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WDR45 mutations may cause a *MECP2* mutation-negative Rett syndrome phenotype

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Mutations in the autophagy-related *WD domain repeat 45* (*WDR45*) gene cause beta-propeller protein-associated neurodegeneration (BPAN), a distinct form of neurodegeneration with brain iron accumulation (NBIA).[1,2] Clinical and imaging features comprise childhood-onset global developmental delay with further regression in early adulthood, progressive dystonia, parkinsonism, stereotypies, and iron deposition in the basal ganglia. Female and the few existing male patients show similar phenotypes, probably because of somatic mosaicism in males and skewed X-chromosome inactivation (XCI) in females, as *WDR45* is located on Xp11.23. To date, about 60 cases have been reported, many of whom had a different initial clinical diagnosis.[3] Hyperkinetic movements and stereotypies overlap with Rett syndrome features, another X-linked disorder most commonly caused by *MECP2* mutations. Indeed, for 7% of the reported cases of BPAN, the initial diagnosis was Rett syndrome,[3] prompting us to perform the first mutational screen of the *WDR45* gene in a large cohort of *MECP2* mutation-negative Rett syndrome patients.

Methods

We sequenced exons 3–12 covering the coding region of *WDR45* (ENST00000356463, NM_0007075) in 40 patients with Rett(-like) syndrome from Serbia, including 2 male patients. All patients had been tested negative for mutations in the *MECP2* gene. In identified sequence change carriers, the XCI pattern was studied using the human androgen receptor assay (HUMARA) as described in reference 4. X-inactivation ratios of less than or equal to 80:20 were considered to represent a random pattern, and ratios greater than 80:20 were considered to indicate a skewed pattern.[4] MutationTaster (mutationtaster.org/) and CADD (cadd.gs.washington.edu/score) were used for *in-silico* prediction analysis. The study was approved by the local ethics committee.

Results

Sanger sequencing of *WDR45* revealed 1 novel, likely pathogenic (c.319_320delCT) and 1 benign (c.20G>A) change in 2 female patients (figure A). Patient A carrying the novel deletion presented with classic Rett syndrome and was 6 years old at the last follow-up. Her early motor development was normal, and she started to sit independently at the age of 7 months and walk at the age of 14 months. She showed no speech development, and microcephaly was noted. At the age of 24 months, she developed bruxism and stereotypic movements, followed by epileptic seizures at the age of 36 months. Family history is negative. The c.319_320delCT deletion (figure A) is situated in exon 6 and is predicted to cause a frameshift and introduce a premature stop codon (p.Leu107Phefs*7) likely leading to nonsense-mediated mRNA decay.

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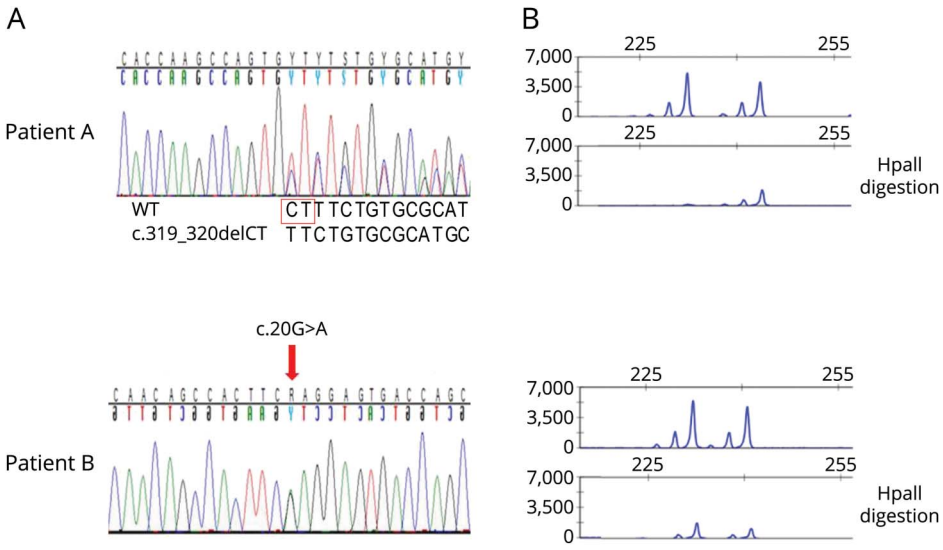
Figure *WDR45* sequence changes in patients A and B



(A) Sanger sequence electropherograms of genomic PCR amplicons of patients A (exon 6, c.319_320delCT, p.L107Ffs*7) and B (exon 3, c.20G>A, p.Arg7Gln). The 2 deleted nucleotides are boxed in red. The arrow above the lower electropherogram marks the exact position of the missense mutation. (B) X-chromosome inactivation (XCI) patterns show skewed XCI for patient A (89:11) and random XCI (56:44) for patient B.

MutationTaster designated this change as disease causing, and the CADD score for this mutation is 28.5. No entry was found in the publicly available databases 1000G (ncbi.nlm.nih.gov/variation/tools/1000genomes/) or ExAC.[5] The HUMARA assay indicated skewed XCI (89:11) (figure B).

No detailed information was available for the carrier of the missense change (patient B) who was lost to follow-up but also presented with typical clinical features of Rett syndrome. Although the c.20G>A (p.Arg7Gln) (figure A) change is also predicted to be disease causing by MutationTaster and CADD (score of 25.4), it was listed in ExAC, with an allele count of 3/86,473.[5] It is important that 2 of the 3 alleles were found in the hemizygous state in male individuals (in 30/30 and 40/43 reads, excluding the possibility of mosaicism).[5] No entry was found in 1000G. XCI was found to be random (56:44) (figure B).

Discussion

Considering the phenotypic overlap between Rett syndrome and BPAN and the fact that 8% of seemingly typical and 42% of atypical Rett syndrome patients screen negative for *MECP2* mutations,[6] we screened a cohort of 40 Serbian patients with Rett(-like) syndrome and found 2 changes in *WDR45*. The deletion c.319_320delCT in patient A is likely causative for BPAN, whereas the missense change c.20G>A in patient B is unlikely to be pathogenic. Although the change occurs in a highly conserved region, the 3 alleles found in the ExAC database harboring this mutation, including 2 hemizygous ones, render it an unlikely genetic cause of BPAN. Furthermore, individuals affected with severe pediatric disease are not part of the ExAC data set.[5] Unfortunately, brain magnetic resonance imaging (MRI) scans of both patients were unavailable and could thus not be reviewed for evidence of brain iron accumulation.

In agreement with previously published observations,[3] our finding emphasizes the need for the *WDR45* gene to be included in panel analyses not only for neurodegeneration with brain iron accumulation but also for patients with Rett (-like) syndrome. Furthermore, the growing evidence for a predominant skewed XCI pattern in BPAN and other X-linked disorders raises the question of the underlying mechanisms regulating XCI, which are still incompletely understood.[1,2,7]

Author contributions

Leonora Kulikovskaja: study concept and design, acquisition, analysis, and interpretation of data, and writing of the first draft. Adrijan Sarajlija: acquisition of data and critical revision of the manuscript for important intellectual content. Dusanka Savic-Pavicevic, Valerija Dobricic, and Christine Klein: study supervision and critical revision of the manuscript for important intellectual content. Ana Westenberger: study concept and design, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content.

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Disclosure

Leonora Kulikovskaja, Adrijan Sarajlija, Dusanka Savic-Pavicevic, and Valerija Dobricic report no disclosures.

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