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ORIGINAL PAPER

A Novel and Mosaic *WDR45* Nonsense Variant Causes Beta-Propeller Protein-Associated Neurodegeneration Identified Through Whole Exome Sequencing and X chromosome Heterozygosity Analysis

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Abstract

Beta-propeller protein-associated neurodegeneration (BPAN) is an X-linked rare dominant disorder of autophagy. The role of *WDR45* has been implicated in BPAN almost exclusively in females possibly due to male lethality. Characterization of distinctive clinical manifestations and potentially the complex genetic determinants in rare male patients remain crucial for deciphering BPAN and other X-linked dominant diseases. We performed whole exome sequencing (WES) followed by segregation analysis and identified a novel nonsense and mosaic variant in *WDR45*, namely NM_007075.3:c.873C>G; p.(Tyr291*) in an affected male at the age of 34. His biphasic medical history was compatible with BPAN, which was characterized by delayed psychomotor development, intellectual disability, and progression into dystonia parkinsonism in his twenties. The variant had an apparently mosaic pattern both in whole exome and Sanger sequencing findings. In order to figure out if mosaicism was restricted to this variant or related to a chromosomal level mosaicism, we used our in-house WES data from 129 unrelated individuals to calculate the threshold values of male and female X chromosome heterozygosity (XcHet) in WES data for our pipeline. A background level of heterozygous variants on X chromosome excluding the pseudoautosomal loci is an observed phenomenon in WES analysis and this level has been used as a quality measure. Herein, we suggest utilization of this measure for detection of digital anomalies of the X chromosome in males by potentially observing a higher XcHet value than the threshold value. This approach has revealed a variant level mosaicism in the affected male, which was further supported with cytogenetic analyses.

Keywords *WDR45* · Mosaicism · Whole exome sequencing · X chromosome heterozygosity · BPAN

BPAN is an X-linked dominant subtype of neurodegeneration with brain iron accumulation (NBIA). The genetic architecture of this extremely rare disorder has been associated with causative variants in *WDR45* identified almost exclusively in females, due to probable male lethality (Haack et al. 2012; Kruer et al. 2012). To date, only 11 male patients have been reported with *WDR45* variants (Abidi et al. 2016;

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Haack et al. 2012, Nakasima et al. 2016; Redon et al. 2017; Spiegel et al. 2016; Takano et al. 2017; Zarate et al. 2016). The clinical presentation of BPAN is marked with a biphasic disease course in early childhood and young adulthood. The first phase is rather static with global developmental delay and intellectual disability. In second phase, the disorder moves on to a progressive mode, in which there is a fast deterioration of motor functions with a sudden onset. Progressive dystonia, parkinsonism, and dementia are the major clinical findings of the second phase (Haack et al. 2012). The characteristic brain 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 (Kruer et al. 2012). Nevertheless, a definitive diagnosis of this syndrome can only be done through genetic testing.

A 34-year-old male patient was admitted to our neurology clinic due to his movement difficulties. His past medical history was indicative of delayed psychomotor development, poor speech, and severe intellectual disability without apparent motor complaints. The parents have reported his moving difficulty, slowness, and contraction in the legs have all developed after the age of 24 years. The movement disorder has progressed rapidly, which was occasionally accompanied by sweating attacks. There was no history of neurological diseases in his family. In his neurological examination, he was neither able to speak nor follow simple commands. Cranial nerves were intact. Risus sardonicus was remarkable. He had postural and target tremors in his hands. Cog wheel phenomenon and rigidity were detected asymmetrically in all extremities. He had bilateral bradykinesia. He could walk without support only in the ante-flexion posture with small steps. It was observed that dystonia was developing in his legs during walking, which caused him to stop walking. Only glabella reflex was brisk among all primitive reflexes. Pyramidal findings were not accompanied to the extrapyramidal signs. He had severe off–on periods for the last 3 years and was using levodopa therapy. In his MRI, there was global cerebral atrophy. T2-weighted hypointense signal intensities in bilateral globus pallidus and substantia nigra demonstrated the iron accumulation. Axial T1-weighted image of substantia nigra showed the specific sign, hyperintense "halo" within a central hypointense band in cerebellar peduncles (Fig. 1).

Blood samples were obtained from family members after receiving written informed consent. The study protocol was approved by the Istanbul University, Faculty of Medicine Ethics Committee (approval number: 2017/133). Whole exome sequencing (WES) was performed for the patient through the commercial service provided by Oxford Gene Technology (OGT; Oxford, UK). Exonic DNA was captured with the SureSelect Human All Exon v6 Kit (Agilent Technologies, Santa Clara, CA) and sequenced on the Illumina HiSeq2000 platform. The sample was sequenced to a mean target coverage of 50 × with 85.97% of bases covered at a depth of > 20 ×. Exome data analysis was performed by our in-house pipeline. Briefly, read alignment to the hg19 reference genome was performed with Burrows–Wheeler aligner and later bam manipulations were conducted using Picard tools (Li and Durbin 2016). Variants were called with GATK Haplotypecaller and annotated using Ensembl Variant Effect

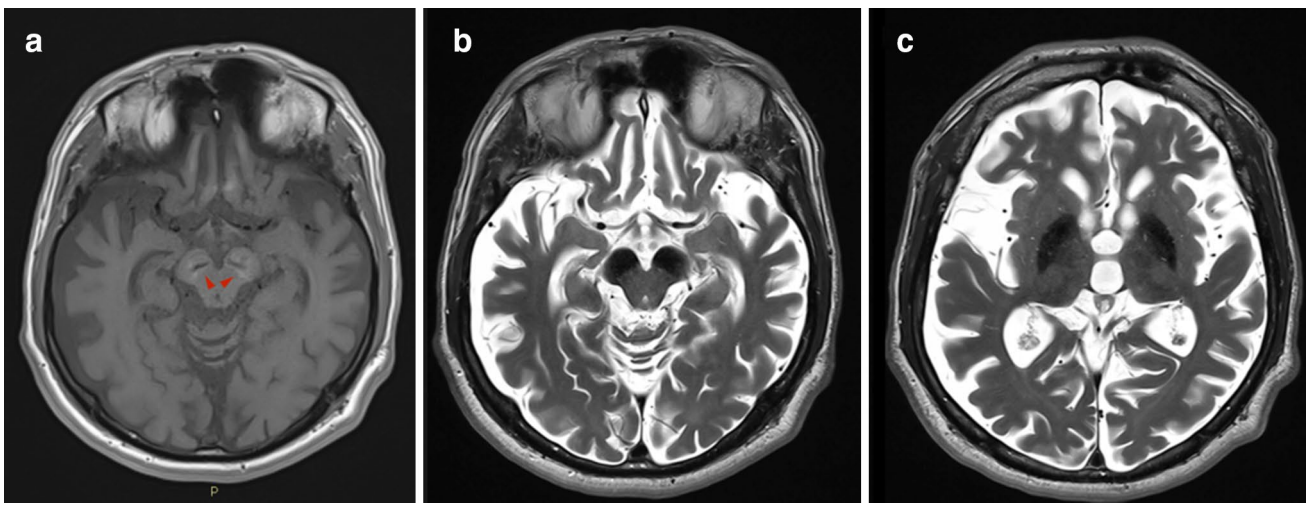

Fig. 1 MRI findings of the patient indicate cerebral and cerebellar atrophy, and iron accumulation in the globus pallidus and in the substantia nigra together with typical pattern for BPAN. **a** T1-weighted axial imaging of substantia nigra demonstrates the hyperintense "halo" surrounding a central linear region of signal hypointensity in the cerebellar peduncles. **b** and **c** T2-weighted axial images demonstrating hypointensity of the substantia nigra (**b**) and the globus pallidus that indicates the iron accumulation (**c**)

Predictor API (Van der Auwera et al. 2013; McLaren et al. 2016). The data were filtered for both recessive and dominant inheritance patterns. These analyses revealed a novel nonsense variant in *WDR45* (NM_007075.3:c.873C>G; p.Tyr291*) in the mosaic state deduced by a 70–30% read in favor of the alternative allele at a depth of 30 reads (Fig. 2a).

NM_007075.3(ENST00000356463):c.873C>G is a novel variant co-localizing with the synonymous rs782557596 (NM_007075.3:c.873C>T) variant. The consequence of this novel transversion is a premature termination codon (PTC) in the penultimate exon of all *WDR45* transcripts annotated in the consensus CDS (CCDS) project (Variant Effect Predictor-Ensembl; ENST00000356463, ENST00000376368, ENST00000376372, and ENST00000634944). According to the current model for translation-dependent nonsense mediated decay (NMD) mechanism, a PTC located more than 50–55 bp upstream of the last exon–exon junction is eligible to NMD (Coban-Akdemir et al. 2018). In this sense, this

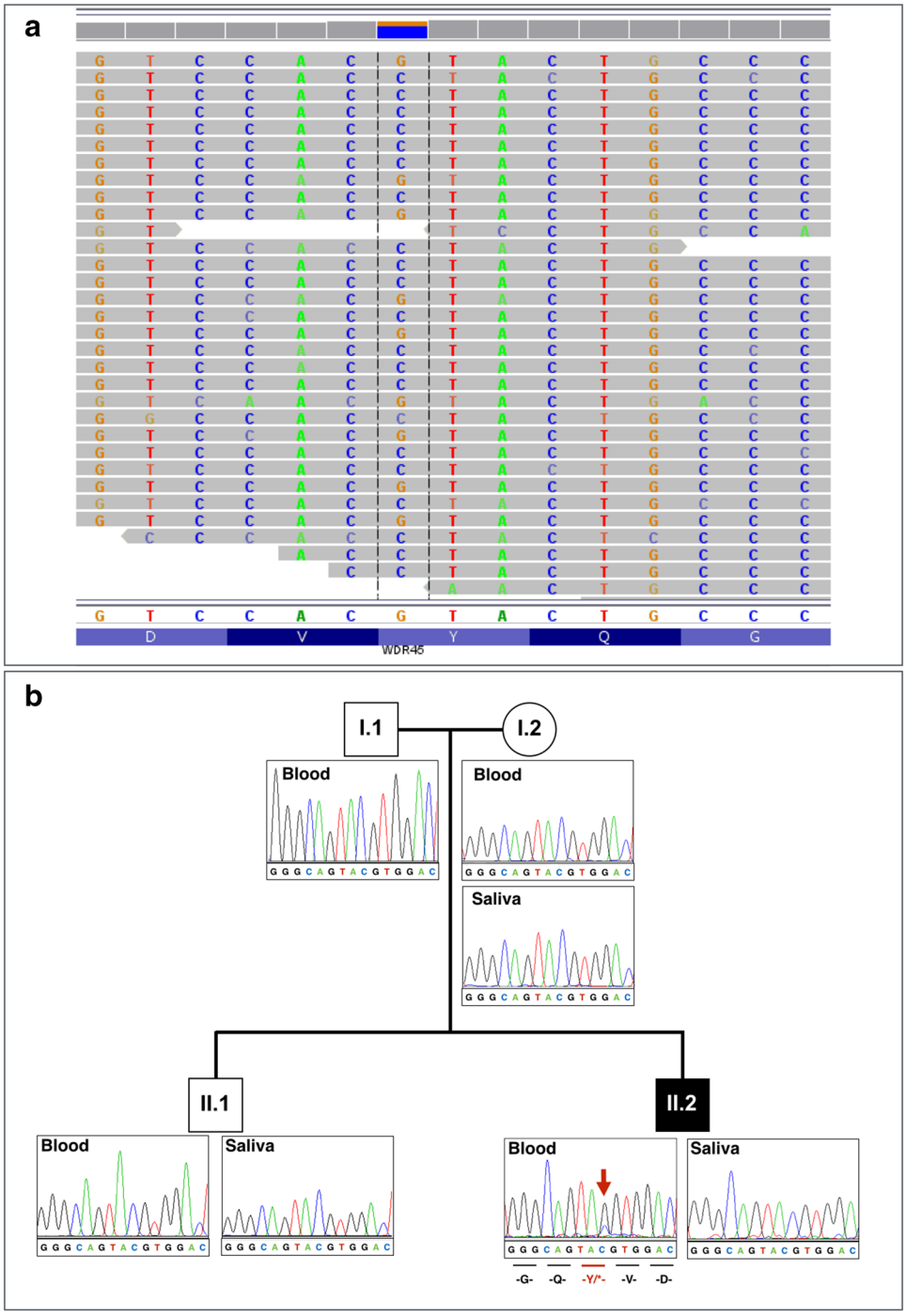

Fig. 2 Identification of the mosaic *WDR45* variant in the affected individual. **a** WES is indicative for a mosaic pattern of the variant (NM_007075.3:c.873C>G) as visualized by IGV in the blood-derived DNA of the patient. **b** Electropherograms show reference reads for all available Sanger sequencing results in the family except for a mosaic pattern in the blood-derived DNA of the patient

novel PTC located 104 bp upstream of the last exon–exon junction makes all four CCDS transcripts eligible to NMD. Therefore, the novel variant potentially creates a null allele and exerts its effect through haploinsufficiency at the mosaic state.

The X-linked dominant nature of BPAN is responsible for the female excess in this NBIA subtype. Accordingly, the pathogenic variants mostly arise de novo in these females and it is possible that the normal allele is silenced due to skewed X-inactivation resulting in loss of protein function (Hor and Tang 2018). The rare males diagnosed with BPAN on the other hand are hemizygous for WDR45 pathogenic variants and are expected to have more severe phenotypes compared to females (Nakashima et al. 2016; Zarate et al. 2016). In infants, severe developmental delay starting with generalized drug-resistant seizures and encephalopathy due to WDR45 pathologies has been reported only in male patients (Abidi et al. 2016; Nakashima et al. 2016; Takano et al. 2017). However, it is hard to elaborate on the phenotypic outcomes of males with mosaic pathogenic WDR45 variants. There is a single and active X chromosome in each cell, which is either harboring the variant or not. The level of mosaicism especially in neurological tissues is a phenomenon that is hard to test but highly distinctive on the phenotype. The seizure-free and less severe BPAN phenotype in our patient may be explained by a relatively low level of mosaicism possibly in neurological tissues. WDR45 encodes WIPI4, the WD-repeat containing protein that functions in ferritin autophagy pathway (Ebrahimi-Fakkari et al. 2016). Depletion of WIPI4 function may disturb iron metabolism in the brain and result in degeneration related to intracellular iron accumulation in delicate brain compartments. The percentage and nature of neurons expressing functional WIPI4 may explain the phenotypic spectrum in mosaic male patients and also females naturally born to be X chromosome mosaics due to X-inactivation.

WDR45 has a processed pseudogene namely, WDR45P1. Therefore, the reads were carefully inspected by the Integrative Genomics Viewer (IGV) (Robinson et al. 2011) in order to discriminate whether they match to WDR45 or WDR45P1. The intron to intron reading within WDR45 has shown that this variant should definitely be annotated within WDR45. Accordingly, WDR45 specific primers were designed for confirmation of this novel variant and its segregation within the family. Intronic primers were designed to amplify exon 11 (ENSE00003463387) of WDR45 to avoid misamplification of WD45P1 (the sequence and PCR conditions of this primer pair are available upon request). We have amplified this region from DNA extracted from a new batch of peripheral blood obtained from the patient after the WES finding. The variant (NM_007075.3:c.873C>G) was confirmed to be mosaic by Sanger sequencing as well, but only in his DNA extracted from blood. DNA of his available family members or his saliva DNA did not exhibit the variant even in low levels (Fig. 2b). The variant was submitted to the freely accessible NCBI ClinVar Database with the SUB4196375 submission number.

X chromosome is hemizygous for the males except for the pseudoautosomal regions (PAR). Nevertheless, WES data has been shown to exhibit certain degree of heterozygosity (X chromosome heterozygosity; XcHet) for regions external to PAR, probably due to low mappability. Predetermined values for XcHet separately for males and females were originally used as quality metrics for WES data and for detection of errors in sample sex status (Taudien et al. 2016). This phenomenon has prompted us to investigate the XcHet value of our patient and decide digitally if he was mosaic at the chromosomal or variant level. At this point, we have determined our baseline XcHet levels from an in-house WES data of 129 individuals (75 females, 54 males). We have prepared a compound variant call format (VCF) file including our patient and then filtered for X chromosome variants excluding PARs. Finally, we have calculated the percentage of XcHet for each individual using python3 pandas package (Danecek et al. 2011; McKinney 2011). The mean values of XcHet were found to be 0.58 ± 0.03 for females and 0.11 ± 0.02 for males. Details for this bioinformatics approach and plotted data can be found in Supplemental Data.

The XcHet value for the patient (0.09) fitted very well with our preset level of XcHet for males. Therefore, we have concluded that he was mosaic only at the variant level, but he did not have any X chromosome digital anomaly or mosaicism at the chromosome level. This finding was further supported by cytogenetic analyses including classical G banding and fluorescence in situ hybridization (FISH). FISH was performed with X (Cytocell Aquarius DXZ1) and Y (Cytocell Aquarius DYZ3) chromosome centromere probes on cells from a 72-h culture of peripheral lymphocytes induced by Phytohemagglutinin. 35 metaphase cells for conventional G banding and 400 cells for FISH were evaluated, which were all consistent with a normal XY chromosome complement. The cytogenetic analyses are presented in supplementary material online.

When all results were evaluated together, the variant was considered to occur de novo and post-zygotic. Analysis of XcHet had been a valuable tool for us to estimate level of mosaicism for this variant. We propose that this metric can be used to detect X chromosome aneuploidies both at mosaic and nonmosaic states. It could be interesting to identify for example XO females or XXY males from WES data only by considering this robust XcHet value. For studies including gross numbers of WES data, it is possible to detect individuals with unexpected X chromosome numbers and reconsider their phenotype and genotype statues. Analysis pipelines may come up with

different number and nature of variants which may interfere with XcHet calculations. We therefore believe that XcHet values should be preset for each pipeline as alternative annotations may lead to variances in XcHet values.

Cognitive inability and dystonia parkinsonism in BPAN patients demonstrate the link between dopamine and WDR45 function. Dopaminergic neurons are responsible for motor and executive functions, reward, motivation, and neuroendocrine control (Civelli and Zhou 2007). Different dopaminergic projections are associated with different functions (Civelli and Zhou 2007). The link between dopamine and WDR45 function is obvious when fetal microarray expression pattern of *WDR45* is analyzed through BrainSpan. Among the parts of cortex, deep gray structures of the cerebrum and midbrain have statistically significant expression pattern. The high expressions of *WDR45* in frontal, prefrontal and insular cortex, corpus striatum, subthalamic nucleus, red nucleus, and substantia nigra pars compacta indicate that the mesocortical and nigrostriatal projections are involved primarily. Frontal-dominant brain atrophy in early-onset male patients' MRIs may reflect the impact of WDR45 expression on dopaminergic mesocortical neuron projections (Takano et al. 2017; Spiegel et al. 2016).

In conclusion, herein we report a novel and de novo nonsense *WDR45* variant in a male patient afflicted with intellectual disability and parkinsonism. The variant is shown to be mosaic not due to a X chromosome digital abnormality using the XcHet metric of WES data. We propose that XcHet, the peculiar entity of WES can be used to identify aneuploidy of sex chromosomes.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval This study was approved by the Ethics Committee of Istanbul Faculty of Medicine, Istanbul University (protocol number 2017/113). Written consent was received from patients family authorizing us to use his medical data in this publication.

Informed Consent Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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Web Resources

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