Ocular Features and Mutation Spectrum of Patients With Familial Exudative Vitreoretinopathy

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Purpose. To investigate the clinical findings in Chinese patients diagnosed with familial exudative vitreoretinopathy (FEVR) and carrying pathogenic mutations.

Methods. One hundred twenty unrelated patients with FEVR were enrolled in this study. Genomic DNA and ophthalmic examinations were collected from all the patients and their available relatives. Targeted next-generation sequencing was performed to detect mutations. In silico programs were used to evaluate the pathogenicity of all the mutations.

Results. Eighty identified mutations were found in 81 unrelated patients (31/81 in *LRP5*, 25/81 in *FZD4*, 12/81 in *TSPAN12*, 8/81 in *NDP*, 4/81 in *KIF11*, and 1/81 in *ZNF408*). Among those mutations, 53 were novel (23/35 in *LRP5*, 15/21 in *FZD4*, 8/11 in *TSPAN12*, 3/8 in *NDP*, 3/4 in *KIF11*, 1/1 in *ZNF408*). Patients with *LRP5, FZD4*, *TSPAN12*, or *NDP* mutations were mainly classified into stage 4 and stage 5 and one-half of patients with *KIF11* mutations were in stage 4. In addition, all the patients in *NDP* group were found to have bilateral symmetry in FEVR stage.

Conclusions. Our results present profound phenotypic variability and a wide mutation spectrum of FEVR in the Chinese population, which could be useful for a precise and comprehensive genetic diagnosis for patients with FEVR in the future.

Keywords: familial exudative vitreoretinopathy, inheritance, ocular manifestation, retinal vascular development

Familial exudative vitreoretinopathy (FEVR) is an inherited retinal disease characterized by incomplete development of retinal vessel and abnormal neovascularization, which was first reported by Criswick and Schepens in 1969.[1] The retinal vascular anomalies in FEVR could result in several secondary changes, including fibrovascular proliferation, vitreous hemorrhage, retinal folding, vitreoretinal traction, macular dragging, and partial or total retinal detachment. The clinical manifestation of FEVR varies widely, ranging from no visual impairment to total blindness, and the severity of ocular symptoms could differ within one family harboring the same mutation or between bilateral eyes of the same individual.[2]

FEVR is clinically and genetically heterogeneous, and it has three inherited forms: autosomal dominant, autosomal recessive, and X-linked recessive. To date, variants in several genes have been identified as causative for FEVR development, including *FZD4*, *LRP5*, *NDP*, *TSPAN12*, *KIF11*, and *ZNF408*. The proteins encoded by the first four genes have been reported to link to the Norrin/β-catenin signaling pathway, which is crucial for the retinal vascular formation during eye development.[3,4] KIF11 is a kinesin family member motor protein localized to spindle microtubules, which is involved in mitotic progression, and its lack could result in severely stunted growth of the retinal vessels.[5] The ZNF408 protein belongs to the family of zinc fingers, and the *ZNF408* mutation leads to retinal vascularization defects and abnormal trunk vascularization in the zebrafish model.[6]

To date, several studies have attempted to explore the genotype–phenotype correlations in Chinese patients with FEVR. Despite the complex relationship, some trends for FEVR have been reported. Wang et al.[7] observed that nearly one-half of patients with *FZD4* mutations showed stage 5 and more than one-half of them displayed asymmetric symptoms, indicating a large-scale degree of phenotypic severity in *FZD4* mutation. Rao et al.[8] found that patients with *LRP5* mutations exhibited broader phenotypic spectrum varying from stage 2 to stage 5, and all patients with *NDP* or truncating mutations were in stage 4 or worse. In addition, patients with FEVR with *NDP*, *TSPAN12*, or *KIF11* mutations seemed to have symmetrical retinopathy bilaterally, but a greater frequency of asymmetry was found in patients with *LRP5* and *FZD4* mutations.[9] Interestingly, Li et al.[10] reported that the phenotype in patients with digenic variants tended to be worse than that with monogenic variants of FEVR-associated genes.

Here, we screened for pathogenic mutations in six genes (*FZD4*, *LRP5*, *NDP*, *TSPAN12*, *KIF11*, and *ZNF408*) in 120

unrelated Chinese patients with FEVR, and then investigated and analyzed their ocular manifestations according to the mutation spectrum.

Methods

Patients

We recruited 120 patients with FEVR from the Department of Ophthalmology at Peking University People's Hospital. Written informed consent was obtained from all participants or parents on behalf of child participants. The collection of clinical and genetic data was approved by the patients and their families. This study was approved by the ethical committee of Peking University People's Hospital and adhered to the tenets of the Declaration of Helsinki. The diagnosis of FEVR was based on the clinical criteria described previously.[11] Patients with a history of premature birth, systemic abnormalities, and ocular trauma were excluded.

Clinical Examinations

Comprehensive ophthalmic examinations, such as indirect ophthalmoscopy, color fundus photography, fluorescein fundus angiography, ocular B-scan ultrasound examinations, and optical coherence tomography, were obtained from all patients and their family members where available. Data for sex, age, and family history were recorded, and ocular symptoms and medical history were reviewed. The severities of the affected eyes were assessed following the clinical classification of FEVR as previously described.[12]

Molecular Analysis

Peripheral blood samples were drawn from all patients and their available relatives, and genomic DNA was extracted using the QIAamp DNA Blood Kit (Qiagen, Germany) and fragmented to generate 350 to 400 bp products. DNA fragments were amplified by PCR and allowed to hybridize with DNA capture probes, which were designed for the targeted genes. The DNA products were eluted and amplified again, and targeted next–generation sequencing was performed using the Illumina HiSeq 2000 (Illumina, Inc., San Diego, CA). A custom-inherited retinal diseases panel based on targeted exome capture technology was previously established and covered the known FEVR genes: *FZD4*, *LRP5*, *NDP*, *TSPAN12*, *KIF11*, and *ZNF408*.

The possible pathogenicity of missense variants was further estimated by using SIFT (http://sift.jcvi.org), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org) online algorithms and via evolutionary conservation analysis.

The Genome Aggregation database (http://gnomad-sg.org/) and the Exome Aggregation Consortium database (http://exac.broadinstitute.org/faq) were used to evaluate the minor allele frequencies in study participants. In addition, identified variants were also evaluated regarding pathogenicity according to the standards and guidelines of American College of Medical Genetics and Genomics.[13]

Analysis of FEVR-Related Studies

We collected the FEVR-related literature on Chinese patients to analyze the spectrum of *FZD4*, *LRP5*, *NDP*, *TSPAN12*, *KIF11*, and *ZNF408* genes. This review process was performed using PubMed and Web of Science for an extensive search. Additionally, related articles and their references were screened to obtain additional and potentially eligible information.

Results

In this study, 81 of 120 patients with FEVR were detected to harbor mutations; 21 were females and 60 were males. The average patients age was 10.64 years (range, 4–40 years). Among the patients carrying mutations (Fig. 1), *LRP5* accounted for the greatest proportion (31/120 [25.83%]), followed by *FZD4* (25/120 [20.83%]), *TSPAN12* (12/120 [10.00%]), *NDP* (8/120 [6.67%]), *KIF11* (4/120 [3.33%]), and *ZNF408* (1/120 [0.83%]). In addition, we retrospectively reviewed reports on Chinese patients with FEVR in the past 5 years,[8,9,14–17] focusing on the mutation spectrum shown in Supplementary Table S1 (number of genes, ≥3). The largest cohort of patients with FEVR collected and screened for genetic analysis by a single clinic in the Chinese literature was in 2018. The age of patients with FEVR ranged from 0 to 56 years, and the percentage of patients with detected variants ranged from 23.00% to 67.40%. Among these domestic reports, the frequency of *LRP5* mutations ranged from 10.00% to 25.93%, *FZD4* mutations ranged from 6.45% to 21.35%, *TSPAN12* mutations ranged from 3.23% to 12.90%, *NDP* mutations ranged from 4.11% to 9.68%, and *KIF11* mutations ranged from 1.61% to 6.74%. One study found the *ZNF408* mutations were identified in 1.80% of patients with FEVR.

Mutations in the *FZD4* Gene

We found 21 mutations in *FZD4* gene from the 25 probands. Of these mutations, six were previously reported[2,18–22] and 15 were novel. The mutations included 15 missense mutations, 2 nonsense mutations, and 4 frameshift mutations (Table 1). The most frequently encountered mutations in *FZD4* was c.205C>T; p.H69Y, detected in five patients (5/25 [20.00%]), followed by the two mutations c.1282_1285delGACA; p.D428Sfs*2 and c.313A>G; p.M105V, each found in three (3/25 [12.00%]) and two patients (2/25 [8.00%]) respectively. In addition, two mutations occurred in the signal sequence portion (Supplementary Fig. S1), three were located in the cysteine-rich domain, one was located in upstream of the first transmembrane domain, 14 were located in the seven transmembrane domains (TMDs), and one was located in KTXXXW motif of the intracellular domain (ICD).

Mutations in the *LRP5* Gene

We identified 35 mutations of *LRP5* gene in the 31 probands; 12 mutations had been reported,[8,15,23–28] and 23 mutations were newly detected. The *LRP5* mutations included 27 missense mutations, 3 nonsense mutations, 2 splicing mutations, 2 duplications, and 1 frameshift mutation (Table 2). The duplication c.55_60dupCTGCTG; p.19_20dulLL was detected in three patients (3/31 [9.68%]), three mutations c.58_60dupCTG; p.20dupL, c.1330C>T; p.R444C and c.4643G>T; p.C1548F, were each found in two patients (2/31 [6.67%]), it is noteworthy that the minor allele frequencies of c.58_60dupCTG; p.20dupL was 0.153 (>10%) in ExAC, and it was classified as

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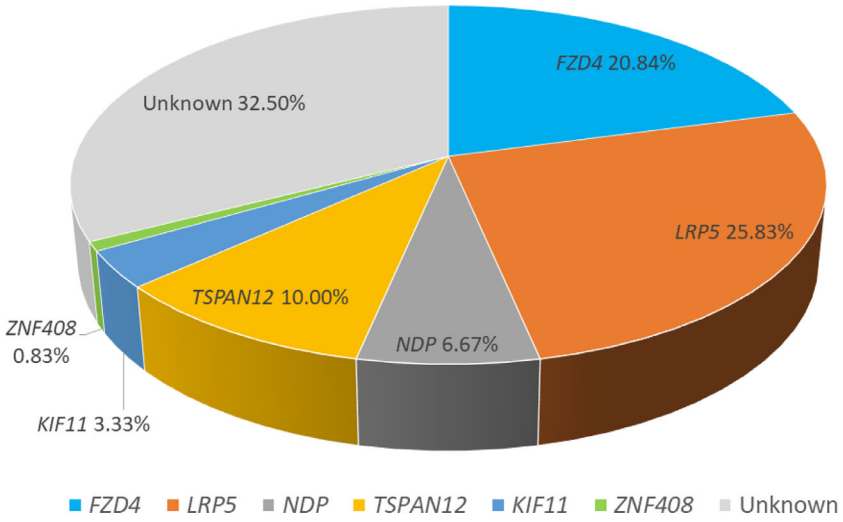

Figure 1. Mutation spectrum of *FZD4*, *LRP4*, *NDP*, *TSPAN12*, *KIF11*, and *ZNF408* in 120 Chinese patients with FEVR.

Table 1. Identified Variants in *FZD4* Gene of Patients With FEVR

Patient ID	Sex	Age	Stage (OD/OS)	Gene	Nucleotide Changes	Protein Changes	SIFT	PP-2	MT	ACMG	ExAC	gnomAD	Reference
P01	F	9	4/4	*FZD4*	c.40_49delCCCGGGGGCG	p.P14Sfs*44	–	–	DC	P	–	0.00000426	Reported
P02	F	7	4/5	*FZD4*	c.42_43delCG	p.G16Rfs*113	–	–	DC	P	–	–	Novel
				FZD4	c.1300A>G	p.M434V	D	PbD	DC	LP	–	–	Novel
P03	M	10	4/4	*FZD4*	c.205C>T	p.H69Y	D	B	DC	U	0.00056200	0.00022400	Reported
P04	F	16	1/1										
P05	M	9	4/3										
P06	M	12	2/2										
P07	M	23	4/1										
P08	M	14	4/2	*FZD4*	c.313A>G	p.M105V	T	PsD	DC	LP	0.00001670	0.00002400	Reported
P09	M	16	4/4										
P10	M	15	4/4	*FZD4*	c.341T>C	p.I114T	D	PsD	DC	LP	–	–	Reported
P11	M	16	4/4	*FZD4*	c.631T>C	p.Y211H	D	B	DC	U	–	0.00002390	Novel
				FZD4	c.1030A>C	p.S344R	D	PbD	DC	U	–	–	Novel
				FZD4	c.1031G>C	p.S344T	D	PbD	DC	U	–	–	Novel
P12	M	12	2/2	*FZD4*	c.684C>A	p.S228R	D	PsD	DC	LP	–	–	Novel
P13	M	5	Normal eye/4	*FZD4*	c.686T>C	p.L229P	D	PbD	DC	LP	–	–	Reported
P14	F	6	4/4	*FZD4*	c.694A>G	p.I232V	T	B	DC	U	–	–	Novel
P15	M	10	4/5	*FZD4*	c.733T>A	p.S245T	T	B	DC	U	–	–	Novel
P16	F	11	1/3	*FZD4*	c.807T>G	p.Y269*	–	–	DC	LP	–	–	Novel
P17	M	8	5/5	*FZD4*	c.877A>G	p.I293V	T	B	DC	LP	–	–	Novel
P18	F	16	4/4	*FZD4*	c.983T>C	p.F328S	D	PbD	DC	U	–	–	Novel
P19	M	10	3/4	*FZD4*	c.1282_1285delGACA	p.D428Sfs*2	–	–	DC	P	0.00000824	0.00001060	Reported
P20	M	9	5/5										
P21	F	11	Artificial eye/5										
P22	M	9	5/1	*FZD4*	c.1328T>C	p.L443P	D	PbD	DC	U	–	–	Novel
P23	M	12	5/2	*FZD4*	c.1387delG	p.A463Hfs*17	–	–	DC	LP	–	–	Novel
P24	F	11	1/5	*FZD4*	c.1482G>A	p.W494*	–	–	DC	LP	–	–	Novel
P25	M	16	1/5	*FZD4*	c.1502T>C	p.L501P	D	PbD	DC	U	–	–	Novel

ACMG, American College of Medical Genetics and Genomics; B, benign; D, damaging; DC, disease causing; LP, likely pathogenic; MT, mutation taster; PbD, probably damaging; Pm, polymorphism; PP-2, Polyphen-2; P, pathogenic; PsD, possibly damaging; T, tolerated; U, uncertain.

a benign mutation. There were nine patients who harbored two heterozygous mutations in *LRP5* gene, one mutation was inherited from the mother and the other one from the father. In addition, 2 mutations were in the signal peptide, 26 mutations occurred in the 4 tandem YWTD-type β-propeller (BP) domains (six in BP1, 12 in BP2, five in BP3, and three in BP4), two were located in the LDLR type A domains, and three were located in the ICD (Supplementary Fig. S2).

Mutations in the *NDP* Gene

We detected eight mutations of *NDP* gene in the eight probands. Among these mutations, five were previously reported[15,29–31] and three were novel. The *NDP* mutations included three missense mutations, four nonsense mutations, and one deletion (Table 3). Only one mutation c.22G>C; p.A8P was located in the signal peptide, the other seven mutations were located in the C-terminal cysteine knot domain (Supplementary Fig. S3).

Table 2. Identified Variants in *LRP5* Gene of Patients With FEVR

Patient ID	Sex	Age	Stage (OD/OS)	Gene	Nucleotide Changes	Protein Changes	SIFT	PP-2	MT	ACMG	ExAC	gnomAD	Reference
P29	M	40	4/4	*LRP5*	c.55_60dupCTGCTG	p.19_20dupLL	–	–	Pm	U	0.00218000	0.00125000	Reported
P30	M	8	2/4										
P26*	F	10	4/4	*LRP5*	c.55_60dupCTGCTG	p.19_20dupLL	–	–	Pm	U	0.00218000	0.00125000	Reported
				LRP5	c.1294T>G	p.W432G	D	PbD	DC	LP	–	–	Novel
P27	M	13	2/4	*LRP5*	c.58_60dupCTG	p.20dupL	–	–	Pm	B	0.15300000	0.08750000	Reported
P28†	M	7	2/2	*LRP5*	c.58_60dupCTG	p.20dupL	–	–	Pm	B	0.15300000	0.08750000	Reported
				LRP5	c.3246C>G	p.Y1082*	–	–	DC	LP	–	–	Novel
P31	M	9	5/5	*LRP5*	c.107T>C	p.L36P	D	PbD	DC	LP	–	–	Novel
P32	M	8	5/5	*LRP5*	c.235T>G	p.W79G	D	PbD	DC	LP	–	–	Novel
P33	M	5	3/4	*LRP5*	c.280C>A	p.Q94K	T	B	DC	U	–	–	Novel
P34	M	13	4/4	*LRP5*	c.542T>C	p.M181T	D	PbD	DC	LP	–	–	Novel
P35‡	M	26	5/5	*LRP5*	c.676G>A	p.G226S	D	PbD	DC	LP	–	–	Novel
				LRP5	c.1123G>A	p.A375T	D	PsD	DC	LP	0.00002480	0.00000796	Reported
P36	F	10	4/4	*LRP5*	c.1057C>T	p.R353W	D	PbD	DC	LP	0.00001670	0.00000399	Novel
P37§	F	10	5/5	*LRP5*	c.1133T>C	p.I378T	D	PsD	DC	LP	–	–	Novel
				LRP5	c.2422G>A	p.D808N	D	PsD	DC	LP	0.00000832	0.00000399	Novel
P38‖	M	7	4/5	*LRP5*	c.1145C>T	p.P382L	D	PbD	DC	P	–	0.00000398	Reported
				LRP5	c.1364C>T	p.S455L	T	PbD	DC	P	–	0.00000401	Reported
P39¶	M	9	5/5	*LRP5*	c.1148T>C	p.L383P	D	PsD	DC	LP	–	–	Novel
				LRP5	c.4105_4106delAT	p.M1369Vfs*2	–	–	DC	LP	0.00000826	0.00000399	Novel
P40	M	15	4/4	*LRP5*	c.1199C>T	p.A400V	T	B	DC	U	0.00029800	0.00013800	Novel
P41	M	8	4/5	*LRP5*	c.1330C>T	p.R444C	D	PsD	DC	LP	0.00002500	0.00000657	Reported
P42	M	10	3/4										
P43#	F	10	4/4	*LRP5*	c.1349G>A	p.R450H	D	PbD	DC	P	–	0.00000400	Reported
				LRP5	c.3245A>G	p.Y1082C	D	PbD	DC	LP	0.00008340	0.00007480	Novel
P44	M	8	5/5	*LRP5*	c.1385G>A	p.R462Q	D	PbD	DC	U	0.00000848	0.00000804	Novel
P45	M	7	3/2	*LRP5*	c.2237G>A	p.R746Q	D	PbD	DC	LP	0.00003320	0.00000657	Reported
P46**	M	6	4/4	*LRP5*	c.2488T>C	p.S830P	D	PbD	DC	LP	–		Novel
				LRP5	c.479C>T	p.P160L	D	PbD	DC	LP	0.00000848	0.00000413	Novel
P62	F	7	1/1	*LRP5*	c.2512C>T	p.R838W	D	PbD	DC	U	0.00000828	0.00000803	Novel
P47	M	7	4/4	*LRP5*	c.2555C>T	p.T852M	D	PbD	DC	LP	–	0.00001310	Reported
P48	F	8	2/5	*LRP5*	c.3237–2A>G	–	–	–	–	U	–	–	Novel
P49	M	7	4/Normal eye	*LRP5*	c.3361A>G	p.N1121D	T	B	DC	LP	0.00066500	0.00039500	Reported
P50	M	7	2/1	*LRP5*	c.3901G>A	p.A1301T	T	B	Pm	U	0.00018500	0.00013100	Novel
P51	M	14	4/4	*LRP5*	c.4488+5G>C	–	–	–	–	U	–	–	Novel
P52	M	11	5/5	*LRP5*	c.4600C>T	p.R1534*	–	–	DC	P	–	–	Novel
P53,	M	11	4/4	*LRP5*	c.4643G>T	p.C1548F	D	PbD	DC	LP	0.00028400	0.00019700	Reported
P54	M	10	2/4										
P55††	F	10	4/4	*LRP5*	c.4670G>A	p.W1557*	–	–	DC	P	–	–	Novel
				LRP5	c.1378G>A	p.E460K	D	PbD	DC	P	–	0.00000657	Reported

ACMG, American College of Medical Genetics and Genomics; B, benign; D, damaging; DC, disease causing; LP, likely pathogenic; MT, mutation taster; PbD, probably damaging; Pm, polymorphism; PP-2, Polyphen-2; P, pathogenic; PsD, possibly damaging; T, tolerated; U, uncertain.

* P26 inherited the mutation c.55_60dupCTGCTG; p.19_20dupLL from her father, who had no signs of FEVR, the c.1294T>G; p.W432G was inherited from her mother, whose information was unavailable.

† P28 inherited the mutation c.58_60dupCTG; p.20dupLL from his father, and the mutation c.3246C>G; p.Y1082* was inherited from his mother; neither parent had signs of FEVR.

‡ P35 inherited the mutation c.676G>A; p.G226S from his father, and inherited the mutation c.1123G>A; p.A375T from his mother; neither parent had signs of FEVR.

§ P37 inherited the mutation c.1133T>C; p.I378T from her father, and inherited the mutation c.2422G>A; p.D808N from her mother, neither parents had signs of FEVR.

‖ P38 inherited the mutation c.1364C>T; p.S455L from his father, and inherited the mutation c.1145C>T; p.P382L from his mother; neither parent had signs of FEVR.

¶ P39 inherited the mutation c.4105_4106delAT; p.M1369Vfs*2 from his father, who had no signs of FEVR, the c.1148T>C; p.L383P was inherited from his mother, who had stage 1 of FEVR.

P43 inherited the mutation c.1349G>A; p.R450H from her father, and inherited the mutation c.3245A>G; p.Y1082C from her mother; neither parent had signs of FEVR.

** P46 inherited the mutation c.479C>T; p.P160L from his father, and inherited the mutation c.2488T>C; p.S830P from his mother; neither parent had signs of FEVR.

†† P55 inherited the mutation c.1378G>A; p.E460K from her father, and inherited the mutation c.4670G>A; p.W1557* from her mother; neither parent had signs of FEVR.

Mutations in the *TSPAN12* Gene

We identified 11 mutations of the *TSPAN12* gene in the 12 probands; three mutations had been reported[14,15,32] and nine mutations were novel. There were six missense mutations, two nonsense mutations, one frameshift mutation, and one splicing mutation in *TSPAN12* (Table 3). The mutation c.765G>T; p.P255P, which was detected in P75 and P76, had a minor allele frequency of 0.803 (>10%) in ExAC and was known as a common single nucleotide polymorphism without pathogenicity/ Additionally, five mutations were located in the TMDs, three mutations were located in the large extracellular loop (ECL-2), and two mutations occurred in the ICD (Supplementary Fig. S4).

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Table 3. Identified Variants in *NDP*, *TSPAN12*, *KIF11*, and *ZNF408* Genes of Patients With FEVR

Patient ID	Sex	Age	Stage (OD/OS)	Gene	Nucleotide Changes	Protein Changes	SIFT	PP-2	MT	ACMG	ExAC	gnomAD	Reference
P56	M	22	4/4	*NDP*	c.22G>C	p.A8P	D	PbD	DC	LP	–	–	Reported
P57	M	4	5/5	*NDP*	c.239_241delCGT	p.80_81delSF	–	–	DC	LP	–	–	Novel
P58	M	4	4/4	*NDP*	c.268C>T	p.R90C	D	PbD	DC	LP	–	–	Reported
P59	M	4	4/4	*NDP*	c.325C>T	p.R109*	–	–	DC	P	–	–	Reported
P60	M	6	5/5	*NDP*	c.343C>T	p.R115*	–	–	DC	P	–	–	Reported
P61	M	9	5/5	*NDP*	c.358T>G	p.Y120D	D	PbD	DC	U	–	–	Novel
P63	M	9	5/5	*NDP*	c.384C>A	p.128C*	–	–	DC	P	–	–	Reported
P64	M	12	2/2	*NDP*	c.388G>T	p.E130*	–	–	DC	LP	–	–	Novel
P65	M	12	5/5	*TSPAN12*	c.95delC	p.S32Lfs*4	–	–	DC	LP	–	–	Novel
P66	F	33	2/1	*TSPAN12*	c.194C>T	p.P65L	T	B	DC	LP	–	0.00000658	Reported
P67	F	10	4/4	*TSPAN12*	c.232G>A	p.G78R	D	PbD	DC	LP	–	–	Novel
P68	M	10	4/4	*TSPAN12*	c.352G>T	p.E118*	–	–	DC	P	–	–	Reported
P69	F	10	4/Normal eye	*TSPAN12*	c.361–2A>G	–	–	–	–	P	–	–	Novel
P70	M	8	5/5	*TSPAN12*	c.559C>A	p.P187T	T	B	DC	U	–	–	Novel
P71	M	12	5/5	*TSPAN12*	c.617G>T	p.C206F	D	PbD	DC	U	–	–	Novel
P72	F	13	1/4	*TSPAN12*	c.689T>A	p.I230N	D	PbD	DC	LP	–	–	Novel
P73	M	4	4/4	*TSPAN12*	c.689T>C	p.I230T	D	PbD	DC	U	0.00002470	0.00002630	Novel
P74	F	6	4/4	*TSPAN12*	c.738G>A	p.W246*	–	–	DC	LP	–	–	Novel
P75,	M	16	1/4	*TSPAN12*	c.765G>T	p.P255P	T	–	Pm	B	0.80300000	0.00000658	Reported
P76	F	6	2/1										
P77	M	4	4/4	*KIF11*	c.308+1G>A	–	–	–	–	P	–	–	Reported
P78	M	8	2/2	*KIF11*	c.388–2A>G	–	–	–	–	U	–	–	Novel
P79	M	4	4/4	*KIF11*	c.1349_1353delGTAAA	p.C450Ffs*2	–	–	DC	P	–	–	Novel
P80	M	7	2/3	*KIF11*	c.2268–4A>G	–	–	–	–	U	–	0.00000657	Novel
P81	M	5	3/4	*ZNF408*	c.1102C>A	p.L368I	D	PbD	DC	U	–	–	Novel

ACMG, American College of Medical Genetics and Genomics; B, benign; D, damaging; DC, disease causing; LP, likely pathogenic; MT, mutation taster; PbD, probably damaging; Pm, polymorphism; PP-2, Polyphen-2; P, pathogenic; PsD, possibly damaging; T, tolerated; U, uncertain.

Mutations in the *KIF11* Gene

We found one reported splicing mutation c.308+1G>A,[15] two novel splicing mutations, c.388–2A>G and c.2268–4A>G, and one novel frameshift mutation c.1349_1353delGTAAA; p.C450Ffs*2 in *KIF11* gene from four probands (Table 3). The frameshift mutation was located in the downstream of kinesin motor domain, which is responsible for proper function of the KIF11 protein.

Mutation in the *ZNF408* Gene

There is only one novel missense *ZNF408* mutation, c.1102C>A; p.L368I, detected in a 5-year-old boy, who displayed bilateral retinal detachment, and he inherited this heterozygous mutation from his mother. The mutated 368 amino acid, which located in the first zinc finger of ZNF408 protein, was conserved among various vertebrates (Supplementary Fig. S5) and predicted to be pathogenic by three in silico programs (Table 3).

Clinical Presentation of Patients With Identified Mutations

Table 4 shows the FEVR stage of patients with mutations in six genes. Most of patients in *FZD4* (21/25, 84.00%), *LRP5* (27/31, 87.10%), *NDP* (7/8, 87.50%), or *TSPAN12* (10/12, 83.33%) group had severe retinopathy (stages 4–5), and patients with *FZD4* mutations presented relatively wider phenotypes that varied from stage 1 to stage 5. One-half of the patients (2/4 [50.00%]) with *KIF11* mutations and one patient carrying *ZNF408* mutation had in stage 4 disease.

Nearly one-half of patients in *FZD4* (12/25 [48.00%]) group presented symmetry of staging (Table 4), while more than one-half of patients with *LRP5* (20/31 [64.52%]), *TSPAN12* (7/12 [58.33%]), or *KIF11* (3/4 [75.00%]) mutations displayed bilateral symmetry. It is noteworthy that all patients with *NDP* (8/8 [100.00%]) mutations showed symmetrical FEVR stage between eyes. Only unilateral FEVR was found in three patients carrying *FZD4*, *LRP5*, and *TSPAN12* mutation, accounting for 3.70% (3/81) of all the cases with identified mutations.

Table 4. Patients at Five Different Stages of FEVR in Different Gene Groups

Stage*	Total	*FZD4*	*LRP5*	*NDP*	*TSPAN12*	*KIF11*	*ZNF48*	Number With Identified Mutation
1	4	1	1	0	0	0	0	2 (50.00%)
2	11	2	2	1	2	1	0	8 (72.73%)
3	4	1	1	0	0	1	0	3 (75.00%)
4	56	12	17	3	7	2	1	42 (75.00%)
5	45	9	10	4	3	0	0	26 (57.78%)
Total	120	25	31	8	12	4	1	81 (67.50%)

* The severity of patients was determined by the highest stage of FEVR in either eye.

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Discussion

The current study comprehensively screened six known genes (*FZD4*, *LRP5*, *NDP*, *TSPAN12*, *KIF11*, and *ZNF408*) in 120 unrelated patients with FEVR, and uncovered 78 of 120 patients (65.00%), except for three cases carrying the benign variants; this percentage in our study is similar to that of that of Wang et al.,[9] who reported that 67.5% of probands had genetic confirmed FEVR.

We found that up to 76 patients (63.33%) were detected to harbor mutations in Norrin/β-catenin signaling genes, including *FZD4*, *LRP5*, *NDP*, and *TSPAN12*; the proteins encoded by these genes are found to had intense interaction with each other, and mechanisms for these cooperative proteins in retinal vascular formation have been explored in recent years.[3,4,33] Norrin acts as a ligand, it could bind to FZD4 receptor or LRP5 coreceptor specifically and form a ternary complex; this process is meditated by TSPAN12 selectively. Then, a downstream β-catenin signaling is initiated, the increasing cytoplasmic β-catenin is translocated to the nucleus and interacts with T-cell factor or lymphoid enhancing factor, resulting in RNA transcription and elongation consequently. In this study, we found that patients with *FZD4*, *LRP5*, *NDP*, and *TSPAN12* mutations were mainly classified into stages 4 and 5 (Table 4), suggesting that Chinese patients with FEVR carrying mutations in the Norrin/β-catenin signaling genes tended to exhibit severe retinopathy.

FZD4 works as the receptor for Wnt or Norrin, and it mainly consists of signal sequence, cysteine-rich domain, seven TMDs, and ICD, which many *FZD4* mutations occur in these functional areas. Compared with the previous studies for *FZD4*-mutated patients from Northern America,[34–37] the detection rates of identified mutations in each area of FZD4 protein was different. Our study found that mutations located in TMDs accounting for 66.67%, whereas the cysteine-rich domain was the most frequently mutated domain in the Northern American population (48.00%), suggesting that the genotypic spectrum for *FZD4* gene in Chinese patients might differ from that of other populations. We also noticed that three *FZD4* mutations, including c.205C>T; p.H69Y, c.1282_1285delGACA; p.D428Sfs*2, and c.313A>G; p.M105V, had been reported as a hotspot in several studies.[7,8,15,36,38] There were five, three, and two patients of this study harboring the three mutations, but the reason for their high frequency in *FZD4* mutations remained unclear. In addition, four patients carrying mutations c.1328T>C; p.L443P, c.1387delG; p.A463Hfs*17, c.1482G>A; p.W494*, and c.1502T>C; p.L501P, which were located in a highly conserved motif (KTXXXW) and its upstream, showed a difference of three or more grades between bilateral eyes (Fig. 2). The KTXXXW motif was important for the activation of canonical Wnt signaling pathway, membrane relocalization, and phosphorylation of Dishevelled,[39,40] although we were unable to determine how the four mutations affect their phenotypes. This finding indicated that *FZD4* mutations, which were considered to cause abnormality of KTXXXW, might result in asymmetric severe retinopathy of patients with FEVR.

LRP5 belongs to the low-density lipoprotein receptor family and interacts with FZD4 synergistically to bind Norrin/Wnt ligands, forming a functional complex to trigger the Norrin/β-catenin or the Wnt signaling pathway. There were 35 mutations in *LRP5* identified from 31 patients, accounting for the greatest proportion in our study. This result is consistent with those of Rao et al.[8] and Li et al.[15] However, some studies have reported the greatest involvement in the *FZD4* gene.[7,9,14,16] Considering the cooperative relationship between FZD4 and LRP5 proteins, a greater number of cases was required to validate whether *FZD4* or *LRP5* accounts for the most frequently gene of FEVR in Chinese population.

Norrin is a secreted signaling factor with the characteristics of autocrine or paracrine, and is constitutively expressed by Müller cells of the retina.[41] This protein contains a signal peptide and a highly conserved C-terminal cysteine knot domain. The known mutation c.22G>C; p.A8P detected in P56 affected the directing localization for Norrin; the other eight mutations were in the C-terminal cysteine knot and might impact its function. In our study, all patients carrying mutations in *NDP* had bilateral symmetry, and most of them (7/8 [87.50%]) had stage 4 or stage 5 disease, highlighting the higher frequency of symmetric severe retinopathy in *NDP* phenotypes. This finding is similar to that of Wang et al.,[9] who suggested that patients with *NDP* mutations might be likely to exhibited symmetrical and severe disease stage between eyes.

The *TSPAN12* gene encodes a 305 amino acid protein, which is the member of the Tetraspanin family. TSPAN12 is involved in the retinal vascular development by promoting the Norrin/β-catenin but not the Wnt/β-catenin signaling pathway.[4] In our study, 6 of 11 mutations in *TSPAN12* were located in the second extracellular loop (ECL-2) and its upstream, and this extracellular loop is responsible for TSPAN12 interacting with FZD4/Norrin and meditating the FZD4 ligand selectivity.[33] Therefore, these *TSPAN12* mutations were considered to affect its function in the activation of Norrin/β-catenin signaling. In addition, the ratio of female-to-male patients in the *TSPAN12* group (6:6) was relatively higher than that of the *FZD4* (8:17), *LRP5* (7:24), or *NDP* (0:8) groups, indicating a high percentage of female individuals among the patients with *TSPAN12* mutations.

KIF11 and *ZNF408* are newly recognized FEVR-associated genes, which are required for mitotic spindle assembly and high affinity DNA binding, respectively,[5,6] and the mechanisms for *KIF11* or *ZNF408* mutations causing abnormal retinal vascularization were independent of Norrin/β-catenin signaling pathway. Here, we detected four *KIF11* mutations and one *ZNF408* mutation from five patients, which each accounted for 3.33% and 0.83% of the cohort, respectively. To date, only a small number of mutations in *KIF11* and *ZNF408* has been reported from patients with FEVR, and the roles of these two proteins in retinal vascular development require further investigation to uncover the involvement of KIF11 or ZNF408 in the pathogenesis of FEVR. In a domestic study of 389 Chinese patients, 8 patients were detected to carry 9 *ZNF408* mutations,[15] which might be the largest sample size of patients with FEVR with *ZNF408* mutations. The ZNF408 protein, which was altered in P81, is composed of 10 Zinc fingers domains with different and still unclear cellular functions; variants in different domains could affect the interaction of ZNF408 with specific targets, thus leading to the dysregulation of different target genes and subsequently are underlying either FEVR or retinitis pigmentosa.[42] Owing to the lack of evidence supporting the pathogenicity of mutation c.1102C>A; p.L368I in FEVR, our study tentatively classified it as a variant with unknown significance.

In conclusion, our study expanded the mutation spectrum of Chinese patients with FEVR, thus contributing to

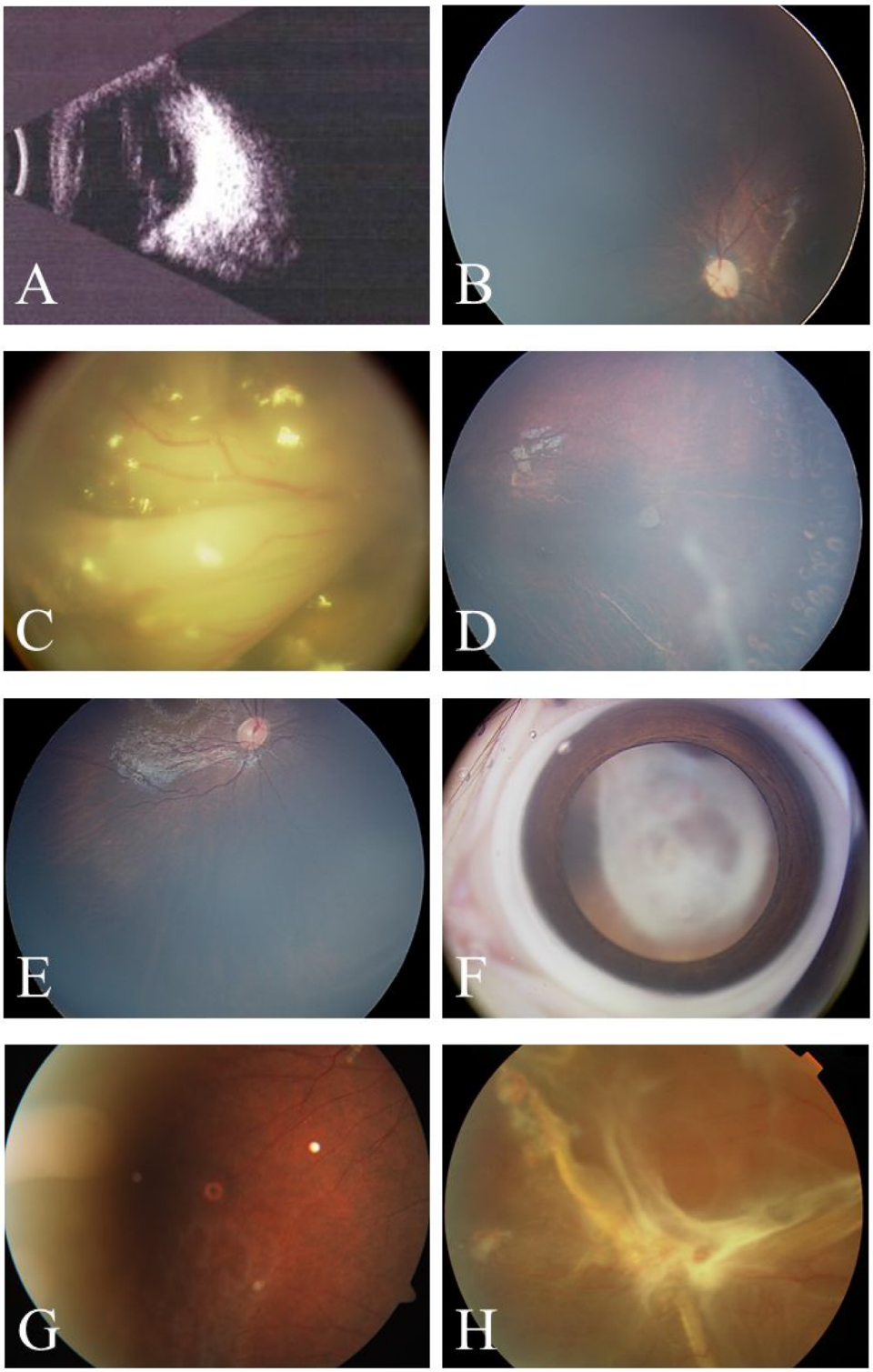

Figure 2. Ophthalmic examinations of four probands who carried four *FZD4* mutations c.1328T>C; p.L443P, c.1387delG; p.A463Hfs*17, c.1482G>A; p.W494*, and c.1502T>C; p.L501P. (**A**, **B**) Ocular B-scan ultrasound and color fundus photography (CFP) showed the stage 5 of right eye and the stage 1 of left eye in P22 respectively. (**C**, **D**) CFP showed the stage 5 of right eye and the stage 2 of left eye in P23. (**E**, **F**) CFP showed the stage 1 of right eye and the stage 5 of left eye in P24. (**G**, **H**) CFP showed the stage 1 of right eye and the stage 5 of left eye in P25.

knowledge of genotype-phenotype relationship in this inherited ocular disease. Owing to the limited number of patients in this study, establishing a comprehensive database with more patient sources is required to further explore the etiology of FEVR, which is helpful for clinical and genetic counselling of FEVR or other retinal vascular diseases.

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