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

Izumi Aida¹, Tetsuo Ozawa², Kentaro Ohta¹, Hidehiko Fujinaka³, Kiyoe Goto¹ and Takashi Nakajima¹

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

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...
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 hyporeflexia 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 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 CoQ10 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 CoQ10.

In 2010, Vermeer et al. reported the first siblings with SCAR10 harboring a homozygous missense mutation (p.Leu510Arg) 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 CoQ10.
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 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.
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 | Sex  | AAO (years) | AALE (years) | Country of origin | Genotype | Cerebellar ataxia | Dysarthria | Nystagmus | Corticospinal tract | Peripheral neuropathy | Epilepsy | Cognitive decline | Conjunctival vessels | Increased CoQ10 level | MRI findings | Reference |
|------|------|-------------|--------------|------------------|-----------|------------------|------------|-----------|-------------------|----------------------|----------|------------------|-----------------------|----------------------|----------------|-----------|
| 1    | Male | 42          | 58           | Japan            | p.Tyr203*, Homo | Yes               | Yes        | No        | N/A               | Decreased vibration sense | No       | No               | No                    | N/A                  | Cerebellar and brain stem atrophy | 11        |
| 2    | Male | 41          | 66           | Japan            | p.Ile166Alafs*3, Homo | Yes               | Yes        | No        | Increased DTRs, Babinski+ | Decreased vibration sense | No       | No               | N/A                   | N/A                  | Cerebellar atrophy | 12        |
| 3    | Female | 37         | 41           | China            | p.Ser415*, Homo | Yes               | Yes        | Yes       | N/A               | Brisk DTRs, Babinski+ | No       | No               | No                    | N/A                  | Cerebellar atrophy | 14        |
| 4    | Male | 36          | 55           | Japan            | p.Glu206Lysfs*17, Homo | Yes               | Yes        | Yes       | Increased DTRs, Spasticity+ | No               | No       | No               | N/A                   | No (serum)           | Cerebellar atrophy | 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.

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

Acknowledgement
The authors thank the patient who provided the data for this report.

References
1. Vermeer S, Hoischen A, Meijer RP, et al. Targeted next-generation sequencing of a 12.5 Mb homozygous region reveals ANO10 mutations in patients with autosomal-recessive cerebellar ataxia. Am J Hum Genet 87: 813-819, 2010.
2. Tsuji T, Cheng J, Tatematsu T, et al. Predominant localization of phosphatidylinerine at the cytoplasmic leaflet of the ER, and its TMEM16K-dependent redistribution. Proc Natl Acad Sci USA 116: 13368-13373, 2019.
3. Bushell SR, Pike AC, Falzone ME, et al. The structural basis of lid spermatic and inactivation in the endoplasmic reticulum scramblase TMEM16K. Nat Commun 10: 3956, 2019.
4. Petkovic M, Oses-Prieto J, Burlingame A, Jan LY, Jan YN. TMEM16K is an interorganelle regulator of endosomal sorting. Nat Commun 11: 3298, 2020.
5. Renaud M, Anheim M, Kamsteeg EJ, et al. Autosomal recessive cerebellar ataxia type 3 due to ANO10 mutations: delineation and genotype-phenotype correlation study. JAMA Neurol 71: 1305-1310, 2014.
6. Bogdanova-Mihaylova P, Austin N, Alexander MD, et al. Anoctamin 10-related autosomal recessive cerebellar ataxia: comprehensive clinical phenotyping of an Irish sibship. Mov Disord Clin Pract 4: 258-262, 2017.
7. Nanetti L, Sarto E, Castaldo A, et al. ANO10 mutational screening in recessive ataxia: genetic findings and refinement of clinical phenotype. J Neurol 266: 378-385, 2019.
8. Balreira A, Boczonadi V, Barca E, et al. ANO 10 mutations cause ataxia and coenzyme Q10 deficiency. J Neurol 261: 2192-2198, 2014.
9. Chamard L, Sylvestre G, Koenig M, Magnin E. Executive and attentional disorders, epilepsy and porencephalic cyst in autosomal recessive cerebellar ataxia type 3 due to ANO10 mutation. Eur Neurol 75: 186-190, 2016.
10. Chamova T, Florez L, Guergueltcheva V, et al. ANO10 c.1150_1151del is a founder mutation causing autosomal recessive cerebellar ataxia in Roma/Gypsies. J Neurol 259: 906-911, 2012.
11. Maruyama H, Morino H, Miyamoto R, Murakami N, Hamano T. Exome sequencing reveals a novel ANO10 mutation in a Japanese patient with autosomal recessive spinocerebellar ataxia. Clin Genet 85: 296-297, 2014.
12. Yoshida K, Miyatake S, Kinoshita T, et al. ‘Cortical cerebellar atrophy’ dwindles away in the era of next-generation sequencing. J Hum Genet 59: 589-590, 2014.
13. Nieto A, Pérez-Flores J, Corral-Juan M, Matilla-Dueñas A, Martínez-Burgallo F, Mottón F. Cognitive characterization of SCAR10 caused by a homozygous c.132dupA mutation in the ANO10 gene. Neurocase 25: 195-201, 2019.
14. Yang S, Chen S, Jiao Y, et al. Autosomal recessive spinocerebellar ataxia caused by a novel homozygous ANO10 mutation in a consanguineous Chinese family. J Clin Neurol 16: 333-335, 2020.
15. Benarroch EE. Anoctamins (TMEM16 proteins): functions and involvement in neurological disease. Neurology 89: 722-729, 2017.