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The evaluation of polyglutamine repeats in autosomal dominant Parkinson's disease

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ABSTRACT

We evaluated the contributions of various polyglutamine (polyQ) disease genes to Parkinson's disease (PD). We compared the distributions of polyQ repeat lengths in 8 common genes (*ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, and *HTT*) in 299 unrelated patients with autosomal dominant PD (ADPD) and 329 normal controls. We also analyzed the possibility of genetic interactions between *ATXN1* and *ATXN2*, *ATXN2* and *ATXN3*, and *ATXN2* and *CACNA1A*. Intermediate-length polyQ expansions (>24 Qs) of *ATXN2* were found in 7 ADPD patients and no controls (7/299 = 2.34% and 0/329 = 0%, respectively; $p = 0.0053 < 0.05/8$ after Bonferroni correction). These patients showed typical L-DOPA-responsive PD phenotypes. Conversely, no significant differences in polyQ repeat lengths were found between the ADPD patients and the controls for the other 7 genes. Our results may support the hypothesis that *ATXN2* polyQ expansion is a specific predisposing factor for multiple neurodegenerative diseases.
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1. Introduction

Several genes other than the "PARK" genes are suspected to be responsible for parkinsonism. Mutations of these genes sometimes confer symptoms that clinically mimic idiopathic Parkinson's disease (PD) and present radiological or pathologic findings characteristic of PD (Klein et al., 2009). These genes include the polyglutamine (polyQ) disease genes: *HTT* (Walker, 2007), *ATXN1* (Dubourg et al., 1995), *ATXN2* (Charles et al., 2007; Furtado et al., 2004; Gwinn-Hardy et al., 2000), *ATXN3* (Lu et al., 2004a; Subramony et al., 2002), *CACNA1A* (Kim et al., 2010), and *TBP* (Kim et al., 2009). Of these genes, it has been suggested that intermediate-length polyQ expansions in *ATXN2* and *TBP* are associated with PD (Charles et al., 2007; Furtado et al., 2004; Kim et al., 2009).

In addition, intermediate-length polyQ expansions (24–33 Qs) in *ATXN2* have recently been suggested as a risk factor for amyotrophic lateral sclerosis (ALS) (Chen et al., 2011; Elden et al., 2010). This observation has inspired several studies investigating how intermediate-length expansions of various polyQ disease genes contribute to neurodegenerative diseases other than those with which they were originally associated (Gispert et al., 2012; Lee et al., 2011b; Ross et al., 2011).

Based on these findings and the suggestion that polyQ diseases may share common pathogenic mechanisms (Al-Ramahi et al., 2007; Bertoni et al., 2011; Chen and Burgoyne, 2012), we hypothesized that polyQ disease genes in general might play a role in PD. We focused on autosomal dominant PD (ADPD) because polyQ neurodegenerative diseases generally have an AD mode of inheritance, and we compared the distribution of polyQ repeat lengths in 8 common genes between ADPD patients and normal controls.

2. Methods

We conducted genetic analyses of *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, and *HTT* in a Japanese cohort with ADPD and normal controls. In this study, we classified the mode of

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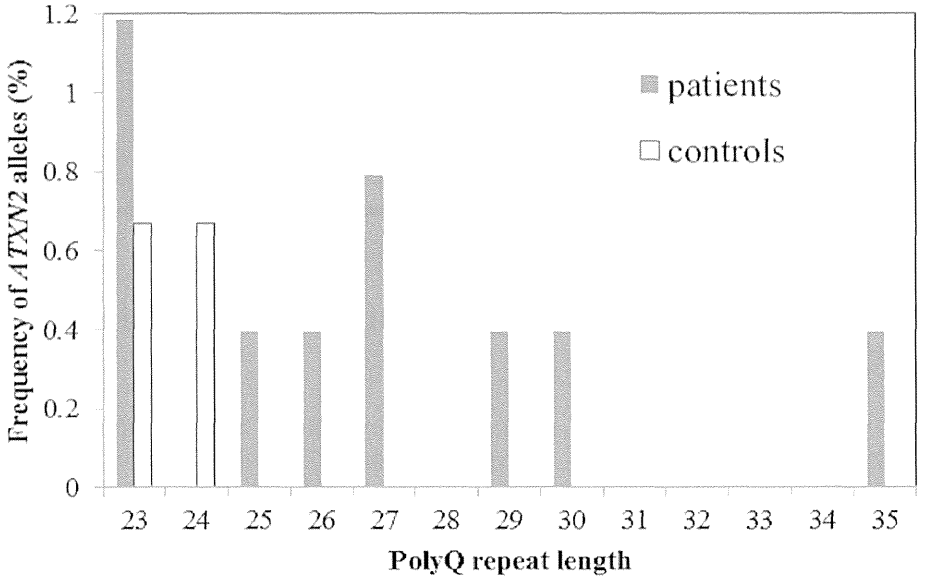

PolyQ length	19	20-21	22	23	24	25	26	27	28	29	30	31-34	35
Patients	1	0	288	3	0	1	1	2	0	1	1	0	1
Controls	0	0	325	2	2	0	0	0	0	0	0	0	0

Fig. 1. The distribution of polyglutamine (polyQ) repeat lengths of *ATXN2* in autosomal dominant Parkinson's disease patients and normal controls. The histogram shows only subjects with ≥23 repeats.

inheritance as autosomal dominant when a family included affected members in 2 consecutive generations. The diagnosis of PD was confirmed by the participating neurologists based on established criteria (Hughes et al., 1992).

We recruited the study subjects from the gene bank of our institution. We selected 299 unrelated patients with ADPD (169 women and 130 men; age at onset [AAO] = 57.7 ± 13.6-year-old [standard deviation], range 17–85 years) from families with unexplained pathogenesis, that is, those with no known pathogenic mutations in the *SNCA*, *PARK2*, *LRRK2*, and *VPS35* genes. A total of 329 healthy unrelated volunteers with no individual or family history of neurodegenerative disease (203 women and 126 men; age at examination = 57.5 ± 11.8-year-old [standard deviation], range 23–88 years) were examined as normal controls. Blood samples were obtained from the patients and controls, all of whom gave informed consent. Our institutional ethics committee approved the genetic study.

DNA was extracted from lymphocytes using standard methods. The polyQ repeat lengths in the polyQ disease genes were detected using capillary electrophoresis with fluorescent 5′-6-fluorescein amidite (FAM)-labeled forward primers. The primer sequences and polymerase chain reaction conditions are described in Supplementary Table 1. The polymerase chain reaction products were mixed with the LIZ-500 size standard (Applied Biosystems, Foster City, CA, USA) and processed on an Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems) for size determination. The sizes of the repeats were determined with GeneMapper 3.7 software (Applied Biosystems).

Statistical analysis was performed using JMP 8 software (SAS Institute, Cary, NC, USA). We evaluated the association between ADPD and the polyQ repeat lengths of each gene using 2-tailed Fisher exact tests, as previously described (Gispert et al., 2012; Lee et al., 2011a; Ross et al., 2011). A p value <0.05/8 after Bonferroni correction was considered significant (8 is for the number of genes investigated in the present study.).

3. Results

3.1. Molecular genetic analysis

The range of repeat lengths in *ATXN2* was between 19 and 35. Most patients (95.6% of patients with ADPD and 98.6% of the controls) had a repeat length of 22, as reported in previous studies (Lee et al., 2011a; Pulst et al., 1996). Of the 253 patients with ADPD, 7 harbored repeat lengths longer than 24, whereas none of the controls did (2.8% and 0%, respectively; $p = 0.0053$, Fig. 1 and Table 1).

No substantial differences in the repeat lengths in *ATXN1*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, or *HTT* were observed between the ADPD patients and controls (Table 1 and Supplementary Fig. 1).

We supplementarily sequenced the entire coding exons and exon and/or intron boundaries of glucocerebrosidase gene (*GBA*) in

Table 1
Fisher exact tests of polyQ repeat lengths between ADPD patients and controls

PolyQ disease gene	PolyQ repeat length	Conventional normal range[a]	Difference between ADPD patients and controls?
ATXN1	21–36 19–35	6–44	No
ATXN2	25–35Qs: 2.3% of ADPD, 0% of control	14–31	Yes, $p = 0.0053$ (<0.05/8), OR = ∞
ATXN3	13–46	11–44	No
CACNA1A	5–18	4–18	No
ATXN7	1–10	4–19	No
TBP	30–40	25–42	No
ATN1	12–36	6–35	No
HTT	15–35	6–34	No

Key: ADPD, autosomal dominant Parkinson's disease; Q, glutamine.
[a] The consensus normal ranges of the polyQ repeat lengths associated with the corresponding disease (e.g., *ATXN1* for SCA1) (Hands et al., 2008; Sequeiros et al., 2010).

the 7 probands with intermediate *ATXN2* polyQ expansion, because rare *GBA* mutations have been considered to be a risk factor for PD (Li et al., 2013; Mitsui et al., 2009); no *GBA* mutation was found in these 7 probands.

3.2. *Pedigree and clinical information for the 7 probands with* ATXN2 *polyQ repeat lengths >24*

Fig. 2 shows the pedigrees of the 7 probands with *ATXN2* polyQ repeat lengths >24 and their families. In family A, AII-2 presented with resting tremor in the bilateral lower extremities and left-dominant bradykinesia, which were responsive to L-DOPA and selegiline. AIII-1, who experienced rigidity and resting tremor predominantly in the left extremities, presented with tongue and jaw tremor (Supplementary Table 2). All these signs were relieved by pramipexole. AIII-3 was reportedly initially diagnosed with essential tremor because her first sign was bilateral postural tremor. She underwent left and right thalamotomy at a 1-year interval. She showed hyperreflexia in the lower extremities, but this symptom was presumably because of cervical spondylosis, for which surgical decompression was performed. AIV-2 and AIV-3, who inherited an intermediate-length polyQ expansion of 35 Qs, were not affected at the time of this study.

In family B, BI-2 was affected at an older age than her offspring, although their genotypes were the same, and all had L-DOPA-responsive parkinsonism with laterality (Supplementary Table 2).

In family C, CII-2 was diagnosed with Parkinson's disease with dementia. Although her parents were consanguineous, her polyQ *ATXN2* lengths were heterozygous (29/22).

All other members of the 7 families showed L-DOPA-responsive parkinsonism with laterality and were free of motor neuron signs, cerebellar ataxia, and saccadic eye movement disorder. None was

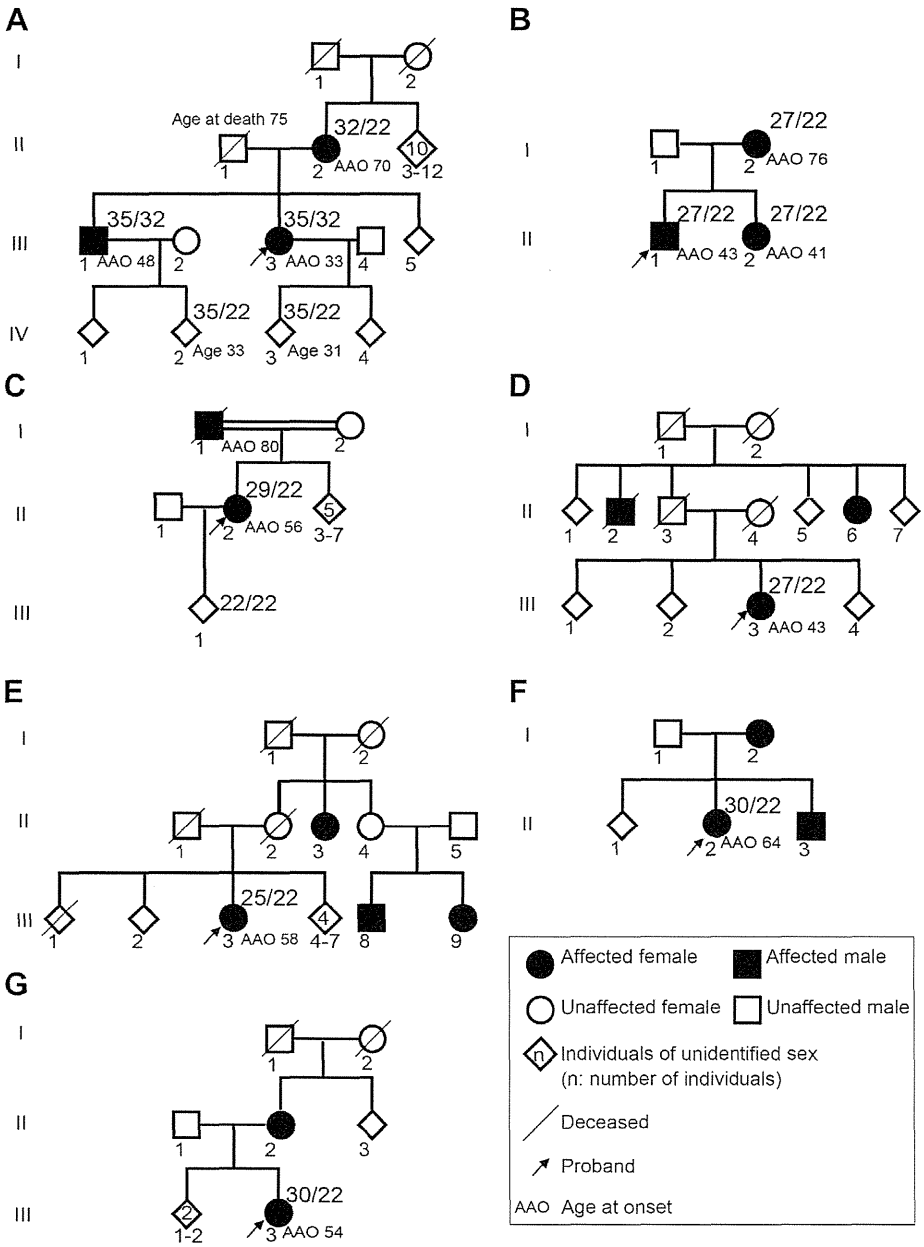

Fig. 2. The pedigrees of 7 families in which the proband has an *ATXN2* polyQ repeat length >24. *ATXN2* repeat lengths are listed previously and to the right of the pedigree symbols of the genotyped individuals.

reported to have any significant brain magnetic resonance imaging abnormality (Supplementary Table 2).

4. Discussion

We investigated the distributions of the polyQ repeat lengths of 8 common polyQ disease genes (*ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, *ATXN7*, *TBP*, *ATN1*, and *HTT*) in patients with ADPD. PolyQ repeat lengths >24 in *ATXN2* were significantly more common in the patients than in the controls. To the best of our knowledge, there have been only 2 similar studies investigating the distribution of *ATXN2* polyQ repeat lengths in PD patients and controls to date (Gispert et al., 2012; Ross et al., 2011). Although both previous studies failed to prove any significant difference, one (Gispert et al., 2012) showed that PD patients tended to have longer repeat lengths, consistent with our results. In the other previous study (Ross et al., 2011), the controls might have included some number of presymptomatic patients because the mean age of the controls was lower than that of the PD patients.

In reference to the recent studies concerning the effect of polyQ repeat length on neurodegenerative disease, we screened for a threshold of the normal *ATXN2* polyQ repeat length around a range from 24 to 34 (Charles et al., 2007; Chen et al., 2011; Elden et al., 2010; Gispert et al., 2012; Lee et al., 2011a, 2011b; Ross et al., 2011). The distribution of our patients differed significantly from that of controls only when the cutoff was set to 25. This may be much lower than the threshold for *ATXN2*-related PD adopted by previous studies (Charles et al., 2007), but it is possible that the cutoff for *ATXN2* polyQ repeat length and its influence on PD may vary from population to population, as is the case for ALS, as indicated in a previous study (Lee et al., 2011b). Such variation of the threshold would be consistent with the observation that previous reports of *ATXN2*-associated PD have mainly been from East Asian populations (Charles et al., 2007; Klein et al., 2009; Lu et al., 2004b; Sun et al., 2011; Wang et al., 2009). Additional factors, such as cis- and trans-acting genetic elements, non-allelic genetic modifiers, and stochastic and environmental factors (Charles et al., 2007; Pulst et al., 2005), might have enhanced the toxicity of *ATXN2* intermediate-length polyQ expansion in our population.

We described the details of family members with *ATXN2* intermediate-length expansions (>24 Qs, Fig. 2 and Supplementary Table 2). These patients generally manifested typical PD phenotypes without motor neuron signs, cerebellar ataxia, or saccadic eye movement disorder, as was stated in previous reports (Furtado et al., 2004; Klein et al., 2009). A correlation between the association of AAO and polyQ repeat length was not clearly present or absent in our patients with repeat lengths of *ATXN2* > 24, as previously observed (Furtado et al., 2002, 2004; Payami et al., 2003; Sun et al., 2011). For example, in family A, members of the third generation had earlier AAOs than did their mother. However, there was a gap between the AAOs of AIII-1 and AIII-3, even though their genotypes were the same. In addition, AIII-1 and AIII-3 had 2 allele expansions (35/32 Qs) instead of a single allele expansion, which might have caused their early onsets (Ragothaman et al., 2004). The 35Q alleles may have been inherited "as is" from AII-1, who reportedly had no neurologic disorder, although it is also possible that an expansion occurred upon transmission. Thus, AAOs might be affected by features other than polyQ repeat length, such as genetic and epigenetic factors.

In the present study, we did not find any association between the ADPD phenotype and the repeat lengths of polyQ disease genes other than *ATXN2*. This result implies that the contribution of *ATXN2* to ADPD is because of the specific effects of this gene rather than the presence of the polyQ expansion itself, as reported in a previous study of ALS (Lee et al., 2011a). This result might appear to be inconsistent with recent reports suggesting that the intermediate polyQ expansion of *TBP* is likely to be a risk factor for PD (Kim et al., 2009; Wu et al., 2004; Xu et al., 2010; Yun et al., 2011). However, because those reports did not provide significant evidence, and because all of these studies were performed in East Asian patients, further evidence should be accumulated.

As a supplementary analysis, we also applied a multiple logistic regression including the product terms *ATXN1* × *ATXN2*, *ATXN2* × *ATXN3*, and *ATXN2* × *CACNA1A* to screen for some interactions among these polyQ disease gene combinations, based on previous studies showing the possibility of interaction among these polyQ genes (Al-Ramahi et al., 2007; Jardim et al., 2003; Lessing and Bonini, 2008; Pulst et al., 2005). However, no significant difference was detected between the PD patients and controls (with a threshold *p*-value of 0.05, Supplementary Table 3).

In conclusion, an intermediate-length polyQ expansion of *ATXN2* is likely to contribute to the pathogenesis of ADPD, either directly causing the PD phenotype or modifying the effects of unknown genes on the PD phenotype. Our results add to the recent finding that intermediate-length polyQ repeat expansions of *ATXN2* may be a contributing factor in multiple neurodegenerative diseases.

Disclosure statement

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2014.01.022.

References

Al-Ramahi, I., Perez, A.M., Lim, J., Zhang, M., Sorensen, R., de Haro, M., Branco, J., Pulst, S.M., Zoghbi, H.Y., Botas, J., 2007. dAtaxin-2 mediates expanded ataxin-1-induced neurodegeneration in a Drosophila model of SCA1. PLoS Genet. 3, e234. http://dx.doi.org/10.1371/journal.pgen.0030234.

Bertoni, A., Giuliano, P., Galgani, M., Rotoli, D., Ulianich, L., Adornetto, A., Santillo, M.R., Porcellini, A., Avvedimento, V.E., 2011. Early and late events induced by polyQ-expanded proteins: identification of a common pathogenic property of polyQ-expanded proteins. J. Biol. Chem. 286, 4727–4741. http://dx.doi.org/10.1074/jbc.M110.156521.

Charles, P., Camuzat, A., Benammar, N., Sellal, F., Destee, A., Bonnet, A.M., Lesage, S., Le Ber, I., Stevanin, G., Durr, A., Brice, A., 2007. Are interrupted SCA2 CAG repeat expansions responsible for parkinsonism? Neurology 69, 1970–1975. http://dx.doi.org/10.1212/01.wnl.0000269323.21969.db.

Chen, X., Burgoyne, R.D., 2012. Identification of common genetic modifiers of neurodegenerative diseases from an integrative analysis of diverse genetic screens in model organisms. BMC Genomics 13, 71. http://dx.doi.org/10.1186/1471-2164-13-71.

Chen, Y., Huang, R., Yang, Y., Chen, K., Song, W., Pan, P., Li, J., Shang, H.F., 2011. Ataxin-2 intermediate-length polyglutamine: a possible risk factor for Chinese patients with amyotrophic lateral sclerosis. Neurobiol. Aging 32, 1925.e1–1925.e5. http://dx.doi.org/10.1016/j.neurobiolaging.2011.05.015.

Dubourg, O., Durr, A., Cancel, G., Stevanin, G., Chneiweiss, H., Penet, C., Agid, Y., Brice, A., 1995. Analysis of the SCA1 CAG repeat in a large number of families with dominant ataxia: clinical and molecular correlations. Ann. Neurol. 37, 176–180. http://dx.doi.org/10.1002/ana.410370207.

Elden, A.C., Kim, H.J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P.J., Rub, U., Auburger, G., Trojanowski, J.Q., Lee, V.M., Van Deerlin, V.M., Bonini, N.M., Gitler, A.D., 2010. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466, 1069–1075. http://dx.doi.org/10.1038/nature09320.

Furtado, S., Farrer, M., Tsuboi, Y., Klimek, M.L., de la Fuente-Fernandez, R., Hussey, J., Lockhart, P., Calne, D.B., Suchowersky, O., Stoessl, A.J., Wszolek, Z.K., 2002. SCA-2 presenting as parkinsonism in an Alberta family: clinical, genetic, and PET findings. Neurology 59, 1625–1627.

Furtado, S., Payami, H., Lockhart, P.J., Hanson, M., Nutt, J.G., Singleton, A.A., Singleton, A., Bower, J., Utti, R.J., Bird, T.D., de la Fuente-Fernandez, R., Tsuboi, Y., Klimek, M.L., Suchowersky, O., Hardy, J., Calne, D.B., Wszolek, Z.K., Farrer, M., Gwinn-Hardy, K., Stoessl, A.J., 2004. Profile of families with parkinsonism-predominant spinocerebellar ataxia type 2 (SCA2). Mov. Disord. 19, 622–629. http://dx.doi.org/10.1002/mds.20074.

Gispert, S., Kurz, A., Waibel, S., Bauer, P., Liepelt, I., Geisen, C., Gitler, A.D., Becker, T., Weber, M., Berg, D., Andersen, P.M., Kruger, R., Riess, O., Ludolph, A.C., Auburger, G., 2012. The modulation of amyotrophic lateral sclerosis risk by ataxin-2 intermediate polyglutamine expansions is a specific effect. Neurobiol. Dis. 45, 356–361. http://dx.doi.org/10.1016/j.nbd.2011.08.021.

Gwinn-Hardy, K., Chen, J.Y., Liu, H.C., Liu, T.Y., Boss, M., Seltzer, W., Adam, A., Singleton, A., Koroshetz, W., Waters, C., Hardy, J., Farrer, M., 2000. Spinocerebellar ataxia type 2 with parkinsonism in ethnic Chinese. Neurology 55, 800–805.

Hands, S., Sinadinos, C., Wyttenbach, A., 2008. Polyglutamine gene function and dysfunction in the ageing brain. Biochim. Biophys. Acta 1779, 507–521. http://dx.doi.org/10.1016/j.bbagrm.2008.05.008.

Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry 55, 181–184.

Jardim, L., Silveira, I., Pereira, M.L., do Ceu Moreira, M., Mendonca, P., Sequeiros, J., Giugliani, R., 2003. Searching for modulating effects of SCA2, SCA6 and DRPLA CAG tracts on the Machado-Joseph disease (SCA3) phenotype. Acta Neurol. Scand. 107, 211–214.

Kim, J.M., Lee, J.Y., Kim, H.J., Kim, J.S., Kim, Y.K., Park, S.S., Kim, S.E., Jeon, B.S., 2010. The wide clinical spectrum and nigrostriatal dopaminergic damage in spinocerebellar ataxia type 6. J. Neurol. Neurosurg. Psychiatry 81, 529–532. http://dx.doi.org/10.1136/jnnp.2008.166728.

Kim, J.Y., Kim, S.Y., Kim, J.M., Kim, Y.K., Yoon, K.Y., Lee, B.C., Kim, J.S., Paek, S.H., Park, S.S., Kim, S.E., Jeon, B.S., 2009. Spinocerebellar ataxia type 17 mutation as a causative and susceptibility gene in parkinsonism. Neurology 72, 1385–1389. http://dx.doi.org/10.1212/WNL.0b013e3181a18876.

Klein, C., Schneider, S.A., Lang, A.E., 2009. Hereditary parkinsonism: Parkinson disease look-alikes—an algorithm for clinicians to "PARK" genes and beyond. Mov. Disord. 24, 2042–2058. http://dx.doi.org/10.1002/mds.22675.

Lee, T., Li, Y.R., Chesi, A., Hart, M.P., Ramos, D., Jethava, N., Hosangadi, D., Epstein, J., Hodges, B., Bonini, N.M., Gitler, A.D., 2011a. Evaluating the prevalence of polyglutamine repeat expansions in amyotrophic lateral sclerosis. Neurology 76, 2062–2065. http://dx.doi.org/10.1212/WNL.0b013e31821f4447.

Lee, T., Li, Y.R., Ingre, C., Weber, M., Grehl, T., Gredal, O., de Carvalho, M., Meyer, T., Tysnes, O.B., Auburger, G., Gispert, S., Bonini, N.M., Andersen, P.M., Gitler, A.D., 2011b. Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients. Hum. Mol. Genet. 20, 1697–1700. http://dx.doi.org/10.1093/hmg/ddr045.

Lessing, D., Bonini, N.M., 2008. Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in Drosophila. PLoS Biol. 6, e29. http://dx.doi.org/10.1371/journal.pbio.0060029.

Li, Y., Sekine, T., Funayama, M., Li, L., Yoshino, H., Nishioka, K., Tomiyama, H., Hattori, N., 2013. Clinicogenetic study of GBA mutations in patients with familial Parkinson's disease. Neurobiol. Aging. http://dx.doi.org/10.1016/j.neurobiolaging.2013.09.019.

Lu, C.S., Chang, H.C., Kuo, P.C., Liu, Y.L., Wu, W.S., Weng, Y.H., Yen, T.C., Chou, Y.H., 2004a. The parkinsonian phenotype of spinocerebellar ataxia type 3 in a Taiwanese family. Parkinsonism Relat. Disord. 10, 369–373. http://dx.doi.org/10.1016/j.parkreldis.2004.03.009.

Lu, C.S., Wu Chou, Y.H., Kuo, P.C., Chang, H.C., Weng, Y.H., 2004b. The parkinsonian phenotype of spinocerebellar ataxia type 2. Arch. Neurol. 61, 35–38. http://dx.doi.org/10.1001/archneur.61.1.35.

Mitsui, J., Mizuta, I., Toyoda, A., Ashida, R., Takahashi, Y., Goto, J., Fukuda, Y., Date, H., Iwata, A., Yamamoto, M., Hattori, N., Murata, M., Toda, T., Tsuji, S., 2009. Mutations for Gaucher disease confer high susceptibility to Parkinson disease. Arch. Neurol. 66, 571–576. http://dx.doi.org/10.1001/archneurol.2009.72.

Payami, H., Nutt, J., Gancher, S., Bird, T., McNeal, M.G., Seltzer, W.K., Hussey, J., Lockhart, P., Gwinn-Hardy, K., Singleton, A.A., Singleton, A.B., Hardy, J., Farrer, M., 2003. SCA2 may present as levodopa-responsive parkinsonism. Mov. Disord. 18, 425–429. http://dx.doi.org/10.1002/mds.10375.

Pulst, S.M., Nechiporuk, A., Nechiporuk, T., Gispert, S., Chen, X.N., Lopes-Cendes, I., Pearlman, S., Starkman, S., Orozco-Diaz, G., Lunkes, A., DeJong, P., Rouleau, G.A., Auburger, G., Korenberg, J.R., Figueroa, C., Sahba, S., 1996. Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat. Genet. 14, 269–276. http://dx.doi.org/10.1038/ng1196-269.

Pulst, S.M., Santos, N., Wang, D., Yang, H., Huynh, D., Velazquez, L., Figueroa, K.P., 2005. Spinocerebellar ataxia type 2: polyQ repeat variation in the CACNA1A calcium channel modifies age of onset. Brain 128 (Pt 10), 2297–2303. http://dx.doi.org/10.1093/brain/awh586.

Ragothaman, M., Sarangmath, N., Chaudhary, S., Khare, V., Mittal, U., Sharma, S., Komatireddy, S., Chakrabarti, S., Mukerji, M., Juyal, R.C., Thelma, B.K., Muthane, U.B., 2004. Complex phenotypes in an Indian family with homozygous SCA2 mutations. Ann. Neurol. 55, 130–133. http://dx.doi.org/10.1002/ana.10815.

Ross, O.A., Rutherford, N.J., Baker, M., Soto-Ortolaza, A.I., Carrasquillo, M.M., DeJesus-Hernandez, M., Adamson, J., Li, M., Volkening, K., Finger, E., Seeley, W.W., Hatanpaa, K.J., Lomen-Hoerth, C., Kertesz, A., Bigio, E.H., Lippa, C., Woodruff, B.K., Knopman, D.S., White 3rd, C.L., Van Gerpen, J.A., Meschia, J.F., Mackenzie, I.R., Boylan, K., Boeve, B.F., Miller, B.L., Strong, M.J., Uitti, R.J., Younkin, S.G., Graff-Radford, N.R., Petersen, R.C., Wszolek, Z.K., Dickson, D.W., Rademakers, R., 2011. Ataxin-2 repeat-length variation and neurodegeneration. Hum. Mol. Genet. 20, 3207–3212. http://dx.doi.org/10.1093/hmg/ddr227.

Sequeiros, J., Seneca, S., Martindale, J., 2010. Consensus and controversies in best practices for molecular genetic testing of spinocerebellar ataxias. Eur. J. Hum. Genet. 18, 1188–1195. http://dx.doi.org/10.1038/ejhg.2010.10.

Subramony, S.H., Hernandez, D., Adam, A., Smith-Jefferson, S., Hussey, J., Gwinn-Hardy, K., Lynch, T., McDaniel, O., Hardy, J., Farrer, M., Singleton, A., 2002. Ethnic differences in the expression of neurodegenerative disease: Machado-Joseph disease in Africans and Caucasians. Mov. Disord. 17, 1068–1071. http://dx.doi.org/10.1002/mds.10241.

Sun, H., Satake, W., Zhang, C., Nagai, Y., Tian, Y., Fu, S., Yu, J., Qian, Y., Chu, J., Toda, T., 2011. Genetic and clinical analysis in a Chinese parkinsonism-predominant spinocerebellar ataxia type 2 family. J. Hum. Genet. 56, 330–334. http://dx.doi.org/10.1038/jhg.2011.14.

Walker, F.O., 2007. Huntington's disease. Lancet 369, 218–228. http://dx.doi.org/10.1016/S0140-6736(07) 60111-1.

Wang, J.L., Xiao, B., Cui, X.X., Guo, J.F., Lei, L.F., Song, X.W., Shen, L., Jiang, H., Yan, X.X., Pan, Q., Long, Z.G., Xia, K., Tang, B.S., 2009. Analysis of SCA2 and SCA3/MJD repeats in Parkinson's disease in mainland China: genetic, clinical, and positron emission tomography findings. Mov. Disord. 24, 2007–2011. http://dx.doi.org/10.1002/mds.22727.

Wu, Y.R., Lin, H.Y., Chen, C.M., Gwinn-Hardy, K., Ro, L.S., Wang, Y.C., Li, S.H., Hwang, J.C., Fang, K., Hsieh-Li, H.M., Li, M.L., Tung, L.C., Su, M.T., Lu, K.T., Lee-Chen, G.J., 2004. 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.

Xu, Q., Jia, D., Wang, J., Guo, J., Jiang, H., Lei, L., Shen, L., Pan, Q., Xia, K., Yan, X., Tang, B., 2010. Genetic analysis of spinocerebellar ataxia type 17 in Parkinson's disease in Mainland China. Parkinsonism Relat. Disord. 16, 700–702. http://dx.doi.org/10.1016/j.parkreldis.2010.08.020.

Yun, J.Y., Lee, W.W., Kim, H.J., Kim, J.S., Kim, J.M., Kim, S.Y., Kim, J.Y., Park, S.S., Kim, Y.K., Kim, S.E., Jeon, B.S., 2011. Relative contribution of SCA2, SCA3 and SCA17 in Korean patients with parkinsonism and ataxia. Parkinsonism Relat. Disord. 17, 338–342. http://dx.doi.org/10.1016/j.parkreldis.2011.01.015.

原　著

Spinocerebellar ataxia type 31（SCA31）の臨床像，画像所見
—Spinocerebellar ataxia type 6（SCA6）との小脳外症候の比較検討—

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要旨：近年原因遺伝子の判明したspinocerebellar ataxia type 31（SCA31）と，同様に純粋小脳失調型であるspinocerebellar ataxia type 6（SCA6）との共通点と相違点を明らかにするため両病型の臨床像とMRI所見について各6例を後方視的に比較検討した．小脳症候は両者に共通したが，小脳症候以外の神経症候はSCA31では難聴のみをみとめたのに対し，SCA6では錐体路症候，精神症状など多岐にわたりより高頻度であった．MRI画像の検討ではSCA31は罹病期間に沿って小脳虫部前葉を中心に萎縮が緩徐に進行するのに対し，SCA6では小脳虫部の萎縮に加え経過10年以下の比較的初期から第4脳室の拡大がめだった．これらの症候や画像所見の特徴は遺伝子診断以前の鑑別の手掛りとなる可能性がある．

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Key words：脊髄小脳失調症（SCA），spinocerebellar ataxia type 31（SCA31），spinocerebellar ataxia type 6（SCA6），MRI

はじめに

Spinocerebellar ataxia type 31（SCA31）は2000年に16q22.1-linked autosomal dominant cerebellar ataxia（16q-ADCA）として疾患概念が確立し[1]，2009年に原因となる遺伝子が明らかとなった新しいSCAである．その発症機序は16番染色体上に存在する2つのことなる遺伝子BEAN1とTK2がイントロンとして共有する位置に異常伸長した$(TGGAA)_n$の5塩基リピートが挿入され，小脳Purkinje細胞の核内にRNA凝集体が蓄積することと判明しており[2]，CAGリピートを原因とする他の多くのSCAとことなる．

これまで本邦で純粋小脳失調を示す遺伝性脊髄小脳変性症（ADCA type III）の鑑別診断を考える上ではspinocerebellar ataxia type 6（SCA6）が筆頭にあげられていたが，SCA31の遺伝子診断が可能となってからはSCA31もまた大きな割合を占めることが判明してきた．従来SCA6はほぼ純粋な小脳失調症を呈するとされるのが一般的ではあるものの，実際にはめまい感，深部腱反射異常，足底反射陽性，痙縮，深部感覚（振動覚）減弱，ジストニーなどの不随意運動，認知機能低下などを呈したという報告も多数みられる[3)~9)]．一方SCA31における小脳外症候は少なく，もっとも最近の総説[10)]においても，臨床的には純粋小脳失調の範疇の患者が多いと報告されている．

最近，当院で今まで病型不明だった純粋型小脳失調症6例が，遺伝子検査でSCA31であることが判明した．ともにADCA type IIIに分類されるSCA6とSCA31との鑑別は一面難しいように思われる．そこで本稿では，SCA31とSCA6の臨床像を小脳症候以外の神経症候をふくめ検討し，両者の鑑別にこれらの症候が寄与するか否かを検討した．

対象と方法

対象は2007年から2012年の間に当院にて診療をおこなったSCA31，SCA6各6例である．通院中の症例は神経内科医による診察を，過去の症例は神経学的診察記録，診察を記録したビデオ画像をもとに，臨床症候を比較し経過中1回でもあれば陽性と判断した．なおSCA6症例の半数は現在通院中でなく，欠損データとなっており，全6例でしらべられなかった項目はしらべた症例中何例という記述としている．解析には年齢，罹病期間，合併する小脳外症候の数にはMann-Whitney検定を，症候の頻度の比較にはFisher検定をもちいた．画像はSCA31とSCA6それぞれ6例のMRI T_2強調画像水平断，矢状断（SCA31はすべてT_2強調画像，SCA6は2例がT_2強調画像，4例がT_1強調画像）を撮像し経験年数25年以上の神経内科専門医2名が独立に判定し，平均化した．病型をふくめ，個々の患者情報を隠して全症例について小脳萎縮，第4脳室拡大，中小脳脚萎縮の有無を評価し所見が

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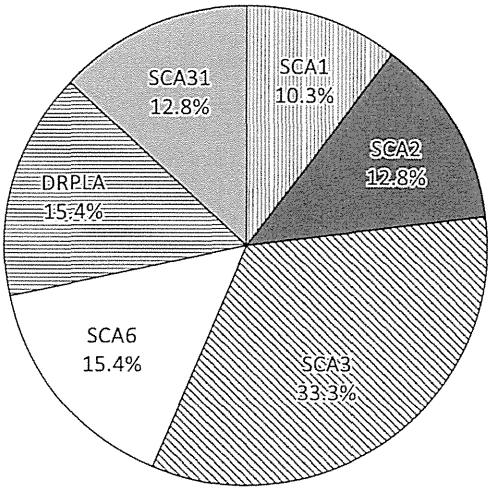

Fig. 1　Percentage of SCA types in our hospital (1998–2012). The graph shows that SCA3 is the most frequent (33.3%) subtype in our hospital. The second most frequent subtypes are DRPLA and SCA6 at the same percentage (15.4%), followed by SCA31 and SCA2 at the same percentage (12.8%) and SCA1 (10.3%). The percentage is calculated with the number of family.

「明らかにあり」，「おそらくあり」，「おそらくなし」，「明らかになし」の4段階の順序変数をもちい，順に+2点，+1点，−1点，−2点と点数化して両病型における平均点をMann-Whitney検定で比較した．遺伝子検査はSCA31の全例とSCA6の1例は東京医科歯科大学で，SCA6の5例は名古屋大学でおこなった．失調の評価にScale for the assessment and rating of ataxia（SARA）を，認知機能評価に改訂版長谷川式簡易知能スケール（HDS-R）をもちいた．

結　果

1．当院におけるSCAの病型の割合（Fig. 1）

最初に当院において，1998年～2012年に診療をおこない遺伝子診断で判明した遺伝性脊髄小脳変性症の内訳を示す．病型の判明した39家系のうち当院ではSCA3がもっとも多く13家系（33.3%）を占め，2番目にDRPLAとSCA6が同率で各6家系（15.4%），続いてSCA31とSCA2が同率で各5家系（12.8%），SCA1が4家系（10.3%）であった．

2．SCA31，SCA6の臨床症候の比較（Table 1）

a．発症年齢

SCA31の発症年齢は平均63.8歳（57～67歳），SCA6の発症年齢は平均40.7歳（28～60歳）で有意差をみとめた．

b．発症時の症候

SCA31の初発神経症候は歩行障害が4例，言語障害が1例，その両者を同時期に自覚したものが1例であった．SCA6の初発神経症候は歩行障害が2例，歩行障害および言語障害を同時期に自覚したものが2例，めまい感が1例，手の震えによる書字障害が1例であった．

c．神経症候

脳神経の所見は，水平方向の眼振はSCA31で全例にみとめたのに対し，SCA6では1例のみにみとめた．下向き眼振はSCA31ではみとめず，SCA6の2例でみとめた．難聴はSCA31では確認できた4例中3例あり，そのうち1例は小脳症候出現より20年以上前の40代から難聴を自覚し，73歳の検査時に両側性に高音域になるにしたがって強い難聴を示した．もう1例では片側の全音域の伝音性難聴と，両側の高音域の難聴がみとめられた．これらの症例の聴性脳幹反応（ABR）のI～V波の潜時はすべて正常であった．SCA6では1例検索したが正常であった．

運動系の所見は，SCA6で四肢筋力低下および筋萎縮をみとめたものが5例中1例あった．SCA31，SCA6ともに全例で経過中に体幹，四肢の小脳失調を呈し，小脳性言語障害をみとめた．筋トーヌスはSCA31の5例で低下していたが，SCA6では低下，正常，亢進がそれぞれ2例ずつ存在した．不随意運動はSCA31では1例のみ振戦をみとめ，SCA6では3例に振戦を，別の1例にミオクローヌスをみとめた．

感覚系では表在覚は全例で正常であった．深部感覚では，振動覚の低下をSCA31の3例，SCA6の5例中4例でみとめ，関節位置覚の障害はSCA6の5例中1例でみとめた．

精神神経症候として，認知機能はSCA31の全例で正常であり，SCA6の3例中1例で低下していた．精神症候として，SCA31の6例では精神症状を示すものはみられなかったが，SCA6では幼稚性，パニック発作，自殺，抑鬱および攻撃性がそれぞれ1例ずつみられ，頻度に有意差をみとめた．

その他の症候として，腱反射はSCA31では低下，正常，亢進がそれぞれ2例ずつ存在したのに対し，SCA6では5例中5例で亢進し有意差をみとめた．Babinski徴候はSCA6の4例中1例のみで陽性であった．自律神経症状はSCA6の1例で排尿障害をみとめた．SCA6ではめまい感を訴えるものが3例中2例存在したが，SCA31では皆無であった．

みとめられた小脳外症候すなわち小脳症状以外の神経症候の数の患者毎の平均値は，SCA31では平均1.5個（0～3個），SCA6では平均4.5個（2～9個）で有意差をみとめた．

d．罹病期間とADLの関係（Fig. 2）

SCA31では罹病期間が長いほどADLが低下していた．SCA6では罹病期間とADLは一定の傾向を示さず，罹病期間が短くても移動に車いすを必要とする患者もみられた．

3．画像所見（Fig. 3）

画像所見を2名の判定者で定性的に検討した．判定者間の一致度は重み付けκ係数＝0.94と高値であった．小脳はいずれの病型でも萎縮をみとめたが，萎縮の程度，部位に差異をみとめなかった．両者とも，とくに小脳虫部の上方で山頂や山腹に相当する部位に萎縮が強い傾向があった．一方，第4脳室の拡大，中小脳脚の萎縮については，SCA6で有意にみとめた．大脳にいずれの病型とも萎縮をみとめなかった．

Table 1　Clinical features of SCA31 and SCA6 patients.

	SCA31		SCA6		Mann-Whitney test
Age at examination	75.33 ± 2.8		55.33 ± 12.7		$p < 0.05$*
Male/Female	M4/F2		M3/F3		—
Age at onset	63.8 ± 4.9		40.7 ± 10.3		$p < 0.05$*
Duration of disease	11.5 ± 5.2		14.7 ± 8.0		$p > 0.05$
Initial symptoms	Unsteadiness of gait	5	Unsteadiness of gait	4	
	Dysarthria	2	Dysarthria	2	
			Vertigo	1	
			Tremors	1	
					Fisher's exact test
Family history	6/6		4/6		0.23
Consanguinity	1/6		0/2		0.75
No. of family	5		6		
<Cerebellar function>					
Upper limb ataxia	6/6		6/6		—
Truncal ataxia	6/6		6/6		—
Lower limb ataxia	6/6		6/6		—
<Cranial nerves>					
Gaze evoked nystagmus					
Horizontal	6/6		1/6		0.0076**
Vertical	0/6		2/6		0.23
Oculomotor disturbance	0/6		0/5		—
Dysarthria	6/6		6/6		—
Dysphagia	3/6		1/6		0.24
Hearing impairment	3/4		0/1		0.5
<Motor functions>					
Muscle weakness	0/6		1/5		0.45
Amyotrophy	0/6		1/4		0.4
Muscular hypotonus	5/6		2/4		0.33
Spasticity	0/6		2/4		0.13
Involuntary movements					
Tremors	1/6		3/6		0.24
Myoclonus	0/6		1/6		0.5
<Sensory>					
Impaired thermal sense	0/6		0/5		—
Reduced vibration sense	3/6		5/5		0.12
Impaired kinesthesia	0/6		2/5		0.18
<Reflexes>					
Deep tendon reflex					
Increased	2/6		5/5		0.045*
Decreased/Lost	2/6		0/5		0.27
Positive Babinski reflex	0/6		1/4		0.4
<Autonomic function>					
Orthostatic hypotension	0/6		0/4		—
Urinary incontinence	0/6		1/4		0.4
<Other symptoms>					
Cognitive impairment	0/6		1/3		0.33
Psychiatric symptoms	0/6		4/5		0.015*
Vertigo	0/6		2/3		0.083
					Mann-Whitney test
No. of extracerebellar symptoms	1.5 ± 0.96		4.5 ± 2.22		$p < 0.05$*

*$p < 0.05$, **$p < 0.01$.

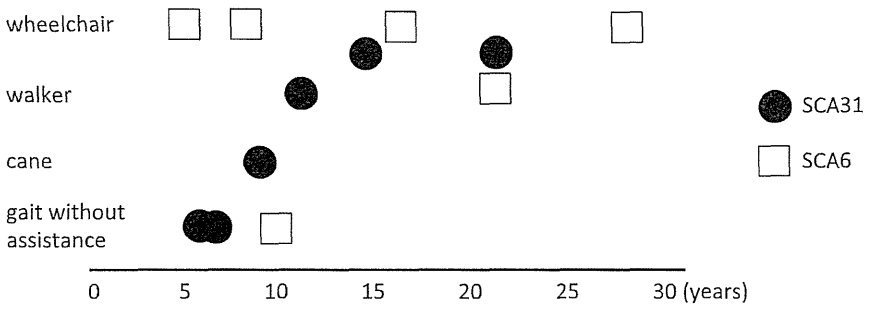

Fig. 2　Clinical course of SCA31 and SCA6.
Gait disturbance slowly progresses with disease duration in SCA31 patients. This trend is unclear in SCA6 and the clinical course varies in each case. A mark indicating patient using both wheelchair and walker is placed in the middle of them.

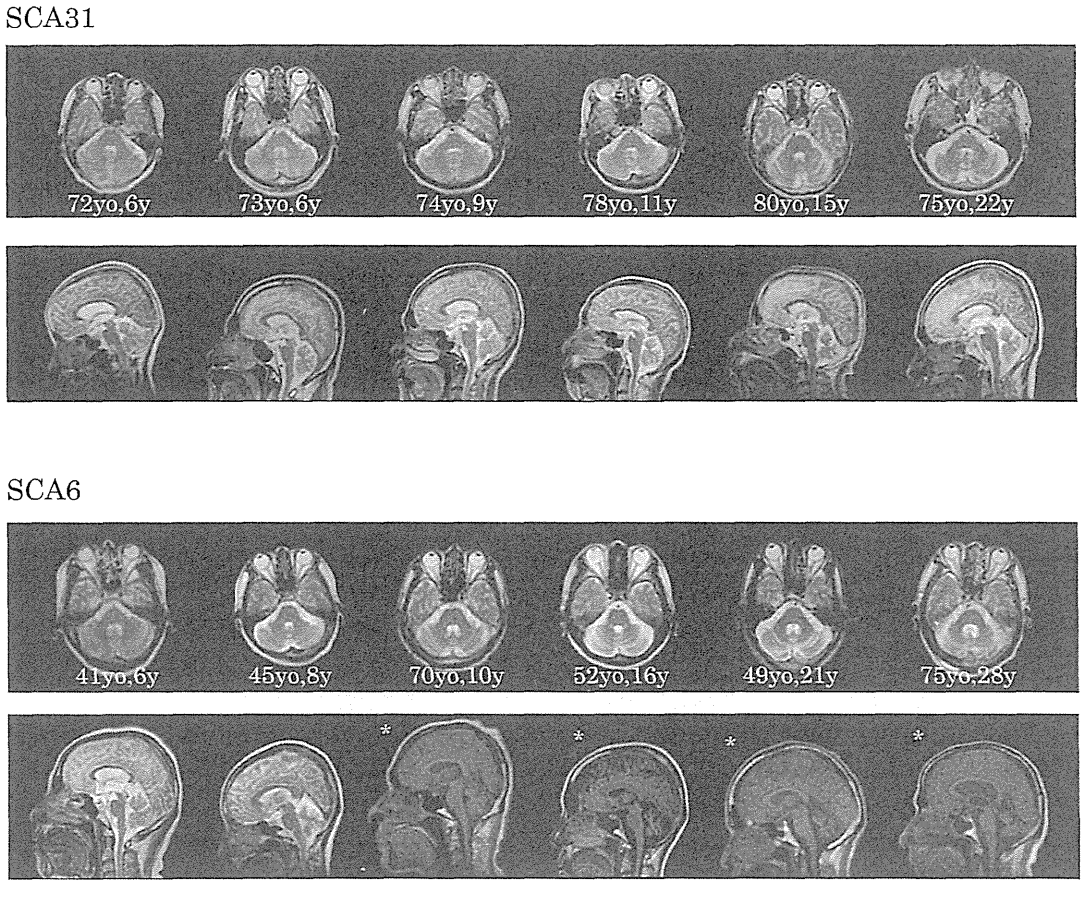

Fig. 3　MRI T_2 weighted image (axial, sagittal 1.5 T; TR 4,600 ms, TE 85 ms) of six SCA31 patients (upper rows) and six SCA6 patients (lower rows). T_1 weighted image (sagittal, TR 520 ms, TE 15 ms) were shown in four SCA6 patients (*).
The numbers in the figure show each patient's age and disease duration at the study. Cerebellar atrophy starts from the upper vermis in SCA31, whereas the 4th ventricle becomes enlarged in SCA6 even in the early stage of disease.

考　察

従来，SCA6は純粋小脳失調を示す例が多いSCAであるといわれてきたが，錐体路症状，錐体外路症状，認知機能低下，めまい感の自覚などを合併したという報告も多い[3)-9)]．今回当院で検討したSCA6においても，Table 1に示すように小脳失調のみを示すものはむしろ少なく，めまい感，深部腱反射異常，足底反射陽性，痙縮，深部感覚（振動覚）低下，不随

意運動，精神症状などの小脳外症候を示した．その点を考慮して，SCA31とSCA6とを比較したところ，患者ごとの小脳外症候の数はSCA31では平均1.5個（0〜3個），SCA6では平均4.5個（2〜9個）で有意差があった．症候別の検討で有意差をみとめたものは水平性眼振と精神症状の頻度であった．有意差のなかったものも既報告と比較して以下に相違点を述べる．

第1に水平性眼振は当院で経験したSCA31では全例でみとめ，SCA6にくらべて有意に多かった（$p = 0.0076$）．一方，SCA6では下向き眼振を呈するものが2例あり，過去の報告にもみられるとおりSCA6に特徴的な所見である．SCA6の水平性眼振については一般に68〜94％と高頻度でみとめるとされている[6)11)12)]．既報告ではSCA31の水平性眼振は5〜55％と当院のものよりも低頻度であった[11)13)〜15)]．また同一家系についてしらべた報告では，眼振のめだたない家系も存在するようである[16)]．これについては今後当地域の症例をさらに集積し，かつ長期に観察して再度検討したいと考えている．

第2に今回SCA31の4例中3例で難聴がみられた．SCA31の難聴の頻度は3.8〜42.9％とされ，SCA31の数少ない小脳外症候であるといわれ[13)16)17)]，両側性の感音性難聴を示すとされている[18)]．難聴はSCA31に特異的ではないとする報告[19)]や，高齢発症の型であることから老人性難聴の合併ではないかと考察している報告[11)]もあるが，今回の検討では小脳症候を発症するより20年以上前の40歳頃に原因不明の難聴で現在まで両側性に持続している症例がみられ，老人性難聴とはことなる経過である．SCA31を発症後に難聴の合併が確認された症例は一定の割合で存在するが，このような難聴の先行を記載しているものは文献を検索しても見当たらなかった．また他の1例では片側の中耳炎の既往によると考えられる伝音性難聴は容易に理解できるが，他側は高音域に強い難聴を示した．ABRの結果からは2例とも脳幹機能，蝸牛の異常は否定的と考えられた．一方，SCA6では難聴の合併は一般的ではない[11)]．

第3に深部感覚（振動覚）障害についてはSCA31，SCA6ともに共通してみられたが，解釈はことなる．SCA31の振動覚障害は半数にみとめられ文献的には10％で合併すると報告がある[14)]が，高齢発症ゆえに年齢による影響が否定できない．これに対しSCA6では比較的若年であるにもかかわらず全例にみとめられた．文献的にも4.3〜58％で合併することが指摘されている[6)18)]．

第4に，認知機能低下はSCA6で3例中1例でみとめられたがSCA31では6例ともまったくみとめなかった．従来ほとんどの報告でSCA31において認知機能低下をきたす頻度は診察時年齢が66.9〜74.5歳と高齢であるにもかかわらず0〜6.1％と低頻度[11)13)14)20)]であった．当院でも診察時平均年齢75.3歳の6例全例で正常であったことは従来の報告に合致しており，SCA31では認知機能低下をきたしにくいと思われる．これに対しSCA6では認知機能低下[5)]やうつ症状を中心とした精神神経症状を示すこと[4)]が報告されている．当院では精神症状の頻度はSCA6で有意に高かった（$p = 0.015$）．認知機能低下や精神症状をともなうSCA6症例での病理学的検索の報告は少ないが，SCA6の原因遺伝子であるCACNA1AのmRNAが小脳のみならず大脳をふくむ中枢神経全体に発現していること[7)]はその原因として考えうるかもしれない．

さらに，SCA6では痙性対麻痺やBabinski徴候などの錐体路徴候や不随意運動など錐体外路症候を示すことも報告されており[3)6)]，その頻度は多く，SCA6で純粋小脳失調を呈するのはごく一部の症例に限られ，多くの症例では発症初期に小脳失調のみを呈しても，進行した病像は多彩な症候が加わるとされる．小脳外症候が出現する時期やその内容，多寡が患者毎にことなることがFig. 2で示したように罹病期間とADLレベルが一定の傾向をもたない理由の一つとなっていると考えた．先にも述べたようにSCA6の病理学的所見は小脳のほか，大脳，脳幹から脊髄にいたるまでほぼ均等に分布しているとされ[7)]，これが小脳外症候を様々な程度で合併する原因となっている可能性について剖検症例を集積し臨床症候と照らし合わせて検討したい．また今回検討した6例のSCA6のCAGリピート数は過去の遺伝子検査結果に記載されていない症例が複数あり，本論文ではリピート数と関連した考察はできなかったが，SCA6ではCAGリピートが多い例で小脳外症候が出やすいという報告[21)]がある一方で関連はないとしているものもみられる[6)]．このような小脳外症候の修飾を受けにくいSCA31では患者間で症候が類似し経過も一定の傾向をもつと考えられる．

つぎに画像について考察する．今回検討したSCA31のMRI画像は，小脳虫部の上方で山頂や山腹に相当する部位から萎縮が進行する点はSCA6と共通の特徴であるが，脳幹の萎縮をみとめない点で経過10年以下の比較的初期から第4脳室の拡大や中小脳脚の萎縮をみとめるSCA6の6例とはことなった所見を呈していた．小脳に限局した萎縮をきたし脳幹は保たれるとする既存のSCA31についての記述とも一致するが[1)13)]，小脳上方の萎縮は他のSCAでもきたしやすい所見であり[22)]，これのみでSCA31を診断することは困難であると考えられる．SCA6についてはこれまでの報告でも虫部の中等度から高度の萎縮，小脳半球の軽度な萎縮をきたすとされており，小脳以外の構造については意見がわかれ，中小脳脚・橋・その他の後頭蓋窩の構造には萎縮はみられないとしているもの[8)23)24)]と，橋の前後径と中小脳脚の径にSCA1, 2, 3, 7には匹敵しない軽度の萎縮を指摘するものがあり[25)]，後者が当院の症例と共通する．しかし，いずれの報告においても画像所見は罹病期間によってことなる可能性があり，今後，縦断的な解析の必要があると思われる．

以上より，SCA31はSCA6に比して，高齢発症で，精神症状，錐体路症候や不随意運動などの小脳以外の症候が少なく均一な臨床群であった．またMRIではSCA6に比して第4脳室拡大や中小脳脚の萎縮の所見が乏しい点が特徴的であった．小脳外症候を注意深く観察し，検討することが両者の鑑別に有用である可能性を提案する．

本報告の要旨は，第133回日本神経学会東海・北陸地方会で発表し，会長推薦演題に選ばれた．

※本論文に関連し，開示すべきCOI状態にある企業，組織，団体はいずれも有りません．

文　献

1) Nagaoka U, Takashima M, Ishikawa K, et al. A gene on SCA4 locus causes dominantly inherited pure cerebellar ataxia. Neurology 2000;54:1971-1975.
2) Sato N, Amino T, Kobayashi K, et al. Spinocerebellar ataxia type 31 is associated with "inserted" penta-nucleotide repeats containing (TGGAA)n. Am J Hum Genet 2009:85;544-557.
3) Kim JM, Lee YL, Kim HJ, et al. The wide clinical spectrum and nigrostriatal dopaminergic damage in spinocerebellar ataxia type 6. J Neurol Neurosurg Psychiatry 2010;81:529-532.
4) Klinke I, Minnerop M, Schmitz-Hübsch T, et al. Neuropsychological features of patients with spinocerebellar ataxia (SCA) types 1, 2, 3, and 6. Cerebellum 2010;9:433-442.
5) Suenaga M, Kawai Y, Watanabe H, et al. Cognitive impairment in spinocerebellar ataxia type 6. J Neurol Neurosurg Psychiatry 2008;79:496-499.
6) Schöls L, Krüger R, Amoiridis G, et al. Spinocerebellar ataxia type 6: genotype and phenotype in German kindreds. J Neurol Neurosurg Psychiatry 1998;64:67-73.
7) 水澤英洋．純粋小脳失調症　脊髄小脳失調症6型を中心に．運動障害 2005;15:77-90.
8) Takahashi H, Ishikawa K, Tsutsumi T, et al. A clinical and genetic study in a large cohort of patients with spinocerebellar ataxia type 6. J Hum Genet 2004;49:256-264.
9) Watanabe H, Tanaka F, Matsumoto M, et al. Frequency analysis of autosomal dominant cerebellar ataxias in Japanese patients and clinical characterization of spinocerebellar ataxia type 6. Clin Genet 1998;53:13-19.
10) 石川欽也，水澤英洋．脊髄小脳失調症31型（SCA31）．神経内科 2013;78:253-256.
11) Yoshida K, Shimizu Y, Morita H, et al. Severity and progression rate of cerebellar ataxia in 16q-linked autosomal dominant cerebellar ataxia (16q-ADCA) in the endemic Nagano area of Japan. Cerebellum 2009;8:46-51.
12) Ikeuchi T, Takano H, Koide R, et al. Spinocerebellar ataxia type 6: CAG repeat expansion in alpha1A voltage-dependent calcium channel gene and clinical variations in Japanese popuration. Ann Neurol 1997;42:879-884.
13) Onodera Y, Aoki M, Mizuno H, et al. Clinical features of chromosome 16q22.1 linked autosomal dominant cerebellar ataxia in Japanese. Neurology 2006;67:1300-1302.
14) Nozaki H, Ikeuchi T, Kawakami A, et al. Clinical and genetic characterizations of 16q-linked autosomal dominant spinocerebellar ataxia (AD-SCA) and frequency analysis of AD-SCA in the Japanese population. Mov Disord 2007;22:857-862.
15) 石川欽也，融　衆太，大和田潔ら．第16番染色体長腕に連鎖する優性遺伝性純粋小脳失調症．神経内科 2004;60:462-468.
16) Owada K, Ishikawa K, Toru S, et al. A clinical, genetic, and neuropathologic study in a family with 16q-linked ADCA type III. Neurology 2005;65:629-632.
17) Ouyang Y, Sakoe K, Shimazaki H, et al. 16q-linked autosomal dominant cerebellar ataxia: a clinical and genetic study. J Neurol Sci 2006;247:180-186.
18) Ishikawa K, Toru S, Tsunemi T, et al. An autosomal dominant cerebellar ataxia linked to chromosome 16q22.1 is associated with a single nucleotide substitution in the 5' untranslated region of the gene encoding a protein with spectrin repeat and Rho guanine-nucleotide exchange-factor domains. Am J Hum Genet 2005;77:280-296.
19) Ikeda Y, Nagai M, Kurata T, et al. Comparisons of acoustic function in SCA31 and other forms of ataxias. Neurol Res 2011;33:427-432.
20) Basri R, Yabe I, Soma H, et al. Spectrum and prevalence of autosomal dominant spinocerebellar ataxia in Hokkaido, the northern island of Japan: a study of 113 Japanese families. J Hum Genet 2007;52:848-855.
21) Sailer A, Houlden H. Recent advances in the genetics of cerebellar ataxias. Curr Neurol Neurosci Rep 2012;12:227-236.
22) Döhlinger S, Hauser TK, Borkert J, et al. Magnetic resonance imaging in spinocerebellar ataxias. Cerebellum 2008;7:204-214.
23) Satoh JI, Tokumoto H, Yukitake M, et al. Spinocerebellar ataxia type 6: MRI of three Japanese patients. Neuroradiology 1998;40:222-227.
24) Butteriss D, Chinnery P, Birchall D. Radiological characterization of spinocerebellar ataxia type 6. Br J Radiol 2005;78:694-696.
25) Murata Y, Kawakami H, Yamaguchi S, et al. Characteristic magnetic resonance imaging findings in spinocerebellar ataxia 6. Arch Neurol 1998;55:1348-1352.

Abstract

Clinical features and MRI findings in spinocerebellar ataxia type 31 (SCA31) comparing with spinocerebellar ataxia type 6 (SCA6)

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Since the discovery of spinocerebellar ataxia type 31 (SCA31) gene, we identified 6 patients whose SCA type had been unkown for a long period of time as having SCA31 in our hospital and realized that SCA31 is not a rare type of autosomal dominant spinocerebellar ataxia in this region. We examined and compared the clinical details of these six SCA31 patients and the same number of SCA6 patients, finding that some SCA31 patients had hearing loss in common while there are more wide range and complicated signs of extra cerebellum in SCA6 such as pyramidal signs, extrapyramidal signs, dizzy sensations or psychotic, mental problems. There is a significant difference in the number of extracerebellar symptoms between SCA31 and SCA6. There are differences also in MRI findings. Cerebellar atrophy starts from the upper vermis in SCA31, as well as some SCA types, whereas the 4th ventricule becomes enlarged in SCA6 even in the early stage of disease. We suggest that these differences in clinical and MRI findings can be clues for accurate diagnosis before gene analysis.

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Key words: spinocerebellar ataxia (SCA), spinocerebellar ataxia type 31 (SCA31), spinocerebellar ataxia type 6 (SCA6), MRI

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SHORT COMMUNICATION

Novel mutations in the *PNPLA6* gene in Boucher-Neuhäuser syndrome

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On whole-exome sequencing, a novel compound heterozygous mutation (c.2923A>G/c.3523_3524insTGTCCG, p.T975A/p.1175_1176insVS) and a novel homozygous one (c.3534G>C, p.W1178C) in the *PNPLA6* gene were identified in sporadic and familial Japanese patients with Boucher-Neuhäuser syndrome (BNS), respectively. However, we did not find any mutations in the *PNPLA6* gene in 88 patients with autosomal recessive hereditary spastic paraplegia (ARHSP). Our study confirmed the earlier report that a *PNPLA6* mutation causes BNS. This is the first report on *PNPLA6* mutations in non-Caucasian patients. Meanwhile, *PNPLA6* mutations might be extremely rare in Japanese ARHSP patients. Moreover, we first found hypersegmented neutrophils in two BNS patients with *PNPLA6* mutations.
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Hereditary spinocerebellar ataxia associated with hypogonadotropic hypogonadism is known as Gordon Holmes syndrome (GHS).[1] GHS with chorioretinal dystrophy is called Boucher-Neuhäuser syndrome (BNS).[2,3] BNS is inherited through autosomal recessive transmission, and is a very rare disease that has been reported in <30 families so far. Recently, GHS with dementia has been reported to be caused by the *OTUD4* and *RNF216* mutations.[4]

Recently, while we revealed several candidate variations causing BNS, *PNPLA6* mutations, which cause spastic paraplegia type 39 with an autosomal recessive mode of inheritance were identified in Caucasian patients with BNS, GHS and spastic ataxia.[5–7] Here we describe a novel compound heterozygous mutation and a novel homozygous one in the *PNPLA6* gene in two Japanese patients with BNS. Furthermore, as hypersegmented neutrophils were reported in two BNS families including our patients,[8,9] we attempted to find hypersegmented neutrophils in BNS genetically proven patients.

We recruited one Japanese sporadic and one familial patient with BNS. Patient 1 (sporadic) was a non-consanguineous kindred. Patient 2 (familial) was a consanguineous kindred with an affected brother and sister. The detailed clinical findings in these patients have been reported elsewhere.[8,10] Patient 1 did not have any mutations in the genes associated with Kallman syndrome (KAL1, FGFR1, PROK2 and PROKR2) or spinocerebeller ataxias (SCA1, SCA2, MJD, SCA6, SCA7, SCA8, SCA12, SCA17, SCA31, DRPLA and ARSACS).

Japan Spastic Paraplegia Research Consortium (JASPAC) has assembled 429 index patients with hereditary spastic paraplegia. We recruited patients with familial progressive spastic paraplegia, consanguinity or a thin corpus callosum. From among these patients, we selected 88 (16 with consanguinity and affected siblings, 20 with only consanguinity, 24 with only affected siblings and 28 with a thin corpus callosum) who were suspected of having autosomal recessive hereditary spastic paraplegia.

The present study was approved by the institutional review boards, and informed consent was obtained from all individuals.

We analyzed the genomic DNA of patient 1 and his parents by whole-exome sequencing. Exome capture was performed using a Sureselect Human All Exon^{XT} V4 Kit (Agilent, Santa Clara, CA, USA), followed by massively parallel sequencing using Illumina HiSeq 2000 (100 bp paired end; Illumina, San Diego, CA, USA). We aligned the exome data with BWA[11] and extracted single-nucleotide variations using GATK.[12] We picked up novel homozygous mutations (patient 1 has a homozygous mutation and his parents have a heterozygous one) and novel compound heterozygous ones (patient 1 has compound heterozygous mutations and his parents have a heterozygous mutation) under the condition of recessive inheritance models by using dbSNP135 (ftp://ftp.ncbi.nlm.nih.gov/snp/organisms/human_9606/ASN1_flat/), 1000 genomes (ftp://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20110521) and the Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/). Then we checked candidate mutations in patient 1 and his parents and patient 2 by Sanger sequencing. Moreover, the missense mutations identified were evaluated using *in silico* algorithms including Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/).

Unfortunately, we could not examine the affected siblings of patient 2. As the 88 patients recruited by the JASPAC had been subjected to

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whole-exome sequencing, we checked whether *PNPLA6* mutations exist or not. Exome capture was performed using an Agilent SureSelect Human ALL Exon 50 Mb Kit (Agilent), followed by massively parallel sequencing using Illumina HiSeq 2000 (100 bp paired end). We aligned the exome data with BWA[11] and extracted single-nucleotide variations using SAMtools.[13] In addition, we performed blood testing in patient 1.

We obtained 10.9, 12.2 and 14.0 Gbp, and 117, 129 and 107 Gbp averaged depth on target for the proband, his father and his mother, respectively. We found no novel homozygous mutations. However, we found one compound heterozygous mutation in patient 1. Only *PNPLA6* was found to be a novel compound heterozygous mutation (c.2923A>G/ c.3523_3524insTGTCCG, p.T975A/ p.1175_1176insVS) in patient 1. On Sanger sequencing, the c.2923A>G mutation was found in patient 1 and his mother, and the c.3523_3524insTGTCCG one was found in patient 1 and his father (Figure 1a). *In silico* analysis was performed for the c.2923 A>G mutation. Polyphen 2 was 'possibly damaging' and SIFT 'damaging'.

In patient 2, we found a novel homozygous mutation (c.3534G>C, p.W1178C) in the *PNPLA6* gene (Figure 1b). Polyphen 2 was 'probably damaging' and SIFT 'damaging'. We could not, however, rule out a pseudo-homozygous state (for example, due to a deletion), as we could not show that each parent had carried one of the two variants in a heterozygous state because of their death.

As we found a novel compound heterozygous mutation and a novel homozygous one in the *PNPLA6* gene in patients 1 and 2, respectively, we concluded that *PNPLA6* mutations had caused BNS in our patients.

In the 88 autosomal recessive hereditary spastic paraplegia patients of the JASPAC, we did not find any non-synonymous *PNPLA6* mutations, insertions or deletions on whole-exome sequencing.

Blood testing of patient 1 revealed 9% of hypersegmented neutrophils. That of patient 2 revealed 28%, as previously reported Umehara *et al.*[8] (Figure 2).

In the present study, we found a novel compound heterozygous mutation and a novel homozygous one in the *PNPLA6* gene in two Japanese patients with BNS. We have confirmed the earlier report that *PNPLA6* mutations cause BNS[6] as same as other studies.[14,15] To date, seven BNS families have been reported to have had *PNPLA6*

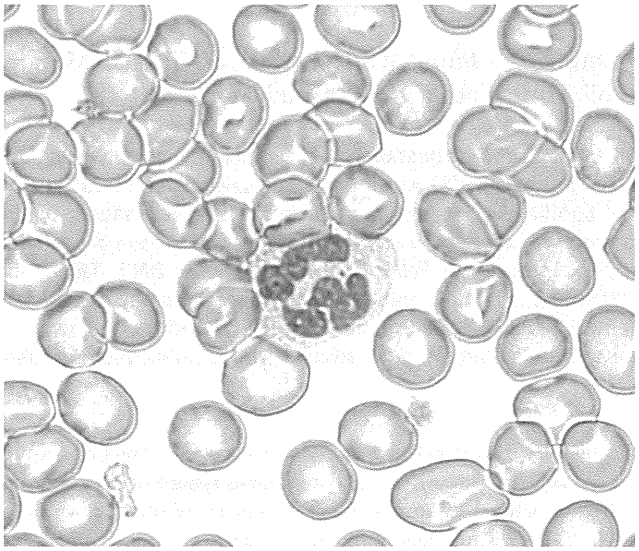

Figure 2 Hypersegmented neutrophils in patient 1. Blood testing of patient 1 revealed 9% hypersegmented neutrophils. As hypersegmented neutrophils were found in patients 1 and 2, Boucher-Neuhäuser syndrome patients with *PNPLA6* mutations might have hypersegmented neutrophils.

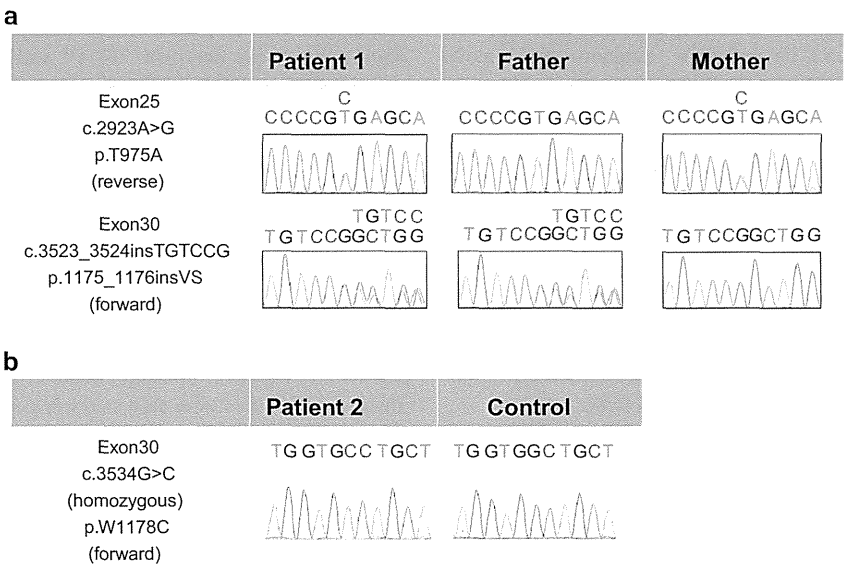

Figure 1 Electropherograms of two Japanese patients with Boucher-Neuhäuser syndrome. (**a**) A novel compound heterozygous mutation (c.2923A>G/ c.3523_3524insTGTCCG) in the *PNPLA6* gene was identified in patient 1. A heterozygous mutation of c.2923A>G was derived from the mother, and that of c.3523_3524insTGTCCG from the father. (**b**) A novel homozygous mutation (pc.3543G>C) in the *PNPLA6* gene was identified in patient 2. Yellow blocks show the positions of missense mutations. Green, red, black and blue indicate A, T, G and C, respectively. Exon 25 is a reverse sequence, and exon 30 a forward one.

Table 1 *PNPLA6* mutations reported so far

Phenotype	*PNPLA mutations*	*Reference*
BNS	p.T975A	Ours
	p.1175_1176insVS	
BNS	p.W1178C (Homozygous)	Ours
BNS	p.V738Qfs*98	Synofzik *et al.*[6]
	p.V1110M	Synofzik *et al.*[16]
BNS	p.S1045L	Synofzik *et al.*[6]
	p.P1122L	
BNS	p.G578W	Synofzik *et al.*[6]
	p.F1066S	
BNS	p.T1058I (Homozygous)	Synofzik *et al.*[6]
BNS	p.Y96X	Tarnutzer *et al.*[14]
	p.R289G	
BNS	c.343-2 A>T	Tarnutzer *et al.*[14]
	p.R1359W	
BNS	p.S1045L	Deik *et al.*[15]
	p.S1173R	
GHS	p.R1031Efs*38	Synofzik *et al.*[6]
	p.R1362G	
GHS	p.S1127C (Homozygous)	Topaloglu *et al.*[18]
GHS	p.D376Gfs*18	Topaloglu *et al.*[18]
	p.R1099C	
GHS	p.R1311W	Topaloglu *et al.*[18]
	p.G832fs*13	
HSP	p.M1012V (Homozygous)	Rainier *et al.*[5]
HSP	p.R890H	Rainier *et al.*[5]
	p.S982fs*37	
HSP	p.R558X	Yoon *et al.*[17]
	Ex.17, 18 deletion	
HSP	p.V263I	Synofzik *et al.*[6]
	p.G840E	
sATX	p.R1031Efs*38	Synofzik *et al.*[6]
	p.V1100G	
OMS/LMS	p.R1099Q	Hufnagel *et al.*[19]
	p.G1176S	
OMS/LMS	p.R1031fs*38	Hufnagel *et al.*[19]
	p.G1129R	
OMS/LMS	c.1973+2 T>G	Hufnagel *et al.*[19]
	p.V1215A	
OMS/LMS	Dup(ex14-20)	Hufnagel *et al.*[19]
	p.V1215A	
OMS/LMS	p.R1031fs*38	Hufnagel *et al.*[19]
	p.G1129R	
OMS/LMS	p.G726R	Hufnagel *et al.*[19]
	p.R1031fs*38	
CA	p.P447L	Fogel *et al.*[20]
	p.Q1200E	

Abbreviaitons: BNS, Boucher- Neuhäuser syndrome; CA, cerebellar ataxia; GHS, Gordon Holmes syndrome; HSP, hereditary spastic paraplegia; LMS, Laurence-Moon syndrome; OMS, Oliver-McFarlane syndrome; sATX, spastic ataxia.

mutations worldwide.[6,14–16] Thus, this is the first report on *PNPLA6* mutations in non-Caucasian patients.

To date, 39 mutations including ours in the *PNPLA6* gene have been identified (Table 1).[5–7,14–20] Although a few mutations (p.S1045L, p.R1031fs*38, p.V1215A and p.G1129R) are shared with some families, most mutations are unique.

PNPLA6 was originally identified as a target enzyme for the poisonous effect of organophosphates.[21] Organophosphates cause a severe neurological disorder in vertebrates known as organophosphate-induced delayed neuropathy, which is characterized by degeneration of long axons in the spinal cord and peripheral nerves leading to paralysis of the lower limbs. *PNPLA6* is primarily expressed in the nervous system and Leydig cells.[22] Brain-specific deletion of *PNPLA6* in mice resulted in severe neuropathologic symptoms concomitant with disruption of the endoplasmic reticulum, vacuolation of nerve cell bodies and abnormal reticular aggregates.[23] Thus, the lysophospholipase activity of *PNPLA6* has a critical function in the maintenance of axonal integrity. The phospholipid esterase domain (EST) is altered on intoxication with organophosphorous compounds.[5,24] EST has an important part in the enzyme activity of *PNPLA6*, and associates with the cell membrane. It is noteworthy that most of the *PNPLA6* gene mutations reported, including ours, exist in the EST.

PNPLA6 mutations cause a broad neurodegenerative spectrum, including disorders such as spastic paraplegia type 39, GHS, spastic ataxia, Oliver-McFarlane syndrome, Laurence-Moon syndrome and cerebellar ataxia in addition to BNS.[5,6,19,20] A study of genotype–phenotype correlations would be required to elucidate the molecular mechanisms underlying *PNPLA6*-related disorders.

As previously reported, 15–28% hypersegmented neutrophils were found in BNS.[7,8] We found hypersegmented neutrophils in patients 1 and 2, indicating that BNS patients with *PNPLA6* mutations might have hypersegmented neutrophils. However, why hypersegmented neutrophils occurred is not clear. Recently, a patient with ataxia and hypogonadism was reported to have hypersegmented neutrophils.[25] The genes that cause hypogonadism and ataxia might also cause hypersegmented neutrophils. Further examinations are required to address this issue.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ACCESSION NUMBERS

The nucleotide sequence data reported are available in the GenBank database under the accession numbers: KJ885304, KJ885305, KJ885306, KJ885307, KJ885308, KJ885309, KJ885310.

1 Holmes, G. A form of familial degeneration of the cerebellum. *Brain* **30**, 466–489 (1907).
2 Boucher, B. J. & Gibberd, F. B. Familial ataxia, hypogonadism and retinal degeneration. *Acta Neurol. Scand.* **45**, 507–510 (1969).
3 Neuhauser, G. & Opitz, J. M. Autosomal recessive syndrome of cerebellar ataxia and hypogonadotropic hypogonadism. *Clin. Genet.* **7**, 426–434 (1975).
4 Margolin, D. H., Kousi, M., Chan, Y. M., Lim, E. T., Schmahmann, J. D. & Hadjvassiliou, M. *et al.* Ataxia, dementia, and hypogonadotropism caused by disordered ubiquitination. *N. Engl. J. Med.* **368**, 1992–2003 (2013).
5 Rainier, S., Bui, M., Mark, E., Thomas, D., Tokarz, D. & Ming, L. *et al.* Neuropathy target esterase gene mutations cause motor neuron disease. *Am. J. Hum. Genet.* **82**, 780–785 (2008).
6 Synofzik, M., Gonzalez, M. A., Lourenco, C. M., Coutelier, M., Haack, T. B. & Rebelo, A. *et al.* PNPL6 mutations cause Boucher-Neuhauser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. *Brain* **137**, 69–77 (2014).
7 Synofzik, M. & Züchner, S. in: *Gene Review* (R) (eds Pagon, R. A., Adam, M. P., Ardinger, H. H., Bird, T. D., Dolan, C. R., Fong, C. T., *et al.*) 1993–2014 (University of Washington, Seattle, WA, 2014).

8 Umehara, T., Yaguchi, H., Suzuki, M., Isozaki, E. & Mochio, S. Are hypersegmented neutropils a characteristic of Boucher-Neuhauser syndrome? *J. Neurol. Sci.* **295**, 128–130 (2010).
9 Jbour, A. K., Mubaidin, A. F., Till, M., El-Shanti, H., Hadidi, A. & Ajlouni, K. M. Hypogonadotrophic hypogonadism, short stature, cerebellar ataxia, rod-cone retinal dystrophy, and hypersegmented neutrophils: a novel disorder or a new variant of Boucher-Neuhauser syndrome? *Med. Genet.* **40**, e2 (2003).
10 Kobayashi, F., Kurihara, Y., Nagasaka, K., Iida, H., Shindo, K. & Takiyama, Y. A patient with cerebellar ataxia, hypogonadotropic hypogonadism and vitelliform macular dystrophy: Boucher-Neuhäuser syndrome [in Japanese]. *Rinsho Shinkeigaku* **50**, 98–102 (2010).
11 Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinfomatics* **38**, 1767–1771 (2010).
12 McKenna, A., Hannna, M., Banks, E., Sivachenko, A., Cibulskis, K. & Kernytsky, A. *et al.* The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* **20**, 1297–1303 (2010).
13 Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J. & Homer, N. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinfomatics* **25**, 2078–2079 (2009).
14 Tarnutzer A. A., Gerth-Kahlert C., Timmann D., Chang D. I., Harmuth F. & Bauer P. *et al.* Boucher-Neuhäuser syndrome: cerebellar degeneration, chorioretinal dystrophy and hypogonadotropic hypogonadism: two novel cases and a review of 40 cases from the literature. *J. Neurol.* **262**, 194–202 (2014).
15 Deik, A., Johannes, B., Rucker, J. C., Sanchez, E., Brodie, S. E. & Deegan, E. *et al.* Compound heterozygous *PNPLA6* mutations cause Boucher-Neuhäuser syndrome with late-onset ataxia. *J. Neurol.* **261**, 2411–2423 (2014).
16 Synofzik, M., Kernstock, C., Haack, T. B. & Shöls, L. Ataxia meets chorioretinal dystrophy and hypogonadism: Boucher-Neuhäuser syndrome due to *PNPLA6* mutations. *J. Neurol. Neurosurg. Psychiatry* (e-pub ahead of print 30 April 2014; doi:10.1136/jnnp-2014-307793).
17 Yoon, G., Baskin, B., Tarnopolsky, M., Boycott, K. M., Geraghty, M. T. & Sell, E. *et al.* Autosomal recessive hereditary spastic paraplegia-clinical and genetic characteristics of a well-defined cohort. *Neurogenetics* **14**, 181–188 (2013).
18 Topaloglu, A. K., Lomnicze, A., Kretzschmar, D., Dissen, G. A., Kotan, L. D. & McArdle, C. A. *et al.* Loss-of-function mutations in *PNPLA6* encoding neuropathy target esterase underlie pubertal failure and neurological deficits in Gordon Holmes syndrome. *J. Clin. Endocrinol. Metab.* **10**, E2067–E2075 (2014).
19 Hufnagel, R. B., Arno, G., Hein, N. D., Hersheson, J., Prasad, M. & Anderson, Y. *et al.* Neuropathy target esterase impairments cause Oliver-McFarlane and Laurence-Moon syndromes. *J. Med. Genet.* (e-pub ahead of print 5 December 2014; doi:10.1136/jmedgenet-2014-102856).
20 Fogel, B. L., Lee, H., Deignan, J. L., Strom, S. P., Kantarci, S. & Wang, X. *et al.* Exome sequencing in the clinical diagnosis of sporadic or familial cerebellar ataxia. *JAMA Neurol.* **71**, 1237–1246 (2014).
21 Johnson, M. K. A phosphorylation site in brain and the delayed neurotoxic effect of some organophosphorus compounds. *Biochem. J.* **111**, 487–495 (1969).
22 Winrow, C. J., Hemming, M. L., Allen, D. M., Quistad, G. B., Casida, J. E. & Barlow, C. Loss of neuropathy target esterase in mice links organophosphate exposure to hyperactivity. *Nat. Genet.* **33**, 477–485 (2003).
23 Akassoglou, K., Malester, B., Xu, J., Tessarollo, L., Rosenbluth, J. & Chao, M. V. Brain-specific deletion of neuropathy target esterase/swisscheese results in neurodegeneration. *Proc. Natl Acad. Sci. USA* **101**, 5075–5080 (2004).
24 Richardson, R. J., Hein, N. D., Wijeyesakere, S. J., Fink, J. K. & Makhaeva, G. F. Neuropathy target esterase (NTE): overview and future. *Chem. Biol. Interact.* **203**, 238–244 (2013).
25 Yoshida, T., Awaya, T., Shibata, M., Kato, T., Numabe, H. & Kobayashi, J. *et al.* Hypergonadotropic hypogonadism and hypersegmented neutrophils in a patient with ataxia-telangiectasia-like disorder: potential diagnostic clues? *Am. J. Med. Genet. A* **167**, 1830–1834 (2014).

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ORIGINAL ARTICLE

A Japanese SCA5 family with a novel three-nucleotide in-frame deletion mutation in the *SPTBN2* gene: a clinical and genetic study

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To date, four families with spinocerebellar ataxia type 5 (SCA5) with four distinct mutations in the *spectrin, beta, nonerythrocytic 2* gene *(SPTBN2)* have been reported worldwide. In the present study, we identified the first Japanese family with SCA5, and analyzed this family clinically and genetically. The clinical features of the five patients in this family revealed late-onset autosomal-dominant pure cerebellar ataxia. We collected DNA samples from the majority of the family members across two generations, and exome sequencing combined with Sanger sequencing revealed a novel heterozygous three-nucleotide in-frame deletion mutation (c.2608_2610delGAG) in exon 14 of the *SPTBN2* gene. This mutation cosegregated with the disease in the family and resulted in a glutamic acid deletion (p.E870del) in the sixth spectrin repeat, which is highly conserved in the *SPTBN2* gene. This is the first three-nucleotide in-frame deletion mutation in this region of the beta-3 spectrin protein highly likely to be pathogenic based on exome and bioinformatic data.
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INTRODUCTION

Spinocerebellar ataxia type 5 (SCA5) is a rare autosomal-dominant pure spinocerebellar ataxia (SCA). SCA5 patients predominantly exhibit limb and gait ataxia (>90%); however, truncal ataxia, sensory deficits, abnormal eye movements, dysarthria and exaggerated deep tendon reflexes are also prevalent (25–90%).[1] Individuals generally struggle with fine motor coordination but rarely require a wheelchair until late in the disease course.[1]

SCA5 is caused by mutations in the *spectrin, beta, nonerythrocytic 2* gene (*SPTBN2*). This gene is primarily expressed in the brain. It encodes the beta-III spectrin protein consisting of 2390 amino acids comprising two calponin homology domains, 17 triple-helical spectrin repeats and a pleckstrin homology domain, and as a cytoskeletal protein is highly expressed in Purkinje cells.[2]

To date, five mutations, that is, three missense (p. L253P, p. T472M and R480W) and two deletion (p.E532_M544del and p.L629_R634delinsW) ones, in the *SPTBN2* gene have been reported in SCA5 families originating from the United States, France, Germany and Norway,[3–6] and an SCA5 patient.[7] In the present study, we report the first Japanese SCA5 family with a novel heterozygous three-nucleotide in-frame deletion mutation in the *SPTBN2* gene.

MATERIALS AND METHODS

Patients

The pedigree is shown in Figure 1a. Eight living family members were interviewed and examined by experienced neurologists, and the diagnosis of familial SCA was made on the basis of the patients' clinical presentations as well as the results of physiological, radiological and biochemical examinations. Unfortunately, we were not able to obtain the consent of other family members to be examined in this study. Brain magnetic resonance imaging (MRI) in four affected members revealed cerebellar atrophy. Molecular analysis of the proband excluded SCA1, SCA2, MJD, SCA6, SCA7, SCA8, SCA12, SCA17, SCA31, SCA36 and DRPLA. The present clinical and genetic study was approved by the institutional review board of the Yamanashi University, and informed consent was obtained from all participating individuals.

Whole-exome sequencing

We carried out whole-exome sequencing of genomic DNA from the proband and the father. Genomic DNA was extracted from blood leukocytes from eight individuals. We used a NEBNest DNA Library Prep Master Mix set (New England Biolabs, Ipswich, MA, USA, E6040) to manufacture the DNA library. Exome capture was performed with a SureSelect Human All Exon V4 kit (Agilent, Santa Clara, CA, USA). Paired-end sequencing was carried out using an Illumina HiSeq 2000, which generated 100-bp reads. Reference sequence data were aligned using a Burrows-Wheeler Aligner.[8] Then single-nucleotide variants were analyzed using a genome analysis tool kit. The additional databases utilized included the Single-Nucleotide Polymorphism Database[9] and the 1000 Genomes Project.[10] As the clinical features of the affected individuals in this family are typical of an autosomal-dominant SCA, we initially filtered for heterozygous variations. We then filtered for those that were shared between the proband and the father. Protein sequence alignment was performed using the PROVEAN[11] and Mutation Taster.[12]

Sanger sequencing

Exon 14 of the *SPTBN2* gene was amplified using the following PCR primer sequences: *SPTBN2* ex14-F: 5′-CTGCTCAACAAGCACACAGC-3′ and

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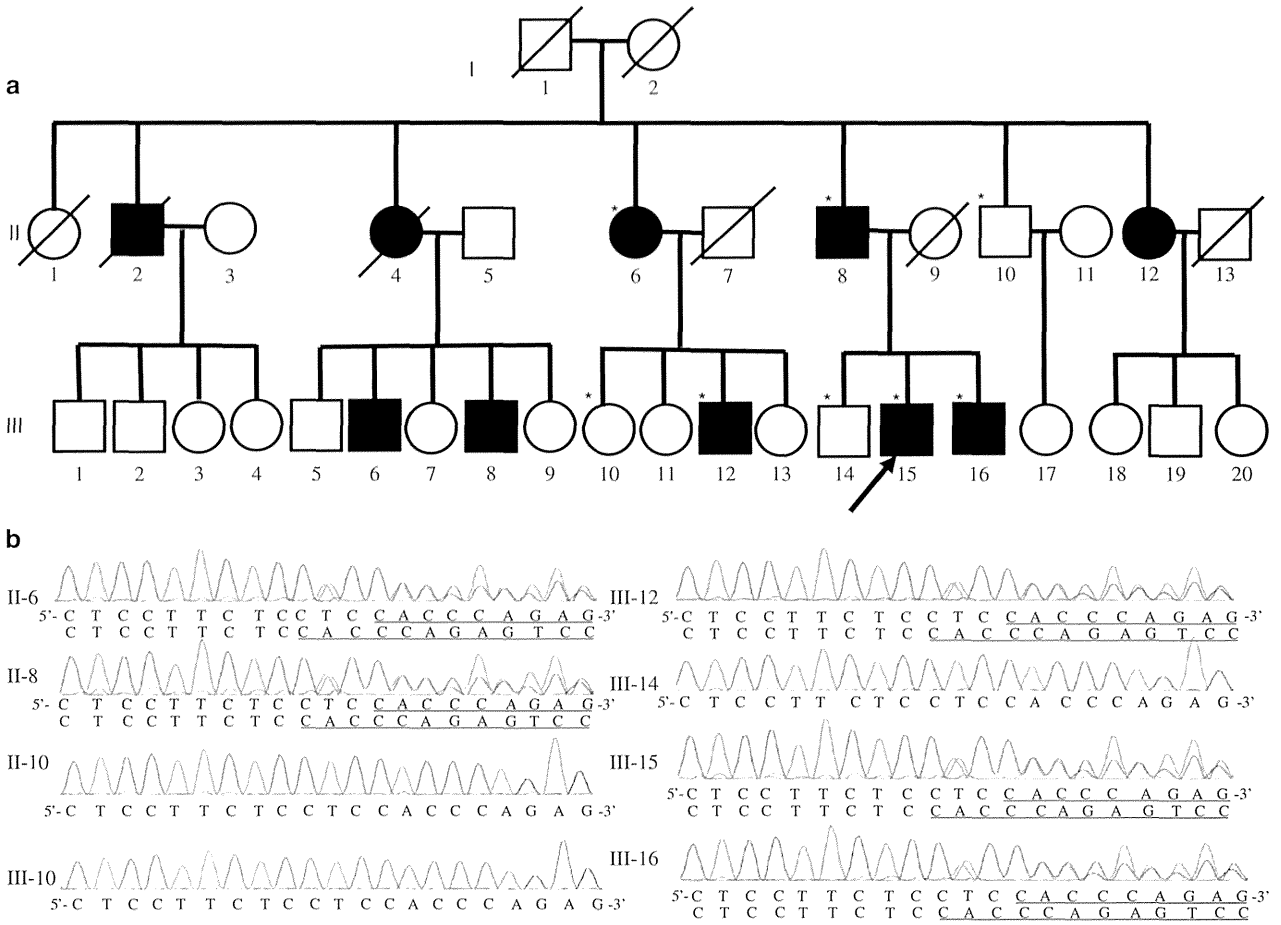

Figure 1 Pedigree of the SCA5 family. (**a**) Squares and circles indicate males and females, respectively. Filled symbols indicate affected individuals, whereas open symbols indicate unaffected individuals. Slashes indicate the people who have died already. Asterisks indicate the individuals who have been examined clinically and molecularly. (**b**) Representative sequencing of the *SPTBN2* gene in part of the family (II-6, II-8, II-10, III-10, III-12, III-14, III-15 and III-16). The mutation cosegregated with the disease.

SPTBN2 ex14-R: 5′-GTTGGCAATGACTGAATCTGG-3′. Sanger sequencing was performed according to an established standard protocol for biotechnology. To determine whether or not the mutation identified in the present study is found in the general population, DNA samples from unrelated control subjects ($n = 90$) were screened.

RESULTS

Clinical features

The proband was a 57-year-old male who presented with poor coordination that he first noted at the age of 51. At the age of 52, he noted mild unsteadiness of gait. Neurological examination at the age of 57 revealed mild dysarthria, dysphagia and incoordination of the lower limbs. He had trouble accomplishing the heel-to-knee test and difficulty in standing with feet together; however, he was able to walk without assistance. Knee tendon reflexes were slightly exaggerated bilaterally; however, Babinski's sign was negative. Eye movements were intact, and no nystagmus was observed. Neither mental retardation nor sensory abnormality was observed.

The clinical features of the five patients in this family are summarized in the Table 1. The ages at onset in the five patients ranged from 50s to 60s. There was no obvious anticipation of age at the onset between the two generations. Limb and trunk ataxia, and cerebellar dysarthria, were the common features in all the affected individuals. Cognitive deterioration, epilepsy, saccadic eye movements, visual disturbance, ophthalmoplegia and peripheral nerve involvement were absent in all the patients. The disease progressed very slowly and the clinical severity correlated with the duration of the disease. Two of the patients (II-6 and II-8) became wheelchair-bound ~20 to 30 years after the disease onset. Brain MRI of our patients revealed moderate to severe atrophy in the cerebellum (Figure 2). Single-photon emission tomography (SPECT) in the proband (III-15) showed blood flow reduction in the cerebellum (Figure 3).

Table 1 Clinical features of the five patients in the SCA5 family

Case	*II-6*	*II-8*	*III-12*	*III-15*	*III-16*
Sex	F	M	M	M	M
Present age	89	87	55	57	52
Age of onset	60	48	54	51	51
Cerebellar atrophy (MRI)	+	+	+	+	+
Limb ataxia	+	+	+	+	+
Dysarthria	+	+	+	+	+
Unsteadiness of gait	w	w	–	+	–

Abbreviations: F, female; M, male; MRI, magnetic resonance imaging; +, positive; –, negative; w, wheelchair-bound.

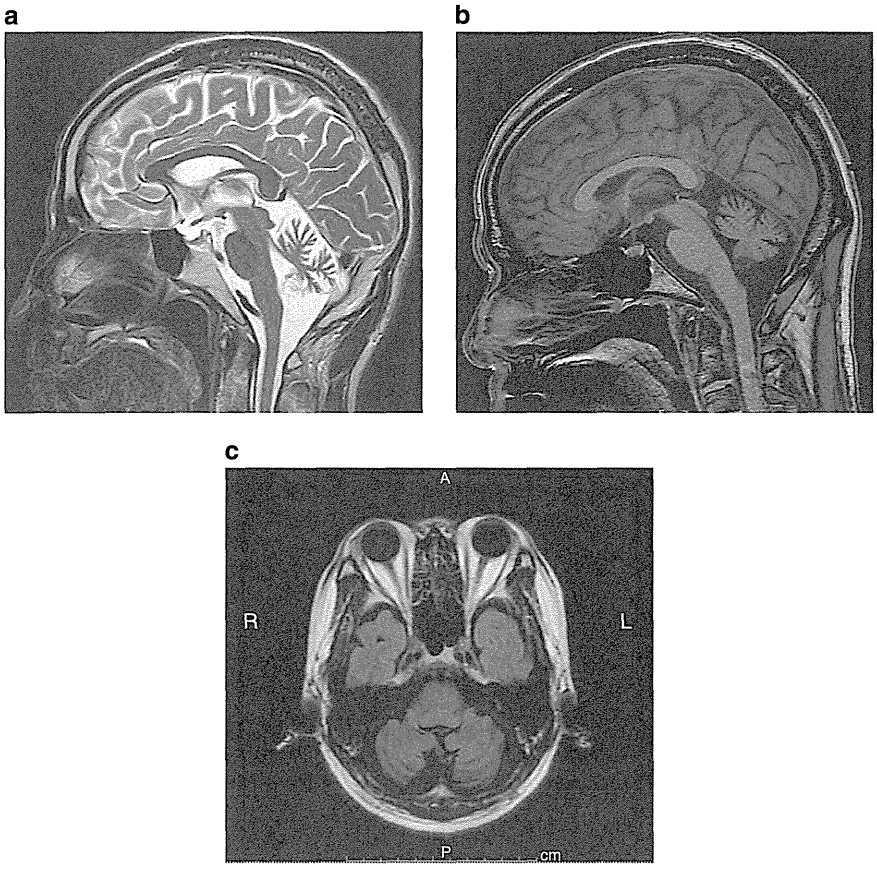

Figure 2 Representative brain MRI findings in the patients. Sagittal brain MRI of III-12 (**a**, T2-weighted) and III-15 (**b**, T1-weighted), and axial brain MRI of III-15 (**c**, T1-weighted) show moderate to severe cerebellar atrophy.

Mutation analysis

We identified 10 692 and 10 641 variations in the proband and the father, respectively, of which 231 and 241, respectively, were novel heterozygous variants. Of these, 183 variants were shared between the proband and the father (Supplementary Data). Once anonymous open reading frames were excluded and genes associated with SCAs were prioritized, we found one likely candidate three-nucleotide in-frame deletion mutation in the *SPTBN2* gene, a gene known to cause SCA5.

On Sanger sequencing, we validated the most possible shared three-nucleotide in-frame deletion mutation in the *SPTBN2* gene as the cause of the disease. This mutation cosegregated faithfully with the disease phenotype in this family (Figure 1b). All the five patients had a heterozygous c.2608_2610delGAG (p.E870del) mutation in the *SPTBN2* gene, which is located at an evolutionarily conserved amino acid (Figure 4). We performed bioinformatic analyses using the PROVEAN and Mutation Taster (see 'Materials and methods' section), which predicted that the detected variant was deleterious and disease-causing, respectively. The variant was not detected in our 180 control chromosomes and was not observed in the databases of normal sequence variations (1000 Genome Project and Single-Nucleotide Polymorphism Database). Thus, the mutation identified was likely to be pathogenic.

DISCUSSION

We presented here the first Japanese case of SCA5 with a highly likely pathogenic mutation in the *SPTBN2* gene. This SCA5 family is the fifth one worldwide. The two deletions (p.E532_M544del and p.L629_R634delinsW) in the *SPTBN2* gene reported earlier in American and French SCA5 families comprise distinct and nonoverlapping in-frame mutations in the third of the 17 spectrin repeats.[3,4] Both of these deletions are predicted to disrupt the triple-helical structure of the spectrin repeat and the conformation of the spectrin tetramer, which likely disrupts the protein structure involving the characteristic leucine and tryptophan residues.[13] The third SCA5 family reported from Germany has a missense mutation (p. L253P) in the *SPTBN2* gene that interferes with the actin-binding site in the second calponin homology domain.[5] The fourth family, of Norwegian descent, has a missense mutation (c.1415C>T) that results in a p. T472M substitution in the second spectrin repeat of the beta-III spectrin protein.[6]

On the other hand, the p.E870del mutation detected in our family is the first one located in the sixth spectrin repeat likely associated with SCA5, resulting in the deletion of one glutamic acid deletion out of a total of 2390 amino acids. Bioinformatic analysis suggests that an amino-acid sequence changed, which might have affected protein features. Similarly, a three-nucleotide in-frame deletion mutation (c.679_681delTTC, p.F227del) in the *KCND3* gene has been reported in another type of autosomal-dominant cerebellar ataxia (SCA22).[14] This mutation leads to the retention of voltage-gated potassium channel Kv4.3 subunits in the cytoplasm, which is consistent with the lack of A-type K^+ channel conductance on whole-cell patch-clamp recording.[14] The function of beta-III spectrin, however, might be much more diverse than that of *KCND3* and rather different from that of *KCND3*.

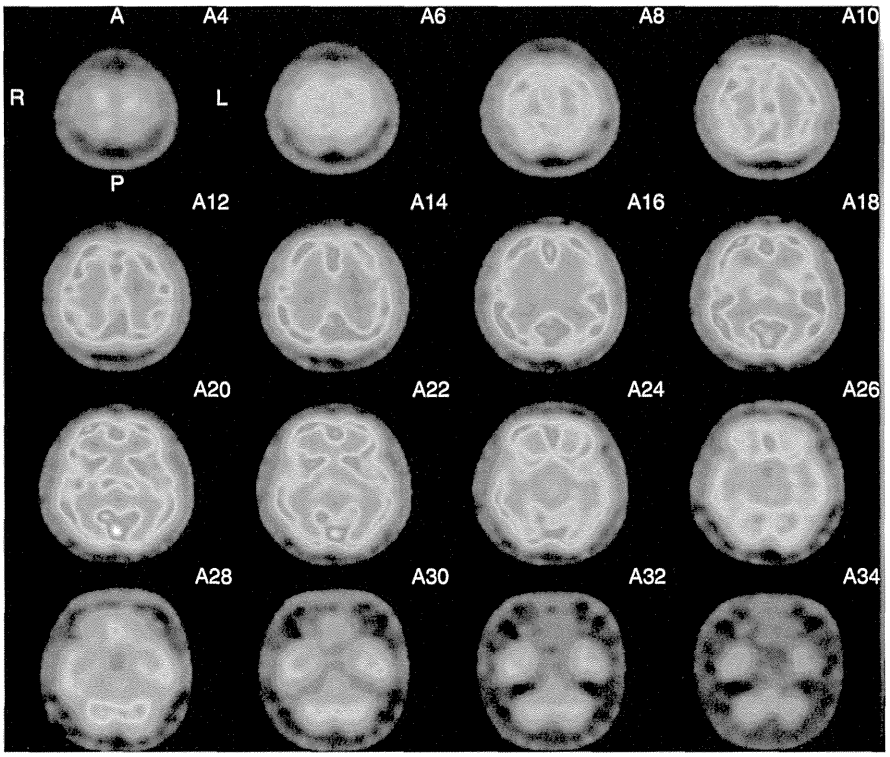

Figure 3 Single-photon emission computed tomography findings. SPECT of a patient (III-15) shows blood flow reduction in the cerebellum.

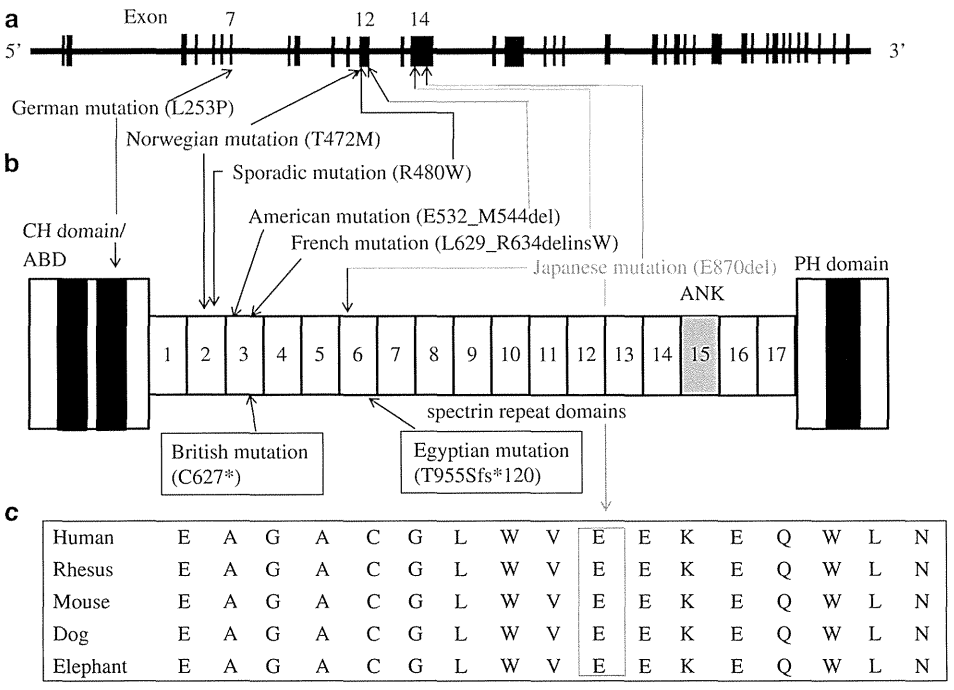

Figure 4 Mutations of the *SPTBN2* gene. (**a**) Illustration of exons (filled rectangles) in the *SPTBN2* gene. (**b**) A schematic representation of the functional domains of β-3 spectrin, modified from the reported representation,[2] is shown along with the positions of all found mutations, exhibiting dominant (upper) or recessive (lower) transmission, so far. CH, calponin homology domain; ABD, actin-binding domain; ANK, ankyrin-binding domain; PH, pleckstrin homology domain. (**c**) Evolutionary conservation of the Japanese SCA5 mutation (p.E870del) among species.