Infantile Developmental Delay Caused by Pathogenic Mutation in WDR45 Gene and Literature Review

QingJun Cao

Shengjing Hospital of China Medical University

Hua Wang

Shengjing Hospital of China Medical University

Liang Huo (✉ Junjunjun198523@163.com)

Shengjing Hospital of China Medical University

Research Article

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Abstract

Background

The reasons of infantile developmental delay are varied and complexity. The mode of BPAN inheritance clinical manifestation and imaging findings of childhood BPAN patients are not typical. The reason of WDR45 mutation is still elusive. This study has the objective to give a comprehensive overview of the genetic and phenotypic spectrum of WDR45 mutations.

Methods

This study recruited 258 infants with developmental delay of the babies from Shengjing Hospital of China Medical University and performed a customdesigned gene panel to capture all exons and 5 base pairs of flanking intronic sequence. We reviewed the WDR45 mutation cases reported before.

Results

We identified two de novo variants in WDR45 mutation and listed the clinical manifestation and treatments. This study summarized variants of the gene WDR45 mutation expression in previous investigations. Childhood patients have the clinical features as generalized developmental delay, seizures, intellectual disorder. By contrast, adolescence and early adulthood patient have the features like developmental regression, dystonia and parkinsonism.

Conclusion

Our study suggest WDR45 mutations cause a series spectrum of neurodegeneration disorders and are most important etiology of BPAN. The developmental delay of infant is not obvious and rarely to be attention. The mode of BPAN inheritance clinical manifestation and imaging findings of infantile BPAN patients are not typical. WDR45 gene mutaion has closely relationship with the function of endoplasmic reticulum and mitochondria.

Introduction

Beta-propeller protein-associated neurodegeneration (BPAN) is a specific neurodegeneration disease with brain iron accumulation, which has the a novo pathogenic variants in the X-linked gene WDR45. The earliest study about BPAN was first defined in 2012 [1] and it has the features with the beginning of childhood developmental delay and evolving to progressive dystonia, dementia, and parkinsonism in late adolescence or early adulthood. Before the WDR45 discovery, the disease was considered as static encephalopathy of childhood with neurodegeneration in adulthood (SENDA). BPAN is one subtype of Neurodegeneration with brain iron accumulation (NBIA), which is a cluster of heterogeneous neurodegenerative diseases characterized by abnormal accumulation of iron in the brain, especially for the basal ganglia [2, 3]. There are 10 types of NBIA, and each type is related with a independent gene disorder. BPAN is known as the NBIA type 5 for WDR45. The prevalence of NBIA disorders is about 1/1 million, and BPAN is only 7% of the NBIA disorders [4]. Brain magnetic

resonance imaging (MRI) of BPAN patients have displayed of iron deposition in the globus pallidus and substantia nigra, cerebral atrophy and cerebellar atrophy [1]. Recently, it is also reported about the hippocampus malformation in BPAN patient [5].

The manifestation of developmental delay in infant period is not obvious, and it is difficult for families to find. The reasons of developmental delay in infant period are varied and complexity. The mode of BPAN inheritance clinical manifestation and imaging findings of childhood BPAN patients are not typical, and the misdiagnosis rate was relatively high. BPAN is a rare and severe disease, so early diagnosis and timely intervention have important clinical significance. The purpose of the study was to offer an overview of BPAN, with the emphasis on clinical manifestation, genetics, and pathogenesis. The assay of WDR45 mutation is vital for prenatal diagnosis and genetic counseling.

Materials And Methods

We recruited 258 infants with developmental delay of the babies from Shengjing Hospital of China Medical University. All patients had developmental delay according to ILAE classification criteria and did not have the history of preterm delivery. Written informed consent was obtained for all patients from their parents or legal guardians. The human research ethics committees of Shengjing Hospital of China Medical University.

We performed a customdesigned gene panel to capture all exons and 5 base pairs of flanking intronic sequence. Before getting the blood samples from the whole families, the informed consent was taken first. We used sonication to sheare the Genomic DNA sample. Then The NimbleGen 2.0 probe sequence capture array of Roche was used to hybridize the sheared DNA. Data analysis is applied as below: Single Nucleotide Polymorphisms (SNPs) and Synonymous changes and that Minor Allele Frequency (MAF) exceed 5% were deleted (<http://www.ncbi.nlm.nih.gov/projects/SNP>). SIFT software were used to filter the nonsynonymous changes.

Results

We identified 2 de novo variants, 1 in female, 1 in male. (Table 1). Two of 258(0.775%) infant individuals with developmental delay were identified by customdesigned gene panel. Two de novo variants resulted in an indel predicted to cause a frameshift alteration. In 1 male individual with intractable seizures and severe developmental delay, we identified a de novo nonsense mutation c19 (exon3) C>T and amino acid disorder of p.R7X,355 (p.Arg7Stop,355) (NM_007075) were confirmed(Figure 1). The infant is the youngest patient in the present reports in the gene database. In 1 female individual, we identified a de novo nonsense mutation c.224 (exon5)C>G and amino acid disorder of p.S75X,287 (p.Ser75Stop,287) were confirmed (without reported) (Figure 2). Both patients had WDR45 gene mutation and their parents were wild-type without the mutation, which suggested that the mutation of the infants are considered as a de novo origin. The distribution of the two WDR45 pathogenic variants was reported in this study (Figure 3).

Table 1. Phenotypic features of patients with WDR45 pathogenic variants

Case, Age, Gender	Mutation (skewed X-inactivation)	Epileptic type	EEG	Neuroimaging, MRI	Other features	Treatment, Medication
Ⅹ, 6m, M	c19 (exon3) C>T p.Arg7Stop,355	Tonic-clonic seizure	The outbreak of extensive extremely high slow wave, sharp slow compound wave, multiple spin slow wave.	Corpus callosum thin, bilateral hippocampus volume small and anterior horn of bilateral ventricles enlarged	Poor eyes contact and sound following, no head control, poor eye contact, no social smile, sleep disorder	VPA(Sodium valproate), LEV(Levetiracetam) TPM(Topiramate)
Ⅹ, 8m, F	c.224 (exon5)C>G p.Ser75Stop,287	N	Slow background wave.	Corpus callosum thin, anterior horn of bilateral ventricles enlarged	Inactively grab objects, cannot sit and crawl, poor eye contact and language	Regular rehabilitation training
N, number; DD/ID, developmental delay or intellectual disorder; Ep, epilepsy; PK, parkinsonism; Dys, dystonia; VPA, valproate; LEV, levetiracetam; TPM, topiramate;						

Two infants had the severe development delay. They are the first children of their mother who had an uneventful pregnancy. The parents of the two individuals were healthy, young and non-consanguineous. They were born in an uneventful natural delivery with full term. Physical examination of the both infants is normal. The height, body weight, head circumference are in the normal range. The blood routine test, plasma ammonia, biochemical tests, plasma lactic acid, disseminated intravascular coagulation (DIC), 25-hydroxyvitamin D3, parathyroid hormone (PTH), blood homocysteine assay, immunoglobulin quantitative, together with urinary and stool test were normal. The other different feature of the individuals is summarized in Table 1.

The male individual had paroxysmal limbs tonic-clonic seizure without disturbance of consciousness or change of complexion, lasting several minutes once. VEEG described the outbreak of extensive extremely high slow wave, sharp slow compound wave, multiple spin slow wave. Cerebral magnetic resonance imaging (MRI) of the male infant showed corpus callosum thin, bilateral hippocampus volume small and anterior horn of bilateral

ventricles enlarged without iron accumulation detected on T1- and T2- weighted (Figure 4). The female individual did not take the initiative to grasp objects and toys after 1 month old. She had insensitive turning over, apathetic facial expressions, looking the hand over and over. She did not have seizure, but with stare blankly. Her VEEG described the background wave was slow. MRI of the female infant showed corpus callosum thin and bilateral temporal lobes extracerebral space widened (Figure 4).

Discussion

We reviewed 78 patients with WDR45 gene mutation in literatures and we expand a new site mutation of WDR45. Most patients had developmental delay or intellectual disability. Childhood patients had epilepsy as the common symptom, and adolescence or early adulthood patients had progressive dystonia and parkinsonism as main clinical features. Studies had been focused on the patients with WDR45 mutation over one year old. It is more important to confirm WDR45 mutation in infants with development delay. The nervous system develops obviously rapid in infancy stage. It is better to give the intervention treatment to infants with the the development delay caused by WDR45 mutation.

The pathogenic WDR45 gene causes the function disorder of Beta-propeller protein, named as BPAN. BPAN was a peculiar genetic mutation disease and has the inherit manner with X-linked dominant. It is a specific neurodegeneration disease with brain iron accumulation. The pathogenic gene of BPAN is WDR45, which Although the inherit manner of BPAN is X-linked dominant associated with WDR45 mutation [1, 42, 56]. The phenotypes are similar in male and female. A pathogenic mutation of WDR45 mutation is fatal to the male embryos, which make the BPAN does not follow the conventional X-linked dominant genetic rule. The incidence was much higher in female than in male, the ratio is about 6.5:1. Haack designed a hypothesis, the mosaicism of WDR45 pathogenic variant lead to both the viability and the phenotype similarity of male patients [1, 10]. Some female patients have X chromosome inactivation pattern [11]. WDR45 gene located on the chromosome Xp11.23, containing 12 exons and the first two exons are noncoding region. WDR45 gene mutations include missense mutations, deletion mutations and splicing mutations. The WDR45 of Mammalian is one of yeast Atg18 orthologs, worm epg-6 ortholog and it was an early significant stage in the formation of autophagosome [12, 13, 41, 43]. One study found that mice with WDR45 knockout (KO) displayed memory and learning disorders, caused by the WDR45 deficiency. WDR45 deficiency damaged the autophagic flux that was caused by swollen axons and SQSTM1- and ubiquitin-positive accumulation aggregates in neurons [13]. Beta-Propeller proteins form one of the largest families of protein structures, with a pseudo-symmetrical fold made up of subdomains called blades [51, 53, 55, 56]. Beta-propeller protein is in a propeller structure interacting with phospholipids and performs the function of cell regulation, including autophagy, cell cycle progression and transcriptional regulation [14, 43, 51, 53, 54]. The patients in our study had WDR45 nonsense mutation c19 (exon3) C>T and c.224 (exon5)C>G. These mutation made the amino acid stop the following normal amino acid coding, thus causing the Beta-Propeller protein disorder.

WDR45 is considered to be involved in autophagy regulation with affecting the functions of endoplasmic reticulum and mitochondria. Hirotomo Saitsu found de novo heterozygous mutations in WDR45 at Xp11.23 in two patients with NBIA by exome sequencing. Protein expression of lymphoblastoid cell lines (LCLs) derived from the patients had severely impaired. Autophagic aberrant structures and autophagic activity disorder were demonstrated in the LCLs in the article [15]. Qihong Xiong reported a 3-year-old Chinese girl with BPAN with a novel de novo mutation (c.1040_1041del, p.Glu347GlyfsTer7) in WDR45 (NM_007075). They analyzed protein

levels of LC3 and p62 in HeLa cells with WDR45 mutation and evidenced that this novel mutation in WDR45 impaired autophagy in cells [16]. Philip Seibler studied the WDR45 mutant fibroblasts and pluripotent stem cell-derived to research the WDR45 function. WDR45 increased cellular iron levels and oxidative stress, with mitochondrial dysfunction, autophagic defects and lysosomal disorder [17]. Bertrand Mollereau proposed the endoplasmic reticulum (ER) stress had the relationship with the autophagy through pathophysiological changing of the wdr45 knockout mice with BPAN [18]. Daniela Stanga proposed that TRAPPC11 mutation can make autophagy disorder with failing to recruit ATG2B-WIPI4/WDR45 to proautophagosomal membrane [19]. Yan G Zhao generated WDR45 knockout mice and research the destruction of WDR45 function can induce poor motor coordination, greatly impaired learning and memory. WDR45 may have the relationship with extensive axon swelling and numerous axon spheroids [20]. Huida Wan found the WDR45 knockout mouse displayed cognitive impairments and neuron loss in the brain, which is related with the endoplasmic reticulum(ER) protein accumulation and ER stress [21].

The clinical manifestation of BPAN has the different characters in the different stages of patient life. When patients with BPAN beginning from childhood (3 months - 8 years old), they always have the developmental delay or intellectual disability. The childhood patient may have the dyskinesia like walking failure and ataxia, language expressive disorder and learning disabilities [44–50, 56]. Epilepsy is the common symptom in the childhood stage, which have the generalized and focal seizure. Infantile spasms can happen to the baby younger than 1 year old [7, 22, 23, 42, 49]. The individual patients can have multiple seizure types and some had the onset of epilepsy with febrile seizure [8, 22, 24, 25, 52]. The severity of epilepsy commonly reduced with age [5, 8, 49]. Some patients have Rett-like features, including stereotyped behaviors, absence of aimful hand motions, bruxism and features of autism spectrum disorder [5, 26–29, 44, 45]. The main clinical features of the patients in adolescence or early adulthood include progressive dystonia and parkinsonism, also with the progressive dementia which is really different from childhood patient [6–8, 55]. Some patients reported had some other manifestation, including sleep disorder, choreiform movement, hypercholesterolemia, visual and hearing disease [6, 11, 30, 55, 56].

MRI findings is particularly important to help doctors considering the diagnosis of BPAN. In the young childhood, MRI displayed nonspecific hypomyelination and thin corpus callosum [30–32, 55]. The adolescence and adulthood patients have the highly specific signal abnormalities in MRI. Globus pallidus and substantia nigra have hypointensity on T2-weighted sequences. The substantia nigra and cerebral peduncles have the hyperintense halo surrounding a central linear band of hypointensity on T1-weighted sequences. All the changings in T2 and T1 are related with iron accumulation and deposition in the cerebral areas [10, 33, 34]. Some articles reported the patients had the cerebral atrophy, cerebellar atrophy and dilated ventricles [10, 33–35, 55]. Fluid attenuated inversion recovery (Flair) and Susceptibility weighted imaging (SWI) displayed bilateral low signal in the substantia nigra [35]. There is no obvious iron accumulation associated MRI findings of brain on FLAIR/T2 sequences in the infantile and young children with BPAN [25].

The treatment for BPAN in childhood should be focused on the rehabilitation training and seizure control to improve the the quality of life [5, 8, 10, 51, 56]. The rehabilitation training in the early stage to improve the global developmental delays and intellectual disability. The early intervention programs can help the BPAN patients with autism to solve social/behavioral difficulties. Anti-epilepsy is also very important, including antiepileptic drugs (AEDs), ketogenic diet (KD) and vagus nerve stimulation (VNS). The young patients families would better be educated to nurse and train the special childhood patients. Psychological counseling in regular is benefit to

the families, to improve the emotional problems of family members and patients. The treatment in adolescents/adults with parkinsonism, dystonia, and spasticity, the usual medication and physical therapy can be considered [10, 34, 36, 53, 56]. The patient with parkinsonism is treated with dopaminergic drugs to improved motor movement and activity interesting. Benzodiazepines, anticholinergics, leatheroids, baclofen can be used to control dystonia, and spasticity. These medicine may aggregate the cognitive impairment of BPAN patients. All patients need to pay attention to ophthalmic follow-up [37, 56]. Even though iron chelating agents has been given to some BPAN patients, the treatment is still in controversy [38–40, 53–56].

In conclusion, Childhood BPAN patients have the clinical features as generalized developmental delay, seizures, intellectual disorder. The developmental delay of infant is not obvious and rarely to be attention. The mode of BPAN inheritance clinical manifestation and imaging findings of infantile BPAN patients are not typical, and the misdiagnosis rate was relatively high. WDR45 gene mutaion has closely relationship with the function of endoplasmic reticulum and mitochondria.

Declarations

Ethical Approval

Not applicable. Th e article was summarized for our patients information in hospital, all the patients with developmental delay need genetic testing, which can help pediatricians and family members understand the definite diagnosis and patient condition. Gene testing belongs to the clinical medical test, do not belongs to the design of experiment, so we do not have the ethical approval. **All the patient's guardians concent to participate the study.**

Concent to participate

All cases were reviewed in our hospital. **All the patient's guardians concent to participate to participate the study.All methods were carried out in accordance with relevant guidelines and regulations (Declaration of Helsinki).**

Concent to Publish

ALL authors concent to publish the manuscript.

Availability of data and materials

All the data and materials are availabilty in ShengJing Hospital of China Medical University.

Competing of Interest

The authors declare that they have no competing interests.

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Author Contributions

Liang Huo designed research; QingJun Cao executed the literature search and analysed data; QingJun Cao wrote the paper, Hua Wang modify the article. all authors edited the final draft.

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Data availability

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

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Figures

A

B

Figure 1

The WDR45 mutation of the male infant The WDR45 mutation of the male infant was de novo nonsense mutation c.19 (exon3) C>T but not his parents.

A

B

Figure 2

The WDR45 mutation of the female infant The WDR45 mutation of the female infant was de novo nonsense mutation c.224 (exon5)C>G but not his parents.

■ A novel mutation
■ Missense mutation
■ Frameshift mutation&Nonsense mutation&Classic splicing mutation

Figure 3

The distribution of WDR45 pathogenic variants The distribution of WDR45 pathogenic variants reported in review studies. We identified two mutation sites c.19 (exon3) C>T and c.224 (exon5)C>G in red. Eleven mutation sites in review studies.

A: male

B: female

Figure 4

MRI findings of two infants with WDR45 mutation MRI findings of two infants: A: MRI of the male patient showed corpus callosum thin, bilateral hippocampus volume small and anterior horn of bilateral ventricles enlarged. B: MRI of the female infant showed corpus callosum thin, bilateral hippocampus volume small and anterior horn of bilateral ventricles enlarged