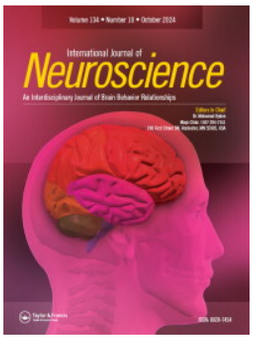

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RESEARCH ARTICLE

Targeted resequencing reveals high-level mosaicism for a novel frameshift variant in *WDR45* associated with beta-propeller protein-associated neurodegeneration

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ABSTRACT

Objectives: Beta-propeller protein-associated neurodegeneration (BPAN) is a rare X-linked dominant neurodegenerative disease, which is characterized by iron accumulation in the basal ganglia. BPAN is associated with pathogenic variation in *WDR45*, which has been reported almost exclusively in females most probably due to male lethality in the hemizygous state.

Methods: Whole exome sequencing (WES) and targeted deep sequencing were performed for a male with a clinical diagnosis of BPAN at the age of 37.

Results: The novel frameshift variant in *WDR45* detected by WES was further analyzed with targeted resequencing to detect a mosaic variant with a level of 85.5% in the blood sample of the proband.

Discussion: Although the main role of *WDR45* remains elusive, recent studies show that *WDR45* may contribute to neurodegeneration through defects in autophagy, iron storage and ferritin metabolism, mitochondria organization, and endoplasmic reticulum homeostasis. The extend of spatiotemporal haploinsufficiency of *WDR45* frameshifting variants caused by mosaicism in males may lead to variable clinical severity, which may be hard to elaborate clinically. Promising genetic analysis strategies using targeted deep sequencing may help determine the clinical outcome of somatic mosaicism in neurological disorders including BPAN. Additionally, we suggest that deep sequencing should be conducted in cerebrospinal fluid samples to provide more reliable results in terms of reflecting the mosaicism level in the brain for future studies.

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WDR45; BPAN; mosaicism; targeted resequencing; WES

Introduction

Neurodegeneration with brain iron accumulation (NBIA) is a clinically and genetically heterogeneous disease group, and several genes have been associated with NBIA forms. Amongst, beta-propeller protein-associated neurodegeneration (BPAN) is the only X-linked dominant subtype of NBIA and mostly occurs with genetic changes in *WDR45* located on chromosome X [1,2]. *WDR45* encodes WD repeat domain phosphoinositide-interacting protein 4 (WIPI4, UniProt: Q9Y484), which has a main role in autophagy [3], furthermore recent studies show that *WDR45* also functions in autophagy-independent pathways, including mitochondrial organization and endoplasmic reticulum (ER) homeostasis [4,5].

Almost 92% of all *WDR45* variations reported to be associated with BPAN are in the form of nonsense, frameshift, cryptic splicing, or deletion, suggesting a null effect *via* nonsense mediated RNA decay mechanism. Those null mutations may reside throughout the gene, which requires full coverage of the region with copy number variation analysis [6].

BPAN is a rare neurological condition, estimated to comprise 7% of all NBIA cases [7]. Yet, it may be speculated that BPAN will actually hold a larger share within all NBIA cases as more cases are recognized

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clinically and confirmed genetically. As an X-linked disorder, its prevalence shows a strong gender bias towards females [6]. XX females are natural born mosaics due to X-chromosome inactivation phenomenon. Accordingly, BPAN females show variable expressivity of the phenotype possibly due to the degree of active X chromosomes carrying the pathogenic *WDR45* variant especially in their neurological tissues. Hemizygous BPAN males, on the other hand, are expected to display the phenotype more severely or to be eliminated due to reduced survival of male embryos. Nevertheless, *de novo* and post zygotic mosaicism detected at least for a subgroup of BPAN males has been attributed to male survival and gender similarity in disease progression [8,9]. Obviously, the conventional tissues used in DNA analysis can only reveal the variation level in extraneurological tissues [1]. Recently, an innovative approach to detect somatic mosaicism in brain using cerebrospinal fluid (CSF) biopsy has been developed [10]. Adaptation of such strategies to measure variant level burden in brain related tissues will elucidate the genotypic contribution of mosaicism on phenotype both in BPAN and other neurological disorders.

The characteristic brain Magnetic Resonance Imaging (MRI) finding is the presence of T2-weighted signal hypointensity in the globus pallidus and hyperintensity in substantia nigra with a hypointense central band. Nigral hypointense central band is the specific MRI sign of BPAN. T2-weighted signal hypointensities in basal ganglia suggesting iron deposition; therefore, BPAN referred to NBIA group [11]. Nevertheless, a definitive diagnosis of this syndrome can only be done through genetic testing.

The typical disease course of BPAN starts with developmental delay and intellectual disability in early childhood. In adolescence or in young adulthood, the disorder suddenly advances into a progressive mode, causing movement and neurocognitive disorders, including parkinsonism, dystonia, and dementia [12]. Although *WDR45* gene involvement is a hallmark for the diagnosis of BPAN, *WDR45* can as well be associated with some other overlapping neurological conditions including Rett-like syndrome, intellectual disability, and epileptic encephalopathy [6].

Herein, we report a novel frameshift and mosaic *WDR45* variant using both whole exome and targeted deep sequencing in a male with a clinical diagnosis of BPAN at the age of 37. Our results are consistent with possibly a postzygotic *de novo* null mutation. We underlie the importance of targeted deep sequencing to determine the exact level of mosaicism, which could easily be missed by conventional WES analysis.

Materials and methods

Subjects and clinical assessments

A family from Turkey with no mention of parental consanguinity has been clinically analyzed at Departments of Neurology in both Hacettepe University and Istanbul University (Figure 1a). Physical and neurological examinations were performed for all available family members, and detailed information on family history was collected. MRI of the brain was performed for the patient. Informed consents were obtained from all family members in accordance with Istanbul University, Istanbul Faculty of Medicine, Clinical Ethics Committee. DNA was extracted from peripheral blood samples of family members using the QIAamp DNA Blood Maxi Kit (Qiagen GmbH, Hilden, Germany).

Whole exome sequencing and segregation analyses

WES was performed for the patient, exonic DNA was captured with the IDT's xGen Exome Research Panel (Illumina, San Diego, CA, USA) and sequenced on the Illumina NovaSeq 6000 platform. Sequence annotation and variant calling were completed in the online SEQ Platform, a cloud-based genomics software, through the service provided by Genomize (Genomize Inc., Turkey). The region encompassing the candidate variant was then visualized manually by Integrative Genomics Viewer (IGV) to detect any possible low-level mosaicism. The variant was reannotated with the ENSEMBL Variant Effector Predictor Tool (VEP) for both detecting the consequence of the variant on current transcript version and retrieving up-to-date population frequency data. Sequence validation and segregation analyses were performed *via* Sanger sequencing using standard procedures. WES data was also used for X-chromosome heterozygosity analysis, as previously described [9].

Targeted deep sequencing of WDR45 variant

Targeted sequencing was applied in an affected male (II.1) and two unaffected siblings (II.2, II.3) (Figure 1a). Amplicon based deep sequencing of the variant region was performed using the specific *WDR45* intronic primers to avoid amplification of *WDR45*-like pseudogenes as controlled using UCSC genome Browser Blat Tool (Forward primer: 5'-AAATGGGGAAAGAGGCAGGG-3' and reverse primer: 5'-GGAGGGAGGAGTCTGAGGTT-3'). Sequencing libraries were prepared using the Nextera DNA Sample Preparation kit (Illumina) and sequenced on a Nextseq 550 (Illumina) with 150 bp paired-end

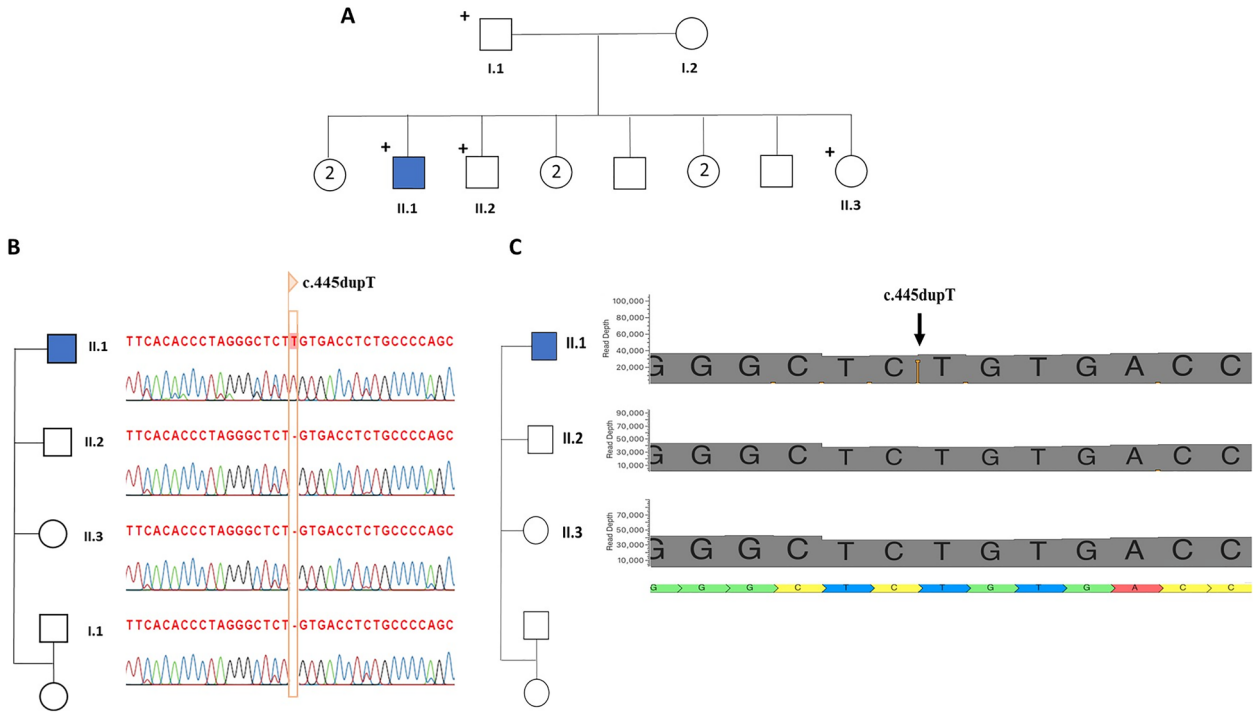

Figure 1. Genetic studies in the family. (a) Pedigree of the family. DNA available for the study is indicated with a plus sign. II.1 has been diagnosed with BPAN. (b) Segregation of NM_001029896.2:c.442dup;p.(Cys148Leufs*2)/NM_007075.4:c.445dup;p.(Cys149Leufs*2). variant within the family. (c) Targeted deep sequencing within the region of the variant in the affected male and his two siblings.

reads. Mapping of the reads was performed against the amplicon sequence.

Results

Clinical presentation

The patient was a 37-year-old male, who was admitted to the outpatient neurology clinic with the complaints of tremor and slowness of movements partially responsive to dopaminergic treatment. His birth was reported to be normal without any complications. His two siblings were healthy. His first observable problems began with speech delay and a general slowness of movements in early childhood. He could not attend school at all due to his intellectual disability. He could only speak with limited number of words to express his needs. Nevertheless, there was a steady course throughout his childhood and beyond. By the age of 30, some behavioral changes were noticed such as a fear of height, insomnia, and agitation at night. Bilateral, slowly progressive upper extremity tremor emerged. He was put on levodopa/carbidopa/entacapone, rasagiline, and sustained-release levodopa with the diagnosis of parkinsonism, but his condition worsened over time. At his first admission to our outpatient clinic, he had bradimimia, moderate to severe asymmetric bilateral bradykinesia and mild to moderate rigidity in all four extremities and resting tremor in the upper extremities. He had postural instability and the gait of the patient, who was usually mobilized with a wheelchair, was unstable and slow. In addition, his family stated that he exhibited stereotypical self-harming behavior, especially when he was alone. In the follow-up, he benefited from dopaminergic treatment after an increase in the dosage but developed L-DOPA-induced dyskinesia, which responded partially to amantadine 200 mg/day. Quetiapine 25 mg/day was helpful for the self-mutilating behaviors. He never had any epileptic seizures, and the current EEG (electroencephalography) was unremarkable. On brain MRI (magnetic resonance imaging), bilateral substantia nigra and globus pallidus showed symmetrical signal drop on T2-W images, susceptibility profound in SWI (Figure 2a, b and d, e). T1-W hyperintense halo surrounding nigral hypointensity was suggestive of BPAN (Figure 2c). Additional findings were cerebellar and cerebral atrophy. Right cerebral hemispheric atrophy was asymmetrically more prominent (Figure 2d, f).

Genetic analyses

WES data analysis revealed a novel frameshift variant in *WDR45*, which is annotated using two reference sequence transcripts valid for *WDR45*: NM_001029896.2:c.442dup;

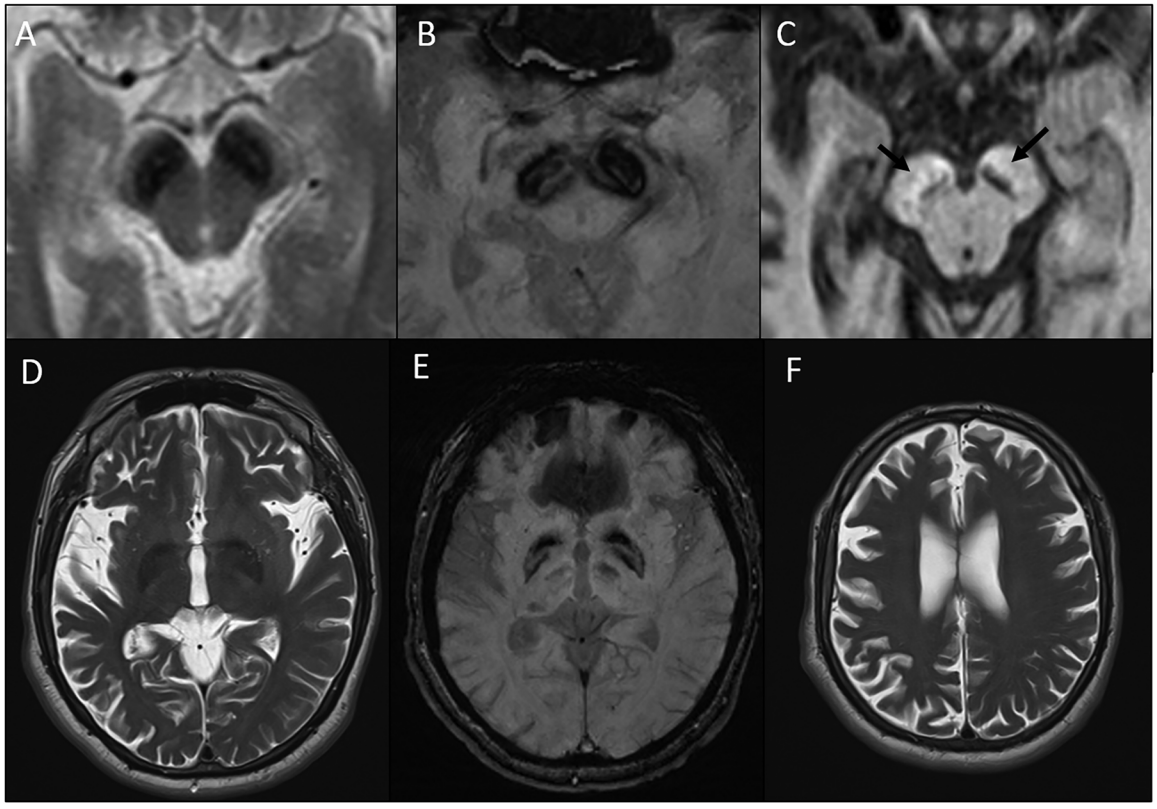

Figure 2. Brain MRI. Axial T2-weighted (W) turbo spin-echo images (a, d, f) and susceptibility-weighted images (b, e), T1-W MPRAGE (c) image. Substantia nigra and globus pallidum show T2 hypointensity and more prominent susceptibility on SWI (a, b and d, e). On T1W image, a hyperintense halo surrounds central hypointensity in the substantia nigra (black arrows, c). Please note that cerebral volume loss is more pronounced in the right hemisphere (d, f).

p.(Cys148Leufs*2) and NM_007075.4:c.445dup; p.(Cys149Leufs*2). We have Sanger sequenced the region to confirm that the variant exists in the patient, and it is absent from all other family members (Figure 1b). The variant was classified as pathogenic with evidence codes of PVS1, PS2, PM2 according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) criteria [13].

Interestingly, an alternative allele frequency of 0.95 in WES data prompted us to perform in-depth analysis of the region with deep sequencing to better understand the extend of this possible variant mosaicism. PCR amplification followed by targeted sequencing has led us to identify that the alternative allele is present in 85.5% of almost 50.000 reads in the region, confirming variant mosaicism. The two family members (II.2 and II.3) used as controls for this analysis had all reference allele reads at the same depth (Figure 1c). XcHet analysis from WES data for the patient was compatible with a single X chromosome, suggesting that the patient had no extra X chromosomes, and the mosaicism was at the variant level.

Discussion

Diagnosis of rare neurological disorders requires collaborative clinical and genetic assessments, as these disorders are heterogenous both clinically and genetically. Advanced brain imaging studies along with high-throughput sequencing technologies and variant interpretation lies in the heart of such assessments. Accordingly, our collective effort has led us to identify mosaicism in *WDR45* for a male patient associated with BPAN.

We have identified a single base duplication in *WDR45* which disturbs the reading frame to cause a premature termination codon (PTC). The variant resides in exon 7/11 of NM_001029896.2 and 8/12 of NM_007075.4, respectively. This PTC resides 527 bp upsteam of the last exon-exon junction in both transcript isoforms. PTCs residing more than 50–55 bp upstream of the last exon–exon junction are reported to be more accessible to translation-dependent nonsense mediated decay (NMD) mechanism [14]. Accordingly, it can be speculated that this variant has a null effect through NMD mechanism, leading to haploinsufficiency in the mosaic state. The variation in the male patient most probably has occurred *de novo* and

postzygotically, but the mother was not available for the study.

The clinical presentation of the patient was typical for BPAN with a steady disease course of developmental delay more prominently affecting speech, mental retardation and later addition of progressive Parkinsonism, hand stereotypies and behavioral problems, namely self-harming behaviors. He did not have any epileptic seizures contrary to majority of cases [15]. Brain MRI was very helpful in differential diagnosis reassuring a need for a conclusive genetic testing for confirmation.

WDR45 encodes a WD40 repeat domain phosphoinositide-interacting protein 4 (WIPI4) that folds into β-propeller structures [16]. WD40 refers to repeat units composed of nearly 40 amino acids that ending with tryptophan (W) and aspartic acid (D) residues [17]. The main function of WDR45/WIPI4 remains elusive. However, as is known, WIPI proteins interact with autophagy proteins and have roles in the biogenesis and maturation of autophagosomes as well as iron storage and ferritin metabolism [18,19]. Additionally, recent studies reveal new important roles in other cellular pathways such as mitochondria organization and ER homeostasis [4–6]. Knockout-mice studies show that the central nervous system (CNS)-specific wdr45$^{-/-}$ mice display impaired learning and memory, poor motor coordination, and axonal swelling, while full-body wdr45$^{-/-}$ mice display ER stress eventually leads to neuronal death [4,5]. Accordingly, like in humans, knockout male mice displayed serious seizures, while knockout female mice were plenty milder. Further, *WDR45*-deficient cells showed impaired mitophagy [5]. Taken together, *WDR45* functions in neuronal and non-neuronal cells and play roles in autophagy as well as the autophagy-independent processes.

Conclusion

Consequently, we have identified a mosaic and novel truncating variant in a male with BPAN. Temporal and spatial haploinsufficiency of this variant in brain tissues probably determines the severity of the condition, which may vary from male lethality *in utero* to survival of BPAN males with phenotypes similar to BPAN females. The potential impact of deep sequencing strategies may underlie the extend of somatic mosaicism in BPAN, which can be missed by conventional next generation sequencing approaches.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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