

RESEARCH REPORT

# Genetic Evaluation to Establish the Diagnosis of X-Linked Familial Exudative Vitreoretinopathy

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**Purpose:** To determine the usefulness of genetic analysis for confirming the diagnosis of X-linked familial exudative vitreoretinopathy (FEVR) and verifying the mode of inheritance. **Methods:** Twenty-seven consecutive patients diagnosed with FEVR were enrolled for genetic analysis. All patients underwent dilated fundus examination. A complete birth, medical, and family history was obtained at the time of examination. Patients were categorized by gender and family history in an effort to identify X-linked FEVR. Participants provided a blood sample for analysis and were evaluated for a mutation in the Norrie's disease gene (*NDP*) by direct sequencing. **Results:** Of the 27 enrolled patients, four male patients had a pedigree consistent with X-linked inheritance and 12 male patients had little or no family history. Two of these 16 patients were found to have a missense mutation in the *NDP* gene. **Conclusions:** We found genetic testing of *NDP* to be helpful in confirming the diagnosis of X-linked FEVR in male patients, especially when limited family history was available. As genetic diagnostics improve, we feel that confirming diagnoses and informing patients better through genetic evaluation and consultation will become more useful in the clinical practice of ophthalmology.

**Keywords** X-linked familial exudative vitreoretinopathy; X-linked FEVR; genetic analysis

## INTRODUCTION

Familial exudative vitreoretinopathy (FEVR) is an inherited disease that is characterized by aberrant retinal development such as peripheral avascular retina, abnormal vascularization with retinal neovascularization, subretinal exudation, an abnormal vitreous composition and vitreoretinal interface, as well as retinal detachment. The differential diagnosis includes retinopathy of prematurity, persistent fetal vasculature syndrome, Coats disease, Norrie's disease, incontinentia pigmenti, and idiopathic retinal detachment. The diagnosis is made by clinical examination, patient history, birth history, and family history and is often difficult to confirm by examination alone. Mild cases are

frequently asymptomatic and, when peripheral avascular retina is the only clinical finding, routine examination may fail to make the diagnosis. Likewise, severe cases with unorganized total retinal detachment may be difficult to distinguish from Norrie's disease, retinopathy of prematurity (if birth history is not available), and severe persistent fetal vasculature syndrome.

The vitreoretinal abnormalities in FEVR are due to aberrant vasculogenesis and subsequent abnormal angiogenesis.<sup>1,2</sup> To date, four distinct loci (*EVR1*, *EVR2*, *EVR3*, *EVR4*) associated with developing FEVR have been identified.<sup>3–6</sup> The causative genes identified at three of these loci play important roles in the pathway necessary for normal retinal development.<sup>1,6,7</sup> This pathway has been identified as the Wnt-receptor: $\beta$ -catenin pathway<sup>1,7,8</sup> and the three gene products act as either a ligand, a receptor, or a co-receptor and are required for activating this pathway.

FEVR demonstrates genetic heterogeneity and can be inherited in an autosomal dominant, autosomal recessive, or X-linked

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fashion. Autosomal dominant FEVR has been associated with mutations in the *Fzd4* gene and the *LRP5* gene.<sup>4,9,10</sup> Mutations in *LRP5* are also responsible for some cases of autosomal recessive FEVR.<sup>11,12</sup> X-linked disease is associated with a mutation in the Norrin gene (*NDP*) at a site distinct from mutations resulting in Norrie's disease.<sup>3,13-15</sup>

The affected patient usually has bilateral but asymmetric involvement. There can be a broad spectrum of clinical presentations within a single family, ranging from asymptomatic to no light perception. Because many affected family members have no symptoms or have decreased vision attributed to other causes, the family history is frequently not helpful. The majority of affected patients present as the sole family member with an eye disease, resulting in the inability to appropriately assign a mode of inheritance. The inability to determine the mode of inheritance impacts patient counseling and may impact management as well. In-vitro fertilization combined with prenatal genetic diagnosis is currently available for some inherited disorders, such as cystic fibrosis, Fanconi's anemia, and retinoblastoma. Pharmacologic agents, such as pegaptanib, are also being investigated as agents useful in managing patients with FEVR and retinopathy of prematurity (ROP). With a greater understanding of the Wnt-receptor: $\beta$ -catenin pathway as well as better understanding the roles of specific gene products, therapies directed at correcting the underlying defect may be developed. Until recently, the only method for more accurately determining heredity involves ophthalmic examination of multiple family members, and this remains the most definitive method to date. With the advent of genetic analysis, screening for specific mutations is becoming more feasible. Genetic evaluation of candidate genes will benefit both the patient and the clinician in diagnosis, management, and family counseling.

We screened 27 patients diagnosed with FEVR for a mutation in the *NDP* gene, with specific interest in male patients with family histories that supported X-linked inheritance or patients lacking sufficient family history to make a determination of inheritance. Out of 27 patients diagnosed with FEVR, 16 were determined as most likely to have a mutation in the Norrin gene product based on gender and family history. Four male patients had family histories demonstrating maternal inheritance and eye disease affecting only males, suggesting X-linkage. Twelve patients were the only known family members to have eye disease or had insufficient family history to make a determination of inheritance. Although other family members were offered examinations, very few opted to have exams, especially family members who were not living in the area. Only one family had an X-linked FEVR established by examinations of multiple family members. Two patients were confirmed as having X-linked FEVR by genetic analysis. This finding definitively establishes the diagnosis of FEVR and aids the families in counseling regarding their other children and future offspring.

TABLE 1  
Staging criteria

Stage	Clinical features
1	Avascular retina without extraretinal vessels
2	Avascular retina with extraretinal vessels A. no exudate B. with exudates
3	Partial RD—fovea spared A. no exudate B. with exudates
4	Partial RD—fovea involved A. no exudate B. with exudates
5	Total RD A. no exudate B. with exudates

RD, retinal detachment.

## MATERIALS AND METHODS

### Patient Enrollment

Patients referred to our practice were recruited to the study through an internal review board (IRB)-approved protocol and consented for participation. Inclusion criteria consisted of a clinical diagnosis of FEVR using the staging criteria listed in Table 1. Patients were then subcategorized by gender and likely mode of inheritance (Table 2). Prior to enrollment, the patients underwent dilated fundus examination at our clinic or had fundus photos forwarded when examination in our clinic was not possible. Detailed birth, medical, and family histories were obtained at the first visit. A total of 27 patients diagnosed with FEVR were enrolled.

### Genetic Analysis

All participants provided a blood sample from which genomic DNA was isolated using the manufacturer's recommended product protocol (Puregene, Gentra Systems Inc., Minneapolis, MN, USA). Polymerase chain reaction (Herculase; Stratagene, La

TABLE 2  
Patient characteristics

Family history	Number of patients		
	Total number	Male patients	Norrin mutation
Autosomal dominant	5	4	0
X-linked	4	4	1 (R121W)
Unknown	18	12	1 (H42R)
Total	27	*16	2

\*Total number of patients with possible mutation in the *NDP* gene.

Jolla, CA, USA) was performed to amplify the *NDP* gene. Primers specific for 5'UTR, exon 2, and exon 3 were used for site-specific amplification, as previously published.<sup>16</sup> Direct sequencing was performed using the Beckman CEQ8000 autosequencer (Beckman-Coulter, Inc., Brea, CA, USA). Female patients diagnosed with FEVR underwent genetic analysis of the *NDP* gene as controls.

## RESULTS

Twenty-seven patients diagnosed with FEVR were enrolled and blood samples obtained for genetic analysis for X-linked FEVR. Of the 27 patients diagnosed with FEVR, 20 were male. Female patients diagnosed with FEVR also had genetic analysis of the *NDP* gene performed to serve as controls. Four male patients had family histories of eye disease affecting only males with maternal inheritance, suggesting X-linkage. Twelve patients were the only family members known to have eye disease or had insufficient family history to make a determination of inheritance. Although other family members were offered examinations, very few opted to have them, especially family members who were not living in the area. Only one family had X-linked

FEVR established by examinations of multiple family members. Sixteen of the 20 male patients were determined to possibly have X-linked FEVR by family history and, therefore, possibly demonstrate a mutation in the Norrin gene product (Table 2). The *NDP* gene was evaluated for mutations by direct sequencing.

Genetic analysis revealed one patient to have a missense mutation in the third exon of the Norrin gene. A cytosine to thymine transition at nucleotide 777 caused an amino acid change at position 121 from an arginine to a tryptophan (Fig. 1). This same mutation was previously reported in a case diagnosed as X-linked FEVR. Furthermore, mutations spanning amino acids 120-126 appear to be a hotspot for mutations resulting in X-linked FEVR. A second patient was found to have an adenine to guanine mutation in the second exon, causing an amino acid change from histidine to arginine at position 42 (Fig. 2). Both mutations have been reported previously in association with X-linked FEVR.<sup>13,14</sup>

The remaining 25 FEVR patients (of the initial 27 patients) were found to have wild-type *NDP* sequences, including the remaining three male patients diagnosed with X-linked FEVR by pedigree.

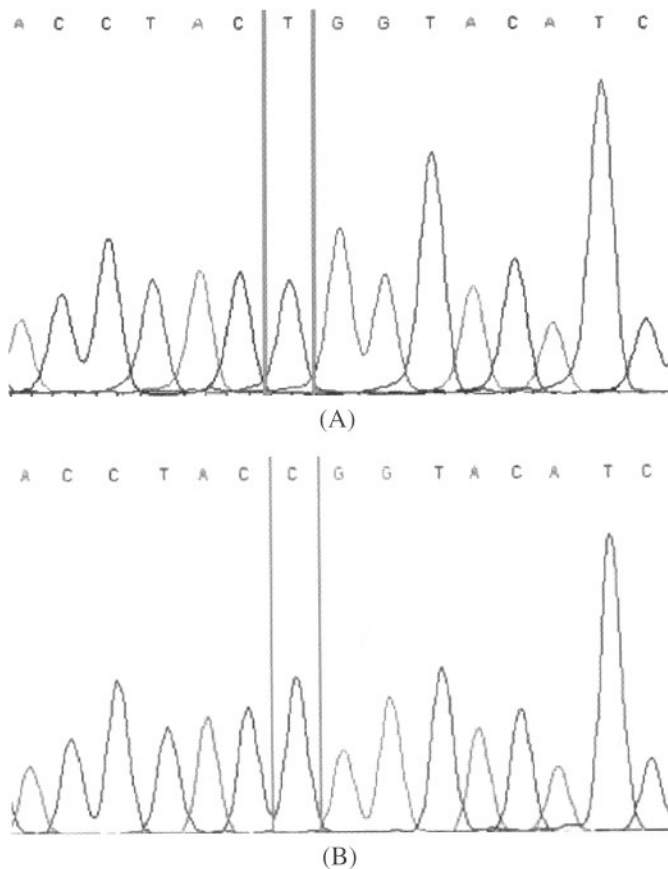


FIG. 1. A) *NDP* sequence result demonstrating a cytosine to thymine transition at nucleotide 769, resulting in R121:W. B) Sequence results from a patient expressing wild-type norrin.

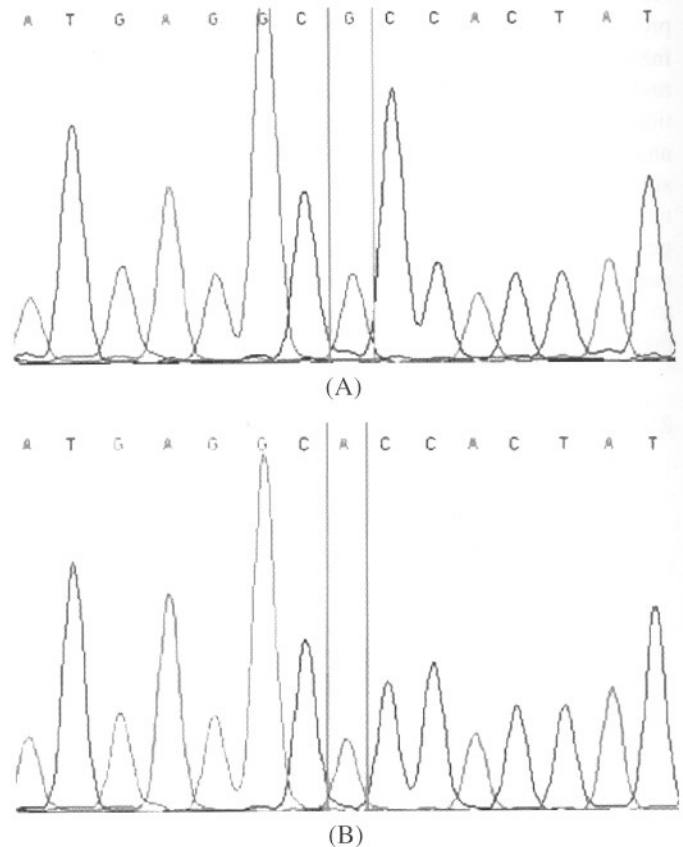


FIG. 2. A) *NDP* sequence results (B) demonstrating an adenine to guanine transition at nucleotide 533, resulting in H42:R. B) Sequence results from a patient expressing wild-type norrin.

## CONCLUSIONS

We found two male patients diagnosed with FEVR to have mutations in the *NDP* gene. One patient had no family history of eye disease, and genetic evaluation both confirmed the diagnosis and established the presence of a heritable mutated gene. The second patient had a family history of eye disease consistent with X-linked inheritance. Interestingly, three patients diagnosed with X-linked FEVR by family history did not demonstrate a mutation in the *NDP* gene. It is possible that mutations in intronic sequences affecting proper splicing or in regulatory sequences altering *NDP* expression may have occurred. Since the incidence of *NDP* mutations occurring in patients diagnosed with X-linked FEVR has not been reported, this finding cannot be answered by our study alone.

Screening of the *NDP* gene will not definitively discriminate between Norrie's disease and FEVR. The possibility of Norrie's disease must be addressed and the final diagnosis determined by other factors, such as age of onset, severity of retinal dysplasia, hearing deficits, CNS deficits, and other systemic abnormalities.

FEVR is a genetically heterogeneous disease that is caused by an aberration in vasculogenesis. The common finding to date is aberrant regulation of vasculogenesis. Genetic mutations identified to date affect genes which interact to activate the Wnt-receptor:  $\beta$ -catenin pathway, a pathway necessary for normal retinal development. Genetic evaluation is becoming more useful in the clinical setting as more associations between genetic mutations and disease are made and screening techniques continue to improve. Many retinal diseases have similar phenotypes and may pose diagnostic challenges. Heritable diseases have a significant impact on patients and their families and require formal genetic counseling. Positive identification of a causative gene mutation facilitates appropriate genetic counseling for the patients and their family. Confirmation of a diagnosis helps the treating physician make better management decisions and better predict the expected course of disease.

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