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【 CASE REPORT 】

Autosomal Recessive Spinocerebellar Ataxia Type 10: A Report of a New Case in Japan

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Abstract:

Autosomal recessive spinocerebellar ataxia of type 10 (SCAR10) is a very rare neurodegenerative disease caused by mutations in the *TMEM16K* (*ANO10*) gene. This disorder is characterized by slowly progressive cerebellar ataxia and pyramidal signs inconstantly associated with cognitive decline, polyneuropathy, epilepsy, and vesicorectal dysfunction. To date, more than 40 cases have been reported in Europe. In contrast, only three cases have been identified in Asian countries. We herein report the third Japanese case of SCAR10 harboring a novel homozygous deletion mutation (c.616delG, p.Glu206Lysfs*17). This case presented with adult-onset slowly progressive spastic ataxia with cerebellar atrophy and mild cognitive decline.

Key words: SCAR10, TMEM16K, ANO10, spasticity, cerebellar ataxia

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Introduction

Autosomal recessive spinocerebellar ataxias (SCARs) are a heterogeneous group of neurodegenerative disorders that are primarily characterized by progressive ataxia with cerebellar atrophy. In addition, SCARs often involve the corticospinal tract, peripheral nerves, and non-nervous systems.

Autosomal recessive spinocerebellar ataxia type 10 (SCAR10, OMIM #613728), also known as autosomal recessive cerebellar ataxia type 3, is a very rare form of SCAR caused by either homozygous or compound heterozygous mutations in the transmembrane protein 16K (*TMEM16K*) gene, which is also called the anoctamin 10 (*ANO10*) gene (1). TMEM16K is an endoplasmic reticulum (ER)-resident lipid scramblase (2, 3). It is presumed that the loss of the TMEM16K function is linked to the development of SCAR10 through impaired endosomal retrograde trafficking and dysfunction in the endolysosomal pathway (4). However, the exact pathogenesis of SCAR10 has not been fully elucidated.

The most common clinical symptoms of SCAR10 are slowly progressive ataxia with marked cerebellar atrophy and pyramidal signs, such as spasticity and hyperreflexia. In addition to these common features, patients with this disorder can have cognitive decline, peripheral neuropathy, epilepsy, bladder and bowel dysfunction, or tortuosity of the conjunctival vessels (1, 5-7). Furthermore, a decrease in muscular or plasma coenzyme Q10 (CoQ10) levels has been observed in some cases (8, 9). In previous reports, most cases developed SCAR10 in adulthood. However, in some, the onset occurred before 10 years old.

More than 40 cases have been reported to date (1, 5-14), and most of them were of European descent. In contrast, only three cases - two in Japan and one in China - have been identified in Asian countries (11, 12, 14). We herein report a Japanese case of SCAR10 due to a novel homozygous single mutation in the *TMEM16K* (*ANO10*) gene.

Case Report

Our patient was a 55-year-old Japanese man. He was born

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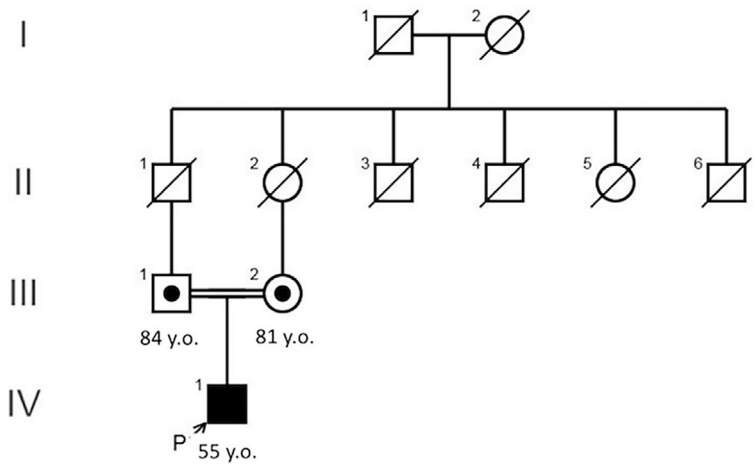

Figure 1. Pedigree of the Japanese family with the SCAR10 harboring c616delG mutation. Square: man, circle: woman, diagonal black line: deceased, black-filled symbol: affected individual, center-dot symbol: asymptomatic carrier, empty symbol: unaffected individual, P (arrow): proband. The parents of the proband are cousins.

to a consanguineous marriage (between cousins), had healthy parents, and had no family history of neurological diseases (Fig. 1). After a normal physical and mental development, he noticed unsteadiness while walking down stairs at 36 years old. Because his walking disorder had slowly progressed, he visited our neurology department at 39 years old. Since then, he has been regularly attending our hospital for rehabilitation and neurological evaluations. Because of further progression of the gait disturbance, he began to use a walker at 51 years old. He had neither episodes of loss of consciousness nor epilepsy.

On a neurological examination, he showed downbeat nystagmus, slurred speech, and limb ataxia. He also showed hyperreflexia in the upper and lower extremities and spasticity in the lower extremities. However, the patient showed no muscle wasting. Both the Hoffman's and Babinski reflexes were negative. Tortuosity of the conjunctival vessels was not observed by an ophthalmic examination. His cognitive function was evaluated using the revised version of Hasegawa's dementia scale (HDS-R), the most widely used brief dementia screening scale in Japan, at 39, 50, and 55 years old. His HDS-R scores declined with age to 28, 20, and 18 out of 30 (cut-off score 20/21). In addition, the Japanese adaptation of the Mini-Mental State Examination (MMSE-J) and the Frontal Assessment Battery (FAB) was performed at 55 years old, with scores of 24 out of 30 (cut-off score 26/27) and 11 out of 18 (cut-off score 11/12), respectively.

The results of nerve conduction studies, needle electromyography, and electric encephalography were normal. Brain magnetic resonance imaging (MRI) revealed marked atrophy of the cerebellum and mild atrophy of the frontal lobes (Fig. 2). Single-photon emission computed tomography revealed a decrease in cerebellar blood flow. In this case, the serum concentration of CoQ_{10} was 395 ng/mL (reference interval: 338-1,340 ng/mL).

After obtaining written informed consent from the patient, we analyzed the genes related to spinocerebellar ataxia (SCA). The gene analysis protocol was approved by the institutional ethics committee of the National Hospital Organization, Niigata National Hospital. The major types of SCA caused by nucleotide repeat expansion, namely SCA1, SCA2, SCA3, SCA6, SCA7, SCA8, SCA10, SCA17, and SCA31, were ruled out by gene testing. Next, we performed next-generation sequencing (NGS) of SCA-related genes using a multi-gene exome panel. NGS revealed a novel homozygous deletion mutation in the *TMEM16K* (*ANO10*) gene (NM_018075.3:c.616delG, p.Glu206Lysfs*17), which was validated by conventional Sanger sequencing (Fig. 3). Based on the clinical features, brain MRI findings, and NGS results, we diagnosed the patient with SCAR10.

Discussion

We encountered a Japanese man with SCAR10 harboring a novel homozygous deletion mutation in the *TMEM16K* (*ANO10*) gene, which induces a premature stop codon at the amino acid position 222 located within transmembrane domain 1. This is the third case of SCAR10 identified in Japan. The phenotype of this patient was consistent with previous reports, although he did not show certain inconstant symptoms of SCAR10, such as peripheral neuropathy, epilepsy, vesicorectal dysfunction, tortuosity of conjunctival vessels, and deficiency of serum CoQ_{10}.

In 2010, Vermeer et al. reported the first siblings with SCAR10 harboring a homozygous missense mutation (p.Leu 510Arg) in the *TMEM16K* (*ANO10*) gene in a consanguineous Dutch family. They further identified three additional mutations in a consanguineous Serbian family (homozygous for c.1150_1151delTT, p.Leu384fs) and in a French family (compound heterozygous for c.1476+1 G>T and c.1604delT, p.Leu535fs) (1). Since then, more than 40 cases of SCAR10 have been reported in European countries (1, 5-10, 13). In

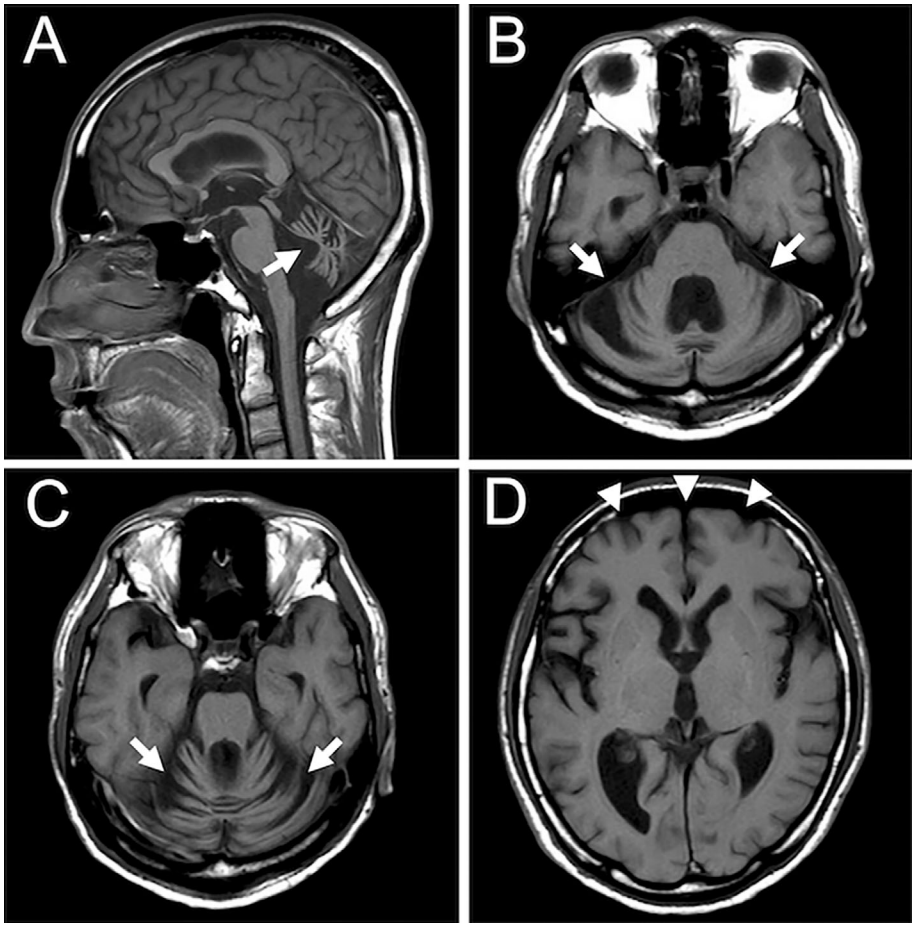

Figure 2. **Magnetic resonance imaging of the brain. All images are T1-weighted images. Mid-sagittal (A) and axial (B, C) images show marked cerebellar atrophy (arrows). Mild atrophy is observed in the frontal lobes (D, arrowheads).**

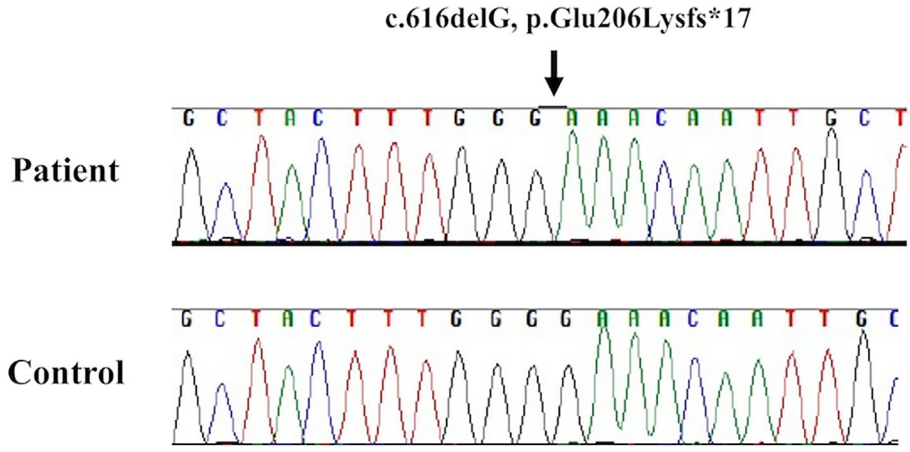

Figure 3. **Results of the *TMEM16K* (*ANO10*) gene mutation analysis. Electropherograms show the mutation of c.616delG (p.Glu206Lysfs*17) in the patient.**

contrast, only three cases, including two from Japan (11, 12) and one from China (14), have been reported in Asian countries to date.

The exact cause of the differences in the number of SCAR10 patients between Europe and Asia is unclear. The relatively high frequency of the c.132dupA mutation in Europe (estimated heterozygote carrier frequency is 1/184) (5) and a c.1150_1151delTT (p.Leu384fs) founder mutation in Roma/Gypsies (10) seem to partly contribute to the regional difference in the number of SCAR10 patients. To correctly compare the prevalence of the disease in Asian and European countries, more active screening of *TMEM16K*

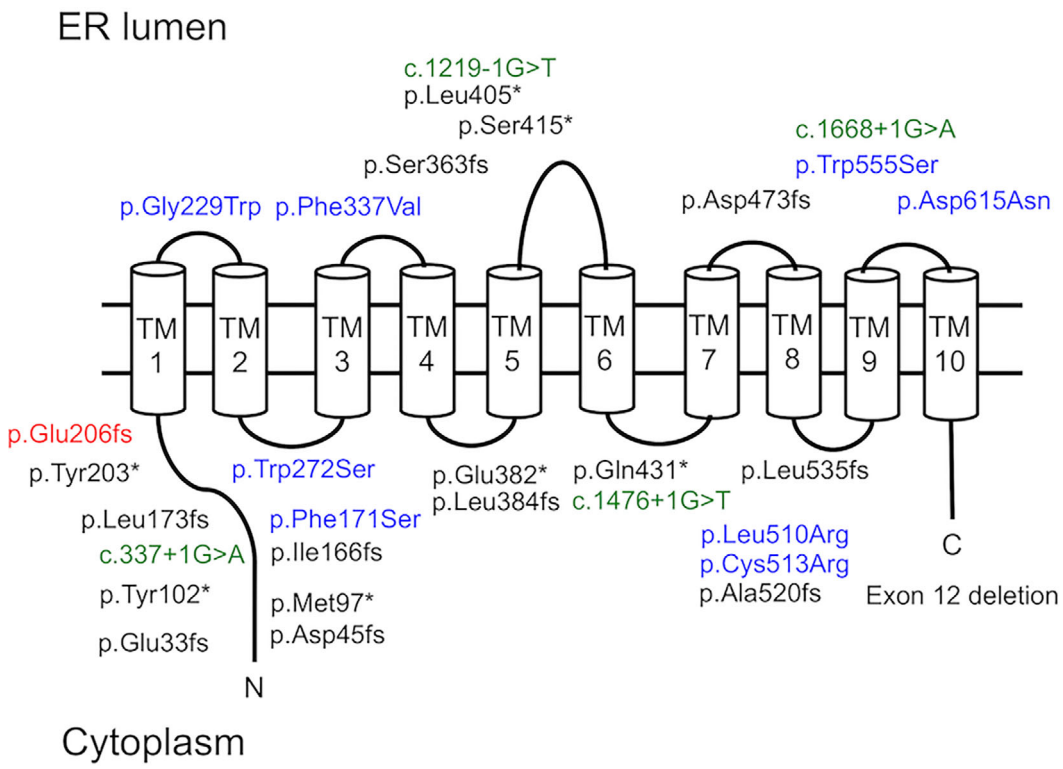

Figure 4. **Schematic structure of the TMEM16K (ANO10) protein and location of the *TMEM16K* (*ANO10*) gene mutations reported in the literature. ER: endoplasmic reticulum, TM: transmembrane domain. The frameshift mutation (c.616delG, p.Glu206fs) identified in this study is shown in red. Other truncating mutations are shown in black. Missense mutations are shown in blue. Splice-site mutations are shown in green. There were no mutational hotspots.**

gene mutations in patients with sporadic or autosomal recessive spastic ataxia should be performed in Asian countries.

TMEM16K (ANO10) is a member of the TMEM16 (ANO) family of proteins, which comprises 10 members (A, B, C, D, E, F, G, H, J, and K) in mammals. TMEM16 (ANO) family proteins are widely expressed in the body and are present in the plasma membrane or intracellular membranes. Many of the family member proteins are calcium-activated lipid scramblases that control the distribution of lipids between the leaflets of biological membranes. These proteins have distinct functions and are involved in various cellular activities, such as regulation of the neuronal cell function, smooth muscle contraction, tumorigenesis, and repair of skeletal muscle cells. Some of these are associated with neuromuscular diseases. For instance, TMEM16E (ANO5) is linked to limb-girdle muscular dystrophy type 2 L and Miyoshi-like disease (Miyoshi muscular dystrophy 3). TEMEM16C (ANO3) is also linked to autosomal dominant dystonia type 24 (15).

Human TMEM16K (ANO10) is an ER-resident calcium-dependent lipid scramblase with 10 transmembrane domains consisting of 660 amino acids (3). Phosphatidylserine (PS), a major phospholipid component of biological membranes, is abundant in the cytoplasmic leaflet and less abundant in the luminal leaflet of the ER membrane. The asymmetric distribution of PS in the ER membrane is disrupted by the scramblase activity of TMEM16K (2). TMEMK16 also acts as an interorganelle regulator of endosomal sorting, and loss of TMEM16K results in impaired endosomal retrograde trafficking and dysfunction in the endolysosomal pathway. It has also been demonstrated that TMEM16K knockout mice display progressive impairment of the neuromuscular function (4).

Truncating mutations, including nonsense and frameshift mutations, are common in SCAR10, although missense mutations and splice-site mutations have also been reported (5, 7, 14). The location of gene mutations is scattered over a wide area in the *TMEM16* (*ANO10*) gene, and no mutational hot spots have been found (Fig. 4). The genotype-phenotype correlation in SCAR10 is not clear (5, 7, 14). Homozygous frameshift mutations c.1150_1151delTT (p.Leu384fs) result in the early onset of symptoms and severe manifestations (1, 10). However, all Asian cases, including our case, which carry homozygous nonsense or frameshift mutations, showed an adult onset and mild to moderate symptoms (11, 12, 14) (Table).

Conclusion

We identified the third SCAR10 patient in Japan by NGS using a multi-gene exome panel. The epidemiology and clinical characteristics of SCAR10 remain unclear, especially in Asian populations. Mutation screening of the *TMEM16K*

Table. Clinical Features of Asian Patients with Autosomal Recessive Spinocerebellar Ataxia Type 10.

Case	Case 1	Case 2	Case 3	Case 4
Sex	Male	Male	Female	Male
AAO (years)	42	41	37	36
AALE (years)	58	66	41	55
Country of origin	Japan	Japan	China	Japan
Genotype	p.Tyr203*, Homo	p.Ile166Alafs*3, Homo	p.Ser415*, Homo	p.Glu206Lysfs*17, Homo
Cerebellar ataxia	Yes	Yes	Yes	Yes
Dysarthria	Yes	Yes	Yes	Yes
Nystagmus	No	N/A	Yes	Yes
Corticospinal tract	N/A	Increased DTRs, Babinski+	Brisk DTRs, Babinski+	Increased DTRs, Spasticity+
Peripheral neuropathy	Decreased vibration sense	Decreased vibration sense	No	No
Epilepsy	Episode of consciousness loss	N/A	No	No
Cognitive decline	No	No	No	Yes
Conjunctival vessels	No tortuosity	N/A	No tortuosity	No tortuosity
Increased CoQ10 level	N/A	N/A	N/A	No (serum)
MRI findings	Cerebellar and brain stem atrophy	Cerebellar atrophy	Cerebellar atrophy	Cerebellar atrophy
Reference	11	12	14	This case

AAO: age at onset, AALE: age at last evaluation, Homo: homozygous, N/A: not available, DTR: deep tendon reflex, Babinski+: positive Babinski sign, Spasticity+: spasticity in the lower extremeties, CoQ10: coenzyme Q10, MRI magnetic resonance imaging

gene using NGS for patients with SCARs will be a useful tool to clarify these cases.

The authors state that they have no Conflict of Interest (COI).

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