De novo variants in WDR45 underlie beta-propeller protein-associated neurodegeneration in five independent families

Xiaojun Tang1 | Xiaoping Lan1 | Xiaozhen Song1 | Wuheng Xu1 | Yuanfeng Zhang2 | Hong Zhang3 | Shengnan Wu1

1Molecular Diagnostic Laboratory, Shanghai Children’s Hospital, Shanghai Jiaotong University, Shanghai, China
2Department of Neurology, Shanghai Children’s Hospital, Shanghai Jiaotong University, Shanghai, China
3Department of Clinical Laboratory, Shanghai Children’s Hospital, Shanghai Jiaotong University, Shanghai, China

Correspondence
Yuanfeng Zhang, Department of Neurology, Shanghai Children’s Hospital, Shanghai Jiaotong University, 355, Luding Road, Shanghai 200062, China. Email: zhangyf1@shchildren.com.cn
Hong Zhang, Department of Clinical Laboratory, Shanghai Children’s Hospital, Shanghai Jiaotong University, 355, Luding Road, Shanghai 200062, China. Email: zhangh@shchildren.com.cn
Shengnan Wu, Molecular Diagnostic Laboratory, Shanghai Children’s Hospital, Shanghai Jiaotong University, 355, Luding Road, Shanghai 200062, China. Email: wushengnan@shchildren.com.cn

Abstract
Background: Beta-propeller protein-associated neurodegeneration (BPAN) is a rare, X-linked dominant neurodegenerative disease mainly characterized by developmental delay, intellectual disability, epilepsy in childhood and dystonia, parkinsonism, dementia in adulthood. BPAN is caused by variants in WD repeat domain 45 (WDR45), which is characterized by iron accumulation in the basal ganglia, however, it may be atypical in early brain MRI.

Methods: Whole exome sequencing was performed for five parents-offspring trios and phenotype-driven data analyses were conducted. All candidate variants were confirmed by Sanger sequencing.

Results: Here, we report five independent children presented variable degree of developmental delay, intellectual disability, and/or epilepsy. Five de novo variants of WDR45 including four novel truncating variants (one splicing variant, two nonsense variants, and one frameshift variant) were identified. Although their early brain MRI showed no obvious iron accumulation, multifocal spikes, or polyspikes in electroencephalograms (EEG) were observed in four patients.

Conclusion: Our study reports four patients with new variants in WDR45, which expands the mutation spectrum of WDR45. In addition, our findings provide an early and precise diagnosis basis of BPAN, which is helpful for accurate genetic counseling and prenatal diagnosis.

KEYWORDS
beta-propeller protein-associated neurodegeneration, exome sequencing, variants, WDR45
Neurodegeneration with brain iron accumulation (NBIA) is a heterogeneous group of progressive neurological disease characterized by cognitive disability, dystonia, and/or parkinsonism. NBIA5, also known as BPAN, constituted 7% of NBIA caused by variants of WDR45 (OMIM: 300526). BPAN is mainly characterized by a biphasic clinical progression of global developmental delay, intellectual disability, epilepsy in childhood and dystonia, parkinsonism, and dementia in adulthood (Haack et al., 2012a). Brain magnetic resonance image (MRI) shows low signal on T2 in the globus pallidus and substantia nigra, indicating iron deposition. However, the early brain MRI may be atypical which is especially conspicuous in older individuals, making it difficult to the early diagnosis of BPAN only by MRI (Hayflick et al., 2013).

To our knowledge, BPAN is an X-linked dominant neurodegenerative disease and most reported patients were female. The clinical course of males is more serious or it usually appears in a somatic mosaic manner, indicating that variants in WDR45 are either fatal or more severe in hemizygous males (Haack et al., 2012a; Zarate et al., 2016). However, since girls with severe phenotypes have also been described, it is suggested that skewing of X-inactivation toward the variants may account for this phenotypic variability (Willoughby et al., 2018).

In recent years, whole exome sequencing has developed rapidly and it has demonstrated its potential clinical application in identifying the pathogenesis of hereditary diseases. Owing to patients’ complex and varied manifestations, achieving an accurate diagnosis is difficult. Therefore, molecular genetic analysis is becoming an effective and necessary tool in the diagnosis of neurodegenerative diseases together with clinical assessment and imaging examination such as MRI.

In this study, five de novo truncating variants in WDR45 were detected in five unrelated patients, with four of them being novel and one being previously reported. Among them, four new variants comprised a new splicing variant, two novel nonsense variants, and a new frameshift variant. Another variant of c.830+1G>A resulting in a splicing defect has been reported in patients with BPAN before. Our results expand the mutation spectrum of WDR45 and the phenotypic characteristics of this X-linked dominant neurodegenerative disease. In addition, early diagnosis by trio exome sequencing may prevent the disease from getting worse and it can provide appropriate symptomatic treatment which is beneficial for patients and their families. Furthermore, more case reports were needed to help to elucidate the function of WDR45 which may be important for understanding the genetic etiology, and then, exploring the treatment of this rare and severe heterogeneity disease.

| Patient ID | WDR45 | Inheritance | Epilepsy | Speech abnormality | Movement abnormality |
|------------|-------|-------------|----------|--------------------|---------------------|
| 1          | c.976+1G>C | De novo     | No       | Yes                | Yes                 |
| 2          | c.830+1G>A | De novo     | Yes      | No                 | Yes                 |
| 3          | c.10C>T | None        | No       | Yes                | Yes                 |
| 4          | c.806del | De novo     | Yes      | Yes                | Yes                 |
| 5          | c.726C>G | De novo     | No       | Yes                | Yes                 |

**TABLE 1** Genotypes and clinical features of five patients with WDR45 variants.
1.1 | Clinical description

This study has been approved by the patient’s parents and the Ethical Committee of Children’s Hospital of Shanghai. Generally, all patients presented variable degree of developmental delay with speech delay and/or motor delay. Clinical features of the five patients are summarized in Table 1.

Patient 1 is a 3-year-old girl born to non-consanguineous and healthy parents via uncomplicated vaginal delivery. At 6 months of age, she first developed a febrile seizure with eye-rolling, cyanotic lips, consciousness lapses, weakness in the limbs, and it attacked five–six times during 1 year. At the age of 3 years, her electroencephalogram (EEG) demonstrated 3–4 Hz spike- and slow-wave bursts on brain especially in sleep (Figure 1a). However, no obvious abnormality was found in the early brain MRI and CT. Subsequently, significant delayed development, poor speech, and gait disturbance were noted. After taken to hospital, she was treated with sodium valproate at 15 months, and then, switched to levetiracetam with oxcarbazepine due to side effects. At present, epileptic has not been well controlled yet and it happened occasionally.

Patient 2 is a 5-year-old boy who was born at full term by cesarean section to healthy non-consanguineous parents. He was first noted to have a febrile seizure with eye-rolling, cyanotic lips, consciousness lapses at 18 months old, and it attacked seven–eight times in 3 years. At the meantime, mild motor delay was occurred. After consultation, he was treated with sodium valproate since July, 2018, but stopped after 2 months by his parents without doctor’s permission. After the drug was discontinued, seizure was still occurred sporadically. At the age of 5 years, EEG demonstrated focal spike or polyspike and slow-wave burst on brain in sleep (Figure 1b).

Patient 3 is a 16-month-old girl born to healthy non-consanguineous parents after an uneventful pregnancy. Growth parameters such as height, weight, and head circumference at birth were normal. She was first noted to present signs of motor developmental delay at 8 months. She did not walk independently and had no oral language at 16 months old. Brain MRI revealed bilateral lateral ventricle broadening, while EEG showed no obvious abnormality.

Patient 4 is a 3-year-old girl who was born at term after an uneventful pregnancy to healthy non-consanguineous parents. The first febrile seizure occurred at 1-year old presenting with eye-rolling, cyanotic lips, no response, upper limb jitter, and it attacked five times during the last and a half year. At the meantime, she had poor language and the brain MRI showed slightly less white matter. At the age of 3 years, her EEG demonstrated 3–4 Hz spike- and slow-wave bursts on brain in sleep and more than 10 episodes of absence were observed during the waking period (Figure 1c).

Patient 5 is a 3-year-old girl with profound developmental delay who was delivered with a history of asphyxiation and her parents were healthy and non-consanguineous. Her first seizure was observed at 11 months. She was able to walk but

FIGURE 1 Electroencephalogram (EEG) in four patients. EEG at the age of 3 years in patient 1 (a), age of 5 years in patient 2 (b), age of 3 years in patient 4 (c), and age of 3 years in patient 5 (d). Multifocal spikes or polyspikes were observed in the four patients.
no speech was developed at 2-year old. At the age of 3 years, brain MRI showed abnormal white matter myelination and EEG demonstrated 3–5 Hz polyspike and slow-wave burst on brain (Figure 1d).

1.2 | Genetic analysis

Genomic DNA for WES and Sanger sequencing from patients and their parents was isolated from whole blood collected
with kit (QIAGEN, Germany) following the manufacturer’s instruction. Whole exome sequencing was performed for five parents-offspring trios and data analysis were conducted as described elsewhere (Feng et al., 2020) with evaluating single nucleotide variants, small insertion/deletions, and copy number variations. Sanger sequencing was performed to confirm all candidate variants from WES (WDR45 for NM_007075.3). The primers used to amplify the variant sequence were WDR45-E11-F(5’ ACTGACCTGACCACCTCTAC 3’) and WDR45-E11-R(5’ TTGTGGAAGGTCCCATCTACG 3’), WDR45-E3-F(5’ TACAGGATAAGCCACACGC 3’) and WDR45-E3-R(5’ TACAGGCGATAACGACGACGC 3’), WDR45-E10-F(5’ ACTGACCTGACCACCTCTAC 3’) and WDR45-E10-R(5’ GGCTGTTCACCTCAACTAT 3’), WDR45-E6-9-F(5’ GTGGAATCTGTCTCCATCC 3’) and WDR45-E6-9-R(5’ CAGTGTGCTACCCCTTACTG 3’). The clinical significance of variants was interpreted according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) recommendations (Richards et al., 2015). Alamut Visual was used to predict the pathogenicity of variants and the protein region function and structure were obtained from UniProt and SWISS-MODEL, respectively.

2 RESULTS

Written informed consent for using the clinical information and genetic findings were obtained from the patients’ parents. The coverage of targeted regions with depths greater than 50× reads is more than 91%. In this study, five different de novo variants of WDR45 including four novel variants were discovered by trio-based whole exome analysis. Sanger sequencing confirmed that these variants were heterozygous in the patients and were absent in their unaffected parents (Figure 2).

Whole exome sequencing identified a total of five distinct truncating variants in WDR45 gene, including four novel variants (c.976+1G>C, c.10C>T, c.806del, and c.726C>G) and one recurrent variant (c.830+1G>A). Trio-based analysis revealed all variants occurred de novo with confirmation by Sanger sequencing. The five WDR45 variants detected in our cohort, comprising one frameshift variant, two nonsense variants, and two canonical splicing variants, are absent from the gnomAD database (http://gnomad-old.broadinstitute.org/).

Two canonical variants, c.976+1G>C adjacent to exon 11 and c.830+1G>A adjacent to exon 10, were detected in patient 1 (Figure 1a) and patient 2 (Figure 1b), respectively. Both variants were predicted to disrupt the canonical splicing donor site and result in potential exons skipping. These variants are expected to result in altered function of WDR45 gene product. A different nucleotide change at the same position of c.976+1G>A has been previously reported in patients of BPAN with severe intellectual disability and developmental delay, strengthening the pathogenicity of c.976+1G>C variant. Notably, another canonical splicing variant c.830+1G>A, previously reported in multiple patients with BPAN, was detected in a mosaic manner with an allele fraction of 53% at 100x coverage in the patient 2. Sanger sequencing confirmed this finding.

Two nonsense variants, c.10C>T and c.726C>G were detected in patient 3 (Figure 1c) and patient 5 (Figure 1e), respectively. Besides, another frameshift variant, c.806del, was detected in patient 4 (Figure 1d). These three variants were predicted to introduce a premature stop codon and were expected to result in the loss of function of the protein of the WDR45 gene. Although these variants have not been reported before, some software predicted these variants were deleterious and they were classified into pathogenic according to the guideline of ACMG.

3 DISCUSSION

Beta-propeller protein-associated neurodegeneration (BPAN) is a form of neurodegeneration with brain iron accumulation caused by variant of WDR45 with highly clinical and genetic heterogeneity (Haack et al., 2012b). It is mainly divided into two periods. The first stage is characterized by global delayed development, intellectual disability, and/or epilepsy in childhood which remains stable until early adulthood. In the second stage, dyskinesia such as progressive dystonia, Parkinsonism, and dementia were occurred in adulthood (Haack, Hogarth, Gregory, Prokisch, & Hayflick, 2013; Haack et al., 2012a; Hayflick et al., 2013). In the early stage, clinical phenotypes may be nonspecific and whole exome sequencing will enable early detection of BPAN in children so that patients can be diagnosed before getting worse (Gregory, Kurian, Haack, Hayflick, & Hogarth, 1993).

As BPAN is an X-linked dominant disease, the majority of diagnosed patients were female and most of male patients were chimera, suggesting that lethality might be saved by the mosaicism (Haack et al., 2012a; Willoughby et al., 2018; Zarate et al., 2016). As patient 2 in a mosaic manner reported previously taking part in many vital biological processes such as cell cycle, signal transduction, gene regulation, apoptosis together.
with other WIPI members (Li & Roberts, 2001; Tsuyuki et al., 2014). And WIPI4 itself is involved in the pathway of autophagy, iron storage, ferritin metabolism, and so on (Zhao et al., 2015). In 2013, WDR45 was first been reported associated with autophagy dysfunction, providing a strong evidence that autophagy defect is closely related to neurodegenerative disorders (Doorn & Kruer, 2013). However, the pathogenesis of BPAN still remains unclear and whether activation of autophagy is a beneficial treatment needs more researches.

WDR45 is made up of a seven-bladed propeller structure containing a conserved motif to interact with phospholipids (Figure 3a) and the variants of c.10C>T, c.806del (p.Asp269Valfs*19), (c) 3D-structure of the variant of c.726C>G (p.Tyr242*). Our results supplement the variants of WDR45 in mutation database and provide some new information on the molecular basis and the phenotypic characteristics of BPAN. Besides, the evidence of iron deposition in the basal ganglia in early brain MRI may be atypical (Russo et al., 2019). Therefore, BPAN should not be excluded if patient accompanied with developmental delay or epilepsy in childhood regardless of whether he showed normal imageological examination.

In recent years, the rapid development of whole exome sequencing and its application in the detection of genetic diseases have brought hope for the clinical diagnosis of a large number of hereditary diseases (Alfares et al., 2018).
Until now, one hundred and forty-eight variants of the \textit{WDR45} gene have been reported already (Figure 4). More cases were needed to be reported to expand the spectrum of \textit{WDR45} germline variants and deep understand this disease.

In this study, five de novo variants of \textit{WDR45} were identified by parent-offspring trio exome sequencing, indicating that the detection of variants using WES can provide a more reliable basis for the final diagnosis. Considering the serious clinical manifestation of patients with BPAN, early diagnosis and regular clinical follow-up may be of great significance for patients to improve their quality of life. In addition, providing some appropriate genetic counseling for patients can help to prevent the disease from worsening and it may provide appropriate symptomatic treatment.

4 CONCLUSION

In conclusion, our study reports five de novo variants with BPAN including four new variants in five independent non-consanguineous Chinese families, which expands the mutation spectrum of \textit{WDR45}. These five children showed typical development delay, intellectual disability, and/or epilepsy. Although their early brain MRI showed no significant iron accumulation, multifocal spikes, or polyspikes were observed in the EEG. The application of trio exome sequencing contributes to the early detection of BPAN, which can help patients get timely genetic counseling preventing the disease from worsening and open the way for preimplantation genetic diagnosis and future prenatal checkups. However, there is no effective treatment for this disease at present, so it is necessary to further explore the pathogenic gene and its function in order to better understand the disease, and then, find out the effective treatment.

ACKNOWLEDGMENTS

We appreciate the patients and their families for their participation. This work was supported by the Shanghai Children’s Hospital Funding (2016YMS001), Joint Research Initiative Shanghai Jiaotong University School of Medicine, the Shanghai Municipal Science and Technology Committee (19411965000 and 18XD1403200), Shanghai Jiao Tong University "Star of Jiao Tong University" Medical-Engineering cross Research Fund (YG2019QNB01).

CONFLICT OF INTEREST

The authors have no conflicts of interests to declare.

AUTHORS CONTRIBUTIONS

XT, HZ, and SW designed the study. XS and WX performed the experiments. XL and YZ analyzed clinical and genetic data. XT and SW wrote the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ORCID

Xiaojun Tang https://orcid.org/0000-0003-0560-7314
Xiaozhen Song https://orcid.org/0000-0002-8767-8732
Shengnan Wu https://orcid.org/0000-0002-2245-2273

REFERENCES

Alfares, A., Aloraini, T., Subaie, L. A., Alissa, A., Qudsi, A. A., Alahmad, A., … Alfadhel, M. (2018). Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. \textit{Genetics in Medicine}, 20(11), 1328–1333. https://doi.org/10.1038/gim.2018.41
Doorn, J. M., & Krue, M. C. (2013). Newly characterized forms of neurodegeneration with brain iron accumulation. \textit{Current Neurology and Neuroscience Reports}, 13(12), 413. https://doi.org/10.1007/s11910-013-0413-9
Feng, J., Lan, X., Shen, J., Song, X., Tang, X., Xu, W., … Wu, S. (2020). A de novo MAPRE2 variant in a patient with congenital symmetric circumferential skin creases type 2. \textit{Mol Genet Genomic Med}, 8(2), e1096. https://doi.org/10.1010/mgg3.1096
Gregory, A., Kurian, M. A., Haack, T., Hayflick, S. J., & Hogarth, P. (1993). Beta-Propeller Protein-Associated Neurodegeneration. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens & A. Amemiya (Eds.), \textit{GeneReviews(R)}. Seattle, WA: University of Washington.
Haack, T. B., Hogarth, P., Gregory, A., Prokisch, H., & Hayflick, S. J. (2013). BPAN: the only X-linked dominant NBIA disorder. \textit{International Review of Neurobiology}, 110, 85–90. https://doi.org/10.1016/B978-0-12-410502-7.00005-3
Haack, T. B., Hogarth, P., Krue, M. C., Gregory, A., Wieland, T., Schwarzmayr, T., … Hayflick, S. J. (2012a). Exome sequencing reveals de novo WDR45 mutations causing a phenotypically distinct, X-linked dominant form of NBIA. \textit{American Journal of Human Genetics}, 91(6), 1144–1149. https://doi.org/10.1016/j.ajhg.2012.10.019
Hayflick, S. J., Krue, M. C., Gregory, A., Haack, T. B., Kurian, M. A., Houlden, H. H., … Hogarth, P. (2013). beta-Propeller protein-associated neurodegeneration: a new X-linked dominant disorder with brain iron accumulation. \textit{Brain}, 136(Pt 6), 1708–1717. https://doi.org/10.1093/brain/awt095
Krue, M. C., Boddart, N., Schneider, S. A., Houlden, H., Bhatia, K. P., Gregory, A., … Hayflick, S. J. (2012). Neuroimaging features of neurodegeneration with brain iron accumulation. \textit{American Journal of Neuroradiology}, 33(3), 407–414. https://doi.org/10.3174/ajnr.A2677
Li, D., & Roberts, R. (2001). WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases. \textit{Cellular and Molecular Life Sciences}, 58(14), 2085–2097. https://doi.org/10.1007/Pi00008038
Richards, S., Aziz, N., Bale, S., Bik, D., Das, S., Gastier-Foster, J., … Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. \textit{Genetics in Medicine}, 17(5), 405–424. https://doi.org/10.1038/gim.2015.30
Russo, C., Ardissone, A., Freri, E., Gasperini, S., Moscatelli, M., Zorzi, G., … Chiapparini, L. (2019). Substantia nigra swelling and dentate nucleus T2 hyperintensity may be early magnetic resonance imaging signs of beta-propeller protein-associated neurodegeneration. *Movement Disorders Clinical Practice, 6*(1), 51–56. https://doi.org/10.1002/mdc3.12693

Tsuyuki, S., Takabayashi, M., Kawazu, M., Kudo, K., Watanabe, A., Nagata, Y., … Yoshida, K. (2014). Detection of WPI1 mRNA as an indicator of autophagosome formation. *Autophagy, 10*(3), 497–513. https://doi.org/10.4161/auto.27419

Willoughby, J., Duff-Farrier, C., Desurkar, A., Kurian, M., Raghavan, A., Study, D. D. D., & Balasubramanian, M. (2018). Functional mRNA analysis reveals aberrant splicing caused by novel intronic mutation in WDR45 in NBIA patient. *American Journal of Medical Genetics Part A, 176*(5), 1049–1054. https://doi.org/10.1002/ajmg.a.38656

Zarate, Y. A., Jones, J. R., Jones, M. A., Millan, F., Juusola, J., Vertino-Bell, A., … Krue, M. C. (2016). Lessons from a pair of siblings with BPAN. *European Journal of Human Genetics, 24*(7), 1095. https://doi.org/10.1038/ejhg.2015.274

Zhao, Y. G., Sun, L., Miao, G., Ji, C., Zhao, H., Sun, H., … Zhang, H. (2015). The autophagy gene Wdr45/Wipi4 regulates learning and memory function and axonal homeostasis. *Autophagy, 11*(6), 881–890. https://doi.org/10.1080/15548627.2015.1047127

**How to cite this article:** Tang X, Lan X, Song X, et al. De novo variants in WDR45 underlie beta-propeller protein-associated neurodegeneration in five independent families. *Molecular Genetics & Genomic Medicine*. 2020;8:e1499. [https://doi.org/10.1002/mgg3.1499](https://doi.org/10.1002/mgg3.1499)