Clinical characteristics and mutation spectrum in 33 Chinese families with familial exudative vitreoretinopathy

Jianbo Mao, Yijing Chen, Yuyan Fang, Yirun Shao, Ziyi Xiang, Hanxiao Li, Shixin Zhao, Yiqi Chen and Lijun Shen

Department of Ophthalmology, Center for Rehabilitation Medicine, Affiliated People’s Hospital, Hangzhou Medical College, Hangzhou, PR China; Department of Retina Center, Affiliated Eye Hospital of Wenzhou Medical University, Hangzhou, PR China; Hangzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University, Hangzhou, PR China

ABSTRACT

Objective: To explore the clinical manifestations and search for the variants of six related genes (LRP5, FZD4, TSPAN12, NDP, KIF11 and ZNF408) in Chinese patients with familial exudative vitreoretinopathy (FEVR), and investigate the correlation between the genetic variants and the clinical characteristics.

Patients and methods: Clinical data, including the retinal artery angle, acquired from wide-field fundus imaging, structural and microvascular features of the retina obtained from optical coherence tomography (OCT) and OCT angiography (OCTA) were collected from 33 pedigrees. Furthermore, mutation screening was performed. Variants filtering, bioinformatics analysis and Sanger sequencing were conducted to verify the variants.

Results: Twenty-one variants were successfully detected in 16 of 33 families, of which 10 variants were newly identified. The proportion of variants in LRP5, FZD4, TSPAN12, NDP and KIF11 was 38.1% (8/21), 33.3% (7/21), 19.1% (4/21), 4.8% (1/21) and 4.8% (1/21), respectively. Three new variants were considered to be pathogenic or likely pathogenic. The FEVR group tended to exhibit a smaller retinal artery angle, higher incidence of foveal hypoplasia and lower vascular density compared to the control group. Patients who harboured variants of FZD4 exhibited greater severity of FEVR than those with LRP5 variants. However, those who harboured LRP5 variants tended to possess lower foveal vascular density.

Conclusions: Six known pathogenic genes were screened in 33 pedigrees with FEVR in our study, which revealed novel variants. These findings enrich the clinical features and mutation spectrum in Chinese patients with FEVR, revealing the genotype-phenotype relationship, and contributing to the diagnosis and treatment of the disease.

KEY MESSAGES

- We identified 21 variants in 5 genes (LRP5, FZD4, TSPAN12, NDP and KIF11) associated with FEVR, 10 of which are novel (three were pathogenic or likely pathogenic).
- The proportion of variants was the highest for the LRP5 gene.
- FZD4 variants may be responsible for greater FEVR severity than LRP5 variants.

Introduction

Familial exudative vitreoretinopathy (FEVR) is a rare inheritable ocular disorder characterized by abnormal retinal vascular growth. It usually occurs in full-term infants and children and can lead to fibrovascular proliferation, vitreoretinal traction, retinal folds and retinal detachment [1]. The clinical manifestations of FEVR vary considerably from no distinct symptoms to vision loss, presenting difficulties for clinical diagnosis and treatment. Fluorescein fundus angiography (FFA) remains the current gold standard for diagnosis; however, it is an invasive and extremely consuming procedure. Currently, wide-field fundus imaging is commonly used because it compensates for the shortcomings of FFA, facilitating 220°–240° imaging of the retina. Optical coherence tomography (OCT) and OCT angiography (OCTA) are used to assess and quantify the structural and functional features of the retinal and choroidal vascular morphology, especially in the macula. These new modalities have found widespread application in the field of ophthalmology.
application for diagnosing and monitoring the progression of FEVR.

The genetic causes of FEVR are numerous. The most common aetiology can be attributed to an autosomal dominant inheritance, but may also include autosomal recessive and X-linked recessive inheritance [2,3]. Currently, 15 genes and one locus are known to be associated with FEVR. Of these, 11 genes, including tetraspanin-12 (TSPAN12), zinc finger protein 408 (ZNF408), catenin alpha 1 (CTNNA1), catenin delta 1 (CTNND1), atonal homolog 7 (ATOH7), RCC1 and BTB domain-containing protein 1 (RCBTB1), integrin-linked kinase (ILK), jagged canonical Notch ligand 1 (JAG1), low-density-lipoprotein receptor-related protein 6 (LRP6), and exudative vitreoretinopathy 3 (EVR3) on chromosome 11p12-13 [4–14], are non-syndromic. The remaining genes are associated with systemic diseases. The proteins encoded by low-density-lipoprotein receptor-related protein 5 (LRP5) are associated with osteoporosis-pseudoglioma syndrome [15], and the Norrie disease protein (NDP) is associated with Norrie disease [16]. The protein encoded by kinesin family member 11 (KIF11) [17] can cause autosomal dominant microcephaly with or without chorioretinopathy, lymphedema or intellectual disability. Catenin beta 1 (CTNNB1) is also an uncommon cause of microcephaly [18]. Frizzled-4 (FZD4) variants can result in hearing deficits and developmental delays [19]. Current research indicates that only around 40–50% of cases of FEVR harbour identifiable genetic variants [20–22], while the relationship between the genotype and clinical manifestations is complex, which are responsible for the challenges in early clinical diagnosis and effective treatment.

In this study, we investigated the genotype and clinical features of FEVR in members of families with and without this disease and compared the results to a healthy control group without any history of FEVR. We collected data using wide-field fundus imaging, OCT, OCTA and FFA, which were performed by the same qualified technician.

We enrolled probands and families associated with FEVR who visited Wenzhou Medical University Affiliated Eye Hospital between September 2017 and November 2021, in addition to the healthy members of the affected families. Normal individuals without an FEVR-related genetic family history or any history of retinal vascular diseases including hypertension, vein occlusions and diabetic retinopathy were enrolled as the control group. All individuals were native Chinese, without any history of premature birth or oxygen inhalation. The patients and controls were matched for age and gender.

**Clinical information collection**

Basic demographic and medical information was collected from each family and comprehensive ophthalmic examinations were conducted for each of the probands and their family members at Wenzhou Medical University Affiliated Eye Hospital. The examinations included intraocular pressure measurement, visual acuity measurement, slit-lamp biomicroscopy, wide-field fundus imaging, OCT, OCTA and FFA, which were performed by the same qualified technician.

Patients were considered to have FEVR on the basis of ophthalmic testing if they exhibited at least one of the following typical clinical findings: (1) peripheral retinal avascularity, (2) severe subretinal exudation, (3) neovascularization, (4) retinal fold or detachment, (5) supraretinal peripheral fibrovascular mass, (6) macular ectopia or (7) vitreous haemorrhage. The examinations of family members can support the diagnosis, and subsequently, family pedigrees were drawn. The severity of each case of FEVR was further assessed and classified according to the grading system devised by Pendergast and Trese [23]. FEVR stages 1 and 2 were denoted as the mild phenotype and stages 3–5 were designated as the severe phenotype.

Wide-field fundus imaging was conducted using the Optos 200Tx (Optos, Marlborough, MA). We measured the retinal artery angle in the participants’ eyes in accordance with the Yugami correlated angle (YCA) defined by Nagura et al. [24]. First, the distance between the optic nerve head (ONH) and the fovea was measured. Subsequently, we drew a circle centred on the ONH with a radius of half the distance between the ONH and the fovea based on calculations performed using the ImageJ software. The points of intersection between the circle and the arteries of the upper and lower arcade were obtained, and the angle enclosed by the two lines joining the intersection points and the ONH was denoted as the retinal artery
angle in the total study population. OCTA was performed using the RTVue XR Avanti AngioVue (Optovue Inc., Fremont, CA) and 3 × 3 mm scans centred on the fovea were obtained for each eye. Spectral domain OCT (Heidelberg Engineering, Heidelberg, Germany) was also performed. The images acquired via the conventional mode were used for structural grading of the fovea, which was classified as the presence or absence of foveal hypoplasia, as described by Thomas et al. [25]. Foveal hypoplasia was characterized by the presence of inner retinal layers in the fovea on spectral domain OCT. Inner retinal thickness (IRT) was defined as the distance between the internal limiting membrane (ILM) and outer border of the inner nuclear layer. Central macular thickness (CMT) was defined as the average thickness of the circle measuring 1 mm in diameter centred on the fovea. The IRT of the fovea and CMT were obtained to quantify the degree of foveal hypoplasia.

**Mutation screening and analysis**

Peripheral blood samples were collected from the individuals and preserved at −80°C before use. Whole-exome sequencing (Peking Tsingke Biotechnology Co., LTD, Peking, China) was performed for the probands who were diagnosed with FEVR and their families. Raw data were filtered using the quality control process. The Genome Analysis Toolkit (https://www.broadinstitute.org/gatk/) was to detect single nucleotide polymorphisms and insertion-deletion in the six known FEVR-associated genes (LRP5, FZD4, TSPAN12, NDP, KIF11 and ZNF408). Finally, SnpEff (http://snpeff.sourceforge.net/SnpEff_manual.html) was used to annotate the information.

The 1000 Genome database (http://www.1000genomes.org/), ExAC (http://exac.broadinstitute.org/), Exome Sequencing Project (https://evs.gs.washington.edu/EVS/) and National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) were searched for each variant to determine if it had been reported previously. Variants that were not found in these databases were considered novel. The potential deleterious effect of each variant was assessed using nine variant bioinformatics tools including SIFT, Poly-phen2HVAR, LRT, Mutation Taster, FATHMM, CADD, GERP+++, phyloP100way_vertebrate, and SiPhy_29way_logOdds. Amino acid sequences corresponding to the alterations in the genotype changes were obtained from the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the European Bioinformatics Institute (https://www.ebi.ac.uk/Tools/msa/tcoffee/).

Furthermore, Sanger sequencing and co-segregation detection were performed for the 33 pedigrees. Finally, the pathogenicity of these variants was evaluated according to the standards and guidelines provided by the American College of Medical Genetics and Genomics (ACMG).

**Data analysis**

The clinical phenotype of the patients was graded and classified [23]. All detected variants were classified according to the type and the number of variants. We further explored the characteristics of the clinical and genomic variations as well as the potential genotypic-phenotypic correlations.

The Statistical Package for the Social Sciences version 23 (SPSS Inc., Chicago, IL) was used for statistical analysis. Normality of distribution of the variables was inspected using the Shapiro–Wilk test. The Student’s $t$-test was used to compare data with normal distributions. Non-normally distributed data of the patients and controls were compared using the Mann–Whitney U test. Nonparametric data were compared using the Kruskal–Wallis H test and chi-squared test. $p$ Values less than 0.05 were considered statistically significant.

**Results**

In this study, the diagnosis and grading of FEVR were performed after a comprehensive clinical analysis based on the results of FFA and genetic screening of 33 families. This study enrolled 127 participants from 33 pedigrees, including 81 patients with FEVR and 46 healthy individuals (Table 1). The FEVR group comprised 40 men and 41 women, whose average age was $22.0 \pm 17.3$ years old. Most patients presented with mild symptoms. The average age of the probands from the 33 FEVR families was $9.1 \pm 11.7$ years old, of which 17 were boys/men and 16 were girls/women. The majority of probands presented with a more severe phenotype, including foveal hypoplasia, compared to their family members. Variants were detected in 48 participants with FEVR, who underwent further phenotype and genotype analysis.

**Number of pedigree variants**

Twenty-one variants were detected in the 33 FEVR families, of which 10 had not been previously reported and are hence considered as novel (Table 2). Variants were detected in only in 16 of the 33 families. Analysis of the family pedigrees (Figure 1) revealed...
Table 1. Clinical phenotype and genotype of participants.

| Features                  | Proband (n = 33) | FEVR (n = 81) | Healthy family members (n = 46) | Normal group (n = 49) |
|---------------------------|------------------|--------------|-------------------------------|-----------------------|
| Age, mean (SD), y         | 9.1 ± 11.7       | 22.0 ± 17.3  | 37.2 ± 15.6                   | 21.3 ± 12.6           |
| Male/Female               | 17/16            | 40/41        | 22/24                         | 20/29                 |
| Variants (numbers of individuals) |                |              |                               |                       |
| With                      | 16               | 40           | 8                             | –                     |
| FZD4                      | 6                | 15           | 0                             | –                     |
| LRP5                      | 2                | 8            | 7                             | –                     |
| NDP                       | 1                | 1            | 1                             | –                     |
| FZD4                      | 3                | 8            | 0                             | –                     |
| KIF11                     | 1                | 1            | 0                             | –                     |
| FZD4 + LRP5               | 1                | 4            | 0                             | –                     |
| LRP5 + FZD4               | 1                | 2            | 0                             | –                     |
| LRP5 ÷ LRP5               | 1                | 1            | 0                             | –                     |
| Without                   | 17               | 41           | 38                            | –                     |
| Ocular symptoms (numbers of individuals) |          |              |                               |                       |
| Retinal detachment        | 10               | 16           | –                             | –                     |
| Retinal breaks            | 5                | 6            | –                             | –                     |
| High                      | 5                | 6            | –                             | –                     |
| Intracocular pressure     |                  |              |                               |                       |
| Cataract                  | 5                | 8            | –                             | –                     |
| Strabismus                | 10               | 12           | –                             | –                     |
| Nystagmus                 | 5                | 9            | –                             | –                     |
| Stages of FEVR (numbers of eyes) |            |              |                               |                       |
| Mild                      | 52               | 137          | –                             | –                     |
| 1                         | 32               | 100          | –                             | –                     |
| 2                         | 20               | 37           | –                             | –                     |
| Severe                    | 14               | 25           | –                             | –                     |
| 3                         | 8                | 8            | –                             | –                     |
| 4                         | 4                | 11           | –                             | –                     |
| 5                         | 2                | 6            | –                             | –                     |
| Foveal Hypoplasia (numbers of eyes) |        |              |                               |                       |
| With                      | 31               | 48           | –                             | –                     |
| Without                   | 19               | 60           | –                             | –                     |
| Previous treatment (numbers of eyes) |          |              |                               |                       |
| Laser/Anti-VEGF           | 21               | 39           | –                             | –                     |
| Incisional surgery        | 11               | 16           | –                             | –                     |

Table 2. Twenty-one variants in LRP5, FZD4, TSPAN12, NDP and KIF11.

| Gene | Family | Type       | Variant              | ESP 6500 | 1000G | ExAC   | Reference   | rs ID     | Heredity   | ACMG ESP |
|------|--------|------------|----------------------|----------|-------|--------|-------------|----------|------------|----------|
| LRP5 | F1     | Missense   | c.95C > T,p.S32L      | 0        | 0     | <0.01% | Reported    | rs755388709 | Maternal | Under certain significance |
| LRP5 | F7     | Missense   | c.4090G > T,p.G1364C  | 0        | 0     | 0      | Novel       | –         | Maternal | Under certain significance |
| LRP5 | F13    | Missense   | c.1996G > T,p.D666N   | 0        | 0.0008| 0.0002 | Reported    | rs180941579 | Paternal | Likely benign |
| LRP5 | F17    | Missense   | c.290C > T,p.A97V     | 0        | 0.0008| 0      | Reported    | rs143433231 | Maternal | Likely benign |
| LRP5 | F18    | Missense   | c.3581G > A,p.K1194H  | 0        | 0.0002| 0      | Reported    | rs201017887 | Maternal | Under certain significance |
| LRP5 | F23    | Missense   | c.3544A > G,p.K1182E  | 0        | 0     | 0      | Reported    | rs950665645 | Paternal | Under certain significance |
| LRP5 | F32    | Missense   | c.1349G > A,p.R450H   | 0        | 0     | <0.01% | Reported    | rs1335197449 | Maternal | Under certain significance |
| LRP5 | F32    | Missense   | c.4215T > G,p.F1405L  | 0        | 0     | 0      | Novel       | –         | Paternal | Under certain significance |
| FZD4 | F1     | Missense   | c.461A > G,p.H154R    | 0        | 0     | 0      | Reported    | rs1334686841 | Maternal | Under certain significance |
| FZD4 | F6     | Missense   | c.1258C > G,p.K414Q   | 0        | 0     | 0      | Novel       | –         | Paternal | Under certain significance |
| FZD4 | F8     | Missense   | c.313A > G,p.M105V    | 0        | 0     | <0.01% | Reported    | rs80358284  | Maternal | Pathogenic |
| FZD4 | F16    | Frameshift | c.1293_1296delAAAGA,p.| 0        | 0     | 0      | Novel       | –         | Patent    | Likely pathogenic |
| FZD4 | F17    | Missense   | c.611T > G,p.D204Y    | 0        | 0     | 0      | Reported    | rs1064794064 | Maternal | Pathogenic |
| FZD4 | F18    | Non-sense  | c.1481G > A,p.W494K   | 0        | 0     | 0      | Novel       | –         | Paternal | Pathogenic |
| FZD4 | F25    | Missense   | c.1250G > G,p.R417Q   | 0        | 0     | 0      | Reported    | rs80358294 | Pathogenic | Pathogenic |
| TSPAN12 | F3 | Missense   | c.461A > G,p.H154R    | 0        | 0     | 0      | Novel       | –         | Paternal | Under certain significance |
| TSPAN12 | F9 | Non-sense  | c.352G > T,p.E118K    | 0        | 0     | 0      | Novel       | –         | Maternal | Pathogenic |
| TSPAN12 | F13 | Missense   | c.697A > G,p.T233P    | 0        | 0     | 0      | Novel       | –         | Paternal | Under certain significance |
| TSPAN12 | F28 | Missense   | c.504G > G,p.W168C    | 0        | 0     | 0      | Novel       | –         | Paternal | Under certain significance |
| NDP  | F14    | Missense   | c.137A > G,p.D46G     | 0        | 0     | 0      | Novel       | –         | Maternal | Under certain significance |
| KIF11| F31    | Missense   | c.1331A > T,p.K444I   | 0        | 0     | 0      | Reported    | rs75975775 | De novo  | Under certain significance |
Figure 1. The 16 family pedigrees with mutations. The individuals enrolled in our study are marked by the horizontal line above the figures. The probands are pointed by the black arrow. The square means male and the circle means female. The figures filled with black refer to FEVR patients and blank figures refer to healthy family members. The healthy carriers are represented by a black dot in the blank figure. F represents a family, V represents a variant, and + indicates a normal allele. V1 and V2 are used to discriminate two genotypes when FEVR patients carry digenic variants.
more than one in three participants carried genetic variants, including 40 patients with FEVR and 8 carriers. A total of 41 patients had single-site variants and 7 patients had variants in two sites, including 6 carrying two types of gene variants and 1 harbouring two variants in LRP5 (Table 1).

Twenty-one variants in five (LRP5, FZD4, TSPAN12, NDP and KIF11) of the six genes associated with FEVR were detected in the 33 families, including 81 patients with FEVR and 41 healthy family members, which accounted for the clinical symptoms of 16 families (16/33, 48.5%). As shown in Figure 2, variants in the LRP5 gene constituted the highest proportion (8/21, 38.1%) of variants, followed by variants in FZD4 (7/21, 33.3%) and TSPAN12 (4/21, 19.1%), respectively. Only one variant was present in NDP and KIF11 (1/21, 4.8% each), and no pathogenic variants were identified in ZNF408.

The distribution of the 10 newly identified variants was as follows: 2 in LRP5 (family 7 and family 32), 3 in FZD4 (family 6, family 16 and family 18), 4 in TSPAN12 (family 3, family 9, family 13 and family 28) and 1 in NDP (family 14). Of the 11 previously reported variants, 6 were identified in LRP5 (family 1, family 13, family 17, family 18, family 23 and family 32), 4 in FZD4 (family 1, family 8, family 17 and family 25), 1 in KIF11 (family 31) (Table 2). Two variants were discovered in five families (family 1, family 13, family 17, family 18 and family 32), respectively. The chromatograms of their sequencing results are depicted in Figure 3.

The variants in TSPAN12 (c.352G>T, p.E118X) and FZD4 (c.1481G>A, p.W494*) were characterized as nonsense variants. A frameshift variant was identified in FZD4 (c.1293_1296delAAGA, p.E431Dfs*2), while the remaining 18 were missense variants. Except for NDP (c.137A>G, p.D46G) and KIF11 (c.1331A>T, p.L444I), the variants of the other three genes were co-segregated with the disease in an autosomal dominant manner.

The pathogenicity of the newly identified variants was further verified using nine bioinformatics tools and amino acid sequence comparisons. According to the guidelines provided by the ACMG, the variants in TSPAN12 (c.352G>T, p.E118X) and FZD4 (c.1481G>A, p.W494*) were perceived as pathogenic. FZD4 (c.1293_1296delAAGA, p.E431Dfs*2) was predicted to be likely pathogenic. The proband of family 9 with the TSPAN12 (c.352G>T, p.E118X) variant, had retinal neovascularization in one eye (and underwent laser treatment), while the other eye was only mildly affected and considered as having stage 1 disease. Peripheral avascular zones were detected in both eyes of the mother. In family 18, the proband and his father harboured an FZD4 (c.1481G>A, p.W494*) variant, and underwent laser treatment for retinal breaks. In family 16, FZD4 (c.1293_1296delAAGA, p.E431Dfs*2) was detected, and the proband developed retinal detachment in one eye and underwent surgical repair, while the contralateral eye was only mildly affected. Her father had peripheral avascular zones in both eyes.

Clinical manifestations

We compared the results of wide-field fundus imaging, OCT and OCTA between the FEVR and control groups (Table 3 and Figure 4). Patients with FEVR tended to have a smaller retinal artery angle on wide-field fundus imaging compared to the controls (p<0.001). The incidence of foveal hypoplasia was higher in patients

Figure 2. The mutation spectrum in the Chinese FEVR cohort. Each coloured box represents the different related genes, showing the proportion of different genes.
with FEVR than that in the controls (p < 0.001). The foveal IRT was significantly higher in eyes with FEVR (p < 0.001). OCTA-acquired vessel densities of the superficial capillary plexus (SCP) and deep capillary plexus (DCP) from the whole retinal image and the parafovea decreased in FEVR (P_{SCP \, \text{whole \ image}} = 0.020, P_{SCP \, \text{parafovea}} = 0.009, P_{DCP \, \text{whole \ image}} < 0.001, P_{DCP \, \text{parafovea}} < 0.001). Additionally, the foveal vessel density in a 300-μm wide region around the foveal avascular zone (FAZ) (FD-300) was lower in the FEVR group (p = 0.001). However, the acircularity index (AI) of the FAZ was greater in eyes with FEVR (p < 0.001). As shown in Table 4, the retinal artery angle was smaller in FEVR eyes with foveal hypoplasia compared to eyes without foveal hypoplasia (p = 0.013). The CMT and IRT of the fovea were significantly higher in eyes with foveal hypoplasia. Moreover, FEVR eyes with foveal hypoplasia exhibited a smaller FAZ area and perimeter, higher AI and lower FD-300. The vascular densities of the DCP on the whole and parafoveal images, and SCP on the parafoveal image were lower, while those of the foveal SCP and DCP were higher in eyes with foveal hypoplasia. The IRT was negatively correlated with the FAZ area (r = −0.745, p < 0.001) and perimeter (r = −0.731, p < 0.001), while the IRT was positively correlated with the vascular density of the SCP (r = 0.501, p = 0.017) and DCP (r = 0.578, p = 0.005) in the fovea. The retinal parameters at different FEVR stages were further analyzed and compared. Foveal hypoplasia was observed in all patients.

Figure 3. The chromatograms of sequencing results in five families carrying two variants. F represents a family, and V represents a variant.
with severe disease compared to patients with mild FEVR. Further statistical analyses were performed for eyes in stages 1 and 2, owing to the small sample size of the severe stages, i.e. stages 3–5. The CMT was higher in stage 2 compared to stage 1 (p = 0.014). The foveal vascular density of the SCP was higher in eyes designated as stage 2 (p = 0.019).

**Genotype–phenotype correlations**

We included 39 participants with FEVR (78 eyes) who harboured variants from amongst the 33 families (Table 5) to investigate the relationship between the causative genes and clinical symptoms of FEVR. Participants with poor image quality were excluded. The parameters of patients with monogenic variants in LRP5, FZD4 and TSPAN12 were compared further due to the sample size. The phenotypic severity varied among the genotypes (P_LRP5-FZD4-TSPAN12 = 0.002) as follows. FZD4 was associated with FEVR of greater severity than LRP5 (P_LRP5-FZD4 = 0.001). The frequency of foveal hypoplasia was the highest in patients with the FZD4 variants; however, the frequency of hypoplasia did not differ significantly among the variants.

The vessel densities of the DCP on the whole and parafoveal images were lower in participants with digenic variants (P_whole image = 0.021; P_parafoveal = 0.022) compared to those with monogenic variants. The AI of the FAZ in patients with TSPAN12 was lower than that in patients with LRP5 (P_LRP5-FZD4-TSPAN12 = 0.013; P_LRP5-TSPAN12 = 0.013). However, patients with FEVR who carried LRP5 variants tended to have a lower foveal vascular density in the SCP and DCP than those harbouring TSPAN12 variants (P_SCP_LRP5-FZD4-TSPAN12 = 0.010; P_DCP_LRP5-FZD4-TSPAN12 = 0.028). The FD-300 was also lower in patients with LRP5 variants compared to those with FZD4 variants (P_LRP5-FZD4-TSPAN12 = 0.024; P_LRP5-FZD4 = 0.028).

### Table 3. The clinical characteristics of eyes with FEVR and control eyes.

| Parameters | FEVR(95% CI) | Control(95% CI) | p Value |
|------------|-------------|----------------|---------|
| Retinal artery angle | | | |
| Age (years) | 23.3 ± 16.4 | 21.3 ± 12.6 | 0.522 |
| Angle (°) | 90.30 ± 19.46 | 122.10 ± 13.34 | <0.001* |
| (86.85–93.76) | (119.06–125.15) | | |
| Macular structure | | | |
| N | 124 | 76 | – |
| Age (years) | 108 | 76 | – |
| With FH/All (%) | 3292 | 5124 | 0.840 |
| CMT | 259.54 ± 56.66 | 248.41 ± 15.31 | 0.399 |
| (247.99–271.08) | (244.91–251.91) | | |
| IRT | 12.47 ± 21.70 | 0.78 ± 4.09 | <0.001* |
| (7.30–16.03) | (0.16–1.71) | | |
| OCTA | | | |
| N | 70 | 76 | – |
| Age (years) | 25.6 ± 18.8 | 21.3 ± 12.6 | 0.334 |
| FAZ Area (mm^2) | 0.27 ± 0.13 | 0.27 ± 0.09 | 0.724 |
| (0.24–0.30) | (0.25–0.29) | | |
| Perimeter (mm) | 2.08 ± 0.55 | 2.06 ± 0.38 | 0.782 |
| (1.95–2.21) | (1.97–2.15) | | |
| AI | 1.18 ± 0.14 | 1.13 ± 0.04 | <0.001* |
| (1.15–1.21) | (1.12–1.14) | | |
| FD-300 (%) | 48.50 ± 6.74 | 51.42 ± 3.82 | 0.01* |
| (46.87–50.13) | (50.55–52.29) | | |
| Whole image VD (%) | | | |
| SCP | 46.75 ± 4.58 | 48.17 ± 2.11 | 0.020* |
| (45.65–47.84) | (47.69–48.65) | | |
| DCP | 47.53 ± 5.44 | 52.51 ± 3.00 | <0.001* |
| (46.23–48.82) | (51.83–53.20) | | |
| Foveal VD (%) | | | |
| SCP | 22.66 ± 9.60 | 20.97 ± 5.49 | 0.721 |
| (20.37–24.95) | (19.72–22.23) | | |
| DCP | 34.48 ± 8.61 | 35.38 ± 6.55 | 0.479 |
| (32.43–36.53) | (33.88–36.87) | | |
| Parafoveal VD (%) | | | |
| SCP | 49.12 ± 4.74 | 50.80 ± 2.52 | 0.009* |
| (47.99–50.25) | (50.22–51.37) | | |
| DCP | 49.39 ± 6.10 | 54.66 ± 3.16 | <0.001* |
| (47.94–50.84) | (53.94–55.38) | | |

*p < 0.05.

N: number of eyes; FH: foveal hypoplasia; CMT: centre macular thickness; IRT: inner retinal thickness; FAZ: foveal avascular zone; AI: acircularity index; FD-300: the foveal vessel density in a 300-μm-wide region around FAZ; VD: vascular density; SCP: superficial capillaryplexus; DCP: deep capillaryplexus.
Discussion

In this study, we employed comprehensive clinical screening and systematic analysis of six genes related to 33 pedigrees of families with FEVR. Ultimately, 21 gene variants of \textit{FZD4}, \textit{LRP5}, \textit{NDP}, \textit{TSPAN12}, \textit{KIF11} and \textit{ZNF408} were identified, which were found to be present in 49.4% (40/81) of patients with FEVR. Ten variants are novel since they have not been reported previously. \textit{LRP5}, which was present in 38.1% of cases in our study, was the most frequently occurring of the five genes with variants associated with FEVR, while \textit{ZNF408} had no pathogenic variants, concordant with previous studies [21]. The gene mutation detection rates in FEVR seem to vary from study to study. Rao et al. [21] reported variants in \textit{LRP5}, \textit{FZD4}, \textit{TSPAN12}, \textit{NDP} and \textit{KIF11} that accounted for 38.7% of patients with FEVR from 31 family pedigrees. Wang et al. [26] showed that up to 51.2% of the families had identifiable variants. It is possible that numerous uncharacterized pathogenic genes for FEVR remain undiscovered. \textit{LRP5}, \textit{FZD4}, \textit{TSPAN12}, \textit{NDP}, \textit{LRP6} and \textit{CTNNB1} have been identified as components of the Norrin/\(\beta\)-catenin signalling pathway. This pathway is highly conserved in biological evolution, playing a significant role in retinal angiogenesis. Recent studies have suggested that \textit{RCBTB1}, \textit{CTNND1}, \textit{CTNNA1}, \textit{ILK} and \textit{DLG1} also participate in the regulation of the Norrin/\(\beta\)-catenin signalling pathway, resulting in abnormal growth of retinal blood vessels. Additionally, Notch ligand \textit{JAG1} was reported to be a novel candidate gene for FEVR. These findings indicate that the pathogenesis of FEVR involves multiple signalling pathways.

The clinical manifestations of FEVR are complex and diverse [27]. We investigated and compared the structural and vessel-related parameters of the retina using wide-angle fundus imaging, OCT and OCTA and found that the retinal artery angle was smaller in eyes with FEVR compared to the control eyes. We speculated that retinal contraction in FEVR may be responsible for this change. Lee et al. [28] used hand-held OCT to investigate the vitreoretinal pathologies in FEVR. These pathologies included temporal and anterior displacement of the retina, significant vitreous capillary adhesion or traction and thickening of the retinal nerve fibre layer at the edge of ONH with protrusion of the adjacent retina. Nagura et al. [24] reported that the YCA was smaller in the eyes with an epiretinal membranes than that in the contralateral eyes. They speculated that contraction of the retina secondary to stretching by the epiretinal membrane was responsible for the decrease in the YCA and deterioration in visual function. There was no significant difference in the retinal artery angle between the mild and severe phenotype groups in our study. The small sample size may account for the absence of detectable differences, and retinal folds and shrinkage were common in several severely affected eyes. This made it difficult to identify and measure certain regions, such as the optic disc and macula. We also found that the incidence of foveal hypoplasia was high in FEVR eyes, especially in those with severe disease. Chen et al. [29] reported that hypoplasia of the inner retinal layer of the fovea occurred in 48.78% of FEVR eyes, which supports our findings. Foveal hypoplasia seemed to be related with angiodysplasia, including a small retina artery angle, small FAZ area and lower FD-300, SCP and DCP. When the FAZ is completely surrounded by parafoveal capillary beds, the cells of the inner retina can be pushed.

![Figure 4. Retinal artery angle, OCTA parameters and incidence of foveal hypoplasia in eyes with FEVR and normal eyes. The FEVR patients had a smaller retinal artery angle (A) than the normal individuals (B). The vascular density of SCP and DCP decreased in the eyes of FEVR patients (C, E) when compared to the normal eyes (D, F). The preserved foveal inner retinal layer was noted in the patients affected by FEVR (G) while the normal individuals had a normal structure (H).](image-url)
centrifugally to form the foveal pit during the maturation of the fovea [30]. In our study, the vascular density of the parafoveal layer and FD-300 were lower in eyes with FEVR. We postulate that this mechanism was impaired in some patients with FEVR, leading to foveal hypoplasia. These results suggest the presence of pathological changes in the fovea in addition to the peripheral retina. Moreover, Zhang et al. [31] reported that the decrease in the vascular density of the SCP was independently related to the severity of FEVR and vision loss. These findings indicate the potential correlation between vascular density and fovea development.

The relationship between the genotype and clinical phenotype of FEVR has been frequently studied and discussed in the literature. Our results showed that the vascular density of the DCP in the whole and parafoveal images was smaller in patients with digenic inheritance of FEVR than that in patients with monoallelic inheritance. Li et al. [32] reported that most eyes of 13 probands with two disease-causing variants had stage 4 (38.46%) or stage 5 (26.92%) disease. A case series detected a heterozygous biallelic variant in FZD4 leading to hearing deficits and developmental delays [19]. In LRPS variants, a higher incidence of severe phenotype was observed in patients with biallelic variants compared to those with monoallelic variants [33]. These findings may be related to the cumulative effect of the multiple and complex signal pathways related to FEVR.

We determined that FEVR of greater severity was more frequently associated with FZD4 compared to LRP5. Foveal hypoplasia was noted in 55% patients with FZD4 variants. Chen et al. [29] searched for variants in 27 FEVR-afflicted families and reported that a preserved foveal IRL or small FAZ area did not occur in patients with FEVR with the LRPS variants, implying that LRPS causes mild phenotypic manifestations, consistent with our results. However, Seo et al. [34] found that an FZD4 variant seemed to result in a milder phenotype compared to LRPS, based on the comparison of FEVR severity and visual acuity in 18 patients. A study including 89 patients with unilateral or bilateral retinal folds found that the former were observed in 87.5% (14/16) and 73.7% (14/19) of patients with LRPS and FZD4 variants, respectively, suggesting that patients with LRPS and FZD4 variants manifested milder phenotypes and a higher frequency of asymmetry [35]. Binocular involvement and severe manifestation occurred in 79.2% (38/48)

### Table 4. The clinical characteristics of different severity of FEVR.

| Parameters          | With FH | Without FH | P     | Stage 1 | Stage 2 | p  |
|---------------------|---------|------------|-------|---------|---------|----|
| Retinal artery angle | N       |            |       |         |         |    |
| Angle (°)           |         | 91.57 ± 17.46 | 0.013* | 91.60 ± 18.24 | 0.895 ± 21.02 | 0.682 |
|                     |         | (73.44–88.78) |       | (87.44–95.77)  | (82.37–97.53)  |    |
| Macular structure   | N       |            |       |         |         |    |
| CMT                 |         | 249.33 ± 17.12 | 0.018 | 248.84 ± 37.82 | 273.04 ± 78.54 | 0.014* |
|                     |         | (244.94–253.71) |       | (238.81–258.88) | (242.58–303.49) |    |
| IRT                 |         | 36.17 ± 22.58 | 0.001* | 8.82 ± 21.04 | 12.60 ± 16.84 | 0.112 |
| (27.74–44.60)       |         | (3.24–14.41)  |       | (5.64–19.56)   |              |    |
| OCTA                | N       |            |       |         |         |    |
| FAZ Area (mm²)      |         | 0.22 ± 0.16 | 0.006* | 0.28 ± 0.13 | 0.25 ± 0.12 | 0.486 |
|                     |         | (0.15–0.29)  |       | (0.24–0.32)   | (0.21–0.30)   |    |
| Perimeter (mm)      |         | 1.84 ± 0.75 | 0.031* | 2.15 ± 0.54 | 1.99 ± 0.46 | 0.224 |
|                     |         | (1.50–2.18)  |       | (1.97–2.33)   | (1.80–2.18)   |    |
| AI                  |         | 1.23 ± 0.20 | 0.020* | 1.20 ± 0.17 | 1.15 ± 0.06 | 0.629 |
|                     |         | (1.14–1.33)  |       | (1.14–1.25)   | (1.13–1.17)   |    |
| FD-300 (%)          |         | 46.04 ± 9.17 | 0.006* | 48.60 ± 4.94 | 50.06 ± 6.68 | 0.450 |
|                     |         | (41.87–50.22) |       | (47.02–50.18) | (48.54–51.57) |    |
| Whole image VD (%)  |         | 46.07 ± 7.14 | 0.940 | 46.31 ± 4.51 | 47.97 ± 2.40 | 0.416 |
|                     |         | (42.98–49.16) |       | (45.89–47.74) | (45.98–48.96) |    |
| DCP                 |         | 44.93 ± 6.07 | 0.005* | 47.89 ± 5.48 | 48.97 ± 4.48 | 0.781 |
|                     |         | (47.38–50.12) |       | (46.11–49.81) | (46.11–48.91) |    |
| Foveal VD (%) SCP   |         | 28.17 ± 13.35 | 0.008* | 21.30 ± 5.54 | 23.87 ± 5.34 | 0.019* |
|                     |         | (22.40–33.94) |       | (18.45–23.74) | (21.97–29.68) |    |
| DCP                 |         | 37.66 ± 12.05 | 0.005* | 33.90 ± 8.69 | 36.03 ± 8.09 | 0.327 |
|                     |         | (32.45–42.88) |       | (31.16–36.65) | (32.69–39.37) |    |
| Parafoveal VD (%)   |         | 47.67 ± 7.22 | 0.008* | 48.94 ± 4.92 | 49.98 ± 2.18 | 0.947 |
|                     |         | (44.55–50.79) |       | (47.39–50.50) | (49.08–50.88) |    |
| DCP                 |         | 46.20 ± 7.09 | 0.042* | 49.99 ± 6.00 | 49.30 ± 5.04 | 0.496 |
|                     |         | (41.14–49.27) |       | (48.09–51.88) | (47.22–51.38) |    |

*p < 0.05.
N: number of eyes; FH: foveal hypoplasia; CMT: centre macular thickness; IRT: inner retinal thickness; FAZ: foveal avascular zone; AI: acircularity index; FD-300: the foveal vessel density in a 300-μm-wide region around FAZ; VD: vascular density; SCP: superficial capillary plexus; DCP: deep capillary plexus.
patients with NDP variants [36]. The proband with NDP in this study also exhibited binocular involvement and underwent surgery. The phenotype in KIF11 was different from other FEVR genes. The retinopathy in patients with FEVR presented as chorioretal dysplasia [37]. The AI of the FAZ obtained from OCTA imaging was significantly lower in TSPAN12 eyes than that in eyes with the LRPS5 variants. The foveal vascular density in the SCP and DCP was lower in FEVR patients with LRPS5 than that in patients harbouring TSPAN12 variants. FD-300 was also lower in eyes with LRPS5 variants compared to eyes with FZD4 and TSPAN12 variants. Thus, we may infer that patients with FEVR with LRPS5 variants exhibited a broader phenotypic spectrum. The variations in the results of different studies may be attributed to the differences in their sample populations. Additionally, environmental factors may influence the phenotypic manifestation for each FEVR variant, and/or there may be epigenetic changes occurring during development or in the process of incomplete gene penetrance [38]. Further studies are warranted to investigate this aspect in detail.

There are some limitations to our study. First, we enrolled patients with FEVR, including probands and all their family members to investigate the clinical characteristics and genotype–phenotype relationships. This strategy could have introduced selection bias into the results. Second, the number of patients with the severe phenotype was relatively small, resulting in an uneven distribution of FEVR grades. Third, some children were too young to undergo examinations that required compliance; thus, the possibility exists that the missing data skewed the results and data analysis. These limitations are expected to be addressed in future research by enrolling a larger sample population.

### Table 5. The clinical characteristics of different genotypes in FEVR cohort.

| Parameters | Monogenic variants (95%CI) | Digenic variants (95%CI) | p Value | LRPS (95%CI) | FZD4 (95%CI) | TSPAN12 (95%CI) | p Value |
|------------|-----------------------------|--------------------------|---------|--------------|--------------|----------------|---------|
| Stages of FEVR | | | | | | | |
| Mild | N=66 | 12 | – | 16 | 30 | 16 | – | 0.002* |
| Severe | N=3 | 4 | 0 | 0 | 2 | 0 | | |
| Retinal artery Angle | N=50 | 8 | – | 15 | 18 | 13 | – | 0.289 |
| Structure Macular CMT | With FH/All (%) | 22/44(50.0%) | 7/12(58.3%) | 0.609 | 4/12(33.3%) | 11/20(55.0%) | 0.05 | 5/10(50.0%) | 0.523 |
| IRT | 265.87±45.27 | 249.67±28.42 | 0.222 | 265.00±60.11 | 260.38±37.08 | 268.70±36.77 | 0.653 | 267.80±36.77 | 0.653 |
| OCTA | Physiological | VD (%) SCP | 21.92±6.26 | 29.11±15.40 | 0.208 | 17.62±2.99 | 21.02±6.55 | 0.010 | 28.68±1.65 | 0.010 |
| | Area (mm²) FAZ | 0.28±0.13 | 0.27±0.21 | 0.468 | 0.25±0.06 | 0.36±0.17 | 0.21±0.03 | 0.113 |
| | Perimeter (mm) | 2.0±0.50 | 1.9±0.89 | 0.664 | 2.32±0.55 | 2.34±0.53 | 1.83±0.18 | 0.175 |
| | AI | 1.19±0.21 | 1.18±0.10 | 0.809 | 1.33±0.35 | 1.14±0.02 | 1.12±0.04 | 0.013* |
| | (1.10–1.29) | (1.10–1.25) | (0.96–1.70) | (1.12–1.15) | (1.07–1.17) | | |
| | FAZ Area (mm²) | 47.72±5.87 | 50.49±4.01 | 0.438 | 42.66±8.06 | 50.45±2.90 | 49.43±1.40 | 0.024* |
| | (44.57–55.43) | (49.92–51.53) | (39.70–51.27) | (40.20–52.87) | (43.70–51.17) | | |
| Whole image | SCP | 46.99±4.37 | 49.82±5.10 | 0.153 | 43.53±6.08 | 48.00±3.30 | 47.32±1.90 | 0.191 |
| | (44.45–48.53) | (45.90–53.75) | (37.16–49.91) | (45.46–50.54) | (45.97–49.67) | | |
| | DCP | 46.85±5.39 | 41.37±5.93 | 0.021* | 46.02±4.72 | 48.02±6.42 | 45.74±4.69 | 0.543 |
| | (44.33–49.37) | (36.81–45.93) | (41.06–50.97) | (43.09–52.96) | (39.92–51.56) | | |
| | FAZ (mm²) | 33.99±8.78 | 37.30±14.76 | 0.501 | 29.78±3.48 | 30.27±11.58 | 40.34±2.10 | 0.034* |
| | (29.88–38.10) | (25.95–48.65) | (26.13–33.44) | (24.36–42.17) | (27.33–42.95) | | |
| Parafoveal area | SCP | 48.56±4.91 | 51.88±4.45 | 0.062 | 45.63±6.92 | 50.47±3.75 | 48.64±2.30 | 0.231 |
| | (46.26–50.86) | (48.46–55.30) | (38.37–52.90) | (47.58–53.35) | (45.79–51.49) | | |
| | DCP | 48.44±5.80 | 42.23±7.57 | 0.022* | 47.78±4.82 | 49.79±7.02 | 46.78±4.87 | 0.399 |
| | (45.72–51.15) | (36.41–48.05) | (42.73–52.84) | (44.39–55.19) | (40.73–52.83) | | |

*p < 0.05.

N: number of eyes; FH: foveal hypoplasia; CMT: centre macular thickness; IRT: inner retinal thickness; FAZ: foveal avascular zone; AI: acircularity index; FD-300: the foveal vessel density in a 300-μm-wide region around FAZ; VD: vascular density; SCP: superficial capillary plexus; DCP: deep capillary plexus.
Conclusions

In summary, we discovered a total of 21 FEVR gene variants, 10 of which have never been reported. LRP5 had the highest proportion of variants. We also reported the retinal artery angle, occurrence of foveal hypoplasia and OCTA features in FEVR, and that the presence of FZD4 variants may lead to more severe FEVR than LRP5 variants. In light of these findings, we suspect that other unknown genes may also cause and/or contribute to the FEVR phenotype that remain to be identified, necessitating future studies that include detailed genetic and clinical research.

Author contributions

L.J.S designed and supervised the study and revised the manuscript. J.B.M, Y.J.C, Y.Y.F and Y.R.S analysed the data and wrote the paper. Z.Y.X and H.X.L evaluated the clinical characteristics of the enrolled patients. J.B.M, Y.J.C, Y.Y.F, S.X.Z and Y.Q.C performed the research. All authors contributed to the article and approved the submitted version.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Data availability statement

Data are available from the corresponding author upon reasonable request.

References

[1] Boonstra FN, van Nouhuys CE, Schuil J, et al. Clinical and molecular evaluation of probands and family members with familial exudative vitreoretinopathy. Invest OphthalmoCommunic. 2019;10(1):5243.

[2] Musada GR, Syed H, Jalali S, et al. Mutation spectrum of the FZD-4, TSPAN12 and ZNF408 genes in Indian FEVR patients. BMC Ophthalmol. 2016;16:90.

[3] Li JK, Fei P, Li Y, et al. Identification of novel KIF11 mutations in patients with familial exudative vitreoretinopathy and a phenotypic analysis. Sci Rep. 2016;6:26564.

[4] Nikopoulos K, Gilsiethen C, Hoischen A, et al. Next-generation sequencing of a 40 Mb linkage interval reveals TSPAN12 mutations in patients with familial exudative vitreoretinopathy. Am J Hum Genet. 2010;86(2):240–247.

[5] Collin RW, Nikopoulos K, Dona M, et al. ZNF408 is mutated in familial exudative vitreoretinopathy and is crucial for the development of zebrafish retinal vasculature. Proc Natl Acad Sci USA. 2013;110(24):9856–9861.

[6] Khan K, Logan CV, McKibbin M, et al. Next generation sequencing identifies mutations in atonal homolog 7 (ATOH7) in families with global eye developmental defects. Hum Mol Genet. 2012;21(4):776–783.

[7] Zhang S, Li X, Liu W, et al. Whole-Exome sequencing identified Dlg1 as a candidate gene for familial exudative vitreoretinopathy. Genet Test Mol Biomarkers. 2021;25(5):309–316.

[8] Downey LM, Keen TJ, Roberts E, et al. A new locus for autosomal dominant familial exudative vitreoretinopathy maps to chromosome 11p12-13. Am J Hum Genet. 2001;68(3):778–781.

[9] Yang M, Li S, Huang L, et al. CTNND1 variants cause familial exudative vitreoretinopathy through the wnt/cadherin axis. JCI Insight. 2022;7(14):e158428.

[10] Zhu X, Yang M, Zhao P, et al. Catenin σ 1 mutations cause familial exudative vitreoretinopathy by overactivating norrin/β-catenin signaling. J Clin Invest. 2021;131(8):e139869.

[11] Li S, Yang M, He Y, et al. Variants in the Wnt co-receptor LRP6 are associated with familial exudative vitreoretinopathy. J Genet Genomics. 2022;49(6):590–594.

[12] Wu JH, Liu JH, Ko YC, et al. Haploinsufficiency of RCBT1 is associated with coats disease and familial exudative vitreoretinopathy. Hum Mol Genet. 2016;25(8):1637–1647.

[13] Park H, Yamamoto H, Mohn L, et al. Integrin-linked kinase controls retinal angiogenesis and is linked to wnt signaling and exudative vitreoretinopathy. Nat Commun. 2019;10(1):5243.

[14] Zhang L, Zhang X, Xu H, et al. Exome sequencing revealed notch ligand JAG1 as a novel candidate gene for familial exudative vitreoretinopathy. Genet Med. 2020;22(1):77–84.

[15] Toomes C, Bottomley HM, Jackson RM, et al. Mutations in LRP5 or FZD4 underlie the common familial exudative vitreoretinopathy locus on chromosome 11q. Am J Hum Genet. 2004;74(4):721–730.

[16] Chen ZY, Battinelli EM, Fielder A, et al. A mutation in the Norrie disease gene (NDP) associated with X-linked familial exudative vitreoretinopathy. Nat Genet. 1993;5(2):180–183.

[17] Robitaille JM, Gillett RM, LeBlanc MA, et al. Phenotypic overlap between familial exudative vitreoretinopathy and microcephaly, lymphedema, and choriorretinal dysplasia caused by KIF11 mutations. JAMA Ophthalmol. 2014;132(12):1393–1399.

[18] Coussa RG, Zhao Y, DeBenedictis MJ, et al. Novel mutation in CTNNB1 causes familial exudative vitreoretinopathy (FEVR) and microcephaly: case report and review of the literature. Ophthalmic Genet. 2020;41(1):63–68.

[19] van der Ende SR, Meyers BS, Capasso JE, et al. Severe familial exudative vitreoretinopathy, congenital hearing loss, and developmental delay in a child with biallelic variants in FZD4. JAMA Ophthalmol. 2022;140(9):889–893.
[20] Chen C, Sun L, Li S, et al. The spectrum of genetic mutations in patients with asymptomatic mild familial exudative vitreoretinopathy. Exp Eye Res. 2020;192:107941.

[21] Rao FQ, Cai XB, Cheng FF, et al. Mutations in LRP5, FZD4, TSPAN12, NDP, ZNF408, or KIF11 genes account for 38.7% of Chinese patients with familial exudative vitreoretinopathy. Invest Ophthalmol Vis Sci. 2017;58(5):2623–2629.

[22] Salvo J, Lyubasyuk V, Xu M, et al. Next-generation sequencing and novel variant determination in a cohort of 92 familial exudative vitreoretinopathy patients. Invest Ophthalmol Vis Sci. 2015;56(3):1937–1946.

[23] Pendergast SD, Trese MT. Familial exudative vitreoretinopathy. Results of surgical management. Ophthalmology. 1998;105(6):1015–1023.

[24] Nagura K, Inoue T, Zhou HP, et al. Association between retinal artery angle and visual function in eyes with idiopathic epiretinal membrane. Transl Vis Sci Technol. 2021;10(9):35.

[25] Thomas MG, Kumar A, Mohammad S, et al. Structural grading of foveal hypoplasia using spectral-domain optical coherence tomography a predictor of visual acuity? Ophthalmology. 2011;118(8):1653–1660.

[26] Wang S, Zhang X, Hu Y, et al. Clinical and genetical features of probands and affected family members with familial exudative vitreoretinopathy in a large Chinese cohort. Br J Ophthalmol. 2021;105(1):83–86.

[27] Tauqeer Z, Yonekawa Y, Retina Service, Massachusetts Eye and Ear Infirmary, Harvard Medical School. Familial exudative vitreoretinopathy: pathophysiology, diagnosis, and management. Asia Pac J Ophthalmol (Phila). 2018;7(3):176–182.

[28] Lee J, El-Dairi MA, Tran-Viet D, et al. Longitudinal changes in the optic nerve head and retina over time in very young children with familial exudative vitreoretinopathy. Retina. 2019;39(1):98–110.

[29] Chen C, Liu C, Wang Z, et al. Optical coherence tomography angiography in familial exudative vitreoretinopathy: clinical features and Phenotype-Genotype correlation. Invest Ophthalmol Vis Sci. 2018;59(15):5726–5734.

[30] Vajzovic L, Hendrickson AE, O’Connell RV, et al. Maturation of the human fovea: correlation of spectral-domain optical coherence tomography findings with histology. Am J Ophthalmol. 2012;154(5):779–789.e2.

[31] Zhang J, Jiang C, Ruan L, et al. Macular capillary dropout in familial exudative vitreoretinopathy and its relationship with visual acuity and disease progression. Retina. 2020; Jun40(6):1140–1147.

[32] Li Y, Peng J, Li J, et al. The characteristics of digenic familial exudative vitreoretinopathy. Graefes Arch Clin Exp Ophthalmol. 2018;256(11):2149–2156.

[33] Chen C, Zhang X, Peng X, et al. Lrp5 biallelic mutations cause a higher incidence of severe phenotype compared with Lrp5 monoallelic mutation. Retina. 2022;42(10):1958–1964. 1

[34] Seo SH, Yu YS, Park SW, et al. Molecular characterization of FZD4, LRP5, and TSPAN12 in familial exudative vitreoretinopathy. Invest Ophthalmol Vis Sci. 2015;56(9):5143–5151.

[35] Wang Z, Chen C, Sun L, et al. Symmetry of folds in FEVR: a genotype-phenotype correlation study. Exp Eye Res. 2019;186:107720.

[36] Chen C, Cheng Y, Zhang Z, et al. Long-term clinical prognosis of 335 infant single-gene positive FEVR cases. BMC Ophthalmol. 2022;22(1):329.

[37] Wang Y, Zhang Z, Huang L, et al. Update on the phenotypic and genotypic spectrum of KIF11-Related retinopathy. Genes (Basel). 2022;13(4):713.

[38] Fei P, Zhang Q, Huang L, et al. Identification of two novel LRP5 mutations in families with familial exudative vitreoretinopathy. Mol Vis. 2014;20:395–409.