

Research Article

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Novel *de novo* Mutation in the Autophagy Gene *WDR45* Causes BPAN in a Chinese Family

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Abstract

Mutations in the *WDR45* gene have recently been identified as causative for the only X-linked beta-propeller protein-associated neurodegeneration (BPAN), a subtype of neurodegeneration with brain iron accumulation (NBIA) with phenotypically and genetically heterogeneous condition. The clinical features include early-onset global developmental delay, progressive accumulation of iron in the basal ganglia, resulting in physical and neurological deterioration. We herein reported a novel mutation (c.1040-1041del) in exon 12 of *WDR45* gene in a 3-year-old Chinese girl, exhibiting developmental delay, seizures, by whole-exome sequencing. Sanger sequencing confirmed the heterozygous mutation was absent in both her parents, and thus it was concluded as a *de novo* frameshift mutation.

Keywords: NBIA; WDR45; BPAN; WES; Autophagy

Introduction

Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group of disorders characterized by abnormal focal accumulation of iron in the basal ganglia and the nigromes, resulting in early-onset global developmental delay and neurological disorders [1]. Advances in genomic sequencing allowed identification of several genes associated with NBIA subtypes, including pantothenate kinase 2 (PANK2), fatty acid 2-hydroxylase (FA2H), phospholipase A2, group VI (PLA2G6); adenosine triphosphate enzyme 13 a2 (ATP13A2), DCAF17, COASY, chromosome 19 open reading frame 12 (C19orf12), Plasma ceruloplasmin (ferric oxidase) (CP); FNS-related tyrosine kinase 1 (FTL1) [2,3]. Recently, mutations in the autophagy gene WDR45 were found to cause the only X-linked subtype of NBIA, beta-propeller protein-associated neurodegeneration (BPAN) [4-8]. The WDR45 gene encodes for a β -propeller scaffolding protein, WIPI4, which is essential for autophagy mechanisms. WIPI4 prevents neurodegeneration by inhibiting the abnormal accumulation of corresponding proteins [9,10]. Herein we report a 3-year old Chinese girl with typical NBIA possessing a novel de novo WDR45 mutation discovered by whole-exome sequencing.

Materials and Methods

Clinical review and family analysis

The clinical records of the patient were reviewed retrospectively, and clinical data such as family history, clinical manifestations, and performed laboratory tests results were collected.

WES and filtering

The molecular study was performed with written informed consent and was approved by the local Institutional Review Board. Peripheral blood was collected from the affected proband and their family members in EDTA tubes and aliquoted for cryopreservation at -80°C immediately after collection. Whole-exome sequencing (WES) and variant calling and filtering were performed as described previously [11]. Basically, blood samples (3-5 ml) were obtained from the proband and her family members (Figure 1). Genomic DNA was extracted using the TIANGEN blood genomic DNA extraction kit following the manufacturer's protocol (Beijing Tiangen, Cat.no.

DP318-03). Whole-exome capture and high-throughput sequencing (HTS) were performed by the Veritas Genetics (Hangzhou, China). Briefly, whole exome was captured using the SeqCap EZ MedExome Target Enrichment Kit (Agilent, California, USA) and sequenced on the Illumina HiSeq2500 platform as 150 bp paired-end runs. The sequencing reads were aligned to the human reference genome (UCSC hg19). Single nucleotide variants (SNVs) and short insertions and deletions (InDels) were functionally annotated and filtered using our inhouse cloud-based rare disease NGS analysis platform with builds in public databases (dbSNP, OMIM, ESP, Clinvar, 1,000 Genomes as previously described [11]. Mutations identified by WES was validated by Sanger sequencing.

Sanger sequencing

The WDR45 mutation found by WES was validated by PCR and then Sanger sequencing. Briefly, the full DNA sequence of the WDR45 gene was downloaded from the NCBI website (NM_007075.3, Genebank ID: 11152). Primers were designed using Primer5 software and synthesized by Shanghai Sangon Biotech. WDR45 gene mutation were analyzed using PCR amplification in combination with Sanger sequencing. PCR were performed under the following reaction conditions: 3 minutes at 95°C followed by 32 cycles for 25 seconds at 94°C, 25 seconds at 58°C, 30 seconds at 72°C, and 1 cycle for 5 minutes for final extension. Primer sequence used were: WDR45f 5'-GGCAGCCTCTGACCTTTTACCAC-3' and WDR45r 5'-AGCTCTGCTGAGTGGAAAGTGG-3'. PCR products were purified using the SanPrep Column DNA Gel Extraction Kit (Sangon Biotech, Shanghai, Cat. No. 112B518131).

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Results

Clinical information review and family pedigree analysis

The patient was a 3-year old girl with non-consanguineous Chinese parents. Her family members, including her parents and her elder sister were apparently healthy and there was no notable family history (Figure 1). She had no perinatal abnormalities and was delivered normally. She was admitted to the First Hospital of Peking University in 2016. She presented with global developmental delay and was unable to stand alone and can barely uses just a few words in Chinese, including "mama" and "baba", showing cognitive and motor developmental delay and lack of fine motor control. The patient had a few febrile seizures. Routine blood and urine tests were normal. Array-CGH analysis was also normal. 24-hour Electro-encephalograms (EEGs) monitoring revealed abnormal background rhythms. Brain MRI revealed evidence of iron deposition in globus pallidus and thin corpus callosum (Supplementary Figure 1).

Genetic analysis

We obtained written informed consent from her parents to perform molecular studies, which were approved by the Institutional Review Board of Peking University First Hospital. To determine the caustic gene mutation of the proband with NBIA, we performed whole-exome sequencing on genomic DNA sample of the proband (II-1). A total of 116,391 genetic variants, including 26,111 non-synonymous changes, were occurred at the coding sequence or the canonical dinucleotide of the splice site junctions. Exonic sequence alterations and intronic variants at exon–intron boundaries, with unknown frequency or minor allele frequency (MAF) <1% and not present in the homozygous state in those databases were retained.

Subsequently, a heterozygous nucleotide variation of c.1040-1041del in exon12 in the *WDR45* gene is identified as the potential disease-causing gene of NBIA (Figure 2A). This variant has not been described in any other databases, including dbSNP, OMIM, ESP, Clinvar, 1,000 Genomes, Human Gene Mutation Database, gnomAD





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Figure 2C: Amino acid sequence alignment of WDR45 around the altered position p.E347, showing high sequence conservation across different species.

YVFTPDGNCNREAFDVYLDICDDEDF*

and ExAc. After validation by Sanger sequencing, this variation was observed only in the proband but not in her family members (Figure 2B and Supplementary Figure 2). *WDR45* c.1040-1041del mutation caused amino acid changes at Glu 347, and resulting in frameshift and premature truncation of the protein (p.Glu347GlyfsTer7) at the highly conserved region (Figure 2C). In addition, no other mutations were observed in the other NBIA-related genes, including *PANK2*, *FA2H*, phospholipase A2, *PLA2G6*, *ATP13A2*, *DCAF17*, *COASY*, *CP and FTL1*. Therefore, we concluded this *de novo* mutation is very likely to cause BPAN in the proband.

Mouse

Discussion and Conclusion

NBIA disorders are extremely rare, having a prevalence of 1/1,000,000 and of these, BPAN which is a X-linked dominant form of NBIA due to heterozygous mutations in WDR45 constitutes only 7%

[1,12]. The phenotype is characterized by a static, global encephalopathy in early childhood with later development of progressive cognitive decline in adolescence and early adulthood. Onset of neurodegeneration is usually heralded by parkinsonism, dystonia, sleep abnormalities, Rett-like syndrome, and epileptic encephalopathies [13,14]. Our patient presented with severe developmental delay, epileptic seizures and also abnormal iron metabolism, whose phenotype is very much corresponding to that of the cases described for BPAN in the literature.

The *WDR45* gene encodes for a β -propeller scaffolding protein that is one of the orthologs of yeast Atg18 and is essential for autophagy [8,10,15]. Lymphoblastoid cell lines (LCLs) derived from the patients showed lower autophagic activity and accumulation of aberrant early autophagic structures, furthermore, Wdr45 deficient mice displayed learning and memory defect and axonal swelling with impaired autophagic flux and accumulation of substrates for autophagic degradation, suggesting that WDR45 functions in maintaining neural homeostasis through its role in autophagy [16]. Loss of WDR45 increased cellular iron levels and impaired mitochondrial and lysosomal functions in patient-origin iPS-derived midbrain neurons cells [17]. The novel *de novo* heterozygous missense mutation (c.1040-1041del) in the *WDR45* gene resulted in a truncated protein (p. Glu347GlyfsTer7) which predicted as damaging by in silico tools. We therefore strongly suspect it to be pathogenic, however, functional studies are needed to further evaluate the pathogenic relevance of this mutation.

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Conflict of Interest

The authors declare no competing financial interests.

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