for autosomal recessive cerebellar ataxia and spastic paraplegia. *Intern Med* **52**, 1629–1633 (2013).
5. Hammer, M. B. *et al.* Exome sequencing: an efficient diagnostic tool for complex neurodegenerative disorders. *Eur J Neurol* **20**, 486–492, doi:10.1111/j.1468-1331.2012.03883.x (2013).
6. de Bot, S. T., Willemsen, M. A., Vermeer, S., Kremer, H. P. & van de Warrenburg, B. P. Reviewing the genetic causes of spastic-ataxias. *Neurology* **79**, 1507–1514, doi:10.1212/WNL.0b013e31826d5fb0 (2012).
7. Tesson, C. *et al.* Alteration of Fatty-Acid-metabolizing enzymes affects mitochondrial form and function in hereditary spastic paraplegia. *Am J Hum Genet* **91**, 1051–1064, doi:10.1016/j.ajhg.2012.11.001 (2012).
8. Schuurs-Hoeijmakers, J. H. *et al.* Mutations in *DDHD2*, encoding an intracellular phospholipase A(1), cause a recessive form of complex hereditary spastic paraplegia. *Am J Hum Genet* **91**, 1073–1081, doi:10.1016/j.ajhg.2012.10.017 (2012).
9. Adzhubei, I. A. *et al.* A method and server for predicting damaging missense mutations. *Nat Methods* **7**, 248–249, doi:10.1038/nmeth0410-248 (2010).
10. Kumar, P., Henikoff, S. & Ng, P. C. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. *Nat Protoc* **4**, 1073–1081, doi:10.1038/nprot.2009.86 (2009).
11. Schwarz, J. M., Rodelsperger, C., Schuelke, M. & Seelow, D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nat Methods* **7**, 575–576, doi:10.1038/nmeth0810-575 (2010).
12. Gonzalez, M. *et al.* Mutations in phospholipase DDHD2 cause autosomal recessive hereditary spastic paraplegia (SPG54). *Eur J Hum Genet* **21**, 1214–1218, doi:10.1038/ejhg.2013.29 (2013).
13. Citterio, A. *et al.* Mutations in *CYP2U1*, *DDHD2* and *GBA2* genes are rare causes of complicated forms of hereditary spastic paraparesis. *J Neurol* **261**, 373–381, doi:10.1007/s00415-013-7206-6 (2014).
14. Magariello, A. *et al.* Further evidence that *DDHD2* gene mutations cause autosomal recessive hereditary spastic paraplegia with thin corpus callosum. *Eur J Neurol* **21**, e25–26, doi:10.1111/ene.12305 (2014).
15. Adzhubei, I., Jordan, D. M. & Sunyaev, S. R. Predicting functional effect of human missense mutations using PolyPhen-2. *Curr Protoc Hum Genet/editorial board, Jonathan L. Haines. et al.* **Chapter 7**, Unit7 20, doi:10.1002/0471142905.hg0720s76 (2013).
16. Sim, N. L. *et al.* SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res* **40**, W452–457, doi:10.1093/nar/gks539 (2012).
17. Brockmann, K. *et al.* Proton MRS profile of cerebral metabolic abnormalities in Krabbe disease. *Neurology* **60**, 819–825 (2003).
18. Jamrozik, Z. *et al.* Late onset GM2 gangliosidosis mimicking spinal muscular atrophy. *Gene* **527**, 679–682, doi:10.1016/j.gene.2013.06.030 (2013).
19. Miyanomae, Y. *et al.* Cerebral MRI and spectroscopy in Sjogren-Larsson syndrome: case report. *Neuroradiology* **37**, 225–228 (1995).
20. van Domburg, P. H. *et al.* Sjogren-Larsson syndrome: clinical and MRI/MRS findings in FALDH-deficient patients. *Neurology* **52**, 1345–1352 (1999).
21. Rossi, A. *et al.* Hypomyelination and congenital cataract: neuroimaging features of a novel inherited white matter disorder. *AJNR. Am J Neuroradiol* **29**, 301–305, doi:10.3174/ajnr.A0792 (2008).
22. Nakajima, K. *et al.* A novel phospholipase A1 with sequence homology to a mammalian Sec23p-interacting protein, p125. *J Biol Chem* **277**, 11329–11335, doi:10.1074/jbc.M111092200 (2002).
23. Inoue, H. *et al.* Roles of SAM and DDHD domains in mammalian intracellular phospholipase A1 KIAA0725p. *Biochim Biophys Acta* **1823**, 930–939, doi:10.1016/j.bbamcr.2012.02.002 (2012).
24. Seelow, D., Schuelke, M., Hildebrandt, F. & Nurnberg, P. HomozygosityMapper--an interactive approach to homozygosity mapping. *Nucleic Acids Res* **37**, W593–599, doi:10.1093/nar/gkp369 (2009).
25. Bahlo, M. & Bromhead, C. J. Generating linkage mapping files from Affymetrix SNP chip data. *Bioinformatics* **25**, 1961–1962, doi:10.1093/bioinformatics/btp313 (2009).
26. Kelley, L. A. & Sternberg, M. J. Protein structure prediction on the Web: a case study using the Phyre server. *Nature Protoc* **4**, 363–371, doi:10.1038/nprot.2009.2 (2009).
27. Mayer, B. J., Hirai, H. & Sakai, R. Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. *Curr Biol: CB* **5**, 296–305 (1995).
28. Withers-Martinez, C., Carriere, F., Verger, R., Bourgeois, D. & Cambillau, C. A pancreatic lipase with a phospholipase A1 activity: crystal structure of a chimeric pancreatic lipase-related protein 2 from guinea pig. *Structure* **4**, 1363–1374 (1996).

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Author contributions

H.D. wrote the main manuscript and prepared the figures and tables. S.T., F.T., K.Y. and N. Matsumoto revised the manuscript and gave conceptual advice. M.U., K.Y. and S.I. collected the clinical data and samples. H.D., Y.F.Y. and S.T. conducted linkage analysis. T.B. and K.T. analyzed PLA_1 activity of the DDHD2 and prepared Figure 3. M.S. and K.O. analyzed the structure of the DDHD2 mutant and prepared Figure 2. H.D., S.M., M.N., Y.T., N. Miyake and H.S. conducted the analysis of the genetic data.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

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Letter to the Editor

Adult-onset vanishing white matter disease with novel missense mutations in a subunit of translational regulator, *EIF2B4*

To the Editor:

Vanishing white matter disease (VWM) is one of the most prevalent inherited leucoencephalopathies with autosomal recessive inheritance. The clinical features of VWM are episodes of rapid neurological deterioration provoked by stresses, such as fever and minor head trauma, during a chronic progressive course (1). Magnetic resonance imaging (MRI) findings that exhibit leukoencephalopathy, with lesions having cerebrospinal fluid (CSF)-like signals, are very characteristic. The phenotypic variation is broad – most cases of VWM develop in early childhood though adult-onset cases have also been reported occasionally. Severe forms start in the prenatal or early infantile period and lead to early death (2). In contrast, much milder variants start in adolescence or adulthood and are characterized by slow disease progression. VWM is caused by mutations in the genes *EIF2B1-5* encoding the subunits of eukaryotic translation initiation factor 2B (eIF2B). Among them, mutations in *EIF2B5* are the most common, accounting for 60–70% of all cases of VWM (3). On the other hand, mutations in *EIF2B4* are 4–14% (4, 5). Here, we describe a patient with adult-onset VWM who carries novel missense mutations in *EIF2B4*.

A 59-year-old Japanese woman presented to our hospital. She noticed gait unsteadiness and forgetfulness at the age of 56, which exacerbated slowly. She had experienced no episodes of rapid neurological symptoms evoked by stresses. Her birth and development during childhood were normal, and she had no ovarian failure. The family history was unremarkable. Her parents did not have a consanguineous marriage. Neurological examinations showed spastic paraparesis, increased bilateral patellar tendon reflexes, and bilateral extensor Babinski signs. Mini-Mental State Examination score was 16. Wechsler Adult Intelligence Scale-third edition (WAIS-III) revealed a low intelligence quotient (verbal IQ of 66, performance IQ of 59, full-scale IQ of 60).

Brain MRI showed symmetric diffuse high-intensity lesions in the deep white matter on T2-weighted images. The lesions in the deep frontal white matter had CSF-like signals on fluid-attenuated inversion recovery (FLAIR) images (Fig. 1a,b). The results of routine laboratory tests were normal. CSF examination showed elevation of protein at 54 mg/dl (normal <40) and increased glycine concentrations at 11.6 μmol/l (normal 7.7 ± 3.5); the latter is considered a biochemical marker for VWM (6).

The clinical course and MRI findings characteristic of VWM prompted us to perform genetic analyses. Sanger sequencing of all exons of the VWM causative genes, *EIF2B1-5*, revealed novel heterozygous missense mutations, c.617T>C (p.Met206Thr) and c.952A>G (p.Ile318Val) in *EIF2B4*. Furthermore, direct nucleotide sequence analysis of the plasmids, in which genomic segments containing c.617T>C and c.952A>G in *EIF2B4* were cloned, revealed that the mutations, c.617T>C (p.Met206Thr) and c.952A>G (p.Ile318Val), were located on different alleles (Fig. 1c).

As shown in Fig. 1d, the amino acid Ile318 of *EIF2B4* is highly conserved among species, and p.Ile318Val mutation is predicted to be probably damaging and disease causing by Polyphen-2, SIFT, and Mutation Taster. On the other hand, the amino acid Met206 is highly conserved among mammals. By SIFT and Mutation Taster, p.Met206Thr is predicted to be damaging and disease causing (Fig. 1d). These mutations were not present in 276 unrelated Japanese control subjects, and not registered in the Human Genetic Variation Database (HGVD), a database of the exome sequencing of 1208 Japanese individuals.

This is the second reported case of adult-onset VWM with mutations in *EIF2B4* (4). The age of 56, when our case developed symptoms, is the oldest among patients with *EIF2B4* mutation thus reported (5). So far, the influence of genotype on the phenotype in *EIF2B4* mutation has not yet been clarified. Revealing the genotype–phenotype relationship is important as it enables clinicians and genetic counselors to provide appropriate information to patients and families. Our case indicates that the heterozygous c.617T>C (p.Met206Thr) and c.952A>G (p.Ile318Val) mutations in *EIF2B4* might be related to the late-onset milder form of VWM.

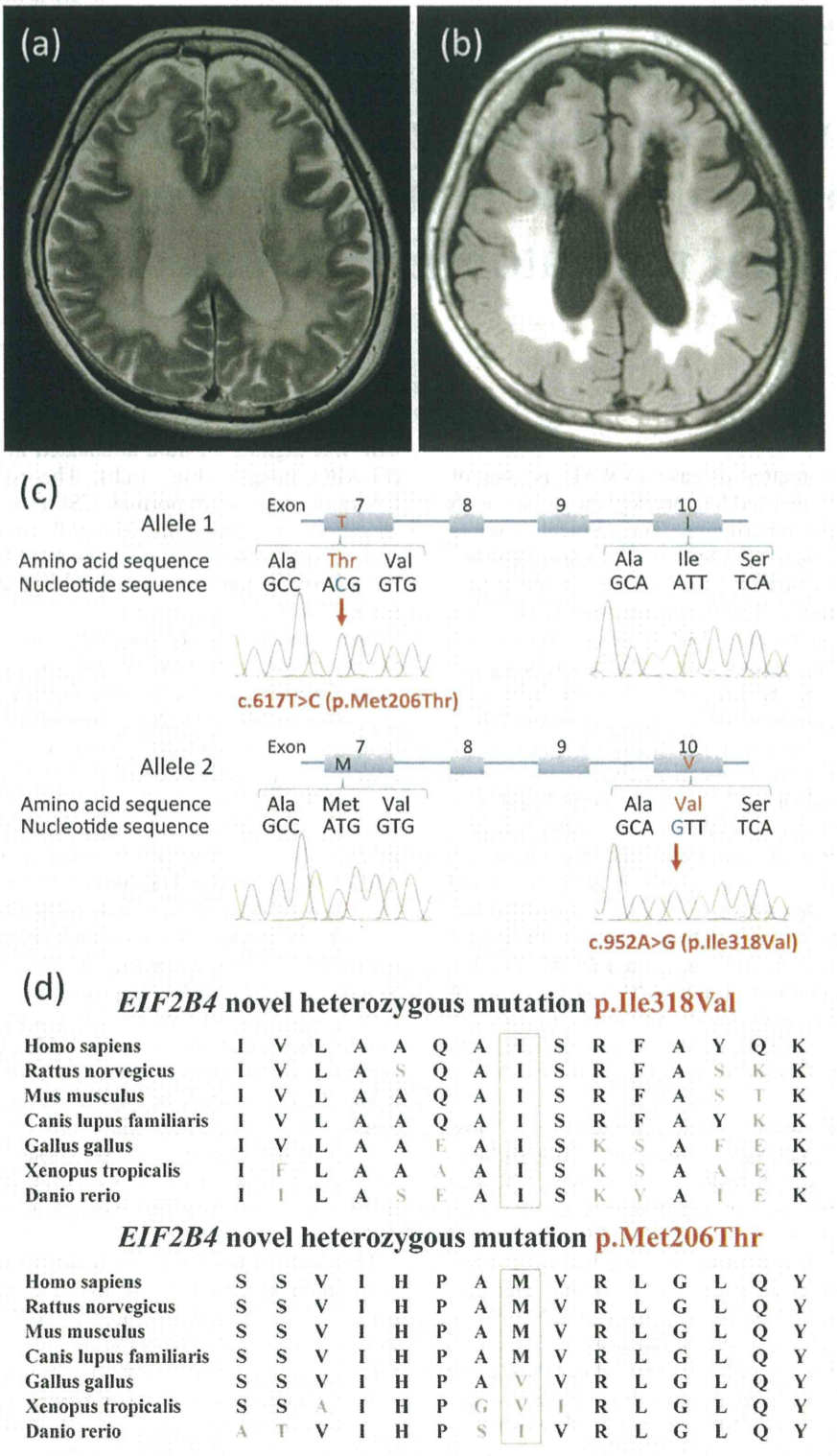

Fig. 1. T2-weighted images show symmetric, diffuse high-intensity lesions in the deep white matter (**a**); replacement by cerebrospinal fluid (CSF) was present predominantly in the deep frontal white matter on FLAIR images (**b**). Heterozygous novel missense mutations, c.617T>C (p.Met206Thr) and c.952A>G (p.Ile318Val) were identified in *EIF2B4* (**c**). The amino acid Ile318 is highly conserved among species, and the amino acid Met206 is highly conserved among mammals (**d**).

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References

1. van der Knaap MS, Barth PG, Gabreels FJ et al. A new leukoencephalopathy with vanishing white matter. Neurology 1997: 48 (4): 845–855.
2. van der Knaap MS, Van Berkel GM, Herms J et al. eIF2B-related disorders: antenatal onset and involvement of multiple organs. Am J Hum Genet 2003: 73 (5): 1199–1207.
3. van der Lei HD, van Berkel CG, van Wieringen WN et al. Genotype-phenotype correlation in vanishing white matter disease. Neurology 2010: 75 (17): 1555–1559.
4. Carra-Dalliere C, Horzinski L, Ayrignac X et al. Natural history of adult-onset eIF2B-related disorders: a multicentric survey of 24 cases. Rev Neurol (Paris) 2011: 167 (11): 802–811.
5. Fogli A, Schiffmann R, Bertini E et al. The effect of genotype on the natural history of eIF2B-related leukodystrophies. Neurology 2004: 62 (9): 1509–1517.
6. van der Knaap MS, Wevers RA, Kure S et al. Increased cerebrospinal fluid glycine: a biochemical marker for a leukoencephalopathy with vanishing white matter. J Child Neurol 1999: 14 (11): 728–731.

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SHORT COMMUNICATION

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

Kishin Koh[1], Fumikazu Kobayashi[1], Michiaki Miwa[1], Kazumasa Shindo[1], Eiji Isozaki[2], Hiroyuki Ishiura[3], Shoji Tsuji[3] and Yoshihisa Takiyama[1]

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*

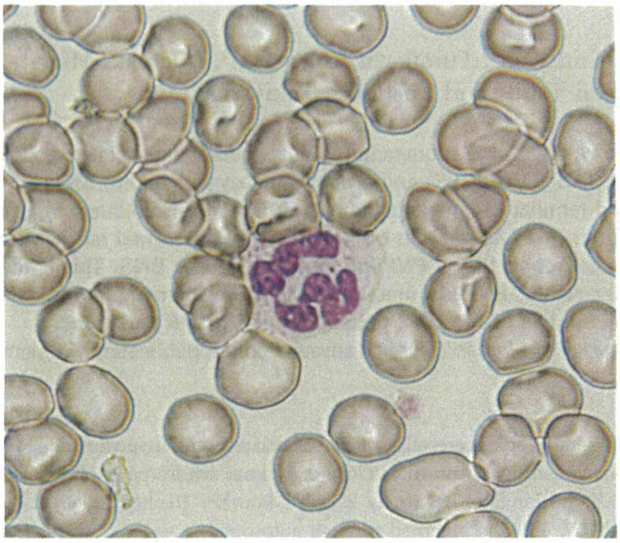

Figure 2 Hypersegmented neutrophils in patient 1. Blood testing of patient 1 revealed 9% hypersegmented neutrophils. As hypersegmented neutrophils were found in patients 1 and 2, Boucher-Neuhäuser syndrome patients with *PNPLA6* mutations might have hypersegmented neutrophils.

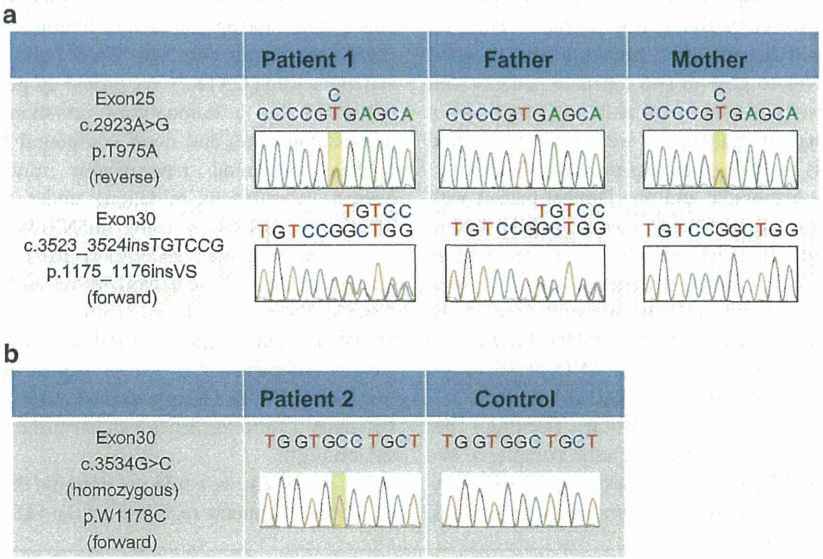

Figure 1 Electropherograms of two Japanese patients with Boucher-Neuhäuser syndrome. (**a**) A novel compound heterozygous mutation (c.2923A>G/ c.3523_3524insTGTCCG) in the *PNPLA6* gene was identified in patient 1. A heterozygous mutation of c.2923A>G was derived from the mother, and that of c.3523_3524insTGTCCG 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.343-2A>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+2T>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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Letter to the Editor

The first Japanese familial case of spinocerebellar ataxia 23 with a novel mutation in the PDYN gene

Keywords:
Spinocerebellar ataxia 23
Head tremor
Hot cross bun (HCB) sign
Parkinsonism
Multiple system atrophy, Prodynorphin (*PDYN*)

Spinocerebellar ataxia (SCA) 23 is a type of spinocerebellar degeneration, and its causative gene, prodynorphin (*PDYN*), has been identified [1]. This degenerative disease is characterized by an ocular motility disorder, gait ataxia, and dysarthria. According to a report on affected Dutch families, onset occurred between the ages of 43 and 56 years. These patients exhibit slow saccades, dysmetria, gait and limb ataxia, and abnormalities in the vibration sense below the knee. Hyperreflexia is commonly observed as well. Postural tremor of the head and upper limbs among some of the family members is diagnosed as essential tremor. The family members also experience mild dementia starting at approximately 50 years of age. Cerebral imaging has shows a high degree of cerebellar atrophy; one patient showed frontotemporal, cerebellar vermis, and bulbospinal atrophy [2].

We report a case of an outpatient (the proband) in our hospital who exhibited clinical manifestations of spinocerebellar degeneration and the "hot cross bun" (HCB) sign on brain MRI; we performed *SCA* genetic analysis on this patient.

The proband (Fig. 1A; II-6) had mild ataxia before the age of 10 years. She exhibited signs such as dysmetria, gait ataxia, dysarthria, and hyperreflexia but no essential tremor, deep sensory disturbance, or distal neuropathy. She was the youngest of six siblings, and her fifth-oldest sister (II-5) had head tremor and symptoms similar to those of the proband. The III-3 was only interviewed by his mother (II-5). His mother said that III-3 had only a minor head tremor. Symptoms of autonomic dysfunction, such as orthostatic hypotension, lower urinary tract symptoms, or intestinal dysfunction, were not observed in these patients.

Brain MRI of the proband showed moderate atrophy of the brain stem and cerebellum (Fig. 1B–D) since the early stage of the disease. Furthermore, brain MRI in the horizontal plane showed the HCB sign in the brain stem (Fig. 1C) and atrophy outside the globus pallidus (Fig. 1D), which are characteristic of the parkinsonism type of multiple system atrophy (MSA-P). Cerebral blood flow scintigraphy performed simultaneously with the MRI showed a greater reduction of blood flow in the brain stem than in the cerebellum (Fig. 1E). For these reasons, she was first diagnosed as olivopontocerebellar-type multiple system atrophy rather than spinocerebellar degeneration.

Genomic DNA samples were extracted from peripheral blood leukocytes from the proband and her sister (II-6 and II-5). Screening for repeat expansions at the SCA1, SCA2, MJD, SCA6, SCA7, SCA8, SCA12, SCA17, SCA31, SCA36, and DRPLA loci was performed in the proband (II-6) using fragment analysis and/or repeat-primed polymerase chain reaction analysis, but we could not observe the expected gene mutations. Next, we performed exome sequencing analysis of the proband. Briefly, exon sequences were enriched using the SureSelect Human All Exon v4 + UTRs Kit and the Illumina Hiseq2000 platform [3]. We then screened the processed DNA for single-nucleotide variants (SNVs) in previously known causative genes of autosomal dominant spinocerebellar ataxia, such as *SPTBN2*, *TTBK2*, *KCNC3*, *PPKCG*, *ITPR1*, *KCND3*, *PDYN*, *FGF14*, and *AFG3L2*. We found one heterozygous novel mutation (p.R213H, c.638A > G) in *PDYN*. The p.R213H mutation was shared by both II-5 and the proband. This mutation was not observed in 422 Japanese control subjects. Analysis of R213H using the PROVEAN and Polyphen-2 database software suggested severe alterations of protein function (PROVEAN score, −4.804 and Polyphen-2 damage score, 1.00). The p.R213H mutation occurred near p.L211S, p.R212W, and p.R215C [2], which also show abnormal protein structures. Amino acid regions 202–230 are completely conserved among mammals (Fig. 2).

The genetic mutation of SCA23 has been described as a disease with onset at middle age and slowly progressing cerebellar degeneration. A neuropathological examination of SCA23 autopsy tissue has revealed neuronal loss in the Purkinje cell layer, dentate nuclei, and inferior olivary nuclei [2]. Additional neurological examination has revealed dysarthria and oculomotor problems, such as slowing saccades and ocular dysmetria [1].

However, in this study, we observed head tremor with the neck turned sideways in addition to cerebellar manifestations in this first Japanese familial case. We analyzed previously reported clinical manifestations other than those evident in our case and observed that the disease appears to have several clinical manifestations, such as neck tremor and typical cerebellar signs alone. This type of neck tremor is highly similar to that observed in Parkinson's disease with tremor. Therefore, on the basis of the observed neck tremor, a careful diagnosis is necessary.

In our case, brain MRI showed the HCB sign. Many previous reports on the HCB sign in brain MRI have indicated the usefulness of slit-like hyperintense T2 signals predominantly in the rear of the lateral putamen, with the HCB sign in the pons, for differential diagnosis of MSA-P and Parkinson's disease [4]. However, even some healthy elderly individuals exhibit an abnormal signal in the

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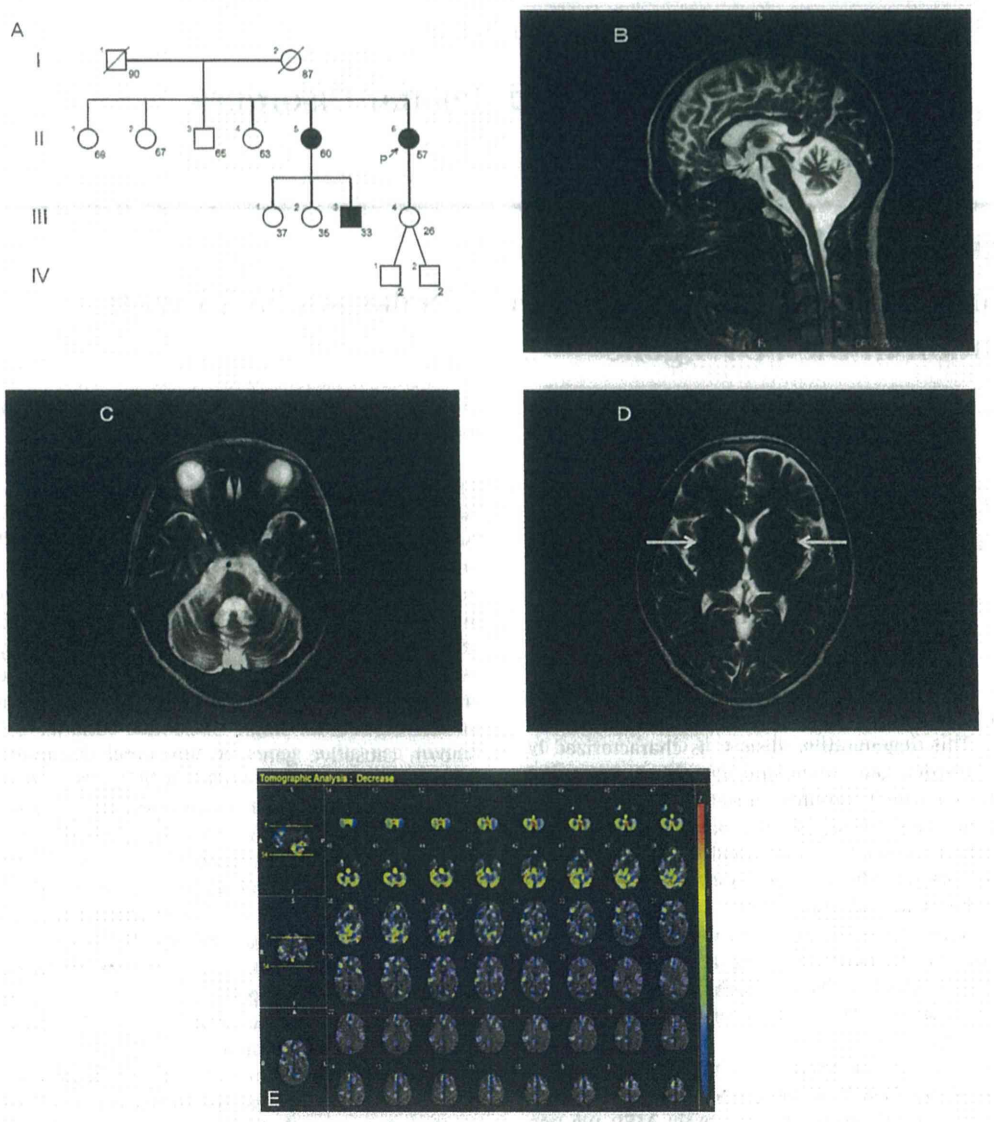

Fig. 1. SCA23 pedigree in Japan. The patient II-6 is the proband (A). Brain T2WI MR sagittal image of the proband (B). A brain T2WI-MR image showing the "hot cross bun" sign as a cruciform. A hyperintense signal can be seen in the atrophied pontine (C). Brain T2MRI in a T2WI MR horizontal image. A hyperintense signal in the rear of the lateral putamen is shown (D: arrow head). Brain single-positron emission computed tomographic three-dimensional stereotactic surface projection analysis (3D-SSP) of the SCA23 proband (E). The cerebellar bloodstream is largely decreased relative to other regions.

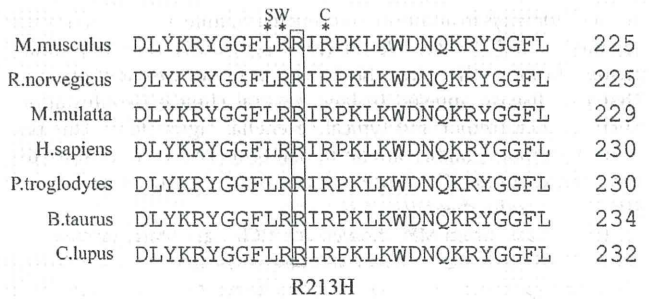

Fig. 2. Amino acid sequence alignment of the *PDYN* gene exon 3 region. Positions 202–230 in *Homo sapiens* are highly conserved across species, including human (*Homo sapiens*; NP_060841.3), chimpanzee (*Pan troglodytes*; XP_519635.2), dog (*Canis lupus*; XP_539946.2), mouse (*Mus musculus*; NP_766341.3), rat (*Rattus norvegicus*; XP_224757.4), chicken (*Gallus gallus*; XP_420453.2), and zebra fish (*Danio rerio*; XP_001333479.2). *The reported mutation position. The sequences were aligned using the NCBI homologene web site (http://www.ncbi.nlm.nih.gov/homologene).

putamen on MRI at ≥1.5T. Therefore, some values of magnetic field strength may yield false positive results [5]. In addition, hyperintense signals in the rear of the lateral putamen are also observed in patients with SCA17 or adult GM1 gangliosidoses; the HCB sign is also detectable in patients with SCA2, SCA7, SCA8, or fragile X-associated tremor ataxia syndrome. As observed in our SCA23 case, genetic testing should be considered for individuals with SCA23 and the HCB sign on brain MRI or hyperintense signals in the rear of the lateral putamen. Recent studies show that the HCB sign in the brain stem is associated with pontocerebellar tract degeneration [5]. In our case, the patient had the HCB sign (Fig. 1C) and atrophy of the pons and brain stem on brain MRI. Therefore, the pontocerebellar tract may have been damaged. In conclusion, we identified a new point mutation (p.R213H) in the *PDYN* gene. Our results suggest that the p.R213H mutation causes severe functional abnormalities. The family of the patient in this case is the first familial case of SCA23 to be identified in Japan.