Familial exudative vitreoretinopathy (FEVR) is a rare Mendelian monogenic disorder resulting in tractional retinal detachment and blindness in adolescents. The disease was first reported by Criswick and Schepens1 in 1969. The primary pathologic process in FEVR is incomplete retinal vascular development on the temporal side of the peripheral retina, which is followed by neovascularization of the retina.2,3 New retinal blood vessels are prone to leakage and rupture, which result in retinal falciform fold, retinal traction with ectopic macula, intraretinal hemorrhage, exudation, followed by subtotal and total retinal detachment, total vision loss and blindness. The clinical manifestations of this disease differ greatly among patients even between the two eyes of the same patient and among affected siblings in the same family, from asymptomatic to complete retinal detachments with blindness.2-4 Many of these retinal abnormalities are similar to retinopathy of prematurity (ROP). However, the affected cases with FEVR, unlike patients with ROP, usually go through a normal gestational period and have no history of low birth weight and supplemental oxygen therapy.5,6 Additionally, detachments often do not occur in FEVR until the first or second decade of life. In fact, the retinal manifestations may appear to remain stable when a patient reaches adulthood in this variable progressive disorder.1

FEVR is inherited as an autosomal dominant, an autosomal recessive, or an X-linked recessive trait. However, autosomal dominant inheritance due to haploinsufficiency is the most prominent form of the disease. To date, six genes have been reported to associate with FEVR, including LRP5, FZD4, TSPAN12, NDP, ZNF408, and KIF11.7-14 The proteins encoded by the first four genes are cooperative in the Wnt/Norrin signaling pathway, which plays a critical role in eye development and angiogenesis. As a result, mutations in these genes can lead to abnormal retinal vascular formation. A recent study15 has demonstrated that Lrp5 knockout (Lrp5−/−) mouse model of FEVR displays significantly delayed retinal vascular development and subsequent pathologic glomeruloid vessels. In addition, as a new candidate gene potentially related to FEVR, novel founder mutations in ZNF408 have been reported in one Dutch family and a Japanese family. Subsequently, knockdown of znf408 in zebrafish indicates that it plays a putative role in retinal vasculogenesis.11 Mutations in KIF11 are reported to be associated with microcephaly, chorioretinopathy, or mental retardation (MCLMR).16 Furthermore, KIF11

Mutations in LRP5, FZD4, TSPAN12, NDP, ZNF408, or KIF11 Genes Account for 38.7% of Chinese Patients With Familial Exudative Vitreoretinopathy

Feng-Qin Rao,1 Xue-Bi Cai,1 Fei-Fei Cheng,1 Wan Cheng,1 Xiao-Long Fang,1 Na Li,2 Xiu-Feng Huang,1 Li-Hong Li,2 and Zi-Bing Jin1

1Division of Ophthalmic Genetics, Lab for Stem Cell & Retinal Regeneration, Institute of Stem Cell Research, The Eye Hospital, Wenzhou Medical University, State Key Laboratory of Ophthalmology, Optometry and Vision Science, Wenzhou, China
2Maternal and Children's Hospital, Kunming, Yunnan, China

Correspondence: Zi-Bing Jin, Division of Ophthalmic Genetics, Lab for Stem Cell & Retinal Regeneration, Institute of Stem Cell Research, The Eye Hospital, Wenzhou Medical University, State Key Laboratory of Ophthalmology, Optometry and Vision Science, Wenzhou 325027, China; jinzb@mail.eye.ac.cn.

F-QR and X-BC contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Submitted: December 19, 2016
Accepted: April 12, 2017

Citation: Rao F-Q, Cai X-B, Cheng F-F, 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;58:2623−2629. DOI: 10.1167/iovs.16-21324
mutations have been recently identified in affected individuals with FEVR by Robitaille et al.13 Besides, there is a phenotypic overlap between the two diseases caused by KIF11 mutations. Other recent studies14,15 have also reported novel KIF11 mutations in FEVR patients.

Despite the individual identifications of the causal genes in previously reported cases, few studies have attempted to screen for the whole panel of causative genes in a defined cohort.14,17,18 Additionally, genotype–phenotype correlation in FEVR has rarely been studied.14,19,20 Thus, the present study was carried out to fill in the knowledge gap by exploring the mutation spectrum and genotype-phenotype correlation in 31 pedigrees of Chinese FEVR patients.

METHODS

Clinical Examinations

This study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient, following the ethical approval from The Eye Hospital of Wenzhou Medical University and Rumning Maternal and Children’s Hospital. We recruited 31 pedigrees with FEVR and 200 healthy controls. All individuals are native Chinese, of full-term birth and normal birth weight. Full ophthalmic examinations were conducted, including visual acuity measurement, slit-lamp examination, detailed fundus photography, and fundus fluorescein angiography (FFA). Each proband and his/ her available family members were assessed by a panel of retinal specialists. The disease severities were further classified and assessed in accordance with the staging system reported by Pendergast et al.4

Comprehensive Mutational Screening

Peripheral blood samples were collected from the 31 pedigrees and preserved in −80°C before use. Genomic DNAs were extracted by using a whole blood DNA extraction kit (Simgen, Hangzhou, China) according to standard protocols. Polymerase chain reactions were performed by using specific primers targeting all open reading frames and the flanking intronic sequences for direct sequencing on genetic sequencer. After the initial denaturation step at 95°C for 5 minutes, the samples were subjected to 35 cycles of amplification at 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds. A final elongation step was performed at 72°C for 5 minutes.

Pathogenicity Evaluation

To evaluate the pathogenicity of the identified mutations, we explored 1000 Genome database (1000G),21 Exome Aggregation Consortium (EXAC),22,23 and in-house data from previous studies. Potential deleteriousness was evaluated from the protein; in the public domain, and multiple protein alignments were used: ClustalW2 (http://www.ebi.ac.uk/ clustalw2/ in the public domain).25 Sequence logos were generated on account of WebLogo3 (http://weblogo.

RESULTS

Clinical Manifestations

In this study, we recruited 31 pedigrees clinically diagnosed with FEVR. Among the probands, 13 were females and 18 were males. Among those, no one had a history of low birth weight or premature birth. Before being diagnosed with FEVR, most probands were found to exhibit other ocular abnormalities, such as strabismus, nystagmus, leukocoria, and the inability to follow moving objects. Fundus photography revealed typical falciform retinal folds and temporal dragging of optic disc and retinal detachment. FFA exhibited incomplete vascularization of the peripheral retina, such as a peripheral avascular zone, especially in patients with unnoticed mild phenotypes. Additionally, a 2-year-old proband (F28, II: 2) was found to have microcephalus, whose head circumference was only 44 cm, 2 standard deviations (SDs) below the mean. The clinical classification for each family is shown in Supplementary Table S1.

Mutations in the FEVR Cohort

We considered a variation as “pathogenic” by the following standards: (1) excluding variants identified in 1000G, ExAC, database of SNP (dbSNP), and 200 normal individuals; (2) excluding variants predicted to be benign by the standards and guidelines from ACMG and 11 bioinformatics tools; and (3) excluding incompatible variants by cosegregation testing. Ultimately, we identified 11 novel mutations and 1 reported mutation in 12 unrelated FEVR families (Fig. 1; Table; Supplementary Fig. S1). Furthermore, we also observed that some affected individuals harbored multiple variants in LRP5 and TSPAN12. However, all these variants were considered as polymorphisms (Supplementary Fig. S2).

In family 15, the index case (F15: III: 3) harbored a nonsense mutation (c.345T>G, p.Y115*) in TSPAN12, which was a newly identified mutation. In family 9, a nonsense mutation (c.1384C>T, p.R462*) was discovered in LRP5. Deletions and insertions were uncovered in family 11 (LRP5, c.2817_2827+1del12bp, p.N940fs), family 21 (NDP, c.52_53ins32bp, p.S29fs), family 20 (FZD4, c.1282_1285del-GACA, p.D428fs), family 23 (FZD4, c.227delA, p.E76fs), and family 28 (KIF11, c.1830_1833delACAA, p.E610fs). Each of them was predicted to cause a frameshift mutation and a prematurely truncated protein. Of the five missense mutations, three occurred in LRP5 gene (c.4205G>A, p.G1402D; c.2257G>C, p.R746P; c.2618A>T, p.K875M) and two in NDP gene (c.195C>G, p.G65W; c.127C>A, p.H43N) (Fig. 2A).

Multiple Sequence Alignment

Amino acid sequences were obtained from National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/ protein; in the public domain), and multiple protein alignments were produced with the ClustalW2 (http://www.ebi.ac.uk/ clustalw2/ in the public domain).25 Sequence logos were generated on account of WebLogo3 (http://weblogo.

Downloaded From: http://arvojournals.org/ on 01/03/2018
### FIGURE 1. Pedigree information. In these pedigrees, the bars over the symbols denote the individuals recruited in the study; filled symbols indicate affected patients with FEVR, the unfilled symbols indicate unaffected individuals, and the dotted symbols denote carriers. M represents a variant, and + indicates normal allele.

### TABLE. Identified Mutations in Five Genes of Twelve Families With FEVR

<table>
<thead>
<tr>
<th>Family</th>
<th>Gene</th>
<th>Type</th>
<th>Mutation</th>
<th>1000G</th>
<th>ExAC</th>
<th>Reference</th>
<th>Cosegregation</th>
<th>ACMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>LRP5</td>
<td>Missense</td>
<td>c.4205G&gt;A, p.G1402D</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Paternal</td>
<td>Likely pathogenic</td>
</tr>
<tr>
<td>F3</td>
<td>LRP5</td>
<td>Missense</td>
<td>c.2237G&gt;C, p.R746P</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Paternal</td>
<td>Likely pathogenic</td>
</tr>
<tr>
<td>F7</td>
<td>LRP5</td>
<td>Missense</td>
<td>c.1384C&gt;T, p.R462*</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Maternal</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>F15</td>
<td>LRP5</td>
<td>Frameshift</td>
<td>c.2817_2827+1del12bp</td>
<td>Novel</td>
<td>Novel</td>
<td>Reported</td>
<td>De novo</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>F21</td>
<td>NDP</td>
<td>Frameshift</td>
<td>c.127C&gt;A, p.H43N</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>De novo</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>F23</td>
<td>NDP</td>
<td>Frameshift</td>
<td>c.127C&gt;A, p.H43N</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>De novo</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>F28</td>
<td>KIF11</td>
<td>Frameshift</td>
<td>c.127C&gt;A, p.H43N</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>De novo</td>
<td>Pathogenic</td>
</tr>
</tbody>
</table>
Mutational analysis of two novel mutations identified in *NDP* gene. (A) Chromatograms of the missense mutation c.195C>G (p.C65W) and c.127C>A (p.H43N) in the *NDP* gene. (B) Structural 3D modeling of NDP missense changes. Wild-type (a) and mutant residues (b, c) were indicated in red and blue, respectively. (C) Conservative amino acid analysis of NDP polypeptides from human to zebrafish, based on ClustalW2 (a) and WebLogo3 (b). The affected amino acid residues were underlined.
**FIGURE 3.** Comparative pathogenic analysis of the five missense mutations. The average of detailed functional predictive scores were calculated and shown in the heatmap after Min-Max normalization, based on the standards and guidelines by American College of Medical Genetics and Genomics and a series of bioinformatics tools. Deeper colors indicate higher pathogenicity. The x-axis represents various variants, while the y-axis depicts diverse pathogenic analysis. The variant in the bottom right (LRP5, p.A1330V) was a nonpathogenic polymorphism, which was used as a negative control.

**FIGURE 4.** Mutation spectrum in Chinese patients with FEVR. (A) The mutation spectrum indicates the proportion of different causal genes observed in the Chinese FEVR cohort. Each colored box represents different genes. △ represents “novel” in 1000G; ◊ represents “novel” in ExAC; and ★ represent “novel” and “reported” in-house data from previous studies, respectively; and ○ represents “pathogenic” and “likely pathogenic,” respectively. In accordance with the guidelines from ACMG. (B) Each colored box represents a mutation type: pink, missense; red, nonsense; blue, frameshift.
Mutation Spectrum

In this study, 12 patients (12/31, 38.7%) were successfully identified with genetic mutations. Among them, mutations in LRP5 (5/31, 16.1%) accounted for 42% of patients with defined mutations, indicating that LRP5 was the most frequent gene mutated in Chinese FEVR patients. The second leading mutation was located in NDP, which corresponded to 25% of patients with defined mutations, whereas mutations in FZD4, TSPAN12, and KIF11 accounted for the rest of the 33% of patients (Fig. 4). These results indicated that genetic factors were responsible for ~61.3% of patients with as yet unknown gene(s) that remain to be identified. Among the six known genes screened in this study, LRP5 and NDP were the predominant genes (67%), while no pathogenic mutations were identified in ZNF408. Through the comprehensive screening, we revealed the mutation spectrum of the six implicated genes in Chinese FEVR patients.

Genotype–Phenotype Correlations

To investigate the relationship between causative genes and FEVR symptoms, we also recruited other affected individuals (apart from the probands) to carry out genotype–phenotype analyses in the 12 families with disease-causing mutations (Supplementary Table S4). We noticed that the affected individuals harboring the LRP5 mutations showed broader phenotypic spectra that varied from stage 2 to stage 5. However, all patients carrying NDP mutations and truncating mutations were correlated with severe phenotypes (stage 4 or above) (Fig. 5). Our results revealed that patients with history of short-term oxygen therapy also showed more severe phenotypes (stage 4 or above). We, therefore, were tempted to hypothesize that short-term oxygen therapy after birth might be a major contributor to genetic causality of FEVR. In addition, owing to relatively small sample size, our findings warrant further investigation.

DISCUSSION

FEVR, a clinically heterogeneous disorder causing tractional retinal detachment at a very young age, is characterized by avascularity in the peripheral retina and compensatory retinal neovascularization. To date, six causative genes have been identified as related to FEVR, including low-density-lipoprotein receptor-related protein 5 (LRP5), frizzled-4 (FZD4), tetrascapnin-12 (TSPAN12), Norrie disease protein (NDP), zinc finger protein 408 (ZNF408), and kinesin family member 11 (KIF11). Previous studies have shown that approximately 35% to 50% of FEVR patients are genetically diagnosed by screening the six known genes. However, the detailed mutation spectrum of each gene was unknown in Chinese individuals. In the present study, we comprehensively screened all known genes in 31 pedigrees and uncovered 38.7% of unrelated FEVR patients harboring mutations in these genes. In addition, we, for the first time, revealed the detailed mutation spectrum in Chinese patients with FEVR.

Among the 31 families, 12 index ones were identified with genetic predispositions (38.7%), including five LRP5 mutations (5/31, 16.1%), three NDP mutations (3/31, 9.7%), two FZD4 mutations (2/31, 6.5%), one TSPAN12 mutation (1/31, 3.2%), and one KIF11 mutation (1/31, 3.2%). Except for the NDP mutations, which resulted in X-chromosome–linked FEVR, mutations in the other five genes cosegregated with the disease in an autosomal dominant manner. It should be noted that among the mutations identified in this study, most were described for the first time in this study.
Genetic Screen of Familial Exudative Vitreoretinopathy

From the clinical examinations of fundus photography and fundus fluorescein angiography, we noticed that retinal folds and detachments were common in severely affected patients, while mildly affected patients displayed avascularity in their peripheral retina. We thus attempted to elucidate whether a genotype–phenotype correlation is present in FEVR. We found that patients with mutations in the LRP5 gene displayed broader phenotypic spectras, from asymptomatic to complete retinal detachments, while in those with NDP mutations, the clinical phenotypes were found to be rather serious. Additionally, relevance between history of oxygen uptake and severe FEVR phenotypes were observed in our study. We found that history of oxygen uptake is probably a significant factor that leads to deterioration of FEVR. As a novel gene related to FEVR, KIF11 mutations were reported to be associated with MCLM. Recently, mutations in KIF11 and their associations with FEVR were reported. Besides, there were phenotypic overlaps between these two diseases that are caused by KIF11 mutations. In addition, we identified one novel truncating mutation in this study (c.1850_1853delACAA, p.E610fs), which broadened the current mutation frequency in this gene.

In conclusion, we performed a comprehensive screening in Chinese patients with FEVR and uncovered the mutation spectrum as well as the complexity of genotype–phenotype correlation, thus extending the current understanding of genetic predispositions in FEVR. However, we found that the detection rate of mutations found in the known genes did not exceed 50% in this study. In light of these results, we suspect that other unidentified genes are likely to be responsible for FEV as well, which warrant further genetic studies.

Acknowledgments

The authors thank all individuals examined and tested in the investigation.

Supported by the National Key Basic Research Program (2013CB967502), National Natural Science Foundation of China (81371059, 81522014), Zhejiang Provincial Key Research and Development Program (2015C03029), NHPPC Grant-in-Aid for Medical and Health Science (201479211), Wenzhou Science and Technology Innovation Team Project (C20150004), MOST Projects Medical and Health Science (201472911), Wenzhou Science and Development Program (2015C03029), NHFPC Grant-in-Aid for the National Key Basic Research Program (2013CB967502), National Natural Science Foundation of China (81371059, 81522014), Zhejiang Provincial Key Research and Development Program (2015C03029), NHPPC Grant-in-Aid for Medical and Health Science (201479211), Wenzhou Science and Technology Innovation Team Project (C20150004), MOST Projects Medical and Health Science (201472911), Wenzhou Science and Development Program (2015C03029), NHFPC Grant-in-Aid for

Disclosure: F.-Q. Rao, None; X.-B. Cai, None; F.-F. Cheng, None; W. Cheng, None; X.-L. Fang, None; N. Li, None; X.-F. Huang, None; L.-H. Li, None; Z.-B. Jin, None.

References