Expanding the genotypic and phenotypic spectrum of Beta-propeller-associated neurodegeneration (BPAN)

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Main Text

Beta-propeller-associated neurodegeneration (BPAN) is a very rare early-onset neurodevelopmental-neurodegenerative disorder due to X-linked dominant mutations of the *WDR45* gene (1, 2). One hundred and twenty-eight BPAN patients were described so far (3). BPAN, also known as neurodegeneration with brain iron accumulation 5 (NBIA5) or "static encephalopathy of childhood with neurodegeneration in adulthood" (SENDA), is characterized by global early psychomotor delay and epilepsy, followed, in young adulthood, by progressive dystonia, parkinsonism, and cognitive deterioration (4). Brain MRI of affected subjects shows iron accumulation in the globus pallidus and substantia nigra. The pathognomonic MRI finding is the T1-weighted mesencephalic hyperintense signal surrounding the substantia nigra. Cerebral and cerebellar atrophy are also frequently observed (5). Most affected subjects are female. The rare finding of *WDR45* mutations in males was initially attributed to the poor viability of hemizygotes. However, recent reports suggest that males carrying a hemizygous *WDR45* mutation can present a different phenotype predominated by epileptic encephalopathy (6). To our knowledge, ninety-seven *WDR45* mutations have been reported in literature to date. The spectrum of variant types comprises 35 frameshift variants, 21 nonsense variants, 19 splice-site variants, 15 missense variants, 3 in-frame deletions, and 3 large deletions. Most of the identified mutations occurred de novo, with very few exceptions (3).

Here we present two novel BPAN cases (subject 1 and 2) each harboring a deleterious *WDR45* variant. The IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) Ethics Committee approved the study. Written informed consent was obtained from all involved subjects.

Subject 1 is a 30-year-old female, only daughter of healthy unrelated parents. Pregnancy and delivery were unremarkable. Familial history was negative for neurological disorders. At 2 years of age, intellectual disability was diagnosed. She started walking at 5 years of age and maintained the ability to walk until the age of 12 when she underwent knee surgery for post-traumatic ligaments rupture. She never acquired a functional language, although comprehension was partially preserved. Her motor and cognitive abilities remained rather stable until the age of 24

when a progressive deterioration of these functions appeared. At 27, the neurological examination showed severe hypertonia of the limbs with hyperreflexia, bilateral Babinski sign, severe cognitive deterioration, and, remarkably, complete ophthalmoplegia without ptosis, which was not reported in the previous examination. Whether this oculomotor abnormality was attributable to oculomotor apraxia, supranuclear gaze palsy or oculomotor nuclear impairment was difficult to assess due to disease severity and the poor collaboration of the patient. Brain MRI revealed a significant symmetrical hypointensity of pallidal nuclei and substantia nigra in T2-weighted sequences. T1-weighted imaging revealed the typical hyperintense signal surrounding substantia nigra. Cerebellar and supratentorial cortico-subcortical atrophy was also observed (Figure A).

Subject 2 is a 44-year old female, second daughter of healthy unrelated parents. No familial history of neurological disorders was reported. Pregnancy and delivery were normal. Psychomotor development was reportedly delayed. She started walking at 18 months. She attended primary school with a dedicated support teacher. At 8 years of age, she developed absence-like seizures, which were effectively treated with sodium valproate. At 10 years of age, she was diagnosed with mild intellectual disability showing a prominent involvement of expressive language. The clinical picture remained stable until the age of 36 when she developed an extrapyramidal syndrome on the right side, characterized by hemiparkinsonism and an abnormal dystonic posture of the foot. Brain MRI showed the typical MRI pattern of BPAN (Figure B). SPECT showed reduced Ioflupane (123I) uptake in the left striatum. She started levodopa therapy with major clinical benefit on parkinsonism; however, after two years, dyskinetic movements appeared on the right side at levodopa dose-peak.

Genetic analysis of subject 1, performed by Sanger sequencing, revealed a de novo heterozygous *WDR45* splice-site mutation c.519+1_3delGTG (NM_007075) (Figure C and D). This mutation was previously reported in a single subject from Japan, displaying a classical BPAN phenotype (7). Transcript analysis on cDNA from blood RNA showed the retention of intron 8 in the proband, probably due to the loss of splice donor site caused by the micro-deletion (Figure C).

Genetic analysis of *WDR45* gene by Sanger sequencing in subject 2 displayed a novel heterozygous frameshift mutation c.968_969delCT → p.323Cfs*18 (NM_007075) (Figure C and D). The parents were not available for blood sampling.

In this report, we present two pathogenic *WDR45* mutations carried by two subjects affected by BPAN. Original relevant findings of this work are the presence of complete ophthalmoplegia in subject 1 and the identification of a novel pathogenic *WDR45* mutation in subject 2. Therefore, this report expands the genotypic and phenotypic spectrum of this very rare neurogenetic disorder.

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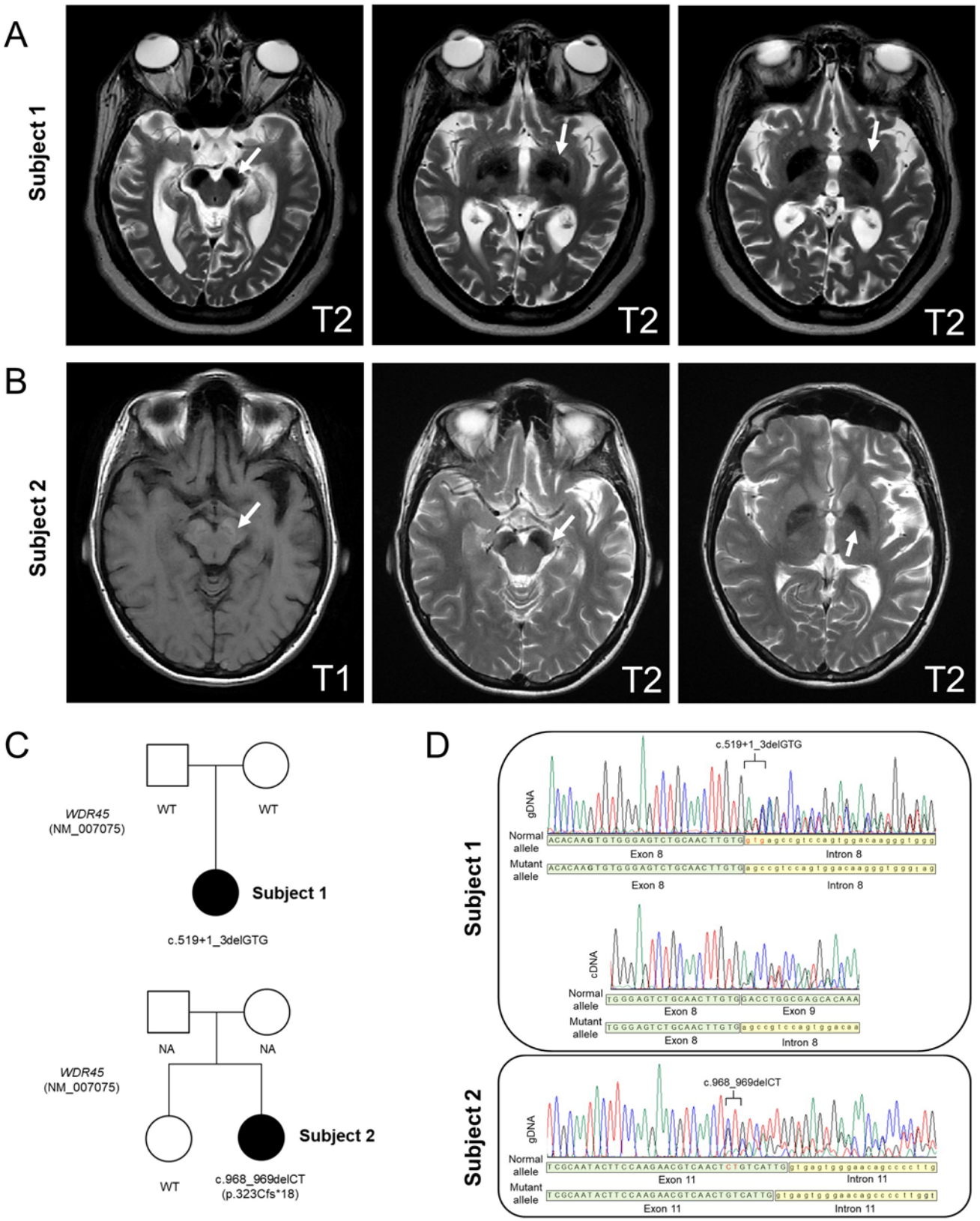
Figure captions

A) Brain MRI of subject 1 displaying hypointensity of the globus pallidus and substantia nigra in T2-weighted sequence. B) Brain MRI of subject 2 showing hypointensity of the globus pallidus and substantia nigra in T2-weighted sequence and the pathognomonic hyperintense rim surrounding substantia nigra in T1-weighted sequence. Arrows indicate iron accumulation in substantia nigra and globus pallidus. C) Pedigrees of the families of subject 1 (upper) and subject 2 (lower) (WT = wild-type, NA = DNA not available). D) Electropherograms show the de novo heterozygous c.519+1_3delGTG *WDR45* mutation carried by subject 1 (upper), the retention of intron 8 on cDNA of subject 1 due to the loss of splice donor site caused by the micro-deletion (middle), and the novel heterozygous frameshift *WDR45* mutation c.968_969delCT, p.323Cfs*18 carried by subject 2 (lower).


A
Subject 1
T2
T2
T2
B
Subject 2
T1
T2
T2
C
WDR45
(NM_007075)
WT
WT
Subject 1
c.519+1_3delGTG
WDR45
(NM_007075)
NA
NA
Subject 2
WT
c.968_969delCT
(p.323Cfs*18)
D
Subject 1
c.519+1_3delGTG
gDNA
Normal allele
Exon 8
Intron 8
Mutant allele
Exon 8
Intron 8
cDNA
Normal allele
Exon 8
Exon 9
Mutant allele
Exon 8
Intron 8
Subject 2
c.968_969delCT
gDNA
Normal allele
Exon 11
Intron 11
Mutant allele
Exon 11
Intron 11