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BRIEF COMMUNICATION

Founder effect of the TTTCA repeat insertions in *SAMD12* causing BAFME1

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Abstract

Benign adult familial myoclonic epilepsy type 1 (BAFME1) in several Japanese and Chinese families has recently been found to be caused by pentanucleotide repeat expansions in *SAMD12*. We identified a Thai family with six members affected with BAFME. Microsatellite studies suggested a linkage to the BAFME1 region on chromosome 8q24. Subsequently, long-read whole-genome sequencing showed the $(TTTTA)_{446}(TTTCA)_{149}$ in intron 4 of *SAMD12* in an affected member. Repeat-primed PCR and long-range PCR revealed that the pentanucleotide repeat expansions segregated with the disease status. Our Thai family is the first non-Japanese and non-Chinese family with BAFME1. SNP array showed that the aberrant repeats had the same haplotype as those previously determined in Japanese and Chinese patients suggesting a common ancestry. The variant is estimated to arise ~12,000 years ago.

Introduction

Benign adult familial myoclonic epilepsy (BAFME) is characterized by adult-onset cortical tremor and generalized seizures, which is inherited in an autosomal dominant manner [1]. This syndrome was first identified in Japan. More than 100 families have now been reported in several countries [2]. Six chromosomal regions have been linked to BAFME including 8q23.3-q24.1 [3], 2p11.1-q12.2 [4], 5p [5], 3q26.32-q28 [6], 16p21.1 [7], and 4q32.1 [7] for BAFME1–BAFME4, BAFME6, and BAFME7, respectively. Recently, the variant of BAFME1 in Japanese and Han Chinese populations was identified to be the TTTTA expansions and TTTCA repeat insertions in an intron of the *SAMD12* gene [7–11]. BAFME2, BAFME3, BAFME4, BAFME6, and BAFME7 are caused by the same pattern of the repeat expansions in *STARD7* [12], *MARCH6* [13], *YEATS2* [14], *TNRC6A* [7], and *RAPGEF2* [7], respectively. In this study, we report the first Thai BAFME1 family who have the same pentanucleotide repeat expansions in *SAMD12*. Interestingly, of all patients who underwent haplotype analysis to date, the variants were on the same haplotype suggesting a founder effect.

Supplementary information The online version of this article (https://doi.org/10.1038/s41431-020-00729-1) contains supplementary material, which is available to authorized users.

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Materials and methods

Subjects

We identified a Thai family with BAFME. Six affected and two unaffected members were recruited (Fig. 1a). Demographic data, clinical manifestations, family history, and electrophysiological examinations including electroencephalograms (EEG), somatosensory-evoked potentials (SSEP), C-reflex, and jerk-locked back averaging method were performed. After informed consent was obtained, blood samples were collected and genomic DNA was

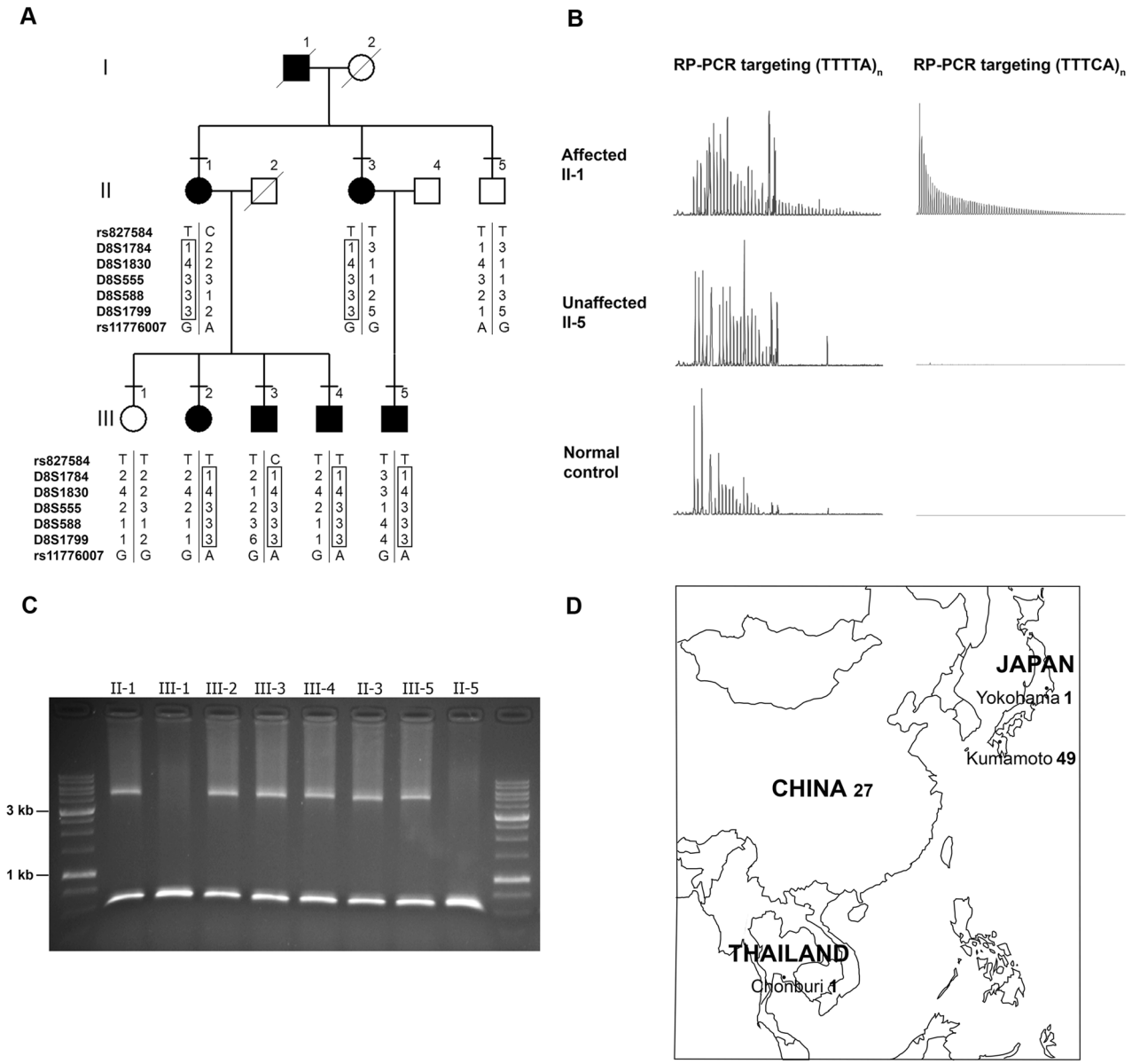

Fig. 1 Identification of TTTCA repeat insertions in *SAMD12* in a Thai BAFME1 family. a Presence of linkage to the BAFME1 region on chromosome 8q24. The haplotype analysis revealed the evidence of linkage between the microsatellite markers D8S1784 and D8S1799. Linkages to BAFME2–4 chromosome regions by typing microsatellite markers D2S2333, D2S2175, D5S630, and D3S1262 were excluded (data not shown). Horizontal bars above each individual symbol indicate that they were studied clinical phenotype and their blood samples were obtained. **b** Repeat-primed PCR to detect TTTTA and TTTCA pentanucleotide repeat expansions. (Left panel) RP-PCR targeting TTTTA repeats shows presence of the TTTTA repeats in the affected, while absence in the unaffected family member and the normal control. (Right panel) RP-PCR targeting TTTCA repeats shows presence of the TTTCA repeats in only the affected family member, while absence in the unaffected member and the normal control. **c** Long-range PCR to determine the size of repeat expansions of each member of the BAFME1 family. The gel revealed 5–6 kb expanded alleles which segregated with the disease in the family. **d** Geographic locations of the families presenting the *SAMD12* $(TTTTA)_{exp}(TTTCA)_{ins}$. Families are located in three countries. Bold numbers represent the number of families (Japan [7, 10], China [8, 9, 11], and Thailand).

extracted from peripheral blood leukocytes using a standard protocol.

Linkage analysis

At the time we started doing the linkage study, four chromosomal regions linked to BAFME were known. We therefore genotyped all eight available members with 18 microsatellite markers (D8S270, D8S1784, D8S1830, D8S555, D8S588, D8S1799, D8S284, D8S272, D2S2333, D2S388, D2S2175, D2S2264, D2S160, D5S630, D5S416, D3S1571, D3S3609, and D3S1262) for BAFME1–4 [3–6]. Details of all markers are shown in the Supplementary Table S4. We typed all fluorescently labeled primers on an ABI Prism 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA) with GeneMapper software (Applied Biosystems). The region would be considered no linkage when none of the alleles of the markers in the region segregated with the disease. On the contrary, the region would be considered promising when at least one of the alleles of any markers in the region segregated with the disease.

Whole-exome sequencing (WES)

Three affected (II-1, III-2, III-5) and one unaffected (III-1) members underwent WES. DNA was enriched by TruSeq Exome Enrichment Kit and sequenced onto Hiseq 2000. Four WES data are openly available in sequence read archive (SRA) accession PRJNA625829. The variants were filtered by the following criteria: (1) present in the heterozygous state, (2) present in the three affected while absent in the unaffected, and (3) present in less than 0.1% of dbSNP135 database.

Array comparative genomic hybridization (aCGH)

To identify the copy number variation (CNV), we used DNA of an affected (III-2) and an unaffected (III-1) member for the Agilent oligonucleotide array-based CGH. DNA copy number changes were measured using Agilent Genomic Workbench v7.0.4.0 software. Aberrations were detected with the ADM-2 algorithm, whose threshold was 6.0. We defined gains and losses over a continuous three probes and a linear log2 ratio average of ≥0.25 or ≤0.25, respectively.

Long-read whole-genome sequencing (LR-WGS)

DNA samples from one affected (III-3) and one control were subjected to WGS using single molecule, real-time (SMRT) sequencing technology with Sequel Sequencing Kit 2.1 and Sequel sequencer (Pacific Biosciences, Menlo Park, CA, USA). The sequencing data were aligned to the reference genome hg19 by using structural variant calling analysis on SMRT Link 5.1.0.26412. Long-read sequencing data are openly available in SRA accession PRJNA547506.

Repeat-primed polymerase chain reaction (RP-PCR)

Using DNA of all family members, the pentanucleotide repeat sequence in an intron of *SAMD12* was amplified by TTTTA and TTTCA RP-PCR with the primers as previously described [7]. RP-PCR products were detected on an ABI Prism 3100 genetic analyzer with Peak Scanner v1.0 software (Applied Biosystems, Foster City, CA, USA).

Long-range PCR (LR-PCR)

Using DNA of all family members, expanded alleles were amplified by LR-PCR with 100–500 ng of genomic DNA, 0.2 µM of each primer (Table S1), 200 µM dNTP mixture, 1X PrimeSTAR GXL buffer, and 2.5 U PrimeSTAR GXL Taq DNA polymerase (Takara Bio Inc., Kusatsu, Japan) in 50 µl. After 5 min at 94 °C, and 30 cycles of 98 °C for 30 s, 58 °C for 30 s, and 68 °C 7 min; PCR products were separated by agarose gel electrophoresis in 1% gel.

Haplotype construction and founder effect analysis

SNP array using HumanOmniZhongHua-8 BeadChip from Illumina was performed in all eight family members. SNP array data are openly available in Gene Expression Omnibus accession GSE148708. We manually constructed the haplotype. The same alleles of each marker in the affected would be considered on the haplotype with the pentanucleotide repeat insertions. The haplotype data used for comparison were obtained from all nine available families (three Japanese, five Chinese, and one Thai) [7, 8].

Estimation of the age of the $(TTTCA)_n$ in intron 4 of *SAMD12*

To estimate the age of this variant, we used the Mutation Dating application (https://shiny.wehi.edu.au/rafehi.h/mutation-dating/) [15]. Dating estimates were determined assuming a correlated genealogy and converted to years by assuming an average of 25 years per generation.

Results

Clinical characterization of patients with BAFME

All six affected family members had tremors and myoclonus of both hands. Four had occasional generalized tonic-clonic seizures precipitated by bright lights or lack of sleep.

None had dementia or cerebellar ataxia. Electrophysiological studies demonstrated long-loop C-reflex and enlarged SSEP. Electroencephalographic showed generalized or multifocal polyspikes and waves with photosensitivity (Table 1). The family pedigree suggested autosomal dominant inheritance (Fig. 1).

Absence of linkage to BAFME2–4 chromosomal regions

The studies, while excluding linkage to chromosome 2p11.1-q12.2, 5p15.31-p15, and 3q26.32-q28 regions, showed a possible linkage to chromosome 8q24 between microsatellite markers D8S1784 and D8S1799 (Fig. 1a), which is the BAFME1 locus.

WES and aCGH failed to identify the causative variant

Eighteen variants from WES met the filtering criteria (Table S2). Subsequently, we did Sanger sequencing of all the variants in all family members; however, no variants segregated with the disease status in the family. aCGH performed from one affected and one unaffected revealed that thirteen CNVs were loss and eight CNVs were gain (Table S3). All CNVs were found in the database of genomic variants as polymorphisms.

Structure of expanded repeats determined by LR-WGS

The sequencing depth of each individual was ~10X. LR-WGS of III-3 showed the configuration of repeat expansion as NC_000008.10:g.119379055_119379157TGAAA[149] TAAAA[446] (ClinVar accession SCV001237462) (Fig. S1) in intron 4 of *SAMD12*.

RP-PCR showed that the TTTTA and TTTCA repeat expansions cosegregate with the disease status in the family

RP-PCR was performed in all family members. The results revealed TTTTA repeat expansions and TTTCA repeat insertions in all six affected individuals (Fig. 1b), while absent in the two unaffected members.

Determining the repeat expansions and the size of TTTTA repeats by LR-PCR

LR-PCR performed in the eight members showed that all six patients showed long allele bands in which the sizes were ~5–6 kb. The normal allele bands of the family members had sizes between 500 and 750 bp (Fig. 1c).

Haplotype analysis and founder effect analysis

SNP array showed that the core haplotype containing the $(TTTCA)_n$ insertion region was found to be shared among all three Japanese, five Chinese, and one Thai pedigrees (Fig. S2), suggesting a common ancestry of the aberrant repeat expansions in these patients from three countries.

Estimation of the age of the $(TTTCA)_n$ in intron 4 of *SAMD12*

A core haplotype in the Thai family identified from the SNP array data was 25.64 Mb in size (chr8: 104,363,338–130,004,216 (hg19)). The region across which this family shared a haplotype in common with previous reported haplotypes [8] spans chr8: 119,165,749–119,511,032 (hg19) (Fig. S2). We estimated that the most recent common ancestor of the BAFME1 lived around 495 generations ago with a 95% confidence interval (CI) between 278 and 709 generations. Assuming a generation time of 25 years, this means that the variant probably appeared 12,375 years ago (95% CI: 6950–17,725 years).

Discussion

We previously identified a Thai family with BAFME4 that is linked to chromosome 3q26.32-q28 [6] and is caused by repeat expansion in *YEATS2* [14]. In this study, we identified another Thai family with BAFME. The microsatellite typing suggested a linkage to the BAFME1 locus. This is the first non-Japanese, non-Chinese BAFME1 family.

Comparing the two families, one with BAFME1 (six affected) and the other BAFME4 (13 affected) [14], in our hands-on experience, they were clinically and neurophysiologically similar. Their seizures were well controlled by valproic acid, levetiracetam, or phenytoin. However, our BAFME1 family had an older average age of onset. For tremors, the average ages were 25 ± 3.6 and 19 ± 1.9 years for BAFME1 and BAFME4, respectively; while for seizures, they were 34 ± 2.5 and 25 ± 1.9 years, respectively.

WES and aCGH failed to identify variants that affect function. Yet, LR-WGS successfully found pentanucleotide repeat expansions in an affected member. RP-PCR confirmed that the repeat expansions segregated with the disease status in the family. LR-PCR revealed the long allele bands in all affected patients, which appeared to be varied in size within the family.

The causative variant, TTTTA repeat expansions and TTTCA repeat insertions in intron 4 of *SAMD12*, is the same as those found in Japanese and Chinese patients [7–11]. Haplotype analysis found that they all shared the same haplotype (Fig. S2) [8]. This suggests a common

Table 1 Clinical manifestation and neurophysiological findings in eight available family members of the Thai family with BAFME.

ID	Year of birth	Sex	Age	Myoclonus (age of onset)	Seizure (age of onset)	Current medication(s)	Seizure control	C-reflexes L (+ or −) (latency; ms) normal = negative	C-reflexes R (+ or −) (latency; ms) normal = negative	N20-P25 L (amplitude; μv) (5.1 ± 1.9)	N20-P25 R (amplitude; μv) (5.1 ± 1.9)	EEG
II-1	1943	F	76	Unknown	35	Dilantin (100) 1 × 1, Depakene (500) 1 × 2	Well controlled	Positive (41.85)	Positive (39)	Positive (14.4)	Positive (12.1)	NA
II-3	1945	F	74	Unknown	35–37	Unknown	Passed away at age of 71	NA	NA	NA	NA	NA
II-5	1950	M	69	None	None	None	No seizures	Negative	Negative	Negative (2.94)	No response	Normal EEG
III-1	1965	F	54	None	None	None	No seizures	Negative	Negative	Negative (2.3)	Negative (3.4)	NA
III-2	1967	F	52	25	27	Keppra (500) 1 × 2	Well controlled	Positive (40.26)	Positive (40.55)	Negative (4.9)	Negative (4.6)	IEDs: bilateral (left > right) polyspikes and waves induced by intermittent photic stimulation
III-3	1970	M	49	28	None	No medication	No seizures	Positive (46.25)	Positive (46.15)	Negative (3.5)	Positive (7.5)	IEDs: generalized polyspikes and waves
III-4	1974	M	45	15	None	No medication	No seizures	Positive (41.8)	Positive (44.65)	Negative (3.4)	Negative (6.4)	NA
III-5	1977	M	42	32	39	Dilantin (100) 3 × 1	Seizure when lack of sleep	Positive (41.05)	Positive (42.95)	Positive (9.3)	Positive (6.9)	NA

NA not available.

ancestral founder in all BAFME1 patients. We estimated that TTTCA repeat insertions occurred 495 generations ago, which corresponds to ~12,000 years. This aligns with the fact that several centuries ago, Chinese migrants came to Thailand, and now up to 10–14% of the Thai population are Chinese [16] (Fig. 1d).

BAFME4 and 6 have been reported in only one family, each. Given that all patients with BAFME1 had the same ancestry, variants causing each of the six types of BAFME would occur only once. This favors the hypothesis that pathogenesis of BAFME is the RNA toxicity of the pentanucleotide repeats, rather than being related to the malfunction of the involved genes.

In summary, we reported the TTTTA repeat expansions and TTTCA repeat insertions in the intron 4 of *SAMD12* in a Thai Family with BAFME. Its haplotype is the same as those found in Japanese and Chinese families suggesting a founder effect.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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