A Novel Homozygous Frameshift WDR81 Mutation associated with Microlissencephaly, Corpus Callosum Agenesis, and Pontocerebellar Hypoplasia

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Abstract

Microlissencephaly is a brain malformation characterized by microcephaly and extremely simplified gyral pattern. It may be associated with corpus callosum agenesis and pontocerebellar hypoplasia. In this case report, we described two siblings, a boy and a girl, with this complex brain malformation and lack of any development. In the girl, exome sequencing of a gene set representing 4,813 genes revealed a homozygous AG deletion in exon 7 of the WDR81 gene, leading to a frameshift (c.4668_4669delAG, p.Gly1557AspfsTer16). The parents were heterozygous for this mutation. The boy died without proper genetic testing. Our findings expand the phenotypic and genotypic spectrum of WDR81 gene mutations.

Keywords
- microlissencephaly
- pontocerebellar hypoplasia
- WDR81 mutation

Introduction

Microlissencephaly is characterized by severe congenital microcephaly, which is associated with smooth brain surface due to abnormal cortical lamination.[1] Phenotypic and genotypic heterogeneity of this brain malformation became more evident in the last decades. Till now, several disease-causing genes, such as NDE1, KATNB1, TUBA1A, TUBB2B, TUBB3, CIT, and ACTG1, have been identified.[2–6] Recently, compound heterozygous mutations in the WDR81 gene have been ascertained in patients with the microcephaly/microlissencephaly-pontocerebellar hypoplasia/atrophy disease spectrum.[7,8] In the present study, we reported two siblings, a boy and a girl, with complex brain malformation of microlissencephaly, corpus callosum agenesis, and pontocerebellar hypoplasia. In the girl, a novel homozygous pathogenic WDR81 variant was identified. Her brother died before having been tested genetically; however, the phenotypic similarities suggested that he had the same mutation.

Case Reports

Patient 1, a boy (►**Fig. 1**) from the first uneventful pregnancy, was born at the 36th gestational week to nonconsanguineous Caucasian parents. His head circumference was 25 cm (–5.2 standard deviation [SD]), weight 2,080 g (–1.5 SD), and length 45 cm (–0.9 SD) at the time of birth. There was no evidence of maternal alcohol and drug abuse, or intrauterine infection. The metabolic workup was normal. Dysmorphic features included sloping forehead, shallow orbits, arched eyebrows, broad nasal root, epicanthi, small nose, anteversion of the nares, prominent upper jaw, and low-set ears. At the age of 10 months his head circumference was 35.5 cm (–8.3 SD), weight 7,780 g (–2.2 SD), and length 68.5 cm (–2.0 SD). He did not acquire any developmental milestones and had spastic quadriplegia. He died at the age of 2 years. Deoxyribonucleic acid (DNA) or tissue samples were unavailable and an autopsy was not performed. On magnetic resonance imaging (MRI) images at the age of 2 weeks, the whole brain was small with a smooth surface, surrounded by an enlarged extracerebral fluid space. The ventricles were deformed and enlarged. The corpus callosum was not recognizable. The brainstem and cerebellum

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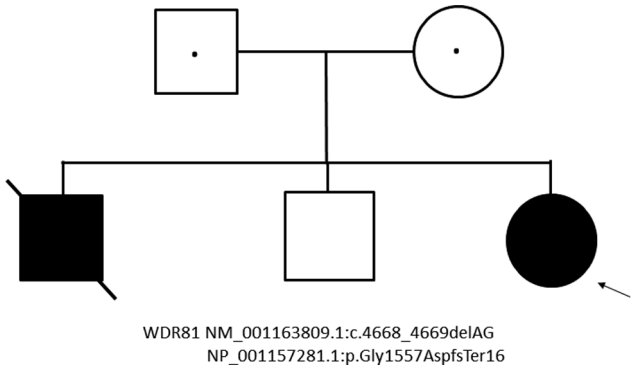

Fig. 1 Pedigree of the family. The WDR81 mutation was segregated in the expected autosomal recessive pattern in all available family members.

were hypoplastic indicating pontocerebellar hypoplasia (►**Fig. 2A–C**). Other organs were not affected. The parents' second child, a boy, is healthy (►**Fig. 1**).

Patient 2, a girl (►**Fig. 1**), was born at term from the third pregnancy after uneventful labor and delivery. At the time of birth, her head circumference was 27 cm (–5.3 SD), weight 3,000 g (–0.8 SD), and length 49 cm (–0.2 SD). She had similar dysmorphic features as Patient 1. At 3 months of age, her occipitofrontal circumference was 30.5 cm (–7.5 SD). She did not show any development and had spastic quadriplegia. The MRI images at the age of 6 days revealed similar abnormalities as seen in Patient 1 (►**Fig. 2D–F**). Other organs were not affected.

Genetic testing was done for Patient 2. Karyotyping was performed by standard protocol, and results showed a normal 46, XX karyotype. Genomic DNA was extracted from peripheral blood samples with the Puregene Kit (Gentra). A homozygous variant in exon 7 of isoform 1 of the WDR81 (NM_001163809.01:c.4668_4669delAG, NP_001157281.1:p.Gly1557AspfsTer16) gene (►**Fig. 3A**, **3B**) was identified by using the Illumina Trusight One Exome Sequencing Panel (Illumina Inc.; San Diego, California, United States), covering the coding region of 4,813 clinically relevant genes. MutationTaster2[9] indicated that this is a disease-causing mutation. The Genome Aggregation Database describes heterozygous p.Gly1557AspfsTer16 variant [dbSNP (rs771116788)] with allele frequency of 2/278488 (0.000007182). Both heterozygous variants were found in the African population, but clinical data, associated with these mutations, were not reported previously. Patient 2 in this report is the first (and so far the only one) in whom this mutation has been confirmed in homozygous state and whose phenotype is reported. Other databases, such as the Human Gene Mutation Database (HGMD Public) and NHLBI Exome Variant Server, do not

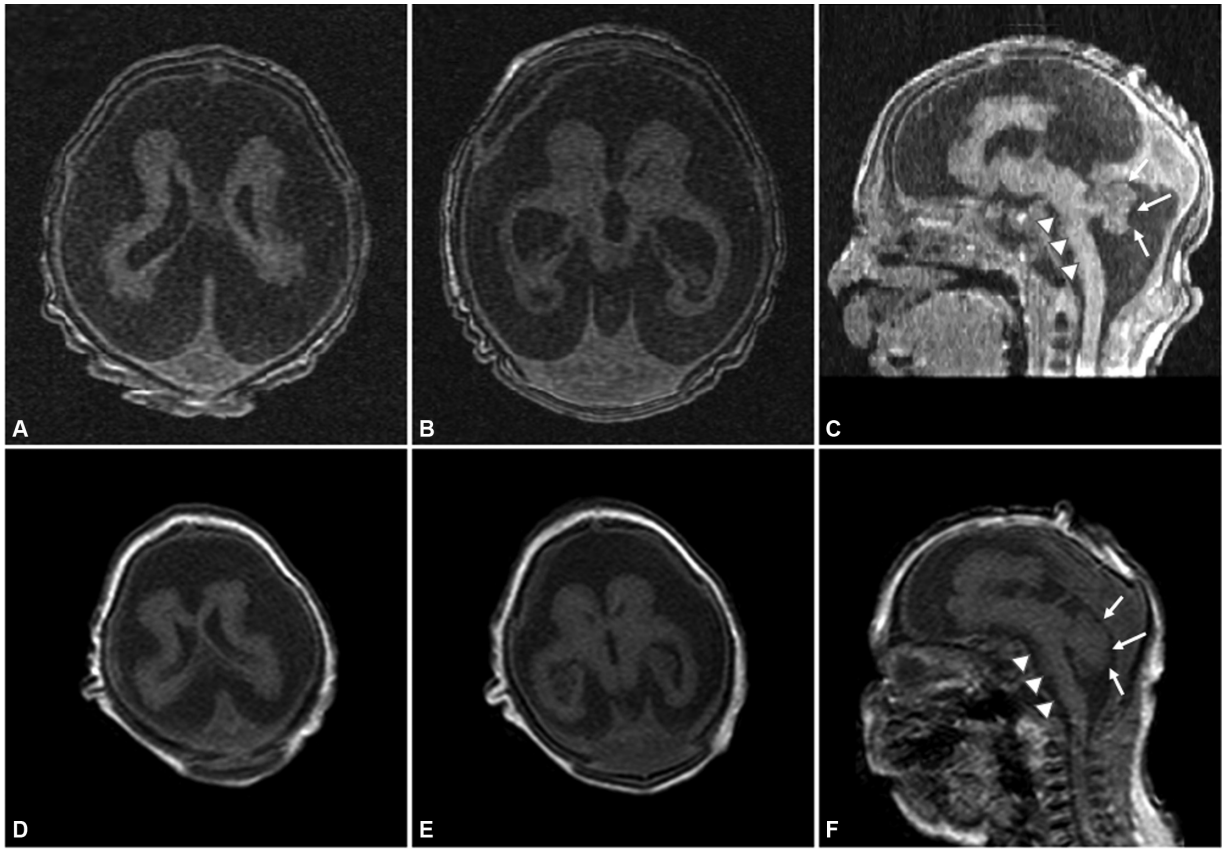

Fig. 2 Brain magnetic resonance imaging (MRI) of the patients. (**A**–**C**) Patient 1. T1-weighted axial (**A**, **B**) and sagittal (**C**) MRI images at the age of 2 weeks. The whole brain is extremely small (microbrain) surrounded by an enlarged extra-axial fluid space. The brain mantle is very thin, gray and white matter cannot be distinguished. Complete agyria can be observed. The corpus callosum is absent and the ventricles are dilated. The brainstem (arrowheads) and cerebellum (arrows) are hypoplastic suggesting pontocerebellar hypoplasia. (**D**–**F**) Patient 2. T1-weighted axial (**D**, **E**) and sagittal (**F**) MRI images at the age of 6 days. Similar malformation pattern can be seen, as in Patient 1.

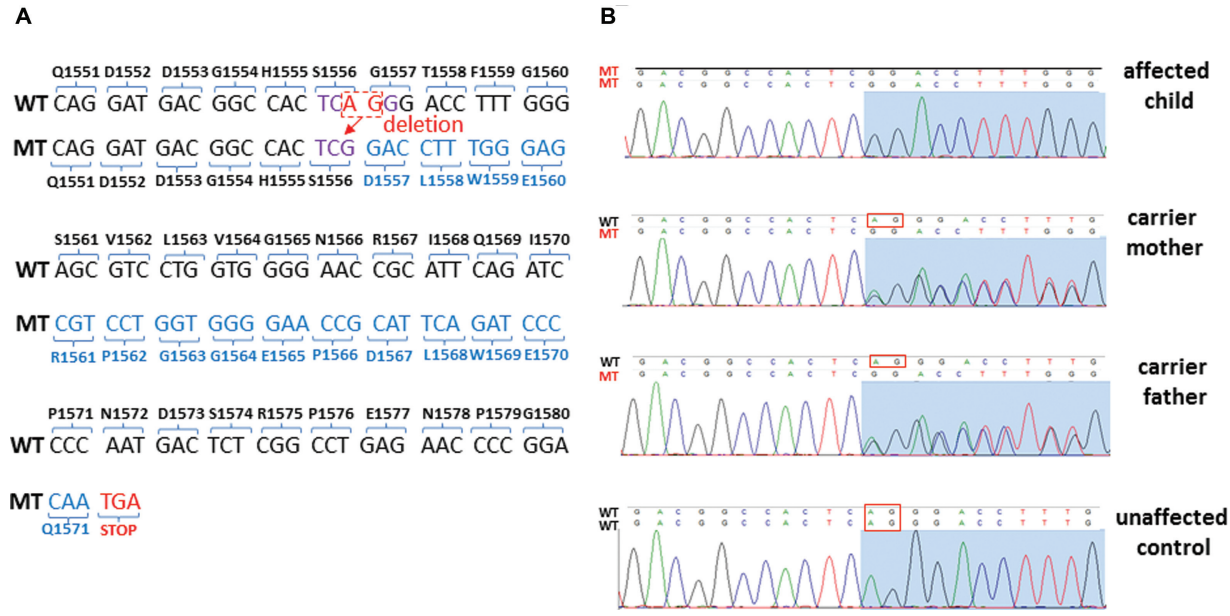

Fig. 3 Molecular testing of the WDR81 gene. (**A**) The predicted effect of the two-nucleotide deletion in WDR81 shows frameshift and premature STOP codon in the mutant (MT) compared with wild-type (WT) sequence. (**B**) The presence of the mutation was confirmed by Sanger sequencing in the affected child and her carrier parents. The red box indicates the deleted WT nucleotides AG, while blue highlight marks the sequence positions of the resulting frameshift in the deoxyribonucleic acid (DNA) chromatograms.

contain any data of this mutation and it was also not found in our in-house clinical exome database of 360 unrelated Hungarian persons. Further, Sanger sequencing confirmed the homozygous mutation in the patient and revealed that the parents were heterozygous for this mutation (ClinVar ID: 635849, ClinVar Accession: VCV000635849.1).

Discussion

Our data suggested a homozygous variant in WDR81 is potentially responsible for the microlissencephaly, corpus callosum agenesis, and pontocerebellar hypoplasia in Patient 2. The same brain malformation was found in her brother (Patient 1) who died without genetic testing. The parents were carriers of this variant.

The WDR81 gene is located on chromosome 17p13.3, consists of 10 exons, and encodes 9 protein isoforms of WD repeat containing protein 81. The longest isoform 1 consists of 1,941 amino acids and contains an N-terminal BEACH (Beige and Chediak–Higashi) domain, a MFS (major facilitator superfamily) domain, and six C-terminal WD40 repeats (WD40 β propeller domain).[10] It is a transmembrane protein with six membrane-spinning domains expressed in all tissues, including neurons in all brain regions.[10,11] In Patient 2, the c.4668_4669delAG mutation caused a frameshift and premature stop codon (p. Gly1557AspfsTer16) in exon 7 of the WDR81 isoform 1 (NM_001163809.01). The mutation may lead either to truncation of the protein with loss of the six C-terminal WD40 repeats and the sixth transmembrane domain (TM6), or nonsense-mediated messenger ribonucleic acid decay.[7]

WD40 repeats are a short structural motif of approximately 40 amino acids, often terminating in a tryptophan-aspartic acid (W-D) dipeptide. WD40 domain-containing proteins have 4 to 16 repeating units forming a β-propeller structure. WD40-repeat proteins are implicated in coordinating multiprotein complex assemblies where the repeating units serve as a rigid scaffold for protein interactions.[12,13] Loss of WD40 repeats may disrupt the interactions of WDR81 protein leading to abnormal network formation.

All the mutations detected so far in WDR81 affected isoform 1 of the protein.[7,8,10,14–16] Novel compound heterozygous nonsense and missense mutations in WDR81, affecting all domains, have been described recently in association with microcephaly/microlissencephaly, callosal hypoplasia/agenesis, and pontocerebellar hypoplasia/atrophy complex in a few patients (►**Table 1**).[7,8] The parents were nonconsanguineous and healthy. WDR81 mutant fibroblasts from the patients showed a significant increase in the mitotic index during prometaphase/metaphase compared with controls, suggesting a delayed prometaphase/metaphase transition.[7] Further experiments in *Drosophila* confirmed that the partial knockdown of the fly orthologue of WDR81 resulted in an increased mitotic index in the neuroblasts form of the central brain, with an increase in the proportion of mitotic cells in prometaphase/metaphase and a decrease in anaphase/telophase, suggesting a delay in the mitotic progression. These mitotic defects may lead to reduced neurogenic cell divisions, alterations of neural cell fates, or to a failure to maintain the progenitor cell population.[7]

Homozygous WDR81 variants in association with fatal congenital hydranencephaly/hydrocephalus have been reported in consanguineous families (►**Table 2**, Patients 2–4).[14,15,17] Another phenotype with intellectual disability, cerebellar hypoplasia/atrophy, with or without quadrupedal locomotion due to truncal ataxia (disequilibrium syndrome 2, CAMRQ2, OMIM #610185), has also been ascertained with homozygous WDR81 variants in two consanguineous families (►**Table 2**,

Table 1 Reported compound heterozygous WDR81 variants causing alterations in isoform 1 of the WDR81 protein

	Gender	Ethnicity	Location	Nucleotide variation	Protein variation	Mutation type	Phenotype	References
1	M	Caucasian	Exon 1 Exon 2	c.1882C > T c.3713C > G	p.Gln628Ter p.Pro1238Arg	Nonsense Missense	Lissencephaly, extremely reduced gyration, thin corpus callosum, dysmyelination, normal cerebellum, enlarged ventricles, and subarachnoid space No development, spastic tetraplegia, generalized dyskinesia, nystagmus	7
2	F (fetus)	Caucasian	Exon 1 Exon 9	c.2834_2837 delTGTT c.5464C > T	p.Phe946Serfs Ter17 p.Arg1822Ter	Frameshift Nonsense	Delayed primary gyration, corpus callosum agenesis, severe hypoplasia of the brainstem and cerebellum	7
3	F	Caucasian	Exon 1 Exon 4	c.1582C > T c.4036_4041dup	p.His528Tyr p.Val1346_Thr 1347dup	Missense Inframe duplication	Gyral simplification, thin corpus callosum, cerebellar atrophy, periventricular gliosis No development, spastic tetraplegia, generalized dyskinesia, nystagmus	7
4	M (+two F fetus siblings)	Caucasian	Exon 1 Exon 1	c.1735G > A c.1358dup	p.Gly579Arg p.Tyr453Ter	Missense Nonsense	Lissencephaly, extremely reduced gyration, thin corpus callosum, normal cerebellum, dysmyelination, enlarged ventricles and subarachnoid space No development, spastic tetraplegia, infantile spasms, dystonia, nystagmus	7
5	F	Caucasian	Exon 3 Exon 9	c.3820_3835del c.5453G > T	p.Pro1274Thr fsTer56 p.Gly1818Val	Frameshift Missense	Cortical atrophy, thin corpus callosum, cerebellar atrophy, dysmyelination No development, spastic tetraplegia, epilepsy	7
6	F	NA	Exon 1 Exon 1	c.84G > A c.1855C > T	p.Trp28Ter P.Arg619Ter	Nonsense Nonsense	Microcephaly, extremely simplified gyral pattern, thin brain tissue, pontocerebellar hypoplasia, corpus callosum agenesis Neonatal seizures, no development	8

Abbreviations: F, female; M, male; NA, not available.

Table 2 Reported homozygous *WDR81* variants causing alterations in isoform 1 of the WDR81 protein

	Gender	Ethnicity	Location	Nucleotide variation	Protein variation	Mutation type	Phenotype	References
1	F (non-cons.)	Caucasian (Hungarian)	Exon 7	c.4668_46 69delAG	p.Gly1557 AspfsTer16	Frameshift	Microlissencephaly Agenesis of the corpus callosum Pontocerebellar hypoplasia Large extra-axial cerebrospinal fluid space No development, spastic tetraplegia	This study
2	Stillbirth, sex NA + M (cons.)	Middle Eastern (Saudi)	Exon 1	c.845G > A	p.Gly282Glu	Missense	Congenital hydrocephalus/hydranencephaly Severe cerebellar hypoplasia (microlissencephaly?) Neonatal death	14,15
3	Parental testing only (cons.)	Middle Eastern (Saudi)	Exon 1	c.850_851del	p.Leu284V alfsTer9	Frameshift	Fatal hydrocephalus (2 cases)	17
4	NA + loss of 2 pregnancies, sex NA (cons.)	Middle Eastern (Saudi)	Exon 1	c.3286C > T	p.Gln1096Ter	Nonsense	Congenital hydrocephalus Cerebellar hypoplasia	15
5	Large cons. family	Turkish	Exon 1	c.2567C > T	p.Pro856Leu	Missense	Hypoplasia of the cerebellum and corpus callosum Intellectual disability Cerebellar ataxia, quadrupedal locomotion (dysequilibrium syndrome, CAMRQ2, OMIM 610185)	10
6	F (cons.)	Middle Eastern (Yemeni/ Emirati)	Exon 4	c.3997C > T	p.Arg1333Ter	Nonsense	Cerebellar hypoplasia/atrophy Global developmental delay Intellectual disability Ataxia (dysequilibrium syndrome, CAMRQ2, OMIM 610185)	16

Abbreviations: cons., consanguinity; F, female; M, male, NA, not available.

Patients 5 and 6).[10,16] Phenotypic similarities to the latter condition were described in mice with missense mutation in the Wdr81 gene.[18]

Thus, in nonconsanguineous families, compound heterozygous, while in consanguineous families, homozygous mutations in WDR81 have been reported until now (►**Tables 1** and **2**). Remarkably, there is no evidence of a relationship between the parents of our patients, yet their offspring(s) had a homozygous mutation (►**Table 2**). The brain malformation spectrum in our patients was more severe than the microlissencephaly/microcephaly spectrum described in the nonconsanguineous patients with compound heterozygous mutations.[7] It was comparable to the consanguineous patients with homozygous mutations and hydranencephaly/congenital hydrocephalus.[14,15,17] Further studies are required to reveal how common this variant is in the Hungarian population and whether any founder effect exists.

WDR81 is a poorly characterized gene and the function of the encoded protein is still unclear. Isoforms 1 to 4 of the protein have been detected in all central nervous system regions in adult wild-type mice[18]; however, the function of these isoforms remains unknown. Particularly no information is available whether any of the isoforms would be able to compensate for the role of mutant isoform 1. Recently, it has been suggested that WDR81 and WDR91 may have roles in endosomal function,[19,20] or autophagy-dependent clearance of ubiquitinated and aggregated proteins.[11] New research efforts might shed light on how WDR81 is involved in neurogenic cell divisions and cell fate regulations.

Ethical Approval

Written informed parental consent had been obtained. The study was approved by the Human Investigation Review Board at Albert Szent-Györgyi Clinical Centre, University of Szeged, Hungary.

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Conflict of Interest

None declared.

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