Clinical and Molecular Evaluation of Probands and Family Members with Familial Exudative Vitreoretinopathy

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PURPOSE. To describe the ophthalmic characteristics and to identify the molecular cause of FEVR in a cohort of Dutch probands and their family members.

METHODS. Twenty families with familial exudative vitreoretinopathy (FEVR) comprising 83 affected and nonaffected individuals were studied. Based on the presence of an avascular zone, the clinical diagnosis was made and biometric data of the posterior pole of 57 patients and family members were obtained by the analysis of fundus photographs and compared with the data of 40 controls. The FZD4, LRP5, and NDP genes were screened for mutations in one affected individual per family. The segregation of the gene variants was studied in the corresponding families.

RESULTS. Forty of 83 individuals showed an avascular zone, the most evident clinical sign of FEVR, five showed major signs of FEVR, and 38 persons were not clinically affected. Compared with the control subjects the patients with FEVR had a significantly larger disc-to-macula distance and a significantly smaller optic disc. In 8 of 20 families, a FZD4 mutation was identified, in 2 a mutation in the LRP5 gene, and in 2 a mutation in the NDP gene. Three known and five novel mutations were identified. Nonpenetrance was observed in 26% of the mutation carriers.

CONCLUSIONS. Significant anatomic differences were identified between the eyes of patients with FEVR with an avascular zone, when compared with those of the control subjects. In patients with an avascular zone, the optic disc was smaller and the disc-to-macula distance larger than in the control subjects. In 60% of the probands, mutations were identified in one of the three known FEVR genes. (Invest Ophthalmol Vis Sci. 2009;50: 4379–4385) DOI:10.1167/iovs.08-3320

Familial exudative vitreoretinopathy (FEVR; MIM 133780) is a hereditary disorder first described by Criswick and Schepens in two families in 1969. The most prominent characteristic of the disease is the aberrant and incomplete vascularization of the peripheral retina. The incomplete vascularization can lead to various complications, such as retinal neovascularization and exudates, vitreous hemorrhage, vitreoretinal traction with deformation of the posterior retina, ectopia of the macula, retinal fold, and retinal detachment. FEVR exhibits strikingly variable phenotypes among patients from the same family and even between the two eyes of one individual. The clinical signs range from hardly detectable vascular anomalies in the peripheral retina in asymptomatic individuals to bilateral retinal detachments leading to blindness.

An early diagnosis of FEVR is important for adequate genetic counseling and prevention and treatment of complications that occur predominantly at a young age. For ophthalmologists there is no clear guideline for the diagnosis of FEVR. In OMIM 133780 (Online Mendelian Inheritance in Man; http://www.ncbi.nlm.nih.gov/Omim/ provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), the striking similarity to retinopathy of prematurity (ROP) is noted, as well as some major signs such as dragged disc, stretched vessels, and falciform folds. During the past decades, several researchers have proposed different classifications of FEVR, based on different criteria. Gow and Oliver proposed a three-stage classification, and they suggested that FEVR is slowly progressive. Others have shown that FEVR often runs a mild nonprogressive course and that considerable visual loss is confined to the more severely affected eyes. Canny and Oliver were the first to describe fluorescein angiography findings in FEVR, and their classification was modified by Laqua, who included fluorescein angiographic findings at each level. An important sign of FEVR is the avascular zone, which is the result of a premature arrest of retinal angiogenesis-vasculogenesis or retinal vascular differentiation, leading to incomplete vascularization of the peripheral retina. Miyakubo et al. emphasized the vascular changes in FEVR and described in their study a grading system with five types based on fluorescein microangiography. In the first three types, the avascular zone is the principal sign; in type one it is the key feature. A classification of FEVR into five stages was also proposed by Pendergast and Trese based on funduscopy. The clinical utility of accurate classification relates to the possibilities of treatment and focuses on the role of exudates.

In one of the more recent studies by Shukla et al., an avascular zone was found in 41.4% of 116 eyes (61 patients), followed by rhegmatogenous retinal detachment, retinal breaks, ectopia of the macula, and exudative retinal detach-
ment. Besides the avascular zone, several posterior pole features have been described such as deformation of the vascular network and temporal ectopia of the macula, similar to the posterior pole in ROP.\textsuperscript{4,7} These features can also be seen without full mydriasis, but when they are subtle, there is no objective grading for these features.

We decided to obtain objective criteria for dragging of the posterior pole and to compare the size of the optic disc and the distance between the optic disc and the fovea in FEVR, based on the presence of an avascular zone, with a control group with nonproliferative diabetic retinopathy. If FEVR can be diagnosed without fluorescein angiography, it would greatly facilitate the identification of patients just by examination of the fundus in full mydriasis. Especially in children, the diagnosis of FEVR should be obtained as early as possible, because serious complications can occur in the first two decades.\textsuperscript{9}

FEVR is a genetically heterogeneous disease that shows X-linked recessive, autosomal dominant, and autosomal recessive modes of inheritance. X-linked recessive FEVR has been associated with mutations in the Norrie disease (NDP) gene.\textsuperscript{11} Autosomal dominant FEVR was initially localized through linkage to markers on the long arm of chromosome 11\textsuperscript{12} and was later found to be associated with mutations in either the FZD4 or the LRP5 gene.\textsuperscript{8,15,14} As an yet unidentified autosomal dominant FEVR gene has been localized to 11p13-p12 in one large family.\textsuperscript{15,16} LRP5 mutations have also been identified in patients with autosomal recessive FEVR.\textsuperscript{17} Norrin, the protein product of NDP, acts as a ligand for and activator of FZD4.\textsuperscript{18} LRP5 is a member of the low-density lipoprotein receptor family, and there is evidence that LRP5 acts as a coreceptor for FZD4.\textsuperscript{19,20} Activation of wild-type FZD4 leads to activation of the noncanonical Wnt/\textit{Ca}\textsuperscript{2+} signaling pathway. FZD4 protein with a loss-of-function mutation may exert a dominant–negative effect through heterodimerization with wild-type proteins and accumulate in the ER.\textsuperscript{22} Homozygous or compound heterozygous loss-of-function mutations in LRP5 are also associated with a recessive osteoporosis-pseudoglioma syndrome, which is characterized by osteoporosis and severe retinal detachments,\textsuperscript{23} whereas heterozygous mutations lead only to osteoporosis. In contrast, gain-of-function mutations in LRP5 have been reported to be responsible for high bone mass disorders.\textsuperscript{24,25} Mutation analysis was undertaken in the probands of each family. When possible, segregation of the mutation in the family was performed.

**Materials and Methods**

**Study Sample**

The project was approved by an accredited Medical Review Ethics Committee (CMO Arnhem-Nijmegen), and the protocol adhered to the tenets of the Declaration of Helsinki. Probands were selected from the patient registers of three centers in The Netherlands (i.e., the Department of Ophthalmology at the Canisius Wilhelmina Hospital and the Radboud University Nijmegen Medical Centre in Nijmegen and the Barltineus Institute for visually impaired children in Zeist) after referrals by an ophthalmologist or general practitioner, and an FEVR diagnosis was made by family history and fundus examination before or after their referral and on the presence of an avascular zone and other major signs of FEVR.

Probands and their (symptomatic and asymptomatic) relatives were contacted regarding participation in the study by phone and, after giving their consent, they received information on FEVR and the research project. On ethical grounds, minors (<18 years of age) were excluded from the project except those in whom DNA studies had already been performed for diagnostic purposes. In total, 85 subjects from 20 families were included in the study. Forty patients with mild, nonproliferative diabetic retinopathy were included and served as control subjects with respect to fundus biometric analyses. These patients were selected at random from a pool of standard control photographs of the Department of Ophthalmology of the Canisius Wilhelmina Hospital.

**Ophthalmologic History and Examination**

Probands and family members had a standardized ophthalmic investigation, consisting of an ophthalmic and general history including gestational age, birth weight, and a family history, as well as an ophthalmic examination. Duration of pregnancy and neonatal birth weight was determined to indicate the possible presence of ROP. ROP can occur in full-term infants, however, rarely without other neonatal events.\textsuperscript{26} The ophthalmic examination included Snellen visual acuity, Amsler test, subjective and objective refraction, estimation of \( k \) angle, slit lamp examination of the anterior segment (cornea, iris, and lens), biomicroscopy of the vitreous and fundus in full mydriasis with special attention for vascularity of the peripheral retina, deformation of the retinal vascular network around the posterior pole, deformation of the optic disc (dragged disc), position of the macula, deformation of the macula, exudates, retinal defects and neovascularization, tortuosity of retinal vessels, hemorrhages, and retinal detachment. Digital fundus photography was performed (probands, family members, and parents with nonproliferative diabetic retinopathy) with a fundus camera (model FF450; Carl Zeiss Meditec, Oberkochen, Germany). For digital imaging and morphometry of the fundus, a computer workstation was used (Winstation 5000; Medivision-OIS, Sacramento, CA). All clinical data were obtained by an experienced ophthalmologist (CEvN). In cases with an avascular zone in the temporal retina, the posterior border of the area was generally located in the equatorial region. Probands and family members with an avascular zone of the temporal peripheral retina were defined to be patients with FEVR.

**Anatomic Characteristics**

Retinal photography and biometry was performed by an experienced optometrist, who was not informed about the clinical data of the probands, their relatives, and the nonproliferative diabetic retinopathy patients. We used the workstation software (Winstation XP, ver. 10.2.61; Medivision-OIS) to calculate the topographic data. Horizontal disc diameter (\( a1 \)), vertical disc diameter (\( a2 \)), and the distance between the temporal margin of the optic disc and the center of the fovea (\( b \)) were measured on the fundus photographs (Fig. 1). Using these measurements we calculated the so-called DM/DD ratio, which has been shown to be a valuable tool in diagnosing mild optic nerve hypoplasia.\textsuperscript{27} DM is the disc-to-macula distance calculated by \( a1/2 + b \) (see Fig. 1); DD is the disc diameter calculated as \((a1 + a2)/2 \). The DM/DD ratio thereby is \((a1/2 + b)/(a1 + a2)/2 \). The measurements were performed for the left and right eyes and means for both eyes were calculated when available.

**Statistical Analysis**

Statistical analyses were performed (Stata ver. 9.0; Stata Corp., College Station, TX). Reported probabilities are two-sided and were considered statistically significant if \( p < 0.05 \). Means and standard deviations for the anatomic measurements were calculated, and measurement values that were more extreme than the total mean value \( \pm 3 \) SD were defined as artifactual outliers. These values were retained but reduced to the next less extreme values to approximate the normal distribution. This method was used once for \( a2 \) in the right eye, twice for \( b \) in the right eye, and once for \( a2 \) in both eyes. Differences in anatomic measurements between groups were tested by using generalized estimated equations (GEEs) to take the correlations within families into account.

**Genotyping**

Blood samples were obtained from 80 patients and family members (\( > 95\% \) of the enrolled subjects). For mutation analysis of the probands, DNA was isolated from blood leukocytes by an automated procedure.
RESULTS

General Characteristics

In the group of 83 probands and family members, the mean age was 37.5 years (SD 17.7). To exclude ROP as a potential confounder in this study, we studied the gestational age for this cohort. For 10 of 83 subjects we had no information on gestational age. Gestational age was 36 weeks or more with no signs of FEVR. This indicates that in 70 of our probands and family members, ROP is not likely to be an explanation for the clinical features.

Principal sign
Avascularity of the peripheral retina (posterior border located in the equatorial region) 40/83

Major signs
Stretched posterior retinal vessels 24/83
Ectopia of macula 22/83
Retinal detachment 11/83
Dragged optic disc 14/83
Demarcation line periphery retina 14/83
Enlargement w angle 13/83
Falciform fold 9/83

Minor signs
Vitreous condensations 42/83
Deformation of macula 15/83
Tortuosity of small vessels 15/83
Exudates 10/83
Retinal bleeding (small) 2/83
Retinal defects (small) 12/83
Neovascularization 4/83
Cataract 20/83

* Patients were counted positive for a given sign if it was present in at least one eye.
and is likely to be pathogenic because it involves a substitution of a conserved amino acid. The p.Met223Lys mutation segregated with the disease, and it concerns also a conserved amino acid. The p.Met223Lys mutation segregated with the disease, and it concerns also a conserved amino acid. The p.Met223Lys mutation segregated with the disease, and it concerns also a conserved amino acid. The p.Met223Lys mutation segregated with the disease, and it concerns also a conserved amino acid.

The analysis of LRP5 revealed a novel mutation in two families, c.2143C>T (p.Arg805Trp) and c.1532A>C (p.Aspl113Ala), both segregating with the disease. The evolutionary conservation of all four amino acids implicated in FEVR is demonstrated in Figure 4. In two isolated (male) patients, previously reported mutations in the NDP gene were identified, c.362G>A (p.Arg121Glu) and c.162G>C (p.Lys54Asn).

In some of the patients, the FZD4 gene harbored two variants that have been described: p.Pro33Ser and p.Pro168Ser. These two variants were found on the same allele that did not cosegregate with FEVR (data not shown).

As can be seen in Figure 3, not all family members carrying pathologic sequence variants had a clinical phenotype. A mutation was identified in 27 of the 83 clinically examined individuals, 20 of whom belonged to the group identified as patients with FEVR based on the presence of an avascular zone. Thus, the overall nonpenetrance for mutations in the coding exons of FZD4, LRP5, and NDP in our population was 26% (7/27).

**DISCUSSION**

The most important clinical characteristic in FEVR, the avascular zone, can mostly be detected by slit lamp biomicroscopy. In this study, we described and quantified posterior retinal findings in FEVR to facilitate the clinical diagnosis and the decision to initiate DNA analyses in family members of patients or in new cases of suspected FEVR. The size of the optic disc as well as the disc-to-macula distance shows a strong correlation with the presence of an avascular zone. Although it may be difficult to see the difference between FEVR and normal eyes just by comparing the posterior poles, measurements of the posterior pole (relatively large DM/DD and b) may be useful to come to a decision for mutation analysis. In most cases, the avascular zone can be seen without fluorescein angiography, and in children the posterior pole can be documented by photography. If it is not possible to observe the periphery of the retina, the posterior pole may give clues for the diagnosis of FEVR. The finding of subtle morphometric changes in the posterior pole may therefore be an additional sign of FEVR.

The values measured in the control group correspond well to values from the literature. The DM/DD ratio normally is 2.67 and in optic nerve hypoplasia, 3.25. In our group of patients, the DM/DD ratio was 2.89, which is between the control and hypoplasia ratios. The finding of subtle differences in the size of the optic nerve head between FEVR individuals and control subjects has not been reported in other studies. A possible explanation would be a mild hypoplasia associated with FEVR. However, the appearance of the optic disc in FEVR

![Figure 2](image-url)

**FIGURE 2.** (A) Left eye of a control subject with mild background diabetic retinopathy: a normal disc-to-macula distance. (B) Left eye of a patient with FEVR: an increased disc-to-macula distance and a stretched configuration of the vascular arcades.
seems to be quite normal and we did not observe the classic characteristics of a dysplastic optic nerve. More research should be done to study these findings.

The products of the three genes, FZD4, LRP5, and NDP, are all components of the amply studied Wnt signaling pathway. The mechanisms by which Wnt signaling is involved in vessel formation are not obvious. The Wnt factors are known to activate a β-catenin-dependent pathway, inducing transcription of target genes capable of stimulating vessel formation (i.e., cyclin D1, c-myc, MMP7, and vascular endothelial growth factor [VEGF]).

Retinal astrocytes and blood vessels influence each other during development. Spindle-shaped astrocyte precursors stimulate the formation of blood vessels on the retinal surface. As blood vessels develop, these precursors mature into adult-appearing astrocytes, which, in turn, induce formation of the blood-retinal barrier.

In ROP, the interaction between the developing vasculature and the maturation of astrocytes may be disturbed by oxygen-related factors such as VEGF and by the non–oxygen-related growth factor IGF-I (insulin-like growth factor). In FEVR, the interaction between developing vasculature and maturation of astrocytes may be disturbed by changes in one of the proteins that play a role in this process, such as VEGF, glial fibrillary acid protein, vimentin, PAX2, or platelet-derived growth factor-α.

Mutations in the known FEVR genes were found in 60% of families, which is the highest mutation detection rate thus far reported. The strict clinical criteria that we used may have
resulted in this high percentage. Clinically, patients from families did not seem to differ significantly in the severity and/or age at onset, whether or not a mutation was found. In our FEVR cohort, we found the FZD4 p.Trp519X mutation in 5 (25%) of 20 families, whereas this variant was found in 2 (2.5%) of 80 families did not seem to differ significantly in the severity and/or age at onset, whether or not a mutation was found. In our FEVR cohort, we found the FZD4 p.Trp519X mutation in 5 (25%) of 20 families, whereas this variant was found in 2 (2.5%) of 80 cases in two Japanese studies, and in 1 (2.5%) of 40 cases in 20 families, whereas this variant was found in 2 (2.5%) of 80 cases in two Japanese studies, and in 1 (2.5%) of 40 cases in 20 families. In our series, these variants were also found on the same allele, but they did not cosegregate with FEVR, which sheds doubts on their pathogenicity. Also, deletions in the promoter area of the NDP gene have been implicated in ROP. In our series, the promoter area of the NDP gene was not analyzed.

Little is known about the penetrance of FEVR, but it was estimated to be 90% by van Nouhuys, based on the presence of asymptomatic parents of patients with FEVR. Toomes et al. found 4 (22.2%) asymptomatic individuals among 18 carriers of carboxyl-terminal LRPS mutations and nonpenetration in 2 (33.3%) of 6 FZD4 mutation carriers. The 26% rate that has been established in this study is in accordance with the results of the two other studies. It reflects the extreme clinical variability in patients with a known pathogenic mutation and stresses the importance of identification of the causative mutation in families with FEVR. Once a mutation is identified, presymptomatic testing can be offered to family members, facilitating early intervention and possibly preventing loss of vision at later ages.

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References


