255	NED	0.80	308	329	310	316
82	D22S1169	FAM	0.78	65	81	67	67
	D22S1163	FAM	0.75	143	159	152	154
	D22S1170	FAM	0.64	197	211	211	211
	D22S1154	FAM	0.72	247	263	259	259
	D22S1174	NED	0.82	121	147	141	145
	D22S277	NED	0.85	161	197	195	195

Chromosome X

Marker Map

Chromosome X



Associated Panels. The following panels cover chromosome X.

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
28	DXS1227	FAM	0.73	79	99	87	93
	DXS990	FAM	0.74	122	132	122	126
	DXS986	FAM	0.77	151	181	163	163
	DXS987	FAM	0.83	205	229	207	217
	DXS993	FAM	0.79	267	293	269	271
	DXS1073	FAM	0.80	306	334	310	316
	DXS8091	VIC	0.78	80	102	82	88
	DXS1106	VIC	0.67	126	140	130	130
	DXS1047	VIC	0.81	156	172	158	166
	DXS1001	VIC	0.82	191	211	196	205
	DXS1068	VIC	0.79	244	264	252	254
	DXS1214	VIC	0.79	284	298	290	290
	DXS8055	VIC	0.65	312	324	314	316
	DXS8051	NED	0.88	104	134	116	120
	DXS8043	NED	0.80	146	180	146	164
	DXS1060	NED	0.84	244	268	248	254
	DXS1226	NED	0.84	280	302	284	298
	DXS991	NED	0.80	313	341	313	327
83	DXS1224	VIC	0.55	160	174	162	162
	DXS7593	VIC	0.74	214	237	218	227
	DXS8009	VIC	0.63	252	266	257	259
	DXS8067	NED	0.73	91	115	103	103
	DXS8019	NED	0.82	160	178	165	169
	DXS8088	NED	0.62	250	266	262	264
84	DXS1223	FAM	0.77	139	161	147	157
	DXS1039	FAM	0.56	177	201	185	189
	DXS8045	FAM	0.54	215	227	220	220
	DXS7108	FAM	0.74	236	258	238	256
	DXS1196	VIC	0.79	212	232	214	216
	DXS1062	NED	0.75	89	115	102	102
	DXS8077	NED	0.72	179	199	187	187
	DXS8064	NED	0.60	214	230	216	220
	DXS1216	NED	0.68	242	256	246	246
85	DXS1205	FAM	0.65	184	202	186	192
	DXS8106	FAM	0.72	264	290	270	278
	DXS8102	VIC	0.56	99	105	100	102
	DXS984	VIC	0.71	160	190	182	184
	DXS8069	NED	0.66	134	148	134	140
	DXS8083	NED	0.73	163	181	165	165
	DXS8020	NED	0.80	216	246	230	236

Panel	Locus	Dye Label	Het	ASR		GT (1347-02)	
86	DXS8080	FAM	0.69	76	104	92	96
	DXS1055	FAM	0.68	142	158	146	150
	DXS8015	FAM	0.77	179	195	189	189
	DXS1061	FAM	0.78	228	248	240	244
	DXS1217	VIC	0.60	235	253	239	247
	DXS8090	VIC	0.77	288	306	298	302
	DXS998	NED	0.58	114	122	116	118
	DXS1059	NED	0.71	184	204	200	202

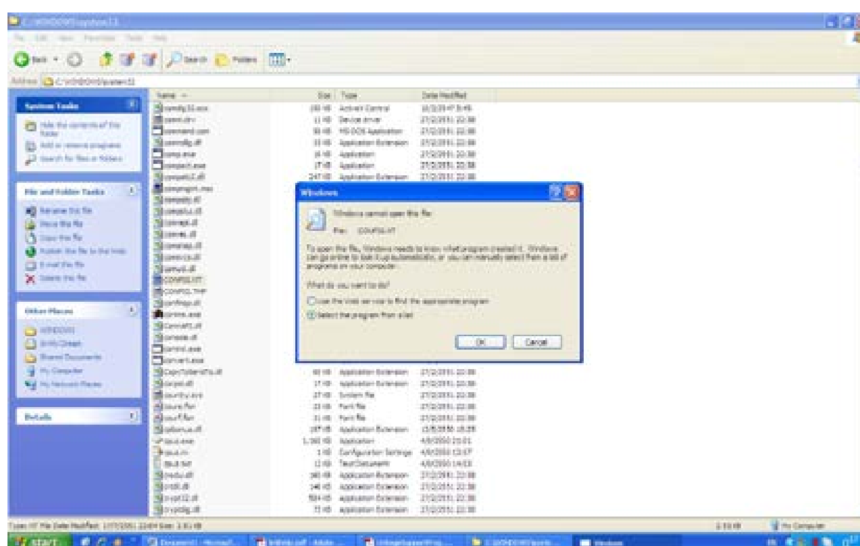
APPENDIX B
MLINK version 5.1

1. Download ftp://linkage.rockefeller.edu/software/linkage/.
2. configuration (configuration version Windows_ Window XP)

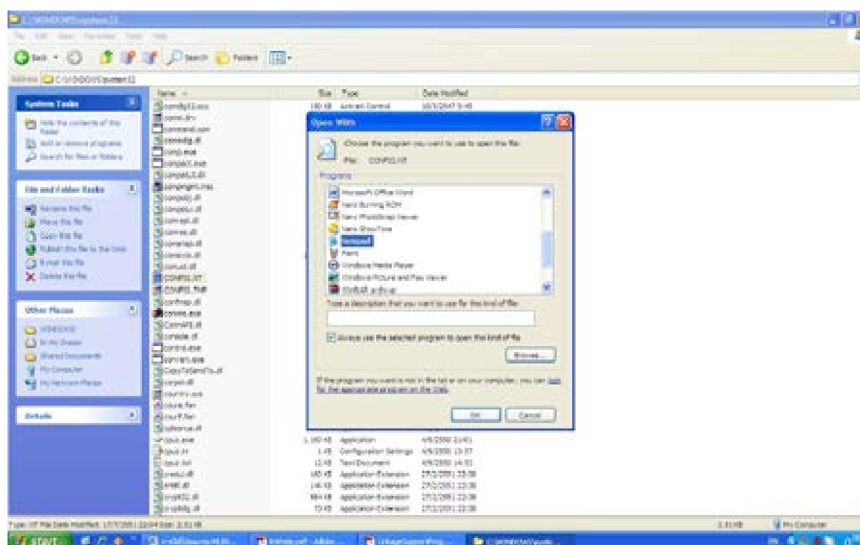
   Window XP

   A. C:\WINDOWS\system32 CONFIG.NT

   Open → Select the program from a list → OK

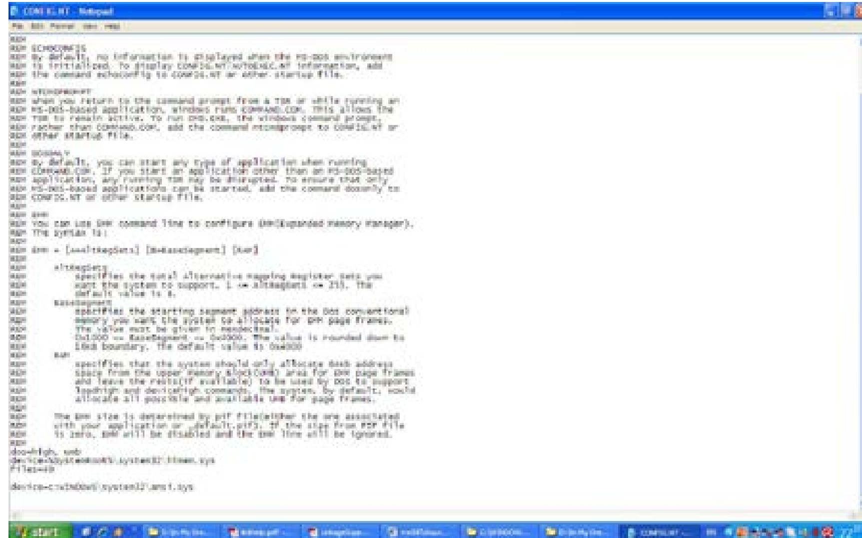


   B. Notepad → OK



C. device=c:\WINDOWS\system32\ansi.sys

Notepad File Save (
LCP)



PEDIGREE FILE (PED FILE)

§ PED FILE Notepad

Column 1	ID
Column 2	ID
Column 3	ID
Column 4	ID
Column 5	ID
Column 6	ID
Column 7	ID (1)
Column 8	ID (2)

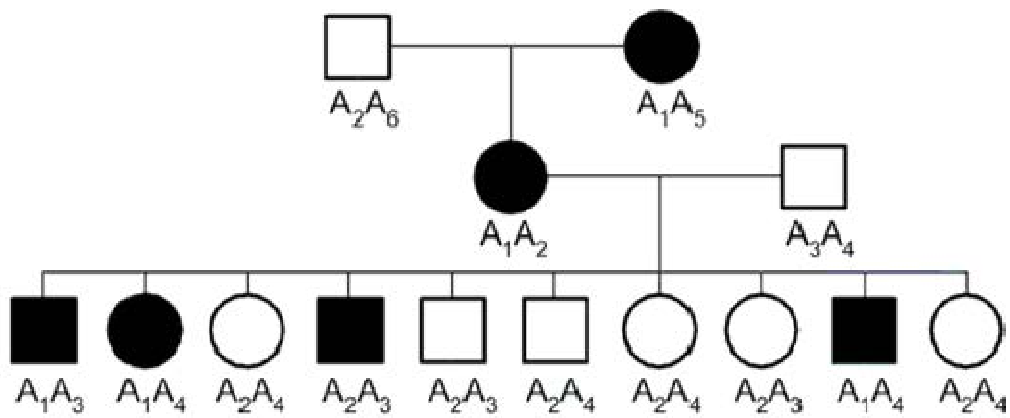
-D - 1

- 2

- ID - 1

- 2

Pedigree

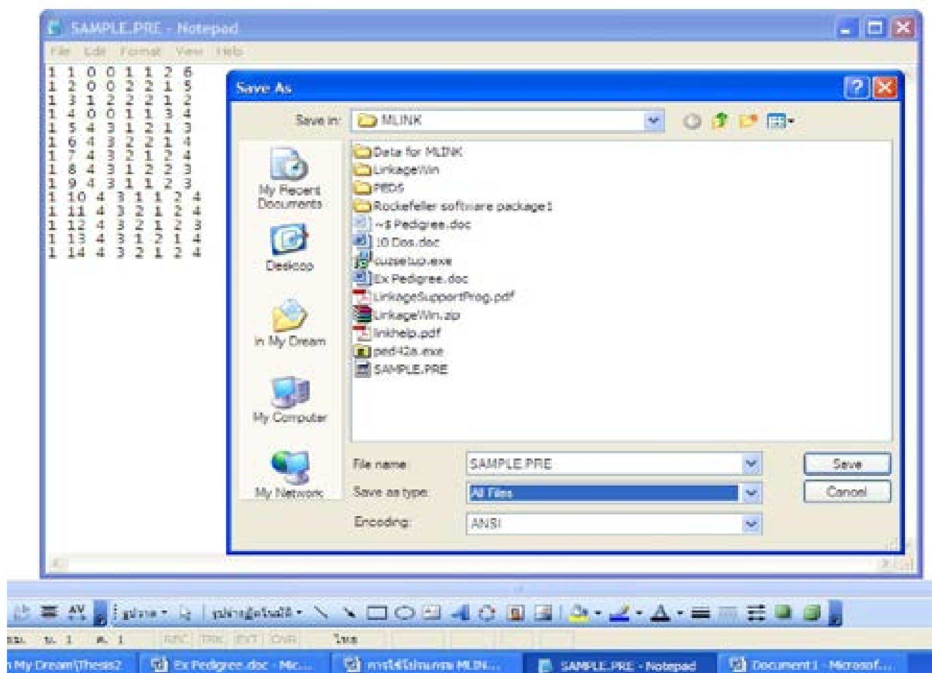


Pedigree PED FILE

1. Notepad

```
SAMPLE.PRE - Notepad
File Edit Format View Help
1 1 0 0 1 1 2 6
1 2 0 0 2 2 1 5
1 3 1 2 2 2 1 2
1 4 0 0 1 1 3 4
1 5 4 3 1 2 1 3
1 6 4 3 2 2 1 4
1 7 4 3 2 1 2 4
1 8 4 3 1 2 2 3
1 9 4 3 1 1 2 3
1 10 4 3 1 1 2 4
1 11 4 3 2 1 2 4
1 12 4 3 2 1 2 3
1 13 4 3 1 2 1 4
1 14 4 3 2 1 2 4
```

File Save as .PRE (save SAMPLE.PRE)



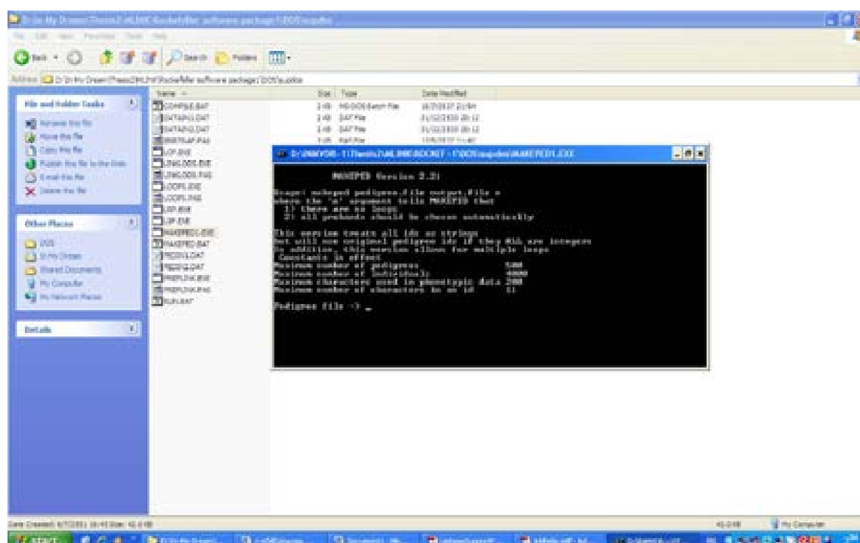
MAKEPED PROGRAM (PRE PED)

MAKEPED

1. MAKEPED

Rockefeller software package1 → DOS → supdos

MAKEPED1.EXE



2. MAKEPED .PRE Enter

3. Output file save .PED (save .PRE)
Enter

4. Does your pedigree file contain any loops? (y/n) → n → Enter
Do you want probands selected automatically? (y/n) → y → Enter

DATAFILE and PREPLINK PROGRAM

1. PREPLINK
Rockefeller software package1 → DOS → supdes → PREPLINK.EXE

2. (k) See or modify loci description_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********** PRESENT STATUS **************
(a) Number of loci                  :     2
(b) Sexlinked                       :     N
(c) Calculate Risk                  :     N
(d) Mutation                        :     N
(e) Haplotype frequencies           :     N
(f) Locus Order                     :     1 2
(g) Interference                    :     N
(h) Recombination sex difference    :     N
(i) Program used                    : MLINK
(j) Recombination values            :
       0.100
*********** OTHER OPTIONS **************
(k) See or modify loci description
(l) See or modify recombination to vary
(m) Read datafile
(n) Write datafile
(o) Exit
****************************************
Press letter to modify or see values
k
```

3. (e) CHANGE LOCUS TYPE_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) allele numbers   GENE FREQS :  0.50000  0.50000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
e
```

4. locus locus 1_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) allele numbers   GENE FREQS :  0.50000  0.50000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
e
ENTER LOCUS TO CHANGE
1
```

5. (c) AFFECTION STATUS_Enter ENTER NEW LOCUS TYPE

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) allele numbers   GENE FREQS :  0.50000  0.50000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
e
ENTER LOCUS TO CHANGE
1
ENTER NEW LOCUS TYPE:
(a) BINARY FACTORS
(b) QUANTITATIVE TRAIT
(c) AFFECTION STATUS
(d) ALLELE NUMBERS
c_
```

6. (a) SEE OR MODIFY A LOCUS_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) affection status GENE FREQS :  0.50000  0.50000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
a
```

7. ENTER LOCUS NUMBER TO SEE OR MODIFY LOCUS (OR 0 TO EXIT) locus locus 1 _Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) affection status GENE FREQS :  0.50000  0.50000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
a
ENTER LOCUS NUMBER TO SEE OR MODIFY LOCUS (OR 0 TO EXIT)
1_
```

8. (c) PENETRANCES_Enter penetrance Autosomal dominant

ENTER NEW PENETRANCES

GENOTYPE 1 1 OLD PEN 0.00000000

0

GENOTYPE 1 2 OLD PEN 0.00000000

1

GENOTYPE 2 2 OLD PEN 1.00000000

1

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************
LOCUS NUMBER : 1
********************************
(a) NUMBER OF ALLELES            :    2
(b) NUMBER OF LIABILITY CLASSES :    1
(c) PENETRANCES :
GENOTYPE   1 1   0.00000000
GENOTYPE   1 2   0.00000000
GENOTYPE   2 2   1.00000000
(d) GENE FREQUENCIES :
  0.50000  0.50000
(e) EXIT
********************************
Press letter to modify values
c
ENTER NEW PENETRANCES
GENOTYPE   1 1 OLD PEN    0.00000000
?
0
GENOTYPE   1 2 OLD PEN    0.00000000
?
1
GENOTYPE   2 2 OLD PEN    1.00000000
?
1
```

9. (d) GENE FREQUENCIES_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************
LOCUS NUMBER : 1
********************************
(a) NUMBER OF ALLELES            :    2
(b) NUMBER OF LIABILITY CLASSES :    1
(c) PENETRANCES :
GENOTYPE   1 1   0.00000000
GENOTYPE   1 2   1.00000000
GENOTYPE   2 2   1.00000000
(d) GENE FREQUENCIES :
  0.50000  0.50000
(e) EXIT
********************************
Press letter to modify values
d
```

10. ENTER 2 NEW GENE FREQUENCIES

    0.01

    0.99

```
D:\NMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************
LOCUS NUMBER : 1
********************************
<a> NUMBER OF ALLELES            :    2
<b> NUMBER OF LIABILITY CLASSES  :    1
<c> PENETRANCES :
GENOTYPE  1 1  0.00000000
GENOTYPE  1 2  1.00000000
GENOTYPE  2 2  1.00000000
<d> GENE FREQUENCIES :
  0.50000  0.50000
<e> EXIT
********************************
Press letter to modify values
d
ENTER  2 NEW GENE FREQUENCIES
0.01
0.99_
```

11. (e) EXIT_Enter

```
D:\NMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************
LOCUS NUMBER : 1
********************************
<a> NUMBER OF ALLELES            :    2
<b> NUMBER OF LIABILITY CLASSES  :    1
<c> PENETRANCES :
GENOTYPE  1 1  0.00000000
GENOTYPE  1 2  1.00000000
GENOTYPE  2 2  1.00000000
<d> GENE FREQUENCIES :
  0.01000  0.99000
<e> EXIT
********************************
Press letter to modify values
e
```

12. (a) SEE OR MODIFY A LOCUS_Enter

```
D:\NMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************************
<1> affection status GENE FREQS :  0.01000  0.99000
<2> allele numbers   GENE FREQS :  0.50000  0.50000
********************************************
<a> SEE OR MODIFY A LOCUS
<b> DELETE LOCUS
<c> ADD LOCUS
<d> CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE <NOT CHROMOSOME ORDER>
<e> CHANGE LOCUS TYPE
<f> RETURN TO MAIN MENU
********************************************
Press letter to modify values
a
```

13. ENTER LOCUS NUMBER TO SEE OR MODIFY LOCUS (OR 0 TO EXIT)

locus locus 2_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) affection status GENE FREQS :  0.01000  0.99000
(2) allele numbers   GENE FREQS :  0.50000  0.50000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
a
ENTER LOCUS NUMBER TO SEE OR MODIFY LOCUS (OR 0 TO EXIT)
2
```

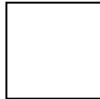
14. (a) NUMBER OF ALLELES_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************
LOCUS NUMBER : 2
*******************************
(a) NUMBER OF ALLELES          :    2
(b) GENE FREQUENCIES :
  0.50000  0.50000
(c) EXIT
*******************************
Press letter to modify values
a_
```

15. alleles (6) _Enter

alleles repeats

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************
LOCUS NUMBER : 2
*******************************
(a) NUMBER OF ALLELES          :    2
(b) GENE FREQUENCIES :
  0.50000  0.50000
(c) EXIT
*******************************
Press letter to modify values
a
ENTER NUMBER OF ALLELES
6_
```



(b) GENE FREQUENCIES_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************
LOCUS NUMBER : 2
********************************
(a) NUMBER OF ALLELES          :    6
(b) GENE FREQUENCIES :
  0.50000  0.50000  0.00000000  0.00000000  0.00000000  0.00000000
(c) EXIT
********************************
Press letter to modify values
b
```

16. 1/NUMBER OF ALLELES

1/6

0.17

0.17

0.17

0.17

0.17

0.15

Enter

17. (c) EXIT

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********************************
LOCUS NUMBER : 2
********************************
(a) NUMBER OF ALLELES          :    6
(b) GENE FREQUENCIES :
  0.17000  0.17000  0.17000  0.17000  0.17000  0.15000
(c) EXIT
********************************
Press letter to modify values
c_
```

18. (f) RETURN TO MAIN MENU_Enter

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
*******************************************
(1) affection status GENE FREQS :  0.01000  0.99000
(2) allele numbers    GENE FREQS :  0.17000  0.17000  0.17000  0.17000  0.17000
0.15000
*******************************************
(a) SEE OR MODIFY A LOCUS
(b) DELETE LOCUS
(c) ADD LOCUS
(d) CHANGE ORDER TO CORRESPOND TO PEDIGREE FILE (NOT CHROMOSOME ORDER)
(e) CHANGE LOCUS TYPE
(f) RETURN TO MAIN MENU
*******************************************
Press letter to modify values
f
```

19. (n) Write datafile

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********** PRESENT STATUS **************
(a) Number of loci                :    2
(b) Sexlinked                     :    N
(c) Calculate Risk                :    N
(d) Mutation                      :    N
(e) Haplotype frequencies         :    N
(f) Locus Order                   :    1 2
(g) Interference                  :    N
(h) Recombination sex difference  :    N
(i) Program used                  : MLINK
(j) Recombination values          :
       0.100
*********** OTHER OPTIONS *************
(k) See or modify loci description
(l) See or modify recombination to vary
(m) Read datafile
(n) Write datafile
(o) Exit
****************************************
Press letter to modify or see values
n_
```

20. SAMPLE.DAT

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
Enter output file name [press ENTER for screen output]
SAMPLE.DAT
```

21. (o) EXIT_Enter

DATAFILE (EXIT DATAFILE)

```
D:\NMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\PREPLINK.EXE
********** PRESENT STATUS ***************
(a) Number of loci                :    2
(b) Sexlinked                     :    N
(c) Calculate Risk                :    N
(d) Mutation                      :    N
(e) Haplotype frequencies         :    N
(f) Locus Order                   :    1 2
(g) Interference                  :    N
(h) Recombination sex difference  :    N
(i) Program used                  :  MLINK
(j) Recombination values          :
      0.100
*********** OTHER OPTIONS ***************
(k) See or modify loci description
(l) See or modify recombination to vary
(m) Read datafile
(n) Write datafile
(o) Exit
****************************************
Press letter to modify or see values
o
```

THE LCP SHELL

1. LCP LCP.EXE

2.

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\LCP.EXE

L I N K A G E   C O N T R O L   P R O G R A M

Input Files

COMMAND file name [PEDIN.BAT] : SAMPLE.BAT
LOG file name [FINAL.OUT] : FINAL.OUT
STREAM file name [STREAM.OUT] : STREAM.OUT
PEDIGREE file name [PEDIN.DAT] : SAMPLE.PED
PARAMETER file name [DATAIN.DAT] : SAMPLE.DAT
Secondary PEDIGREE file name [] :
Secondary PARAMETER file name [] :

CTRL/A - Abort   CTRL/H - Help   CTRL/Z - Exit
```

Page Down (PGDN)

3. General pedigrees_PGDN

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\LCP.EXE

L I N K A G E   C O N T R O L   P R O G R A M

Pedigree Options

General pedigrees : <-_
Three-generation pedigrees :
Experimental cross pedigrees :

CTRL/A - Abort   CTRL/H - Help   CTRL/Z - Exit
```

4. MLINK_PGDN

```
D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\LCP.EXE

L I N K A G E   C O N T R O L   P R O G R A M

General Pedigree Analysis Options

LODSCORE :
ILINK :
LINKMAP :
MLINK : <-_

CTRL/A - Abort   CTRL/H - Help   CTRL/Z - Exit
```

5. Lod score table_PGDN

D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\LCP.EXE

L I N K A G E C O N T R O L P R O G R A M

MLINK - Test Options

Specific evaluation :
Lod score table : <-_
Multiple pairwise Lod table :

CTRL/A - Abort CTRL/H - Help CTRL/Z - Exit

6. No sex difference_PGDN

D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\LCP.EXE

L I N K A G E C O N T R O L P R O G R A M

MLINK - Sex Difference Options

No sex difference : <-_

CTRL/A - Abort CTRL/H - Help CTRL/Z - Exit

7. Locus order 1 2_PGDN (Other recomb.)

D:\INMYDR~1\Thesis2\MLINK\ROCKEF~1\DOS\supdos\LCP.EXE

L I N K A G E C O N T R O L P R O G R A M

MLINK - Lod Table Specification

Command Screen

Locus order [] : 1 2
Recombination fractions [.0] : .0
Recombination varied [1] : 1
Other recomb. [.01 .05 .1 .2 .3 .4] : .01 .05 .1 .2 .3 .4

CTRL/A - Abort CTRL/H - Help CTRL/Z - Exit

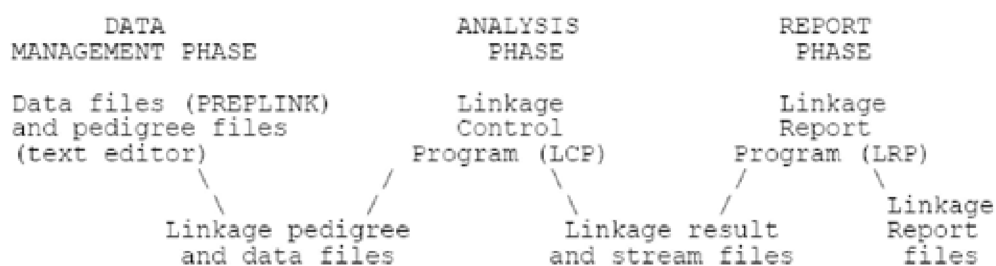
8. CTRL/Z

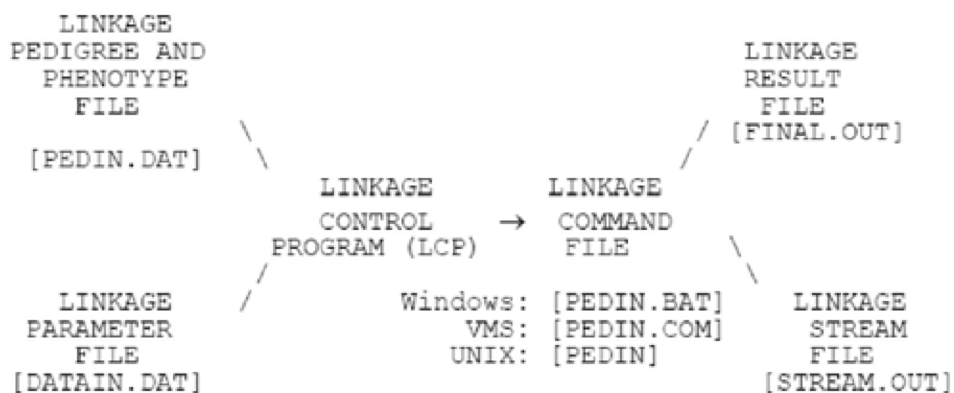
9. SAMPLE.BAT

10. SAMPLE.BAT

FINAL.OUT STREAM.OUT

11. FINAL.OUT LOD score


DATA
MANAGEMENT PHASE
ANALYSIS
PHASE
REPORT
PHASE
Data files (PREPLINK)
and pedigree files
(text editor)
Linkage
Control
Program (LCP)
Linkage
Report
Program (LRP)
Linkage pedigree
and data files
Linkage result
and stream files
Linkage
Report
files


LINKAGE
PEDIGREE AND
PHENOTYPE
FILE
[PEDIN.DAT]
LINKAGE
CONTROL
PROGRAM (LCP)
LINKAGE
COMMAND
FILE
Windows: [PEDIN.BAT]
VMS: [PEDIN.COM]
UNIX: [PEDIN]
LINKAGE
RESULT
FILE
[FINAL.OUT]
LINKAGE
PARAMETER
FILE
[DATAIN.DAT]
LINKAGE
STREAM
FILE
[STREAM.OUT]

BIOGRAPHY

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Education

She received her bachelor degree with a second class honor in Medical Technology from Faculty of Allied Health Science, Chulalongkorn University in 2006. She got a Royal Golden Jubilee (RGJ) Ph.D. Scholarship from the Thailand Research Fund (TRF) and participated in Inter-department of Biomedical Sciences, Faculty of Graduate School, Chulalongkorn University since 2007.

Research Grants

1. 90's Anniversary Chulalongkorn University, Rachadaphisek Somphot Grants, Chulalongkorn University, Bangkok, Thailand.
2. The Royal Golden Jubilee Ph.D Program, Thailand Research Fund (TRF).

Publications

1. Two novel *CTNS* mutations in cystinosis patients in Thailand. **Yeetong P**, Tongkobpetch S, Kingwatanakul P, Deekajondat T, Bernardini I, Suphapeetiporn K, Gahl WA, Shotelersuk V. Gene. 2012 Mar.
2. Three novel mutations of the IRF6 gene with one associated with an unusual featuresin Van der Woude syndrome. Yeetong P, Mahatumarat C, Siriwan P, Rojvachiranonda N, Suphapeetiporn K, Shotelersuk V. Am J Med Genet A. 2009 Nov;149A(11):2489-92
3. Identification of mutations in the SRD5A2 gene in Thai patients with male pseudohermaphroditism. Sahakitrungruang T, Wacharasindhu S, Yeetong P, Snabboon T, Suphapeetiporn K, Shotelersuk V. Fertil Steril. 2008 Nov;90(5):2015. e11-5.