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A NOVEL VARIANT OF THE *NDP* GENE RESULTS IN NORRIE DISEASE IN A VIETNAMESE PATIENT

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ABSTRACT

Norrie disease is an X-linked recessive inherited disorder caused by pathogenic variants in the *NDP* gene. Common symptoms of Norrie include abnormal ophthalmological features, such as retinal detachment, cataract, iris atrophy, and corneal opacity, which could result in peripheral vascular disorder and behavioral, learning, or hearing impairments. This study reports the case of a two-month-old male infant who presented with severe ocular manifestations, including retinal detachment, vitreoretinal proliferation, cataract, and microphthalmia. Recently, molecular diagnosis played a crucial role in clarifying the underlying genetic causes of not only retinal disorders but also Norrie disease. In this study, we employed whole-exome sequencing (WES) to detect the pathogenic mutation of the patient. WES analysis of the proband identified a novel hemizygous frameshift variant in the *NDP* gene, c.77delC (p.T26Ifs*15), which is associated with Norrie disease and classified as likely pathogenic according to the ACMG/AMP guidelines. Concurrently, WES revealed a known heterozygous nonsense variant in the *ATP7B* gene, c.314C>A (p.S105*), associated with Wilson syndrome. Segregation analysis within the family by Sanger sequencing showed that the proband inherited the *NDP* c.77delC variant from his asymptomatic heterozygous mother, and the *ATP7B* c.314C>A variant from his heterozygous father. The proband's sister was negative for both variants. The family received genetic counselling regarding the diagnosis and implications of the identified variants for future offspring. This case emphasizes the clinical value of integrating detailed ophthalmological assessment with comprehensive genomic testing to elucidate overlapping early-onset retinal phenotypes. These findings contribute to understanding the genetic etiology of severe pediatric retinal disorders in Vietnam and underscore the utility of comprehensive genetic testing like WES in achieving the precise molecular diagnosis.

Keywords: FEVR, *NDP* gene, Norrie disease, retinal disorder, Sanger sequencing, WES.

INTRODUCTION

Norrie disease (ND, OMIM: 310600) is a rare X-linked recessive disorder that affects the growth of the neural retina's vasculature (Le *et al*., 2023), with the estimated prevalence of about 1/100,000 (Wang *et al*., 2022). Most affected patients are males, while females are usually asymptomatic (Huang *et al*., 2017). These occurrences are secondary to spontaneous somatic mutations, non-random X-chromosome inactivation, or a mutation on both X chromosomes that occur in consanguineous families (Shastry *et al*., 1999; Sims *et al*., 1997). This disease is characterized by fibrous and vascular alterations in the retina at birth, which worsen throughout the course of childhood and adolescence and result in varying degrees of visual impairment (Donnai *et al*., 1988). The typical symptom of ND patients is blindness related to abnormal features such as retinal detachment, cataract, iris atrophy, corneal opacity, or pseudoglioma (Rivera-Vega *et al*., 2005; Wu *et al*., 2007). Beyond ocular features, ND patients may also develop learning or behavioral issues, peripheral vascular disease, and hearing loss (Sudha *et al.*, 2018).

ND is caused by pathogenic variants in the *NDP* gene, which encodes Norrin, a small and secreted protein belonging to the cystine-knot growth factor superfamily (Hayashi *et al.,* 2021; Sudha *et al.*, 2018). Norrin is predominantly expressed in the retina and inner ear that acts as a ligand binding to Frizzled-4 receptors and activating the Wnt/β-catenin signaling pathway (Ohlmann and Tamm, 2012; Ye *et al*., 2009). It is thought that Norrin is particularly important for the specialization of retinal cells and the creation of the blood supply to the retina (De Silva *et al*., 2021). The *NDP* gene spans 28 kb on chromosome Xp11.3, consists of three exons, of which exons 2 and 3 encode the full-length Norrin protein. More than 160 pathogenic variants have been reported, ranging from single-nucleotide substitutions and small deletions or insertions to regulatory region variants (Jia and Ma, 2021). Structurally, the *NDP* gene contains two major domains, a highly conserved cysteine-knot motif that comprises most of the protein and an N-terminal signal peptide that facilitates extracellular export (Le *et al*., 2023).

In recent years, whole-exome sequencing (WES) has been shown to be an effective technology for molecular diagnosis of hereditary ocular diseases (Dan *et al*., 2022; Lu *et al*., 2022; Trang *et al*., 2022; Gong *et al*., 2022). In this study, WES was employed to detect disease-causing variants in an infant presenting with severe ocular manifestations, followed by Sanger sequencing for validation and the familial segregation analysis.

MATERIALS AND METHODS

Subject

The patient (ID: EVR-32) diagnosed with familial exudative vitreoretinopathy (FEVR) disease by ophthalmologists at the National Eye Hospital (VNEH) in Hanoi, Vietnam was enrolled in this study, along with his family members. A clinical ocular examination was performed on this patient, including visual acuity test, ophthalmoscopy, and ocular ultrasound. The approval of this study was provided by the Institutional Review Board of the Institute of Genome Research, Vietnam Academy of Science and Technology (No.4-2024/NCHG-HĐĐĐ).

After receiving counseling about the benefits of comprehensive genetic sequencing using WES, the patient's parents agreed to provide informed consent for genetic testing.

Genomic DNA extraction

Peripheral blood samples (2 ml) from the patient and his family member were collected into tubes containing ethylene-diaminetetraacetic acid (EDTA) anti-coagulant and stored at -20°C until use. Genomic DNA was extracted from blood samples using GeneAll® Exgene™ Blood SV mini 250p Kit (Korean), following the manufacturer's guidelines.

Whole-exome sequencing

WES was performed on the Illumina NovaSeq 6000 system (Illumina, USA) according to the manufacturer's instructions, generating 150-bp paired reads. The Agilent SureSelect v6 kit was used for exome capture. The average exome sequencing depth exceeded 100X, with a minimum coverage of 30X across all targeted regions. The data generated were quality-assessed, compared, and sequence-processed using FastQC tools, BWA v0.7.19 (r1273), and Picard tools (http://broadinstitute.gith-ub.io/picard/) based on the human genome reference GRCh37/hg19. Gene variant annotation was performed using Genome Analysis Toolkit (GATK) v4.6.2.0. Missense variants were functionally predicted by SIFT and MutationTaster (Trang *et al*., 2022), while SnpEff and SnpSift v5.4 tools were applicable for frameshift variants. Variants reported as benign or non-pathogenic in the ClinVar database were excluded from the screening list. The remaining variants considered to be potentially pathogenic were screened according to the following criteria: (i) gene variants associated with FEVR and *NDP*-related retinal disorder; (ii) variants reported as pathogenic/likely pathogenic in the ClinVar database; (iii) variants with a MAF (Minor Allele Frequency) <0.1% according to population databases including 1000 Genomes phase 3 and Exome Sequencing Project 6500; (iv) priority was given to variants predicted to be "damaging" or "disease-causing". Variants were considered pathogenic if they met the criteria of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) (Richards *et al*., 2015) and the known genetic pattern of disease.

Sanger sequencing

Sanger sequencing was used to test and confirm the pathogenic variant in the patient and his father, mother, and sister by using the ABI PRISM® BigDye™ Terminator v3.1 Cycle Sequencing kit (Thermo Scientific, USA) on the ABI 3500 (xL) Genetic Analyzer system (Applied Biosystems, USA). The DNA regions containing exon 2 of the *NDP* and *ATP7B* genes were amplified using the primer pairs in Table 1. PCR conditions were as follows: 95°C/5 min; (95°C/30 sec; 58°C/30 sec; 72°C/30 sec) x 35 cycles and 72°C/8 min. The PCR products were bidirectionally sequenced and analyzed by SnapGene software. The reference sequence of the human *NDP* and *ATP7B* genes was available with numbers NM_000266.4 and NM_000053.4, respectively, in the National Library of Medicine (NCBI) GenBank.

Table 1. Primers used for Sanger sequencing.

Gene	Exon	Sequence (5'-3')	Amplicon size (bp)	Accession number
NDP	2	F: GGATCCTAGGAGGTGAAGCC R: TGGCTTCTTGCCTGTTTCTG	314	NM_000266.4
ATP7B	2	F: CAATGGAGCTGACACAGGAC R: TGGCTATGAAGGTGGTCTGG	359	NM_000053.4

RESULTS AND DISCUSSION

Clinical findings of the patient

A 2-month-old male infant was admitted to the VNEH in 2023 for evaluation of complex ocular symptoms. A visual acuity test revealed corneal opacification in both eyes of the proband. Ocular ultrasound (B-scan) identified bilateral cataract (Figure 1A), followed by retinal detachment with associated vitreoretinal proliferation (Figure 1B). Axial length measurements were 15.2 mm in the right eye and 15.3 mm in the left eye, both significantly below the normal mean of approximately 19 mm for this age, confirming bilateral microphthalmia. The initial clinical diagnosis was FEVR. He was the second child in his family with no reported history of ocular disorders. Consequently, the proband and his parents were referred to genetic testing and counseling.

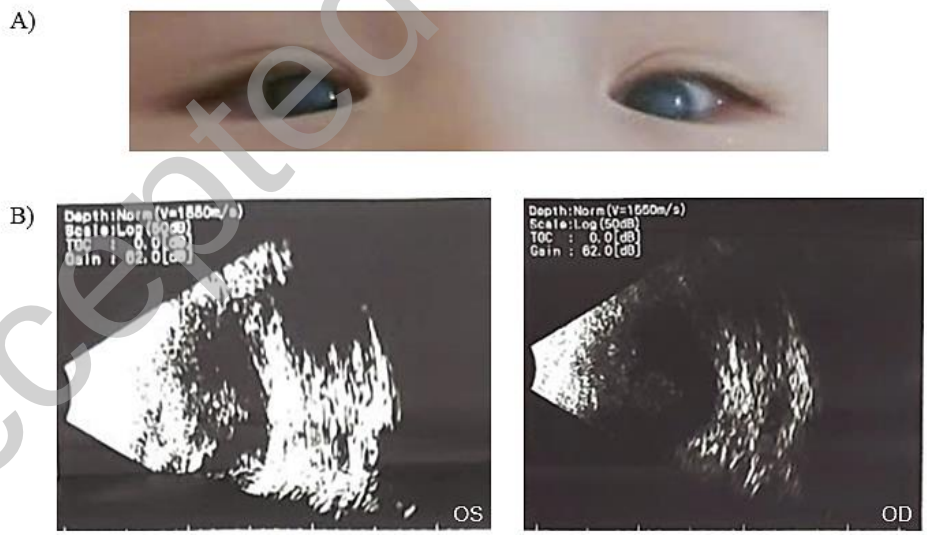

Figure 1. Clinical expression of the affected patient. (A) Ophthalmology phenotype. (B) Ocular ultrasound result (OS - left eye, OD - right eye).

Genetic findings

WES was performed on the proband's genomic DNA. Analysis identified a frameshift variant, c.77delC (p.T26Ifs*15), in the exon 2 of the *NDP* gene (Table 2). This variant is highly deleterious due to the

deletion of a single nucleotide, which introduces a frameshift at codon 26, substituting threonine with isoleucine and introducing a premature termination codon (PTC) 15 residues downstream. Consequently, the mutant transcript is predicted to undergo nonsense-mediated mRNA decay (NMD), as the PTC is located early in the coding sequence of exon 2 and upstream of the final exon–exon junction. If the transcript escapes NMD, a truncated Norrin protein lacking critical functional domains, such as the cysteine-knot domain essential for receptor binding, structural stability, and activation of the Wnt/β-catenin signaling pathway, may be produced. The *NDP* c.77delC variant is novel because it is absent from ClinVar and the Human Gene Mutation Database (HGMD). Thus, according to the ACMG/AMP guidelines, the c.77delC variant was classified as likely pathogenic, based on the very strong (PVS1) and moderate (PM2) evidence criteria. Concurrently, WES identified a known pathogenic variant in *ATP7B*, c.314C>A (p.S105*), in exon 2. Variants in *ATP7B* gene cause Wilson disease, an autosomal recessive disorder of copper metabolism that can lead to progressive, irreversible hepatic and neurological damage if untreated. Despite the absence of a Wilson diagnosis in the proband's family, carrier testing for this variant is clinically warranted given the disease's severity, the efficacy of early intervention, and its documentation in the Vietnamese pediatric populations (Huong *et al*., 2022; Pham *et al*., 2017).

Segregation of these two variants within the family was examined by Sanger sequencing (Figure 2). The results confirmed the proband's genotype as hemizygous *NDP* c.77delC variant and heterozygous *ATP7B* c.314C>A variant, consistent with the WES data. His mother (I-2) was identified as a heterozygous carrier for the *NDP* variant, while his father (I-1) carried the heterozygous *ATP7B* variant. The proband's sister (II-1) was negative for both variants.

Table 2. WES analysis of the proband.

Gene	Variant change		Variant type	Zygosity	Clinvar	HGMD
	cDNA	Amino acid				
NDP	c.77delC	p.T26Ifs*15	Frameshift	Hemizygous	-	-
ATP7B	c.314C>A	p.S105*	Stop gain	Heterozygous	Pathogenic	-

Pathogenic variants in the *NDP* gene cause a phenotypic spectrum ranging from severe the Norrie disease to FEVR, with considerable variability in phenotype (Luvisi and Blair, 2025). In this study, the clinical ocular features of the proband were extremely complex at the early age of onset, including vitreoretinal proliferation, corneal opacification, retinal detachment, cataract, and microphthalmia, which are consistent with Norrie disease phenotype. In contrast, severe FEVR often presents later in infancy or childhood, with variable expressivity, incomplete peripheral retinal vascularization, and a broader clinical spectrum that may preserve useful vision in some individuals. These manifestations were much more severe than those previously reported in the four patients carrying the *NDP* pathogenic variants,

which have been detected at our center to date (Trang *et al*., 2022). This is likely to be a consequence of the null mutation causing incorrect synthesis of Norrin, leading to protein function impairment, especially in male individuals who carry only a single copy of the gene. The truncating or loss-of-function variants in *NDP* are more commonly associated with the classic phenotype of Norrie, whereas certain missense variants may retain partial protein function and present with FEVR-like features, as shown in the ClinVar database.

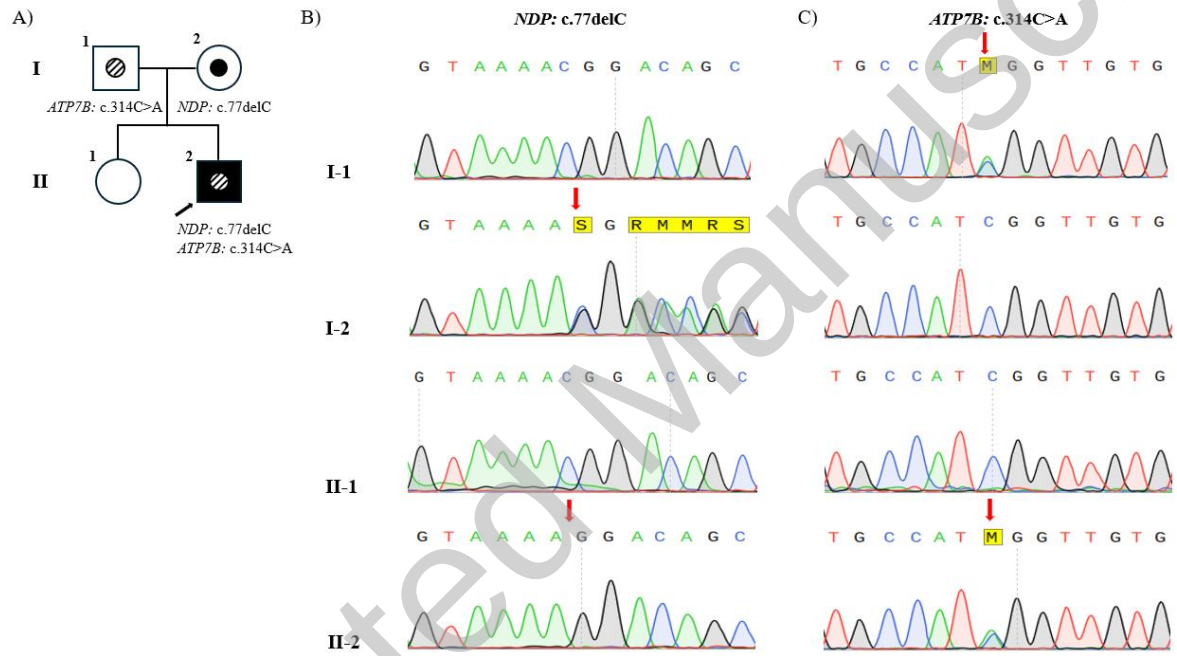

Figure 2. Molecular genomic analysis of familial pedigree in the proband. (A) Pedigree showing the inheritance of the *NDP* and *ATP7B* variants, in which the proband is denoted with an arrow symbol (black). Squares and circles represent males and females. Black shading indicates Norrie disease (*NDP* variant). A black dot denotes a heterozygous *NDP* carrier, while a hatched dot denotes a heterozygous *ATP7B* carrier. (B) and (C) Sanger sequencing chromatograms of the *NDP* and *ATP7B* variants compared between the parents, sister, and affected patient, the mutated nucleotide position was indicated with a red arrow.

The identification of a novel hemizygous *NDP* variant in this family has direct and significant implications for genetic counseling. First, the proband's mother was confirmed to be a heterozygous carrier. As an X-linked recessive disorder, each son of a carrier mother has a 50% risk of inheriting the pathogenic variant, while each daughter has a 50% chance of becoming a carrier. The proband's sister tested negative for the familial *NDP* variant, which provides reassurance and relieves her of future reproductive concerns related to this condition. Second, the father of proband is only carrier of *ATP7B* c.314C>A (p.S105*) variant and does not have Wilson disease and the mother has been confirmed not to carry the identified variant. Therefore, there is no risk of having an affected child with Wilson disease associated with this variant

in this family. The incidental finding of a heterozygous *ATP7B* variant, a known pathogenic founder variant in the Vietnamese population, adds another layer of complexity to genetic counseling.

This case underscores the importance of pre- and post-test genetic counseling when comprehensive genomic testing such as WES is employed. Unsolicited or secondary findings, particularly those with well-established clinical actionability, must be disclosed and managed according to updated ACMG/AMP recommendations. We therefore advocate that genomic testing for pediatric retinal disorders should always be embedded within a genetic counseling framework, involving a multidisciplinary team of ophthalmologists, clinical geneticists, and genetic counselors.

CONCLUSION

To summarize, we identified a novel hemizygous variant of the *NDP* gene in chromosome X that was the genetic cause of Norrie disease in a male infant via WES. This finding expands the mutational spectrum of *NDP* and contributes to the understanding of inherited retinal disorders in the Vietnamese population. Another heterozygous *ATP7B* variant was also detected, which is not related to the proband's clinical phenotype but has implications for carrier counseling within the family. In addition, this study is limited by the single-case present and absence of functional validation; therefore, further studies are suggested to confirm the biological effect of the variant.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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