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Original Article

Genetic analysis in a Chinese BAFME pedigree indicates the possibility of an unidentified locus

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Abstract: Benign adult familial myoclonic epilepsy (BAFME) is a rare adult-onset epilepsy syndrome with shivering-like tremor, myoclonus and seizure as core symptoms. BAFME has been mapped on 8q23.3-q24.1, 2p11.1-q12.2, 5p15.31-p15.1 and 3q26.32-3q28 in different families demonstrating genetic heterogeneity so far. We reported a large Chinese BAFME pedigree excluded linkage to chromosome 8q23.3-q24.1 and 2p11.1-q12.2 before. Recently, we further followed up this family and conduct electrophysiological examinations comprehensively. Linkage analysis was performed with 16 selected microsatellites encompassing the 4 above-mentioned locus. We re-investigated 46 individuals in this Chinese BAFME family and 21 individuals were diagnosed as BAFME. The possibility of this BAFME family linked with chromosome 2p11.1-q12.2, 3q26.32-3q28 or 5p15.31-p15.1 was excluded when assuming a dominant mode of inheritance. For 8q23.3-q24.1, the obtained maximum-LOD score and maximum-HLOD score were 1.717 and 1.717 at maker D8S1804. But no co-segregation of any haplotype with disease status was found by haplotype analysis. We inferred that the BAFME-causative gene in the Chinese pedigree may be located on an unidentified locus, indicating the genetic heterogeneity of BAFME.

Keywords: Benign adult familial myoclonic epilepsy (BAFME), epilepsy, genetic heterogeneity, genetic linkage

Introduction

BAFME is a rare, autosomal dominant epilepsy syndrome with adult-onset shivering-like tremor, cortical myoclonus and seizure as core symptoms [1, 2]. Generally speaking, BAFME manifests with a nonprogressive course and good response to anti-epileptic drugs [3-5]. Nearly 100 worldwide-distributed BAFME families have been reported under different names to date, such as BAFME, familial adult myoclonic epilepsy (FAME), familial cortical myoclonic tremor with epilepsy (FCMTE), familial cortical tremor and epilepsy (FCTE), and autosomal dominant cortical myoclonus and epilepsy (ADCME). BAFME demonstrates genetic heterogeneity and 4 loci (2p11.1-q12.2, 3q26.32-3q28, 5p15.31-p15.1 and 8q23.3-q24.1) have been mapped [6-9]. However α2-adrenergic receptor subtype B (α2B-AR) and Ubiquitin protein ligase E3 component n-recognin 5 (UBR5) were identified as possible causative genes in Italy and Japanese families respectively, no study replicated them so far [10, 11]. We herein re-investigated a large Chinese BAFME family reported before and conducted genetic analysis [12].

Subjects and methods

Study subjects and clinic data

This study was approved by the Ethics Committee of Xiangya Hospital, Central South University. All participants provided signed, informed consent. This study adhered to the tenets of the Declaration of Helsinki.

A large Chinse BAFME pedigree from Anhui Province (People's Republic of China) was reported before [12]. BAFME were diagnosed

Table 1. Clinical and electrophysiological features of patients in Chinese BAFME pedigree

NO.	Gender	Age (y)	Onset ages (y)			EEG	SEPs	AEDs
			Myoclonus	Tremor	GTCS			
V4	F	81	45	45	45	+	+	PB
V19	F	74	-	35	34	+	+	N
V22	M	58	40	40	-	+	+	N
V28	M	68	-	33	50	+	+	N
V29	M	65	-	34	-	+	+	N
V30	M	63	-	32	-	+	+	N
V31	F	59	-	32	45	+	+	N
V35	F	62	48	48	51	+	+	PB
VI3	M	56	40	40	-	+	+	VPA
VI4	M	51	32	37	33	+	+	PB
VI9	F	33	20	20	-	+	+	N
VI14	F	46	36	36	-	+	+	N
VI16	M	40	34	36	-	+	+	N
VI17	M	38	16	16	16	+	+	PB
VI21	F	41	39	31	-	+	+	N
VI22	M	36	33	33	-	+	+	N
VI28	F	47	33	33	35	+	n.d	N
VI32	M	43	29	-	29	+	n.d	N
VII1	F	33	27	-	-	+	+	N
VII14	M	18	16	16	-	+	+	N
VII17	F	24	17	-	-	+	+	N

F, female; M, male; SEPs, somatosensory evoked potentials; AEDs, antiepileptic drugs; n.d., not done; PB, phenobarbital; VPA, valproic acid; y, year; +, present or performed; -, not observed or none.

according to the criteria adopted from the Hitomi's study [13]. History inquiry and physical examination of each case were conducted by two neurology physician simultaneously. Clinical data such as onset age of myoclonus, cortical tremor or generalized tonic-clonic seizures (GTCS), the frequency of GTCS summarized in **Table 1**. All patients underwent magnetic resonance imaging (MRI) scanning, electroencephalograph (EEG) and somatosensory evoked potential (SEP) tests.

Genetic markers genotyping

In this Chinese BAFME pedigree, 18 family members were studied for linkage analysis. For each participant, 5 ml peripheral blood was collected to extract genomic DNA. Marshfield Genetic Maps (http://www.bli.uzh.ch/BLI/Projects/genetics/maps/marsh.html) and published literatures were referred to select microsatellite markers [6-9]. And loci investigated were D8S1784, D8S1779, D8S1694, D8S514, D8S1804, D2S388, D2S2216, D2S2264, D2S1897, D5S580, D5S486, D5S1380, D5S2096, D3S1571, D3S3609, D3S1602, and D3S1262. Fluorescence based semi-automated methods were used to genotype these microsatellite markers on an ABI 373A automated DNA sequencer (PE Applied Biosystems, Inc.). Sequences of corresponding primers were obtained from NCBI (National Center for Biotechnology Information) website (http://www.ncbi.nlm.nih.gov/probe). PCR conditions were 30 cycles of 94°C for 1 min, specific annealing temperature for each primer for 1 min, and 72°C for 1 min. PRISM®GeneMapper™ Software Version 3.0 (Applied Biosystems) was used to analyses allelic sizes.

Linkage analysis and haplotype analysis

MERLIN program 1.1.2 (Center for Statistical Genetics) [14] was used to implement the parametric two-point linkage analysis under autosomal dominant inheritance model: penetrance was set at 0.01-0.99. Therefore two-point Log odds (LOD) scores and heterogeneity LOD (HLOD) scores were yielded. Furthermore, haplotype analysis was conducted.

Results

Clinic data and electrophysiological examinations

We re-investigated 46 individuals in a Chinese BAFME family, which is consistent with the autosomal dominance inheritance even if consanguineous marriage existed in cousins between III1 and III2 (**Figure 1**). The clinic data and electrophysiological examination were described in **Table 1**. Totally 21 individuals were diagnosed as BAFME, including 11 individuals experienced GTCS seizures, 16 individuals suffered cortical tremor and 13 individuals suffered myoclonus. The average onset-age with cortical tremor, myoclonus and GTCS are 33.1 years (range 16-48 years), 31.6 years (range 16-48 years) and 37.6 years (range 16-51 years), respectively. Furthermore, onset-ages

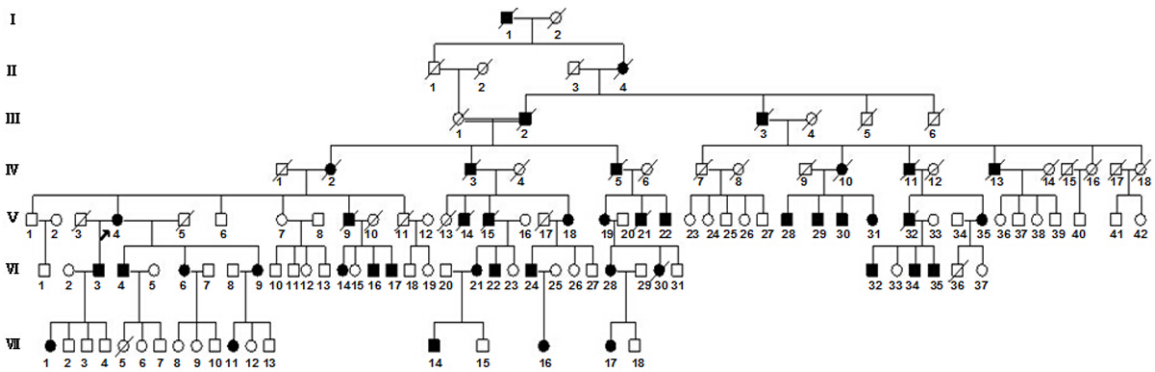

Figure 1. A Chinese pedigree with BAFME.

Table 2. LOD and HLOD Scores for BAFME versus each markers

Markers	LOD Scores	ALPHA Scores	HLOD Scores
D2S388	-5.552	0.000	0.000
D2S2216	-2.210	0.000	0.000
D2S2264	-2.668	0.000	0.000
D2S1897	-3.602	0.000	0.000
D3S1571	-7.820	0.000	0.000
D3S3609	-8.428	0.000	0.000
D3S1602	-8.468	0.000	0.000
D3S1262	-8.504	0.000	0.000
D5S580	-0.956	0.000	0.000
D5S486	-1.840	0.000	0.000
D5S1380	-1.445	0.000	0.000
D5S2096	-1.427	0.000	0.000
D8S1784	-3.165	0.000	0.000
D8S1779	-4.182	0.000	0.000
D8S1694	0.314	1.000	0.314
D8S514	1.601	1.000	1.601
D8S1804	1.717	1.000	1.717

of core symptoms decrease in successive generations which indicated possibly genetic anticipation. Four patients were treated with either Valproic acid or Phenobarbital. Therefore, these clinical symptoms were ameliorated effectively.

All 21 patients manifested with normal background activity and interictal generalized epileptiform discharges in EEG. The large amplitude ("giant SEP") of complex P25-N33 as well as the long latency reflex (C-reflex) was presented in 16 affected cases, but not in the healthy subjects. All 21 BAFME patients received the examination of MRI and nothing especial found except one with encephalomalacia.

Linkage analysis

LOD scores for every microsatellite marker were generated by two-point linkage analysis with recombination fraction values (θ) set from 0.00 to 0.50. The results of two-point LOD scores between the disease phenotype and each marker loci and haplotype analysis for these markers are shown in **Table 2** and **Figure 2**, respectively. The possibility of this BAFME family linked with chromosome 2p11.1-q12.2, 3q26.32-3q28 and 5p15.31-p15.1 were excluded when assuming the mode of autosomal dominant inheritance. For 8q23.3-q24.1, the maximum-LOD score (1.717) and maximum-HLOD score (1.717) were obtained at maker D8S1804 with θ=0.00. But we conducted haplotype analysis and found that no co-segregation of any haplotype with disease status in this BAFME family.

Discussion

Although BAFME hitherto was not recognized by the International League against Epilepsy, Nearly 100 families has been well-described and studied [15]. 2p11.1-q12.2 and 8q23.3-24 were considered major loci for the origin of European and Asian, respectively [4, 6, 7]. Recently 8q23.3-24 and 5p15.31-p15.1 was found associated with Chinese BAFME families [16, 17]. 3q26.32-3q28 was only reported in a large BAFME family from Thailand [9]. T Kato et al. identified UBR5 gene mutation in a Japanese BAFME family with only 5 patients. UBR5 is located in 8q22.3, which is close to the reported locus linked to Japanese BAFME families [10]. But this report was not replicated by other studies so far. De Fusco M et al. identified α2B-

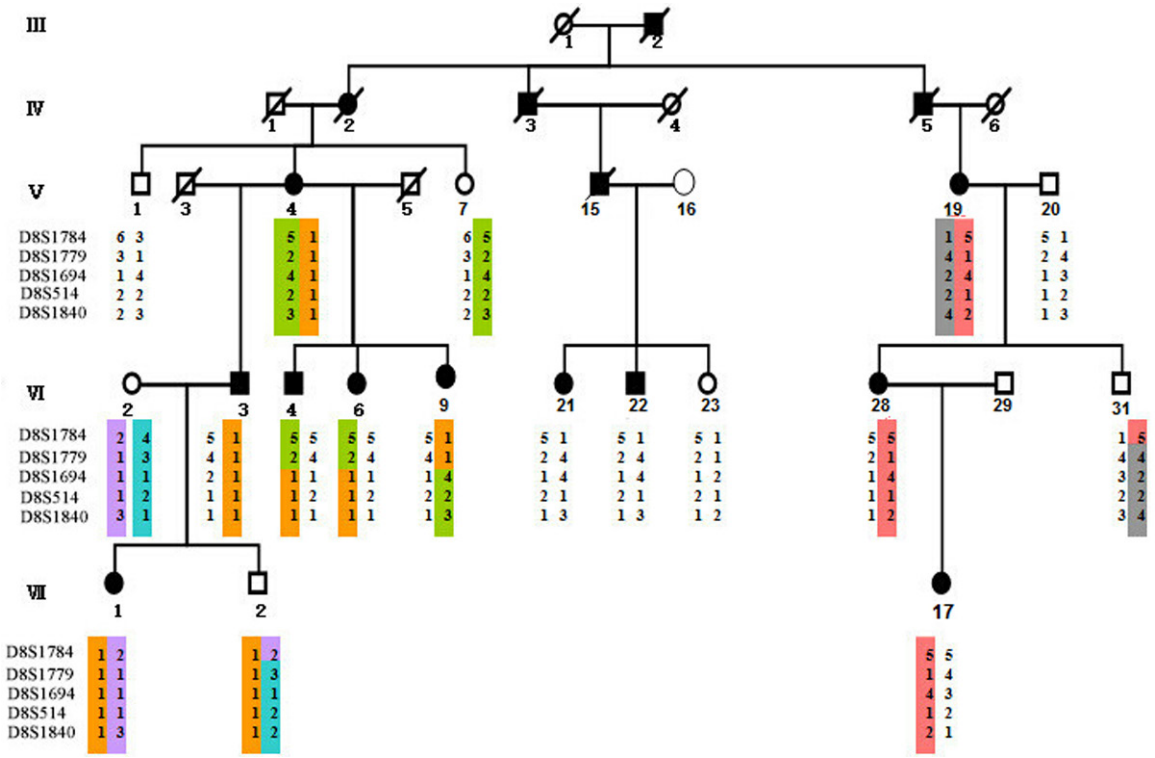

Figure 2. Structure of Chinese BAFME pedigree and transmitted haplotype at the 8q23.3-q24.1 locus. Family members were genotyped for five microsatellite markers mapping on chromosome 8q.

AR gene as the causative gene of two Italian ADCME families meanwhile they did not detect any mutation of ADRA2B in other BAFME family mapped on 2p11.1-q12.2 [11]. So they proposed that ADCME and BAFME may be two distinct clinical entities.

In 2005, we firstly reported and studied this large Chinese BAFME pedigree [12]. By linkage analysis, the possibility linked with 2p11.1-q12.2 and 8q22.3 was eliminated. Ten years later, we investigated this pedigree and found some "normal" individuals in 2005 had converted to patients with clinical manifestations (for example: V22, 31, VI9, 16, 21, 22, VII1, 14, 17). The exact clinical history data of some patients in the follow-up had been learned from other members, or related electrophysiological examinations were reexamined in part clearly presented positive results. In this Chinese large BAFME family, electrophysiologic studies revealed tremor origins as a result of cortical hyperexcitability. Myoclonic tremor and seizure responded well to VPA or PB. We can confirm the diagnosis of BAFME by clinical and electrophysiological features of this pedigree. Furthermore, we found genetic anticipation phenotypes of this family, which is analogous to those observed in Japanese pedigrees [18-20]. But results of our linkage analysis indicated another unidentified locus may be responsible for this Chinese pedigree. Genome-wide scan linkage analysis and exosome sequencing would contribute to identify the causative gene.

In summary, the BAFME-causative gene for the Chinese pedigree may be located on an unidentified locus, indicating the genetic heterogeneity of BAFME.

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Disclosure of conflict of interest

None.

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