

Clinical and Molecular Evaluation of Proband 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 Schepens¹ in two families in 1969. The most prominent characteristic of the disease is the aberrant and incomplete vascularization of the peripheral retina.² 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 Oliver³ 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.⁴ Canny and Oliver² were the first to describe fluorescein angiography findings in FEVR, and their classification was modified by Laqua,⁵ who included fluorescein angiographic findings at each level.

An important sign of FEVR is the avascular zone,^{4,6,7} which is the result of a premature arrest of retinal angiogenesis-vasculogenesis or retinal vascular differentiation, leading to incomplete vascularization of the peripheral retina.^{4,6,8} Miyakubo et al.⁷ 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 Trese⁹ 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.,¹⁰ 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-

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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.^{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.⁴

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.¹¹ Autosomal dominant FEVR was initially localized through linkage to markers on the long arm of chromosome 11¹² and was later found to be associated with mutations in either the *FZD4* or the *LRP5* gene.^{8,13,14} An as yet unidentified autosomal dominant FEVR gene has been localized to 11p13-p12 in one large family.^{15,16} *LRP5* mutations have also been identified in patients with autosomal recessive FEVR.¹⁷ Norrin, the protein product of *NDP*, acts as a ligand for and activator of *FZD4*.¹⁸ *LRP5* is a member of the low-density lipoprotein receptor family, and there is evidence that *LRP5* acts as a coreceptor for *FZD4*.¹⁹⁻²¹ Activation of wild-type *FZD4* leads to activation of the noncanonical Wnt/Ca²⁺ 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.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,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.^{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 Departments of Ophthalmology at the Canisius Wilhelmina Hospital and the Radboud University Nijmegen Medical Centre in Nijmegen and the Bartiméus 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, 83 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.²⁶ The ophthalmic examination included Snellen visual acuity, Amsler test, subjective and objective refraction, estimation of κ 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 patients 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.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 <0.05 . Means and standard deviations for the anatomic measurements were calculated, and measurement values that were more extreme than the total mean value ± 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

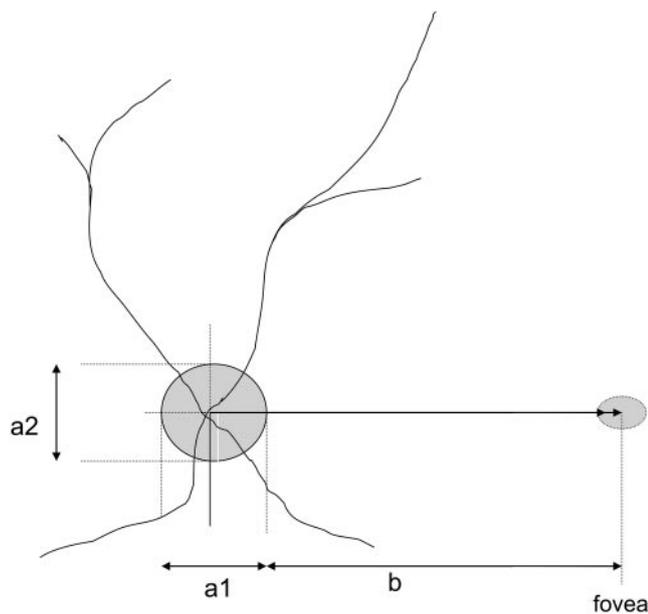


FIGURE 1. Measurements of anatomic characteristics: a1, horizontal disc diameter; a2, vertical disc diameter; b, distance between temporal edge of optic disc and fovea centralis; DM = $a1/2 + b$; DD = $(a1 + a2)/2$; DM/DD = $(a1/2 + b)/[(a1 + a2)/2]$.

with the magnetic bead platform from Chemagen AG (Baeswider, Germany). The *FZD4* (2 exons, 6 amplicons), *LRP5* (23 exons, 23 amplicons), and *NDP* (3 exons, 3 amplicons) genes were scanned for mutations by using standard sequencing methodologies on a genetic analyzer (model 3730; ABI) with primers flanking the coding exons and the adjacent splice sites. Exon 5 of *LRP5* could not be analyzed as several intronic PCR primers failed to amplify an *LRP5*-specific product. For *NDP*, the splice site of the noncoding exon 1 was also included in the analysis. Primers and PCR conditions are available on request. In families in which a genetic variant in one of the genes was identified, DNA of all available affected and nonaffected family members was analyzed for segregation of the specific variant by analysis of the relevant exons using the same conditions.

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 normal birth weight in 70 probands and family members. Three patients had gestations of less than 36 weeks. One patient had a gestational age of 33 weeks at birth and an *FZD4* mutation, and one patient (from a twin pregnancy) had a gestational age of 32 weeks. A third person had a gestational age of 35 weeks at birth and 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.

All 83 individuals were clinically examined, and the presence of an avascular zone and major and minor signs were registered (Table 1). Forty individuals showed an avascular zone in either one or both eyes. A further five individuals had severe bilateral signs of FEVR, such as falciform folds or ablatio retinae, that made it difficult to observe an avascular zone. In 29 individuals, fundus examination was negative in both eyes. In nine individuals, the presence of an avascular zone was not

evident enough during funduscopy or photography to be recorded. Therefore, in our group of 83 persons we were able to confirm the diagnosis FEVR in 45 (54%) individuals based on the presence of an avascular zone.

To analyze the more subtle posterior pole changes reported in FEVR, the patients were selected on the basis of the temporal peripheral avascular zone, and their anatomic posterior pole characteristics were compared to the same characteristics in a control group. The anatomic characteristics of the study sample are presented in Table 2. Fundus photography was performed in 57 (107 eyes) of the 83 individuals (mean age, 41.1 years, SD 15.3; 25 men/32 women) and in all 40 patients with nonproliferative diabetic retinopathy (mean age, 64.3 years, SD 11.9; 25 men/15 women).

A slight difference was found between the right and left eyes in the patient and diabetic retinopathy groups, and therefore the anatomic values were not compared with values from the literature. All values differed significantly between the individuals with an avascular zone in one or both eyes and the diabetic retinopathy group (Table 2). The diameters of optic discs are smaller in patients with FEVR and the disc-to-macula distances larger. The DM/DD ratio is high compared with the diabetic retinopathy group, which indicates an increased disc-to-macula distance in FEVR (Fig. 2). These differences were much less pronounced in the probands and family members selected on the basis of the presence of a pathogenic mutation in one of the FEVR genes. These variations arise because of the admixture of affected subjects and those without penetrance in the mutation-positive group (Table 2).

Mutation Analysis

In Figure 3 the pedigrees of the 12 of 20 families in whom a mutation was identified are given, together with the segregation of the mutations. Mutations in the *FZD4* gene were found in 8 (40%) of 20 families, with the previously reported²⁸ c.957G>A (p.Trp319X) mutation as the most frequent one (5/20). The other mutations identified in *FZD4* were a novel nonsense mutation c.1448G>A (p.Trp496X) and two novel missense mutations, c.1333A>C (p.Thr445Pro) and c.668T>A (p.Met223Lys), each of which was found in a single family. The p.Thr445Pro mutation in family W05-224 is a de novo mutation

TABLE 1. Clinical Signs in FEVR and Their Prevalence in Study Population

Signs	Patients/Total*
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 κ 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.

TABLE 2. Group-Specific Mean of Biometric Data

Group	OD				OS				OU			
	a1	a2	b	DM/DD	a1	a2	b	DM/DD	a1	a2	b	DM/DD
Patients with diabetic retinopathy	1.65 (40; 0.15)	1.74 (40; 0.16)	3.60 (40; 0.25)	2.63 (40; 0.26)	1.66 (40; 0.16)	1.75 (40; 0.16)	3.44 (40; 0.25)	2.53 (40; 0.26)	1.65 (40; 0.14)	1.74 (40; 0.15)	3.52 (40; 0.24)	2.58 (40; 0.25)
Probands and family members with mutation	1.57 (13; 0.16)	1.71 (13; 0.13)	3.94** (13; 0.59)	2.88** (13; 0.35)	1.56* (8; 0.14)	1.65 (8; 0.07)	3.50 (8; 0.39)	2.68 (8; 0.33)	1.59 (7; 0.15)	1.66 (7; 0.11)	3.47 (7; 0.27)	2.64 (7; 0.28)
Probands and family members with an avascular zone in one or both eyes	1.49** (27; 0.15)	1.65* (27; 0.13)	3.94*** (27; 0.53)	3.00*** (27; 0.38)	1.48*** (20; 0.12)	1.59*** (20; 0.12)	3.62 (20; 0.41)	2.85** (20; 0.32)	1.48*** (19; 0.12)	1.60*** (19; 0.12)	3.67 (19; 0.39)	2.87** (19; 0.32)

Data in parentheses are the number of individuals and SD; a1, horizontal diameter of disc; a2, vertical diameter of disc; b, disc to macula distance; DM/DD, disc-to-macula distance divided by disc diameter. *P*-values are based on GEE for comparison of group mean versus mean in patients with diabetic retinopathy: * $P < 0.01-0.05$; ** $P < 0.001-0.01$; *** $P \leq 0.001$.

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 analysis of *LRP5* revealed a novel mutation in two families, c.2413C>T (p.Arg805Trp) and c.1532A>C (p.Asp511Ala), 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)²⁹ and c.162G>C (p.Lys54Asn).³⁰

In some of the patients, the *FZD4* gene harbored two variants that have been described: p.Pro33Ser and p.Pro168Ser.^{31,32} 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.^{27,33} The DM/DD ratio normally is 2.67 and in optic nerve hypoplasia, 3.25.²⁷ 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

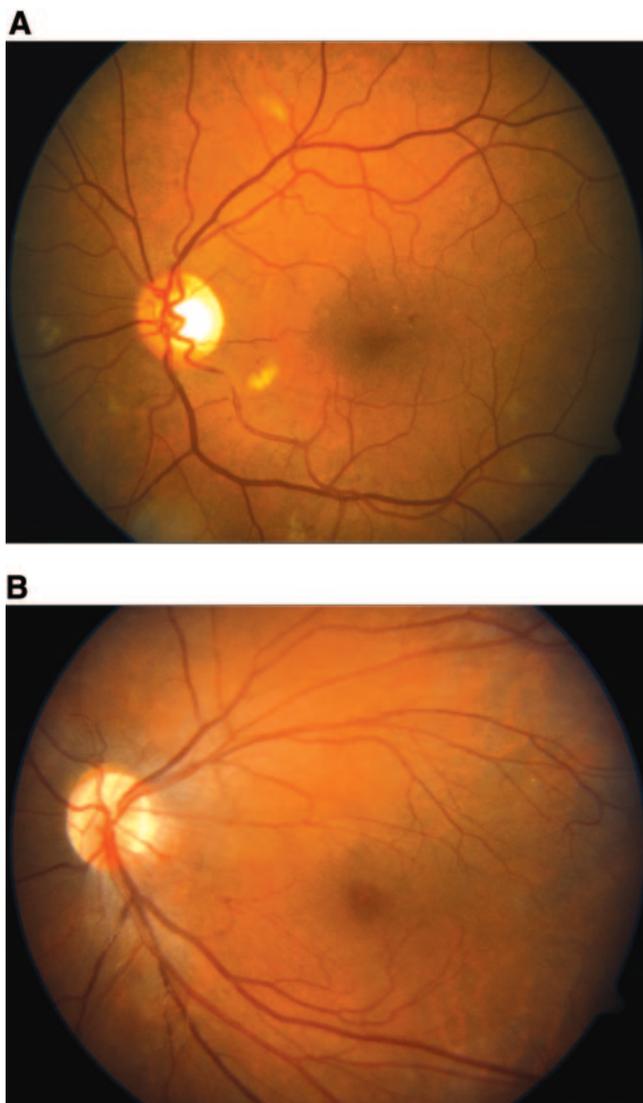


FIGURE 2. (A) Left eye of a control subject with mild background diabetic retinopathy: a normal disc-to-macula distance. (B) Left eye of a patient with FEVR: an increased disc-to-macula distance and a stretched configuration of the vascular arcades.

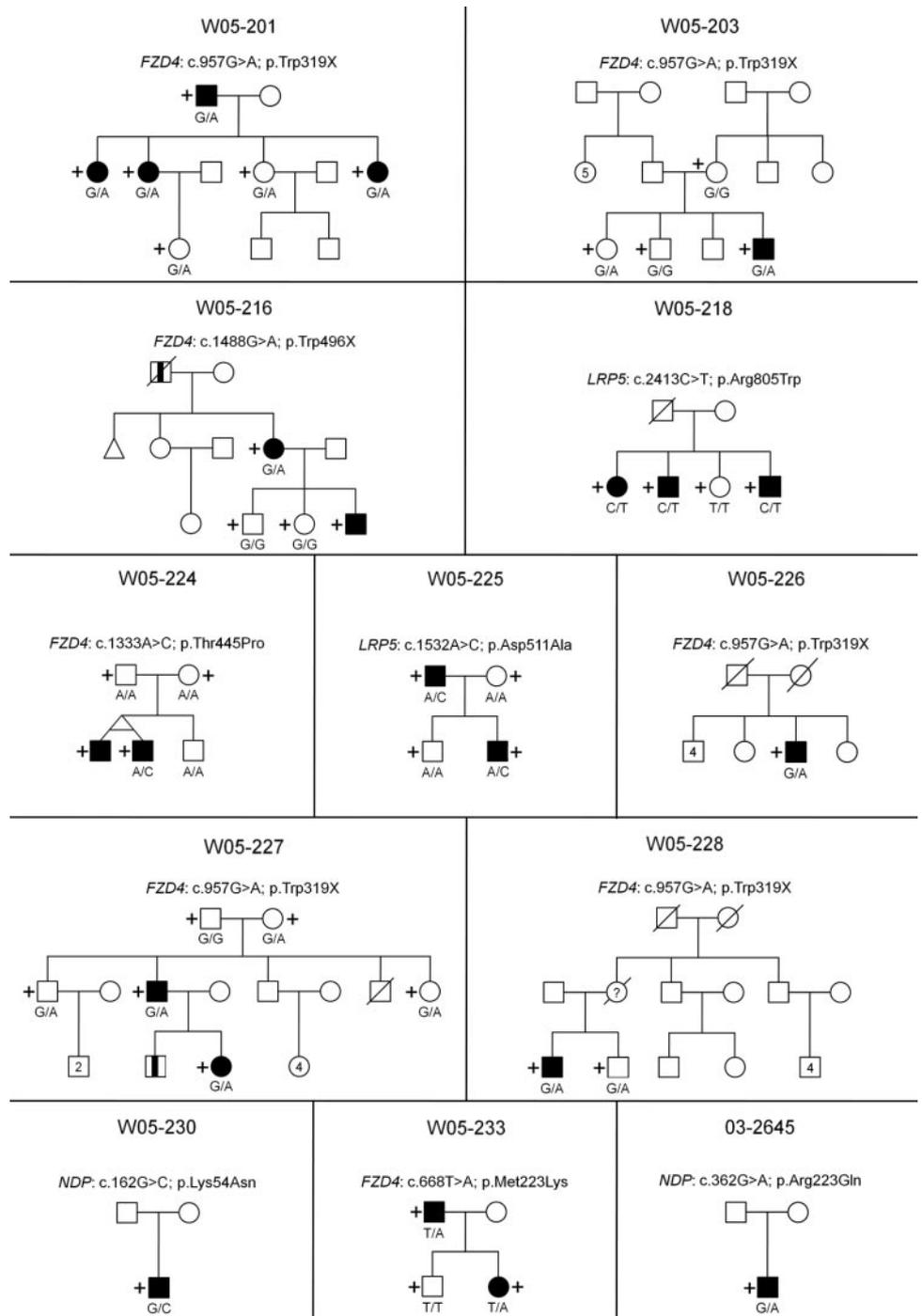


FIGURE 3. FEVR families with mutations in *FZD4*, *LRP5*, and *NDP*. *Open symbols*: clinically unaffected; *filled symbols*: clinically affected family members (presence of avascular zone); +, clinically evaluated; labels below the symbols are the genotype with respect to the familial mutation. *FZD4* mutations: nomenclature based on GenBank reference number NM012193.2; *LRP5* mutations: nomenclature based on GenBank reference number NM002335.1; *NDP* mutations: nomenclature based on GenBank reference number NM000266.1.

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]).³⁴ 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.³⁵

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).³⁶⁻³⁸ 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

LRP5		511	FZD4		445	
Mus musculus	TSLGWPNGLAID	DLQEGKLYWGDA	521	Homo sapiens	MVKIGVFSVLYTVPATCVIACYF	456
Rattus norvegicus	TSLGWPNGLAID	DLQEGKLYWGDA	522	Equus caballus	MVKIGVFSVLYTVPATCVIACYF	456
Homo sapiens	TSLGWPNGLAID	DLQEGKLYWGDA	522	Bos taurus	MVKIGVFSVLYTVPATCVIACYF	456
Papio anubis	ASLGWPNGLAID	DLQEGKLYWGDA	519	Rattus norvegicus	MVKIGVFSVLYTVPATCVIACYF	457
Bos taurus	ASLGWPNGLAID	DLQEGKLYWGDA	709	Mus musculus	MVKIGVFSVLYTVPATCVIACYF	456
Gallus gallus	TSLGWPNGLAID	DLQEGKLYWGDA	521	Gallus gallus	MVKIGVFSVLYTVPATCVIACYF	444
Danio rerio	TSLGWPNGLAID	DIYIAGKLYWGDA	499	Xenopus laevis	MVKIGVFSVLYTVPATCVIACYF	442
consensus	:*****:	* .*****		Consensus	*****	

LRP5		805	FZD4		223	
Mus musculus	TNCMTLVDKVGR	RANDLTIDYADQ	815	Homo sapiens	SRSAKEFTDIWMAVWASLCFIST	234
Rattus norvegicus	TNCMTLVDKVGR	RANDLTIDYADQ	816	Equus caballus	SRSAKEFTDIWMAVWASLCFIST	234
Homo sapiens	TNCMTLVDKVGR	RANDLTIDYADQ	816	Bos taurus	SRSAKEFTDIWMAVWASLCFIST	234
Papio anubis	TNCMTLVDKVGR	RANDLTIDYADQ	813	Rattus norvegicus	SRSAKEFTDIWMAVWASLCFIST	235
Bos taurus	TNCMTLVDKVGR	RANDLTIDYADQ	1003	Mus musculus	SRSAKEFTDIWMAVWASLCFIST	234
Gallus gallus	TNSITLVDKVGR	RANDLTIDYADQ	815	Gallus gallus	SRSAKEFTDIWMAVWASLCFIST	222
Danio rerio	SNIVTLVDKVGR	RANGLTIDYIDQ	793	Xenopus laevis	NRLSKEFTDIWMAVWASLCFIST	220
consensus	:*	:*****.*	**	Consensus	.*	:*****.*

FIGURE 4. Alignment of novel mutations in FZD4 and LRP5 using CLUSTALW2 (EBI). GenBank numbers. FZD4 protein alignment: *Homo sapiens* NP_036325.2; *Equus caballus* XP_001489854.1; *Bos taurus* XP_874584.2; *Rattus norvegicus* NP_072145.1; *Mus musculus* NP_032081.3; *Gallus gallus* NP_989430.1; *Xenopus laevis* NP_001083922.1 LRP5 protein alignment: *Mus musculus* NP_032539.1; *Rattus norvegicus* NP_001099791.2; *Homo sapiens* NP_002326.2; *Papio anubis* NP_001128671.1; *Bos taurus* XP_614220.3; *Gallus gallus* NP_001012915.1; and *Danio rerio* XP_696943.3.

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.Trp319X mutation in 5 (25%) of 20 families, whereas this variant was found in 2 (2.5%) of 80 cases in two Japanese studies,^{28,32} and in 1 (2.5%) of 40 cases in the United Kingdom.⁸ Other nonsense mutations were found only once in our series of 20 families. In one family, the missense mutation p.Trp445Pro in the *FZD4* gene was found as a de novo mutation, indicating that it is likely to be causative. Furthermore, Trp445 is conserved in chordates as distantly related as humans and frogs and resides in one of the transmembrane domains of the protein. The other mutation in the *FZD4* gene, p.Met223Lys, concerns a significant change of an evolutionary conserved amino acid, and it segregates with the disease in the family. Both mutations in the *LRP5* gene, p.Asp511Ala and p.Arg805Trp, segregated with the FEVR phenotype in the families, and both are likely to be pathogenic because evolutionary conserved amino acids are involved. Both mutations in the *NDP* gene were identified in isolated male patients. The *NDP* p.Lys54Asn mutation was previously identified by Kondo et al.⁵⁰ in a patient with typical FEVR; the p.Arg121Gln mutation has been identified in Norrie disease.¹¹

ROP is a condition that mimics FEVR, because a comparable set of clinical features is exhibited. Eighty-four percent of our study group were born after 36 weeks of gestation, with normal birth weight, and without adverse neonatal events, which excludes the possibility of ROP as a major cause of the disease. In ROP two variants of *FZD4* (p.Pro33Ser and p.Pro168Ser) have been suggested to be associated with the disease.⁵¹ 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,⁴ based on the presence of asymptomatic parents of patients with FEVR. Toomes et al.¹⁴ found 4 (22.2%) asymptomatic individuals among 18 carriers of carboxyl-terminal *LRP5* mutations and nonpenetrance in 2 (33.3%) of 6 *FZD4* mutation carriers.⁸ 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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