A Novel TTBK2 Mutation in a Chinese Pedigree with Spinocerebellar Ataxia 11

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Short Report

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Abstract

Spinocerebellar ataxia type 11 (SCA11) is a rare disease and tau tubulin kinase 2 (TTBK2) gene was the causative gene. To date, only seven SCA11 families have been reported. Here, we reported a Chinese SCA11 pedigree with cerebellar ataxia. Both patients in the family demonstrated typical clinical features of cerebellar ataxia and cerebellar atrophy on brain MRI. A novel heterozygous duplicated mutation (c.1211_1217dupAGGAGAA) of TTBK2 gene was identified in the proband using whole-exome sequencing (WES), which resulted in frameshift mutation and formed a premature stop codon (p. N406Kfs*47). The mutation was detected in the proband's affected brother, and his unaffected mother, who with a lower percentage of the mutation and considered as an asymptomatic mutation carrier. Our study delineated the genotypic spectrum of SCA11.

Introduction

Hereditary ataxia (HA) is a group of rare genetic neurodegenerative diseases, including autosomal dominant cerebellar ataxias (ADCAs), autosomal recessive cerebellar ataxias (ARCAs), X-linked cerebellar ataxias, and mitochondrial cerebellar ataxias (*1*). ADCAs are alternatively called spinocerebellar ataxias (SCAs) and show highly heterogeneous. Multiple genes and loci have been linked to SCAs. Abnormal trinucleotide or pentanucleotide repeat expansions in the corresponding genes are the main pathogenic genotypes of SCAs, including SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 31, 36, and dentatorubral-pallidoluysian atrophy (DRPLA) (*2, 3*). Other genotypes can also be found in SCAs, including insertions and/or deletions, missense, and nonsense mutations. These mutations are the causes of SCA5, 11, 13, 14, 15/16 and 27, 28 (*4*).

SCA11 was firstly designated as one of the subtypes of SCAs with the clinical features of pure progressive cerebellar ataxia in 1999 (*3*). The clinical features of SCA11 show slowly aggravated cerebellar disorders, such as ataxia of the gait, trunk, upper or lower extremity, also including dysarthria and nystagmus (*5*). Life expectancy of SCA11 patients is normal (*6*). Tau tubulin kinase 2 (*TTBK2*) gene was identified as the causative gene of SCA11 in 2007 (*7*). TTBK2 protein contains a serine/threonine kinase domain and a conserved C-terminal located domain, interacting with the inositol/IP3 pathway reported by previous researches (*8, 9*). Studies also showed that phosphorylation of tau protein and TAR DNA-binding protein 43 (TDP-43) by TTBK2 likely played an important role in degenerative diseases(*10*).

In China, SCA3 is the most common autosomal inherited cerebellar ataxia, while SCA11 is exceedingly rare (*11*). Here, we reported a Chinese pedigree with SCA11 carrying a novel *TTBK2* mutation.

Materials And Methods

Subjects

All examined individuals were from the Chinese SCA11 family. All investigations in this study had obtained informed consent and were approved by the ethics committee of Fujian Medical University Union Hospital.

Clinical data collection

The proband and the family members were studied by history of the diseases and physical examinations, then the brain magnetic resonance imaging (MRI) was completed. The SCA11-affected individuals also underwent tests for electromyography (EMG).

Genetic testing

Genomic DNA of the subjects was extracted from peripheral blood leukocytes using a TIANamp Genomic DNA Kit (Tiangen) according to the standard protocols. PCR and capillary electrophoresis were performed for genetic screening of SCA1, 2, 3, 6, 7, 8, 10, 12, 17, 36, DRPLA and Friedreich ataxia. The variant of proband in *TTBK2* were screened by whole-exome sequencing (WES) on Illumina Hiseq sequencer (Illumina Inc., San Diego, CA). The identified variants were Sanger sequenced for confirmation on an ABI 3730 Genetic Analyzer (Foster City, CA, USA). Segregation analysis was performed within the family by sanger sequencing.

Results

Clinical Description

The pedigree of this non-consanguineous family was shown in Figure. 1A. The proband (II-3), a Chinese boy born in 2006, was transferred to our department in 2021. He developed progressive slurred speech, balance, and gait problems in the past three years. He could walk independently but fell sometimes. He had poor grades in physical tests. Gradually, he suffered tremor in both hands and had trouble in writing. Neurological examination revealed dysarthria, bilateral nystagmus, and cerebellar ataxia; signs of cerebellar ataxia including positive finger-to-nose test and heel-knee-shin test, and inability to do tandem walking. His muscle strength was normal (5/5). The tendon reflexes were increased, and the sensations were normal. The Babinski signs were not elicited bilaterally. The MMSE score was 21/30, which indicated that he was mild cognitive impairment.

The older brother (II-2) born in 2005. He developed progressive slurred speech in the past three years. He had gait disturbance but could walk without an aid. He also had poor grades in physical tests. Neurological examination revealed dysarthria, bilateral nystagmus, and gait indicative of cerebellar ataxia, showing unstable to do the finger-to-nose test with left hand and tandem walking. The muscle strength of limbs was normal (5/5). The tendon reflexes were normal. The Babinski reflexes were negative bilaterally. Sensations were intact. The MMSE score was 28/30.

Their father (I-1), mother (I-2), and older sister (II-1) had no neurological symptoms and were normal in neurological examination.

The brain magnetic resonance imaging (MRI) of the proband (II-3) and his older brother (II-2) showed severe cerebellum atrophy, sulcus and cistern of cerebellum were widened and deepened (Figure. 1B and 1C). There was no obvious abnormality in the brain MRI of their mother (I-2) (Figure. 1D). Electromyography (EMG) of II-3 and II-2 were normal.

Genetic analysis

Genetic screening of the proband for repeat expansions forSCA1, 2, 3, 6, 7, 8, 10, 12, 17, 36, DRPLA, and Friedreich ataxia was performed and did not find any repeat expansions. Then whole-exome sequencing (WES) was performed and revealed a novel heterozygous 7-base duplicated variant in *TTBK2*, c.1211_1217dupAGGAGAA, which was confirmed by Sanger sequencing (Figure. 1E). The variant resulted in a frameshift mutation, forming a premature stop codon (p. N406Kfs*47). Further Sanger sequencing in the pedigree identified the same heterozygous p. N406Kfs*47 variant in the proband's older brother (II-2) and his asymptomatic mother (I-2). No variants of *TTBK2* were detected in his father (I-1) or his sister (II-1). Therefore, we analyzed percentage of the heterozygous variant in II-3, II-2, and I-2, which revealed a lower percentage in the asymptomatic mother (I-2) (0.19) compared to the proband (II-3) (0.39) and his brother (II-2) (0.38).

Discussion

SCA11 is a very rare progressive degenerative disease. The diagnosis of SCA11 mainly depends on clinical features and identification of mutations in *TTBK2*. We reported a Chinese SCA11 family with two affected patients carrying a novel *TTBK2* variant and an asymptomatic mutation carrier.

Both patients in this family developed typical clinical features of cerebellar ataxia around the age of 12 years and showed severe cerebellum atrophy on brain MRI. The heterozygous variant p. N406Kfs*47 was novel. According to the ACMG guideline, this variant could be graded as a pathogenic variant and was supported by the following evidence. First, pathogenic mechanism of the SCA11 causative TTBK2 mutations was loss of function, and this duplicated variant was a frameshift mutation (PVS1) (*12*). Second, this duplicated variant was presented neither in the 1000-ethnicity-matched control group, nor in the gnomAD and ExAC databases (PM2) (*13*).

Interestingly, the proband's asymptomatic mother (I-2) of the proband also carried the p. N406Kfs*47 mutation and showed no abnormalities on brain MRI. We hypothesized that percentage of the *TTBK2* variant in I-2 might be too low to result in clinical features or I-2 was chimeric, whose mosaic rate of the variant in the central nervous system was quite low.

To date, only seven SCA11 families have been reported. The clinical and genetic features of these patients are summarized in Table 1. All pedigrees had a familial history. Four pedigrees were identified in European countries, Denmark, Britain, France, and Germany, while three pedigrees were found in Asian countries, Pakistan, and China (*6, 7, 14, 15*). The pedigree reported in this study was the third SCA11 family in Chinese. Age of onset of patients with SCA11 ranged from early teens to forties, except the patient in the Danish family showed symptoms from the age of 4. All patients presented with lower-limbs onset ataxia; some may also have a bulbar onset at the same time. Most patients with SCA11 had no upper motor neuron signs, except active or hyperactive tendon reflexes were reported in the Pakistani family and the first Chinese family. All patients had no sensory involvements.

All the *TTBK2* mutations identified so far were heterozygous. Except the p. V1097A missense mutation in exon 15 found in the first Chinese family, the other *TTBK2* mutations were all frameshift mutations due to insertions and/or deletions (indels) of 1-2 bases pairs localized in the exon 12. The TTBK2 p. N406Kfs*47 mutation found in this study was a frameshift mutation due to a longer 7bp duplicated variant (c.1211_1217dupAGGAGAA), further delineating the genotypic spectrum of the rare SCA11 disease. All the mutations localized in the conserved C-terminal located domain of TTBK2 and distributed in the N-terminal of the region except the p. V1097A mutation (Figure. 1F). The *TTBK2* mutations either leaded to nonsense-mediated decay of the mutant transcripts or caused premature stop codons in the mRNA, producing truncated TTBK2 protein in different lengths. The nonsense-mediated decay or truncated TTBK2 protein significantly reduced kinase activity and also acted as a dominant negative allele that interfered with the function of normal TTBK2 protein (*7*). Furthermore, functional experiments should be done to determine the pathogenic mechanism of this duplicated mutation of *TTBK2* in the SCA11 family and provide more genetic information of this rare disease.

Declarations

Acknowledgments

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Ethical Approval

All investigations in this study had obtained informed consent and were approved by the ethics committee of Fujian Medical University Union Hospital.

Competing interests

The authors have no conflicts of interests.

Author Contributions

Y-Q. L., J-M. C., and Z-Y. Z. contributed to the design of the study. J-M. C. and Y-L. H. contributed to data acquisition. Y-Q. L. and J-M. C. contributed to data analysis. Y-Q. L. and Z-Y. Z. contributed to drafting the text and preparing the figure. All authors reviewed the manuscript.

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Availability of data and materials

The dataset used and analyzed for this study is available from the corresponding author upon request.

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Tables

Table 1. Summary of genetic and clinical features of patients with SCA11

Exon	Nucleotides Change	Amino acids change	Population	Gender	Familial history	Age of onset (y)	Site of onset	Cerebellum signs	UMN signs	Sensor involve
12	c.1205_1207delinsA	p.T402Kfs*48	Danish	Female	Yes	4	Lower limbs	Ophthalmoplegia, dysarthria, gait ataxia	No	No
12	c.1211_1217dupAGGAGAA	p. N406Kfs*47	Chinese	Male	Yes	12	Lower limbs	Nystagmus, gait ataxia	No	No
12	c.1284_1285delAG	p.E429Dfs*21	Pakistani	N/A	Yes	11	N/A	Nystagmus, dysarthria, gait ataxia	Hyperactive reflexes	No
12	c.1284dupA	p.E429Rfs*22	Chinese	Male	Yes	13	Lower limbs	Dysarthria, ophthalmoplegia, nystagmus, gait ataxia	Hyperactive reflexes	No
12	c.1306_1307delGA	p.D436Yfs*14	German	Male	Yes	44	Lower limbs	Dysarthria, ophthalmoplegia, nystagmus, gait ataxia	No	No
12	c.1306_1307delGA	p.D436Yfs*14	French	Male	Yes	48	Bulbar, lower limbs	Dysarthria, gait ataxia	No	No
12	c.1329_1330insA	p.R444Tfs*7	British	N/A	Yes	N/A	N/A	N/A	N/A	No
15	c.3290T>C	p.V1097A	Chinese	Female	Yes	44	Bulbar, lower limbs	Dysarthria, nystagmus, gait ataxia	Active reflexes	No

UMN: upper motor neuron; MRI: magnetic resonance imaging; N/A: not available.

Figures

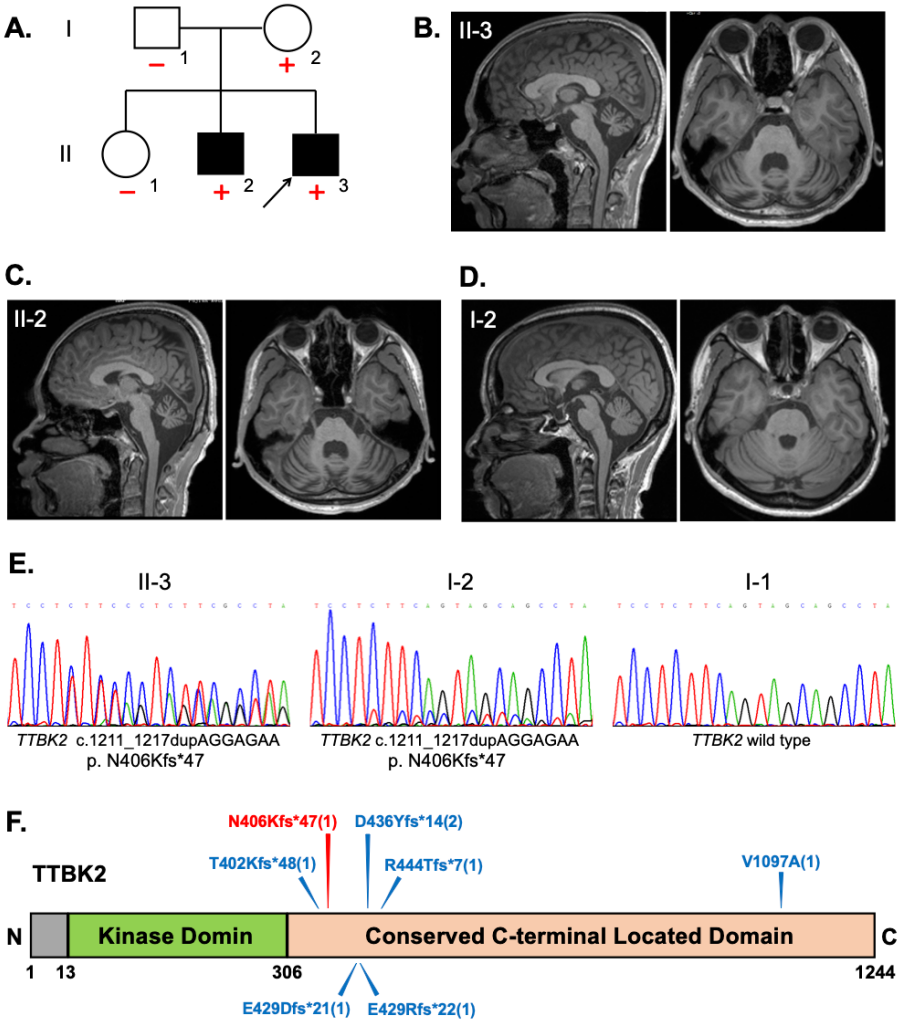

Figure 1

Clinical features of the pedigree and genetic analysis of the *TTBK2* p. N406Kfs*47 mutation. (A) The pedigree of the family with SCA11. +: with p. N406Kfs*47 mutation in *TTBK2*; -: with no mutation in *TTBK2*. (B) The sagittal and axial T1 images of brain MRI of the proband (II-3) demonstrated severe cerebellar atrophy. (C) The sagittal and axial T1 images of brain MRI of the proband's brother (II-2) demonstrated severe cerebellar atrophy. (D) The sagittal and axial T1 images of brain MRI of the proband's mother (I-2) demonstrated no obvious abnormality. (E) Sanger sequencing of the proband (II-3), his mother (I-2), and his father (I-1). (F) Schematic graph of the TTBK2 coding region, showing the position of mutations reported in previous reports (blue) and our research (red). Number "(1)" means this mutation has been reported in one case, number "(2)" means this mutation has been reported in two cases.