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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.

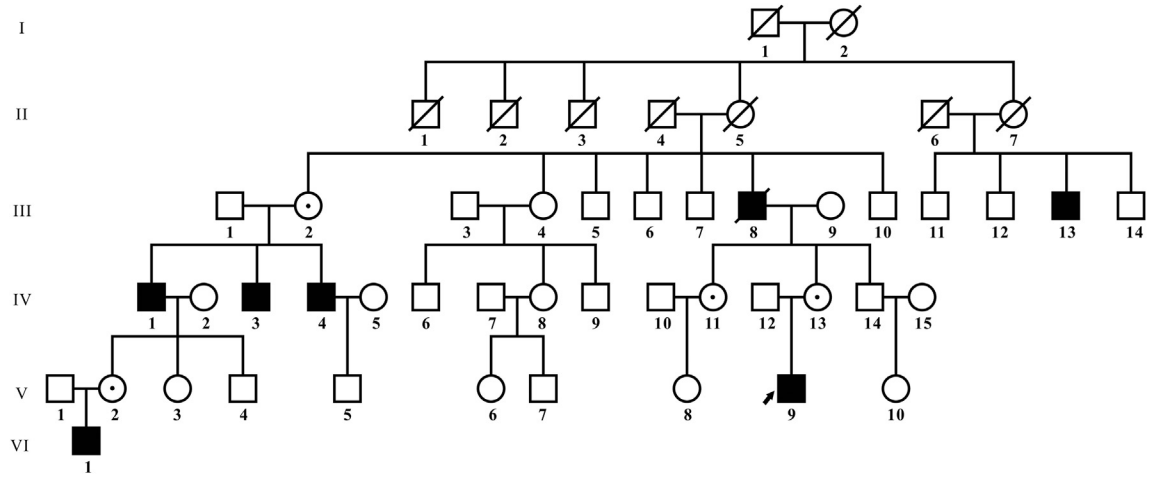

Fig. 1. Pedigree of the familial exudative vitreoretinopathy (FEVR) family reported in this study. The affected FEVR patients (black square), asymptomatic heterozygous mutation carriers (dot).

Table 1
Primers and annealing temperatures used for *NDP* amplification.

Exon	Sequence (5′–3′)	Annealing temperature (°C)	Amplicon size (bp)
2	F: 5′TGGGGTGGAATGGATGACAG 3′ R: 5′TTCTTGCCTGTTTCTGAGGG 3′	54.6	316
3	F: 5′CCATGAAAGCCTGGTCCCTA 3′ R: 5′GAATATGCAGAGTCCCGGGA 3′	58.7	360

NDP = Norrie disease pseudoglioma.

3. Results

3.1. Clinical examination

This family had seven blind patients. Six patients, including the proband V9 and III3, IV1, IV3, IV4, and VI1, were checked for existing symptoms. All patients showed weak eyesight. Spotlight reaction was weak and progressive with blindness at the age of 3–4 years old. All patients had signs of a cataract. III3, IV1, IV3, and IV4 affected individuals also had a sign of glaucoma; the vision of those patients was sharp decline after the transient high intra-ocular pressure with a severe headache. Eye examination for the proband V9 (17 years old): vision oculus dexter (OD) showed light perception with best-corrected visual acuity (BCVA): 11.0/−2.50 × 10° → counting fingers (CF)/30 cm, vision oculus sinister (OS): 20/400, BCVA: −4.00/−2.00 × 70° → 20/60. Intraocular pressure: OD: 9 mmHg; OS: 13 mmHg. Opacity was found in both eye lenses, the cortex, the gray of the posterior capsule, and gray flocculent in the vitreous body. Fundus: retinal vascular abnormalities, expansion, and straight peripheral in both eyes. There was retina fold in the posterior pole of right eye, and macular ectopic, vitreous retinal adhesion along with tractional hole formation in the left eye. Ocular ultrasound: retina fold in the right eye, unsmooth peripheral wall of eyeball (suspicious hole) in the left eye. Both eyes presented with vitreous opacity. FFA test: the various vascular branches of posterior pole retina, the temporal peripheral retinal showing nonperfusion zones along with abnormal new blood vessels and fluorescence leakage. The fundus examination was consistent with the FEVR. The diagnosis was FEVR oculus uterque (OU), complicated cataract OU, high myopia and amblyopia OD, and retina fold OD. Patient VI1 was a 2-year-old young child, full-term normal delivery, no history of oxygen uptake, eye exam: vision OD showed light perception, vision OS: 0.1, only distinguish the first line of visual chart. The other detailed clinical manifestations of affected individuals are showed in Table 2.

3.2. Mutation analysis

A missense mutation c.310A>C in exon 3 of *NDP* was detected in the proband (V9) and his three uncles (IV1, IV3, IV4). This mutation resulted in a lysine to glutamine substitution at position 104 (p. Lys104Gln) (Fig. 2). The proband's mother (IV13), aunt (IV11), and his grandmother (III2) were confirmed as carriers. The unaffected individuals (III14, IV11, IV14, V4, and V8) were not detected for this mutation. This missense mutation was not observed in normal Chinese humans of the 1000 Genomes Project.

This missense mutation position is located in the C-terminal cystine knot-like domain of Norrin. As can be seen in Fig. 3, the 104 codon lysine is a highly conserved amino acid across different species.

4. Discussion

In this work, we report a large family with a history of typical X-linked FEVR. A novel mutation (c.310A>C, P. Lys104Gln) in the *NDP* was found in four hemizygous-

Table 2
The ophthalmology manifestations of affected individuals.

Patients	III13		IV1		IV3		IV4		V9		VI1	
Age (y)	70		50		46		42		17		2	
Oculus sinister/dexter	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD
Vision	NLP	NLP	LP	NLP	LP	NLP	NLP	NLP	0.05	LP	0.1	LP
IOP (mmHg)	15	12	16	18	15	13	11	9	13	9	11	12
Cornea	T	T	T	T	T	T	T	T	T	T	T	T
Ophthalmoscope test												
Retinal vascular abnormalities	+	+	+	+	+	+	+	+	+	+	+	+
Retina fold	+	+	−	+	−	+	+	+	−	+	−	+
Retinal detachment	+	+	−	+	−	+	+	+	−	−	−	−
Retinal tear	−	−	−	+	−	−	−	+	+	−	−	−
Retinal deposits	+	−	−	+	+	+	−	+	−	−	−	−
Macular ectopic	−	−	−	−	−	+	−	+	−	+	−	−
Lens opacity	+	+	+	+	+	+	+	+	+	+	+	+
Vitreous opacity	+	+	+	+	+	+	+	+	+	+	+	+
FFA test												
Abnormal new blood vessels	−	+	−	−	−	+	−	+	+	+	−	−
Fluorescence leakage	−	+	−	−	+	−	−	+	+	+	−	−
Nonperfusion zones of temporal	+	+	+	+	+	+	+	+	+	+	+	+

FFA = fundus fluorescein angiography; IOP = intraocular pressure; LP = light perception; NLP = no light perception; OS = oculus sinister; OD = oculus dexter; T = transparent; − = absence of trait; + = presence of trait.

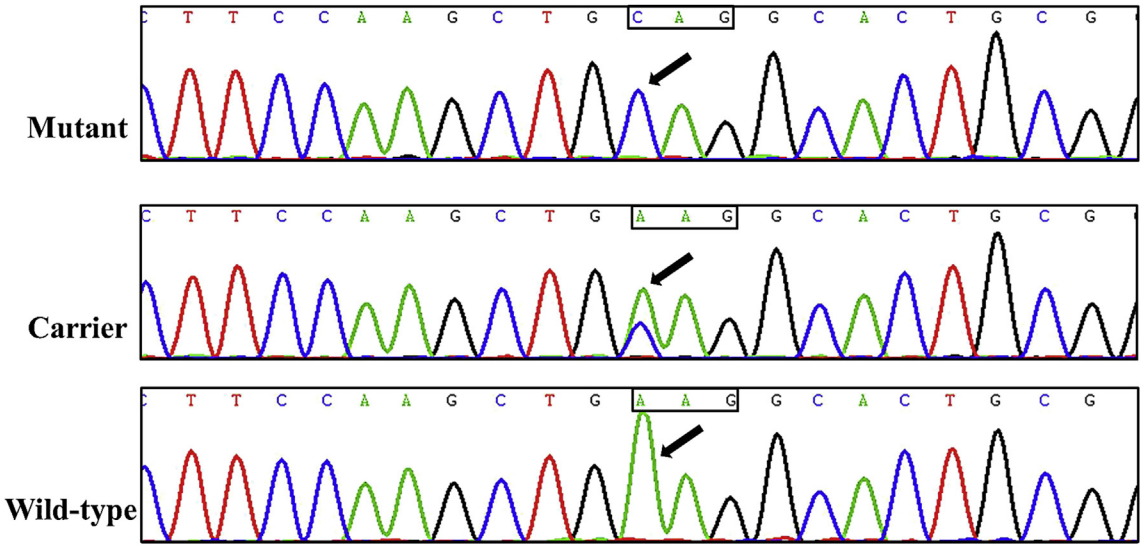

Fig. 2. Partial nucleotide sequences of *NDP* exon 3. An affected male shows a missense mutation c. 310A>C (upper panel). The arrow indicates nucleotide change that causes substitution of lysine residue (AAG) with glutamine (CAG) at position 104 of *NDP* (p.Lys104Gln). Middle panel shows the mutation in heterozygous state from a carrier. Lower panel is a normal *NDP* sequence. NDP = Norrie disease gene.

affected male individuals and three heterozygous-unaffected female individuals. This missense mutation is located in the C-terminal cystine knot-like domain of Norrin. Mutations affecting this domain appear to cause more severe phenotypes.[11] Comparative analysis shows that the 104 codon lysine is a highly conserved amino acid across different species. It suggests that any mutation at this codon may lead to a deleterious effect.

Previously, the same missense mutation was reported in one patient with less severe Norrie disease.[12] This result suggests that X-linked FEVR and Norrie disease can share the codon mutation in the same gene. There are some similarities in clinical symptoms between X-linked FEVR and Norrie disease, such as retinal traction, retinal folds, and retinal detachment. In typical Norrie disease, the characteristic manifestation is bilateral congenital blindness within the 1st year of life. However, in this family, the vision for the seven affected individuals was normal at birth, and blindness in both eyes did not occur at the same time. Two additional distinctions are that 40% of Norrie patients also develop progressive sensorineural deafness and 50% of patients have mental retardation in early childhood.[13] In contrast, the affected individuals of this family were found to have neither mental retardation nor hearing abnormality. Finally, the severe deterioration of the eye in X-linked FEVR is blindness. However, deterioration of the eye in Norrie disease is continuous, and atrophy of the eye globe is a severe characteristic. Therefore, this family can be confirmed as FEVR rather than Norrie disease. Our results suggest that X-linked FEVR and Norrie disease may be allele-dependent.

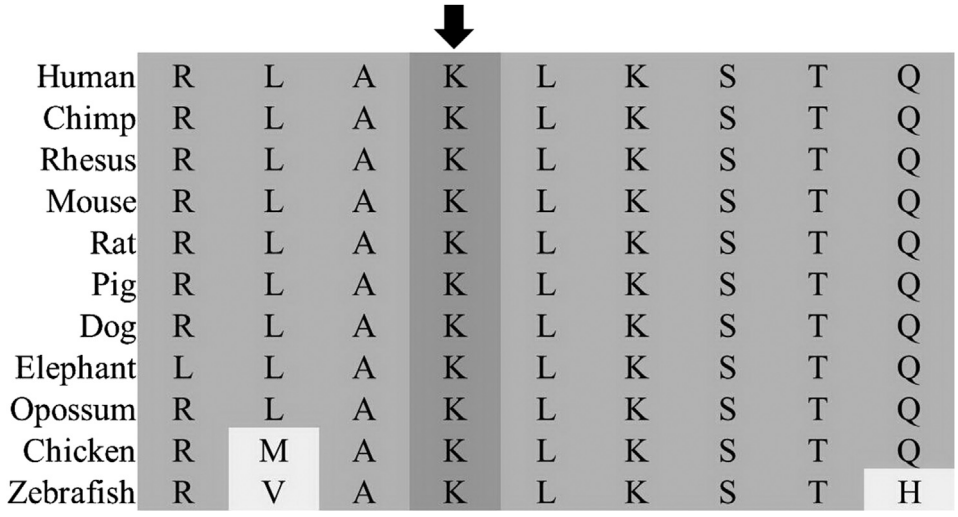

Fig. 3. Protein sequence alignment of human *NDP* with its orthologues. Conserved amino acid residues are shaded. The position of the missense mutations p.K104Q is indicated. NDP = Norrie disease gene.

Table 3
NDP sequence missense mutations that are likely to be familial exudative vitreoretinopathy (FEVR).

No.	Nucleotide variant	Effect	Exon	Reference
1	c.53T>A	p.I18K	2	14
2	c.112C>T	p.R38C[a]	2	13,15
3	c.122G>A	p.R41K[a]	2	16–17
4	c.125A>G	p.H42R	2	16,18
5	c.162G>C	p.K54N	2	14,19
6	c.174G>T	p.K58N[a]	2	16,20
7	c.181C>A	p.L61I	3	21
8	c.220C>T	p.R74C[a]	3	22–23
9	c.287G>A	p.C96Y[a]	3	23–24
10	c.307C>G	p.L103V	3	25
11	c.328T>G	p.C110G	3	26
12	c. 338G>A	p.G113D	3	27
13	c.344G>T	p.R115L	3	14
14	c.359A>G	p.Y120C	3	16
15	c.361C>T	p.R121W[a]	3	28–29,12,21
16	c.362G>A	p.R121Q[a]	3	13,19,20
17	c.362G>T	p.R121L	3	30
18	c.370C>T	p.L124F	3	3

[a] The mutations have been suggested to be associated with X-linked FEVR and Norrie disease.

More than 100 nucleotide variants have been reported for *NDP*. Most of the mutations cause Norrie disease; only a small percentage of mutations were reported in X-linked FEVR (Table 3). To date, seven *NDP* mutations (p.R38C, p.R41K, p.K58N, p.R74C, p.C96Y, p.R121W, and p.R121Q) have been suggested to be associated with X-linked FEVR and Norrie disease. Interestingly, Allen et al[22] reported a Syrian family with the R74C *NDP* mutation; one affected individual IV-33 was diagnosed with X-linked FEVR, whereas another affected individual IV-31 was diagnosed with Norrie disease. The same mutation clearly can lead to two different phenotypes or diseases. This heterozygous phenotype has also been observed in many other datasets. Obviously, epigenetic and other unidentified factors are also involved in determining the phenotypic expression of X-linked FEVR and Norrie disease. Of course, *NDP* variations play a key role in X-linked FEVR and Norrie disease, and examining other genetic variations may help explain the difference between X-linked FEVR and Norrie disease.

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