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Identification of a novel mutation in PLA2G6 gene and phenotypic heterogeneity analysis of PLA2G6-related neurodegeneration

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ABSTRACT

Introduction: This study reports a novel mutation site of the phospholipase A2 group VI (PLA2G6) gene, and analyzes the information of 67 previously published cases to elucidate PLA2G6 phenotype-genotype variations.
Methods: We collected clinical data and examined gene mutation sites from one Chinese patient with adult-onset ataxia and her family. Next-generation sequencing (NGS) and Sanger sequencing were used to verify possible mutations. PolyPhen-2, SIFT, and MutationTaster were used to predict their pathogenicity. For analyzing the distribution frequency of the mutation, 597 healthy controls were recruited. We also analyzed the clinical and genetic information of 67 cases from 23 studies in Pubmed database.
Results: A novel compound heterozygous mutation of the PLA2G6 gene, c.1648delC and c.991G > T, was found in the Chinese patient, and classified as pathogenic. The c.1648delC variation was absent in ExAC, 1000G, dbSNP databases and the 597 healthy controls. Of the 67 cases, 29 presented ataxia. The signs of cerebellar atrophy appeared in the MRIs of most patients, while signs of iron accumulation were absent in older-aged patients with a compound heterozygous mutation. Thirty-eight patients showed no ataxia. A negative or mild extrapyramidal symptom accompanied by a low age, a homogenous mutation, while moderate or severe extrapyramidal symptoms were associated with an old age and a compound heterozygous mutation.
Conclusion: A novel compound heterozygous mutation of the PLA2G6 gene, c.1648delC and c.991G > T, is associated with adult onset ataxia. Phenotype-genotype variations of PLA2G6 are predicted to be caused by the loss of protein or enzyme activity of phospholipase-2.

1. Introduction

The phospholipase A2 group VI gene (*PLA2G6*) is located on chromosome 22q13.1, is 6.0 Mb in size, and has 17 exons. The encoded protein is a Ca^{2+}-independent A2 phospholipase enzyme that catalyzes the release of fatty acids from phospholipids and plays a role in phospholipid remodeling, release of arachidonic acid and leukotrienes, and Fas-mediated apoptosis. *PLA2G6* mutations are linked to a rare heterogenous autosomal recessive disease, phospholipaseA2-associated neurodegeneration (PLAN). Its three clinical phenotypes are: infantile neuroaxonal dystrophy (NAD), neurodegeneration with brain iron accumulation (NBIA), and early-onset dystonia-parkinsonism (PARK/DYT-PLA2G6) [1]. NAD can either have an early onset (INAD) or a late onset (ANAD). The former occurs at 6 months to 3 years of age, while the latter occurs at 4 years of age or later. Clinical signs of NAD include dystonia, cognitive impairment, cerebral ataxia, and spastic quadriparesis. NBIA is characterized by progressive extrapyramidal symptoms, intellectual impairment, and excessive iron deposition in the brain. PARK/DYT-PLA2G6 usually occurs at under 30 years of age. Its clinical symptoms include dystonia, impaired cognition, psychosis, dysarthria, and pyramidal tract signs. However, clinical heterogeneity and expanded phenotype have been constantly reported, hinting at the complexity of *PLA2G6* mutation-related diseases.

Clinical data were collected, and a pedigree map was constructed based on the clinical information of a Chinese patient with ataxia symptoms and her family. Targeted NGS and Sanger sequencing were used for the genetic and bioinformatics analyses. Whole-exome sequencing was performed in 597 healthy controls. A novel compound heterozygous *PLA2G6* mutation was identified. To investigate the correlation between the clinical phenotype and genetic mutation, 67 cases with *PLA2G6* mutations from 23 previously conducted studies were analyzed.

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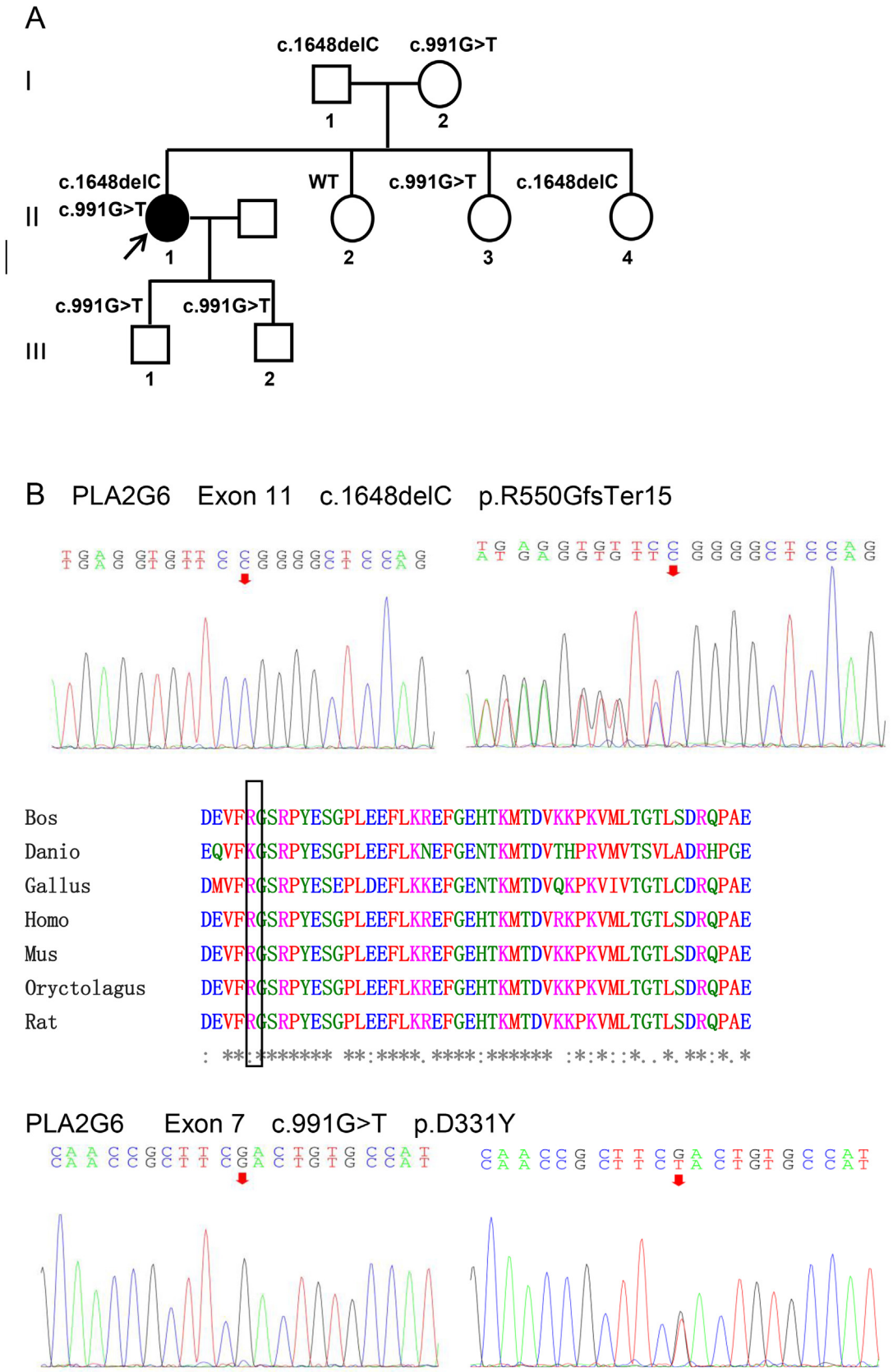

Fig. 1. Figure legends. Pedigree chart of the Chinese family: The squares indicate males, the circles indicate females, the black symbols indicate the affected individual, and the arrows indicate the proband. The detailed mutation site for each individual is labeled on the graph (A). Chromatogram of p.R550Gfs*15 and p.D331Y mutations of PLA2G6: The left panel in the chromatogram depicts the reference sequence. The right panel represents a heterozygous mutated sequence. Conservation of the PLA2G6 protein residues targeted by the mutation identified in patients according to the Uniprot database. The closest homologs of the PLA2G6 protein were aligned using ClustalW (B). MRI graph of the proband in the Chinese Family: Axial FLAIR and sagittal T1-weighted image of the patient shows severe cerebellar atrophy (arrows) with widening of the cerebellar folia. There are no signs of iron deposition at the globus pallidus (C).

2. Methods

2.1. Study subjects

The study was approved by the Ethics Committee of the First Affiliated Hospital of the Zhengzhou University School of Medicine. The affected individual with ataxia was from the Neurology Department of the First Affiliated Hospital of Zhengzhou University in 2017; she was being investigated along with the unaffected individuals from the same Chinese family. As a control group, 597 healthy individuals of Chinese ancestry who went to our hospital for a regular physical examination were consecutively recruited from Mar 1st to May 30th in 2018, whose age ranged from 17 years old to 80 years old. According to the principles of the Declaration of Helsinki, an informed consent was provided by all the participants in writing, as defined by the Ethics Committee. The PubMed database was searched for related literature in English published before August 2018, including case reports, letters, correspondence, brief communications, and cohort studies, using "phospholipase A2 group VI gene mutation", "*PLA2G6* gene mutation", "phospholipase A2 group VI gene" AND "mutation" or "*PLA2G6* gene" AND "mutation" as search terms. A total of 209 articles were identified. After reviewing the title and abstracts, 46 articles for which full text was available were chosen. After reading the full text and discarding the articles as reviews, or those about basic experiments or single heterozygous mutations, 23 studies involving 67 cases were included in our study. Two investigators independently completed the process of searching and identifying the articles, and disagreements were resolved by discussion or involvement of a third reviewer when necessary.

2.2. Clinical assessment

Clinical and demographic information of all members in the Chinese family was obtained. Detailed physical examinations were conducted by a senior neurologist. For the proband, venous blood samples were collected for laboratory tests, 3.0 T magnetic resonance imaging (MRI) and standard electroencephalogram (EEG) examinations were conducted, and electromyography examinations were performed for measuring nerve conduction, amplitude, and evoked responses. The clinical information of the 67 cases was obtained from the 23 studies that were included and compared.

2.3. Genetic investigations

Blood samples were collected from all the family members and 597 healthy controls. Genomic DNA was extracted from peripheral EDTA-treated blood samples using a Blood Genomic Extraction Kit (Qiagen, Hilden, Germany). A customized panel was designed to cover 225 genes of ataxia using the online SureDesign tools (Agilent technologies, USA), with the OMIM and HGMD databases as a reference. A target gene NGS was performed in the probands according to the standard protocol reported earlier [2]. Sanger sequencing was performed to verify the potential variants filtered. Cosegregation analysis was carried out in families with genetic mutations. SIFT, PolyPhen-2, and MutationTaster were used to predict the pathogenicity of the identified variants. For 597 controls, whole-exome sequencing was conducted to find the distribution frequency of the mutations. The information of gene variations of the 67 cases was also collected from the literature.

2.4. Statistical analysis

The Statistical Product and Service Solutions (SPSS) 19.0 software was used to process data, and the collected data were expressed as means ± SD. The two-sample independent *t*-test and x^2 test were used to analyze the data from the two groups. $P < 0.05$ indicated a statistically significant difference.

3. Results

3.1. Clinical data

The Chinese family comprised native residents belonging to the southwest region of the Henan Province. The marriage between the parents was not consanguineous, and the family history was unremarkable. Eight members were investigated, one of whom showed cerebellar ataxia. A pedigree was drawn (Fig. 1A). The proband was a 31-year-old woman who came to our hospital with a persistent and gradually deteriorating dizziness. She complained about feeling imbalanced while standing and feeling like she was falling backwards for about one year. She also complained of weakness, but bradykinesia, tremors, dystonia, cognitive decline, seizures, and autonomic symptoms were absent. She was depressed. Her medical history showed normal perinatal and early developmental milestones. The physical examinations revealed normal limb strength, muscular tension, and intelligence, while the tendon reflexes were hypersensitive. Bilateral Hoffmann, Rossolimo, and Babinski signs were positive. The rapid rotation, finger-nose, and heel-knee-tibia tests were normal, but the Romberg test with open and closed eyes yielded positive results, and walking in a straight line was unsuccessful. Magnetic resonance imaging revealed the signs of cerebellar atrophy, rather than the signs of iron deposition in the brain. Other signs such as white matter lesions, cerebral cortex atrophy, and abnormal cerebral tissue softening were also absent (Fig. 1C). Electroencephalogram examinations were normal, but the electromyogram examination showed a delayed conduction of the bilateral pyramidal tract in the leg. The laboratory tests showed normal blood cell numbers, as well as normal kidney and liver function. Furthermore, the levels of serum tumor markers and related antibodies, homocysteine and B vitamins, lipids, glucose, and electrolytes were normal. The index of rheumatic immunity was also normal.

3.2. Genetic data

Targeted NGS technology was used for analyzing the customized panel of ataxia-related genes. Using the SureSelect target enrichment system, an average capture efficiency rate of 52.47% was achieved. Of these, 99.7% sequences were aligned with the reference genome. The sequencing depth was over 100-fold in almost all samples. Overall, 98.45% of the target regions achieved a 10-fold coverage, and 97.68% achieved a 20-fold coverage, demonstrating the high throughput capacity of this approach. A total of 132 genetic variants including SNPs, non-coding region variants, synonymous mutations, missense mutations, and frameshift mutations were identified. After filtering, the number of candidate variants was reduced to 9. Sanger sequencing further identified novel compound heterogeneous *PLA2G6* mutations, c.1648delC and c.991G > T, in the proband. The frameshift mutation of c.1648delC in exon 11 was a novel heterogeneous mutation, resulting in a p.R550GfsTer15 variation; this is the first time where this mutation was reported to be associated with PLAN. The mutation was absent in the 1000G, ExAC, and dbSNP databases, and in the 597 controls. Another mutation, a c.991G > T mutation in exon 7, caused a variation of p.D331Y and was included in the HGMD Pro and PubMed databases. This mutation showed a frequency of 0.00004752 in the ExAC database, 0.001 in the 1000G database, 0.0002 in the dbSNP database, and 0.00335 in the 597 controls. A co-segregation assessment showed the compound heterogeneous mutation to be segregated with regards to the disease in the proband, as clinically unaffected members were either heterozygotes or wild-type individuals (Fig. 1A). According to ACMG standards, PolyPhen-2, SIFT, and MutationTaster predicted the c.991G > T mutation to be deleterious, while c.1648delC was a likely pathogenic variant. The two mutated regions were also found to be highly conserved among species (Fig. 1B).

3.3. Information from 67 cases

A total of 67 cases from 23 studies were included in this research [2–24]. Detailed clinical and gene mutation data were collected. Twenty-nine patients showed symptoms of ataxia, and 38 patients showed no ataxia. Almost of all patients had a homozygous mutation or compound heterozygous mutation except two patients (only found a single heterozygous mutation), and there was a significant difference between the distributions of the mutation type in the two groups ($x^2 = 4.869$, $P = 0.036$). The patients with a symptom of ataxia had a higher frequency of compound heterozygous mutation (15/29) than those without ataxia (8/36). The onset ages were 9 months–41 years. There was a tendency that a low age was usually accompanied by a homozygous mutation, while older ages were associated with compound heterozygous mutations (11.4 ± 12.4, 17.6 ± 12.4, respectively; $P = 0.060$). The difference of age also appeared when patients were compared by their symptoms; patients with ataxia had a younger onset age, while patients without ataxia had an older onset age (7.4 ± 8.4, 17.5 ± 13.6, respectively; $P < 0.001$). In the ataxia group, a homogenous mutation was more linked with a younger age than a compound heterozygous mutation (3.9 ± 5.3, 11.0 ± 9.6, respectively, $P = 0.020$), and most of the subjects showed cerebellar atrophy in their MRI scans, except two patients with a homozygous mutation of c.2239C > T. However, the signs of iron accumulation only appeared in the MRI scans of some patients, and was not found in any older-aged patients (ranging from 19 to 31 years old) with a compound heterozygous mutation. In the group without ataxia, the patients with negative or mild extrapyramidal symptoms were usually young (ranging from 9 months to 4 years old) and had a homogenous mutation, with cerebellar atrophy being visible in the MRI scan. On the other hand, moderate or severe extrapyramidal symptoms appeared in older-aged patients (ranging from 18 to 41 years old), some of whom had a compound heterozygous mutation. More detailed data are listed in Table 1 and Table 2.

4. Discussion

Clinical heterogeneity of PLAN has been widely reported in recent years, which suggests a great variability in PLAN phenotypes. For example, a phenotype similar to spastic paraplegia was found by identifying a *PLA2G6* mutation [3]. One study has reported a Chinese pedigree of *PLA2G6* gene mutation with familial cortical myoclonic tremor and epilepsy [25]. The variety of *PLA2G6* mutations was also noted. Thus far, approximately 40 types of mutations of *PLA2G6* were found, most of which are missense, non-sense, and shear site mutations. The two allele mutations associated with PLAN are homozygous and compound heterozygous mutations [1]. The amount of phenotype-genotype variability has attracted attention from several scientific studies that aim to explore the correlation between genotypes and their corresponding phenotypes, among which the changes in protein or enzyme activity of phospholipase A2 and the PLAN phenotypes arising from *PLA2G6* mutations are noteworthy.

Our own study demonstrated another phenotype characterized by adult onset ataxia and a lack of extrapyramidal symptoms, with MRI scans that showed cerebellar atrophy rather than iron accumulation. For this, the clinical and genetic data of the family were analyzed. The initial panel for NGS was designed for analyzing gene mutations associated with ataxia; we identified a novel compound heterozygous *PLA2G6* gene mutation from this panel. This new phenotype-genotype variation led us to conduct further investigation. We collected data from 67 cases reported in 23 studies for further analyses. Among them, a total of 29 patients showed ataxia, with an onset age that was lower than that of patients without ataxia. The patients with a homozygous *PLA2G6* mutation had an average age of 3.9 years. Their brain MRIs generally showed cerebellar atrophy and iron accumulation. We classified these patients as NAD (or ANAD) and NBIA. However, the patients with an older onset age, the oldest patient being of 31 years, demonstrated a compound heterozygous mutation and lack of iron accumulation in their brain MRIs. The extrapyramidal symptoms

Table 1
PLA2G6 genotype-phenotype variants of the cases with ataxia from 23 studies.

Study	N	Ataxia	CF	MT	V	AO	EPS	PS	CI	IA	CA
Ref [2]	2	+	no	Compound heterozygosity	c.991G > T/c.1077G > A	8	+ +	–	+ + +	–	+
					c.991G > T/c.1077G > A	19	+ +	–	+ + +	–	+
Ref [3]	3	+	no	Compound heterozygosity	c.1117G > A/c.1511C > T	7	+	+ + + +	+ +	+	+
					c.911G > T/c.1982C > T	29	+ + +	–	–	±	+
					c.911G > T/c.2218G > A	31	–	+ + +	–	–	+
Ref [6]	1	+	no	Homozygosity	c.680C > T	1.5	+ + +	+ + +	+ +	–	+
Ref [7]	1	+	no	Homozygosity	c.609 894del286	1	+ +	+ + +	+ + +	+	+
Ref [8]	2	+	no	Compound heterozygosity	c.991G > T/c.1077G > A	8	+ +	–	+ +	–	+
					c.991G > T/c.1077G > A	19	+ + +	–	+ +	–	+
Ref [9]	11	+	yes	Homozygosity	c.1125delA	9 m	–	–	+	+	+
					c.1911delC	1	+ +	–	+	+	+
					c.1911delC	1	–	–	+	–	+
					c.1772 GA	1	+ + +	–	+	+	+
					c.1772 GA	1	+	–	+	+	+
					c.1933CT	1	–	–	+	+	+
					c.2218 GA	1	+ +	–	+	+	+
					c.673CT	3	+ + +	–	+	+	+
					c.673CT	5	+	–	+	+	+
					c.673CT	6	+	–	+	+	+
					c.673CT	6	–	–	+	+	+
Ref [10]	1	+	no	Compound heterozygosity	c.1903C > A/c.1602G > A	4	+ + +	+ +	+ +	–	+
Ref [11]	2	+	no	Compound heterozygosity	c.1674del/c.2370T.G	19 m	+ + +	+ +	+ +	–	+
					c.1674del/c.2370T.G	1	+ + +	+ +	+ +	–	+
Ref [12]	3	+	no	Homozygosity	c.2239C > T	9	–	+ + +	–	+	–
				Homozygosity	c.2239C > T	21	–	+ + +	–	+	–
				Compound heterozygosity	c.1898C > T/c.1765_1768delTCTG	7	–	+ +	–	–	+
Ref [18]	1	+	no	Compound heterozygosity	c.1946G > A/c.1912G > A	9	+	+	NA	+ +	+
Ref [19]	1	+	no	Compound heterozygosity	c.1187-2A > G/c.1612C > T	3	+ + +	–	+	+	+
Ref [20]	1	+	no	Compound heterozygosity	c.1077G > A/c.1634A > G	8	–	–	+	+	+

N, number of cases; CF, consanguineous family; MT, mutation type; V, variant; AO, onset age; EPS, extrapyramidal symptoms; PS, pyramidal signs; CI, cognitive impairment; IA, iron accumulation; CA, cerebellar atrophy; +, positive; -, negative; NA, not available.

Table 2
PLA2G6 genotype-phenotype variants of the cases without ataxia from 23 studies.

Study	N	Ataxia	CF	MT	V	AO	EPS	PS	CI	IA	CA
Ref [2]	1	–	yes	Homozygosity	c.991G > T	30	+ +	–	–	–	–
Ref [3]	1	–	no	Compound heterozygosity	c.668C > T/c.1915G > A	27	+ +	+ +	–	+	+
Ref [4]	1	–	no	Homozygosity	c.991G > T	37	+ +	–	–	–	–
Ref [5]	2	–	no	Compound heterozygosity	c.758G > T/c.2341G > A	27	+ + +	–	+ + +	–	–
					c.109C > T/c.2321G > T	23	+ + +	–	+	–	–
Ref [7]	2	–	yes	Homozygosity	c.2222G > A	26	+ + +	+	+ +	–	–
					c.2222G > A	29	+ +	+ +	+ + +	–	+
Ref [12]	1	–	no	Homozygosity	c.1786C > T	4	–	+ + +	–	–	+
Ref [13]	4	–	no	Homozygosity	c.2222G > A	26	+ + +	+ +	+	–	–
					c.2222G > A	22	+ +	+	+ +	–	–
					c.2222G > A	25	+ + +	+	+	–	–
					c.2222G > A	23	+ + +	+ +	+	–	–
Ref [14]	4	–	yes	Homozygosity	c.1894C > T	21	+ + +	+ +	+ +	–	–
					c.1894C > T	22	+ + +	+ +	+ +	–	–
					c.1894C > T	25	+ + +	+ +	+ +	–	+
					c.2239C > T	18	+ + +	+ +	–	–	–
Ref [15]	2	–	yes	Homozygosity	c. 991G > T	36	+	NA	–	–	–
					c. 991G > T	36	+	NA	–	–	–
Ref [16]	1	–	no	Homozygosity	c.2239C > T	27	+ +	–	–	–	–
Ref [17]	2	–	no	Compound heterozygosity	c.610-1G > T/c.1627C > T	41	+ + +	–	+	–	–
					c.610-1G > T/c.1627C > T	36	+ +	–	+	+	–
Ref [18]	11	–	NA	Homozygosity	c.2221C > T	21 m	+	+	–	–	+
				Heterozygosity	c.671T > C	17 m	+	+	–	+	+
				Homozygosity	c.1039G > A	1.5	+	+	–	–	+
				Homozygosity	c.208C > T	21 m	+	+	–	–	+
				Homozygosity	c.985C > T	1.5	+	+	–	–	+
				Homozygosity	c.238G > A	8	+	+	–	+ +	+
				Homozygosity	c.671T > C	3	+	+	–	–	+
				Heterozygosity	c.2030G > T	11 m	+	+	–	–	+
				Homozygosity	c.847G > A	1	+	+	–	–	+
				Homozygosity	c.1613G > A	1.5	+	+	–	+	+
				Homozygosity	c.1471C > T	9 m	+	+	–	–	+
					c.1547C > T						
Ref [21]	1	–	yes	Homozygosity	c.1483C > T	1.5	+	–	+	–	+
Ref [22]	1	–	no	Homozygosity	c.2277-1G > C	15 m	+	–	+ +	–	+
Ref [23]	3	–	no	Compound heterozygosity	c.216C > A/c.1904G > A	20	+ + +	–	+	+	–
					c.1354C > T/c.1904G > A	25	+ +	–	+	–	–
					c.1354C > T/c.1904G > A	35	+ +	–	+	–	–
Ref [24]	1	–	no	Homozygosity	c.3 G > T	1.5	+	–	+	–	–

N, number of cases; CF, consanguineous family; MT, mutation type; V, variant; AO, onset age; EPS, extrapyramidal symptoms; PS, pyramidal signs; CI, cognitive impairment; IA, iron accumulation; CA, cerebellar atrophy; +, positive; -, negative; NA, not available.

appeared only partially in these patients. As a result, it was difficult to classify these patients into any category of PLAN because of the older onset age for ANAD, the lack of iron accumulation in the brain for NBIA, and the ataxia symptoms for PARK/DYT-PLA2G6.

The protein encoded by the *PLA2G6* gene is a Ca^{2+}-independent phospholipase A2 (iPLA2), a multifaceted enzyme essential for membrane remodeling in neurons; this enzyme plays a role in phospholipid remodeling, signal transduction, cell proliferation, and endoplasmic reticulum stress-mediated apoptosis. This enzyme causes the release of free fatty acids and lysophospholipids by catalyzing the hydrolysis of the sn-2 fatty acyl bond of phospholipids [4]. Multiple studies have verified that iPLA2 deficiency may alter membrane permeability, fluidity, and ion homeostasis, thereby causing mitochondrial abnormalities, including mitochondrial respiratory chain dysfunction, reduced ATP synthesis, abnormal mitochondrial morphology, as well as increased lipid peroxidation levels [15]. In fact, different types of *PLA2G6* mutations have been identified in both homozygous and heterozygous conditions, leading to different levels of protein activity, suggesting a correlation between the genotype and the resulting variable phenotype [16]. This study investigated the phenotype-genotype variability of *PLA2G6* mutations and the possible reasons for this variability. Gregory et al. [1] found that mutations leading to absence of a protein were associated with more severe infantile neuroaxonal dystrophy-type clinical phenotypes, while compound heterozygous missense mutations resulted in a protein with some residual enzyme function and were correlated with a less severe phenotype of neurodegeneration. One study on Chinese individuals with early-onset Parkinson's disease further verified the partial loss of enzyme function due to novel *PLA2G6* mutations occurring in a heterozygous form [8]. Shi et al. found that D331Y mutation in the *PLA2G6* gene resulted in partial loss of phospholipase A2 activity and the phenotype of Parkinson's disease, compared to the loss of phospholipase A2 activity resulting from the S519A mutation [4]. From the aforementioned studies, we can conclude that mutations leading to complete absence of a protein are associated with a severe phenotype, while compound heterozygous mutations with residual protein activity are associated with a less severe phenotype. However, the detailed signal pathways for how the site variations of mutation and the changes in phospholipase A2 protein affect the clinical phenotype of PLAN have not been elucidated completely; further studies are required to explore this aspect.

Author contributions

Yan Ji, MD, design and conceptualized study; acquired and analyzed the data; drafted and revised the manuscript for intellectual content.

Yusheng Li, MD, conceptualized study, drafted the manuscript for intellectual content, study supervision.

Changhe Shi, MD, acquisition and analysis of data, administrative, technical, and material support

Yuan Gao, MD, critical revision of the manuscript for important intellectual content.

Jing Yang, PhD, drafting of the manuscript.

Dongyi Liang, MD, acquisition of data.

Zhihua Yang, PhD, acquisition of data.

Yuming Xu, PhD, MD, study conception, design, and organization, analysis and interpretation of data, critical revision of the manuscript, study supervision.

Disclosure

Dr. Xu reports no disclosures. Dr. Ji reports no disclosures. Dr. Yusheng Li, Dr. Changhe Shi, Dr. Yuan Gao, Dr. Jing Yang, Dr. Dongyi Liang and Dr. Zhihua Yang report no disclosures.

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