FZD4 in a Large Chinese Population With Familial Exudative Vitreoretinopathy: Molecular Characteristics and Clinical Manifestations

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Purpose. The purpose of this study was to establish a genotype-phenotype correlation of familial exudative vitreoretinopathy (FEVR) caused by *FZD4* gene mutations.

Methods. Six hundred fifty-one probands and their family members were recruited based on a clinical diagnosis of FEVR between 2015 and 2021 at Zhongshan Ophthalmic Center. Ocular examinations were performed in all participants. Targeted gene panel sequencing and whole-exome sequencing were performed in the probands, and Sanger sequencing was used to verify the mutations and segregation analysis was performed in the family members.

Results. Fifty-one *FZD4* mutations (24 novels and 27 known) were detected in 84 families. Of these 168 eyes with FEVR, the eyes at stages 1, 2, 3, 4, and 5 were 29 (17.3%), 15 (8.9%), 19 (11.3%), 55 (32.7%), and 12 (7.1%), respectively. Exact stage of 38 (22.6%) eyes could not be determined. The FEVR phenotypes were more severe in the probands than the phenotypes in the family members ($P < 0.001$). The families were divided into two groups, probands that inherited the variant from the mother, and probands that inherited the variant from the father. In addition, the FEVR stage differences between these two groups were different ($P < 0.05$). Despite the mutations being located in different domains of *FZD4*, no significant differences were identified among the domains in terms of FEVR staging, retinal folds, retinal detachment, temporal midperipheral vitreoretinal interface abnormality, and foveal hypoplasia.

Conclusions. The *FZD4* probands had severer phenotype than the family members, and the FEVR stage difference was greater between the probands and mothers than that between the probands and fathers.

Keywords: familial exudative vitreoretinopathy, FZD4, clinical manifestation, Chinese population, domain, genotype-phenotype correlation

Familial exudative vitreoretinopathy (FEVR; MIM: 133780) is a rare, inherited form of vitreoretinopathy, characterized by a deficiency in peripheral retina vascularization and secondary complications due to retinal ischemia, such as vitreoretinal traction with deformation of the posterior retina, vitreous hemorrhage, retinal fold, and retinal detachment. It was first described by Criswick and Schepens in 1969,[1] and it has since been linked to several genes.

To date, 9 genes have been associated with the development of FEVR: frizzled class receptor 4 (*FZD4*; MIM: 604579),[2] tetraspanin 12 (*TSPAN12*; MIM: 613138),[3] low-density lipoprotein receptor-related protein 5 (*LRP5*; MIM: 603506),[4] norrin cystine knot growth factor NDP (*NDP*; MIM: 300658),[5] zinc finger protein 408 (*ZNF408*; MIM: 616454),[6] kinesin family member 11 (*KIF11*; MIM: 148760),[7] catenin β-1 (*CTNNB1*; MIM: 116806),[8] jagged 1 (*JAG1*; MIN: 601920),[9] and catenin α-1 (*CTNNA1*; MIM: 116805).[10] Several inheritance patterns have been identified in FEVR, including autosomal dominant (AD), autosomal recessive (AR), and X-linked recessive (XL) patterns. AD is the most common inheritance pattern of FEVR, typically featuring complete penetrance and highly variable expressivity. In most previous studies, mutations in *FZD4*, *TSPAN12*, *LRP5*, *KIF11*, *ZNF408*, *CTNNB1*, *JAG1*, and *CTNNA1* caused the AD inheritance pattern of FEVR,[2–9] whereas the AR pattern was observed in *LRP5*.[4] *NDP* is responsible for XL FEVR.[5] The known gene mutations can explain only approximately 50% of FEVR cases.[11]

The *FZD4* gene, a member of the frizzled (FZ) gene family, is located on chromosome 11q14.2. It contains 2 coding exons and encodes a 537–amino acid protein with the N-terminal cysteine-rich (CRD) domain, transmembrane domain 7, and the C-terminal S/T-X-V motif.[12] CRD is an extracellular cysteine-rich domain at the amino terminus of FZ proteins, considered the determination in binding specificity for Wnt ligands.[13] Transmembrane domain 7 is conserved among all members of the FZ gene family, whereas the C-terminal S/T-X-V motif is conserved among some members of the FZ gene family.[14] The domains are essential for the initiation of canonical Wnt pathways and for the phosphorylation of disheveled (Dvl) proteins.[15] The *FZD4* protein is a soluble protein that can activate the Wnt

signaling pathway,[12] playing a crucial role in retinal angiogenesis.[2] Although *FZD4* disease mutations in FEVR were first identified in 2002,[2] a clear genotype-phenotype relationship has yet to be established. In FEVR, a highly variable expressivity may be observed between the eyes of the same patient and among different patients who carry the same mutations in the same genes.

In this study, we examined *FZD4* gene mutations in a relatively large Chinese cohort of patients with FEVR, and we aimed to identify genotype-phenotype correlations.

Methods

The present study is a cross-sectional study, conducted in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Board (IRB) of Zhongshan Ophthalmic Center (2014MEKY048), Sun Yat-sen University, Guangzhou, China. The FEVR families were recruited from the patients referred to our hospital from January 2014 to April 2021. A total of 651 probands were recruited. Written informed consent was obtained from the subjects or their guardians before the clinical data and blood samples were collected. Comprehensive ophthalmic examinations were performed, including slit-lamp biomicroscope, intraocular pressure (IOP) measurement, binocular indirect ophthalmoscopy, fundus photography, and fundus fluorescein angiography (FFA). The clinical diagnostic criteria of FEVR, as well as the severity staging, were based on Trese et al.'s reports.[16] To eliminate the possible interference of retinopathy of prematurity, participants were excluded based on the following criteria: gestational age of less than 32 weeks (or a neonatal birth weight of less than 2000 g) and a history of oxygen inhalation and other ocular diseases. The clinical diagnosis of FEVR was made by two pediatric retinal specialists independently. If there was any disagreement between them, an experienced retinal specialist (author D.X.) gave the final diagnosis.

DNA samples were extracted from the peripheral whole blood of each individual using the methods used in our previous study.[17] In the probands, targeted gene panel (TGP) sequencing was performed from January 1, 2015, to December 31, 2017, and whole-exome sequencing (WES) was performed from January 1, 2018, to May 8, 2021. Sanger sequencing was used for verifying the variants via next generation sequencing and segregation analysis in the available family members. The Human Gene Mutation Database (HGMD: http://www.hgmd.cf.ac.uk/ac/index.php) and the Genome Aggregation Database (gnomAD: (https://gnomad.broadinstitute.org), as well online algorithms PolyPhen2, Mutation Taster, and SIFT were used in the bioinformatic analysis of the variants.

Statistical analysis was performed using SPSS software (IBM SPSS Statistics 25; IBM Corp., Armonk, NY, USA). Data were described using median and interquartile range in non-normal distribution cases. Chi-squared and Fisher's exact tests were used to compare the categorical data. Statistical significance was set at $P < 0.05$.

Results

Demographic Characteristics of Probands With *FZD4* Mutations

In this study, we identified that 84 unrelated probands with clinical diagnosis of FEVR had *FZD4* mutations, as did 50 family members. Of the 84 probands, 54 were boys and 30 were girls. The mean age at the time of diagnosis was 7.6 years (range = 1 month to 43 years; Table 1).

Table 1. Demographic Data of the Probands and Family Members With *FZD4* Mutations

	Probands	Family Members	*P* Value
Number	84	50	0.279
Male, *n*, %	54	27	
Female, *n*, %	30	23	
Age, y, mean ± SD	7.6 ± 9.9	30.0 ± 13.4	<0.001*
FEVR stage			<0.001
1	29	45	
2	15	28	
3	19	12	
4	55	3	
5	12	4	
Others	38	1	
Asymmetry			<0.001
Low	34	44	
Medium	9	6	
High	18	0	
Retinal folds, *n* (%)	55 (32.7)	3 (2.9)	<0.0001
Retinal detachment, *n* (%)	18 (10.7)	5 (4.9)	0.118
TEMPVIA			0.079
Obtuse type	18 (10.7%)	4 (3.9%)	
Acute type	15 (8.9%)	6 (5.9%)	
Foveal hypoplasia	28 (16.7%)	0 (0%)	<0.0001

* The *P* values were calculated by Student's *t*-test. The rest of the *P* values were calculated by the chi-square test, for those columns with case number <5, Fisher exact test was used.

FEVR, familial exudative vitreoretinopathy; TEMPVIA, temporal midperipheral vitreoretinal interface abnormality.

A low asymmetry referred to a difference of one grade or none between contralateral eyes. A medium asymmetry referred to a difference of two grades between contralateral eyes. A high asymmetry referred to a difference of three, four, or five grades between contralateral eyes.

FZD4 Mutations

Fifty-one pathogenic variants in *FZD4* were detected, including 24 (45.1%) novel and 27 (54.9%) known variants.[11,17–28] The 51 pathogenic variants included 23 missense, 13 deletion, 6 nonsense, 3 insertion, 2 splicing, 1 copy number, 1 delins, and 2 frameshift variants. The c.313A>G was the most common variant, detected in 12 probands; followed by c.1282_1285del, detected in 10 probands; c.1589G>A, detected in 6 probands; and copy number variant (CNV; exons 1 and 2 deletion) detected in 4 probands (see Table 2).

The mutations were divided into 4 groups according to the domains they were located in: (1) CRD (amino acids 42–167), (2) transmembrane domain 7 (amino acids 209–509), (3) amino acids 495–537 in the C-terminus region of the *FZD4* protein (encompasses K-T/S-XXX-W,[29] a PDZ binding motif, and S/T-X-V, a PDZ recognition motif), and (4) others (see Fig. 1).[30] The region of amino acids 495 to 537 is deemed critical with respect to its structure, function, and involvement in FEVR.[31] The amino acids belonging to both the transmembrane domain 7 group and the amino acids 495 to 537 group were assigned to the latter group. Of the 51 mutations detected in this study, 22 were found in CRD, 17 in transmembrane domain 7, there were 3 in amino acids 495 to 537 at the C-terminus region, and 6 in others (see Fig. 1). Two mutations were identified in the intron, causing no change to the amino acids. One was CNV (exons 1 and 2 of *FZD4*).

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Table 2. Pathogenic Variants Detected in FZD4

No	Number	Exon	Nucleotide Changes	Protein Change	Type	1000G	ExAC	SIFT	Poly-Phen2	CADD	REVEL	Mutation Taster	Reference
1	1	1	c.49_50insCCCGGGGGCG	p.Val17Alafs*116	Insertion	0	0	–	–	–	–	–	Novel
2	2	1	c.107G>A	p.Gly36Asp	Missense	0	0	D	B	0.51388	0.374	A	[23]
3	2	1	c.118G>T	p.Glu40*	Nonsense	0	0	T	P	0.09039	0.511	–	[11]
4	1	1	c.133T>C	p.Cys45Arg	Missense	0	0	D	D	0.80669	0.967	D	[17]
5	1	1	c.134G>A	p.Cys45Tyr	Missense	0	0	D	D	0.88401	0.944	D	[24]
6	1	1	c.133T>A	p.Cys45Ser	Missense	0	0	D	P	0.72502	0.95	D	[17]
7	1	1	c.141dup	p.Ile48Hisfs*82	Insertion	0	0	–	–	–	–	–	Novel
8	1	1	c.158G>C	p.Cys53Ser	Missense	0	0	D	D	0.82476	0.986	D	[17]
9	1	1	c.169G>C	p.Gly57Arg	Missense	0	0	D	D	0.88966	0.93319	D	Novel
10	1	1	c.182C>T	p.Thr61Ile	Missense	0	0	D	D	0.87976	0.93	D	[18]
11	3	1	c.205C>T	p.His69Tyr	Missense	0.0002	0.000562	D	B	0.76753	0.663	A	[22]
12	1	1	c.223G>A	p.Ala75Thr	Missense	0	0.000009	D	D	0.9522	0.875	D	[17]
13	1	1	c.260del	p.Gln87Argfs*46	Deletion	0	0	–	–	–	–	–	Novel
14	1	1	c.264C>A	p.Tyr88*	Nonsense	0	0	–	–	–	–	–	[21]
15	1	1	c.268T>C	p.Cys90Arg	Missense	0	0	D	D	0.85883	0.965	D	[17]
16	1	1	c.284A>T	p.Gln95Leu	Missense	0	0	D	B	0.50873	0.487	D	Novel
17	1	1	c.285G>A	p.Gln95Gln	Synonymous	0	0	–	–	–	–	–	Novel
18	12	2	c.313A>G	p.Met105Val	Missense	0	0.000017	T	P	0.09039	0.511	A	[25]
19	1	2	c.316_317dup	p.Thr107Alafs*27	Frameshift	0	0	–	–	–	–	–	Novel
20	1	2	c.341T>C	p.Ile114Thr	Missense	0	0	D	P	0.71563	0.851	D	[20]
21	1	2	c.351C>G	p.Cys117Trp	Missense	0	0	D	D	0.39612	0.563	D	Novel
22	1	2	c.380del	p.Arg127Profs*6	Deletion	0	0	–	–	–	–	–	[29]
23	1	2	c.451C>T	p.Gln151*	Nonsense	0	0	–	–	–	–	–	Novel
24	1	2	c.456C>A	p.Asn152Lys	Missense	0	0	T	D	0.23802	0.533	D	Novel
25	1	2	c.485del	p.Pro162Glnfs*33	Deletion	0	0	–	–	–	–	–	Novel
26	1	2	c.541T>C	p.Cys181Arg	Missense	0	0	T	B	0.4704	0.77591	A	[28]
27	1	2	c.551_552del	p.Val184Glyfs*5	Deletion	0	0	–	–	–	–	–	Novel
28	1	2	c.579G>A	p.Trp193*	Nonsense	0	0	–	–	–	–	–	Novel
29	1	2	c.631T>C	p.Tyr211His	Missense	0	0	D	B	0.43228	0.35	D	[19]
30	1	2	c.716T>C	p.Leu239Pro	Missense	0	0	D	D	0.70572	0.727	D	[26]
31	1	2	c.757C>T	p.Arg253Cys	Missense	0	0	D	D	0.87568	0.595	D	[20]
32	1	2	c.930C>G	p.Tyr310*	Nonsense	0	0	–	–	–	–	–	Novel
33	2	2	c.957G>A	p.Trp319*	Nonsense	0	0	–	–	–	–	–	[22]
34	1	2	c.974T>G	p.Leu325Arg	Missense	0	0	D	D	0.70193	0.76	D	Novel
35	1	2	c.975_978del	p.Thr326Glyfs*31	Deletion	0	0	–	–	–	–	–	[17]
36	1	2	c.1000-1001insCTCA	p.Lys334Thrfs*6	Insertion	0	0	–	–	–	–	–	Novel
37	1	2	c.1034_1054del	p.Ser345_Ala351del	Deletion	0	0	–	–	–	–	–	[17]
38	1	2	c.1155del	p.Asp385Glufs*46	Frameshift	0	0	–	–	–	–	–	Novel
39	1	2	c.1181C>T	p.Pro394Leu	Missense	0	0	D	D	0.79179	0.896	D	Novel
40	10	2	c.1282_1285del	p.Asp428Serfs*2	Deletion	0	0	–	–	–	–	–	[18]
41	1	2	c.1293_1296del	p.Glu431Aspfs*2	Deletion	0	0	–	–	–	–	–	Novel
42	1	2	c.1310T>C	p.Ile437Thr	Missense	0	0	D	D	0.64757	0.943	D	[20]
43	1	2	c.1328_1332del	p.Leu443Hisfs*14	Deletion	0	0	–	–	–	–	–	Novel
44	1	2	c.1475del	p.Gly492Alafs*21	Deletion	0	0	–	–	–	–	–	[17]
45	1	2	c.1478-79insAT	p.Met493Ilefs*21	Deletion	0	0	–	–	–	–	–	Novel
46	1	2	c.1492_1502del	p.Ala498Serfs*33	Deletion	0	0	–	–	–	–	–	Novel
47	1	2	c.1498del	p.Thr500Leufs*13	Deletion	0	0	–	–	–	–	–	[23]
48	6	2	c.1589G>A	p.Gly530Glu	Missense	0	0.000173	D	P	0.46364	0.787	D	[20]
49	1	Intron 1	c.286-3G>C	–	Splicing	0	0	–	–	–	–	Splice site change	Novel
50	4	1-2	CNV (exon1-2 deletion)	–	CNV	0	0	–	–	–	–	–	[27]
51	1	Intron 1	c.286-2A>G	–	Splicing	0	0	–	–	–	–	Splice site change	Novel

T, tolerant; B, benign; D, damaging or disease causing; P, possibly damaging, A, disease causing automatic; N, polymorphism; P, polymorphism.

CNV, copy number variants.

Clinical Data of Probands

All probands underwent comprehensive ophthalmic examination. Of 168 eyes, 29 (17.3%) were at stage 1, 15 (8.9%) were at stage 2, 19 (11.3%) were at stage 3, 55 (32.7%) were at stage 4, 12 (7.1%) were at stage 5, and 38 (22.6%) eyes had vitreous hemorrhages (VHs) or cataracts and could not be assigned to a FEVR stage. Typical fundus changes of retinal folds were found in 55 (32.7%) eyes. Asymmetry (a stage difference equal to or greater than 2) was found in 27 (32.1%) probands.

Clinical Data Between Family Members

Fundus images of the 50 family members with *FZD4* variants were assigned to FEVR stages. Of these, 45 (45.0%) eyes were in stage 1, 28 (28.0%) were in stage 2, 12 (12.0%) were in stage 3, 3 (3.0%) were in stage 4, 4 (4.0%) were in stage 5, and 7 (7%) were without prominent vascular changes. Rhegmatogenous retinal detachment (RRD) was observed in one family member (Fig. 2). Asymmetry (a stage difference equal to or greater than 2) was found in six (12.0%) family members. A stage difference of one was detected in

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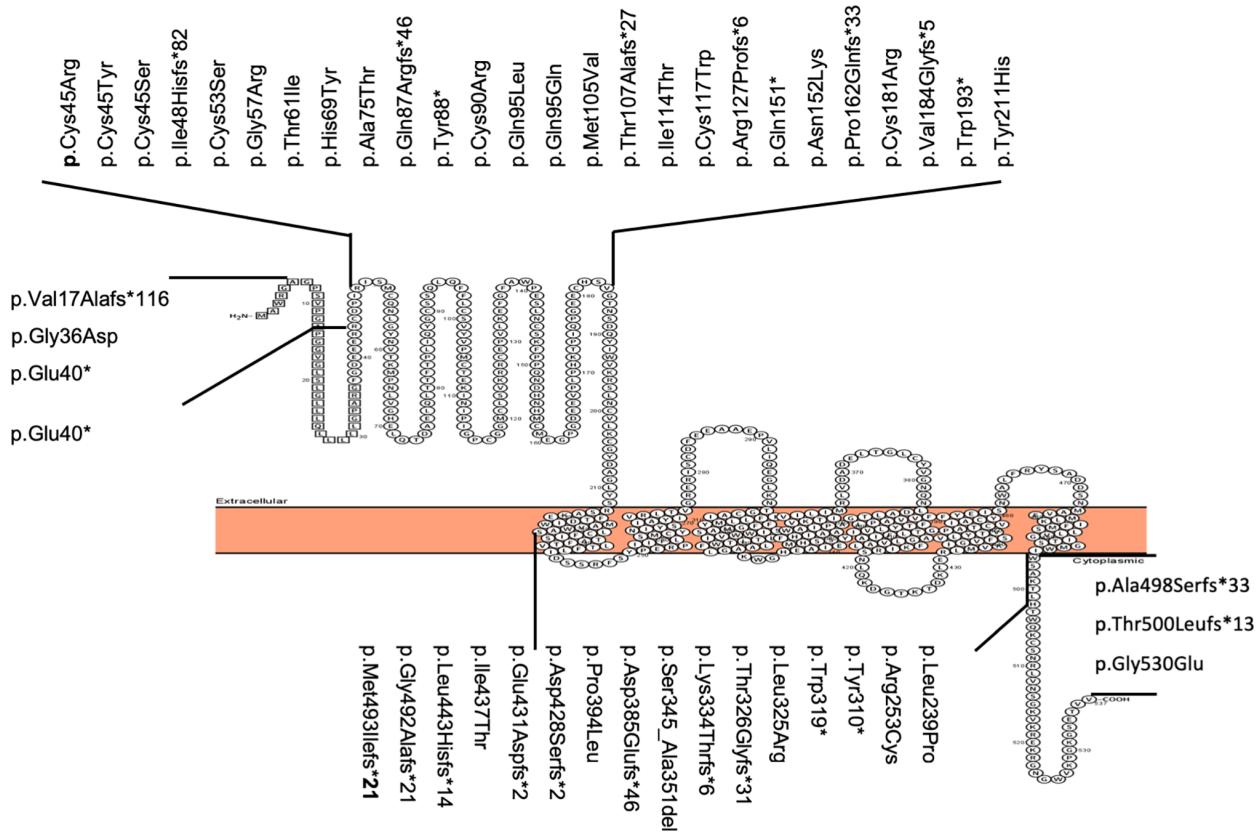

Figure 1. Schematic diagram of *FZD4* domains and mutations detected in this study.

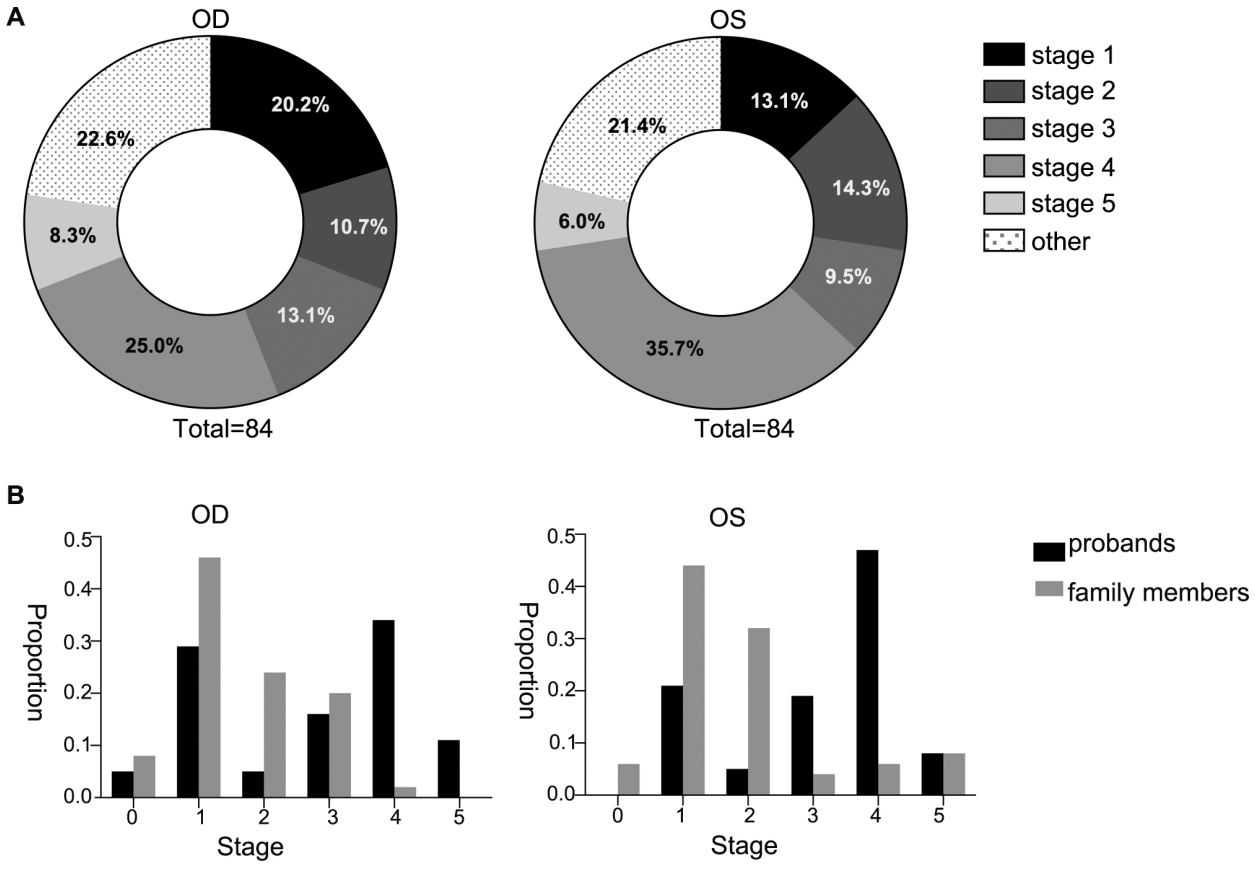

Figure 2. FEVR stages of bilateral eyes. (**A**) FEVR stages of all the probands. (**B**) FEVR stages between probands and family members. OD, right eye; OS, left eye.

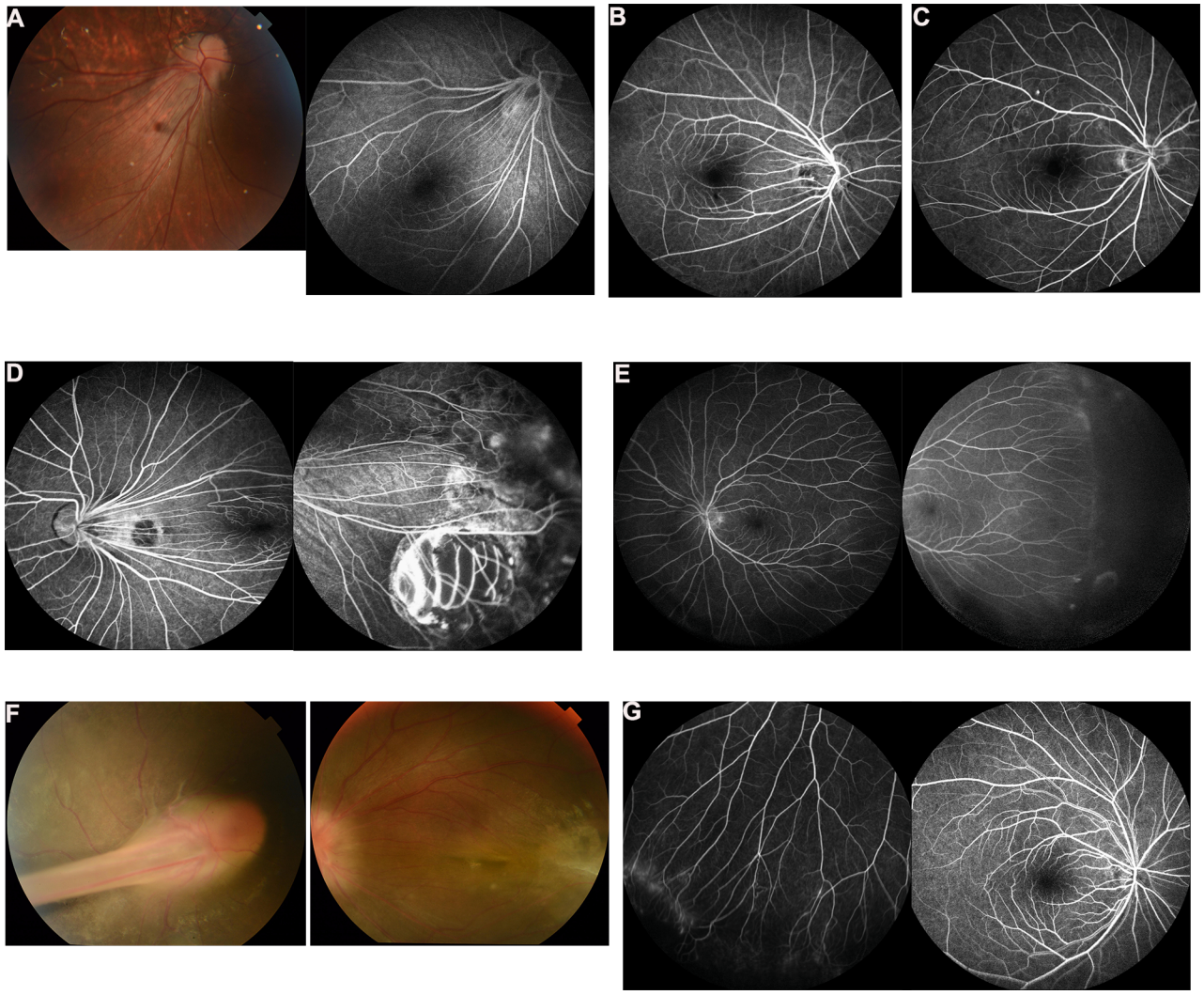

Figure 3. Fundus images of probands and family members. **A**, **B**, and **C** are from the family DX684 with the c.313A>G (p.Met105Val) variant. (**A**) Fundus image and fundus fluorescein angiography (FFA) of the proband DX684 indicates prominent macular dragging and ectopic macula. (**B**) FFA of the brother of DX684 indicates supernumerary branching. (**C**) FFA of the mother of DX684 indicates supernumerary branching. (**D**) FFA of the mother of DX684 was unremarkable. **D** and **E** are from the family DX342 with the c.1034_1054del (p.Ser345_Ala351del) variant. **D** FFA of the proband DX342 indicates macular dragging, ectopic macula, supernumerary branching, and coloboma of the choroid. (**E**) FFA of the father of DX342 indicates peripheral avascular retinal area and supernumerary branching. **F** and **G** are from the family XDW41 with the c.1000-1001insCTCA (p.Lys334Thrfs*6) variant. (**F**) Fundus images of the proband XDW41 indicate retinal folds in the right eye, macular dragging, and ectopic macula in the left eye. (**G**) FFA of the mother of XDW41 indicates supernumerary branching.

14 (28.0%) subjects. The severity of FEVR between probands and family members was analyzed. The probands had more severe FEVR phenotypes than the family members ($P < 0.001$; see Fig. 2, Fig. 3), and they displayed more asymmetry than the family members (see Table 1).

The families were further divided into two groups, probands that inherited the variant from the mother were assigned to group A ($n = 16$), and probands that inherited the variant from the father were assigned to group B ($n = 10$). Only those parent-child trios with clinical and genetic data were included in this subgroup analysis. If the bilateral eyes had different severity, the eyes with the higher stage were assigned as the stage of the patients. The FEVR stages of the probands between groups were not significantly different ($P = 0.469$). Then, we compared the FEVR stage differences between the probands and the parents (in group A, the stage difference = the FEVR stage of the proband – the FEVR stage of the mother; in group B, the stage difference = the FEVR stage of the proband – the FEVR stage of the father). The FEVR stage difference of group A was larger than group B (group B, $P < 0.05$; Table 3).

Table 3. Phenotype Comparison Between Probands and Parents

Stages Difference	0	1	2	3	4	5
Stages difference between probands and mothers, *n*	1	5	8	0	1	1
Stages difference between probands and fathers, *n*	0	7	0	1	1	1
P value	0.015					

Clinical Data of Probands with the Same Variants

The phenotypes of probands with the same variants were further analyzed. The 6 probands with the c.1589G>A vari-

Table 4. Phenotypes Comparison Between Probands With the Same Mutations

Mutation	c.1589G>A	c.1282_1285del	c.313A>G	c.205C>T	CNV (Exon1-2)
No. of probands	6	10	12	3	4
Stages					
0	0	0	0	0	0
1	0	1	0	0	0
2	1	0	0	0	0
3	1	1	2	0	0
4	2	5	4	1	4
5	0	2	1	0	0
Others	2	1	5	0	0

Others, rhegmatogenous retinal detachment or vitreous hemorrhage which cannot classify the FEVR stages.

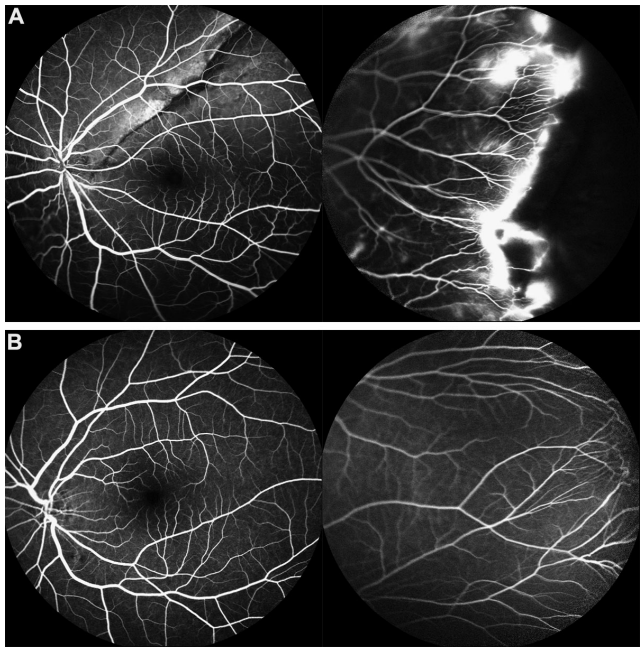

Figure 4. Representative fundus images of probands with the same variant, 1589G>A (Gly530Glu). (**A**) FFA of QT2446 indicates rhegmatogenous retinal detachment. (**B**) FFA of QT2341 indicates supernumerary branching.

ant were at stages 2 and 4. The 10 probands with the c.1282_1285del variant ranged from stage 1 to stage 5. The 12 probands with the c.313A>G variant ranged from stage 3 to stage 5. The phenotypes of the probands with the c.205C>T and CNV (exon 1–2) variants were all at stage 4 (see Table 4, Fig. 4). The result indicated the genetic heterogeneity of *FZD4*, even the probands with the same variant, of the phenotypes were different.

Clinical Data Comparison Between Domains and Mutation Types

To further explore the potential relationship between genotype and phenotype, we analyzed the relationship between the domains and phenotypes. The chi-squared test was used for intergroup comparison, and no statistical difference was observed among the four groups ($P = 0.725$). The analysis also considered clinical phenotype staging, presence of retinal folds, retinal detachment, temporal midperipheral vitreoretinal interface abnormality (TEMPVIA),[32] and foveal hypoplasia. The phenotypes of the bilateral eyes were analyzed separately, and no significant difference was observed among the domains (see Table 5).

Table 5. Phenotype Comparison Between Different *FZD4* Domains

Domains	Others, *n*	CRD, *n*	7 Transmembrane Domains, *n*	Amino Acids 495–537, *n*	*P* Value
Stages					0.725
1	5	8	9	2	
2	2	5	5	4	
3	0	8	7	2	
4	8	18	13	4	
5	1	6	3	1	
Folds					0.657
Yes	8	30	29	10	
No	8	16	14	5	
Retinal detachment					0.574
Yes	13	47	37	13	
No	1	7	6	3	
TEMPVIA					0.740
Yes	3	5	7	0	
No	3	4	7	2	
Foveal hypoplasia					1.000
Yes	2	5	4	0	
No	4	11	8	2	

TEMPVIA, temporal mid-peripheral vitreoretinal interface abnormality.

CRD, cysteine-rich domain.

Discussion

FEVR is a clinically heterogeneous disorder causing severe visual impairment at a very young age, which can greatly reduce the patient's quality of life.[21,33] *FZD4* is an essential link in the known signaling pathways of FEVR. The contribution of *FZD4* to FEVR varies between populations. In 2 Chinese studies with a sample size of more than 50, *FZD4* mutations accounted for the greatest proportion of FEVR cases.[18,34] Therefore, it is important to study the relationship between genotype and phenotype in FEVR caused by *FZD4* mutations. To the best of our knowledge, this study drew from the largest cohort of patients with FEVR in the literature to date, describing the phenotypes of 84 families with FEVR with *FZD4* pathogenic variants.

Phenotypes of *FEVR* Genes

Until now, nine genes have been reported to cause FEVR; however, the phenotypes differed between the genes. FEVR is divided into five stages, based on retinal vascular changes.[16] Seo et al. reported that carrying a mutation in *FZD4* resulted in a milder phenotype than carrying mutations in other genes.[34] However, probands with FEVR in stages 4 or 5 accounted for 39.8% of all proband eyes in this study, which is divergent with the finding of Zhao et al.'s study, in which 82.8% were in stages 4 or 5.[35] Patients in Zhao et al.'s and our study were both recruited from Chinese populations, which may suggest a racial bias in the phenotype severity observed in these studies.

Phenotype Variability: Clinical Differences Between Parents and Probands

Phenotype heterogeneity was common in patients with *FZD4* mutations—not only in probands with the same mutations but also in members of the same family. The

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probands presented more severe phenotypes than the family members. Kashani et al. reported that 76% of the probands in their study were at stage 3, 4, or 5 of FEVR; however, only 21% of family members were at stage 3, 4, or 5 in their study.[36] Zhao et al. demonstrated that, in families with *FZD4* mutations, 45.71% of probands were in stage 5, whereas 65.85% of family members were at stage 1.[35] Efforts have been made to explain such variability; however, little has been determined from previous studies. The main consideration is to determine whether the cause is cumulative effects or selection bias. We favor selection bias. Kashani et al. suggested that the prevalence of FEVR is underestimated in the general population[36] (only when a patient has a problem with their vision, do they seek medical attention). Most often, the proband has the most severe phenotype. The phenotype analysis of patients' siblings will be helpful to rule out cumulative effects. Further studies are needed to determine the phenotype heterogeneity of patients with *FZD4* mutations.

In this study, we first found that the FEVR stage difference between the mothers and the probands was greater than that between the fathers and the probands, which may indicate the important role of FZD4 in placental development. *FZD4* played a crucial role in Wnt pathway,[12] and the Wnt signaling has been reported to be important in placental development.[37] Fzd5, a member of the FZ gene family, was reported to function in regulating trophoblast differentiation and sites of chorionic branching morphogenesis.[30] The possible conjecture may be as follows: during pregnancy, negative influences of impaired placental development caused by *FZD4* mutations of mothers affected the placental oxygen transportation and impaired angiogenesis. This could be called "double attack" to the retinal angiogenesis, one is from his own FZD4 mutation, and the other one is caused by the FZD4 mutation from the mother. Therefore, clinical phenotype of probands inherited from their mothers will be more severe than that from their fathers.

Function of the Domains

In this study, most *FZD4* mutations carried by probands were inherited from the parents, and only four were spontaneous mutations. This strongly suggests that when one offspring presents an *FZD4* mutation, timely and comprehensive ophthalmological examination of family members becomes essential. This will aid in the early identification of asymptomatic patients. There is no doubt that early identification, together with early intervention and follow-up, can improve clinical outcomes. Of the 51 mutations identified in the present study, 24 were novel, extending the spectrum of the *FZD4* mutations underlying FEVR. Of the 51 mutations, 22 (43.1%) were identified in CRD, 17 (33.3%) in transmembrane 7, 3 (5.9%) in amino acids 495 to 537 at the C-terminus region, and 6 (11.8%) in others. This suggests that CRD plays an essential role in FEVR caused by *FZD4* mutations.[24] However, FEVR caused by mutations in CRD is no more serious than FEVR caused by anomalies in other domains ($P > 0.05$).

No obvious genotype-phenotype correlation was observed. The autosomal dominant feature of *FZD4* mutations may be caused by haploinsufficiency, which is consistent with previous studies.[17,24]

In summary, we evaluated the genotype-phenotype correlation of patients with FEVR with *FZD4* variants in a relatively large Chinese FEVR cohort. The results were as follows: (a) the phenotype was relatively severe, with 39.8% of eyes in FEVR stage 4 or 5; (b) the probands had more severe phenotypes than the family members; (c) the FEVR stage difference was greater in maternal inheritance than paternal inheritance; and (d) the phenotypes were not correlated to the location of the mutations, and phenotype heterogeneity was observed in patients with *FZD4* mutations.

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