Whole Exome Sequencing Revealed Three Novel Variants in TSPAN12 and LRP5 Genes for Two Families with Familial Exudative Vitreoretinopathy

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Research Article

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Abstract

Background: Familial exudative vitreoretinopathy (FEVR) is a complex form of blindness-causing retinal degeneration. This study investigated the potential disease-causing variant and molecular basis in two Chinese families with FEVR.

Methods: All family members underwent detailed ophthalmological examinations, including best-corrected visual acuity, fundus examination, and fluorescein fundus angiography. Whole exome sequencing, bioinformatics analysis, and Sanger sequencing of available family members were used to confirm the disease-causing gene variant.

Results: The proband F1-II:1, his mother, and proband F2-II:1 were diagnosed with FEVR based on clinical symptoms, fundus manifestation, and fundus fluorescein angiography. Whole exome sequencing showed that the proband F1-II:1 had a novel heterozygous variant in the *TSPAN12* gene, c.236T>G p. (Met79Arg), which may disturb the transmembrane domain of the TSPAN12 protein. The variant was cosegregated with the phenotypes of FEVR in the family. The proband F2-II:1 carried novel compound heterozygous variants, c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp), in the *LRP5* gene, which may disturb the second β -propeller motif of the LRP5 protein. These three variants were classified as likely pathogenic variants. The variants were predicted to be damaging or deleterious using multiple lines of prediction algorithms; they were not frequently found in multiple population databases. The residues affected by these variants are highly conserved among different species. The three novel variants may be potential disease-causing variants in the two families.

Conclusions: These results expand the spectrum of variants in *TSPAN12* and *LRP5* genes and enrich the understanding of the molecular etiology of FEVR.

1 Background

Familial exudative vitreoretinopathy (FEVR, OMIM: 133780) is a clinically and genetically heterogeneous inherited ophthalmic disorder (1, 2). It is characterized by incomplete retinal vascular development and pathological neovascularization (3). Patients usually complain of reduced visual acuity or blindness in early childhood. The fundus can exhibit peripheral retinal avascularization, falciform retinal folds, macular ectopia, retinal exudate, retinal neovascularization, and retinal detachment (4). However, some patients may not complain of any visual impairment; they may only exhibit peripheral avascularization (5). The reported prevalence is approximately 0.11% in newborns (6), but, the actual prevalence may be underestimated because some patients are asymptomatic and demonstrate peripheral retinal involvement only (7).

FEVR can be inherited in autosomal dominant, autosomal recessive, or X-linked manners; the most common mode of inheritance is autosomal dominant (8). Thus far, the following eleven genes have been reported to cause FEVR: norrin (*NDP*, OMIM, 300658) (9), frizzled 4 (*FZD4*, OMIM, 604579) (10), low density lipoprotein receptor-related protein 5 (*LRP5*, OMIM, 603506) (11), tetraspanin 12 (*TSPAN12*, OMIM, 613310) (12), catenin beta 1 (*CTNNB1*, OMIM, 116806) (13), zinc finger protein 408 (*ZNF408*, OMIM, 616454) (14), atonal homolog 7 (*ATOH7*, OMIM, 609875) (15), kinesin family member 11 (*KIF11*, OMIM, 148760) (16), RCC1 and BTB domain containing protein 1 (*RCBTB1*, OMIM, 607867) (17), jagged 1 (*JAG1*, OMIM, 601920) (18), and α -catenin (*CTNNA1*, OMIM) (19). Moreover, one locus, EVR3, which maps to 11p13-p12, can also lead to FEVR; its causative gene has not been fully identified (20). Among these pathogenic genes, *FZD4*, *LRP5*, and *TSPAN12* are the most common disease-causing genes related to FEVR (21). The first five genes are involved in the Norrin or Wnt/ β -catenin signaling pathway and have functions in cell adhesion, migration, and signaling (22).

Although increasing numbers of gene variants have been identified using next generation sequencing technology, these reported gene variants are responsible for only 50–60% of FEVR cases. Moreover, some patients may exhibit rapid progression without correct diagnosis and intervention. Thus, it is imperative to ascertain genetic etiology and achieve accurate diagnosis for affected patients, especially patients who are asymptomatic and exhibit peripheral retinal involvement alone. In this study, two families were diagnosed with FEVR based on clinical manifestations. We performed whole exome sequencing of probands and Sanger sequencing of available family members to elaborate the underlying disease-causing gene variant.

2 Methods

2.1 Participants

The study was authorized by the medical ethics committee of Henan Provincial People's Hospital and conformed to the tenets of the Declaration of Helsinki. All participants were consecutively recruited in our hospital. Before all examinations, informed consent was obtained from each participant (or parents/guardians of participants ≤ 18 years of age).

2.2 Clinical examinations

Detailed premature delivery history, oxygen uptake history, family history, and birth weight information were acquired for the probands. Exhaustive ophthalmological examinations were completed, including best-corrected visual acuity, intraocular pressure, slit-lamp microscopy, ophthalmoscopy, fundus photography, and fundus fluorescein angiography (FFA). All participants underwent pupillary dilation with a mixture of 0.5% phenylephrine hydrochloride and 0.5% tropicamide eye drops (Santen Pharmaceutical, Osaka, Japan). Fundus photography was performed with a VISUCAM 200 digital fundus camera (Carl Zeiss Meditec AG, Jena, Thuringia, Germany) or Optos Daytona ultrawide field system (Optos PLC, Dunfermline, United Kingdom). FFA was acquired using SPECTRALIS Engineering systems (Heidelberg Engineering Ltd, Hertfordshire, United Kingdom) or Optos Daytona ultrawide field system (Optos PLC) with 20% fluorescein solution (Guangzhou Pharmaceutical Holdings Limited, Guangzhou, China). FEVR was diagnosed based on previously reported criteria (23).

2.3 Whole exome sequencing

Genomic DNA samples were prepared from peripheral blood of all participants using a TIANamp Genomic DNA Kit (TIANGEN Biotech, Beijing, China). Library construction was performed from proband samples using xGen Exome Research Panel (Integrated DNA Technologies, Coralville, Iowa, United States).

Samples were sequenced on a HiSeq platform (Illumina, San Diego, California, United States) using a whole exome sequencing protocol, in accordance with the manufacturer's instructions. Sequence data were analyzed for corresponding ophthalmologic inherited genes, especially inherited retinal disease genes; sequences were aligned using Burrows-Wheeler Aligner (<http://bio-bwa.sourceforge.net/>). Variant calling and nomenclature complied with the recommendations of the Human Genome Variation Society (<http://www.hgvs.org/>). Variant annotation was performed in accordance with American College of Medical Genetics (ACMG, <https://www.acmg.net/>) guidelines.

2.4 Raw reads filtering

After whole exome sequencing, raw reads were filtered to remove duplicates, then aligned to the hg19 (GRCh37) human genome reference sequence. Quality control was recalibrated by Picard Mark Duplicates (<http://sourceforge.net/projects/picard/>), Genome Analysis Toolkit (<https://gatk.broadinstitute.org/hc/en-us>), and SAM tools (<http://samtools.sourceforge.net/>). Variants were validated and analyzed preferentially if they met the following previously reported criteria (24–26): (1) minor allele frequency of the variant < 0.01 in the 1000 Genomes Project database (<http://www.internationalgenome.org/>), Exome Aggregation Consortium database (<http://exac.broadinstitute.org/>), Genome Aggregation database (<http://gnomad.broadinstitute.org/>), and an in-house Chinese individuals database; (2) variant location in an exon region or canonical splicing intron region that affected transcription splicing; (3) damaging or deleterious variant prediction using Sorting Intolerant From Tolerant (<http://sift.jcvi.org/>), Polymorphism Phenotyping (<http://genetics.bwh.harvard.edu/pph2/>), ClinPred (<https://sites.google.com/site/clinpred/>), Likelihood Ratio Test (<http://www.genetics.wustl.edu/jflab/lrt>), Mutation Taster (<http://www.mutationtaster.org/>), Mutation Assessor (<http://mutationassessor.org/r3>), Functional Analysis Through Hidden Markov Models (<http://fathmm.biocompute.org.uk/>), and Protein Variation Effect Analyzer (<http://provean.jcvi.org>); (4) highly conserved variant prediction using Genomic Evolutionary Rate Profiling (<http://mendel.stanford.edu/SidowLab/downloads/gerp>); (5) other reported pathogenic variant that did not meet the above criteria (e.g., high minor allele frequency variant, deep-intronic variant, or synonymous single nucleotide variant).

2.5 In silico analysis

Preferentially selected variants were validated and cosegregated by Sanger sequencing, performed using an 3500xL Dx Genetic Analyser (Applied Biosystems, Foster City, California, United States) with ABI BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Primers were designed with Primer3 (<http://primer3.ut.ee/>) to amplify *TSPAN12* and *LRP5* gene fragments. The primer information is provided in Supplementary Table 1. Consensus sequences corresponding to proband sequences were downloaded from national center for biotechnology information (<https://www.ncbi.nlm.nih.gov/>). All sequences were analyzed using SeqMan II software in the Lasergene software package (DNASTAR, Madison, Wisconsin, United States). Evolutionary conservation among different species for single nucleotide variants were analyzed using MegAlign software in Lasergene software package (DNASTAR). Genomic and protein structures were schematically represented using IBS 1.0 software (<http://ibs.biocuckoo.org>).

3 Results

3.1 Clinical manifestation

Proband F1-II:1 was a 24-year-old man who complained of mild reduced visual acuity in the left eye; his best-corrected visual acuity (BCVA) was 1.0 in both eyes. Fundus examination of both eyes showed increased numbers of peripheral retinal vessels with straightened morphology. FFA demonstrated retinal leakage, retinal avascularization, straightened vessels, and increased numbers of vessels in both peripheral retinas (Figure 1A). Two years later, the patient complained of worsened visual acuity in the left eye; his BCVA values were 1.0 and 0.8 in the right and left eyes, respectively. The right eye exhibited a fundus appearance similar to the findings during the previous examination; the left eye exhibited a more severe fundus appearance than had been observed during the previous examination, particularly involving falciform retinal folds and peripheral retinal exudates (Figure 1B). Because the patient experienced anaphylaxis during the first FFA examination, he could not complete the follow-up FFA examination. The patient's father and mother did not complain of any symptoms. His father's FFA findings were normal (Figure 1C), while his mother demonstrated peripheral retinal avascularization in both eyes and mild peripheral retinal leakage in the left eye (Figure 1D).

Proband F2-II:1 was a 30-year-old woman who complained of severely reduced visual acuity in the right eye. Her BCVA values were 0.1 and 0.8 in the right and left eyes, respectively. Fundus examination showed severe retinal detachment in the right eye; thus, the patient underwent pars plana vitrectomy. Additionally, FFA demonstrated increased numbers of peripheral retinal vessels and peripheral retinal leakage in both eyes; it showed post-vitrectomy appearance in the right eye (Figure 1E). Both of the proband's parents did not complain of any symptoms, and they exhibited normal FFA appearance.

Both probands had no history of premature delivery, problems with oxygen uptake, or low birth weight; neither proband had clinically significant family medical history. All participants had no systematic complaints or extraophthalmic abnormalities that could be identified through conventional examinations (e.g., short stature or microcephaly). The ophthalmic features of all participants are summarized in Table 1. Based on clinical manifestations, proband F1-II:1, his mother, and proband F2-II:1 were diagnosed with FEVR.

Table 1

Clinical features of all participants in the study

3.2 Sequencing and in silico analysis results

The mean sequencing depth in proband F1-II:1 was 118.52, with 30X coverage over 98.60% of the target region. In total, 70.76 million reads mapped to the

No.ID	Gender	Symptom	Age at (year)		BCVA		Fundus Examination		FFA	
			Onset	Exam	OD	OS	OD	OS	OD	OS
F1-I:1	Male	No	NA	50	1.0	1.0	Normal	Normal	Normal	Normal
F1-I:2	Female	No	NA	48	1.0	1.0	PRA	PRA	PRA	PRA, PRL
F1-II:1	Male	VD	18	24	1.0	0.8	PRA, IPRV	FRF, PRE, PRA, IPRV	PRA, PRL, IPRV	PRA, PRL, IPRV
F2-I:1	Male	No	NA	46	1.0	1.0	Normal	Normal	Normal	Normal
F2-I:2	Female	No	NA	45	1.0	1.0	Normal	Normal	Normal	Normal
F2-II:1	Female	VD	20	30	0.1	0.8	PoRD, IPRV	IPRV	PoRD, IPRV, PRL	IPRV, PRL

Abbreviations: F: Family; BCVA: best-corrected visual acuity; NA: not available; OD: right eye; OS: left eye; FFA: fluorescein fundus angiography; VD: vision decline; FRF: falciform retinal folds; PRE: peripheral retinal exudates; PRA: peripheral retinal avascularization; PoRD: postoperative retinal detachment; IPRV: increased peripheral retinal vessel; PRL: peripheral retinal leakage. Note: For anaphylaxis during the first FFA examination, the proband F1-II:1 could not complete the follow-up FFA examination, appearance on FFA was 2 years before appearance on fundus examination.

human reference genome and 86,877 variants were called. Sequencing information regarding the proband is summarized in Table 2. Whole exome sequencing and Sanger sequencing showed that the proband had a novel heterozygous variant c.236T>G p. (Met79Arg) in the *TSPAN12* gene. Sanger sequencing of the proband's mother also revealed the c.236T>G p. (Met79Arg) variant; his father exhibited a normal genotype. Sanger sequencing chromatographs of the family are shown in Figure 2A. The variant cosegregated with the FEVR phenotypes in the family. The pedigree of the family is presented in Figure 2B. We concluded that the variant was inherited in an autosomal dominant manner. The variant information is summarized in Table 3. In particular, the variant was located in exon 4 of the *TSPAN12* gene; this was predicted to affect the transmembrane domain of the TSPAN12 protein. Schematic representations of the genomic and protein structures are shown in Figure 2C and 2D.

The mean sequencing depth of proband F2-II:1 was 155.29, with 30X coverage over 96.77% of the target region. In total, 82.98 million reads mapped to the human reference genome and 86,987 variants were called. Sequencing information regarding the proband is summarized in Table 2. Whole exome sequencing and Sanger sequencing showed that the proband carried novel compound heterozygous variants, c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp), in the *LRP5* gene. Sanger sequencing chromatographs of the proband are shown in Figure 2A. The pedigree of the family is presented in Figure 2B. Information regarding the variants is summarized in Table 3. In particular, the variants were located in exons 6 and 8 of the *LRP5* gene, respectively; both residues affected by the variants were located in the second β -propeller motif of the LRP5 protein. Schematic representations of the genomic and protein structures are shown in Figure 2C and 2D.

Table 2

Whole exome sequencing information of probands in this study

ID	Mean depth	Percentage of covered target region (%)		No. of mapped reads (million)	No. of total variants	No. of single nucleotide variants	No. of insertions/deletions
		20X	30X				
F1-II:1	118.52	99.42	98.60	70.76	86,877	72,135	14,521
F2-II:1	155.29	97.44	96.77	82.98	86,987	72,218	14,757

Table 3

Information regarding variants in *TSPAN12* and *LRP5* genes detected in this study

ID	Gene	Transcript	Nucleotide change	Amino acid change	Variant type	Exon	State	ACMG
F1-II:1	<i>TSPAN12</i>	NM_012338	c.236T>G	p.(Met79Arg)	missense	E4	Het	LP
F2-II:1	<i>LRP5</i>	NM_002335	c.1210G>A	p.(Gly404Arg)	missense	E6	Het	LP
F2-II:1	<i>LRP5</i>	NM_002335	c.1612C>T	p.(Arg538Trp)	missense	E8	Het	LP

Abbreviations: Het, heterozygous; LP, likely pathogenic; ACMG, American College of Medical Genetics.

These three variants were predicted to be damaging or

deleterious, using multiple lines of prediction algorithms; they were not frequently found in ethnically matched populations in multiple population databases. Predictive functional effects and population distribution frequencies are summarized in Table 4. (The table is too wide for A4 or Letter landscape page, it was uploaded as an additional file). The variants were classified as likely pathogenic, based on ACMG guidelines. Evolutionary conservation alignment showed that the variants were highly conserved among different species (Figure 2E). Based on Sanger sequencing and bioinformatics analysis, we inferred that c.236T>G p. (Met79Arg) in the *TSPAN12* gene was the potential disease-causing variant in family 1; compound variants, c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp), in the *LRP5* gene were potential disease-causing variants in family 2.

Table 4. Predictive functional effects and population distribution frequencies of three variants

Gene	Variation	SIFT	PolyPhen2 HDIV	PolyPhen2 HVAR	ClinPred	LRT	Mutation Taster	Mutation Assessor	FATHMM	PROVEAN	GERP	1000 Genom
<i>TSPAN12</i>	c.236T>G	0.02	0.106	0.148	0.85504239	0.000	1.000	0.69	-1.26	-3.19	5.99	NA
<i>LRP5</i>	c.1210G>A	0.00	1.000	0.999	0.99769127	0.000	1.000	3.49	-5.61	-7.15	2.87	NA
<i>LRP5</i>	c.1612C>T	0.02	0.034	0.032	0.69098313	0.463	1.000	3.75	-4.15	-5.18	2.05	NA

Note: SIFT, Sorting Intolerant From Tolerant, it ranges from 0 to 1, deleterious (<0.05), tolerated (>=0.05), lower values are more deleterious; PolyPhen2 HDIV, probably damaging (0.453-0.956), benign (<=0.452), higher values are more deleterious; PolyPhen2 HVAR, probably damaging (>=0.909), possibly damaging (0.447-0.909), higher values are more deleterious; ClinPred, deleterious (>=0.5), tolerated (<0.5), higher values are more deleterious; LRT, Likelihood Ratio Test, lower values are more deleterious; Mutation Assessor, higher values are more deleterious; FATHMM, Functional Analysis Through Hidden Markov Models, lower values are more deleterious; Variation Effect Analyzer, it ranges from -14 to 14, -14~2.5, deleterious, -2.5~14, neutral, lower values are more deleterious; GERP, Genomic Evolutionary Rate Index, higher values are more conserved and deleterious. Abbreviations: NA: not available; ExAC, Exome Aggregation Consortium database; GnomAD, Genome Aggregation Database.

4 Discussion

In this study, we enrolled two probands with reduced vision who were diagnosed with FEVR based on clinical symptoms, as well as fundus and FFA examinations. We performed whole exome sequencing, Sanger sequencing validation, cosegregation analysis, functional prediction, population distribution analysis, and evolutionary conservation alignment. Our results suggested that novel variants—c.236T>G p. (Met79Arg) in the *TSPAN12* gene, as well as c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp) in the *LRP5* gene—were potential disease-causing variants in two probands. Except the c.1210G>A p. (Gly404Arg) variant was reported to cause osteoporosis-pseudoglioma syndrome in another patient (27), the other two variants have not been associated with any disease.

Clinical symptoms and fundus appearances can vary distinctly among patients and genetic backgrounds in patients with FEVR; in some instances, disease presentation can vary between eyes in a single patient (28). The proband F1-II:1 exhibited different appearances between eyes, such that the right eye demonstrated normal vision and mild fundus abnormality, while the left eye demonstrated mild reduced vision and moderate fundus abnormality. Moreover, the progress of disease was asynchronous between eyes: the right eye showed minimal progression, while the left eye showed progression with falciform retinal folds and peripheral retinal exudates at the 2-year follow-up. Distinct fundus findings were also present in proband F2-II:1, such that the right eye showed severe retinal detachment, while the left eye showed mild abnormality. Although the two probands has similar disease courses and were of similar age, their symptom severities and fundus appearances were different.

NDP, *FZD4*, *LRP5*, and *TSPAN12* gene variants can impair the Norrin or Wnt/ β -catenin signaling pathways, which are responsible for angiopoiesis during retinal development (29). In the canonical Wnt/ β -catenin pathway, *FZD4* and *LRP5* form a ternary complex as a coreceptor; Wnt binds to the coreceptor and activates downstream β -catenin signaling (30). In the Norrin/ β -catenin pathway, *NDP* binds to the coreceptor and activates downstream β -catenin signaling with the *TSPAN12* auxiliary component (31). When these signaling pathways are activated, β -catenin translocates to the nucleus and interacts with the T-cell factor/lymphoid enhancing factor family of transcription factors, thus initiating RNA transcription and elongation (32, 33).

The *TSPAN12* gene encodes the *TSPAN12* protein, which contains four-pass transmembrane domains and four cysteines in the second extracellular region, forming two extracellular loops and an intracellular loop. Xiao et al. reported that variants in the second extracellular loop comprised 38% of 40 causative variants (34). Variants in transmembrane domains and extracellular regions can severely impair function, variants in the C-terminal end can moderately impair function, and variants in the N-terminal end can slightly impair function. The transmembrane domains provide a scaffold for extracellular loops to change conformation and interact with *FZD4* for allosteric modulation. The variant c.236T>G p. (Met79Arg) is located in the transmembrane domain and may disrupt the domain structure of the *TSPAN12* protein, potentially preventing *TSPAN12* incorporation into the receptor complex and destabilizing the *NDP*/*FZD4*/*LRP5* interaction (35).

The *LRP5* gene encodes the *LRP5* protein, which contains a putative signal peptide, four β -propeller motifs at the amino terminal that alternate with four epidermal growth factor-like repeats, three low-density lipoprotein receptor-like repeats, a single transmembrane domain, and a cytoplasmic domain (36). Xiao et al. reported that variants in the first, second, and third β -propeller epidermal growth factor domains comprised 12%, 38%, and 17% of 58 causative variants, respectively (34). Although the exact functions of these domains are unknown thus far, studies of *LRP6* (with strong homology and similar function to *LRP5*) showed that the first and second β -propeller motifs, the third and fourth β -propeller motifs formed tandems to function respectively (37). Variants located in the second β -propeller motif may destroy the stable structure of first two β -propellers and interrupt their interactions with *NDP* or *FZD4*. The c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp) variants are located in the second β -propeller motif of the *LRP5* protein; therefore, these two variants may impair the second motif and cause the first and second β -propeller motifs tandems to become inactive.

Although we found three novel disease-causing variants in two FEVR families, there were some limitations in this study. First, we only speculated that variants were potential disease-causing based on clinical manifestations, whole exome sequencing, and bioinformatics analysis. Second, the parents of proband F2-II:1 did not undergo complete Sanger sequencing because peripheral blood samples were unavailable. We plan to validate the pathogenicity of the three variants by in vivo and in vitro analyses, and we will attempt to complete Sanger sequencing of available family members in a future study.

5 Conclusion

In conclusion, through whole exome sequencing and bioinformatics analysis, we identified a variant in the *TSPAN12* gene and compound variants in the *LRP5* gene in two families with FEVR. To our knowledge, this is the first report regarding c.236T>G p. (Met79Arg) in the *TSPAN12* gene, as well as c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp) in the *LRP5* gene, as potential disease-causing variants for FEVR. These results expand the spectra of variants in the *TSPAN12* and *LRP5* genes; they also enrich the understanding of the molecular etiology of FEVR. We presume that these findings will provide insights regarding accurate diagnosis, family genetic counseling, and future gene therapy for FEVR.

Abbreviations

FEVR, familial exudative vitreoretinopathy; *TSPAN12*, tetraspanin 12; *LRP5*, low density lipoprotein receptor-related protein 5; FFA, fundus fluorescein angiography; BCVA, best-corrected visual acuity; ACMG, American College of Medical Genetics.

Declarations

Ethics approval and consent to participate

The study was authorized by the medical ethics committee of Henan Provincial People's Hospital and conformed to the tenets of the Declaration of Helsinki.

Patient consent for publication

Before all examinations, written informed consent for the publication of any associated data and accompanying images was obtained from each participant (or parents/guardians of participants ≤ 18 years of age).

Availability of materials and data

The sequencing data used and/or analyzed during the current study are available at the following URL: <https://www.ncbi.nlm.nih.gov/bioproject/752456> or in the BioProject database under accession number PRJNA752456.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Concept and design: Zongming Song; Acquisition, analysis, or interpretation of data: Dongdong Wang, Zixu Huang, Qianqian Shi, Miao Zheng, Yuanyuan Xiao; Drafting of the manuscript: Handong Dan; Critical revision of the manuscript: Zongming Song; Statistical analysis: Handong Dan; Administrative, technical, or material support: Yuanyuan Xiao, Zixu Huang, Zongming Song. All authors read and approved the final manuscript.

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Figures

Figure 1

Clinical manifestations of two families with FEVR. (A) FFA manifestation of proband F1-II:1 demonstrated retinal leakage, retinal avascularization, straightened vessels, and increased numbers of vessels in the peripheral retina in both eyes. White arrows indicate peripheral retinal leakage. (B) Fundus appearance of proband F1-II:1. Fundus appearance of both eyes demonstrated increased numbers of peripheral retinal vessels with straightened morphology; fundus appearance of the left eye also showed falciform retinal folds and peripheral retinal exudates. White arrows indicate falciform retinal folds and black arrows indicate peripheral retinal exudates. (C) FFA findings were normal in the father of proband F1-II:1. (D) FFA examination of the mother of proband F1-II:1 showed peripheral retinal avascularization in both eyes and mild peripheral retinal leakage in the left eye. White arrows indicate peripheral retinal avascularization. (E) FFA examination of proband F2-II:1 demonstrated increased numbers of peripheral retinal vessels and peripheral retinal leakage in both eyes, as well as post-vitrectomy appearance in the right eye. White arrows indicate peripheral retinal leakage. Abbreviations: OD, right eye, OS, left eye.

Figure 2

Sequencing and in silico analysis results of two families. (A) Sanger sequencing chromatographs of two families. Proband F1-II:1 and his mother carried the c.236T>G variant in the *TSPAN12* gene, whereas his father did not carry the c.236T>G variant. Proband F2-II:1 carried c.1210G>A and c.1612C>T variants in the *LRP5* gene. Arrows denote mutant bases. (B) Pedigrees of two families. Circles denote unaffected females, boxes denote unaffected males, dark symbols indicate affected participants, and arrows denote probands. (C) Schematic representations of genomic structures of *TSPAN12* and *LRP5*. Variant c.236T>G was located in exon 4 of the *TSPAN12* gene, while variants c.1210G>A and c.1612C>T were located in exons 6 and 8 of the *LRP5* gene, respectively. Numbers below diagrams indicate corresponding exon numbers, parts of exons are omitted. (D) Schematic representations of protein structures of *TSPAN12* and *LRP5*. Variant c.236T>G p. (Met79Arg) was located in the transmembrane domain of the *TSPAN12* protein. Blue band denotes transmembrane domain, pink band denotes the first (small) extracellular loop, yellow band denotes intracellular loop, and red band denotes the second (large) extracellular loop. Both c.1210G>A p. (Gly404Arg) and c.1612C>T p. (Arg538Trp) variants were located in the second β -propeller motif of the *LRP5* protein. Yellow band denotes signal peptide, pink bands denote β -propeller motifs, red bands denote epidermal growth factor repeats, green bands denote low density lipoprotein receptor-like repeats, and blue band denotes transmembrane domain. (E) Evolutionary conservation of the three variants among different species. Residues affected by these variants are highly conserved. The box indicates non-conserved amino acid residues.

Supplementary Files

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