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BRIEF COMMUNICATION

PLA2G6-associated neurodegeneration presenting as a complicated form of hereditary spastic paraplegia

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Abstract

PLA2G6-associated neurodegeneration (PLAN) comprises heterogeneous neurodegenerative disorders, including infantile neuroaxonal dystrophy, neurodegeneration with brain iron accumulation 2B, and Parkinson disease 14 (PARK14). In addition, very recently, *PLA2G6* mutations have been reported to represent a phenotype of hereditary spastic paraplegia (HSP). In this study, we screened 383 HSP families to clarify the frequency of *PLA2G6* mutations in the Japan Spastic Paraplegia Research Consortium, and revealed the clinical characteristics of HSP with *PLA2G6* mutations. We found three families with compound heterozygous mutations of the *PLA2G6* gene, c.517 C > T/c.1634A > G, c.662 T > C/c.991 G > T, and c.1187-2 A > G/c.1933C > T, and one family with a homozygous mutation of the *PLA2G6* gene, c.1904G > A/c.1904G > A. All three families with compound heterozygous mutations presented a uniform phenotype of a complicated form of HSP with infantile/child-onset spastic paraplegia, cerebellar ataxia, and mental retardation. On the other hand, the family with a homozygous mutation presented a late-onset complicated form of HSP with parkinsonism. This study may extend the clinical and genetic findings for PLAN.

Introduction

Hereditary spastic paraplegias (HSPs) are characterized by various inherited disorders in which weakness and spasticity of the lower extremities are the predominant symptoms [1]. HSPs are classified into a pure or complicated form according to the absence or presence of complications, respectively. The clinical presentations of complicated forms include ataxia, mental retardation, parkinsonism, dementia, and neuropathy. To date, the causative genes or loci of HSPs, from SPG1 to SPG79, have been reported. Furthermore, other diseases, including Charcot-Marie-Tooth disease, Parkinson disease (PD), amyotrophic lateral sclerosis, and spinocerebellar ataxia include spastic paraplegia as one of their clinical presentations, emphasizing that a lot of causative genes responsible for HSPs and other neurological diseases overlap. Therefore, gene analysis is necessary to confirm the causative genes of HSPs.

Phospholipase A2 group VI (*PLA2G6*) encodes a calcium-independent phospholipase. Mutations of it cause *PLA2G6*-associated neurodegeneration (PLAN), including infantile neuroaxonal dystrophy 1 (INAD, OMIM #256600), neurodegeneration with brain iron accumulation

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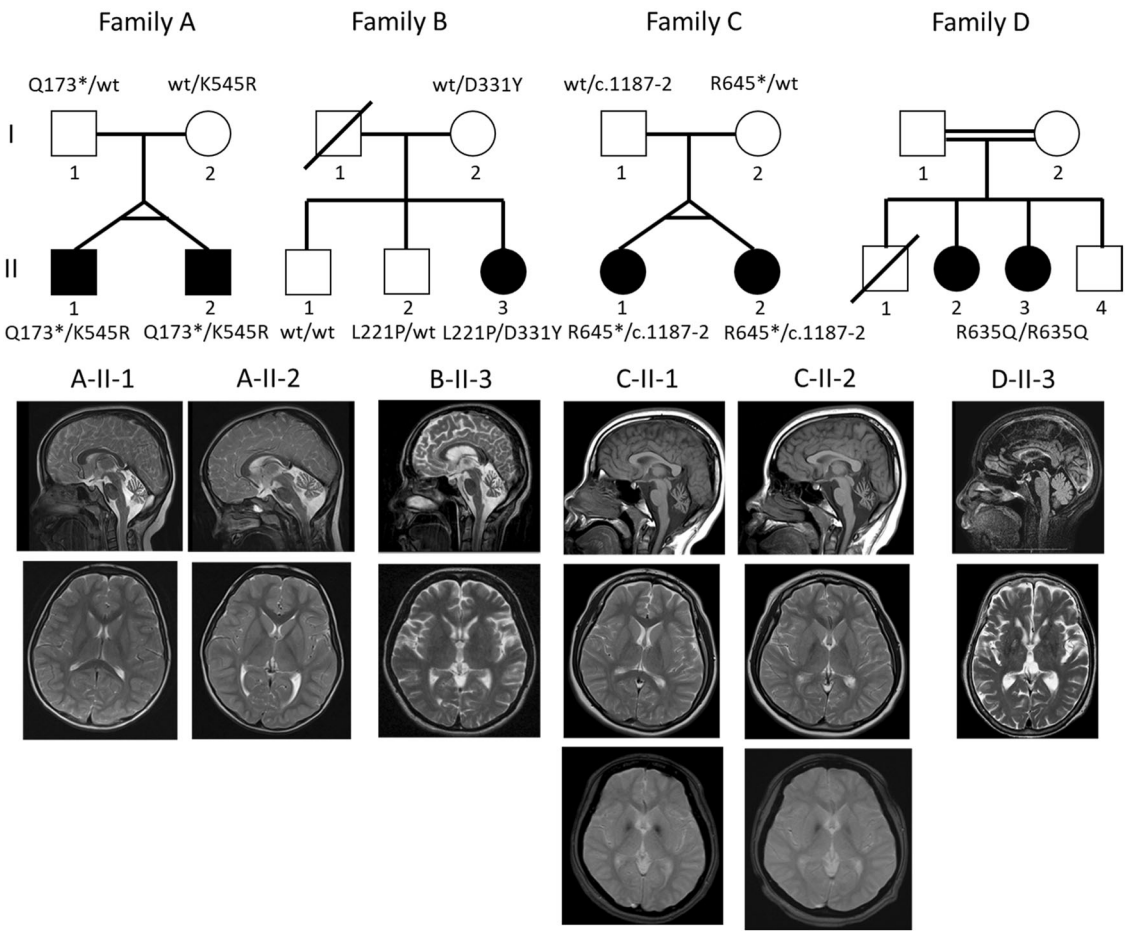

Fig. 1 **a** Pedigree charts and mutations. Squares and circles indicate males and females, respectively. Filled symbols indicate affected individuals, whereas open symbols indicate unaffected individuals. The slash and double lines indicate deceased and consanguineous marriage, respectively. wt wild type. **b** Brain MRI of patients. Five patients (A-II-1 and 2, B-II-3, and C-II-1 and 2) showed mild cerebellar atrophy. C-II-1 and 2 showed hypointensity of the globus pallidum on T2* MRI

2B (NBIA, OMIM #610217), and dystonia-parkinsonism [2, 3]. Very recently, *PLA2G6* mutations were reported to be a cause of spastic paraplegias [4, 5]. Therefore, we screened our HSP patients to find HSPs associated with *PLA2G6* mutations, and to reveal the frequencies and clinical findings.

Clinical and genetic study

In this study, 383 HSP patients were recruited through the Japan Spastic Paraplegia Research Consortium (JASPAC), which was established in 2006 to clarify the molecular epidemiology of HSPs in Japan and the pathogenesis of HSPs. The JASPAC collected Japanese patients who exhibited leg spasticity with/without other complications, including mental retardation, parkinsonism, and others. Furthermore, the JASPAC collected patients having affected parents or children, probands having an affected brother or sister, probands having parents of consanguineous marriage, and probands showing a complicated form with normal parents. This study was approved by our institutional review board, and written informed consent was obtained from all the participants.

We performed whole-exome analysis on the 383 Japanese patients to find *PLA2G6* mutations, and confirmed the mutations in the probands and their families by Sanger sequencing. Known genes, SPG1-79, were excluded by whole-exome sequencing. Exon capture was performed using a Sureselect Human All Exon V4 + UTRs Kit, followed by massively parallel sequencing using an Illumina HiSeq 2000 (100 bp paired end). We aligned the exome data with a Burrows-Wheeler Aligner, and extracted single-nucleotide variations and short insertion/deletions using SAMtools [6]. We referred to the NCBI Reference Sequence (NM_003560.2) for *PLA2G6*.

We found six patients with *PLA2G6* mutations in four Japanese families. The family trees and gene analysis results are shown in Fig. 1. Four probands (patients A-II-1, B-II-3,

Table 1 Genetic findings in four families with *PLA2G6* mutations

	Family AMutation 1	Family AMutation 2	Family BMutation 1	Family BMutation 2	Family CMutation 1	Family CMutation 2	Family DMutation
Position on Chr22	38539204 G > A	38516874 T > C	38536124 A > G	38528924 C > A	38524439 T > C	38511635 G > A	38511664 C > T
MutationAmino-acid change	c.517 C > T p. Q173*	c.1634A > G p. K545R	c.662 T > C p. L221P	c.991 G > T p. D331Y	c.1187-2 A > G-	c.1933C > T p. R645*	c.1904G > A p. R635Q
Reference	–	Known [2, 8]	–	Known [9]	Known [10]	Known [11]	Known [12]
Prediction by Polyphen-2	–	Probably damaging	Benign	Possibly damaging	–	–	Probably damaging
Prediction by SIFT	–	Deleterious	Neutral	Deleterious	–	–	Deleterious
CADD score (deleterious > 20)	41	28.4	20.2	27.1	24.8	43	32
Allele frequency in 1261 in-house controls	0	0	0	0.00040	0	0	0.00040
Allele frequency in iJGVD	0	0	0	0	0	0	0

SIFT Sorting Intolerant From Tolerant, *CADD* Combined Annotation Dependent Depletion, *iJGVD* Integrative Japanese Genome Variation Database

C-II-1, and D-II-3) had the p.Q173*/p.K545R, p.L221P/p.D331Y, p.R645*/c.1187-2 A > G, and p.R635Q/p.R635Q mutations, respectively. According to the co-segregation study, unaffected individuals A-I-1, A-I-2, B-I-2, B-II-2, C-I-1, and C-I-2 had a heterozygous mutation of p.Q173*, p.K545R, p.D331Y, p.L221P, c.1187-2 A > G, and p.R645*, respectively. These results indicate that patients A-II-1, A-II-2, B-II-3, C-II-1, and C-II-2 had compound heterozygous mutations of *PLA2G6*. Mutations of p.Q173* and p.L221P are not registered in ExAC [7] (accessed on 18 June 2018), Human Genetic Variation Database [8] (accessed on 18 June 2018), Integrative Japanese Genome Variation Database [9] (accessed on 18 June 2018), or 1261 in-house data. Other mutations have been reported to be causative mutations for NBIA, INAD, and PD [10–14]. We evaluated the functional prediction of *PLA2G6* mutations by means of in silico algorithms using PolyPhen-2 [15], SIFT [16], and the PHRED-like CADD score [17] (Table 1).

A summary of the clinical findings is presented in Table 2. The patients in families A, B, and C exhibited infantile/child-onset spastic paraplegia with cerebellar atrophy and mental retardation, this being similar to the clinical spectrum of atypical neuroaxonal dystrophy. Meanwhile, a female patient (D-II-3) showed adult-onset spastic paraplegia with parkinsonism. Her blood serum was positive for anti-human T-lymphotropic virus (HTLV)-1 antibody but her cerebrospinal fluid (CSF) was negative. Moreover, her elder sister (D-II-2) had spastic paraplegia similar to her (D-II-3). Brain magnetic resonance imaging (MRI) showed cerebellar atrophy in all infantile/child-onset patients, i.e., A-II-1, A-II-2, B-II-3, C-II-1, and C-II-2. T2* MRI revealed hypointensity of the globus pallidus in C-II-1 and C-II-2, this being characteristic of PLAN. However, brain MRI showed no abnormalities in the adult-onset patient (D-II-3).

Discussion

In this study, we found six patients with *PLA2G6* mutations in four families among 383 HSP patients on exome sequencing. HSP caused by *PLA2G6* mutations was found in 1.05% (4/383) of the patients with the clinical diagnosis of HSP in Japan. Three families exhibited a uniform phenotype of infantile/child-onset complicated HSP with mental retardation, and cerebellar ataxia, this being similar to atypical neuroaxonal dystrophy. Furthermore, this study revealed six asymptomatic individuals, indicating that loss of function of PLA2G6 causes PLAN.

We found seven mutations comprising two novel ones (p.Q173* and p.L221P), two causing INAD (c.1187-2 A > G and p.R645*), two causing Parkinson disease (p.D331Y

Table 2 Clinical findings in four families with *PLA2G6* mutations

Patient	A-II-1	A-II-2	B-II-3	C-II-1	C-II-2	D-II-3
Age of onset (y.o.)	Infantile	Infantile	10	1	1	66
Gender	Male	Male	Female	Female	Female	Female
Initial symptoms	Gait impairment	Gait impairment	Gait impairment	Mental retardation	Mental retardation	Gait impairment
Pyramidal signs	+	+	+	+	+	+
Cerebellar ataxia	+	+	+	−	−	−
Extrapyramidal signs	−	−	+	−	−	+
Mental retardation	+	+	+	+	+	−
Cerebellar atrophy on brain MRI	+	+	+	+	+	−
Modified ranking scale	4	4	5	4	4	2

+ positive, − negative

and p.R635Q), and one causing NBIA (p.K545R) [10–14]. These mutations were reported to be compound heterozygous ones, indicating that the INADs ones were caused by c.1187-2 A > G/p.R538C and p.R645*/p.L354P, the PD ones by p.D331Y/p.M358Ifs* and p.R635Q/p.Q452*, and the NBIA ones by p.K545R/p.M358Ifs* [10–14]. We found that mutations of PD and NBIA also caused a spectrum of atypical neuroaxonal dystrophy in this study.

On the other hand, we describe a late-onset spastic paraplegia patient (D-II-3) with extrapyramidal signs with a positive anti-HTLV-1 antibody result for blood serum and a negative one for CSF. Although patients with HTLV-1 infection sometimes exhibit HTLV-1-associated myelopathy (HAM) showing spasticity, they must have positive anti-HTLV-1 antibody results for both blood serum and CSF. In addition, patients with HAM do not show parkinsonism, and the elder sister also showed spastic paraplegia. Thus, we diagnosed her as having HSP. The average onset age of dystonia-parkinsonism is 20s–30s [3]. However, that for D-II-3 was 66 years. This might indicate that she has a new phenotype of late-onset PLAN as a pure form of HSP.

Brain T2* MRI was performed in one family. It showed hypointensity of the globus pallidus, which is one of the characteristic findings for PLAN. This indicates that brain T2* MRI may be useful for examining the possibility of PLAN. Thus, brain T2* MRI would be useful for differential diagnosis of HSPs when a patient shows spastic paraplegia with cerebellar atrophy and mental retardation.

PLA2G6 mutations lead to elevated mitochondrial lipid peroxidation and mitochondrial dysfunction [18]. However, what causes the phenotype differences has not been clarified. Therefore, we should perform a phenotype-genotype correlation study to elucidate the molecular mechanism underlying PLAN.

Accession numbers

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Salinas S, Proukakis C, Crosby A, Warner TT. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. Lancet Neurol. 2008;7:1127–38.
2. Morgan NV, Westaway SK, Morton JE, Gregory A, Gissen P, Sonek S, et al. PLA2G6, encoding a phospholipase A2, is mutated in neurodegenerative disorders with high brain iron. Nat Genet. 2006;38:752–4.
3. Paisan-Ruiz C, Bhatia KP, Li A, Hernandez D, Davis M, Wood NW, et al. Characterization of PLA2G6 as a locus for dystonia-parkinsonism. Ann Neurol. 2009;65:19–23.
4. Ozes B, Karagoz N, Schüle R, Rebelo A, Sobrido MJ, Harmuth F, et al. PLA2G6 mutations associated with a continuous clinical

spectrum from neuroaxonal dystrophy to hereditary spastic paraplegia. Clin Genet. 2017;92:534–9.
5. Chen YJ, Chen YC, Dong HL, Li LX, Ni W, Li HF, et al. Novel PLA2G6 mutations and clinical heterogeneity in Chinese cases with phospholipase A2-associated neurodegeneration. Park Relat Disord. 2018;49:88–94.
6. Koh K, Ishiura H, Beppu M, Shimazaki H, Ichinose Y, Mitsui J, et al. Novel mutations in the *ALDH18A1* gene in complicated hereditary spastic paraplegia with cerebellar ataxia and cognitive impairment. J Hum Genet. 2018 [Epub ahead of print].
7. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91.
8. Higasa K, Miyake N, Yoshimura J, Okamura K, Niihori T, Saitsu H, et al. Human genetic variation database, a reference database of genetic variations in the Japanese population. J Hum Genet. 2016;61:547–53.
9. Nagasaki M, Yasuda J, Katsuoka F, Nariai N, Kojima K, Kawai Y, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat Commun. 2015;6:8018.
10. Guo S, Yang L, Liu H, Chen W, Li J, Yu P, et al. Identification of novel compound mutations in PLA2G6-associated neurodegeneration patient with characteristic MRI imaging. Mol Neurobiol. 2017;54:4636–43.
11. Lu CS, Lai SC, Wu RM, Weng YH, Huang CL, Chen RS, et al. PLA2G6 mutations in PARK14-linked young-onset parkinsonism and sporadic Parkinson's disease. Am J Med Genet B Neuropsychiatr Genet. 2012;159B:183–91.
12. Riku Y, Ikeuchi T, Yoshino H, Mimuro M, Mano K, Goto Y, et al. Extensive aggregation of α-synulein and tau in juvenile-onset neuroaxonal dystrophy: an autopsied individual with a novel mutation in the PLA2G6 gene-splicing site. Acta Neuropathol Commun. 2013;1. https://doi.org/10.1186/2051-5960-1-12.
13. Paisán-Ruiz C, Li A, Schneider SA, Holton JL, Johnson R, Kidd D, et al. Widespread Lewy body and tau accumulation in childhood and adult onset dystonia-parkinsonism cases with PLA2G6 mutations. Neurobiol Aging. 2012;33:814–23.
14. Yoshino H, Tomiyama H, Tachibana N, Ogaki K, Li Y, Funayama M, et al. Phenotypic spectrum of patients with PLA2G6 mutation and PARK14-linked parkinsonism. Neurology. 2010;75:1356–61.
15. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7:248–9.
16. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. PLoS ONE. 2012;7:e46688.
17. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper G, Shendure J, et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet. 2014;46:310–5.
18. Kinghorn KJ, Castillo-Quan JI, Bartolome F, Angelova PR, Li L, Pope S, et al. Loss of PLA2G6 leads to elevated mitochondrial lipid peroxidation and mitochondrial dysfunction. Brain. 2015;138:1801–16.