

## ORIGINAL ARTICLE

# A Japanese SCA5 family with a novel three-nucleotide in-frame deletion mutation in the *SPTBN2* gene: a clinical and genetic study

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To date, four families with spinocerebellar ataxia type 5 (SCA5) with four distinct mutations in the *spectrin, beta, nonerythrocytic 2* gene (*SPTBN2*) have been reported worldwide. In the present study, we identified the first Japanese family with SCA5, and analyzed this family clinically and genetically. The clinical features of the five patients in this family revealed late-onset autosomal-dominant pure cerebellar ataxia. We collected DNA samples from the majority of the family members across two generations, and exome sequencing combined with Sanger sequencing revealed a novel heterozygous three-nucleotide in-frame deletion mutation (c.2608\_2610delGAG) in exon 14 of the *SPTBN2* gene. This mutation cosegregated with the disease in the family and resulted in a glutamic acid deletion (p.E870del) in the sixth spectrin repeat, which is highly conserved in the *SPTBN2* gene. This is the first three-nucleotide in-frame deletion mutation in this region of the beta-3 spectrin protein highly likely to be pathogenic based on exome and bioinformatic data.

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## INTRODUCTION

Spinocerebellar ataxia type 5 (SCA5) is a rare autosomal-dominant pure spinocerebellar ataxia (SCA). SCA5 patients predominantly exhibit limb and gait ataxia (>90%); however, truncal ataxia, sensory deficits, abnormal eye movements, dysarthria and exaggerated deep tendon reflexes are also prevalent (25–90%).<sup>1</sup> Individuals generally struggle with fine motor coordination but rarely require a wheelchair until late in the disease course.<sup>1</sup>

SCA5 is caused by mutations in the *spectrin, beta, nonerythrocytic 2* gene (*SPTBN2*). This gene is primarily expressed in the brain. It encodes the beta-III spectrin protein consisting of 2390 amino acids comprising two calponin homology domains, 17 triple-helical spectrin repeats and a pleckstrin homology domain, and as a cytoskeletal protein is highly expressed in Purkinje cells.<sup>2</sup>

To date, five mutations, that is, three missense (p. L253P, p. T472M and R480W) and two deletion (p.E532\_M544del and p.L629\_R634delinsW) ones, in the *SPTBN2* gene have been reported in SCA5 families originating from the United States, France, Germany and Norway,<sup>3–6</sup> and an SCA5 patient.<sup>7</sup> In the present study, we report the first Japanese SCA5 family with a novel heterozygous three-nucleotide in-frame deletion mutation in the *SPTBN2* gene.

## MATERIALS AND METHODS

### Patients

The pedigree is shown in Figure 1a. Eight living family members were interviewed and examined by experienced neurologists, and the diagnosis of familial SCA was made on the basis of the patients' clinical presentations as

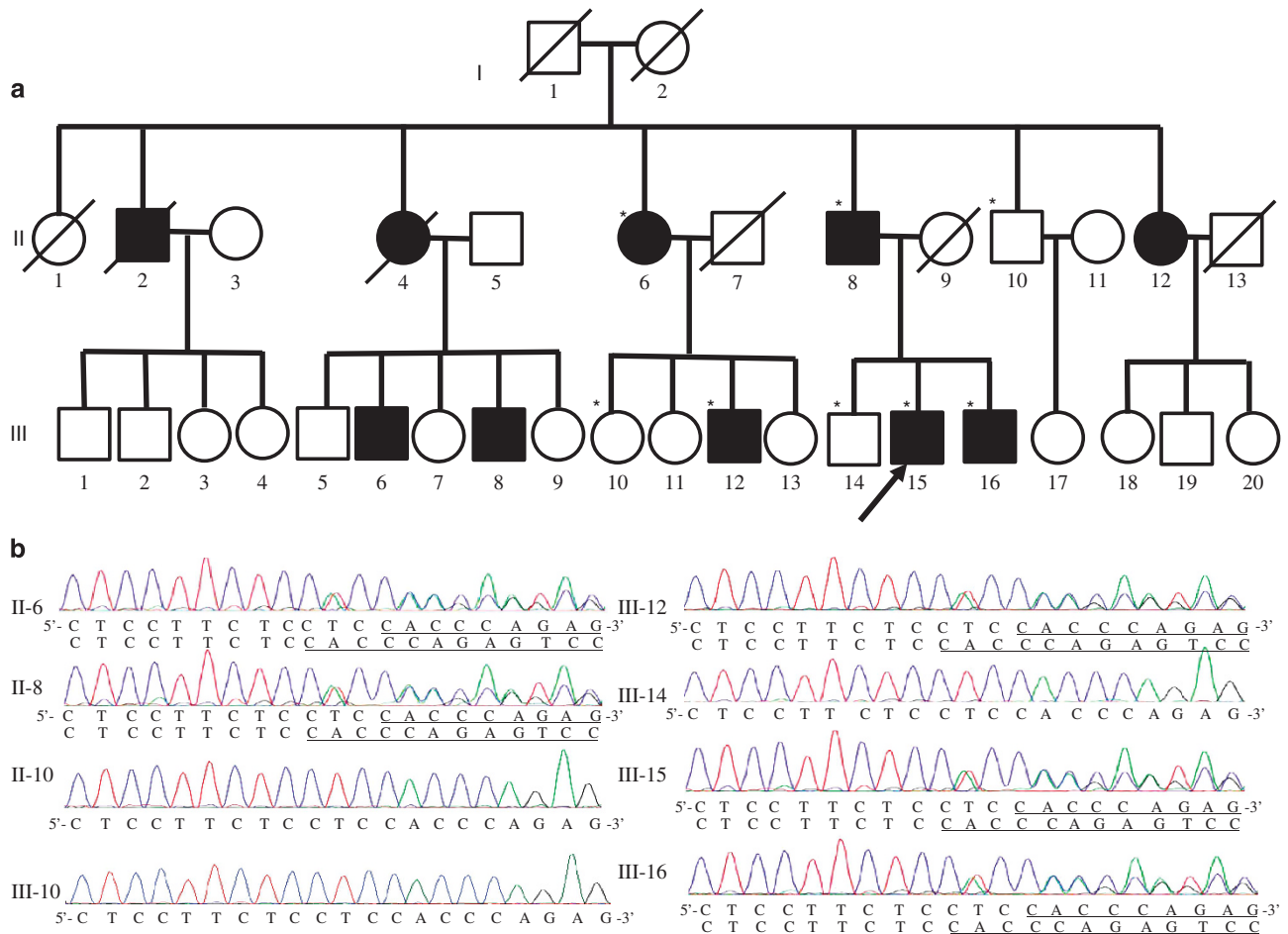
well as the results of physiological, radiological and biochemical examinations. Unfortunately, we were not able to obtain the consent of other family members to be examined in this study. Brain magnetic resonance imaging (MRI) in four affected members revealed cerebellar atrophy. Molecular analysis of the proband excluded SCA1, SCA2, MJD, SCA6, SCA7, SCA8, SCA12, SCA17, SCA31, SCA36 and DRPLA. The present clinical and genetic study was approved by the institutional review board of the Yamanashi University, and informed consent was obtained from all participating individuals.

### Whole-exome sequencing

We carried out whole-exome sequencing of genomic DNA from the proband and the father. Genomic DNA was extracted from blood leukocytes from eight individuals. We used a NEBNext DNA Library Prep Master Mix set (New England Biolabs, Ipswich, MA, USA, E6040) to manufacture the DNA library. Exome capture was performed with a SureSelect Human All Exon V4 kit (Agilent, Santa Clara, CA, USA). Paired-end sequencing was carried out using an Illumina HiSeq 2000, which generated 100-bp reads. Reference sequence data were aligned using a Burrows-Wheeler Aligner.<sup>8</sup> Then single-nucleotide variants were analyzed using a genome analysis tool kit. The additional databases utilized included the Single-Nucleotide Polymorphism Database<sup>9</sup> and the 1000 Genomes Project.<sup>10</sup> As the clinical features of the affected individuals in this family are typical of an autosomal-dominant SCA, we initially filtered for heterozygous variations. We then filtered for those that were shared between the proband and the father. Protein sequence alignment was performed using the PROVEAN<sup>11</sup> and Mutation Taster.<sup>12</sup>

### Sanger sequencing

Exon 14 of the *SPTBN2* gene was amplified using the following PCR primer sequences: *SPTBN2* ex14-F: 5'-CTGCTCAACAAGCACACAGC-3' and



**Figure 1** Pedigree of the SCA5 family. (a) Squares and circles indicate males and females, respectively. Filled symbols indicate affected individuals, whereas open symbols indicate unaffected individuals. Slashes indicate the people who have died already. Asterisks indicate the individuals who have been examined clinically and molecularly. (b) Representative sequencing of the *SPTBN2* gene in part of the family (II-6, II-8, II-10, III-10, III-12, III-14, III-15 and III-16). The mutation cosegregated with the disease.

*SPTBN2* ex14-R: 5'-GTTGGCAATGACTGAATCTGG-3'. Sanger sequencing was performed according to an established standard protocol for biotechnology. To determine whether or not the mutation identified in the present study is found in the general population, DNA samples from unrelated control subjects ( $n = 90$ ) were screened.

## RESULTS

### Clinical features

The proband was a 57-year-old male who presented with poor coordination that he first noted at the age of 51. At the age of 52, he noted mild unsteadiness of gait. Neurological examination at the age of 57 revealed mild dysarthria, dysphagia and incoordination of the lower limbs. He had trouble accomplishing the heel-to-knee test and difficulty in standing with feet together; however, he was able to walk without assistance. Knee tendon reflexes were slightly exaggerated bilaterally; however, Babinski's sign was negative. Eye movements were intact, and no nystagmus was observed. Neither mental retardation nor sensory abnormality was observed.

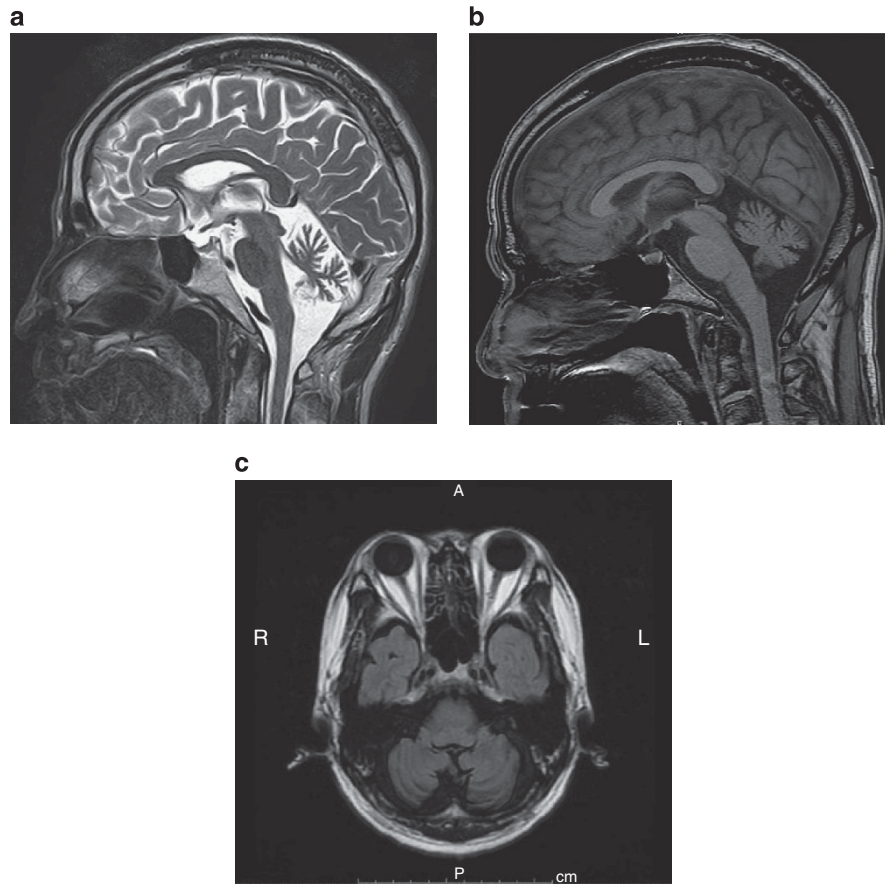
The clinical features of the five patients in this family are summarized in the Table 1. The ages at onset in the five patients ranged from 50s to 60s. There was no obvious anticipation of age at the onset between the two generations. Limb and trunk ataxia, and cerebellar dysarthria, were the common features in all the

**Table 1** Clinical features of the five patients in the SCA5 family

| Case                     | II-6 | II-8 | III-12 | III-15 | III-16 |
|--------------------------|------|------|--------|--------|--------|
| Sex                      | F    | M    | M      | M      | M      |
| Present age              | 89   | 87   | 55     | 57     | 52     |
| Age of onset             | 60   | 48   | 54     | 51     | 51     |
| Cerebellar atrophy (MRI) | +    | +    | +      | +      | +      |
| Limb ataxia              | +    | +    | +      | +      | +      |
| Dysarthria               | +    | +    | +      | +      | +      |
| Unsteadiness of gait     | w    | w    | -      | +      | -      |

Abbreviations: F, female; M, male; MRI, magnetic resonance imaging; +, positive; -, negative; w, wheelchair-bound.

affected individuals. Cognitive deterioration, epilepsy, saccadic eye movements, visual disturbance, ophthalmoplegia and peripheral nerve involvement were absent in all the patients. The disease progressed very slowly and the clinical severity correlated with the duration of the disease. Two of the patients (II-6 and II-8) became wheelchair-bound ~20 to 30 years after the disease onset. Brain MRI of our patients revealed moderate to severe atrophy in the cerebellum (Figure 2). Single-photon emission tomography (SPECT) in the proband (III-15) showed blood flow reduction in the cerebellum (Figure 3).



**Figure 2** Representative brain MRI findings in the patients. Sagittal brain MRI of III-12 (a, T2-weighted) and III-15 (b, T1-weighted), and axial brain MRI of III-15 (c, T1-weighted) show moderate to severe cerebellar atrophy.

### Mutation analysis

We identified 10 692 and 10 641 variations in the proband and the father, respectively, of which 231 and 241, respectively, were novel heterozygous variants. Of these, 183 variants were shared between the proband and the father (Supplementary Data). Once anonymous open reading frames were excluded and genes associated with SCAs were prioritized, we found one likely candidate three-nucleotide in-frame deletion mutation in the *SPTBN2* gene, a gene known to cause SCA5.

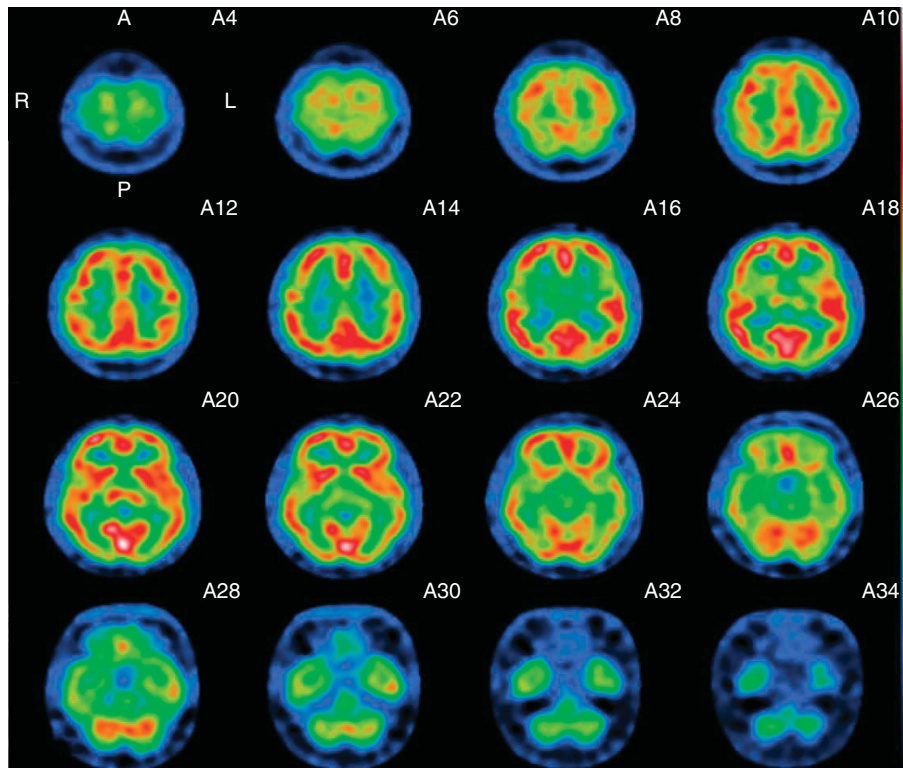
On Sanger sequencing, we validated the most possible shared three-nucleotide in-frame deletion mutation in the *SPTBN2* gene as the cause of the disease. This mutation cosegregated faithfully with the disease phenotype in this family (Figure 1b). All the five patients had a heterozygous c.2608\_2610delGAG (p.E870del) mutation in the *SPTBN2* gene, which is located at an evolutionarily conserved amino acid (Figure 4). We performed bioinformatic analyses using the PROVEAN and Mutation Taster (see 'Materials and methods' section), which predicted that the detected variant was deleterious and disease-causing, respectively. The variant was not detected in our 180 control chromosomes and was not observed in the databases of normal sequence variations (1000 Genome Project and Single-Nucleotide Polymorphism Database). Thus, the mutation identified was likely to be pathogenic.

### DISCUSSION

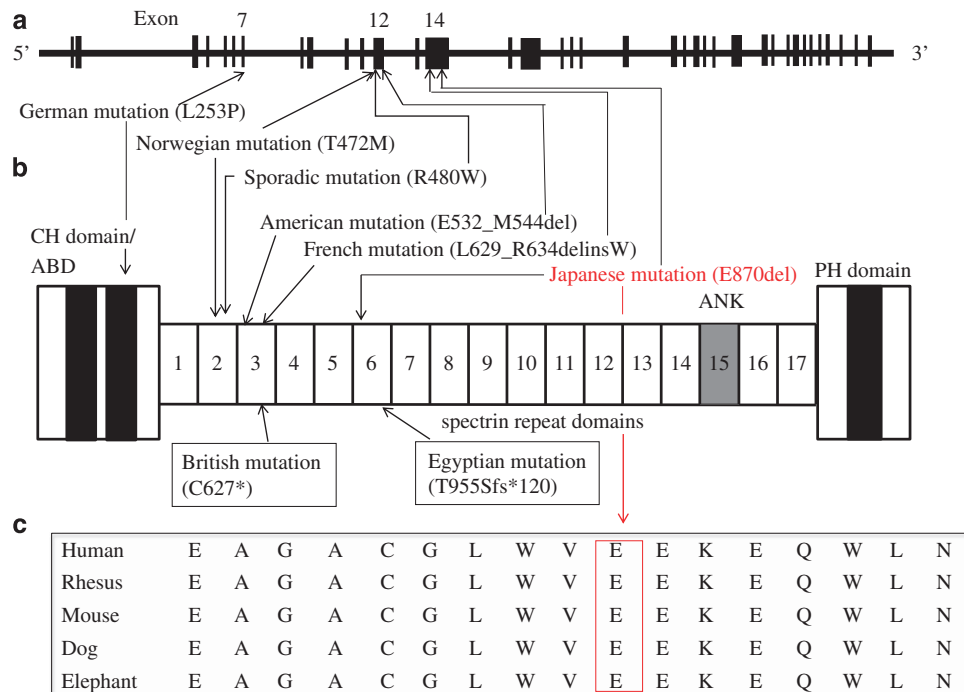
We presented here the first Japanese case of SCA5 with a highly likely pathogenic mutation in the *SPTBN2* gene. This SCA5 family is the fifth one worldwide. The two deletions (p.E532\_M544del and

p.L629\_R634delinsW) in the *SPTBN2* gene reported earlier in American and French SCA5 families comprise distinct and nonoverlapping in-frame mutations in the third of the 17 spectrin repeats.<sup>3,4</sup> Both of these deletions are predicted to disrupt the triple-helical structure of the spectrin repeat and the conformation of the spectrin tetramer, which likely disrupts the protein structure involving the characteristic leucine and tryptophan residues.<sup>13</sup> The third SCA5 family reported from Germany has a missense mutation (p. L253P) in the *SPTBN2* gene that interferes with the actin-binding site in the second calponin homology domain.<sup>5</sup> The fourth family, of Norwegian descent, has a missense mutation (c.1415C>T) that results in a p. T472M substitution in the second spectrin repeat of the beta-III spectrin protein.<sup>6</sup>

On the other hand, the p.E870del mutation detected in our family is the first one located in the sixth spectrin repeat likely associated with SCA5, resulting in the deletion of one glutamic acid deletion out of a total of 2390 amino acids. Bioinformatic analysis suggests that an amino-acid sequence changed, which might have affected protein features. Similarly, a three-nucleotide in-frame deletion mutation (c.679\_681delTTC, p.F227del) in the *KCND3* gene has been reported in another type of autosomal-dominant cerebellar ataxia (SCA22).<sup>14</sup> This mutation leads to the retention of voltage-gated potassium channel Kv4.3 subunits in the cytoplasm, which is consistent with the lack of A-type K<sup>+</sup> channel conductance on whole-cell patch-clamp recording.<sup>14</sup> The function of beta-III spectrin, however, might be much more diverse than that of *KCND3* and rather different from that of *KCND3*.



**Figure 3** Single-photon emission computed tomography findings. SPECT of a patient (III-15) shows blood flow reduction in the cerebellum.



**Figure 4** Mutations of the *SPTBN2* gene. (a) Illustration of exons (filled rectangles) in the *SPTBN2* gene. (b) A schematic representation of the functional domains of  $\beta$ -3 spectrin, modified from the reported representation,<sup>2</sup> is shown along with the positions of all found mutations, exhibiting dominant (upper) or recessive (lower) transmission, so far. CH, calponin homology domain; ABD, actin-binding domain; ANK, ankyrin-binding domain; PH, pleckstrin homology domain. (c) Evolutionary conservation of the Japanese SCA5 mutation (p.E870del) among species.



These mutations in the *SPTBN2* spectrin repeats may affect the structure, binding properties and overall function of the protein, and also lead to SCA5. To date, no curative treatment for autosomal-dominant spinocerebellar ataxias has been found. Therefore, in addition to its value in genetic counseling, genetic knowledge of the relevant loci might help to identify the possible targets of pharmaceutical interventions.

Clinical presentations similar to those in our family have been reported in other SCA5 families.<sup>3–6</sup> The age at the onset is typically from the third to fifth decade, with highly variable symptoms that worsen over time. The major characteristics of SCA5 are slow progression with patients remaining ambulatory even after decades of suffering from the disease and having an approximately normal longevity.<sup>15</sup> The MRI findings in four patients were pronounced cerebellar atrophy, but the brainstem remained intact, in accordance with the major clinical presentation. Our study suggests that SCA5 should be considered in a pure form of autosomal-dominant cerebellar ataxia when SCA6 or SCA31 is excluded, particularly in Japan.

A single infantile-onset SCA5 patient with a heterozygous missense mutation (c.1438C>T, p.R480W) in the *SPTBN2* gene has been reported.<sup>7</sup> The parents of the patient were healthy and nonconsanguineous. This is a much more severe phenotype with ataxia and global development delay.<sup>7</sup> Meanwhile, a homozygous mutation in the *SPTBN2* gene has been reported in a consanguineous family with childhood developmental ataxia and cognitive impairment.<sup>16,17</sup> Thus, recessive mutations in the *SPTBN2* gene might cause a more severe disorder than SCA5, that is, resembling one infantile-onset SCA5.<sup>7</sup> This suggests that beta-III spectrin has an important role in both development and cognition in addition to its function in the cerebellum. Cognitive impairment is an integral part of a recessive ataxic syndrome, and the condition is called spectrin-associated autosomal-recessive cerebellar ataxia type 1 (SPARCA1).<sup>13</sup> The identification of SPARCA1 and normal heterozygous carriers of the stop codon in *SPTBN2* provides insights into the mechanism underlying the molecular dominance in SCA5 and demonstrates that the cell-specific repertoire of spectrin subunits underlies a novel group of disorders, namely, the neuronal spectrinopathies, which include SCA5, SPARCA1 and a form of West syndrome.<sup>16</sup>

In summary, there are increasing numbers of *SPTBN2* mutations being reported, and we added a novel *SPTBN2* mutation to the 'spectrinopathies', which will be useful for elucidating the molecular mechanism underlying the 'spectrinopathies'.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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*Accession codes:* The nucleotide sequence data reported are available in the GenBank database under the accession number KJ567653.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)