Familial exudative vitreoretinopathy (FEVR) is a severe hereditary retinal disorder characterized by defects in retinal vascular development. To date, six genes have been reported to be responsible for this disease, including LRP5, FZD4, TSPAN12, NDP, ZNF408, and KIF11. The purpose of our study was to investigate the genetic defects in Chinese patients with FEVR through mutational analyses of 31 pedigrees.

**Methods.** Clinical data and peripheral blood were collected from 31 pedigrees with FEVR. All coding sequences and intron/exon junctions were amplified and sequenced comprehensively, followed by cosegregation testing to verify suspected variants in the family members. Finally, we assessed clinical relevance of the identified mutations, according to the standards and guidelines from the American College of Medical Genetics and Genomics.

**Results.** Twelve index cases (12/31, 38.7%) were confirmed to harbor mutations in the known genes, including one previously reported mutation and 11 novel mutations. Among the detected mutations, LRP5 accounted for the largest proportion with a mean mutation rate of 16.1% (5/31, 16.1%), followed by NDP (3/31, 9.7%), FZD4 (2/31, 6.5%), TSPAN12 (1/31, 3.2%), and KIF11 (1/31, 3.2%). All the novel changes were predicted to be pathogenic by a series of bioinformatics analyses.

**Conclusions.** We comprehensively screened six known disease-causing genes in 31 pedigrees with FEVR and achieved a clear picture of the mutation spectrum in Chinese patients with FEVR, which highlights the importance and utility of clinical genetic diagnosis.

**Keywords:** familial exudative vitreoretinopathy, mutations, genetic diagnosis, genotype-phenotype correlations
mutations have been recently identified in affected individuals with FEVR by Robitaille et al.12 Besides, there is a phenotypic overlap between the two diseases caused by KIF11 mutations. Other recent studies13,14 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 Kunming 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 at –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 (Supplementary Table S1). PCR products were then purified and cloned into the pMega vector (Simgen, Hangzhou, China) and transformed into DH5α E. coli for sequencing. The identified sequences were used as probes to detect variants in all available family members. The identity of each sequence was confirmed by direct sequencing. Additionally, the identified mutations were verified in 1000 healthy controls to exclude polymorphisms.

Pathogenicity Evaluation

To evaluate the pathogenicity of the identified mutations, we explored 1000 Genome database (1000G),21 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 LR5 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 LR5. Deletions and insertions were uncovered in family 11 (LR5, c.2817_2827+1del112bp, p.N940fs*), family 21 (NDP, c.52_53ins32bp, p.S29fs), family 20 (Fzd4, c.1282_1285delGACA, 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 LR5 gene (c.4205G>A, p.G1402D; c.2237G>C, p.R746P; c.2618A>G, p.Y874D) and two in NDP gene (c.195C>G, p.G65W; c.1273C>A, p.H43N) (Fig. 2A). Structural modeling of the NDP protein (Fig. 2B) demonstrated that both G65W and H43N were located in the first antiparallel β-sheet, which was required for receptor binding. Additionally, both mutated amino acids were highly conserved residues from human to zebrafish (Fig. 2C).

In accordance with the guidelines from ACMG, one missense variant was perceived as “pathogenic” and four missense variants were predicted to be “likely pathogenic.” The pathogenicity was further verified by 11 bioinformatics tools (Fig. 3; Supplementary Table S3). Taken together, we identified 12 mutations in different genes in 12 unrelated FEVR families and 11 of them were described for the first time.
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>
<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>
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<tr>
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<td>LRP5</td>
<td>Missense</td>
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<td>Novel</td>
<td>Novel</td>
<td>Paternal</td>
<td>Likely pathogenic</td>
</tr>
<tr>
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<td>Missense</td>
<td>c.2237G&gt;C, p.R746P</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Maternal</td>
<td>Likely pathogenic</td>
</tr>
<tr>
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<td>LRP5</td>
<td>Missense</td>
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<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Maternal</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>F9</td>
<td>LRP5</td>
<td>Nonsense</td>
<td>c.1384C&gt;T, p.R462*</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Paternal</td>
<td>Pathogenic</td>
</tr>
<tr>
<td>F11</td>
<td>LRP5</td>
<td>Frameshift</td>
<td>c.2817_2827+1del12bp</td>
<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>Maternal</td>
<td>Pathogenic</td>
</tr>
<tr>
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<td>Nonsense</td>
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<td>Novel</td>
<td>Novel</td>
<td>Novel</td>
<td>De novo</td>
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<tr>
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<td>Pathogenic</td>
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<tr>
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<td>Novel</td>
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<td>Novel</td>
<td>Maternal</td>
<td>Pathogenic</td>
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<tr>
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<td>De novo</td>
<td>Pathogenic</td>
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</table>
FIGURE 2. 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 represented various variants, while the y-axis depicted 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 \( \text{LRP5} \) (5/31, 16.1%) accounted for 42% of patients with defined mutations, indicating that \( \text{LRP5} \) was the most frequent gene mutated in Chinese FEVR patients. The second leading mutation was located in \( \text{NDP} \), which corresponded to 25% of patients with defined mutations, whereas mutations in \( \text{FZD4}, \text{TSPAN12}, \) and \( \text{KIF11} \) 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, \( \text{LRP5} \) and \( \text{NDP} \) were the predominant genes (67%), while no pathogenic mutations were identified in \( \text{ZNF408} \). 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 \( \text{LRP5} \) mutations showed broader phenotypic spectra that varied from stage 2 to stage 5. However, all patients carrying \( \text{NDP} \) 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 neoangiogenesis. To date, six causative genes have been identified as related to FEVR, including low-density-lipoprotein receptor-related protein 5 ( \( \text{LRP5} \) ), frizzled-4 ( \( \text{FZD4} \) ), tetraspanin-12 ( \( \text{TSPAN12} \) ), Norrie disease protein ( \( \text{NDP} \) ), zinc finger protein 408 ( \( \text{ZNF408} \) ), and kinesin family member 11 ( \( \text{KIF11} \) ). 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.\(^{14,17,18}\) 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 \( \text{LRP5} \) mutations (5/31, 16.1%), three \( \text{NDP} \) mutations (3/31, 9.7%), two \( \text{FZD4} \) mutations (2/31, 6.5%), one \( \text{TSPAN12} \) mutation (1/31, 3.2%), and one \( \text{KIF11} \) mutation (1/31, 3.2%). Except for the \( \text{NDP} \) 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 spectra, 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 MCLMR. 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.1830_1833delACAA, 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 FEVR as well, which warrant further genetic studies.

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