The First Case of Spinocerebellar Ataxia Type 8 in Monozygotic Twins

Jun Sawada, Takayuki Katayama, Takashi Tokashiki, Shiori Kikuchi, Kohei Kano, Kae Takahashi, Tsukasa Saito, Yoshiki Adachi, Yuji Okamoto, Akiko Yoshimura, Hiroshi Takashima, and Naoyuki Hasebe

Abstract:
Spinocerebellar ataxia type 8 (SCA8) is a rare hereditary cerebellar ataxia showing mainly pure cerebellar ataxia. We herein report cases of SCA8 in Japanese monozygotic twins that presented with nystagmus, dysarthria, and limb and truncal ataxia. Their ATXN8OS CTA/CTG repeats were 25/97. They showed similar manifestations, clinical courses, and cerebellar atrophy on magnetic resonance imaging. Some of their pedigrees had nystagmus but not ataxia. These are the first monozygotic twins with SCA8 to be reported anywhere in the world. Although not all subjects with the ATXN8OS CTG expansion develop cerebellar ataxia, these cases suggest the pathogenesis of ATXN8OS repeat expansions in hereditary cerebellar ataxia.

Key words: Spinocerebellar ataxia type 8 (SCA8), monozygotic twin, CTA/CTG repeats, magnetic resonance imaging

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Introduction
Spinocerebellar ataxia type 8 (SCA8) is an autosomal dominant cerebellar ataxia showing progressive relatively pure cerebellar ataxia (1, 2). Gait ataxia and cerebellar dysarthria are common initial symptoms, and clinical findings include nystagmus, limb incoordination, hyperactive tendon reflexes, spasticity, and diminished vibration perception (3).

This disease is caused by a trinucleotide CTG repeat expansion lying in the 3'-untranslated region of the gene called the ataxin 8 opposite strand (ATXN8OS) in human chromosome 13q21-21.33 (1). While normal alleles contain 15 to 50 repeats, expanded alleles contain 71-1,300 repeats or more (1, 4-6). The ATXN8OS CTG expansion appears to be toxic; however, not all subjects with the CTG expansion develop cerebellar ataxia. The phenotype of SCA8 varies more widely than those of the other types of SCA, and its spectrum variance is not well established (7). Furthermore, the CTG expansion has been detected in patients with other neuropsychiatric diseases, such as other neurodegenerative diseases (8-11) and psychiatric diseases (12), as well as in healthy controls (13). These findings have resulted in controversy surrounding testing for the ATXN8OS CTG expansion in ataxic individuals (14).

We herein report the first cases of ataxia in Japanese monozygotic twins with ATXN8OS gene expansions.

Case Report

Case 1 (III-5 in Fig. 1)
This patient was a Japanese woman who developed a staggering gait at 26 years old. She then noted disturbances in speech and swallowing. These symptoms gradually progressed, and she visited our hospital at 32 years old. She had histories of Kawasaki disease and meningitis at 3 and

1Division of Neurology, Department of Internal Medicine, Asahikawa Medical University, Japan, 2Department of Neurology, National Hospital Organization Okinawa Hospital, Japan, 3Department of Neurology, National Hospital Organization Matsue Medical Center, Japan and 4Department of Neurology and Geriatrics, Kagoshima University Graduate School of Medical and Dental Sciences, Japan
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17 years of age, respectively.

A neurological examination revealed a disturbance in saccadic eye movement, horizontal nystagmus, slurred speech, tongue peripheral atrophy, and ataxia of the limbs and trunk. She had the highest score in the Mini-Mental State Examination (30/30). Laboratory findings showed no marked abnormalities in peripheral blood counts or general biochemical tests. Her thyroid function was normal, and anti-thyroid antibody was negative. Vitamin B1, B12, and E and folate levels were not reduced, while lactate and pyruvate levels were not elevated. Brain magnetic resonance imaging (MRI) revealed atrophy of the cerebellum (Fig. 2A, B). Brain scintigraphy was not conducted.

**Case 2 (III-4 in Fig. 1)**

The older sister of Case 1, who was identified as a monozygotic twin at birth, had noted a mild disturbance in speech when she was a senior high school student. She also developed a staggering gait at 25 years old. She was admitted to our hospital at 31 years old. A neurological examination revealed a disturbance in saccadic eye movement, nystagmus in the left gaze, slurred speech, tongue peripheral atrophy, and ataxia of the limbs and trunk. Brain MRI showed atrophy of the cerebellum (Fig. 2C, D). Cerebral blood flow scintigraphy ($^{99}$ Tc-ethyl cysteinate dimer) revealed a de-

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**Figure 1.** Family tree of the present cases. Black circles indicate patients expressing cerebellar ataxia manifestations (Case 1: III-5, Case 2: III-4). I-3, III-1, and IV-4 presented with nystagmus but not ataxia.

**Figure 2.** Brain magnetic resonance imaging (MRI) of the twin cases (A, B: case 1, C, D: case 2). These images show atrophy of the cerebellar vermis and hemisphere (A, C: T2-weighted axial view, B, D: T1-weighted sagittal view).
creased uptake in the bilateral cerebellum (Fig. 3).

These monozygotic sisters were born in Tokyo. They had lived together until they were senior high school students (18 years old). They then moved to different prefectures and married and gave birth at around the same time.

Their maternal grandfather (I-3) and brother (III-1) as well as the second son (IV-4) of case 2 were noted to have nystagmus by their family. Other family members apart from the monozygotic sisters have not yet presented with ataxia or undergone brain MRI or a genetic analysis (Fig. 1).

Genetic testing

• Genomic DNA extraction

The genomic DNA of the patients was extracted from peripheral blood using a Gentra Puregene Blood kit (QIAGEN, Valencia, CA, USA) according to the manufacturer’s instructions. The protocol was reviewed and approved by the Institutional Review Board of Kagoshima University. All patients and family members provided their written informed consent to participate in this study.

• A triplet-repeat expansion analysis of known SCA genes and whole-exome sequencing

We screened for the expansion of triplet repeats associated with SCAs (SCA1, -2, -3, -6, -7, -8, -12, -31, and DRPLA) by standard polymerase chain reaction (PCR) methods using the GeneScan analysis software program with an ABI Prism 3130xl Genetic Analyzer (Foster City, CA, USA) as described previously (15). We also performed whole-exome sequencing on Case 1 to confirm the effects of other genes. Regarding the use of genomic DNA, 10-ng samples were amplified using the Ion AmpliSeq™ Exome RDY Kit (Thermo Fisher Scientific, Waltham, MA, USA), and libraries were prepared using the Ion Xpress™ Barcode Adapters 1-16 Kit according to the manufacturer’s instructions. Adaptor-ligated amplicon libraries were purified using the Agencourt AMPure XP System (Beckman Coulter Genomics, Danvers, MA, USA). The concentration of the library was assessed using the Applied Biosystems® StepOne™ Real-Time PCR system and Ion Library TaqMan™ Quantitation Kit, and equimolar amounts of each library were pooled together and run on an Ion Chef (Thermo Fisher Scientific, Inc.) for emulsion PCR and loaded onto a
PI v3 chip. This chip was run on the Ion Proton sequencing system according to the manufacturer’s instructions.

**Data analysis and variant interpretation**

Using the Torrent Suite software program, ver. 5.8.0, reads were aligned to the human reference genome (build GRCh37/hg19) and to the BED file designed using the Ion AmpliSeq Designer. DNA variants were called with the Torrent Variant Caller plug-in. The read depth and percentage of reads mapped to the target region (reads on target) were calculated using the Coverage Analysis plug-in.

We confirmed all previously reported pathogenic mutations by referencing the Human Gene Mutation Database Professional 2019.1 (https://portal.biobase-international.com/hgmd/pro). In addition, we checked all variants against global databases, including the 1,000 Genomes (http://www.internationalgenome.org), the Exome Sequencing Project (http://evs.gs.washington.edu/EVS), and the Exome Aggregation Consortium (http://exac.broadinstitute.org/), as well as against Japanese databases, including the integrative Japanese Genome Variation Database (iJGVD; https://ijgvd.mega bank.tohoku.ac.jp) and the Human Genetic Variation Database (http://www.hgvd.genome.med.kyoto-u.ac.jp). We also checked the variants against our in-house whole-exome sequencing database of individuals with non-SCAs. In silico analyses of variants were performed using SIFT (http://sift.jcvi.org), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2), PROVEAN (http://provean.jcvi.org/index.php), Mutation Assessor (http://mutationassessor.org), and Condel (http://bg.upf.edu/fannsdb). We completed the annotation process using the CLC Genomic Workbench software and an in-house R script. All suspected variants were validated using Sanger sequencing and interpreted according to the American College of Medical Genetics and Genomics standards and guidelines (16).

**Results**

A gene analysis of the sisters showed the expansion of ATXN8OS CTA/CTG repeats as 25/97 repeats (Fig. 4), but no abnormalities were noted in other ataxia loci, including SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, SCA17, and SCA31, or dentatorubropallidolysian atrophy. Based on these findings, the sisters were diagnosed with SCA8. Although they were treated with taltirelin hydrate, their ataxia gradually progressed.

Whole-exome sequencing revealed four rare variants in ataxia-related genes: FRMD7, CACNA1 G, STUB1, and UBR4 (Table). Difficulties were associated with evaluating the pathogenic significance of these rare gene variants because other family members besides the monozygotic sisters had not undergone a genetic analysis.

**Discussion**

The clinical presentation of SCA8 patients is characterized by cerebellar ataxia of varying severity, and ataxia occurs alone or in combination with other features. The identification of a triplet repeat expansion of the ATXN8OS gene on chromosome 13q21 in a genetic analysis has been critical for confirming a diagnosis of SCA8 (1). However, genetic testing must be interpreted with caution due to several diagnostic pitfalls.

Expanded ATXN8OS CTA/CTG repeats may rarely coexist with the expansion of SCA1, SCA3, SCA6, and SCA31 CAG repeats (3, 14, 17, 18); however, our genetic analysis ruled out this possibility. Furthermore, expanded ATXN8OS CTA/CTG repeats have been detected in other neurodegenerative diseases, including Parkinson’s disease (9, 19), multiple system atrophy (8), progressive supranuclear palsy (11), amyotrophic lateral sclerosis (19, 20), and Alzheimer’s disease (10), as well as psychiatric diseases (12) and even in healthy controls (13). Therefore, abnormal expansions alone are not sufficient to make a diagnosis of SCA8. In addition, incomplete penetrance of the ATXN8OS gene may result in difficulty reaching a diagnosis of SCA8 and lead to unusual and varied manifestations. With the accumulation of newly reported cases, it has become apparent that the clinical presentation of SCA8 varies (7). A disturbance in saccadic eye movement, nystagmus, dysarthria, and ataxia of the limbs and trunk, which are common manifestations of SCA8, were observed in our cases.

The cut-off value for the triplet length that genetically separates normal from affected individuals is controversial in SCA8 (5). The majority of symptomatic SCA8 patients had expansion lengths between 45 and 109, with greater than 80 repeats being frequently reported (7, 21). While 99% of

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**Figure 4.** PCR results for the triplet repeat region of spino-cerebellar ataxia (SCA) 8. The normal specimen (Lane 1) showed 25/26 repeats, and the positive control (Lane 4) showed 92/99 repeats. Case 1 (Lane 2) and Case 2 (Lane 3) examined in the present study both showed 23/95 repeats.
control subjects were shown to have an ATXN8OS gene CTA/CTG repeat length of between 2 and 37 (22). 0.5% of control subjects in Scotland had composite triplet repeat lengths of greater than 91 (23). Although some difficulties are associated with establishing a definite cut-off value for an abnormal repeat length, a repeat length of at least 50 is expected for affected individuals based on the largest published ATXN8OS case series to date (21). However, most studies in various countries, including Japan, suggest a repeat length greater than 80 to be in the abnormal range (3, 7). Based on these findings, the repeat size of 97 in our cases appeared to be pathogenic.

The incidence of SCA8 in Japanese ataxic subjects is rare and differs by area (1.1%-4.5%) (3, 4, 24). A survey of SCA patients in Hokkaido Prefecture, the northernmost region of Japan, detected no SCA8 patients (25). Among patients with sporadic cerebellar ataxia in a Japanese single-hospital cohort, abnormalities in spinocerebellar ataxia type 6 were the most common (36%), and the incidence of SCA8 was 3.6% (24). Aydin et al. reported that 5 out of 440 (1.1%) unrelated German patients suspected to be SCA-negative for the most common SCA subtypes (SCA1-3, SCA6, SCA7, and SCA17) had expanded ATXN8OS CTA/CTG alleles with 92-129 repeats (26). Since the phenotype of SCA8 markedly varies, and given that the spectrum of presentation has not yet been well defined, the diagnosis of SCA8 may frequently be missed (7). The incidence of SCA8 may thus be more frequent than current estimations.

Since monozygotic twins are considered to be genetically identical, discordance in the disease phenotype between monozygotic twins has been used in genetic research to clarify the contribution of genetic and environmental factors to disease development. However, recent studies have shown that monozygotic twins differ both genetically and epigenetically (27). Monozygotic twins that are phenotypically discordant for monogenic diseases have been described in cases of Huntington’s disease, familial Alzheimer’s disease, and SCA (28-30). In the SCA group with the CAG expansion forms, monozygotic twin cases showed different clinical features, with SCA2 and dentatorubropallidoluysian atrophy presenting in different twins (31, 32). In our case, the monozygotic sisters had lived in different locations and environments but had very few clinical differences and similar ages at the onset. Therefore, genetic factors, including the ATXN8OS repeat expansion, may affect disease development more intensely than environmental factors.

We performed whole-exome sequencing on ataxia-related genes for Case 1, which revealed four rare variants. These genes (FRMD7, CACNA1 G, STUB1, and UBR4) have been implicated in congenital nystagmus, SCA42, SCA 48 and SCA 16, and episodic ataxia, respectively (33-36). Evaluating the pathogenic significance of these rare gene variants has proven difficult because no family members other than the monozygotic sisters had undergone a genetic analysis. The pathogenic significance of these four rare variants therefore currently remains unclear; however, it cannot be denied
that these mutations may have affected the phenotype of the monozygotic sisters and other family members. For example, the nonsense mutation in FRMD7 should affect male family members who present with nystagmus because mutations in FRMD7 cause X-linked idiopathic congenital nystagmus (33). Further studies of ataxia-related genes, including in vivo experiments, and their influence on clinical manifestations are needed.

Genetic counseling is one of the most sensitive issues for SCA8 because of the complexity of its inheritance, low penetrance, and clinical variety. Family members (I-3, III-1, and IV-4) of the present cases had nystagmus but not ataxia. Although a genetic analysis of these family members has not yet been performed, they may have expanded ATXN8OS CTA/CTG repeats. Long-term follow-up and appropriate genetic counseling for the family members are needed.

In conclusion, we herein report the first cases of SCA8 in monozygotic twins anywhere in the world. SCA8 often exhibits intra-family differences in clinical manifestations, and there is controversy surrounding the pathogenesis of the ATXN8OS CTG expansion; however, these twin cases suggest the pathogenesis of ATXN8OS CTG expansions in hereditary cerebellar ataxia. A long-term observational study on these monozygotic twin cases and their family members will be important for clarifying the contributions of genetic factors to SCA8.

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

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References
1. Koob MD, Moseley ML, Schut LJ, et al. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat Genet 21: 379-384, 1999
2. Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurol 9: 885-894, 2010
3. Hu Y, Hashimoto Y, Ishii T, et al. Sequence configuration of spinocerebellar ataxia type 8 repeat expansions in a Japanese cohort of 797 ataxia subjects. J Neurol Sci 382: 87-90, 2017.
4. Ikeda Y, Shizuka M, Watanabe M, Okamoto K, Shoji M. Molecular and clinical analyses of spinocerebellar ataxia type 8 in Japan. Neurology 54: 950-955, 2000
5. Ikeda Y, Dalton JC, Moseley ML, et al. Spinocerebellar ataxia type 8: molecular genetic comparisons and haplotype analysis of 37 families with ataxia. Am J Hum Genet 75: 3-16, 2004
6. Todd PK, Paulson HL. RNA-mediated neurodegeneration in repeat expansion disorders. Ann Neurol 67: 291-300, 2010
7. Gupta A, Jankovic J. Spinocerebellar ataxia 8: variable phenotype and unique pathogenesis. Parkinsonism Relat Disord 15: 621-626, 2009
8. Factor SA, Qian J, Lava NS, Hubbard JD, Payami H. False-positive SCA8 gene test in a patient with pathologically proven multiple system atrophy. Ann Neurol 57: 462-463, 2005
9. Wu YR, Lin HY, Chen CM, et al. Genetic testing in spinocerebellar ataxia in Taiwan: expansions of trinucleotide repeats in SCA8 and SCA17 are associated with typical Parkinson’s disease. Clin Genet 65: 209-214, 2004
10. Sobrido MJ, Cholfin JA, Perlman S, Pulst SM, Geschwind DH. SCA8 repeat expansions in ataxia: a controversial association. Neurology 57: 1310-1312, 2001
11. Samukawa M, Hirano M, Saigoh K, et al. PSP-Phenotype in SCA 8: Case Report and Systemic Review. Cerebellum 18: 76-84, 2019
12. Lindgren JB, Yuan OP, Schalling M, et al. Long repeat tracts at SCA8 in major psychosis. Am J Med Genet 96: 873-876, 2000
13. Vincent JB, Neves-Pereira ML, Paterson AD, et al. An unstable trinucleotide-repeat region on chromosome 13 implicated in spinocerebellar ataxia: a common expansion locus. Am J Hum Genet 66: 819-829, 2000
14. Roda RH, Schindler AB, Blackstone C. SCA8 should not be tested in isolation for ataxia. Neurol Genet 3: e150, 2017
15. Hirano R, Takashima H, Okubo R, et al. Fine mapping of 16q-linked autosomal dominant cerebellar ataxia type III in Japanese families. Neurogenetics 5: 215-221, 2004
16. Li MM, Datto M, Duncanvege EI, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 19: 4-23, 2017
17. Sulek A, Hoffmann-Zacharska D, Zdzienicka E, Zaremba J. SCA8 repeat expansion coexists with SCA1--not only with SCA6. Am J Hum Genet 73: 972-974, 2003
18. Izumi Y, Maruyama H, Oda M, et al. SCA8 repeat expansion: large CTA/CTG repeat alleles are more common in ataxic patients, including those with SCA6. Am J Hum Genet 72: 704-709, 2003.
19. Kim JS, Son TO, Yoon J, Ki CS, Cho JW. Non-Ataxic Phenotypes of SCA8 Mimicking Amyotrophic Lateral Sclerosis and Parkinson Disease. J Clin Neurol 9: 274-279, 2013
20. Hirano M, Samukawa M, Isano C, Saigoh K, Nakamura Y, Kusunoki S. Noncoding repeat expansions for ALS in Japan are associated with the ATXN8OS gene. Neurol Genet 4: e252, 2018
21. Schols L, Bauer I, Zuhlike C, et al. Do CTG expansions at the SCA8 locus cause ataxia? Ann Neurol 54: 110-115, 2003
22. Brusco A, Cagnoli C, Franco A, et al. Analysis of SCA8 and SCA12 loci in 134 Italian ataxic patients negative for SCA1-3, 6 and 7 CAG expansions. J Neurol 249: 923-929, 2002
23. Zeman A. Spinocerebellar ataxia type 8 in Scotland: genetic and clinical features in seven unrelated cases and a review of published reports. J Neurol Neurosurg Psychiatry 75: 459-465, 2004
24. Sakakibara R, Tateno F, Kishi M, et al. Genetic Screening for Spinocerebellar Ataxia Genes in a Japanese Single-Hospital Cohort. J Mov Disord 10: 116-122, 2017
25. Sasaki H, Yabe I, Yamashita I, Tashiro K. Prevalence of triplet repeat expansion in ataxia patients from Hokkaido, the northernmost island of Japan. J Neurol Sci 175: 45-51, 2000
26. Aydin G, Dekomien G, Hoffjan S, Gerding WM, Epplen JT, Arning L. Frequency of SCA8, SCA10, SCA12, SCA36, FXTAS and C9orf72 repeat expansions in SCA patients negative for the most common SCA subtypes. BMC Neurol 18: 3, 2018
27. Kelelaar MR, Hofstra RMW, Hayden MR. What monozygotic twins discordant for phenotype illustrate about mechanisms infl-
28. Sturrock A, Leavitt BR. The clinical and genetic features of Huntington disease. J Geriatr Psychiatry Neurol 23: 243-259, 2010.
29. Brickell KL, Leverenz JB, Steinbart, et al. Clinicopathological concordance and discordance in three monozygotic twin pairs with familial Alzheimer’s disease. J Neurol Neurosurg Psychiatry 78: 1050-1055, 2007.
30. Durr A. Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet neurrol 9: 885-894, 2010.
31. Anderson JH, Christova PS, Xie TD, Schott KS, Ward K, Gomez CM. Spinocerebellar ataxia in monozygotic twins. Arch Neurol 59: 1945-1951, 2002.
32. Sato K, Yunoki T, Morimoto N, et al. Differential clinical features in a pair of monozygotic twins with dentatorubropallidoluysian atrophy. Eur J Neurol 18: e100-101, 2011.
33. Tarpey P, Thomas S, Sarvathanan N, et al. Mutations in FRMD7, a newly identified member of the FERM family, cause X-linked idiopathic congenital nystagmus. Nat Genet 38: 1242-1244, 2006.
34. Coutelier M, Blesneac I, Monteil A, et al. A Recurrent Mutation in CACNA1 G Alters Cav3.1 T-Type Calcium-Channel Conduction and Causes Autosomal-Dominant Cerebellar Ataxia. Am J Hum Genet 97: 726-737, 2015.
35. Genis D, Ortega-Cubero S, San Nicolas H, et al. Heterozygous STUB1 mutation causes familial ataxia with cognitive affective syndrome (SCA48). Neurology 91: e1988-e1998, 2018.
36. Conroy J, McGettigan P, Murphy R, et al. A novel locus for episodic ataxia: UBR4 the likely candidate. Eur J Hum Genet 22: 505-510, 2014.

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