Mutation Spectrum of the \textit{LRP5}, \textit{NDP}, and \textit{TSPAN12} Genes in Chinese Patients With Familial Exudative Vitreoretinopathy

Miao Tang, Limei Sun, Andina Hu, Miner Yuan, Yu Yang, Xuening Peng, and Xiaoyan Ding

State Key Laboratory of Ophthalmology, Retina Division, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, Guangdong, China

Correspondence: Xiaoyan Ding, State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, 54, S Xianhe Road, Guangzhou 510060, China; dingxiaoyan@gzzoc.com.

MT and LS contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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\textbf{Purpose.} \textit{LRP5}, \textit{NDP}, and \textit{TSPAN12} are known to be associated with familial exudative vitreoretinopathy (FEVR). In this study, a comprehensive mutation screening for the three genes was performed in patients with a clinical diagnosis of FEVR in Han Chinese.

\textbf{Methods.} Genomic DNA and clinical data were collected from 100 probands and their family members. Sanger sequencing was performed to screen for \textit{LRP5}, \textit{NDP}, and \textit{TSPAN12} mutations and phenotype-genotype correlation was analyzed.

\textbf{Results.} There were 23 causative mutations identified in 23 unrelated probands (10/23 in \textit{LRP5}, 8/23 in \textit{TSPAN12}, and 5/23 in \textit{NDP}). Apart from \textit{NDP} mutations, only two \textit{LRP5} mutations inherited in an autosomal recessive manner. Among the 23 causative mutations, 13 were novel variants (4/10 in \textit{LRP5}, 6/8 in \textit{TSPAN12}, and 3/5 in \textit{NDP}). According to the modified classification system, statistical significance was observed in the distribution of mutated genes ($P = 0.049$). None of the causative mutations was found in group I FEVR. Probands with \textit{LRP5} or \textit{NDP} mutations were mainly categorized into group III and IV, \textit{TSPAN12} mutations were mainly observed in probands with group IV and V FEVR.

\textbf{Conclusions.} The detection rate for mutations in the three known genes was 23%. Mutations in \textit{LRP5} and \textit{TSPAN12} were more frequent, accounting for 10% and 8%, respectively. The \textit{NDP} mutations were only identified in 6% in this cohort. There were 13 novel variants found, which provided a deeper understanding of this disease. Potential phenotype-genotype correlation was observed in the modified system. \textit{TSPAN12} mutations might lead to the most severe phenotype.

Keywords: familial exudative vitreoretinopathy, mutational analysis, phenotype-genotype correlation

\section*{Familial exudative vitreoretinopathy (FEVR) is a genetic disorder affecting the development of retinal vasculature. The primary feature in FEVR is referred to as a premature arrest of vascularization in the peripheral retina. The subsequent ischemia and hypoxia can induce neovascularization and variable secondary complications, including vascular leakage, fibrovascular proliferation, traction of the posterior pole structure, macular dragging, retinal fold, retinal tears, and partial or total retinal detachment. The severity of clinical manifestation ranges from the absence of visual impairment to total blindness. FEVR is genetically heterogeneous and has been reported to have autosomal dominant (adFEVR; Mendelian Inheritance in Man [MIM# 133780], autosomal recessive (arFEVR; MIM# 133780), and X-linked recessive (XL-FEVR; MIM# 305390) inheritances. To date, mutations in the \textit{FZD4} (MIM# 604579)\textsuperscript{1} have been shown to contribute to the dominant FEVR. The \textit{TSPAN12} (MIM# 613138) and \textit{LRP5} (MIM# 605506) mutations are believed to be inherited as dominant or recessive trait.\textsuperscript{2,5} While mutations in the \textit{NDP} (MIM# 300658) gene have been found in X-linked FEVR.\textsuperscript{5} Moreover, mutations in the \textit{ZNF408} (MIM# 616468),\textsuperscript{6} \textit{KIF11},\textsuperscript{6} \textit{RCBTB1},\textsuperscript{7} and \textit{CTNNB1} (MIM# 617572)\textsuperscript{8} have been linked to FEVR patients in recent studies.

The proteins, encoded by the \textit{FZD4}, \textit{LRP5}, \textit{NDP}, and \textit{TSPAN12} genes, are involved in the Wnt/Norrin-$\beta$-catenin signaling pathways that are critically required for retinal angiogenesis during eye development.\textsuperscript{11,12} As an atypical Wnt ligand, Norrin activates the canonical Wnt pathway through its specific binding to the Frizzled4 receptor and Lrp5/6 coreceptor,\textsuperscript{11} which is selectively enhanced by Tetraspanin 12.\textsuperscript{12,13} It produces a downstream signal that stabilizes the cytosolic $\beta$-catenin. The increasing $\beta$-catenin translocates into the nucleus and interacts with the lymphoid enhancer factor/T-cell factor (LEF/TCF) transcription factor, which induces the transcription of target genes.\textsuperscript{14}

In this study, sequence analysis of \textit{LRP5}, \textit{NDP}, and \textit{TSPAN12} was performed in 100 unrelated Chinese individuals with clinical diagnosis of FEVR and their families. A comprehensive mutation spectrum for \textit{LRP5}, \textit{NDP}, and \textit{TSPAN12} in China was determined.

\section*{Methods}

\subsection*{Patients}

Written informed consent in accordance with the guidelines of the Declaration of Helsinki was obtained from the participating individuals or their guardians before the clinical data and DNA
samples were collected. All procedures were approved by the
internal review board of the Zhongshan Ophthalmic Center,
Sun Yat-sen University. There were 100 probands recruited and
had a final diagnosis of FEVR. Patients with a gestational age of
less than 38 weeks or a neonatal birth weight of less than 2000
grams were excluded, to eliminate the possible presence of
retinopathy of prematurity. The clinical diagnosis of FEVR
was described in a previous study and EZDI mutations were
found in 21/100 individuals.15 There were 70 ethnically
matched individuals without history of ocular diseases
recruited as controls. Comprehensive fundus examinations
were performed, and no signs of retinal diseases were
observed.

Genetic Analysis
Tissue samples (blood or buccal swabs) were obtained from
each participant for genomic DNA extraction using the
standard protocols of the QIAGEN QIAamp DNA Blood Kit
(Qiagen, Hilden, Germany) and the Epicenter Master Pure
Complete DNA and RNA Purification Kit (Illunina, Madison,
WI, USA). For mutational analysis, the coding exons of
LRP5, NDP, TSPAN12, and their flanking intronic sequences
were amplified with PCR. The resulting amplicons were analyzed
with Sanger sequencing. Direct sequencing was performed
with an ABI BigDye Terminator Cycle Sequencing Kit using an
ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster
City, CA, USA). The reference sequences of LRP5 (GenBank
NG_015853.1 for gDNA, NM_001291902.1 for mRNA), NDP
(GenBank NG_009832.1 for gDNA, NM_000266.3 for mRNA),
and TSPAN12 (GenBank NG_025203.1 for gDNA,
NM_012358.3 for mRNA) were used for the identification of
variants.

In Silico Analyses
Protein alignments for novel missense mutations were
performed using the online COBALT program. Four online
algorithms were used to estimate the pathogenicity of the
missense changes, including SIFT, PolyPhen-2, Align GVGD,
and Mutation Taster. For splice site mutations, another four
online algorithms were used to predict their potential
outcome, including Human Splicing Finder, NNSPLICE,
F SPLICE, and NetGene2. For secondary structural analysis of
mutant protein, PsiPred v.3.3 was used.

Cloning Sequencing
Heterozygous frameshift mutations were confirmed by cloning
sequencing. The target fragments covering the mutation sites
were amplified with PCR. The PCR products were gel purified
by using the Universal DNA Purification Kit (TIANGEN, Beijing,
China) and cloned into pMD19-T (Takara BIO, Shiga, Japan)
according to the manufacturer’s instructions. Escherichia coli
DH5α was transformed with the resulting plasmids and cultured
in Luria-Bertani (LB) agar plates with ampicillin at
37°C for 12 to 14 hours. A single colony was placed in 5 mL of
liquid LB medium with ampicillin and grown overnight in the
37°C incubator shaker for 12 to 14 hours. The plasmids were
isolated from the suspension with the TIANprep Mini Plasmid
Kit (TIANGEN), and Sanger sequencing was used to confirm
the mutant and wild-type alleles.

Statistics
All analyses were performed using SPSS software version 16.0
(IBM Corp., Armonk, NY, USA). The independent samples t-test
was used for normally distributed continuous variables, while
the Kruskal–Wallis test was used for the nonnormally
distributed continuous variables. A P value of less than 0.05
was considered statistically significant.

RESULTS
In this study, 23 potentially pathogenic mutations were
identified in 23 unrelated FEVR probands, including one
patient harboring compound heterozygous mutations in
LRP5 (c.1058G>A;[92-2A>C]) and two unrelated individuals
carried the same reported LRP5 mutation (c.4488+1T>G).
Apart from NDP mutations, only two LRP5 mutations inherited
as an autosomal recessive trait. The remaining nucleotide
changes in LRP5 and TSPAN12 were heterozygous and
inherited as autosomal dominant pattern (Table 1). Of the 23
probands with LRP5, NDP, and TSPAN12 mutations, nine had a
family history of FEVR and 14 were isolated cases (Fig. 1).

Among these 23 variants, 13 of them were novel (4/10 in
LRP5, 6/8 in TSPAN12, and 5/5 in NDP). None of these novel
mutations was detected in 70 ethnically matched control
individuals. These variants were not found in current online
databases of human sequence variants, including the Ensembl,
dbSNP, ExAC databases, genomeAD, and The Human Gene
Mutation Database (HGMD). Chromatograms of these novel
variants and clone sequencing of heterozygous insertion and
deletion mutations are shown in Figure 2.

Apart from these diseases causing mutations, 20 synonym-
ous variants in LRP5 and TSPAN12 were excluded from this
study (data not shown). Six known variants in LRP5 were
considered as benign because the minor allele frequency was
higher than 0.1% in the East Asian population (Supplementary
Table S1). Besides that, three variants were considered as
uncertain significance (Supplementary Table S2). Novel variant
c.286-1G>T was located in the splice acceptor site of exon 4 in
TSPAN12. It was predicted to damage the normal acceptor
splice site by three algorithms (NNSPLICE, FSPLICE, and
NetGene2). The 4-year-old male proband showed a retinal fold
in his right eye, however, the same variant was found in his
healthy father. The c.1512G>T (p.(W504C)) variant in LRP5
previously identified in an osteoporosis-pseudoglioma syn-
drome (OPPG) family with compound heterozygous mutations
(p.(W504C);[W478R]) and members with a single p.(W504C)
variants only exhibited low bone mineral density.16 The
heterozygous p.(W504C) was detected in our index patient
with an ectopic macula and her healthy mother. Additionally,
the splice site variant c.3637+4C>T (rs746451473), located in
the splice donor site of exon 16 of LRP5, was found in a
sporadic male patient. Three of four algorithms considered it as
benign.

Mutations Detected in LRP5
Three novel heterozygous missense mutations were found. The
c.1318A>T (p.[I440F]) was found in a 9-month-old girl with
retinal fold in both eyes, her mother and elder sister carried the
same mutation and were diagnosed as FEVR. The isolectine at
codon 440 was conserved among various species (Fig. 3A) and
three online programs predicted this change as pathogenic.
The c.1942G>A (p.[V648I]) mutation was detected in a 19-
year-old male with asymmetric manifestation. FEVR associated
rhegmatogenous retinal detachment was observed in his right
eye while an ectopic macula was found in the left eye. His
healthy mother did not carry the same mutation. P.(V648I) was
predicted as pathogenic variant by SIFT and Mutation Taster.

The valine was highly conserved (Fig. 3B) and was replaced by
predicted as pathogenic variant by SIFT and Mutation Taster.

the patient harboring compound heterozygous mutations in
LRP5, was used for normally distributed continuous variables, while
dragging in the left eye. The hydrophilic cysteine was replaced by hydrophobic phenylalanine. The cysteine at codon 913 was highly conserved from human to various species (Fig. 3C) and the substitution was predicted as pathogenic by all algorithms.

The heterozygous change c.1582G>T (p.[R395W]) was found in a 7-month-old boy with a retinal fold in the left eye and in his affected father with peripheral vascular deficiencies detected by fundus fluorescein angiography. The c.4733C>T mutation was predicted as pathogenic by all algorithms. The conserved arginine at codon 395 was replaced by hydrophobic tryptophan. Align GVGD and Mutation Taster predicted it as pathogenic. The amino acid changes with probabilities >0.05 are predicted to be deleterious.

Two LRP5 mutations inherited as an autosomal recessive pattern. The homozygous mutation c.1183C>T (p.[R353Q]) was previously reported in patients with OPPG. No skeletal problem was reported in patients with OPPG.17 No skeletal problem was reported in this family. The novel c.92-2A>G mutation located in the splice acceptor site of exon 2 and the most common outcome for a splice acceptor site mutation was a deletion of the following exon. Four algorithms predicted the loss of natural splice site.

Two recurrent mutations were found in our study. The splice site mutation c.4488+2T>G was found in two unrelated probands with retinal folds and segregated among their families. The c.4087G>A (p.[R353Q]) mutation was previously reported in a Chinese family.18 was identified in a 3-year-old boy with falciform retinal fold in the right eye. His healthy parents did not carry the same mutation.

**Mutations Detected in TSPAN12**

Three novel frameshift mutations were found. The single base pair insertion c.438_439insT caused a frameshifting resulting in 11 incorrect amino acids after codon 146 and a premature termination at codon 158 (p.[R353Q]). The proband was a 1-month-old girl with total retinal detachment and massive vitreous proliferation in her left eye and macular ectopic in her right eye. Her father with bilateral retinal folds carried the same

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**Table 1. Causative Mutations Identified in 100 Probands With Familial Exudative Vitreoretinopathy**

<table>
<thead>
<tr>
<th>Family ID</th>
<th>Gene</th>
<th>Allele Status</th>
<th>cDNA Change</th>
<th>Protein Change</th>
<th>SIFT</th>
<th>PolyPhen2</th>
<th>Align GVGD</th>
<th>Mutation Taster</th>
<th>Source</th>
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<td>Hemi</td>
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<td>p.(I66K)</td>
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<td>Probably damaging</td>
<td>Class C55</td>
<td>Disease causing</td>
<td>Reported</td>
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<td>57</td>
<td>NDP</td>
<td>Hemi</td>
<td>c.203A&gt;C</td>
<td>p.(H68P)</td>
<td>0.14, T</td>
<td>Benign</td>
<td>Class C65</td>
<td>Disease causing</td>
<td>Novel</td>
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<td>Hemi</td>
<td>c.281A&gt;T</td>
<td>p.(H94L)</td>
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<td>p.(R121Q)</td>
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<td>c.354delG</td>
<td>NA</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
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<td>c.1058G&gt;A</td>
<td>p.(R353Q)</td>
<td>0.14, T</td>
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<td>Class C35</td>
<td>Disease causing</td>
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</tr>
<tr>
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<td>Disease causing</td>
<td>Novel</td>
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<tr>
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<td>p.(I440F)</td>
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<td>p.(E528K)</td>
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<td>Class C55</td>
<td>Disease causing</td>
<td>rs749683290</td>
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<td>LRP5</td>
<td>Hetero</td>
<td>c.1942G&gt;A</td>
<td>p.(V648I)</td>
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<td>p.(T1578M)</td>
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<td>Disease causing</td>
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<td>2T&gt;C</td>
<td>p.(MIT)</td>
<td>0, D</td>
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<td>Novel</td>
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<td>46G&gt;C</td>
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<td>Disease causing</td>
<td>Novel</td>
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<td>50</td>
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<td>Hetero</td>
<td>c.438+49insT</td>
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<td>NA</td>
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<td>65delC</td>
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<td>48</td>
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<td>916-918</td>
<td>p.(3066ext*35)</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tr>
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**Family ID | Gene | Allele Status | cDNA Change | Protein Change | FSPLICE | NetGene2 | NNSPLICE | Human Splicing Finder | Source |
<table>
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<td>Hetero</td>
<td>c.92-2A&gt;C</td>
<td>p.?</td>
<td>LNSS</td>
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<td>LNSS</td>
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<td>Hetero</td>
<td>c.4488+2T&gt;G</td>
<td>p.?</td>
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<td>150-1G&gt;A</td>
<td>p.?</td>
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<td>Hetero</td>
<td>285+1G&gt;A</td>
<td>p.?</td>
<td>WE</td>
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<td>LNSS</td>
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<td>469-1G&gt;A</td>
<td>p.?</td>
<td>LNSS</td>
<td>LNSS</td>
<td>LNSS</td>
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</tbody>
</table>

**WE:** without effect; **LNSS:** loss of the natural splice site; **NPS:** new predicted splice site; **Hetero:** heterozygous; **Homo:** homozygous; **Hemi:** hemizygous; **NA:** not available; **D:** deleterious; **T:** tolerated.

*One patient harbored compound heterozygous mutations in LRP5.
†Two unrelated probands carried the same mutation.
**FIGURE 1.** Schematic pedigrees of the families with causative mutations. Arrows indicate proband; open symbols, clinically unaffected; solid symbols, clinically affected; +, clinically evaluated; +/-, homozygous mutation; +/-, heterozygous mutation; --/--, wild type.
novel mutation. A 1-bp deletion c.655delC was identified in a singleton. This novel mutation caused a frameshift resulting in four incorrect amino acids after codon 218, followed by premature termination at codon 223 (p.Q219Nfs*5). The female patient developed total retinal detachment in her left eye during early childhood, which resulted in no light perception. She visited our clinic at the age of 44 with decreased vision in the right eye, and a retinal fold with remarkable temporal exudates was observed. Her brother and father were reported to have the congenital cataract from childhood.

A novel stop loss mutation, c.916_918+3delTAAAA, was identified in a 4-year-old boy with dragged macular in his right eye. His mutation-carrying mother manifested bilateral esotropia with characteristic peripheral avascular retina. The 6-bp deletion removed the termination codon (TAA) at the end of exon 8 and the extra three bases in the following intron (AAA). It was predicted to abolish the original stop codon 306 (*306E).
and resulted in an elongated protein with 34 additional amino acids at the C-terminus (p.*306E). To establish whether the stop loss mutation could affect the structure of Tetraspanin12, PsiPred v.3.3 was used to analyze the secondary structure of wild-type and mutant protein. According to the prediction, two extra α-helices and one new β-sheet would be generated, hypothetically, the additional amino acids would alter the structure of Tetraspanin 12 (Fig. 4).

Two novel splice acceptor mutations were found. The c.150-1G>A mutation, located in the splice acceptor site of exon 4, was identified in a 10-year-old boy with bilateral retinal folds and his asymptomatic father. Four algorithms revealed the creation of a novel splice site, which would result in a one nucleotide deletion in the mRNA, and thereby would lead to a shift in the open reading frame and protein truncation. The proband showed asymmetric manifestation with worse visual acuity in his right eye due to retinal fold involving the macula. The second splice acceptor mutation, c.469-1G>A, was identified at the end of intron 6. All four online tools predicted the loss of natural splice site. The proband was a 5-year-old boy with bilateral retinal folds. His healthy parents did not carry the same mutation.

The novel c.2T>C (p.[M1T]) was identified in a 17-year-old male patient. The proband was noticed to have FEVR associated rhegmatogenous retinal detachment in his right eye, which pars plana vitrectomy was performed. Falciform retinal fold was observed in his left eye. His asymptomatic father exhibited with typical FEVR features of bilateral peripheral avascularization; however, no DNA sample was available for mutational analysis. The methionine at codon 1 was highly conserved from human to other spices (Fig. 3D), and this mutation led to the replacement of a hydrophobic residue by a hydrophilic threonine residue. This change was predicted to be deleterious in all four online programs.

The missense mutation c.464G>C (p.[R155T]), was found in a patient with asymmetric manifestation. This 28-year-old male patient suffered total blindness in the right eye with retinal detachment during early childhood, while his left eye was asymptomatic. Peripheral retinal capillary nonperfusion was observed by fluorescein angiography. P.(R155T) was never

Figure 3. Amino acid conservation of novel missense variations. (A) P.(I440F) in LRP5, (B) p.(V648I) in LRP5, (C) p.(C913F) in LRP5, (D) p.(H68P) in NDP, and (F) p.(H94L) in NDP.
reported as the causative mutation in FEVR and the minor allele frequency of this change was lower than 0.1%.

A recurrent mutation c.285+1G>A, located in the splice donor site of intron 5, was identified in a full-term infant, and cosegregated among his family. The boy visited our clinic 1 week after birth and fundus screening showed bilateral tractional retinal detachment with pronounced peripheral subretinal exudation and hemorrhage. After the intravitreal injection of Ranibizumab in both eyes, the subretinal exudate and hemorrhage were absorbed, however a falciform retinal fold was presented in the left eye. This mutation was previously reported in a Nigerian female with autosomal recessive FEVR, who carried two heterozygous TSPAN12 mutations, c.[146C>T],c.285+1G>A].5

Mutations Detected in NDP
Two novel missense mutations were detected. The c.203A>C (p.[H68P]) mutation was found in a 35-year-old male with ectopic macular in the right eye. The hydrophobic histidine at codon 68 was conserved across majority of the species (Fig. 3E). This mutation changed the hydrophobic alkaline histidine into the hydrophobic proline residue. No family member was available for fundus examinations and molecular analysis. The c.281A>T (p.[H94L]) was identified in a 3-month-old male infant with bilateral retinal fold. The characteristic features of FEVR, including peripheral avascular zone and retinal exudates were observed in both eyes. No family member had complained about ocular symptoms and his healthy mother was heterozygous for this mutation. The histidine at codon 94 was highly conserved from human to various species (Fig. 3F), and this mutation led to the replacement of a hydrophilic alkaline histidine by a hydrophobic leucine residue. Both p.[H68P] and p.[H94L] were predicted to be deleterious by two algorithms (Align GVGD and Mutation Taster).

The novel c.334delG mutation was found in a 6-month-old boy, who exhibited bilateral tractional retinal detachment. The single base pair deletion was predicted to cause a frameshift after codon 112 and generate a prolonged protein at the C-end. The novel c.334delG mutation was found in a 6-month-old boy, who exhibited bilateral tractional retinal detachment. The single base pair deletion was predicted to cause a frameshift after codon 112 and generate a prolonged protein at the C-end. No family member was available for fundus examinations. 

TABLE 2. Phenotype–Genotype Analysis in Trese’s Staging System

<table>
<thead>
<tr>
<th>Stage</th>
<th>N</th>
<th>FZD4</th>
<th>LRP5</th>
<th>TSPAN12</th>
<th>NDP</th>
<th>N with Causative Mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>4 (45.2%)</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5 (35.7%)</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (25.0%)</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>13</td>
<td>8</td>
<td>3</td>
<td>2</td>
<td>26 (60.5%)</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>8 (38.1%)</td>
</tr>
</tbody>
</table>

* Patients were staged in the basis of the highest stage of FEVR in either eye.

DISCUSSION

After screening the coding and flanking regions of LRP5, NDP, and TSPAN12 for mutations, 23 pathogenic mutations were found in 25 unrelated FEVR probands, considering our previously reported study,15 there were 44 probands with causative mutations staged according to the Trese’s staging system.24 In Trese’s system, 35 (35/44) patients with causative mutations in these four genes were staged into severe FEVR (stage 3–5), including 15 (15/21) in FZD4, 10 (10/10) in LRP5, 7 (7/8) with TSPAN12, and 3 (3/5) with NDP mutations. However, statistical significance was not established in this classification system (P = 0.411) (Table 2).

After analyzing the clinical features of the 100 probands, a modified approach based on the Trese’s staging system was addressed. Probands with asymptomatic FEVR, in whom the diagnosis of FEVR was only confirmed by fundus fluorescein angiography, were categorized into group I. Patients with vitreous hemorrhage due to retinal neovascularization were categorized into group II. Patients staged into group I or II visited our clinic for the first time mainly in adolescent or adulthood. Patients with an ectopic macula were categorized into group III, while patients with retinal folding were categorized into group IV. Patients with severe total tractional retinal detachment at least in one eye were categorized into group V.

Combined with FZD4 mutations in these probands, we reported before, most of the probands (86.4%, 38/44) with pathogenic variants were categorized into group III and IV, including 9 cases in group III and 29 in group IV. None of the FZD4, LRP5, TSPAN12, and NDP mutation was found in probands with group 1 FEVR, and only one proband was categorized into group II (1/21 in FZD4 mutations). Five patients were staged into group V (5/44). Among the five probands, three of them carried the TSPAN12 mutation (3/5) and one of them harbored the NDP variants (1/5), while only one patient with compound heterozygous mutation in LRP5 exhibited extremely severe FEVR (1/5). According to the modified system, statistical significance was observed in the distribution of four genes (P = 0.049) (Table 3).
Table 3. Phenotype–Genotype Analysis in Modified Classification System

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>FZD4</th>
<th>LRP5</th>
<th>TSPAN12</th>
<th>NDP</th>
<th>N With Causative Mutation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>II</td>
<td>21</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1 (25.0)</td>
</tr>
<tr>
<td>III</td>
<td>48</td>
<td>15</td>
<td>8</td>
<td>4</td>
<td>2</td>
<td>29 (60.4)</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>5 (29.4)</td>
</tr>
</tbody>
</table>

P = 0.049

* Patients were staged in the basis of the highest stage of FEVR in either eye.

al screening of four confirmed genes that caused FEVR. Compared with the previous study of a large cohort of 98 FEVR patients in Caucasian persons, the detection rates of causative mutations in Wnt/Norrin signal pathway were similar with the previous study, accounting for 46% (42/92). However, the involvement of each gene was different. LRP5 was the most frequently mutated gene in Caucasian population (19.6%) and FZD4 mutations accounted for 15.2%.25 The reported mutation frequencies in the LRP5, TSPAN12, and NDP gene varied in different populations. The frequency of LRP5 mutations ranged from 10% to 22.2%,19,25–28 TSPAN12 mutations were identified 2% to 10% in patients with autosomal dominant or autosomal recessive FEVR,2,3,25,26,28–32 while the frequency of NDP mutations ranges from 6.5% to 12.5%.19,25,26,33,34 FEVR in Han Chinese may have a unique mutation spectrum with a larger involvement of LRP5.

In summary, this study provided a comprehensive understanding of the mutation spectrum of FEVR-associated genes of FEVR in Wnt/Norrin pathway. Mutation screening of known causative mutations in Wnt/Norrin signal pathway were similar with the prior reports, we suggested the mutation might lead to the most severe phenotype in FEVR.

In this investigation, we sequenced four confirmed genes that caused FEVR. Compared with the previous study of a large cohort of 98 FEVR patients in Caucasian persons, the detection rates of causative mutations in Wnt/Norrin signal pathway were similar with the previous study, accounting for 46% (42/92). However, the involvement of each gene was different. LRP5 was the most frequently mutated gene in Caucasian population (19.6%) and FZD4 mutations accounted for 15.2%.25 The reported mutation frequencies in the LRP5, TSPAN12, and NDP gene varied in different populations. The frequency of LRP5 mutations ranged from 10% to 22.2%,19,25–28 TSPAN12 mutations were identified 2% to 10% in patients with autosomal dominant or autosomal recessive FEVR,2,3,25,26,28–32 while the frequency of NDP mutations ranges from 6.5% to 12.5%.19,25,26,33,34 FEVR in Han Chinese may have a unique mutation spectrum with a larger involvement of FZD4, instead of LRP5.

To investigate the potential genotype and phenotype correlation in probands with causative mutations, patients were grouped according to the modified system. None of the causative mutations was found in group I FEVR. Probands with FZD4, LRP5, and NDP mutations were mainly categorized into group III and IV. Probands with TSPAN12 mutations were observed in group III, IV, and V, mainly in group IV and different with the prior reports, we suggested TSPAN12 mutation might lead to the most severe phenotype in FEVR in Han Chinese.

In summary, this study provided a comprehensive understanding of the mutation spectrum of FEVR-associated genes of FEVR in Wnt/Norrin pathway. Mutation screening of known genes would provide invaluable information for diagnosis and genetic counseling, especially for patients without family history or with atypical presentations. Further analysis of novel causative genes may explain some mild clinical findings such as group I FEVR patients, and definitely will contribute to our understanding of the molecular mechanisms of retinal angiogenesis, which is crucial for FEVR and other retinal vascular disease.

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References


Figure 5. Mutation spectrum of FZD4, LRP5, NDP, and TSPAN12 in Chinese patients with FEVR.


