Journal of Epileptology • 2022 • 30 • 10.2478/joepi-2022-0001

A patient with Beta-Propeller Protein-Associated Neurodegeneration: a new missense mutation of the *WDR45* gene

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Received May 17, 2022
Accepted for publication on-line June 15, 2022
Published on-line July 01, 2022

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SUMMARY

Introduction. Beta-propeller protein-associated neurodegeneration (BPAN) is a neurodegenerative disorder; its estimated prevalence is 2 to 3 per million individuals. All published cases of BPAN have been sporadic, with a clear female predominance and mutations in various exons of the *WDR45* gene.

Case presentation. The study aimed to confirm the diagnosis of BPAN in a 9-year-old girl with a developmental delay since early childhood complicated with intellectual disability, lack of speech and febrile and non-febrile tonic-clonic seizures. The patient also had autistic symptoms as well as some Rett-like symptoms: stereotypical movements of the hands–twisting objects, putting hands in the mouth.

Discussion. Clinical exome analysis and Sanger sequencing of the proband have been performed to confirm the diagnosis. The novel heterozygous missense mutation c.755T>C of the *WDR45* «autophagy» gene was revealed. Sanger sequencing of the trio (proband and parents) proved the *de novo* nature of mutation; its clinical significance has been defined as probably pathogenic. Thus, we report a new missense variant of the *WDR45* gene in a girl with a clinical picture of BPAN. The use of NGS made it possible to get a correct diagnosis during rather a short period before the second debilitating phase of the disease started so that the physicians and the family would have time to prepare and hopefully choose the way to resist.

Key words: epilepsy • *WDR45* gene • BPAN • NBIA • developmental delay

INTRODUCTION

Beta-propeller protein-associated neurodegeneration (BPAN), first described by Haack et al. in 2012, is the only known X-linked NBIA form (NBIA5, OMIM: 300894) caused by mutations in the *WDR45* gene (Haack et al., 2013). Neurodegeneration with brain iron accumulation (NBIA) presents a group of inherited neurological diseases with progressive extrapyramidal symptoms and focal iron accumulation generally observed in the globus pallidus and substantia nigra (Hayflick et al., 2013). BPAN is the most common NBIA disorder; its estimated prevalence is 2 to 3 per million individuals (Wilson et al., 2021). Not more than 100 BPAN cases carrying *WDR45* mutation have been described worldwide by 2021 (Cong et al., 2021).

* The work was carried out as part of the Innovative Biotechnologies program for 2021–2025, as well as with the support of a grant for postgraduate students of the National Academy of Sciences of Belarus for 2022.

All published cases of BPAN have been sporadic, with a clear female predominance and mutations in various exons of the *WDR45* gene (Wilson et al., 2021; Cong et al., 2021). Here we report a 9-year-old girl with BPAN having *de novo* mutation c.755T>C not formerly detected in *WDR45*. The patient presents several typical features of BPAN as well as some RLS peculiarities.

CASE PRESENTATION

The patient is a 9-year-old girl delivered after a full-term pregnancy to non-consanguineous parents. Her birth weight was 3750 g, and Apgar scores were 8/9. She had no family history of mental or neurological disorders. The girl developed with a delay in motor skills: she sat independently at 10–11 months, began to crawl at 12–13 months, and walked unsupported at two years of age. Her gait was unstable. She also had a developmental speech disorder: babbling at the age of one year without further progress.

Intellectual disability became apparent around the age of two. Stereotypical hand movements in the form of "twisting objects in hands", and "putting in the mouth" appeared after one year. Despite the presence of constant stereotyped movements, the girl has not lost the ability to use her hands. Since the age of 6, flexion contractures have been detected in the elbow joints, and a year later, scoliosis was diagnosed.

Epilepsy started at one year and seven months in the form of febrile tonic-clonic seizures. Three years later non-febrile tonic-clonic seizures appeared, as well as shuddering in the limbs on awakening with a partial impairment of consciousness. Epileptic seizures stopped at the age of 5 years whilst the patient was taking valproic acid 30 mg/kg and levetiracetam 50 mg/kg. At the age of 7, antiepileptic drugs were withdrawn, and there has been no relapse of epilepsy to date.

According to EEG data, at the age of 4, during passive wakefulness, the main rhythm was 4.4–5 Hz and an amplitude of up to 110 μV in the central-parietal-occipital leads. With the onset of sleep, epileptiform activity in the form of sharp-slow wave complexes arose in the right frontotemporal-parietal leads. As sleep deepened, the high-amplitude spike-polyspike-wave epileptiform activity of a high index was recorded in the frontotemporal leads maximum under F8–T4. (Fig. 1a). At 9 years after four years of epileptic seizures termination and two years since the discontinuation of antiepileptic drugs, according to EEG data, epileptiform activity persisted in the form of sharp-slow wave complexes in the right frontal centrotemporal leads, periodically with diffuse spread (Fig. 1b).

At the age of nine, the girl had severe mental retardation and lack of speech. Self-service skills were missing. The patient had autistic symptoms – 19 points on the CASD scale. Constant stereotypical movements of the hands (twisting objects, putting hands in the mouth) were typical. Neither oculomotor nor bulbar abnormalities were detected. Muscle tone in the limbs was slightly dystonic. Tendon reflexes were increased, and Babinsky's symptom was positive on both sides. There were slight flexion contractures in the elbow joints and scoliotic spine deformity. She walked on her own, with support on a wide base. Some notable features of the phenotype were evident: low hair growth on the forehead, hypertelorism, fused eyebrows, low-set ears, and a protruding lower jaw, but there was no microcephaly (Fig. 2). Her cardiovascular and respiratory systems, as well as abdominal organs, were normal. There were no changes in biochemical laboratory tests.

When the patient was 5 years old, signs of peripheral pigmentary retinal dystrophy of both eyes were detected: pigment disorganisation and many small grouped linearly pigmented foci along the fundus periphery.

MRI scans at the age of eight years (Fig. 3) revealed iron deposits in the substantia nigra and globus pallidus. Neither abnormal myelination nor atrophy of the corpus callosum and the brainstem was identified.

Considering the iron accumulation in the basal nuclei and substantia nigra, epilepsy and impaired intellectual disabilities, we clinically suspected the Beta-propeller protein-associated neurodegeneration in the proband. Therefore, the patient was referred for a genetic examination to look for mutations in the *WDR45* gene.

DISCUSSION

Genomic DNA was isolated from the buccal epithelium of the proband and parents by phenol/chloroform extraction.

We performed the next-generation sequencing (NGS) of proband's DNA using Illumina's TruSight One Expanded sequencing panel on NextSeq 550 (Illumina Inc., USA). Sample preparation was fulfilled using the Illumina DNA Prep with Enrichment kit. Quality filtering, alignment against the reference genome NCBI build 37 (UCSC hg19), and variant calling were carried out with DRAGEN applications. Variants were annotated by ANNOVAR software using RefGene, InterVar,

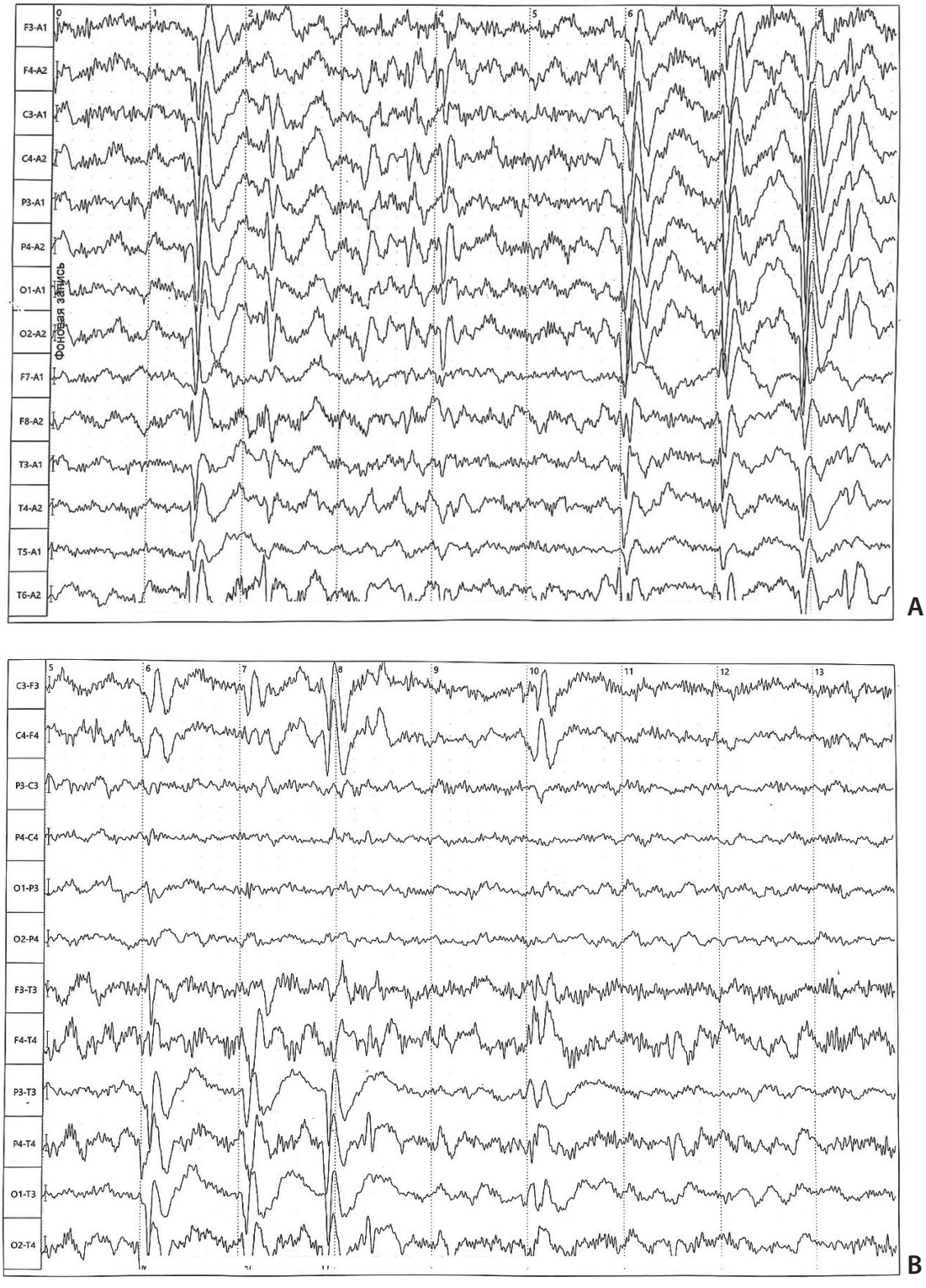

Figure 1. A – Spike-polyspike-wave epileptiform activity of a high index in the right frontotemporal leads (4 y.o). B – Sharp-slow wave complexes in the right fronto-centrotemporal leads, periodically with diffuse spread (9 y.o).

Figure 2. The patient is nine years old. The stereotypical movements of the hand (twisting objects, putting hands in the mouth) are observed.

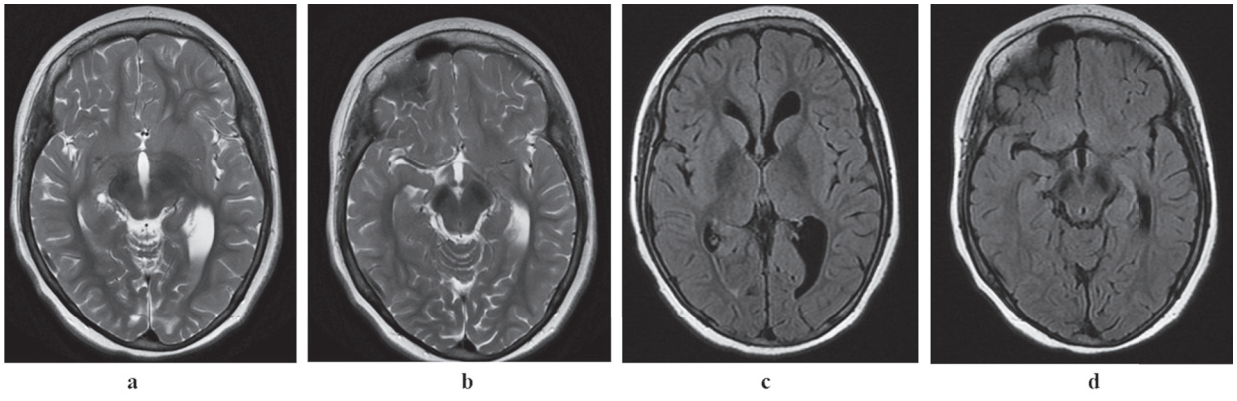

Figure 3. Iron accumulation in the substantia nigra and globus pallidus in T2-weighted (a, b) and FLAIR (c, d) scans

NHLBI Exome Sequencing Project (ESP), gnomAD, 1000 Genomes Project, REVEL, ClinVar, dbSNP, and dbNSFP databases. Annotated variants were filtered based on their frequency, location, and in silico score of pathogenicity and further investigated for compliance with the studied clinical phenotype.

All putative variants were classified in accordance with the guidelines of the American College of Medical Genetics and Genomics (Richards et al., 2015). The Sanger sequencing (ABI 3500 Genetic Analyzer) was performed for variant confirmation and family genotyping.

A heterozygous missense variant c.755T>C in exon 9 of the *WDR45* gene (NM_001029896.2, Xp11.23) was detected in proband. The variant leads to amino acid substitution p.Leu252Pro and has not been previously described in the literature or the genetic databases. It is likely to change an evolutionarily conserved amino acid residue (PhyloP100way = 8.37) and highly impact protein function (class C65 by Align-GVGD). Multiple lines of computational evidence support a deleterious effect of c.755T>C on the gene product: SIFT = 0; PolyPhen-2 HumVar = 0.996; Mutation Taster = 1.000; CADD = 27.6; Revel = 0.739.

Family genotyping revealed that the variant appeared *de novo* (Fig. 4).

A diagnosis of NBIA5 (OMIM: 300894) is proposed for a patient with a set of special clinical features and

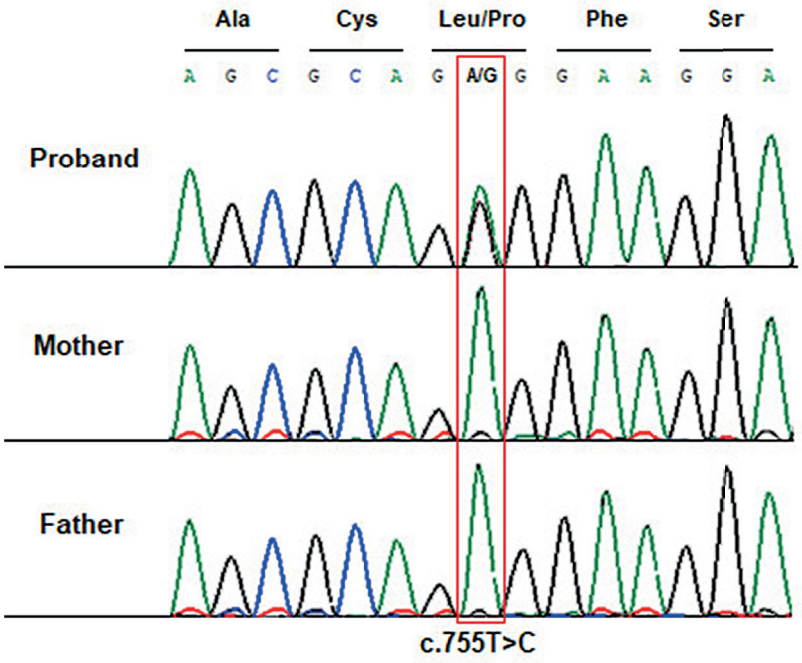

Figure 4. Sanger sequencing demonstrated that the proband is heterozygous for the c.755T>C variant of the WDR45 gene (NM_001029896.2). *De novo* origin was shown with the unaffected patient's mother and father.

definite MRI pictures. So far, 10 different genes are associated with various NBIA subtypes (Tello et al., 2018). At the same time, overlapping clinical features exist between some of the subtypes (Cong et al., 2021; Wang et al., 2019). Taking into account these items, it is no wonder that most individuals suffering from BPAN, or NBIA type 5, had, on average, a 16-years delay from the moment of clinical presentation to diagnosis (Adang et al., 2020). To avoid misdiagnosis and to speed up diagnostics, genetic testing has been widely used since 2013. BPAN was associated with *WDR45* pathogenic mutations (Hayflick et al., 2013).

WDR45 encodes a WD40-repeat-containing phosphatidylinositol-3-phosphate-binding protein, also called WIPI4. It is predicted to fold into a β-propeller structure containing 7 WD40-repeat regions. The group of this protein family is involved in various essential biological processes, including signal transduction, cell cycle progression, gene regulation, etc. WIPI4 is especially important for autophagy, an intracellular degradation system (Li, Roberts, 2001). This process consists of packing cytoplasmic components into autophagosomes and delivering them to lysosomes for subsequent degradation. WIPI4 is thought to be primarily involved in phosphatidylinositol 3-phosphate binding and phagophore expansion and/or closure (Cong et al., 2021). In addition, it has been shown to regulate autophagy through the AMPK and mTOR upstream pathways and possibly to control the size and maturation of nascent autophagosomes (Ji et al., 2021).

It is assumed that in patients with mutations in *WDR45*, the autophagic flow is impaired; consequently, aberrant autophagic structures accumulate in cells (Saitsu et al., 2013). Although loss-of-function *WDR45* mutations are considered causative for BPAN, cases of missense changes with various disease severity were described (Cong et al., 2021). To date, nearly 20 missense variants in the *WDR45* have been reported as pathogenic or likely pathogenic; they provoke either BPAN, ID or DDE. The currently known pathogenic variants leading to BPAN are not concentrated in a specific region or domain but are localised throughout the *WDR45* gene and often occur in the area of WD40-repeats. WD40-repeats are known to act as scaffolds for protein interactions and reversible formation of protein complexes (Smith, 2008) therefore pathogenic variants in the *WDR45* may prevent normal action of WD40 and thus result in the *WDR45*-associated disease phenotype (Li, Roberts, 2001; Ji et al., 2021; Chard et al., 2019).

The missense variant c.755T>C is localised in the area of WD40-repeats of *WDR45* – the WD6 domain. This variant can be considered pathogenic based on the

following ACMG criteria: *de novo* variant in the family without a history of BPAN or other NBIA (PS2); absence in population databases Exome Sequencing Project, 1000 Genomes Project, and Exome Aggregation Consortium (PM2); the variant is located in a region of the *WDR45*, where benign changes are extremely rare (PM1); the results of more than three programs for predicting pathogenicity *in silico* confirm the pathogenic effect of missense change on protein (PP3); missense variant in a gene with a low rate of benign missense variation and in which benign missense variants are implicated in disease mechanisms (PP2); typical clinical phenotype observed in *WDR45* mutation carriers (PP4).

CONCLUSION

In this case report, we describe a nine-year-old girl with profound ID, epilepsy, Rett-like stereotypical limb movements, pigmentary retinopathy combined with iron accumulation in globus pallidus and substantia nigra. Specific clinical features observed led us to the presumed diagnosis of NBIA type 5, which was confirmed during exome sequencing. The data currently available indicate that the c.755T>C (p.Leu252Pro) missense *de novo* variant (not reported before) leads to the disabling disease phenotype and adds one more patient to the growing BPAN cohort.

ACKNOWLEDGMENTS

We thank the patient's family for their kind consent to prepare this article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Adang L.A., Pizzino A., Malhotra A., Dubbs H., Williams C., Sherbini O., et al.: *Phenotypic and Imaging Spectrum Associated With WDR45*. Pediatric Neurology, 2020, 109: 56–62.

Chard M., Appendino J.P., Bello-Espinosa L.E., Curtis C., Rho J.M., Wei X.C., et al.: *Single-center experience with Beta-propeller protein-associated neurodegeneration (BPAN); expanding the phenotypic spectrum*. Molecular Genetics and Metabolism Reports, 2019, 20: 100483.

Cong Y., So V., Tijssen M.A.J., Verbeek D.S., Reggiori F., Mauthe M.: *WDR45, one gene associated with multiple neurodevelopmental disorders*. Autophagy, 2021, 12: 1–16.

Haack T.B., Hogarth P., Gregory A., Prokisch H., Hayflick S.J.: *BPAN: the only X-linked dominant NBIA disorder*. International Review of Neurobiology, 2013, 110: 85–90. doi: 10.1016/B978-0-12-410502-7.00005-3

Hayflick S.J., Kruer M.C., Gregory A., Haack T.B., Kurian M.A., Houlden H.H., et al.: *Beta-propeller protein-associated neurodegeneration: a new X-linked dominant disorder with brain iron accumulation*. Brain, 2013, 136: 1708–17.

Ji C., Zhao H., Chen D., Zhang H., Zhao Y.G.: *β-propeller proteins WDR45 and WDR45B regulate autophagosome maturation into autolysosomes in neural cells*. Current Biology, 2021, 31: 1666–1677.e6.

Li D., Roberts R.: *WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases*. Cell Mol Life Sci., 2001, 58(14): 2085–2097.

Richards S., Aziz N., Bale S., Bick D., Das S., Gastier-Foster J., et al.: *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*. Genetics in Medicine, 2015, 17: 405–424.

Saitsu H., Nishimura T., Muramatsu K., Kodera H., Kumada S., Sugai K., et al.: *De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood*. Nat. Genet., 2013, 45: 445–449.

Smith T.F: *Diversity of WD-Repeat proteins*. Subcell Biochem, 2008, 48: 20–30.

Tello C., Darling A., Lupo V., Pérez-Dueñas B., Espinós C.: *On the complexity of clinical and molecular bases of neurodegeneration with brain iron accumulation*. Clin. Genet., 2018, 93: 731–740.

Wang Z.B., Liu J.Y., Xu X.J., Mao X.Y., Zhang W., Zhou H.H., et al.: *Neurodegeneration with brain iron accumulation: Insights into the mitochondria dysregulation*. Biomedicine & Pharmacotherapy, 2019, 118: 109068.

Wilson J.L., Gregory A., Kurian M.A., Bushlin I., Mochel F., Emrick L., et al.: *Consensus clinical management guideline for beta-propeller protein-associated neurodegeneration*. Dev. Med. Child. Neurol., 2021, 63: 1402–1409.