Molecular Vision 2012; 18:2438-2446 <http://www.molvis.org/molvis/v18/a256>
Received 22 December 2011 | Accepted 2 October 2012 | Published 4 October 2012

Identification of *FZD4* and *LRP5* mutations in 11 of 49 families with familial exudative vitreoretinopathy

Huiqin Yang, Shiqiang Li, Xueshan Xiao, Panfeng Wang, Xiangming Guo, Qingjiong Zhang

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, P. R. China

Purpose: To identify mutations in *FZD4* and *LRP5* in 49 Chinese families with familial exudative vitreoretinopathy (FEVR) and to reveal the mutation spectrum and frequency of these genes in the Chinese population.
Methods: Clinical data and genomic DNA were collected for patients from 49 families with FEVR. The coding exons and adjacent intronic regions of *FZD4* and *LRP5* were amplified with polymerase chain reaction, and the resulting amplicons were analyzed with Sanger sequencing.
Results: Eleven mutations were detected in 11 of the 49 families (22.4%), including five mutations in the *FZD4* gene in six families and six mutations in the *LRP5* gene in five families. Of the 11 mutations, eight were novel. Two families had the same *FZD4* mutation, and one family had compound heterozygous mutations in *LRP5*. The phenotypes of the patients with the mutations showed great variability.
Conclusions: Our findings provide an overview of the mutation spectrum and frequency of *FZD4* and *LRP5* in Chinese patients with FEVR and emphasize the complexity of FEVR mutations and phenotypes.

Familial exudative vitreoretinopathy (FEVR, MIM 133780) is a hereditary disorder resulting from a developmental anomaly of the retinal vessels that may be stationary or progressive [1]. Patients with FEVR exhibit highly variable manifestations, ranging from asymptomatic to complete blindness. Progressive vascular anomalies impair vision due to various complications such as retinal neovascularization, exudates, fibrovascular proliferation, retinal folds, optic disc dragging, and retinal detachment [2]. Some minimally affected individuals may be detected only with fluorescein angiography of the peripheral retina, which exhibits avascularization and a nonperfusion zone [3].

Mutations in at least four genes have been identified as responsible for autosomal dominant (the *FZD4*, *LRP5,* and *TSPAN12* genes) [4–7], autosomal recessive (the *LRP5* gene) [8], or X-linked (the *NDP* gene) [9,10] FEVR. The encoded proteins of these four genes are involved in the wingless (Wnt) signaling pathway, which monitors retinal vascular development [7,11–14]. To date, several mutations have been identified in the four genes in patients with FEVR [15]. However, such studies in Chinese patients are limited [16,17]. To better understand the molecular defects underlying FEVR in the Chinese population, we performed a mutation screening of *FZD4* and *LRP5* in 49 Chinese families with FEVR and identified mutations in 11 families.

Correspondence to: Dr. Qingjiong Zhang, Ophthalmic Genetics & Molecular Biology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54 Xianlie Road, Guangzhou 510060, China. Phone: (+86)-20-87330422; FAX: (+86)-20-87333271; email: zhangqji@mail.sysu.edu.cn or qingjiongzhang@yahoo.com

METHODS

Patients: Written informed consent in accordance with the guidelines of the Declaration of Helsinki was obtained from the participating individuals or their guardians before the clinical data and genomic samples were collected. Ethical approval was provided by the Internal Review Board of the Zhongshan Ophthalmic Center, China. Probands from the 49 families with FEVR were collected from our Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Center. Of the 49, 15 had a familial history of FEVR, and 34 were isolated cases. The clinical diagnosis of FEVR was as previously described [18–21]. *TSPAN12* mutations in the 49 families were excluded with Sanger dideoxy sequencing as described previously [21].

Genetic analysis: Genomic DNA was prepared from venous leukocytes. The primer sequences used to amplify the coding exons and the adjacent intronic sequences of *FZD4* and *LRP5* are listed in Appendix 1. Touchdown polymerase chain reaction was performed, with the annealing temperature commencing at 64 °C, then decreasing by 0.5 °C after each cycle for the first 15 cycles, and finally being maintained at 57 °C for the remaining 21 cycles. Sequencing was performed with an ABI BigDye Terminator Cycle Sequencing Kit, v3.1, using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences from the patients and the consensus sequences from the NCBI human genome database (*FZD4*: NC_000011.9 for gDNA, NM_012193.2 for mRNA, and NP_036325.2 for protein; *LRP5*: NC_000011.9 for gDNA, NM_002335.2 for mRNA, and NP_002326.2 for protein) were aligned by using the SeqManII program of the Lasergene package (DNAstar, Madison, WI). Each variation was

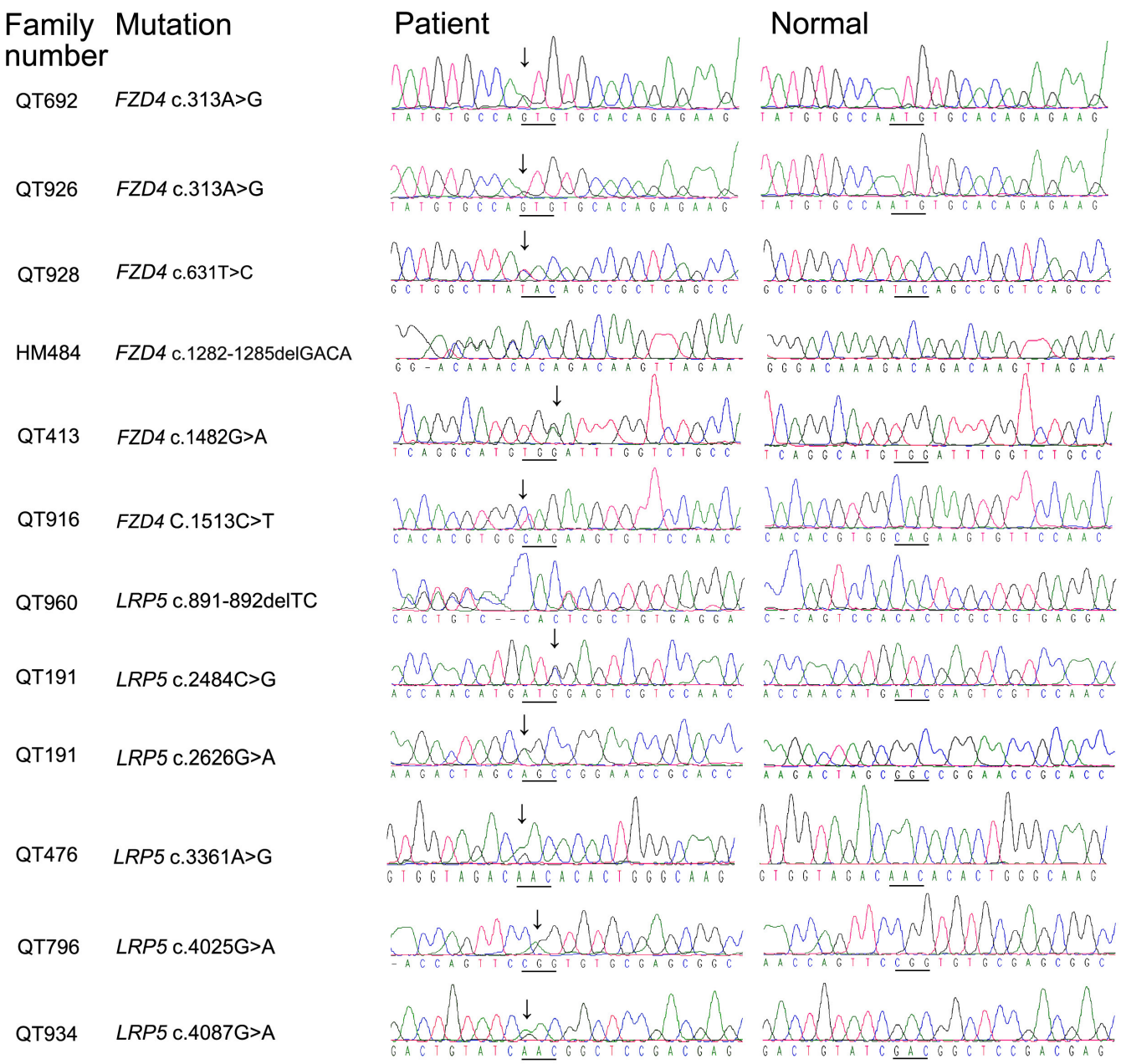

Figure 1. Eleven mutations identified in *FZD4* and *LRP5* genes of 49 families with FEVR. The columns from left to right display the family number, the mutation designation, and sequence chromatography from patients and normal controls.

initially confirmed with bidirectional sequencing and then evaluated in 192 chromosomes from 96 normal controls. The mutations were described according to the recommendations of the Human Genomic Variation Society (HGVS).

Information assessment of missense mutations: Nonsynonymous substitutions were further analyzed by using a set of programs aimed at predicting the effect of the substitution at the protein level:

Sequence alignments with protein orthologs were used to determine whether an amino acid at the mutation position was evolutionarily conserved or not. Substitutions at evolutionarily conserved positions/sites are more deleterious than those at evolutionarily unconserved positions [22].

Blosum62—Blosum62 is an amino acid substitution scoring matrix. Missense mutations had a lower fraction of nonconservative changes (negative blosum62 scores)

Table 1. Mutations identified in the FZD4 and LRP5 genes of the families with FEVR.

Family number	Gene/exon	DNA change	Allele status	Protein change	Computational prediction		Occurrence in		Note
					Blosum 62	PolyPhen	families	controls	
QT692,QT926	*FZD4/2*	c.313A>G	Hetero	p.Met105Val	5→-2	probably damaging	2/49	N/A	Known [25]
QT928	*FZD4/2*	c.631T>C	Hetero	p.Tyr211His	7→2	benign	1/49	0/96	Novel
HM484	*FZD4/2*	c.1282–1285delGACA	Hetero	p.Asp428SerfsX2	N/A	N/A	1/49	0/96	Novel
QT413	*FZD4/2*	c.1482G>A	Hetero	p.Trp494*	N/A	N/A	1/49	0/96	Novel
QT916	*FZD4/2*	c.1513C>T	Hetero	p.Gln505*	N/A	N/A	1/49	N/A	Known [18]
QT960	*LRP5/5*	c.891–892delTC	Hetero	p.Arg298Leu fsX2	N/A	N/A	1/49	0/96	Novel
QT191	*LRP5/11*	c.2484C>G	Hetero	p.Ile828Met	4→1	probably damaging	1/49	0/96	Novel
QT191	*LRP5/12*	c.2626G>A	Hetero	p.Gly876Ser	6→0	probably damaging	1/49	0/96	Novel
QT476	*LRP5/15*	c.3361A>G	Hetero	p.Asn1121Asp	6→1	possibly damaging	1/49	N/A	Known [27]
QT796	*LRP5/19*	c.4025G>A	Hetero	p.Arg1342Gln	5→1	probably damaging	1/49	0/96	Novel
QT934	*LRP5/19*	c.4087G>A	Hetero	p.Asp1363Asn	6→1	probably damaging	1/49	0/96	Novel

Abbreviations: Hetero: Heterozygous, N/A: Not available.

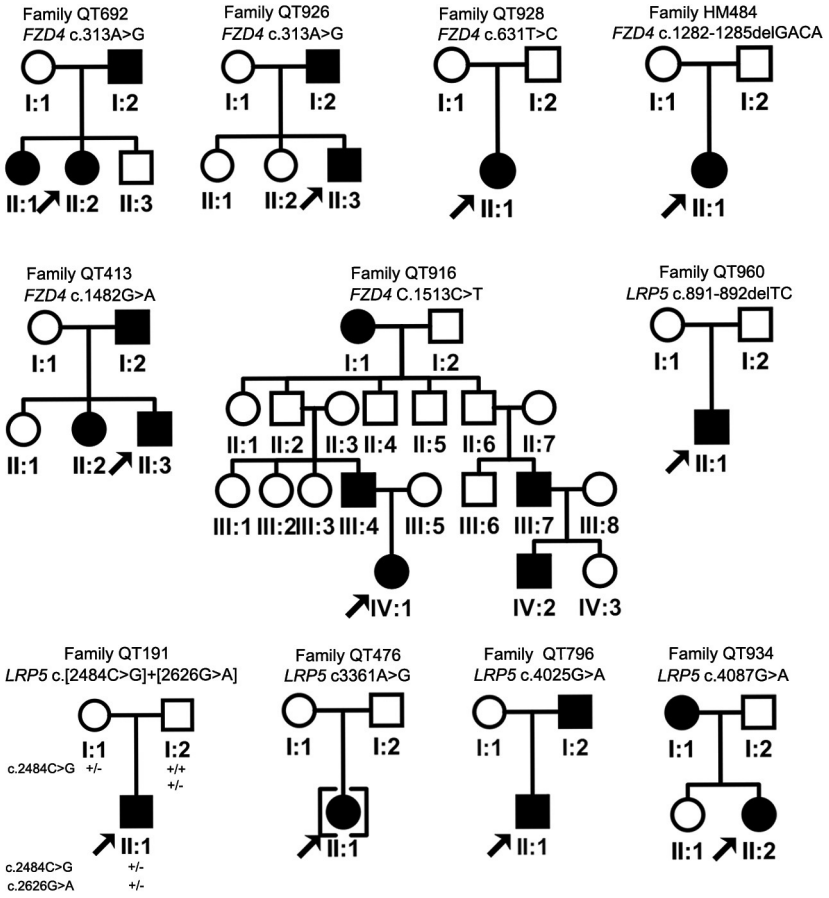

Figure 2. Pedigrees of 11 families with *FZD4* or *LRP5* mutations. A + sign represents a normal allele, and a - sign indicates a variant. The proband in family QT191 had compound heterozygous mutation, while his mother had a heterozygous c.2484C>**G**: variant and his father had a heterozygous c.2626G>A variant. The squares brackets around II:1 in family QT476 indicated an adopted proband.

compared with that predicted from randomly distributed nonsynonymous single nucleotide polymorphisms, suggesting that blosum62 values predict deleterious function [23].

PolyPhen—PolyPhen is a sequence homology–based online tool used to predict the functional impact of a substitution. PolyPhen predicts how damaging a particular variant may be by using a set of empirical rules based on sequence, phylogenetic, and structural information about a particular variant [24].

RESULTS

Mutations detected: Eleven heterozygous mutations (Figure 1), including eight novel and three known mutations, were identified in 11 of the 49 (22.4%) families with FEVR, including five *FZD4* mutations in six families and six *LRP5* mutations in five families (Table 1). Of the 11 families with *FZD4* and *LRP5* mutations, six had a familial history of FEVR, and five were isolated cases (Figure 2). Two families had the same *FZD4* mutation, and one family had compound heterozygous mutations in *LRP5*.

Of the 11 mutations, seven were missense, two were nonsense, and two were frameshift deletions. The eight novel mutations were not detected in 192 chromosomes of 96 normal controls. All five novel missense changes affected evolutionarily conserved residues (Figure 3), and four of the five were predicted to be pathogenic (Table 1). The cosegregation of the mutation in additional family members who were screened is shown in Table 2.

Phenotypes: All 11 probands and their affected relatives with *FZD4* or *LRP5* mutations had ocular changes typical of FEVR (Table 2). Individuals with mutations may be asymptomatic or blind, with visual acuity ranging from normal to no light perception. Fundus changes varied significantly in the different patients, with mildly affected individuals showing brush-like or increased branching of the peripheral vessels, peripheral avascular zone, peripheral fibrous proliferation, and/or straightening of the temporal arcades (Figure 4). These signs were also prevalent in the "healthy eye" of the probands or affected relatives, especially under examination with fluorescein angiography. The affected eyes of the probands and the relatives showed more severe ocular changes, including

temporal dragging of the optic disc, falciform retinal folds, neovascularization, exudates, tractional retinal detachment, and/or retrolenticular fibrotic masses.

DISCUSSION

In this study, 11 mutations in *FZD4* and *LRP5* were detected in 11 families with FEVR but were not present in 96 normal individuals. Based on the results of segregation analysis in the family members and the functional prediction of the mutations, these mutations appear to be the cause of FEVR in the Chinese patients.

The phenotypes of all the patients with *FZD4* or *LRP5* mutations were closely related to the developmental anomalies observed in the retinal vessels and the resulting complications. However, we documented great variability in the clinical signs between the right and left eyes of the same patient, among different affected members of the same family, and between different families. We have not identified specific phenotypes that can establish a genotype-phenotype correlation for different mutations in the same gene or for mutations in different genes.

So far, several mutations have been identified in the *FZD4* and *LRP5* genes of patients with FEVR. Mutations in *FZD4* have been detected in 5%–40% of families with FEVR [18,19,25–29], whereas those in *LRP5* have been identified in 12%–18% of families [18,26,27]. In our study, the *FZD4* and *LRP5* mutations were identified in 11 of 49 families, in which *TSPAN12* mutations have been excluded by our previous study [21]. We detected *FZD4* mutations in 9.6% (5/52) of families with FEVR and *LRP5* mutations in 11.5% (6/52) if the three families with *TSPAN12* mutations are taken into account [21]. In summary, mutations in *FZD4*, *LRP5*, and *TSPAN12* were not detected in a large proportion of families (73.1%, 38 of the 52 families) in our case series. For those families in which we failed to detect mutations, a small number might have mutations in the intronic or regulatory regions of these genes, which could not be detected with the strategies used in this study. Mutation in the *NDP* gene has been excluded in the remaining 38 families in our recent study [30]. It is more likely that additional genes involved in FEVR have yet to be discovered. Other components in the Wnt/Norrin signaling pathway might be potential candidates for further screening since all four known FEVR causative genes encode proteins involved in this pathway. Moreover, the samples in which mutations were not identified will be good targets for identifying additional FEVR genes with next-generation sequencing and exome sequencing in the near future.

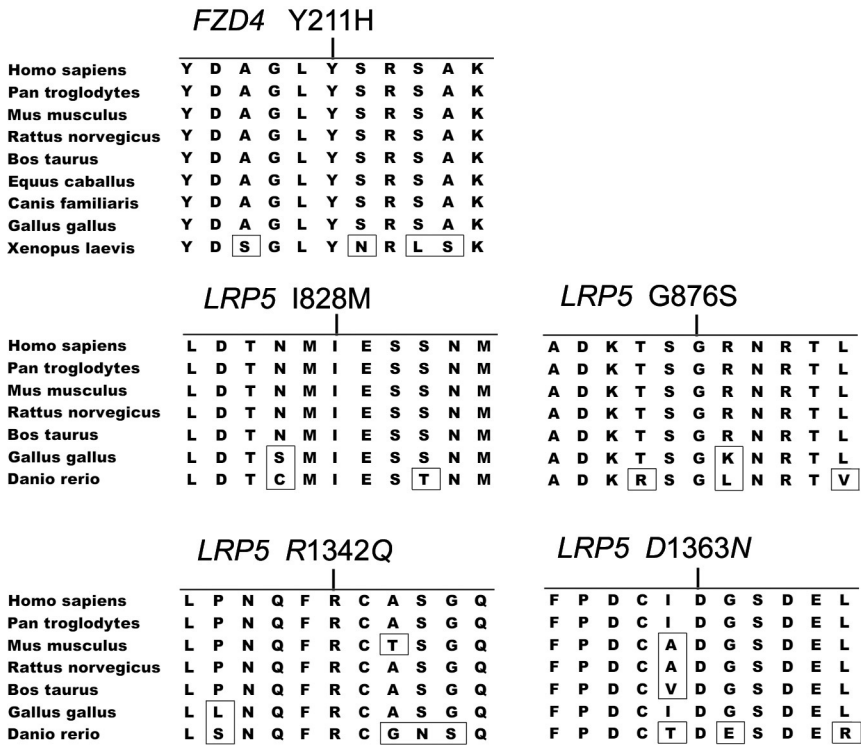

Figure 3. Protein alignment for the novel missense mutations identified in *FZD4* and *LRP5*. Nonconserved amino acid residues are boxed. The residues with mutations are highly conserved. *FZD4* orthologs included *Homo sapiens* (NP_036325.2), *Pan troglodytes* (XP_001175326.1), *Mus musculus* (NP_032081.2), *Rattus norvegicus* (NP_072145.1), *Bos taurus* (NP_001193198.1), *Equus caballus* (XP_001489854.1), *Canis familiaris* (XP_848753.1), *Gallus gallus* (NP_989430.1), and *Danio rerio* (XP_002664771.1). The *LRP5* orthologs are from *Homo sapiens* (NP_002326.2), *Pan troglodytes* (XP_508605.2), *Mus musculus* (NP_032539.1), *Rattus norvegicus* (NP_001099791.2), *Bos taurus* (XP_614220.3), *Gallus gallus* (NP_001012915.1), and *Danio rerio* (NP_001170929.1).

Table 2. Clinical information on the patients with *FZD4* or *LRP5* mutations.

Family number	ID/Sex/Age	Mutation (gene/DNA)	Best vision (right; left)	Main phenotypes	
				Right eye	Left eye
QT692	II:2/F/20y	FZD4/c.313A>G	0.3; 0.3	IBPV	RD, AZ, PFP, NV
	I:2/M/52y	FZD4/c.313A>G	1.0; 1.0	IBPV	IBPV
	II:1/F/21y	FZD4/c.313A>G	1.0; 1.0	IBPV	IBPV
QT926	II:3/M/5y	FZD4/c.313A>G	N/A	IBPV	TDOD
	I:2/M/36y	FZD4/c.313A>G	FC; 1.0	IBPV, AZ, FPF	IBPV, AZ
QT928	II:1/F/4y	FZD4/c.631T>C	N/A	TDOD	TDOD, PFP
HM484	II:1/F/4y	FZD4/c.1282–1285delGACA	0.3; 0.1	STA	TDOD, PFP
QT413	II:3/M/9y	FZD4/c.1482G>A	0.02; 0.8	RFM, LD	AZ, PFP, BPV
	I:2/M/39y	FZD4/c.1482G>A	1.0; 1.0	AZ, BPV, NV	AZ, BPV, NV
	II:2/F/14y	FZD4/c.1482G>A	0.6; 0.8	RD,TDOD	STA
QT916	IV:1/F/2y	FZD4/c.1513C>T	N/A	TDOD	FPF
	III:4/M/26	FZD4/c.1513C>T	N/A	IBPV	TDOD, BPV, AZ, PE
QT960	II:1/M/2mo	LRP5/c.891–892delTC	NLP; NLP	RFM, RD,MC, FAC	RFM, RD,MC, FAC
QT191	II:1/M/5mo	LRP5/c.[2484C>G]+[2626G>A]	NLP; NLP	RFM, SCP	RFM, SCP
QT476	II:1/F/1y	LRP5/c.3361A>G	HM; HM	TDOD	RFM
QT796	I:2/M/24y	LRP5/c.4025G>A	LP; 0.2	RFM	AZ
	II:1/M/5mo	LRP5/c.4025G>A	N/A	NYS, MC, RFM	NYS, MC, RFM, SCP
QT934	II:2/F/6mo	LRP5/c.4087G>A	HM; HM	RFM	RFM
	I:1/F/30y	LRP5/c.4087G>A	0.8;0.8	IBPV	IBPV

Abbreviations: M: Male, F: Female, y: Years old, mo: Months, FC: Finger counting, HM: Hand move, LP: Light perception, NLP: No light perception, N/A: Not available, IBPV: Increased branching of peripheral vessels, RD: Retinal detachment, PFP: Peripheral fibrous proliferation, NV: Neovascularization, TDOD: Temporal dragging of optic disc, AZ: Avascular zone, FRF: Falciform retinal fold, STA: Straightening of temporal arcades, RFM: Retrolenticular fibrotic mass, LD: Lens dislocation, BPV: Brushlike peripheral vessels, PE: Peripheral exudates, MC: Microcornea, FAC: Flat anterior chamber, SCP: Stretched ciliary process. NYS: Nystagmus.

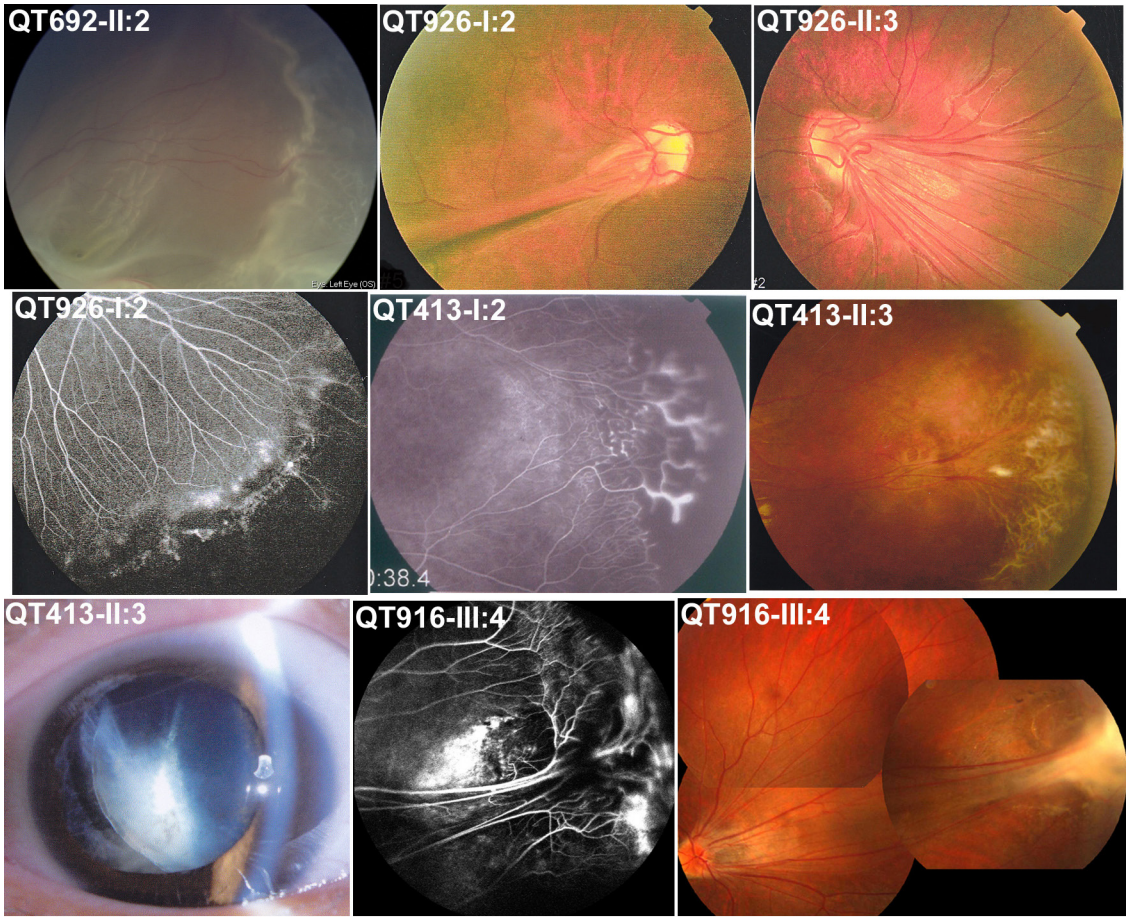

Figure 4. Ocular changes in affected individuals with an *FZD4* or *LRP5* mutation. The individual ID is indicated on the top left of each picture, which is the same as in Figure 2 and Table 2. Signs of FEVR included retinal detachment (top left), falciform retinal fold (top middle), temporal dragging of optic disc (top right), peripheral avascular zone and brush-like peripheral vessels (middle left), shell-like peripheral vessel terminatio and neovascularization (center), peripheral fibrovascular proliferation (middle right), lens dislocation (bottom left), peripheral exudates (bottom middle), temporal dragging of optic disc, and peripheral fibrous proliferation (bottom right).

APPENDIX 1.

Primers used for PCR amplification and sequencing of *FZD4* and *LRP5* Abbreviations: E: Exon, F: Forward, R: Reverse, bp: Base pair. *FZD4*-E2 was amplified in three overlapping segments A, B, and C. Primer sequences for *LRP5*-E4 obtained from Gong et al. [31]. Sequencing primer for *LRP5*-E10-F is another primer TTCCTCCTCACCTGCTG. To access the data, click or select the words "Appendix 1." This will initiate the download of a compressed (pdf) archive that contains the file.

ACKNOWLEDGMENTS

The authors thank all patients and family members for their participation. This study was supported by the National Science Fund for Distinguished Young Scholars (30,725,044) and the Fundamental Research Funds of State Key Lab of Ophthalmology, Sun Yat-sen University.

REFERENCES

1. Criswick VG, Schepens CL. Familial exudative vitreoretinopathy. Am J Ophthalmol 1969; 68:578-94. [PMID: 5394449].
2. van Nouhuys CE. Signs, complications, and platelet aggregation in familial exudative vitreoretinopathy. Am J Ophthalmol 1991; 111:34-41. [PMID: 1985487].
3. Ober RR, Bird AC, Hamilton AM, Sehmi K. Autosomal dominant exudative vitreoretinopathy. Br J Ophthalmol 1980; 64:112-20. [PMID: 7362811].
4. Robitaille J, MacDonald ML, Kaykas A, Sheldahl LC, Zeisler J, Dube MP, Zhang LH, Singaraja RR, Guernsey DL, Zheng B, Siebert LF, Hoskin-Mott A, Trese MT, Pimstone SN, Shastry BS, Moon RT, Hayden MR, Goldberg YP, Samuels ME. Mutant frizzled-4 disrupts retinal angiogenesis in

familial exudative vitreoretinopathy. Nat Genet 2002; 32:326-30. [PMID: 12172548].

5. Toomes C, Bottomley HM, Jackson RM, Towns KV, Scott S, Mackey DA, Craig JE, Jiang L, Yang Z, Trembath R, Woodruff G, Gregory-Evans CY, Gregory-Evans K, Parker MJ, Black GC, Downey LM, Zhang K, Inglehearn CF. Mutations in lrp5 or fzd4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. Am J Hum Genet 2004; 74:721-30. [PMID: 15024691].

6. Nikopoulos K, Gilissen C, Hoischen A, van Nouhuys CE, Boonstra FN, Blokland EA, Arts P, Wieskamp N, Strom TM, Ayuso C, Tilanus MA, Bouwhuis S, Mukhopadhyay A, Scheffer H, Hoefsloot LH, Veltman JA, Cremers FP, Collin RW. Next-generation sequencing of a 40 mb linkage interval reveals tspan12 mutations in patients with familial exudative vitreoretinopathy. Am J Hum Genet 2010; 86:240-7. [PMID: 20159111].

7. Poulter JA, Ali M, Gilmour DF, Rice A, Kondo H, Hayashi K, Mackey DA, Kearns LS, Ruddle JB, Craig JE, Pierce EA, Downey LM, Mohamed MD, Markham AF, Inglehearn CF, Toomes C. Mutations in tspan12 cause autosomal-dominant familial exudative vitreoretinopathy. Am J Hum Genet 2010; 86:248-53. [PMID: 20159112].

8. Jiao X, Ventruto V, Trese MT, Shastry BS, Hejtmancik JF. Autosomal recessive familial exudative vitreoretinopathy is associated with mutations in lrp5. Am J Hum Genet 2004; 75:878-84. [PMID: 15346351].

9. Berger W, van de Pol D, Warburg M, Gal A, Bleeker-Wagemakers L, de Silva H, Meindl A, Meitinger T, Cremers F, Ropers HH. Mutations in the candidate gene for norrie disease. Hum Mol Genet 1992; 1:461-5. [PMID: 1307245].

10. Chen ZY, Battinelli EM, Fielder A, Bundey S, Sims K, Breakefield XO, Craig IW. A mutation in the norrie disease gene (ndp) associated with x–linked familial exudative vitreoretinopathy. Nat Genet 1993; 5:180-3. [PMID: 8252044].

11. de Iongh RU, Abud HE, Hime GR. Wnt/frizzled signaling in eye development and disease. Front Biosci 2006; 11:2442-64. [PMID: 16720326].

12. Xu Q, Wang Y, Dabdoub A, Smallwood PM, Williams J, Woods C, Kelley MW, Jiang L, Tasman W, Zhang K, Nathans J. Vascular development in the retina and inner ear: Control by norrin and frizzled-4, a high-affinity ligand-receptor pair. Cell 2004; 116:883-95. [PMID: 15035989].

13. Ai M, Heeger S, Bartels CF, Schelling DK. Clinical and molecular findings in osteoporosis-pseudoglioma syndrome. Am J Hum Genet 2005; 77:741-53. [PMID: 16252235].

14. Qin M, Kondo H, Tahira T, Hayashi K. Moderate reduction of norrin signaling activity associated with the causative missense mutations identified in patients with familial exudative vitreoretinopathy. Hum Genet 2008; 122:615-23. [PMID: 17955262].

15. Nikopoulos K, Venselaar H, Collin RW, Riveiro-Alvarez R, Boonstra FN, Hooymans JM, Mukhopadhyay A, Shears D, van Bers M, de Wijs IJ, van Essen AJ, Sijmons RH, Tilanus MA, van Nouhuys CE, Ayuso C, Hoefsloot LH, Cremers FP. Overview of the mutation spectrum in familial exudative vitreoretinopathy and norrie disease with identification of 21 novel variants in fzd4, lrp5, and ndp. Hum Mutat 2010; 31:656-66. [PMID: 20340138].

16. Jia LY, Li XX, Yu WZ, Zeng WT, Liang C. Novel frizzled-4 gene mutations in chinese patients with familial exudative vitreoretinopathy. Arch Ophthalmol 2010; 128:1341-9. [PMID: 20938005].

17. Zhang K, Harada Y, Wei X, Shukla D, Rajendran A, Tawansy K, Bedell M, Lim S, Shaw PX, He X, Yang Z. An essential role of the cysteine-rich domain of fzd4 in norrin/wnt signaling and familial exudative vitreoretinopathy. J Biol Chem 2011; 286:10210-5. [PMID: 21177847].

18. Toomes C, Bottomley HM, Scott S, Mackey DA, Craig JE, Appukuttan B, Stout JT, Flaxel CJ, Zhang K, Black GC, Fryer A, Downey LM, Inglehearn CF. Spectrum and frequency of fzd4 mutations in familial exudative vitreoretinopathy. Invest Ophthalmol Vis Sci 2004; 45:2083-90. [PMID: 15223780].

19. Nallathambi J, Shukla D, Rajendran A, Namperumalsamy P, Muthulakshmi R, Sundaresan P. Identification of novel fzd4 mutations in indian patients with familial exudative vitreoretinopathy. Mol Vis 2006; 12:1086-92. [PMID: 17093393].

20. Kondo H, Qin M, Kusaka S, Tahira T, Hasebe H, Hayashi H, Uchio E, Hayashi K. Novel mutations in norrie disease gene in japanese patients with norrie disease and familial exudative vitreoretinopathy. Invest Ophthalmol Vis Sci 2007; 48:1276-82. [PMID: 17325173].

21. Yang H, Xiao X, Li S, Mai G, Zhang Q. Novel tspan12 mutations in patients with familial exudative vitreoretinopathy and their associated phenotypes. Mol Vis 2011; 17:1128-35. [PMID: 21552475].

22. Leabman MK, Huang CC, DeYoung J, Carlson EJ, Taylor TR, de la Cruz M, Johns SJ, Stryke D, Kawamoto M, Urban TJ, Kroetz DL, Ferrin TE, Clark AG, Risch N, Herskowitz I, Giacomini KM. Natural variation in human membrane transporter genes reveals evolutionary and functional constraints. Proc Natl Acad Sci USA 2003; 100:5896-901. [PMID: 12719533].

23. Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. Characterization of single-nucleotide polymorphisms in coding regions of human genes. Nat Genet 1999; 22:231-8. [PMID: 10391209].

24. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. A method and server for predicting damaging missense mutations. Nat Methods 2010; 7:248-9. [PMID: 20354512].

25. Kondo H, Hayashi H, Oshima K, Tahira T, Hayashi K. Frizzled 4 gene (fzd4) mutations in patients with familial exudative vitreoretinopathy with variable expressivity. Br J Ophthalmol 2003; 87:1291-5. [PMID: 14507768].

26. Boonstra FN, van Nouhuys CE, Schuil J, de Wijs IJ, van der Donk KP, Nikopoulos K, Mukhopadhyay A, Scheffer

H, Tilanus MA, Cremers FP, Hoefsloot LH. Clinical and molecular evaluation of probands and family members with familial exudative vitreoretinopathy. Invest Ophthalmol Vis Sci 2009; 50:4379-85. [PMID: 19324841].

27. Qin M, Hayashi H, Oshima K, Tahira T, Hayashi K, Kondo H. Complexity of the genotype-phenotype correlation in familial exudative vitreoretinopathy with mutations in the lrp5 and/or fzd4 genes. Hum Mutat 2005; 26:104-12. [PMID: 15981244].

28. MacDonald ML, Goldberg YP, Macfarlane J, Samuels ME, Trese MT, Shastry BS. Genetic variants of frizzled-4 gene in familial exudative vitreoretinopathy and advanced retinopathy of prematurity. Clin Genet 2005; 67:363-6. [PMID: 15733276].

29. Robitaille JM, Zheng B, Wallace K, Beis MJ, Tatlidil C, Yang J, Sheidow TG, Siebert L, Levin AV, Lam WC, Arthur BW, Lyons CJ, Jaakkola E, Tsilou E, Williams CA, Weaver RG Jr, Shields CL, Guernsey DL. The role of frizzled-4 mutations in familial exudative vitreoretinopathy and coats disease. Br J Ophthalmol 2011; 95:574-9. [PMID: 21097938].

30. Yang H, Li S, Xiao X, Guo X, Zhang Q. Screening for NDP Mutations in 44 Unrelated Patients with Familial Exudative Vitreoretinopathy or Norrie Disease. Curr Eye Res 2012; [PMID: 22563645].

31. Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Jüppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML. Osteoporosis-Pseudoglioma Syndrome Collaborative Group. LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 2001; 107:513-23. [PMID: 11719191].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 4 October 2012. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.