論　文

A novel missense variation (E308D) of *SPTBN2* in a Japanese patient with cerebellar ataxia

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*SPTBN2*にミスセンス変異を認めた脊髄小脳失調症

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【要　　旨】

本研究は、日本人の脊髄小脳失調症(Spinocerebellar ataxia: SCA)症例の遺伝解析研究である。本症例は臨床的にSCAタイプ5に矛盾せず、頭部ＭＲＩ画像では小脳萎縮が認められ、脳幹は保たれていた。遺伝学的解析により、SCA5の原因遺伝子であるβ−IIIスペクトリン遺伝子(*SPTBN2*)の新規非同義変異(c. 924G>C, p. E308D)が認められた。11番染色体に位置する本遺伝子は細胞骨格たんぱく質をコードしており、Ｅ308Ｄ変異はホットスポットに局在していた。日本人健常者507人を検討した結果、9人が同様の変異を有していた。したがって、SCA5の責任遺伝子内のミスセンス変異であるＥ308Ｄは責任変異ではない可能性が示唆された。

【キーワード】

脊髄小脳失調症5型　　常染色体優伝　　*SPTBN2*　　点変異　　E308D

Abstract

We describe a 54-year-old man with spinocerebellar ataxia (SCA). This patient presented clinically as SCA type 5 (SCA5). Brain MRI demonstrated cerebellar atrophy without brainstem involvement. Genetic analysis detected a novel nonsynonymous variant (c. 924 G>C, p. E308D) in *SPTBN*, the causative gene of SCA5. However, this variant was observed in nine of Japanese 507 unaffected controls, suggesting that E308D was not causative mutation but associated with SCA5.

Keywords

Spinocerebellar ataxia type 5 (SCA5), Autosomal dominant, SPTBN2, Point mutation, E308D

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Introduction

Spinocerebellar ataxia type 5 (SCA5, MIM：600224) is an autosomal dominant spinocerebellar ataxia (SCA) caused by mutations in the gene encoding β-III spectrin (*SPTBN2*, MIM：604985), which is located at 11q13.[1)2)] To date, eight pathogenic mutations (six heterozygous and two homozygous mutations), one possibly pathogenic mutation, and twelve single nucleotide polymorphisms including eight protein polymorphisms have been reported in *SPTBN2*.[2)-8)] Here, we report a SCA case with a Glu 308 Asp (E 308 D) variation in *SPTBN2*.

Materials and Methods

Exome sequencing

The Exome sequencing was conducted as previously described.[9)]

Sanger sequencing

The nucleotide sequences of the SNV region in the *SPTBN2* gene were evaluated by individual Sanger sequencing as previously described.[10)]

The nucleotide sequences of primers used in PCR and sequencing are

5'-TGAGGGGTCTTACTGCCCTA-3' as the forward primer and

5'-GTGCGGTAGGAGTTGAAGGA-3' as the reverse primer.

Case presentation

A 54-year-old Japanese man noticed gait disturbance at age 53. His elder brother deceased at age 62 was a patient with SCA. There is no familial record of ataxic symptoms in his parents who have passed away. On neurological examination, he showed bilateral horizontal gaze nystagmus that was inhibited by fixation, slightly slurred speech, slightly impaired vibration sense in the lower limbs, and mild

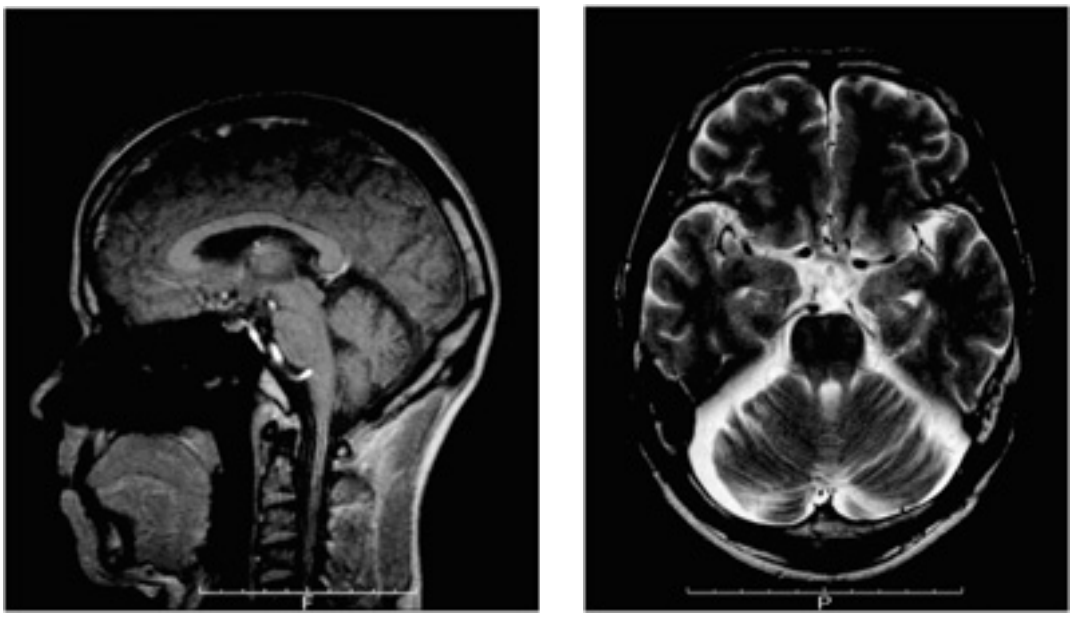

Figure 1　Brain MRI showing atrophy of the cerebellum without brainstem involvement

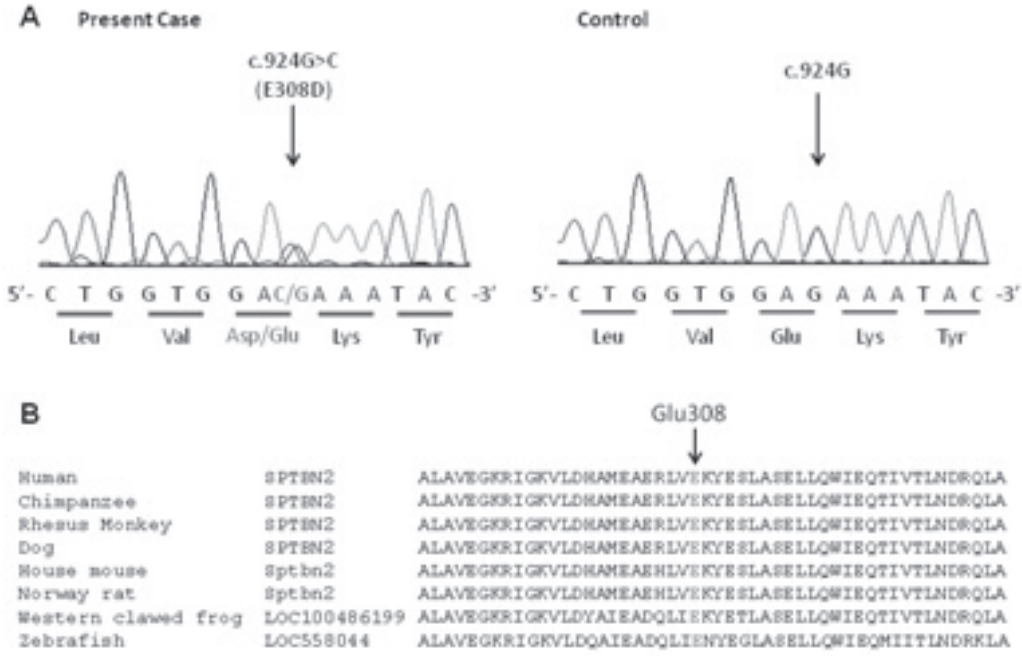

Figure 2 (A) A G-to-C substitution was detected at position 924, causing a glutamic acid-to-aspartic acid substitution (E308D).
(B) Alignment of amino acid sequences of SPTBN2 and counterparts in the region containing the E308D mutation among species. The 308th glutamic acid is highly conserved.

limb and truncal ataxia. Muscle tone of lower limbs was slightly hypertonic. Deep tendon reflexes were brisk, except for the Achilles tendon reflex. The Babinski sign was equivocal bilaterally. Muscle strength and autonomic functions were intact. Brain MRI demonstrated mildly cerebellar atrophy without brainstem involvement (Figure 1). Upon treatment with taltirelin (10 mg/day), the progression of the disease is very slow. Six years later, he can walk without any assistance and keep working.

He gave written informed consent for a genetic study. This study was approved by the Ethics Committees of Kurume University School of Medicine and Kyushu University, Faculty of Medicine. We found that the number and sequence of CAG and CTG repeats are normal in genes responsible for SCA1 (MIM：164400), SCA2 (MIM：183090), SCA3 (MIM：109150), SCA6 (MIM：183086), SCA7 (MIM：164500), SCA8 (MIM：698768), SCA12 (MIM：604326), SCA17 (MIM：607136), and DRPLA (MIM：125370). He did not have a -16C-T variation in the *PLEKHG4* gene (MIM：609526). Upon exome analysis, there were no novel nonsynonymous mutation in *TTBK2* (MIM：611695), *PRKCG* (MIM：176980), *KCNC3* (MIM：176264), *ITPR1* (MIM：147265), *IFRD1* (MIM：603502), *KCND3* (MIM：605411), *PDYN* (MIM：131340), *EEF2* (MIM：130610), *FGF14* (MIM：601515), *AFG3L2* (MIM：604581), *ODZ3* (MIM：610083), and *TGM6* (MIM：613900). Apart from that, we identified a novel heterozygous nonsynonymous single nucleotide variant, c. 924G>C, p. E308D in the *SPTBN2* gene (Figure 2A). Although the

PolyPhen-2 score of p.E308D is 0.084, the glutamic acid at the relevant position is broadly conserved in species from zebrafish to human (Figure 2B). After we examined 507 unrelated control samples (1014 chromosomes), c.924G>C was detected on nine chromosomes (0.89%).

Discussion

β-III spectrin is highly expressed in Purkinje cells, where it participates in the membrane stabilization of the glutamate transporter EAAT4.[11] Moreover, β-III spectrin is essential for the recruitment and maintenance of ankyrin R which has a critical role in modulating neuronal sodium channel activity and hence neuron excitability at the plasma membrane of Purkinje cell dendrites.[12]

At first, we diagnosed this patient with SCA5 for four reasons. First, this disease is likely to be an autosomal dominant SCA because there is no consanguineous marriage in this family. In addition, his elder brother was suffering from SCA. Second, genetic analyses denied the possibility of other autosomal dominant SCAs. Third, brain MRI of the present case demonstrated cerebellar atrophy and intact brainstem structures which coincides with the MRI of autosomal dominant SCA5.[13] Last, as shown in the Figure 3, residue 308 is located in the hotspot region for causative mu-

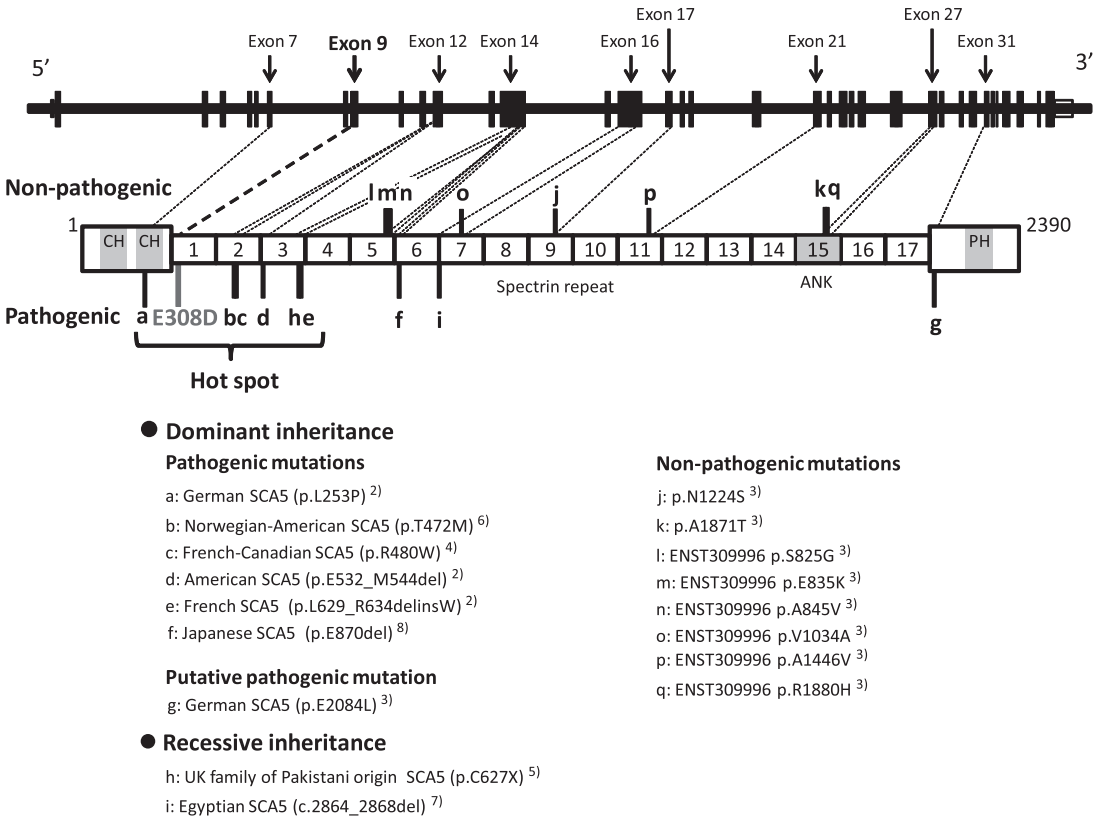

Figure 3　Illustration of previous mutation reports of *SPTBN2*. The upper row showed non-pathogenic mutations. The lower row showed pathogenic or possibly pathogenic mutations. E308D is located on hotspot for pathogenic mutations. CH: calponin homology domain, ANK: ankyrin binding domain, PH: pleckstrin homology domain

tations of SCA5 which have been demonstrated to alter the stability of EAAT4 and ankyrin R at the plasma membrane.[11)12)]

While it is possible that E308D is a partially penetrant amino-acid change with an allele frequency of 0.89% among control samples, we find it more likely that this variant is non-causative.

In conclusion, there might be many phenotypically silent nonsynonymous variants in *SPTBN2*. Further studies are necessary to clarify the pathogenesis of SCA5 through E308D. Also, clinicians should use caution in diagnosing SCA5 through genetic testing of *SPTBN2*.

Conflicts of interest/disclosures

We declare that we have no financial or other conflicts of interest in relation to this research and its publication. This work was supported by KAKENHI (Grant-in-Aid for Scientific Research (C)) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT). This work was also partly performed in the Cooperative Research Project Program of the Medical Institute of Bioregulation, Kyushu University.

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