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Brief Communication

# A novel missense mutation of NDP in a Chinese family with X-linked familial exudative vitreoretinopathy

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**Abstract**

Familial exudative vitreoretinopathy (FEVR) is a hereditary ocular disorder characterized by a failure of peripheral retinal vascularization. In this report, we describe a novel missense mutation of the Norrie disease gene (*NDP*) in a Chinese family with X-linked FEVR. Ophthalmologic evaluation was performed on four male patients and seven unaffected individuals after informed consent was obtained. Venous blood was collected from the 11 members of this family, and genomic DNA was extracted using standard methods. The coding exons 2 and 3 and their corresponding exon−intron junctions of *NDP* were amplified by polymerase chain reaction and then subjected to direct DNA sequencing. A novel missense mutation (c.310A>C) in exon 3, leading to a lysine-to-glutamine substitution at position 104 (p.Lys104Gln), was identified in all four patients with X-linked FEVR. Three unaffected female individuals (III2, IV3, and IV11) were found to be carriers of the mutation. This mutation was not detected in other unaffected individuals. The mutation c.310A>C (p.Lys104Gln) in exon 3 of *NDP* is associated with FEVR in the studied family. This result further enriches the mutation spectrum of FEVR.


*Keywords:* Chinese; familial exudative vitreoretinopathy; mutation; Norrie disease pseudoglioma; X-linked

## 1. Introduction

Familial exudative vitreoretinopathy (FEVR) is a genetically-heterogeneous disorder characterized by abnormal vascularization of the peripheral retina, which can result in retinal detachment and severe visual impairment. The most prominent characteristics of the disease result from the incomplete and aberrant vascularization of the peripheral retina, retinal blood-vessel differentiation,[1] or both. The latter can lead to various complications, such as retinal neovascularization and exudates, vitreous hemorrhage, vitreoretinal traction, ectopia of the macula, and retinal folds and detachments. The clinical signs in affected individuals can be diverse, ranging from hardly detectable vascular anomalies in the peripheral retina in asymptomatic individuals to bilateral retinal detachments leading to blindness. Patients with mild symptoms show little or no change in visual acuity. Fundus fluorescein angiography (FFA) examination reveals a small area of no vascular perfusion around the retinal periphery, a common feature in all affected individuals among the family. FEVR is a typical Mendel single-gene disease that was first described by Criswick and Schepens in 1969,[2] and has since become a well-recognized and extensively studied condition.

Conflicts of interest: The authors declare that they have conflicts of interest related to the subject matter or materials discussed in this article.

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To date, mutations in genes encoding Norrin [encoded by Norrie disease pseudoglioma (*NDP*)] for X-linked recessive form,[3] and Frizzled 4 (*FZD4*), low density lipoprotein receptor related protein 5 (*LRP5*), Tetraspanin-12 (*TSPAN12*), and zinc finger protein 408 (*ZNF408*) for autosomal dominant (AD) form have been shown to cause FEVR.[4–7] A few families with *LRP5* and *TSPAN12* autosomal recessive (AR) inheritance related to FEVR have also been documented.[8,9]

Each of these encoded proteins is a component of the Norrin/β-catenin signaling pathway (also referred to as the Norrin / Frizzled-4 pathway).[10] The *NDP* locus maps to chromosome Xp11.4, spans 28kb, and comprises three exons; however, only exons 2 and 3 of *NDP* are translated into a 133 amino-acid protein Norrin. Norrin acts as a ligand for the *LRP5*, *FZD4*, and *TSPAN12* coreceptors that activate canonical Wnt signaling.[10] Wnt signaling plays an important role in eye organogenesis and angiogenesis.[10] Mutations in *NDP* disrupts the Wnt signaling pathway, directly leading to FEVR.

Here we describe a large typical four-generation FEVR family with a total of 40 family members. All seven affected patients were male. Mutation analysis identified a novel mutation in *NDP* that caused the X-linked FEVR.

## 2. Methods

### 2.1. Participants

The FEVR family was from the northern area of the Henan province in China (Fig. 1). The proband (V9) was born in 1997 after normal pregnancy. He was diagnosed with FEVR at 10 years of age. During genetic consulting, we found that the family had seven members with similar symptoms. These family members were then examined at the Eye Institute in the People's Hospital of Henan Province. The clinical diagnosis of FEVR was made based on the following criteria: (1) positive family history with seven male-only affected individuals; (2) ophthalmic examination confirming bilateral vitreous opacity, retina surrounding no vascular zone, merging heterotopy of macula, retinal fold, and retinal detachment; and (3) absence of history of premature labor and oxygen uptake.

Informed consent was obtained from all individuals tested after explanation of the nature and possible consequences of the study, and the research adhered to the tenets of the Declaration of Helsinki. Ethical approval was obtained from the People's Hospital of Henan Provincial Ethics Committee.

Peripheral venous blood (EDTA-K2 anticoagulant; 5 mL per individual) was collected from 11 family members, including four patients (IV1, IV3, IV4, and V9) and seven unaffected individuals (III2, III14, IV11, IV13, IV14, V4, V8, and V10).

### 2.2. Genetic analysis

Genomic DNA was extracted from peripheral blood lymphocytes by standard procedures using a Qiagen Blood kit (QIAGEN, Germantown, MD, USA). The two exons (exons 2 and 3) and the corresponding intron–exon boundaries of the *NDP* gene were amplified by polymerase chain reaction (PCR). Primer sequences and annealing temperatures are listed in Table 1. Each 25-μL PCR amplification reaction contained 1X buffer, 150 ng of genomic DNA, 0.2 mM of each dNTP, 2-U Taq polymerase, 1 mM of forward and reverse primers, and 1.5-mM MgCl2. PCR products were analyzed in 1.5% agarose gels. Amplified products were excised and purified using QIA quick PCR Purification kit (QIAGEN, Germantown, MD, USA). Sequencing was performed using an ABI Big Dye terminator cycle sequencing kit (v3.1) on an ABI 3730 Genetic analyzer (Applied Biosystems, Foster City, CA, USA). The proband (V9) was sequenced first for mutation identification. The sequencing results were compared with the human reference sequence from the University of California, Santa Cruz (UCSC) 2013 Human Genome Assembly. A missense mutation was found in exon 3 of *NDP*. To confirm this mutation, the exon 3 of *NDP* from the other individuals (affected individuals IV1, IV3, and IV4; unaffected individuals III2, III14, IV11, IV13, IV14, V4, V8, and V10) were then amplified and sequenced.

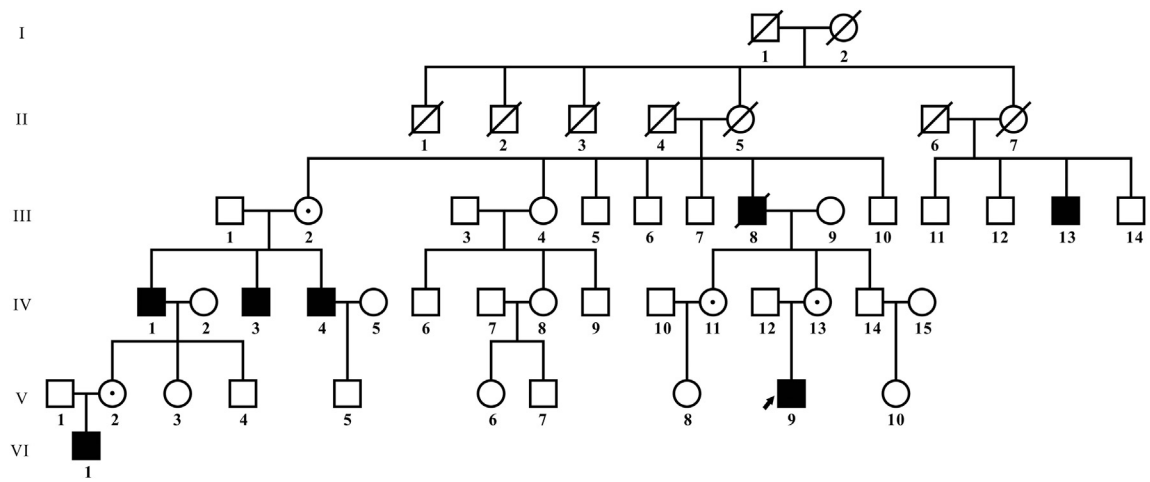


Fig. 1. Pedigree of the familial exudative vitreoretinopathy (FEVR) family reported in this study. The affected FEVR patients (black square), asymptomatic heterozygous mutation carriers (dot).

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