Title

DNA analysis of benign adult familial myoclonic epilepsy reveals associations between the pathogenic TTTCA repeat insertion in *SAMD12* and the non-pathogenic TTTTA repeat expansion in *TNRC6A*

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Conflict of Interest Statement

The authors have nothing to disclose.

Abstract

Benign adult familial myoclonic epilepsy (BAFME) is an autosomal dominant disease characterized by adult-onset tremulous hand movement, myoclonus, and infrequent epileptic seizures. Recently, intronic expansion of unstable TTTCA/TTTTA pentanucleotide repeats in *SAMD12*, *TNRC6A,* or *RAPGEF2* were identified as pathological mutations in Japanese BAFME pedigrees. To confirm these mutations, we performed a genetic analysis on 12 Japanese BAFME pedigrees. A total of 143 participants, including 43 familial patients, five suspected patients, three sporadic non-familial patients, 22 unaffected familial members, and 70 unrelated controls, were screened for expanded abnormal pentanucleotide repeats in *SAMD12, TNRC6A, RAPGEF2, YEAT2, MARCH6,* and *STARD7*. DNA samples were analyzed using Southern blotting, long-range polymerase chain reaction (PCR), repeat-primed PCR and long-range PCR followed by Southern blotting. Of the 51 individuals with clinically diagnosed or suspected BAFME, 49 carried a *SAMD12* allele with an expanded TTTCA/TTTTA pentanucleotide repeat. Genetic and clinical anticipation was observed. As in previous reports, the one patient with homozygous mutant alleles showed more

severe symptoms than the heterozygous carriers. In addition, screening for expanded pentanucleotide repeats in *TNRC6A* revealed that the frequency of expanded TTTTA repeat alleles in the BAFME group was significantly higher than in the control group. All patients who were clinically diagnosed with BAFME, including those in the original family reported by Yasuda, carried abnormally expanded TTTCA/TTTTA repeat alleles of *SAMD12*. Patients with BAFME also frequently carried a TTTTA repeat expansion in *TNRC6A*, suggesting that there may be unknown factors in the ancestry of patients with BAFME that make pentanucleotide repeats unstable.

Keywords: TTTCA/TTTTA pentanucleotide repeats, *SAMD12*, anticipation, *TNRC6A*

1 INTRODUCTION

Benign adult familial myoclonic epilepsy (BAFME) is a rare autosomal dominant disorder proposed by Yasuda in 1991 (1). BAFME is characterized by adult onset hand tremor, myoclonus, and rare seizures, and has been described as a non-progressive course without cerebellar ataxia or dementia. Historically, various names have been given to the disease, including BAFME, hereditary tremor with epileptiform seizures (2), heredofamilial tremor and epilepsy (3), cortical tremor (4), familial essential myoclonus and epilepsy (5), familial adult myoclonic epilepsy (FAME) (6), familial benign myoclonus epilepsy of adult onset (7), familial cortical tremor with epilepsy (8), autosomal dominant cortical myoclonus and epilepsy, and familial cortical myoclonic tremor with epilepsy (FCMTE) (9).

In 1999, we performed a linkage analysis using Yasuda's pedigree, and identified a significant linkage on chromosome 8q23.3-q24.11, within 8 cM (10). In 2011, we reconfirmed the BAFME-linked region and performed fine mapping of the BAFME locus (11). As a result, the BAFME-linked region was found to be within an approximately 7.16 Mb span on chromosome 8q23.3-q24.13. However, no causative

92 mutation could be identified in the BAFME-linked region. At that time, we concluded
93 that causative mutations for BAFME might exist in the noncoding regions, such as
94 introns and intergenic regions.
95 As we predicted, the causative mutations for BAFME were located in intronic
96 regions. Recently, the expanded TTTCA/TTTTA pentanucleotide repeats in *SAMD12*
97 (8q24.11-q24.12), *TNRC6A* (16p12.1), *RAPGEF2* (4q32.1), *YEAT2* (3q27.1), and
98 *MARCH6* (5p15.2), and the ATTTC pentanucleotide repeats in *STARD7* (2q11.2) were
99 reported as pathological mutations in BAFME (12–15). The pathological mutation in
100 *SAMD12* causes RNA foci, including UUUCA repeats, in the brain in particular.
101 Anticipation was also reported for *SAMD12*, *TNRC6A*, *RAPGEF2*, and *STARD7*.
102 In the present study, we therefore analyzed the number of these pentanucleotide
103 repeats in affected and nonaffected individuals from 12 Japanese BAFME families,
104 including the largest BAFME family in the world, which was initially reported by
105 Yasuda (1). In some cases, the amount of genomic DNA (gDNA) was low, and some
106 samples had deteriorated in quality due to long-term storage, making it difficult to
107 determine the repeat length. Various analysis methods were therefore considered, and

one combining long-range polymerase chain reaction (long-range PCR), repeat-primed PCR (RP-PCR), and long-range PCR followed by Southern blotting (PCR-Southern blotting) was selected, because it could be performed with a relatively small amount of gDNA and facilitated detection more effectively than conventional Southern blotting. In addition, we investigated the relationship between the expansion of pentanucleotide repeats and phenotypic variation in BAFME.

2 METHODS

2.1 Standard protocol, approvals, registrations, and patient consent

All participants gave written informed consent. The research protocol and consent form were approved by the relevant institutional review boards of Kagoshima University.

2.2 Diagnosis

Yasuda reported that BAFME was characterized by the following features: (i) autosomal dominant inheritance; (ii) tremulous finger movement or myoclonus of the extremities after adolescence; (iii) infrequent epileptic seizures; (iv) polyspike and wave

abnormalities on examination by electroencephalogram (EEG) and marked photosensitivity; (v) enlarged cortical components of somatosensory evoked potential (SEP); (vi) enhanced long-loop reflex (C-reflex); (vii) positive spikes preceding myoclonus ascertained using the jerk-locked averaging method; and (viii) a benign non-progressive course without cerebellar ataxia or dementia (1,10). Kobayashi *et al.* recently presented diagnostic criteria (16), which were generally consistent with the features proposed by Yasuda. Among the clinical features proposed by Yasuda, we clinically diagnosed individuals who showed (ii), (iii), and/or (viii) as BAFME, and together, they strongly suggested (i). For patients where the electrophysiological information for features (iv) to (vii) could be obtained, a clinical diagnosis of BAFME was confirmed. In the pedigrees of BAFME patients with a clinical diagnosis, the five patients with clinically suspected BAFME included three asymptomatic individuals (III-11 of pedigree 4, IV-3 of pedigree 6, and II-2 of pedigree 7) who only showed abnormalities after examination by EEG; another individual (II-9 of pedigree 10) with tremor only when fatigued; and the other individual (II-11 of pedigree 10) with temporary tremor in the past.

140

141 **2.3 Participants**

142 We enrolled 100 healthy Japanese controls who were unrelated to the known BAFME
143 families, three Japanese patients who had been clinically diagnosed with BAFME (two
144 males and one female) but were also unrelated to the known BAFME families, and
145 members of 12 Japanese pedigrees, including 43 individuals with clinically diagnosed
146 BAFME (21 males and 22 females), five individuals with clinically suspected BAFME,
147 and 22 nonaffected individuals (Figure 1, Table S1). For pedigree 9, the family tree was
148 unknown. One of the pedigrees, the family reported by Yasuda, whose BAFME genetic
149 linkage was analyzed by Mikami *et al.* (10) and Mori *et al*. (11), included 16 individuals
150 with clinically diagnosed BAFME (nine males and seven females) and 11 nonaffected
151 family members (six males and five females) (Figure 1, pedigree 1).

152

153 **2.4 DNA analysis**

154 The gDNA was extracted from peripheral leukocytes using standard methods. We
155 analyzed a total of 143 participants, including 43 familial patients, five BAFME

suspected patients, three sporadic non-familial patients, 22 unaffected familial members, and 70 unrelated controls, using PCR, RP-PCR, and fragment analysis. Long-range PCR targeting *SAMD12* was performed for all affected individuals. For PCR, RP-PCR, and long-range PCR to detect the abnormally expanded TTTCA/TTTTA repeats in *SAMD12, TNRC6A, RAPGEF2, YEATS2*, and *MARCH6*, and the ATTTC repeats in the *STARD7* locus, we used the same methodology as employed in previous studies (12–15,17). The fragment analysis was conducted using an ABIPRISM 3130 Avant Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). For three family members from pedigree 5, the long-range PCR for *SAMD12* was performed with 5–20 ng gDNA and primers 5′-CTTGAGCCCCAGACAAGAAT and 5′-TGCTACTGTAAAAAGATAAACAAAATG, because the reported primers did not work. After 1 min at 98 °C, DNA samples underwent 30 cycles (98 °C for 10 s, 60 °C for 15 s, and 68 °C for 10 min). Long-range PCR for *TNRC6A* was performed with specific primer pairs (Table S2). All long-range PCR products were separated by electrophoresis in a 0.8% agarose gel.

The gDNA from some patients was not fragmented, and this was analyzed via Southern blotting analysis as described by Ishiura *et al*. (12). The probes were detected using LAS-1000 (Fujifilm, Tokyo, Japan) or FUSION-SOLO.7S.WL (Vilber Lourmat, Marne-la-Vallée, France) imaging systems.

To detect the single nucleotide polymorphisms (SNPs) showing the founder effect in previous reports (12,17) in 27 representatives of each family and to evaluate the CAG repeat region of *TNRC6A* for patients with an expanded TTTTA repeat in *TNRC6A*, PCR was performed using standard methods with specific primers (Table S2).

2.5 Statistical analysis

A paired *t*-test was used to compare the abnormally expanded TTTCA/TTTTA repeat lengths of the cases in the parent and offspring generations. We calculated Pearson's correlation coefficient for repeat length and age of onset; of the mother's age at childbirth and the mother–offspring differences in repeat length; of parent–offspring differences in repeat lengths and parent–offspring differences in age of onset; and of parent–offspring differences in age of onset, as well as two-tailed *P* values. Fisher's

exact test was used to compare the number of patients with an expanded pentanucleotide repeat allele in *TNRC6A* between affected and non-affected individuals. *P* values of < 0.05 were considered to be significant.

Analyses of relationships between repeat length and symptoms were performed only on individuals with sufficient information. Individuals without DNA samples but with information on the age of onset were included in the data analysis for age of onset.

3 RESULTS

3.1 Diagnosis

A total of 51 individuals were examined, comprising 46 clinically diagnosed with BAFME and five suspected BAFME cases. Typical RP-PCR and Southern blot analysis results are shown in Figure 2.

In *SAMD12*, the long-range PCR and Southern blot analysis, together with the RP-PCR results, revealed that the abnormally expanded TTTCA/TTTTA alleles were heterozygously or homozygously present in 45 and one, respectively, of the patients who had been clinically diagnosed with BAFME, including sporadic three patients with

BAFME. In addition, in the 90 nonaffected individuals, including 22 nonaffected BAFME family members, no abnormally expanded TTTCA/TTTTA alleles were detected. Four nonaffected subjects carried an expanded TTTTA repeat (> 100) allele, but they had no TTTCA pentanucleotides. Based on these results, the insertion of TTTCA pentanucleotide repeats completely co-segregated with clinically diagnosed BAFME patients. The expansion of pathological pentanucleotide repeats in *TNRC6A*, *RAPGEF2, YEATS2, MARCH6,* and *STARD7* were not observed in patients with *SAMD12* mutations. Three of the five individuals suspected to have BAFME were heterozygous carriers of the abnormally expanded TTTCA/TTTTA allele of *SAMD12* and the other two (III-11 in pedigree 4 and IV-3 in pedigree 6) did not. Therefore, for these two individuals, the same analysis was performed on *TNRC6A*, *RAPGEF2*, *YEATS2, MARCH6,* and *STARD7,* but neither individual carried expanded alleles. We confirmed the molecular diagnosis of BAFME in the 16 affected individuals from the Yasuda's family when the clinical definition of BAFME was proposed (Figure 3).

3.2 Repeat length of *SAMD12* in normal and BAFME subjects

219 The distribution of expanded TTTCA/TTTTA repeat lengths in *SAMD12* is shown in
220 Figure 2C. The abnormally expanded TTTCA/TTTTA alleles gave 47 discrete long-
221 range PCR products with a range of 516 (3.39 kbp) to 2363 (12.63 kbp) repeats and a
222 median of 1146 repeats (6.54 kbp) (mean ± SD = 1250 ± 462.2 repeats, 7.09 ± 2.29
223 kbp). The long-range PCR and RP-PCR revealed that the size of the TTTTA repeats in
224 the 186 alleles from controls ranged from 14 to 1050 repeats. Two individuals were not
225 included in the above analysis: the gDNA from individual III-7 in pedigree 1 was of
226 insufficient quality for long-range PCR, and individual II-6 in pedigree 12 carried
227 homozygous mutations.
228
229 **3.3 Repeat lengths within *SAMD12* and age of onset**
230 Figure 4 shows the relationship between the abnormally expanded TTTCA/TTTTA
231 repeat length of *SAMD12* and patient age at the time of symptom onset (excluding the
232 one patient with homozygous abnormally expanded TTTCA/TTTTA repeat alleles). Our
233 analysis included only patients with this data available. The age of onset of myoclonic
234 tremor was 30.63 ± 11.53 (10–56) and that of epilepsy was 36.16 ± 11.43 (20–58). We

found a significant negative correlation between the length of TTTCA/TTTTA repeats and age of onset of myoclonic tremor (n = 27, r = −0.46, $P = 0.017$) and of either myoclonic tremor or epilepsy (n = 31, r = −0.52, $P = 0.003$). A moderate correlation between the repeat length and age of onset of epilepsy (n = 25, r = −0.39, $P = 0.054$) was also found (Figure 4A–C).

3.4 Parent–offspring differences in repeat lengths within *SAMD12*

To evaluate the instability of TTTCA/TTTTA repeat lengths in the BAFME alleles by generation, we studied the change in repeat length in 20 parent–offspring pairs. A change in repeat length was found in all pairs, indicating that the TTTCA/TTTTA repeat is remarkably unstable. The 20 parent–offspring transmissions yielded 19 increases in length and one decrease, resulting in an average change of 0.97 kbp (range, −0.88 to 3.08 kbp, $t_0 = 4.65$, $P < 0.001$) (Figure 5A). A previous study reported that expansions tended to be larger in maternal transmissions than in paternal transmissions (12), but our study could not form this conclusion due to the small sample size of paternal transmissions (only three pairs).

We found a moderate positive correlation between the mother's age at childbirth and mother–offspring differences in abnormally expanded TTTCA/TTTTA repeats (n = 10, r = 0.48, $P = 0.16$) (Figure 4D).

3.5 Relationship between parent–offspring differences in repeat lengths within *SAMD12* and differences in age of onset

To clarify the genetic and clinical anticipation, we analyzed relationship between parent-offspring differences in repeat lengths within *SAMD12* and differences in age of onset. Of the 15 parent–offspring pairs with a definite age of onset of myoclonic tremor, the onset of symptoms in the child generation of 14 pairs occurred significantly earlier than in the parent generation (seven paternal and seven maternal transmissions) ($t_0 = 3.6$, $P = 0.003$) (Figure 5B). Eight pairs (seven maternal and one paternal) out of these could be genetically analyzed and the results revealed that increased TTTCA/TTTTA repeat lengths in the parent–offspring transmissions were associated with an earlier age of onset in the offspring. Although the offspring in four maternally transmitted cases (III-10 and III-15 in pedigree 1, III-2 in pedigree 2, and III-3 in pedigree 11) who

showed increases in TTTCA/TTTTA repeats (increases of 165, 85, 143, and 479 repeats, respectively) had no myoclonic tremor at sampling, they had not yet reached their parent's age of onset. Therefore, we could not analyze these four parent–offspring pairs. In addition, because of absence of samples, we could not include three other pairs.

All offspring with maternally transmitted BAFME (n = 7) had earlier myoclonic tremor onset than did their mothers (average, 19.4 years earlier; range, 3–41 years). Moderate correlations between differences in the repeat length and parent–offspring differences in age of onset of myoclonic tremor (n = 8, r = 0.64, $P = 0.089$), epilepsy (n = 6, r = 0.65, $P = 0.16$) and either myoclonic tremor or epilepsy (n = 9, r = 0.62, $P = 0.075$) were found. Both the age of onset of epilepsy and the age of onset of either myoclonic tremor or epilepsy was significantly younger in the offspring than in their mothers (n = 10, $t_0 = 2.8$, $P = 0.019$, and n = 16, $t_0 = 4.1$, $P < 0.001$, respectively) (Figure 5C, D).

3.6 Genetic diagnostic analysis of Yasuda's family

We performed a genetic diagnostic analysis of 27 members (16 affected and 11 nonaffected) of the family identified by Yasuda for whom gDNA was available (pedigree 1 in Figure 1; Figure 3). Except for individual III-7, long-range PCR or Southern blotting were available for the diagnosis of BAFME. Due to poor quality and small quantities of gDNA, only RP-PCR results were available for the diagnosis of III-7. All patients in the family who were clinically diagnosed as having BAFME carried the abnormally expanded TTTCA/TTTTA repeat allele of *SAMD12*. In addition, we found longer repeat alleles in all offspring-generation samples than in those from all parent generations (n = 8, average ± SD = +0.62 ± 0.18 kbp). The paternal and maternal differences in repeat length were 0.35 and 0.56 kbp (n = 2), and 0.43–0.82 kbp (n = 6, average ± SD = +0.67 ± 0.16), respectively. For the age of onset of myoclonic tremor, clinical anticipation was observed in all parent–offspring transmissions. Regarding the age of onset of epilepsy, except for the II-6–III-4 transmission, clinical anticipation was observed in all parent–offspring transmissions. The paternal and maternal differences in the age of onset were −4 (n = 1) and −27 to −3 years (n = 4, average ± SD = −14.5 ± 10.62), respectively.

3.7 Genetic diagnostic analysis of the homozygous patient

We identified one patient, II-6 from pedigree 12, with homozygous expansion alleles. She developed an epileptic seizure at the age of 24 and presented with myoclonic tremor in all four limbs, refractory epilepsy, progressive cognitive decline, and cerebellar ataxia. In addition to the progression of cerebellar ataxia, she developed gait disturbance at 57 years of age because the myoclonic tremor spread throughout her legs. Electrophysiological tests revealed giant somatosensory evoked potential (gSEP) and C-reflex. Although a molecular diagnosis was not performed, other family members, including her parents, showed the typical symptoms of BAFME. Only II-6 showed symptoms as severe as progressive myoclonus epilepsy. The repeat length of II-6 was 5.77 kbp (992 repeats), not much different from other patients with heterozygous BAFME mutations. Nevertheless, she had a more severe clinical presentation than patients with heterozygous BAFME mutations.

3.8 Expansion of the TTTTA repeat in *TNRC6A*

There was a significant difference in the frequency of TTTTA repeat expansion in the *TNRC6A* gene between in BAFME patients and in controls ($p = 0.0009$, Fisher's exact test). We found that 15 unrelated affected individuals carried five (16.7%) expanded TTTTA repeat alleles of *TNRC6A* out of 30 alleles. On the other hand, a total of 107 nonaffected individuals, including seven unrelated nonaffected family members and 100 healthy controls, showed that only three (1.4%) expanded TTTTA repeat alleles of *TNRC6A* out of the 214 alleles. However, the TTTTA repeat expansion of *TNRC6A* and TTTCA repeat expansion of *SAMD12* were not completely linked in some parent–offspring pairs (II-4–III-3 and II-24–III-14 in pedigree 1, II-2–III-1 in pedigree 7, and I-8–II-9 and –II-10 in Figure 1). The insertion of a TTTCA repeat was not observed in either group. The expanded TTTTA alleles gave nine discrete long-range PCR products with a range of 53 (0.98 kbp) to 605 (3.74 kbp) repeats and a median of 345 repeats (2.44 kbp) (mean ± SD = 358 ± 155.5 repeats, 2.50 ± 0.78 kbp).

3.9 Founder effect

329 The seven SNPs, rs7464659, rs6994270, rs2515029, rs9643124, rs10086119, rs7832475
330 and rs4876828, in previous reports for genetic founder effect analysis were shared
331 among 25 of 27 subjects and pedigrees of the previous reports (Table S3)(12,17). The
332 other two subjects possibly shared the founder effect, but because they have no other
333 family members, we could not identify heterozygous SNPs. In other words, this
334 indicated a founder effect shared between our pedigrees and pedigrees in previous
335 studies of Japan and China.
336
337 **3.10 CAG triplet repeat in *TNRC6A***
338 *TNRC6A* is an abbreviation for Trinucleotide Repeat Containing Adapter 6A, which has
339 a CAG triplet repeat in an exon. We focused on CAG repeat in the *TNRC6A* as a genetic
340 modifier of the BAFME symptoms. We performed Sanger sequencing to detect
341 expansion of the triplet repeat for 47 patients with BAFME who carried a
342 pentanucleotide repeat expansion in *SAMD12*. Individuals III-6 in pedigree 1 and II-3 in
343 pedigree 4 could not be analyzed due to low quality gDNA for PCR analysis. No

patients showed an expansion of the CAG repeat. A correlation in the instability between intronic pentanucleotide repeats and exonic triplet repeats could not be shown.

4 DISCUSSION

Our analysis of a specific TTTCA/TTTTA repeat sequence in the BAFME gene revealed that patients in the offspring generation had significantly larger expansions than their parents. In addition, a significant negative correlation was found to exist between repeat length and age of onset. These findings suggest that progressive increases in the TTTCA/TTTTA repeat length in successive generations provide a molecular explanation for the anticipation observed in BAFME. Our finding of anticipation corresponds with the results previously reported by Ishiura *et al*. (12) and others (17–19), and enabled us to make an accurate diagnosis and genetic prediction for many of the family members at risk: two family members (II-9 and II-11 in pedigree 10 in Figure 1) who were asymptomatic at sampling but were found to carry BAFME alleles do now exhibit BAFME symptoms. Taken together with previous reports, our results both support the hypothesis that the TTTCA/TTTTA repeat expansion is directly

involved in the pathogenesis of BAFME, and also indicate that the phenotypic variation of BAFME depends on the TTTCA/TTTTA repeat length in the BAFME loci. Ishiura *et al.* reported that expansions tended to be larger after maternal transmission than after paternal transmission (12). In this study, however, we found that a moderate correlation existed between the parent–offspring repeat length difference and the mother's age when the child was born (Figure 4D). This indicates that the repeat expansion in maternal transmission includes a more complex mechanism involving the senescence of primary oocytes.

In the present study, only the parent–offspring generation in pedigree 7 showed a greater length of the abnormally expanded TTTCA/TTTTA repeat allele in the mother (II-2) than in her daughter (III-1). Strangely, the mother was asymptomatic at the time of sampling, although the daughter had already presented with both epilepsy and myoclonic tremor. In this case, despite the fact that the repeat length had decreased in the course of mother–daughter transmission, there was clinical anticipation. This atypical phenomenon may have been caused by the presence of somatic mosaicism between leukocytes and neurons, or unknown modifiers, although further analysis will

376 be required in order to address this situation. As for the pathogenesis of BAFME,
377 Ishiura *et al*. revealed that RNA-mediated toxicity—in particular, expanded UUUCA
378 repeat-mediated toxicity—is the mechanism underlying the pathogenesis of BAFME via
379 nuclear RNA foci that include the UUUCA repeat (12). Furthermore, the TTTCA
380 pentanucleotide was not detected even in some normal samples that had the abnormal
381 expansion of TTTTA repeats in *SAMD12*, although Cen *et al.* reported that the TTTCA
382 pentanucleotide was always detected in BAFME patients, even though the range of
383 expanded TTTTA repeat in *SAMD12* was 25–44 repeats (17). Therefore, one possible
384 explanation for the atypical relationship between repeat length and phenotype in the
385 mother–daughter pair in pedigree 7 is that the daughter carries a shorter allele with
386 TTTTA repeats but a longer allele with TTTCA repeats, which may result in shorter
387 allele TTTCA/TTTTA repeat lengths than mother.
388 We studied one patient (II-6) from pedigree 12 who carried the abnormally
389 expanded TTTCA/TTTTA repeat alleles homozygously. Other members of pedigree 12
390 who had BAFME showed typical BAFME symptoms, and individual II-6 also showed
391 the typical electrophysiological findings for BAFME (i.e., gSEP and C-reflex).

392 However, she presented with progressive cerebellar ataxia, refractory epilepsy, and
393 progressive cognitive decline, which were resistant to medication. Ishiura *et al.*
394 previously reported similar patients with homozygous BAFME mutant alleles. These
395 findings suggest that patients with homozygous BAFME mutant alleles present
396 progressive myoclonus epilepsy-like symptoms with a gene dosage effect.
397 We initially tried to perform Southern blotting for all patients to evaluate the
398 length of the repeat alleles. However, because of the small quantities of gDNA available
399 or poor quality due to long-term storage, 27 out of 49 samples from suspected and
400 confirmed BAFME patients could not be included in the Southern blotting analysis.
401 Various other analysis methods were therefore considered, and one combining long-
402 range PCR, RP-PCR, and PCR-Southern blotting was selected because it could be
403 performed with a relatively small amount of gDNA and facilitated detection more
404 effectively than conventional Southern blotting. Whereas the Southern blotting required
405 5–10 μg of adequate-quality gDNA, long-range PCR in combination with RP-PCR
406 required only 25–50 ng gDNA to determine the length of the abnormally expanded
407 TTTCA/TTTTA repeats. In addition, PCR-Southern blotting was a useful method for

confirming the existence of TTTCA repeats in the abnormally expanded alleles. For example, it was difficult to confirm whether the inheritance was heterozygous or compound heterozygous in Case 2 in pedigree 9, because the shorter allele, which was observed in long-range PCR, was also expanded relative to that of typical healthy controls (Figure S4). However, the PCR-Southern blotting method, using only 50 ng gDNA, revealed that the shorter allele had no TTTCA repeat insertions, indicating a heterozygous mode of inheritance in this case.

Yasuda originally reported BAFME family (pedigree 1 in Figure 1; Figure 3) in 1991 (1). This family has been used for genetic linkage studies of BAFME (10,11). The abnormally expanded TTTCA/TTTTA repeat alleles in *SAMD12* were previously identified in three members of Yasuda's family (pedigree 1 in Figure 1; patients II-24, III-14, and III-15 in this study) (12). In the present study, we confirmed that all BAFME patients from this family for whom we obtained gDNA also carried the abnormally expanded TTTCA/TTTTA repeat alleles in *SAMD12*, and that the mutation completely co-segregated with BAFME. In two parent–offspring pairs (II-9–III-7 and II-24–III-14 in pedigree 1), the parents exhibited epilepsy, but epilepsy was not observed in their

offspring. However, the ages of onset of epilepsy in the parents were 49 (II-9) and 53 (II-24), and the ages of the offspring at the time of investigation were 30 (III-7) and 25 (III-14) years old, so we suspect that the offspring had not yet reached the age at which symptoms will appear.

Although a significant genetic linkage on chromosome 8 was found in the Yasuda's family in the previous studies (10,11), the other families were possible genetic linkage outside of chromosome 8. In addition, because patients carrying *TNRC6A*, *RAPGEF2*, *YEATS2*, *MARCH6* or *STARD7* mutations presented BAFME similar symptoms, we screened these mutations for possible symptom modifier. Interestingly, we found that the TTTTA repeat expansion of *TNRC6A* occurred with a significantly higher frequency in patients with BAFME who carried an abnormal expanded TTTCA/TTTTA repeat allele of *SAMD12*. This suggests that the Japanese ancestral founder of the affected individuals may have acquired instability in the repeat sequences by some mechanism, leading to simultaneously carrying both expansions. The segregation distortion caused by long repeats of TTTTA in DNA sequences may have led to an uneven distribution of the BAFME mutation and TTTTA repeats, except for in

parent–child transmissions where the BAFME mutation did not co-segregate with the expansion of TTTTA in *TNRC6A* (II-4 with III-3 and II-24 with III-14 in pedigree 1, II-2 with III-1 in pedigree 7, and I-1 with II-1, I-8 with II-9, and I-8 with II-11 in pedigree 10 in Fig.1). There were no obvious differences in clinical symptoms between individuals with BAFME with or without the TTTTA repeat expansion in *TNRC6A*. There were no changes in the repeat sequences within *RAPGEF2*, *YEATS2*, *MARCH6*, and *STARD7* or in the CAG triplet repeat of *TNRC6A*, but there may be abnormalities in other repeat sequences scattered throughout the entire gene. Further research may reveal these mechanisms in the future.

In conclusion, the screening of *SAMD12* for expanded TTTCA/TTTTA pentanucleotide repeats was performed using molecular diagnostic methods, which correctly diagnosed a total of 51 confirmed or suspected BAFME patients. The results showed anticipation at the molecular and clinical levels in parent–offspring transmissions in accordance with previous studies. In particular, we found that the non-pathogenic TTTTA repeat expansion in *TNRC6A* was found with significantly higher frequency in patients with BAFME who carried a pathogenic abnormal expanded

TTTCA/TTTTA repeat allele of *SAMD12*. Further analysis is required to clarify the causes of the overlap of the two kinds of pentanucleotide repeat expansions on different chromosomes and its clinical consequences.

Acknowledgements

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Conflict of Interest Statement

The authors declare no conflict of interest.

Supplementary information is available at *Journal of Human Genetics*'s website.

References

1. Yasuda T. Benign Adult Familial Myoclonic Epilepsy (BAFME). Kawasaki Med J. 1991;17(1–4):1–13.

2. Inoue S. one pedigree of hereditary tremor with epileptiform seizures. seishin-shinkeigaku zasshi. 1951;53:33–7.

3. Kudo J, Kudo T, Yamauchi T. Seven families of heredofamilial tremor with epilepsy. ClinNeurol. 1984;24:1–8.

4. Ikeda A, Kakigi R, Funai N, Neshige R, Kuroda Y, Shibasaki H. Cortical tremor: a variant of cortical reflex myoclonus. Neurology. 1990;40:1561–5.

5. Inazuki G, Naito H, Ohama E, Kawase Y, Honma Y, Tokiguchi S, et al. A Clinical Study and Neuropathological Findings of a Familial Disease with Myoclonus and Epilepsy -The Nosological Place of Familial Essential Myoclonus and Epilepsy (FEME) -. seishin-shinkeigaku zasshi. 1990;92(1):1–21.

6. Uyama E, Tokunaga M, Murakami T, Kuwano A, Kondo I, Uchino M. Familial adult myoclonus epilepsy: a new phenotype of autosomal dominant myoclonic epilepsy. Ann Neurol. 1996;40:505.

7. Okino S. Familial benign myoclonus epilepsy of adult onset: A previously unrecognized myoclonic disorder. J Neurol Sci. 1997;145:113–8.

8. Okuma Y, Shimo Y, Shimura H, Hatori K, Hattori T, Tanaka S, et al. Familial cortical tremor with epilepsy: An under-recognized familial tremor. Clin Neurol Neurosurg. 1998;100(1):75–8.

9. Guerrini R, Bonanni P, Patrignani A, Brown P, Parmeggiani L, Grosse P, et al. Autosomal dominant cortical myoclonus and epilepsy (ADCME) with complex partial and generalized seizures: A newly recognized epilepsy syndrome with linkage to chromosome 2p11.1-q12.2. BRAIN. 2001;124:2459–75.

10. Mikami M, Yasuda T, Terao A, Nakamura M, Ueno S, Tanabe H, et al. Localization of a Gene for Benign Adult Familial Myoclonic Epilepsy to Chromosome 8q23.3-q24.1. Am J Hum Genet. 1999;65(3):745–51.

11. Mori S, Nakamura M, Yasuda T, Ueno SI, Kaneko S, Sano A. Remapping and mutation analysis of benign adult familial myoclonic epilepsy in a Japanese pedigree. J Hum Genet [Internet]. 2011;56(10):742–7. Available from: http://dx.doi.org/10.1038/jhg.2011.93

503 12. Ishiura H, Doi K, Mitsui J, Yoshimura J, Matsukawa MK, Fujiyama A, et al.
504 Expansions of intronic TTTCA and TTTTA repeats in benign adult familial
505 myoclonic epilepsy. Nat Genet [Internet]. 2018;50(4):581–90. Available from:
506 http://dx.doi.org/10.1038/s41588-018-0067-2
507 13. Yeetong P, Pongpanich M, Srichomthong C, Assawapitaksakul A, Shotelersuk
508 V, Tantirukdham N, et al. TTTCA repeat insertions in an intron of YEATS2 in
509 benign adult familial myoclonic epilepsy type 4. Brain. 2019;142(11):3360–6.
510 14. Florian RT, Kraft F, Leitão E, Kaya S, Klebe S, Magnin E, et al. Unstable
511 TTTTA/TTTCA expansions in MARCH6 are associated with Familial Adult
512 Myoclonic Epilepsy type 3. Nat Commun. 2019;10(1):1–14.
513 15. Corbett MA, Kroes T, Veneziano L, Bennett MF, Florian R, Schneider AL, et al.
514 Intronic ATTTC repeat expansions in STARD7 in familial adult myoclonic
515 epilepsy linked to chromosome 2. Nat Commun. 2019;10(1):1–10.
516 16. Kobayashi K, Hitomi T, Matsumoto R, Watanabe M, Takahashi R, Ikeda A.
517 Nationwide survey in Japan endorsed diagnostic criteria of benign adult familial

myoclonus epilepsy. Seizure [Internet]. 2018;61(May):14–22. Available from: https://doi.org/10.1016/j.seizure.2018.07.014

17. Cen Z, Jiang Z, Chen Y, Zheng X, Xie F, Yang X, et al. Intronic pentanucleotide TTTCA repeat insertion in the SAMD12 gene causes familial cortical myoclonic tremor with epilepsy type I. BRAIN. 2018;141(8):2280–8.

18. Lei XX, Liu Q, Lu Q, Huang Y, Zhou XQ, Sun HY, et al. TTTCA repeat expansion causes familial cortical myoclonic tremor with epilepsy. Eur J Neurol. 2018;0:1–6.

19. Zeng S, Zhang M, Wang X, Hu Z, Li J, Ki N, et al. Long-read sequencing identified intronic repeat expansions in SAMD12 from Chinese pedigrees affected with familial cortical myoclonic tremor with epilepsy. J Med Genet. 2018;0:1–6.

TITLES AND LEGENDS TO FIGURES

Figure 1. Pedigrees analyzed in this study. BAFME patients are indicated by filled black symbols. Family members with suspected BAFME who showed only EEG abnormalities, transient tremor, fatigue-induced tremor, or a combination of these symptoms are represented by filled gray symbols. Symbols with black dots represent individuals whose gDNA samples were available. The familial relationships among the individuals in pedigree 9 are unknown. The number of TTTTA repeat in *TNRC6A* are shown in red for parent–offspring transmission in which no linkage was observed.

Figure 2. The results of repeat-primed PCR (RP-PCR) (A) and Southern blotting (B) analyses for patients heterozygous and homozygous for BAFME mutant alleles. The left and right sides of the RP-PCR results show the results of fragment analysis after amplification of the TTTTA repeat and TTTCA repeat, respectively (A). Patients with heterozygous mutant BAFME alleles (top panels) and patients with homozygous mutant alleles (middle panels) showed the abnormally expanded TTTTA repeats and the abnormally expanded TTTCA insertion. The TTTCA insertion was not detected in the

548 controls, and the peak for TTTTA was up to 218 bp (the lowest bands). Southern blot
549 analysis with hybridization probes targeted to TTTTA revealed that there was a normal
550 band and an abnormally expanded band in patients with a heterozygous mutant allele,
551 and that there were abnormally expanded bands but no normal bands in the patient with
552 homozygous mutant alleles (B). Distribution of TTTCA/TTTTA repeat lengths in the
553 abnormal chromosomes of BAFME patients. These numbers of repeats are in the range
554 of 516–2363, forming 3.39–12.63 kbp repeat lengths (C).
555
556 **Figure 3.** Molecular diagnosis of BAFME patients from the family in pedigree 1. This
557 family is the largest family with BAFME in the world, and was used by Yasuda to
558 propose the BAFME disease concept. The middle row shows the results of long-range
559 PCR, and the lower row shows the results of Southern blotting analysis. The expansion
560 band lengthens in the process of transmission from parent to offspring. The gDNA from
561 individual III-7 was of extremely poor quality, so Southern blotting and long-range PCR
562 were not possible. Repeat-primed PCR, however, was able to detect the abnormally
563 expanded TTTCA/TTTTA repeats in this patient (data not shown).

564

565 **Figure 4.** The correlation between the repeat length and the age of onset of myoclonic

566 tremor (A), epilepsy (B) and either myoclonic tremor or epilepsy (C), and the

567 correlation between parent–offspring differences in repeat length and the mothers' age

568 at childbirth (D). All combinations of factors showed correlations.

569

570 **Figure 5.** Differences in repeat length (A), age at onset of myoclonic tremor (B), age at

571 onset of epilepsy (C), and age at onset of either myoclonic tremor or epilepsy (D)

572 between parents and their offspring. The 20 parent–offspring transmissions analyzed

573 here yielded 19 length increases and one decrease, resulting in an average change of

574 0.97 kbp (range, –0.88 to 3.08 kbp, $t_0 = 4.65$, $P < 0.001$) (A). Of the 15 parent–offspring

575 pairs analyzed here, the onset of myoclonic tremor occurred at an earlier age for the

576 offspring than for the parent in 14 pairs (seven paternal and seven maternal

577 transmissions) ($n = 15$, $t_0 = 3.6$, $P = 0.003$) (B). The age at onset of epilepsy in the

578 offspring was significantly earlier than that of their mothers ($n = 10$, $t_0 = 2.8$, $P = 0.019$)

579 (C). The age at onset of either myoclonic tremor or epilepsy in the offspring was also

580 significantly earlier than that of their mothers (n= 16, $t_0 = 4.1$, $P < 0.001$) (D). The black

581 and gray lines indicate paternal and maternal transmissions, respectively. *$P < 0.05$,

582 **$P < 0.01$, ***$P < 0.001$.

583

584 **Figure S4.** The results of long-range PCR (A) and long-range PCR followed by

585 Southern blotting with a DIG-$(TGAAA)_9$ probe (B). The shorter band of Case 2 from

586 pedigree 9 was clearly longer than the normal bands of the other samples (A). In the

587 long-range PCR followed by Southern blotting, the DIG-$(TGAAA)_9$ probe detected

588 whether the bands included TTTCA (B). Case 2 from pedigree 9 carried TTTCA in the

589 longer band (arrowhead 1) and no TTTCA in the shorter band (arrowhead 2).

590

Table 1 Details of TTTTA pentanucleotide repeat expansion in *TNRC6A*

TTTTA expansion in *TNRC6A*	Negative	Positive	Total
BAFME with TTTCA insertion in *SAMD12*	25	5	30
Controls	209	3	212

Controls vs. BAFME with TTTCA insertions in *SAMD12*; $P = 0.0009$ (Fisher's exact test)

Figure 1

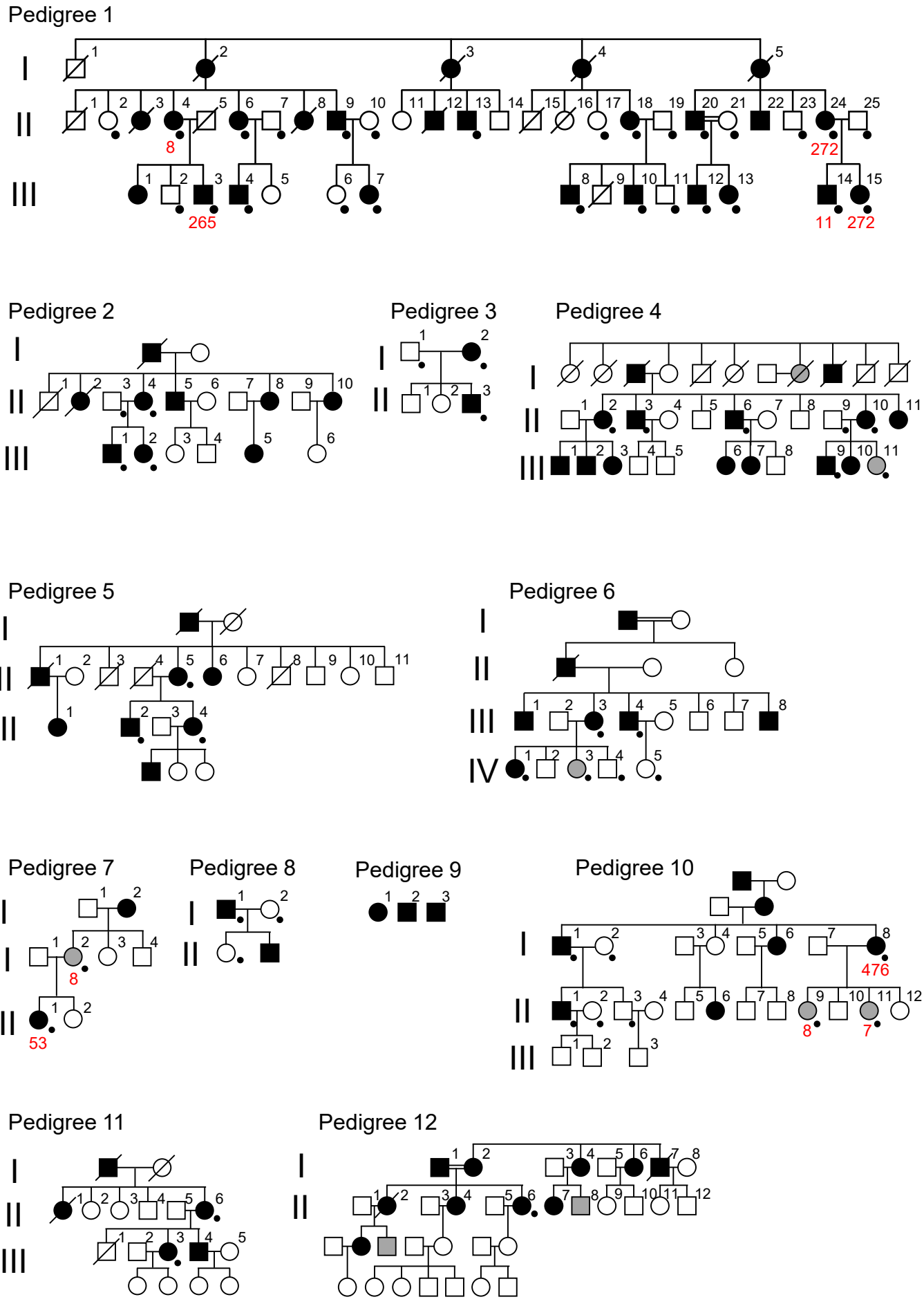

Pedigree 1
I
II
III
8
265
272
11
272
Pedigree 2
I
II
III
Pedigree 3
I
II
Pedigree 4
I
II
III
Pedigree 5
I
II
III
Pedigree 6
I
II
III
IV
Pedigree 7
I
II
III
8
53
Pedigree 8
I
II
Pedigree 9
Pedigree 10
I
II
III
476
8
7
Pedigree 11
I
II
III
Pedigree 12
I
II

Figure 2

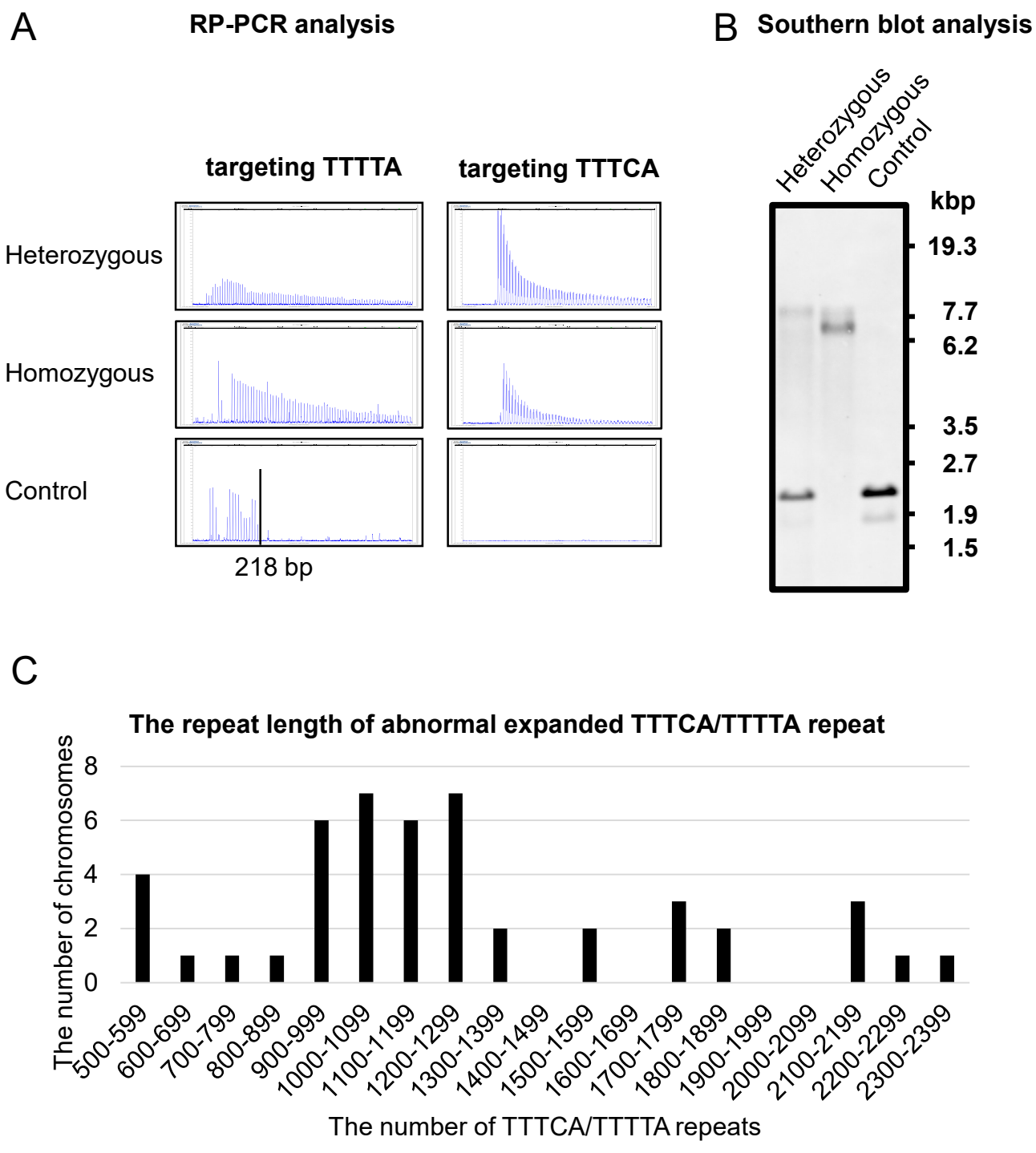

A
RP-PCR analysis
targeting TTTTA
targeting TTTCA
Heterozygous
Homozygous
Control
218 bp
B
Southern blot analysis
Heterozygous
Homozygous
Control
kbp
19.3
7.7
6.2
3.5
2.7
1.9
1.5
C
The repeat length of abnormal expanded TTTCA/TTTTA repeat
The number of chromosomes
0
2
4
6
8
500-599
600-699
700-799
800-899
900-999
1000-1099
1100-1199
1200-1299
1300-1399
1400-1499
1500-1599
1600-1699
1700-1799
1800-1899
1900-1999
2000-2099
2100-2199
2200-2299
2300-2399
The number of TTTCA/TTTTA repeats

Figure 3

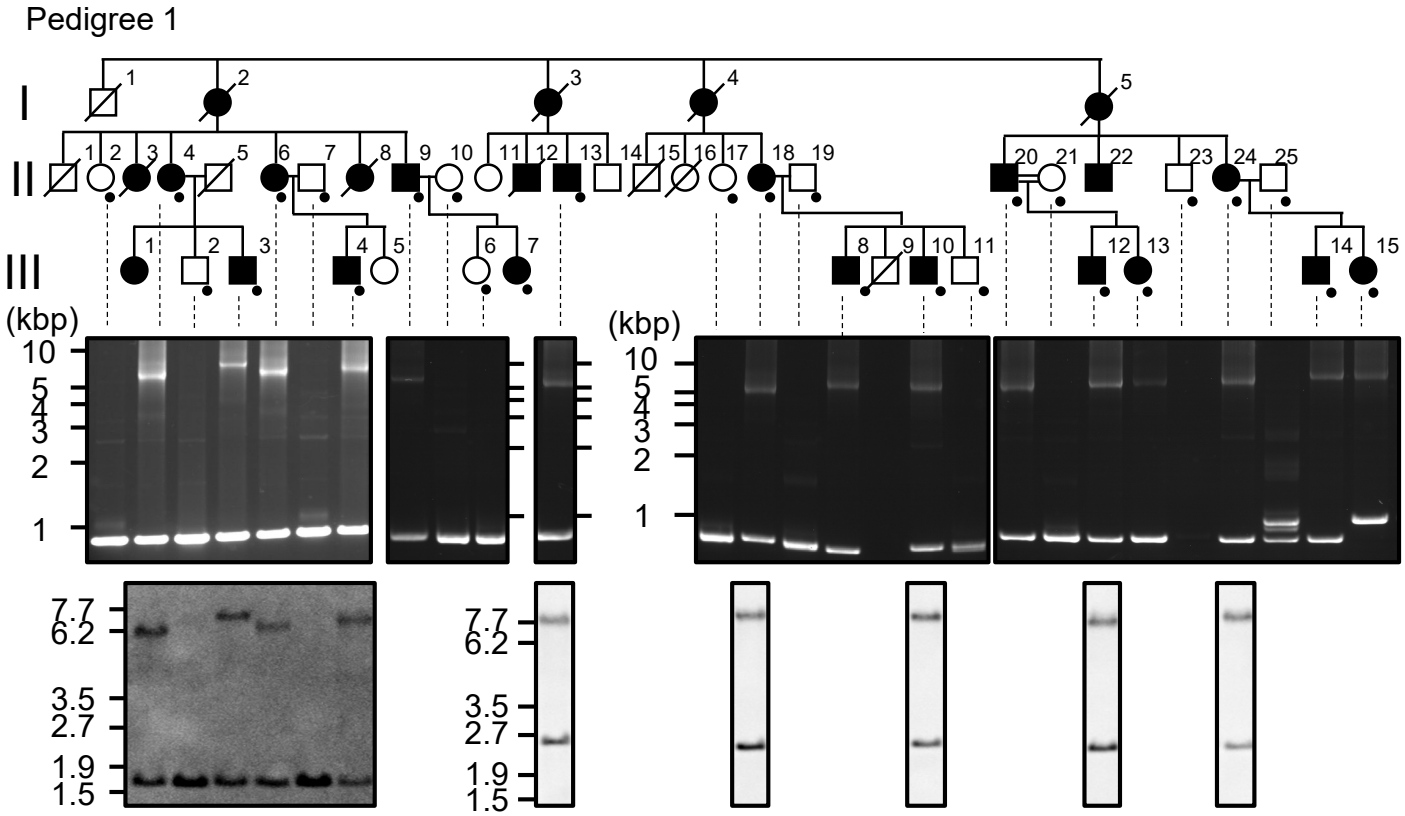


Figure 4

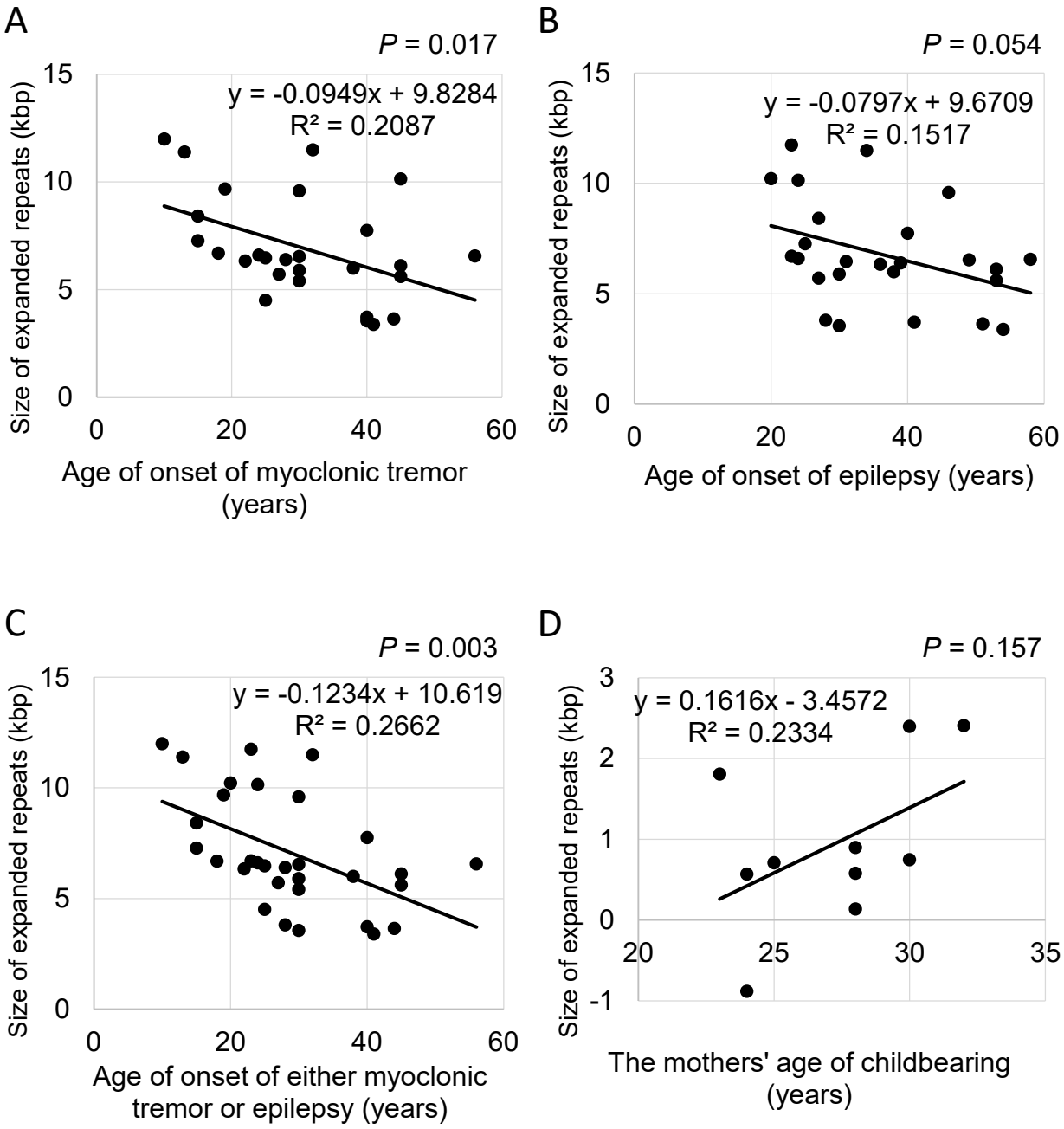

A
P = 0.017
y = -0.0949x + 9.8284
R² = 0.2087
Size of expanded repeats (kbp)
Age of onset of myoclonic tremor (years)
B
P = 0.054
y = -0.0797x + 9.6709
R² = 0.1517
Size of expanded repeats (kbp)
Age of onset of epilepsy (years)
C
P = 0.003
y = -0.1234x + 10.619
R² = 0.2662
Size of expanded repeats (kbp)
Age of onset of either myoclonic tremor or epilepsy (years)
D
P = 0.157
y = 0.1616x - 3.4572
R² = 0.2334
Size of expanded repeats (kbp)
The mothers' age of childbearing (years)

Figure 5

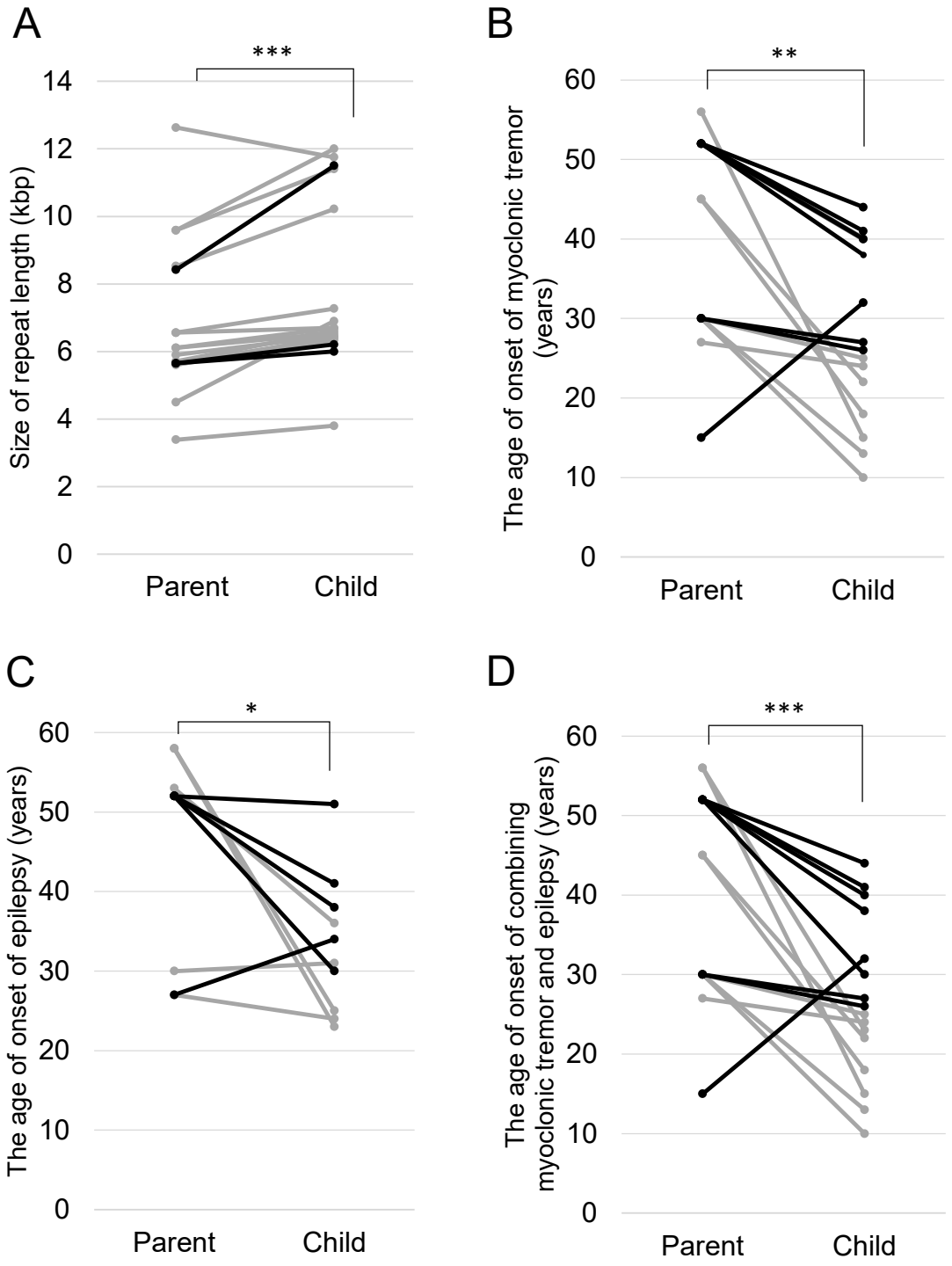

A
***
Size of repeat length (kbp)
14
12
10
8
6
4
2
0
Parent
Child
B
**
The age of onset of myoclonic tremor (years)
60
50
40
30
20
10
0
Parent
Child
C
*
The age of onset of epilepsy (years)
60
50
40
30
20
10
0
Parent
Child
D
***
The age of onset of combining myoclonic tremor and epilepsy (years)
60
50
40
30
20
10
0
Parent
Child

Table S1 Details of patients diagnosed with BAFME and suspected to be carriers of BAFME

				age of onset									
Ped	Pt	A	S	MT	E	SY	EEG	gSEP	CR	others	site of MT	*SAMD12*†	*TNRC6A*‡
1	II-4	67	F	27	27	27	PSWC, PES	+	+	VEP; L	U/E, L/E	979	8
	III-3	39	M	24	24	24	PSWC, PMR	+	+		U/E	1160	265
	II-6	65	F	30	30	30	PSWC	+	+	VEP; L	U/E, L/E, abdomen	1018	9
	III-4	41	M	25	31	25	mild S	+	+	VEP; L	U/E	1132	16
	II-9	57	M	30	49	30	S	+	+		U/E	1147	8
	III-7	30	F	26	-	26	PSWC	+	+		U/E	N/A	N/A
	II-13	54	M	38	38	38	PSWC	+	+	VEP; L	U/E, L/E	956	12
	II-18	65	F	45	53	45	PSWC	+	+		U/E	960	11
	III-8	43	M	22	36	22	S	+	+	VEP; L		1107	12
	III-10		M									1125	10
	II-20		M									969	11
	III-12	47	M									1038	11
	III-13	37	M									1080	12
	II-24	53	F	45	53	45	PSWC	+	+		U/E	1060	272
	III-14	25	M	18	-	18	SWC	+	+		U/E, L/E	1176	11
	III-15		F									1145	272
2	II-4	59	F	56	58	56	abnormal					1149	10
	III-1	33	M	15	25	15	abnormal					1292	10
	III-2	30	F	-	23	23	abnormal					1179	10
3	I-2	71	F									968	11
	II-3	41	M									1118	14
4	II-2	61	F	44	51	44	PS (+)	+				560	12
	II-3	62	M	40	41	40	slow, PS (+)	+				582	N/A
	II-6	65	M	40	30	30	slow, PS (+)	+				535	15
	II-10	54	F	41	54	41	PS (+)	+				516	15
	III-9		M	18	29	29	HV (+)	+				598	14
	III-11		F	-	-		abnormal					-	15
5	II-5		F	64	64	64	slow, PS (+)	+				1076	19
	III-2		M	55	64	55	slow, PS (+)	+				1146	11
	III-4		F	38	43	38	slow, PS (+)	+				1221	11
6	III-3	57	F		> 20	> 20						1543	12
	IV-1	37	F	-	20	20	PS (+)	+				1882	8
	IV-3		F	-	-	-	HV (+), PS (+)	-				-	8
	III-4	61	M	45	24	24	S, PS (+)	high		§		1867	12
7	II-2	53	F	-	-	-	SWC, PS (+)	+				2363	6

	III-1	29	F	-	23	23	S, SWC, PS (+)	+				2188	53
8	I-1	75	M	30	-	-	-	+	+	¶	U/E, L/E, trunk	921	9
9	1		F									1192	10
	2		M									1069	345
	3		M									1777	12
10	I-1	61	M	15	27	15						1522	435
	II-1	34	M	32	34	32	PSWC					2138	500
	I-8		F	30	46	30	S					1756	476
	II-9	23	F	13	< 42	13	slow					2118	8
	II-11	14	F	10						††		2238	7
11	II-6	69	F	25	-	25					U/E, L/E, trunk	738	9
	III-3	39	F	21		21					U/E, L/E	1217	9
12	II-6	60	F	24	24	24	S, SW	+	+		U/E, L/E	992	12
13			F	19	> 19							1774	13
14			M									1215	605
15			M	40	40	40	slow	+				1389	13

Ped: pedigree, Pt: patient, A: age at sampling, S: sex, MT; myoclonic tremor, E: epilepsy, SY: symptom, EEG: electroencephalogram, gSEP: giant somatosensory evoked potential, CR: enhanced long-loop reflex, PSWC: polyspike and wave complex, PES: photo-evoked spike, PMR: photomyoclonic response, SWC: spikes and wave complex, S: spike, SW: sharp and wave, PS: photic stimulation, HV: hyper ventilation, VEP; L: visual evoked potential large, U/E: upper extremities, L/E: lower extremities, N/A: not available

A blank entry indicates absence of information

Pedigrees 13–15 represent three patients clinically diagnosed with BAFMR but unrelated to the known BAFME families

†: approximately size of TTTCA/TTTTA repeat in *SAMD12*

‡: approximately size of TTTTA repeat in *TNRC6A*

§: visual seizure

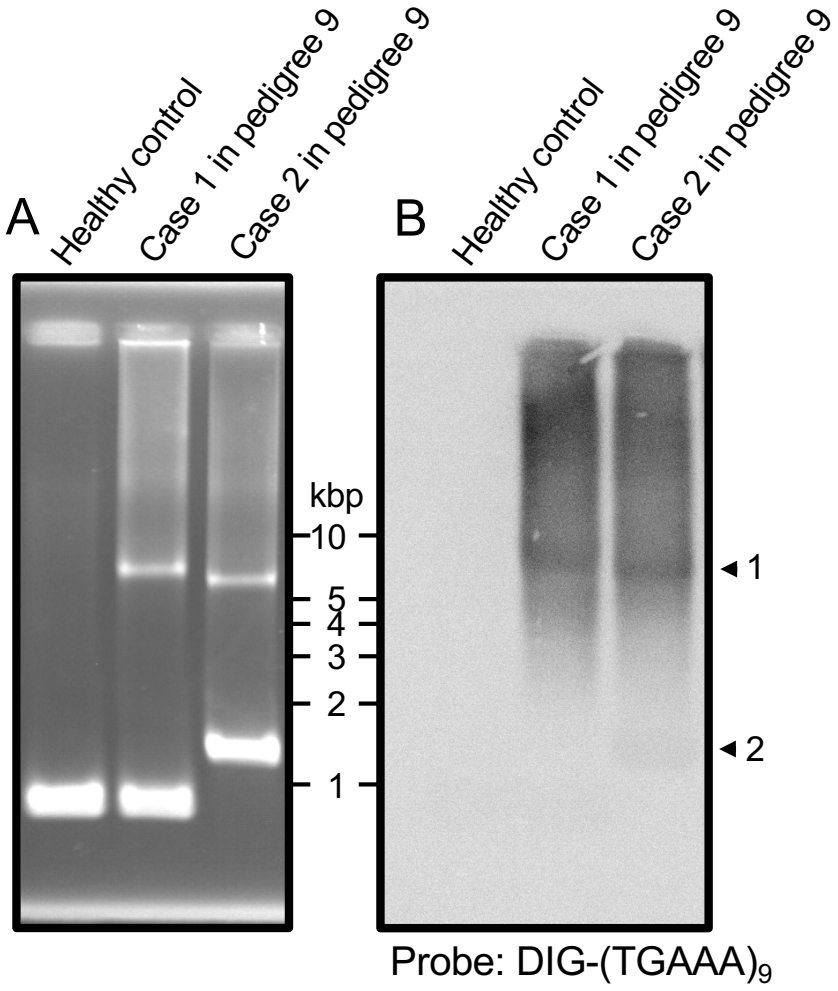
¶: gait disturbance, writing disorder

††: temporary tremor at age of elementary school, developed tremor at age of 33

Table S2 Primers for the PCR and long-range PCR amplifications

TNRC6A_LF	5’-GCAAGGGCTCAAGAATGCTGGTGGAC-3’
TNRC6A_LR	5’-TGATCCCAGCTGCCACTTCCAACTCA-3’
rs7464659-F	5’-TTCAAGGGGCTCTCTTGCTT-3’
rs7464659-R	5’-TAGCAGAAGTTGTGGCCCAA-3’
rs6994270-F	5’-TGTGGAAGACAGTGTGGCAA-3’
rs6994270-R	5’-CCAGCCCACGTTTTCCTTTA-3’
rs2515029-F	5’-ACAATGTTGCAAGGGCTGAC-3’
rs2515029-R	5’-TGCATTGGGTTAGCTGTGCA-3’
rs9643124-F	5’-TGGCAGGAAGTGAGATTGGA-3’
rs9643124-R	5’-AAGTCAACTGCGGTGAAGCT-3’
rs10086119-F	5’-TGTGACGCATTATGTGTGCC-3’
rs10086119-R	5’-TGGTGGTGCATGCCTGTAAT-3’
rs7832475-F	5’-GTCAGAATTCTGGCCCGTGA-3’
rs7832475-R	5’-AGTAGCTGGGACTATGGGCA-3’
rs4876828-F	5’-TCTGGAAGGAAAAGGCAGCC-3’
rs7832475-R	5’-TGGCCAATGGAATGCTAGCA-3’
TNRC6A_CAG_F	5’-AGTCATTGCGAGTTCCCTGG-3’
TNRC6A_CAG_R	5’-CTTCACGAGGATACCGAGGC-3’
TNRC6A_CAGrepeat_F	5’-TGGCTAATCTTTTCCACCCCT-3’
TNRC6A_CAGrepeat_R	5’-TGATGATAAGGTGTGAGTCTCGT-3’

Figure S3


A
Healthy control
Case 1 in pedigree 9
Case 2 in pedigree 9
kbp
10
5
4
3
2
1
B
Healthy control
Case 1 in pedigree 9
Case 2 in pedigree 9
1
2
Probe: DIG-(TGAAA)9