Splicing Variant of WDR37 in a Case of Neurooculocardiogenitourinary Syndrome

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Case Report

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Abstract

Background

Neurooculocardiogenitourinary syndrome (NOCGUS), a multisystemic syndrome characterized by motor disorder, intellectual disability, seizures, abnormal brain structure, ocular diseases, and cardiac diseases, has been reported with missense variant of WD repeat-containing protein 37 (WDR37) in humans. This report aimed to identify the cause of neurooculocardiogenitourinary syndrome (NOCGUS) in an affected patient.

Case presentation

We identified a de novo intronic 4-bp deletion of WDR37, c.727-27_727-24del, which were predicted to cause abnormal splicing by SpliceAI, in the patient with NOCGUS. Reverse transcription polymerase chain reaction (RT-PCR) revealed intron retention of 63 base pairs before exon 10 in messenger RNA, which was predicted to insert 21 additional aberrant amino acids (p.S242_I243insLCQKKLISRKCLFWPSLWQQ). The patient had novel phenotypes, anal atresia, and polycystic kidney, in addition to intellectual disability, seizures, cerebellar vermian anomaly, and coloboma, which are typical in NOCGUS. We did not observe motor impairments or cardiovascular anomalies.

Conclusion

This is the first reported case of NOCGUS with the splicing variant of WDR37, which manifests with distinctive but variable features. Our findings may expand a possible phenotypic expression of NOCGUS.

Background

WD repeat-containing protein 37 (WDR37) is a member of the WD40 repeat and a 494-amino acid protein that contains seven WD domains in the C terminus.\(^1\) WDR37 forms homodimers and is localized to the intermediate filaments\(^2,\)\(^3\); however, its function is uncertain. The missense variants may cause their effects through a potential dominant-negative mechanism based on the findings of zebrafish mutants.\(^1\) In humans, missense variants in WDR37 cause neurooculocardiogenitourinary syndrome (NOCGUS; OMIM ID: 618652), an autosomal dominant multisystemic disorder characterized by variable features such as specific facies; ocular coloboma; significant neurological deficits with brain malformations; anomalies of cardiovascular, genitourinary, skeletal, and gastrointestinal systems; and postnatal growth failure.\(^1,\)\(^2,\)\(^4,\)\(^5\) To date, no frameshift, or nonsense variants in WDR37 have been reported in humans. We report the case of a patient with NOCGUS who had a splicing variant of WDR37 and whose features were less severe than those of previously reported cases of missense variants in WDR37.
METHODS

Genetics investigations

Whole-exome sequencing of the patient was performed, and data analyzed as previously published. Segregation of \textit{WDR37} variants was examined in Sanger sequencing of Trio DNA. Reverse transcription polymerase chain reaction (RT-PCR) was performed to confirm aberrant splicing (methodology detailed in eAppendix 1).

Case presentation

The patient, aged 6 years, and 11 months, was the second child of unrelated healthy parents. His elder brother was healthy. At 31 weeks of gestation, ultrasonography had revealed a polycystic kidney and enlarged lateral ventricles of the brain. The patient was delivered by cesarean section at 37 weeks of gestation, without asphyxia. His birth weight was 2622 g (−0.4 SD), his height was 47.0 cm (+ 0.1 SD), and his head circumference was 33.8 cm (+ 0.1 SD). He had midlevel anal atresia with a rectobulbar urethral fistula and underwent sigmoid colostomy the day after birth. He also had facial dysmorphisms (Fig. 2A,B), right heterochromia iridum, left anterior segment dysgenesis (Peters' anomaly and sclerocornea) with secondary glaucoma, left polycystic kidney, and a sacral dimple, with which no lipoma or tethered cord was observed on spinal magnetic resonance imaging (MRI). Brain MRI at 8 days of age showed enlarged lateral ventricles, periventricular heterotopia, malrotated hippocampi, hypoplastic and posteriorly rotated cerebellar vermis, enlarged retrocerebellar space, and mega cisterna magna (Fig. 2C-F). Chromosomal G-banding revealed a karyotype of 46,XY, 16qh+, which is considered a normal variant.

The patient underwent posterior sagittal anorectoplasty at age 7 months, and the colostomy was closed at age 11 months. He showed global developmental delay, with head control at 6 months, sitting alone at 12 months, and walking without support at 4 years and 1 month. Electroencephalograms at age 4 months and age 2 years and 4 months were normal. At age 4 years and 1 month, monthly focal impaired awareness seizures with rough breathing appeared, followed by nausea for 20 s, which disappeared after administration of carbamazepine. At age 5 years and 1 month, his developmental quotient score on the Japanese Enjoji Scale was 27. He verbalized no meaningful words but could follow simple instructions. He exhibited an autistic spectrum disorder with obsessive interests. His weight was 19.4 kg (−0.8 SD), his height was 114.0 cm (−1.0 SD), and his head circumference was 48 cm (−2.6 SD) at age 6 years and 11 months.

Genetic analyses

We performed case-only whole exome sequencing for the patient and found a novel intronic variant, c.727−27_727-24del, in \textit{WDR37} (NM_014023.4), in which a splicing change was predicted by SpliceAI (loss [donor] 0.14, gain [acceptor] 0.44). Sanger sequencing confirmed that this variant was a de novo development (Fig. 1A). This variant was absent from 218 in-house Japanese control exome data and public databases including the Genome Aggregation Database v3.1.1 (http://gnomad.broadinstitute.org/)
and 38KJPN Allele Frequency Panel (https://jmorp.megabank.tohoku.ac.jp/). RT-PCR with cDNA derived from the patient and a healthy control showed that two different-sized products were amplified in the patient (Fig. 1B, bottom; A full-length gel is presented in eAppendix 2). Sequencing analysis of cloned PCR products revealed that the c.727-27_727-24del variant caused 63-bp retention of intron 9 sequences in messenger RNA (mRNA), leading to an in-frame 21 amino acid insertion (Fig. 1C). According to the American College of Medical Genetics Standards and Guidelines, this variant was classified as a likely pathogenic (PS2, PM2, PM4). These findings suggest that this WDR37 variant was probably the cause of NOCGUS in this case.

Discussion and Conclusions

To date, 13 patients with NOCGUS, aged 6 weeks to 30 years (median 7 years) have been reported in association with nine missense variants of WDR37. The frequencies of phenotypes of each of these 13 patients are illustrated in Fig. 3. Neurological, ocular, and craniofacial anomalies were found in all patients with NOCGUS, including our patient; however, not all patients exhibited cardiovascular, gastrointestinal, genitourinary, or skeletal features. Our patient did not exhibit cardiovascular and skeletal features, but he had anal atresia and polycystic kidney, which had not been reported previously. Patients with NOCGUS caused by pathogenic WDR37 variants have distinctive but variable features.

The abnormal shape of the cerebellum is common among patients with WDR37 variants, as in our patient. The Dandy–Walker variant has been reported in 4 patients. Four other patients (probands 2–5) were reported by Kanca et al. to have cerebellar vermis hypoplasia; however, brain MRI in these patients depicted an enlarged tegmentovermian angle and an obtuse fastigial recess, both of which are the most significant qualitative measures for confirming the Dandy–Walker malformation phenotype. In total, of 12 patients, 9 (75%), including our patient, had the Dandy–Walker malformation phenotype, which is characteristic of patients with WDR37 variants. Our patient also had periventricular heterotopia and malrotated hippocampi, which have been reported in 1 and 2 patients with WDR37 variants, respectively.

An abnormal gyration pattern, including polymicrogyria, has been reported in a few patients (4 of 11 [36%]) with WDR37 variants; this number is smaller than that reported with cerebellar malformation. Although the expression pattern of WDR37 has not been fully elucidated in the human fetal brain, WDR37 might be more essential to the fetal development of the cerebellar vermis than to that of the cerebrum. WDR37 might be more crucial for cerebral function postnatally, inasmuch as intellectual disability is a quintessential feature in patients with WDR37 variants and microcephaly develops postnatally in most patients, including ours.

Six of the nine variants of WDR37 in 10 patients were located between the coiled-coil domain and the first WD40 repeats (eAppendix 3). The in-frame insertion p.(Ser242_Ile243insLCQKCLKSRKCLFWPSLWQQ) was located between the second and third WD40 repeats of WDR37. Two patients with missense variants between the second and third WD40 repeats had all seven cardinal features of NOCGUS including limited, or no ambulation; however our patient did not have motor disturbance, sensorineural hearing loss,
cardiovascular anomalies, and skeletal anomalies. The latter findings were not found in our patient. Animal models of the WDR37 mutant suggest a potential dominant-negative mechanism of action by the missense variants. The splicing variant of our patient may differently affect the WDR37 function compared to missense variants at adjacent positions, but this must be confirmed by functional studies.

In conclusion, our findings indicate the pathogenicity of the splicing variant of WDR37 and expand the phenotypic spectrum of NOCGUS.

Declarations

Acknowledgement

We thank the patient and his family for participating in this research.

Ethics approval and consent to participate

The study was approved by the Institutional Review Board Committee at Showa University School of Medicine and Hamamatsu University School of Medicine based on the Declaration of Helsinki.

Informed consent was obtained from participant’s parents.

Consent for publication

The patient’s parents provided written informed consent.

Availability of data and materials

The anonymized data are available from the corresponding author upon reasonable request. The datasets generated and analysed during the current study are available in ClinVar, and submission ID of datasets is SUB13562015.

Competing interests

The authors declare no competing interests.

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Authors’ contributions

MK took responsibility for the study concept and design. JS and MK contributed to clinical diagnosis and examination of the recruited patients. MN and HS performed the genetic analyses. MS and MK wrote the
first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

References


Figures
Figure 1

Genetic analysis of *WDR37* variant

(A) Sanger sequencing of the patient and his parents revealed that the c.727-27-727-24del variant was a de novo development. The 4-bp deletion (CTTT) is highlighted with a red box. (B) Schematic representation of the *WDR37* gene structure (top) and electrophoresis of reverse-transcription polymerase...
chain reaction (RT-PCR) with complementary DNA (cDNA) derived from the patient and from a control (bottom). Black boxes, lines, and light blue dashes and arrows denote the coding exons, introns, and RT-PCR primers, respectively. The electrophoresis revealed two bands of different sizes (black and red arrows) in the patient; the wider band (white asterisk) was digested by T7 endonuclease I, which indicated that it was a heteroduplex. WT, wild-type, VT, variant. (C) Schematic representation (top) and electropherograms (bottom) of WDR37 cDNA sequences of WT and variant, respectively. Electropherograms of the variant show a 63-bp retention of the intron 9 sequences, leading to an in-frame 21–amino acid insertion between exons 9 and 10 (red box). Black lines, gray boxes, and orange box denote spliced introns, exons, and retained sequence, respectively.
Figure 2

Clinical and radiological features of Proband.

(A and B) The patient with the $WDR37$ splicing variant has broad nasal bridge, downslanted palpebral fissure, downturned corners of the mouth, and thin upper lip. (C-F) Brain magnetic resonance images at 8 days of age. Axial T2-weighted images (C, D) show mild dilation of lateral ventricles, particularly the body
and posterior horn, and bilateral periventricular heterotopia (arrowheads). The T1-weighted coronal image (E) shows cavum septi pellucidi and malrotation of bilateral hippocampi. The T1-weighted midsagittal image (F) shows thinning of the corpus callosum, mildly posteriorly malrotated hypoplastic cerebellar vermis, enlarged fourth ventricle, and mega cisterna magna.

Figure 3
Bar diagram showing the frequencies of major clinical features in patients with \textit{WDR37} variants.

An asterisk (*) signifies a feature that our patient had. ASD = atrial septal defect; PDA = patent ductus arteriosus; VSD = ventral septal defect.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- eAppendix1.docx
- eAppendix2.docx
- eAppendix3WDR37.pptx