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# Pentanucleotide Repeat Insertions in *RAI1* cause Benign Adult Familial Myoclonic Epilepsy Type 8

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Supporting Data

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## Abstract

**Background:** Benign adult familial myoclonic epilepsy (BAFME) is an autosomal dominant disorder characterized by cortical tremors and seizures. Six types of BAFME, all caused by pentanucleotide repeat expansions in different genes, have been reported. However, several other BAFME cases remain with no molecular diagnosis.

**Objectives:** We aim to characterize clinical features and identify the mutation causing BAFME in a large Malian family with 10 affected members.

**Methods:** Long-read whole genome sequencing, repeat-primed PCR and RNA studies were performed.

**Results:** We identified TTTTA repeat expansions and TTTCA repeat insertions in intron 4 of the *RAI1* gene that co-segregated with disease status in this family. TTTCA repeats were absent in 200 Malian controls. In the affected individuals, we found a read with only nine TTTCA repeat units and somatic instability. The *RAI1* repeat expansions cause the only BAFME type in which the disease-causing repeats are in a gene associated with a monogenic disorder in the haploinsufficiency state, i.e., Smith-Magenis syndrome (SMS). Nevertheless, none of the Malian patients exhibited symptoms related to SMS. Moreover, leukocyte RNA levels of *RAI1* in six Malian BAFME patients were no different from controls.

**Conclusions:** These findings establish a new type of BAFME, BAFME8, in an African family and suggest that haploinsufficiency is unlikely to be the main pathomechanism of BAFME.

### Keywords

BAFME8; Long-read sequencing; *RAI1* ; TTTCA repeat insertions; haploinsufficiency

## Introduction

Benign adult familial myoclonic epilepsy (BAFME), also known as autosomal dominant cortical myoclonus and epilepsy (ADCME), familial adult myoclonic epilepsy (FAME), and familial cortical myoclonic tremor with epilepsy (FCMTE),[1] is characterized by cortical tremors, myoclonus, and infrequent seizures that progress slowly. Diagnosis is based upon

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clinical and electrophysiological criteria.[2] The six autosomal dominant types of BAFME are classified by their underlying genes, i.e., *SAMD12* (BAFME1),[3] *STARD7* (BAFME2),[4] *MARCHF6* (BAFME3),[5] *YEATS2* (BAFME4),[6] *TNRC6A* (BAFME6),[3] and *RAPGEF2* (BAFME7),[3] these types of BAFME are caused by TTTTA repeat expansions followed by TTTCA repeat insertions in an intron of the affected gene. Only one Chinese pedigree with BAFME has been found to harbor a different repeat motif $(TTTGA)_n$, inserted at the same site as the known $(TTTCA)_n$ insertion in *SAMD12*.[7] BAFME5 is distinguished from the 6 other BAFMEs as a distinct neurological disorder caused by a homozygous frameshift mutation in the *CNTN2* gene[8] It is inherited in an autosomal recessive fashion and characterized by the onset of seizures in adolescence followed by the development of cortical myoclonic tremors.

BAFME types 1, 4, 6 and 7 have been reported in various Asian populations including Japanese,[3, 9] Chinese,[10–12] Sri Lankan, Indian[13] and Thai.[6,14] BAFME2 was reported in Caucasian and middle eastern populations including Italian, Australian/New Zealander, French, Spanish, Iraqi of Sephardic Jewish ancestry, South African of European ancestry, and Syrian.[4] BAFME3 has been found in French and Dutch populations.[5] BAFME1, 2, 3, and 7 have been reported in more than one independent family. The pentanucleotide repeat expansions in BAFME1 are located on the same haplotype, suggesting a founder effect in Asian populations.[3, 10, 13, 14] No BAFME case has been reported in individuals of African descent.

Although several families with BAFME have been identified, the behavior of the repeats when they are passed on from parent to offspring has not been studied. Long-read sequencing technology (LRS) not only helps identify causative variants, particularly tandem repeat expansions,[15] in unsolved genetic diseases,[16] but it can also determine the exact number of repeat units. Consequently, it can characterize the behavior of the repeats when applied to a multigenerational family.

In this study, we identified TTTTA repeat expansions and TTTCA repeat insertions in intron 4 of the *RAI1* gene in a large Malian pedigree, establishing a new entity of BAFME8 found in an African family. In addition, BAFME8 is the only BAFME type in which the repeats are found in a gene in which haploinsufficiency causes a disorder, suggesting that loss-of-function of the host gene is not the mechanism of BAFME8.

## Methods

### Subjects

This study was approved by the Ethics committee of the Faculty of Medicine and Dentistry of the University of Sciences, Techniques and Technologies of Bamako (USTT-B), Mali. 27 family members, 10 affected and 17 unaffected (individuals with * in Figure 1), were enrolled after giving a written consent and assent for children and the disabled. All available family members underwent a thorough neurological examination. Eight presented with symptoms consistent with benign adult familial myoclonic epilepsy, and two siblings from a consanguineous marriage had both benign adult familial myoclonic epilepsy and peripheral neuropathy symptoms. An EEG was performed on all patients while brain imaging and

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blood chemistries were obtained from available patients. In addition, the patients with peripheral neuropathy underwent nerve conduction studies (NCS). Genomic DNA was extracted from peripheral blood using the Gentra Puregene Blood DNA Kit C (Qiagen, Germantown, MD). DNA was obtained from 200 unaffected Malian individuals to serve as controls for RNA studies.

## Long-read sequencing (LRS)

DNA samples were subjected to whole-genome sequencing using single-molecule real-time (SMRT) sequencing technology. SMRTbell libraries were prepared using the SMRTbell Express Template Prep Kit 2.0 (P/N 100–938-900) and SMRTbell Enzyme Clean-up Kit 2.0 (P/N 101–932-600). Fragments below 8 kb were eliminated using BluePippin. Finally, SMRTbell libraries were sequenced on the Sequel II system, and raw subreads were processed through the CCS workflow (PacBio SMRTLink version 10.0) to generate HiFi reads with a minimum estimated quality value (QV) of 20 (phred-scaled, corresponding to an accuracy of 99%).

First, Pacbio HiFi ccs reads were mapped to human reference genome GRCh38 using LAST software according to the instructions on https://github.com/mcfrith/last-rna/blob/master/last-long-reads.md. In brief, a reference genome was indexed with the command lastdb -P8 -uNEAR -R11 -c GRCh38 GRCh38.fa. Next, the rates of insertion, deletion, and substitutions between reads and the genome were determined with the command last-train -P8 -Q0 GRCh38 myseq.fastq > myseq.par. Reads were then aligned to the reference genome with the command lastal -P8 -p myseq.par GRCh38 myseq.fastq | last-split | gzip > output.maf.gz. Finally, changes in the length of tandem repeats were found using tandem-genotypes tools[17] with the command tandem-genotypes -g hg38.refGene.txt.gz simpleRepeat.txt.gz output.maf.gz > tandemgenotype_output.txt where hg38.refGene.txt.gz and simpleRepeat.txt.gz were downloaded from UCSC genome browser.

Initially, we specifically searched for the repeat expansion in the sixth previously known loci (GRCh38 BAFME1 chr8:118366813–118366918 (*SAMD12*), BAFME2 chr2:96197067–96197124 (*STARD7*), BAFME3 chr5:10356339–10356411 (*MARCHF6*), BAFME4 chr3:183712177–183712226 ( *YEATS2*), BAFME6 chr16:24613439–24613532 (*TNRC6A*), BAFME7 chr4:159342527–159342618 (*RAPGEF2*)) by analyzing long-read data.

Subsequently, in order to identify the repeat expansion region, we retrieved the TTTTA repeats that were expanded in both cases (III-7 and III-14) but absent in 208 unaffected controls in our in-house database. Then we inspected for the presence of the TTTCA repeat insertion in both cases.

## Repeat-prime PCR (RP-PCR)

The pentanucleotide repeat sequence in intron 4 of *RAI1* was amplified by TTTTA and TTTCA RP-PCR with the sets of primers shown in Supplementary Table 1. PCR for TTTTA and TTTCA was carried out with 200 ng of genomic DNA, 0.8 μM of each primer, 0.2 mM dNTP mixture, 1x PCR buffer containing 15 mM $MgCl_2$, 1x Q-Solution, 2.5 U HotStarTaq DNA polymerase (Qiagen) in 50 μl. Touchdown PCR was performed with the following

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conditions: first, 15 min at 95 ºC, then 19 cycles at 94 ºC for 1 min, 61 ºC for 1 min, and 72 ºC 1 min with the annealing temperature gradually reduced (0.5 ºC per cycle), then 19 cycles at 94 ºC for 1 min, 51 ºC for 1 min and 72 ºC for 1 min, and finally, 72 ºC for 10 min. The RP-PCR products were detected on an ABI Prism 3100 genetic analyzer (Applied Biosystems) with Peak Scanner Software V1.0 (Applied Biosystems).

### RNA extraction, reverse transcription, and sequencing of the complementary DNA

To determine the existence of the mutant RNA, the total RNA of six affected family members, six unaffected family members, and three healthy controls, was collected using Tempus Blood RNA tubes (Thermo Fisher Scientific), isolated using the Tempus Spin RNA Isolation kit (Thermo Fisher Scientific), and reverse-transcribed using the ProtoScript® II First Strand cDNA Synthesis Kit (NEB, Ipswich, MA, USA). Forward and reverse primers were designed to flank the heterozygous SNP rs3818717, located in the *RAI1* coding sequence before the repeats, which was identified in III-7 from the LRS data (Figure 3C). PCR was performed using the primers listed in Supplementary Table 2 (Figure 3D). The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, Ohio) and sent for direct sequencing at Macrogen Inc. (Seoul, Korea). Analyses were performed by Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI).

### Real-time quantitative PCR (RT-qPCR)

For quantitative gene-expression analysis, RT-qPCR was performed using a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific). The amounts of mRNA were calculated using the $2^{-\quad Ct}$ method, normalized against housekeeping genes Beta-actin. RT-qPCR reactions were performed using the TaqMan method (TaqMan ID# Hs00430773_m1 *RAI1* NM_030665 ex 2–3, Hs01554690_m1 *RAI1* NM_030665 ex 3–4, Hs1060665_gl_m1 *ACTB*). All data were analyzed using the StepOnePlus™ Software (Applied Biosystems).

## Results

### Clinical findings

A large Malian family of the Bambara ethnic group was evaluated for evolutive jerk and tremor-type limb movements. The pattern of the disease distribution was consistent with autosomal dominant inheritance (Figure 1). The mean age of disease onset was 37.7 years (range: 7 – 68 years) and that of diagnosis was 46.7 years (range: 17 – 70 years). The presenting symptoms were predominantly tremor (60%) and generalized tonic clonic seizures (20%). Over time, patients had difficulty with daily activities such as opening jars, writing, signing papers, driving, and walking, with many having frequent falls. Some patients reported speech impairment and episodes of generalized tonic clonic seizures. Overall, symptoms were exacerbated by emotion and during flu-like episodes. Eventually, patients had to quit or change their jobs because of increasing disabilities. Neurological examination revealed tremor and impaired coordination including dysmetria, dysdiadochokinesia and difficulty with tandem gait and nystagmus (90%), dysarthria (70%), and myoclonic jerks of the limbs (60%). While 50% had brisk reflexes in all four extremities, 70% of patients had distal sensory loss including pinprick and vibration, more

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marked in the lower limbs. Members of the family exhibited wide variability in disease presentation and severity.

In addition to these symptoms, distal muscle weakness, tingling and atrophy in all limbs, hypoesthesia with stocking and glove distribution, proprioceptive ataxia, foot drop, and hammer toes, consistent with peripheral neuropathy, were apparent in two siblings of consanguineous parents (Patients IV-37 and IV-42).

None of the patients displayed cognitive impairment. EEG showed focal and generalized paroxysmal abnormalities in 50% of the affected individuals, while brain imaging was normal in all available patients. Clonazepam and sodium valproate were introduced in the most severely affected patients, leading to some improvement in symptoms. The clinical and laboratory findings are summarized in Table 1.

### Identification of the repeat expansions by LRS

DNA samples from two affected members (III-7 and III-14) were used for whole genome PacBio HiFi long-read sequencing. The total outputs were 34.8 and 60.1 Gb, respectively. Our search in the sixth previously known loci found no TTTTA repeat expansions and TTTCA repeat insertions. Because TTTTA repeat expansions and TTTCA repeat insertions in introns caused all six previously identified BAFME types (BAFME1–4 and BAFME6–7), we used tandem-genotypes to detect the reads that contained expansions. HiFi whole genome sequencing of 208 unaffected controls in our in-house database were used for comparison.

Two reads of III-14 and six reads of III-7 revealed repeat expansions in intron 4 of the *RAI1* gene (Figure 2A and Supplementary Table 3). The expanded repeats are estimated to be $(\mathrm{TTTTA})_{278\text{–}715}(\mathrm{TTTCA})_{9\text{–}334}$ (Supplementary Table 3). The unexpanded (short) alleles of patients range from 19 to 41 repeat units (Supplementary Figure 2).

None of the 208 controls had this pentanucleotide repeat expansion. The distribution of the number of TTTTA repeat units among all 208 controls ranges between 16 and 22 repeat units. The highest number of repeats observed is 20 repeat units (Supplementary Figure 3).

### The TTTTA repeat expansions and TTTCA repeat insertions co-segregate with disease status in the family

RP-PCR detected the presence of the TTTTA and TTTCA repeats in intron 4 of *RAI1* in 10 affected family members and in none of 17 unaffected family members tested (Figure 2B and Supplementary Figure 4). Among the 200 Malian controls, no TTTCA repeat insertions were found, while eight controls showed TTTTA repeat expansions.

### LRS in the remaining eight affected members

DNA samples from the remaining eight affected members (III-5, III-12, III-21, III-23, IV-3, IV-12, IV-37, IV-42) were used for PacBio HiFi whole genome long-read sequencing and analyzed using tandem genotypes software. The total outputs for each sample ranged from 17.5 to 71.8 Gb, with a mean of 33.8 Gb. Reads with repeats were found in six of eight affected members (III-5, III-12, III-21, III-23, IV-12, IV-37) (Supplementary Table 3). While

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RP-PCR showed the patterns of TTTTA and TTTCA repeat expansions, LRS of IV-3 and IV-42 did not identify any reads with repeat expansion. The total number of reads was 16 and the median (25th–75th percentile) numbers of repeat units for TTTTA and TTTCA were 636.5 (539.25–700) and 19.5 (14.25–70.75), respectively. We identified the shortest TTTCA repeat units (nine repeats) in one read of III-14.

### Reverse transcription – PCR (RT-PCR)

The RT-PCR experiment did not show any difference in the band size between six affected family members, six unaffected family members and three Malian controls (Figure 3E). Furthermore, from whole genome sequencing, four out of six patients (III-7, IV-3, IV-37, IV-42) were heterozygous for C/T at the rs3818717; and the electropherograms of their complementary DNA (cDNA) showed both C and T (Figure 3F), indicating that *RAI1* was expressed from both alleles.

### Quantitative expression analysis

Real-time quantitative PCR (RT-qPCR) studies were carried out using the TaqMan assay for the *RAI1* exon 2–3 and 3–4 junctions, showing no significant difference in transcript levels among six affected individuals (III-7, III-21, III-23, IV-3, IV-37, IV-42), six unaffected (III-6, IV-7, IV-15, IV-17, IV-20, IV-22), and three Malian healthy controls (Figures 3A and 3B).

### Identification of two single gene disorders in two family members by LRS

Since two members (Patients IV-37 and IV-42) had peripheral neuropathy symptoms and electro-physiological patterns consistent with axonal sensory and motor neuropathy which were not parts of BAFME, we sought for other pathogenic variants. HiFi ccs reads were called using DeepVariant.[18] Due to the history of consanguineous marriage, we sought homozygous variants and found that both were homozygous for a one base-pair substitution, c.1A>G (GRCh38 chr1:2412502) in *PEX10* (NM_153818.2), leading to initiator codon loss (p.Met1?). Biallelic pathogenic variants in *PEX10* are known to cause peroxisome biogenesis disorders.[19] LRS revealed that the variant co-segregated with the disease status in this family (Supplementary Table 4).

## Discussion

In this study, we established BAFME type 8 as a new disease entity and successfully identified the 7$^{th}$ BAFME gene[3–6] in a Malian BAFME family. While other BAFME types have been found in Asian or Caucasian families, this represents the first African family with BAFME. The repeat expansions of BAFME8 are in intron 4 of the *RAI1* gene and co-segregate with disease status in this large family with 10 affected members.

Patients with BAFME8 have symptoms similar to those of other types of BAFME. In addition, seven affected members of the Malian family had distal sensory loss, a symptom not previously reported in other BAFME types. Whether this symptom is a part of the BAFME spectrum or due to nutritional deficiency or other causes requires further study.

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Several mechanisms associated with BAFME have been suggested. First, RNA toxicity could occur when the causative repeats form RNA foci, consisting of UUUCA repeats, in the nuclei of cortical neurons and cerebellar Purkinje cells; these were found in BAFME1 patients' autopsied brains.[1,3] Alternatively, haploinsufficiency could occur for the gene that contains the repeats. Finally, repeat-associated non-AUG (RAN) could disrupt normal function; RAN is the non-canonical protein synthetic process wherein peptide synthesis begins at the site of the expanded repeats in the absence of an AUG codon. Notably, none of the pentanucleotide repeat expansions in previously reported BAFME types has been found in a gene whose haploinsufficiency causes a single gene disorder. Whether only one or several combined mechanisms are involved in the pathogenesis of BAFME remains to be determined.

The causative variants in BAFME8 show the same pattern as those underlying the other six types of autosomal dominant BAFME, i.e., TTTTA repeat expansions and TTTCA repeat insertions in an intron. However, the pentanucleotide expansions observed in BAFME8 are unique in that they occur in a gene (*RAI1*) in which haploinsufficiency is known to cause a monogenic disease (Smith-Magenis Syndrome, or SMS). *Retinoic Acid-Induced 1* (*RAI1*), located on chromosome 17p11.2, is a circadian regulator that modulates *CLOCK* gene expression,[20] SMS is a genetic disorder characterized by distinctive physical features including developmental delay, cognitive impairment, and typical behavioral disorder.[21] Notably, none of our 10 patients exhibited symptoms of SMS. In addition, RNA studies using leukocytes demonstrated no significant difference in transcript levels between six affected, six unaffected, and three healthy Malian controls. These findings suggest that haploinsufficiency of host genes is unlikely to be the pathomechanism of BAFME. Other possible mechanisms include RNA toxicity and non-AUG start sites.[22]

We demonstrated that the *RAI1* repeat expansions in blood cells are somatically unstable, with the number of repeats varying extensively in the same patients (Supplementary Table 3); this is consistent with a previous observation in a family with BAFME3.[5] However, our contention is limited by the use of only 1–2 flow cells for HiFi sequencing, which may yield only a small number of reads with the repeats. In addition, whether a new configuration ($TTTTA_{exp}GGGGT_{ins}GGGAT_{ins}TTTCA_{ins}$) found in one read of III-23 (Supplementary Figure 1) plays any role in the disease pathogenesis needs further studies.

The TTTTA repeats in BAFME8 (chr17:17,808,359–17,808,460 GRCh38) are located near AluJB (chr17:17,808,467–17,808,738), with only 7 base pairs separating them. This is consistent with previous studies that have shown TTTTA repeats to be typically adjacent to one or more Alu repeats.[23]

In one of two reads of III-14, only nine TTTCA repeat units were detected. This is the smallest number ever reported; the previously reported shortest repeat unit was 14.[24] TTTCA repeat units of another read numbered 46. Whether the insertion of nine TTTCA repeat units is pathogenic requires further investigation.

Interestingly, Patients IV-37 and IV-42 had both BAFME and clinical and electrophysiological features of axonal sensorimotor neuropathy. In addition to the repeat

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expansions in *RAI1*, they also had a homozygous one base-pair substitution, c.1A>G, in *PEX10* leading to initiator codon loss (p.Met1?). The c.1A>G variant was previously identified in a patient with mild Zellweger spectrum disorder who was compound heterozygous for c.1A>G and c.199C>T.[25] Therefore, the neuropathy symptoms represent another entity caused by a homozygous variant in a different gene rather than being a phenotypic extension of BAFME.

In summary, we have identified a new disease, BAFME8, in an African family that results from a pentanucleotide TTTTA repeat expansion followed by a TTTCA repeat insertion in intron 4 of the *RAI1* gene, expanding the genetic spectrum of BAFME. The fact that none of the patients had symptoms of SMS and all had the normal *RAI1* RNA levels makes haploinsufficiency of the host gene unlikely to be the pathomechanism. Moreover, HiFi sequencing of all affected members identified the shortest TTTCA repeat units, a new repeat configuration, and the expanding behavior of the repeats when passed to next generations.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data Availability Statement

All datasets generated during the current study are not publicly available but are available from the corresponding author on reasonable request.

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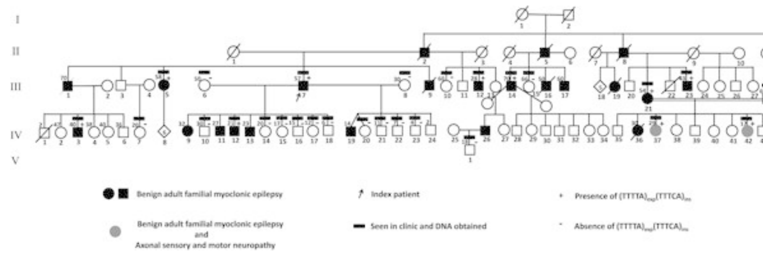


**Figure 1.**

A large Malian pedigree with 10 affected and 17 unaffected family members, consistent with an autosomal dominant inheritance. Affected members are represented by black symbols. A square represents a male, a circle represents a female, and a diamond represents an individual with unspecified or unknown sex.

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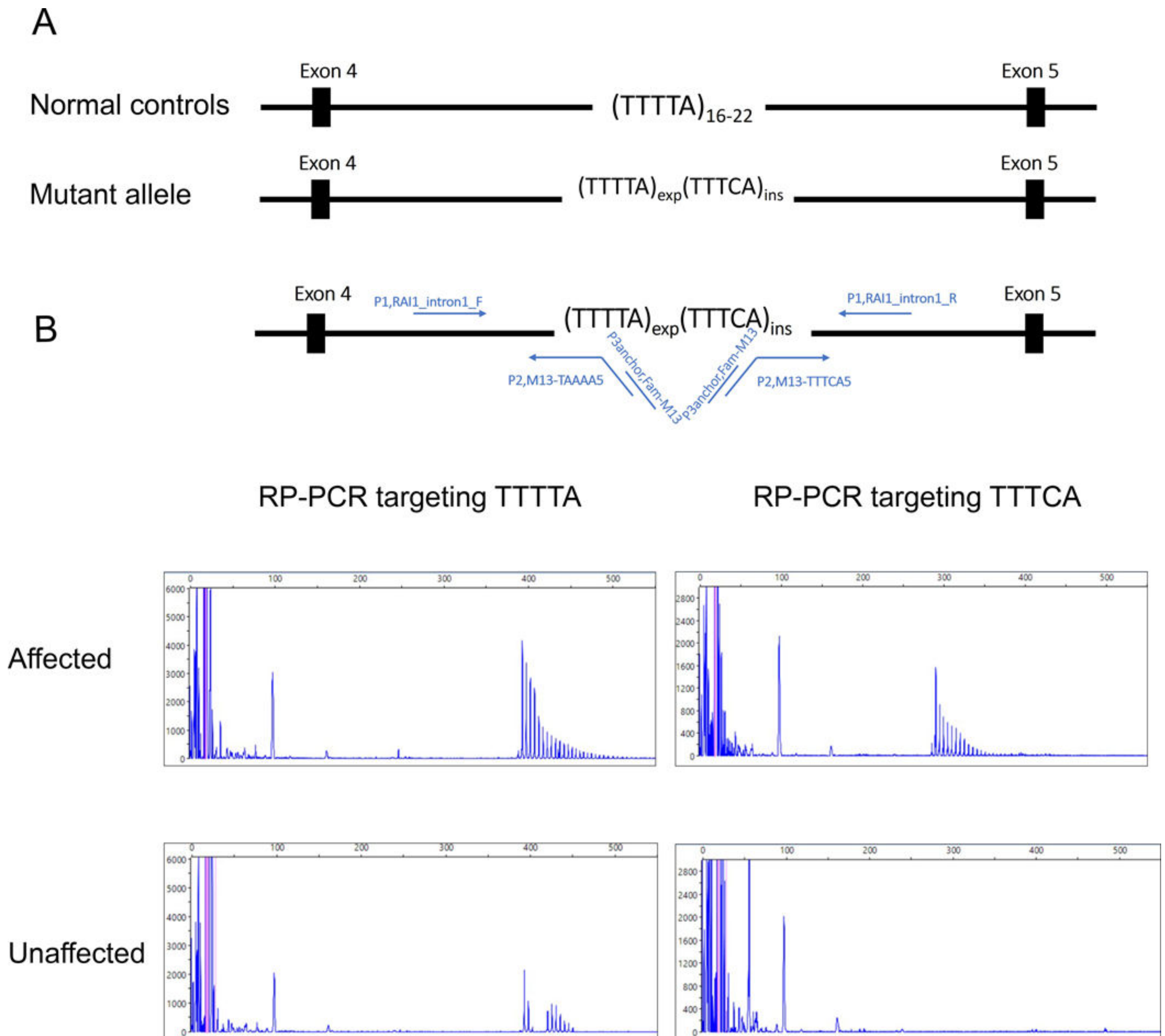


**Figure 2.**

Identification of disease-causing variant. **(A)** A schematic representation of exon 4, intron 4 and exon 5 of the *RAI1* gene. The mutant allele has TTTTA repeat expansions and TTTCA repeat insertions. **(B)** RP-PCR for TTTTA repeat expansions and TTTCA repeat insertions. The primer sets P1-F, P2-TAAAA5 and P3anchor were designed to amplify the TTTTA repeats, whereas P2-TTTCA5, P1-R and P3anchor targeted the TTTCA repeats. The RP-PCR results showed that the affected individual had TTTTA repeat expansions and TTTCA repeat insertions, which were not found in unaffected individuals.

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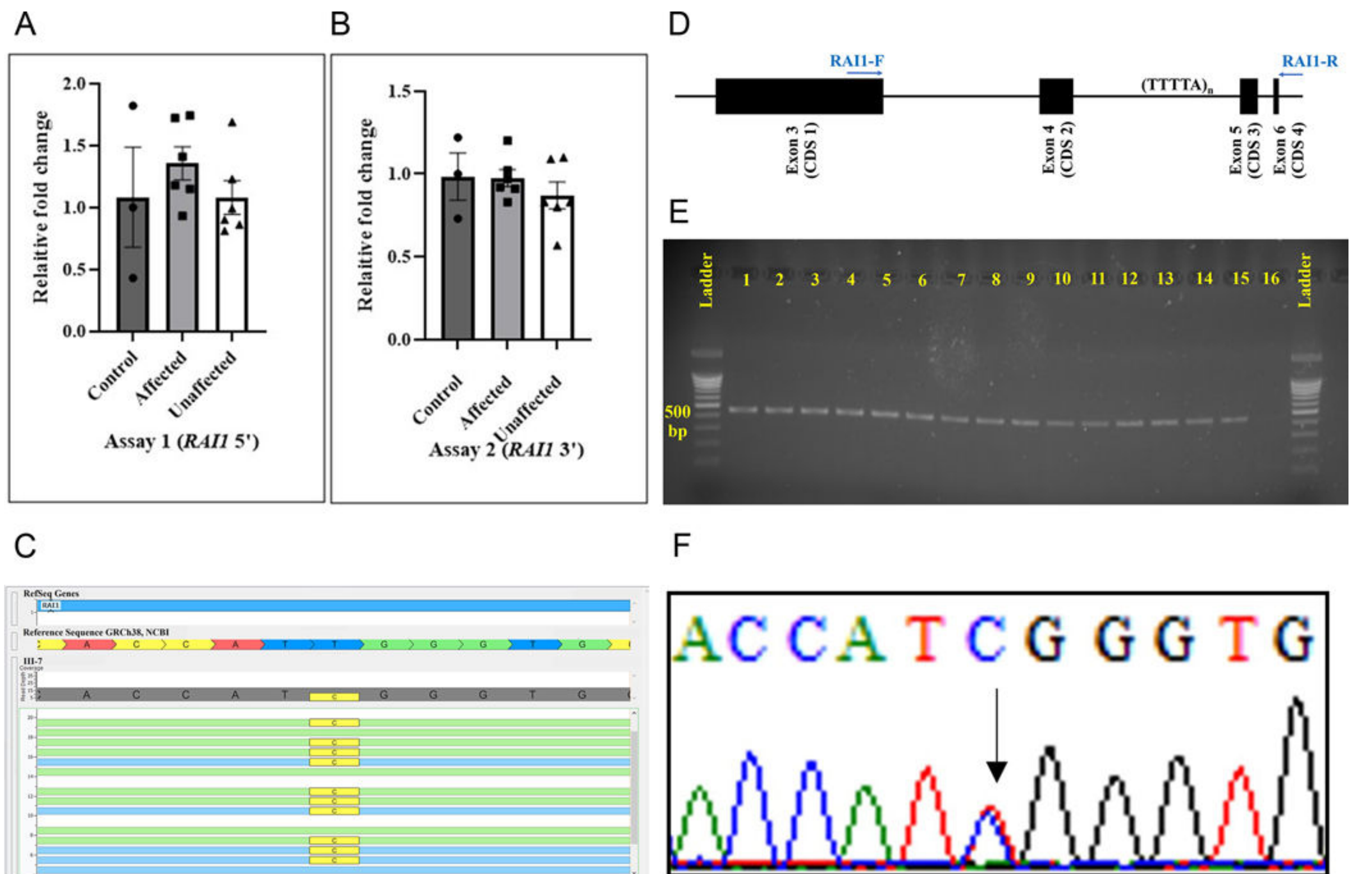


**Figure 3.**

*RAI1* RNA studies. **(A-B)** Lymphoblastoid cells from six affected family members were analyzed by qRT-PCR to compare mRNA expression with that of six unaffected family members and three Malian unaffected controls. Two independent *RAI1* Taqman probes were used for RNA expression (Assay 1- Hs00430773_m1; Assay 2- Hs01554690_m1). The Y-axis shows the relative fold change of *RAI1* expression in affected, unaffected, and controls, normalized to Beta Actin (*ACTB*). Error bars indicate ±1SD. No significant difference in mRNA expression was found between the affected and unaffected family members and the Malian controls. **(C)** The BAM file for patient III-7 exhibited heterozygosity for the rs3818717 (C/T) variant, which was utilized for designing primers for the reverse transcription – PCR (RT-PCR). **(D)** Forward (RAI1-F) and reverse (RAI1-R) primers were designed between exons 3 and 6. **(E)** Agarose gel electrophoresis was performed on PCR products from six affected family members (Lane 3=III-7, Lane 4=III-21, Lane 5=III-23, Lane 6=IV-42, Lane 7=IV-3 and Lane 9=IV-37), six unaffected family members (Lane 8=IV-7, Lane 11=III-6, Lane 12=IV-15, Lane 13=IV-22, Lane 14=IV-17 and Lane 15=IV-20), three Malian controls (Lane 1=C1 and Lane 2=C2, Lane 10=C3) and negative control (Lane 16). No differences in band size were observed among the samples. **(F)** The electropherogram of the cDNA of patient III-7 showed heterozygosity for the rs3818717 (C/T). Four of six patients (III-7, IV-3, IV-37, IV-42) had the rs3818717 variant, confirming that the single band on the gel in Figure 3E represented the products of two alleles.

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## Table 1:

Phenotypic and genetic findings in patients with BAFME8

| Patients | Clinical examination findings | | | | | | | | | | | | | Laboratory findings | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age (yr) | Sex | Age of onset (yr) | First symptom | Tremor | Myoclonic jerks | Dysarthria | Coordination | Tendon reflexes | Nystagmus | GTCS | Distal sensory loss | Skeletal deformity | EEG | *RAI* variant |
| III-5 | 58 | F | 48 | Myoclonic jerks | None | Yes | None | Impaired | Brisk | No | Yes | Yes | None | Abnormal | $TTTTA_n$; $TTTCA_n$ |
| III-7 | 57 | M | 45 | Tremor | Yes | Yes | Yes | Impaired | Brisk | Yes | None | Yes | None | Abnormal | |
| III-12 | 28 | M | 27 | Tremor | Yes | None | None | Normal | Brisk | No | None | None | None | Normal | |
| III-14 | 70 | M | 68 | Tremor | Yes | None | Yes | Impaired | Normal | Yes | None | None | None | Abnormal | |
| III-21 | 54 | F | 44 | GTCS | Yes | Yes | Yes | Normal | Normal | Yes | Yes | Yes | None | Normal | |
| III-23 | 43 | M | 18 | Tremor | Yes | Yes | Yes | Impaired | Brisk | Yes | None | Yes | None | Abnormal | |
| IV-3 | 40 | M | 35 | Tremor | Yes | Yes | Yes | Impaired | Brisk | Yes | None | Yes | None | Normal | |
| IV-12 | 21 | M | 7 | Tremor | Yes | Yes | No | Normal | Normal | No | None | None | None | NR | |
| IV-37 | 29 | F | 17 | Unbalance | Yes | None | Yes | Impaired | Reduced/ Absent | Yes | None | Yes | Yes | Normal | |
| IV-42 | 17 | F | 7 | Tremor | Yes | None | None | Impaired | Reduced/ Absent | *Yes* | None | Yes | Yes | Abnormal | |

NR: not recorded at patient request; GTCS: generalized tonic clinic seizures.