**Review Article**

**Familial Exudative Vitreoretinopathy-Related Disease-Causing Genes and Norrin/β-Catenin Signal Pathway: Structure, Function, and Mutation Spectrums**

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Familial exudative vitreoretinopathy (FEVR) is a hereditary ocular disorder characterized by incomplete vascularization/abnormality of peripheral retina. Incomplete and aberrant vascularization leads to various complications, including retinal neovascularization and exudates, retinal fold and detachments, vitreous hemorrhage, and macular ectopia, ultimately leading to total blindness.

FEVR is genetically heterogeneous and can be inherited as a dominant, recessive, or X-linked trait. The dominant form is the most common mode of inheritance. So far, mutations in at least 9 genes have been attributed to the development of FEVR including *NDP, FZD4, LRP5, TSPAN12, ZNF408, KIF11, RCBTB1, CTNNB1,* and *JAG1* [2–10]. The proteins encoded by the first four genes are cooperative in the Norrin/β-catenin signaling pathway (also named as Norrin/Frizzled-4 pathway) and showed intense interaction with each other [11]. So, this review specially focused on the mutation spectrums of these genes.

The mechanisms of *NDP, FZD4, LRP5,* and *TSPAN12* in retinal vascular had been intensively investigated during the past years. The *Ndp* knockout mouse exhibited superficial retinal vasculature development delay and was unable to form deep retinal vasculature [12]. Similarly, *Fzd4* played a central role in vascular development in the eye and ear. Knockout of *Fz4* has been shown to affect vascular development both in retinal and in inner ear and cause retinal stress [13, 14]. Compared with *Fzd4* or *Ndp* knockout mice,
Lrp5 knockout mice showed many milder vascular defects, in which attenuated retinal vessels and capillaries lacking lumen structure was observed [15, 16]. Afterwards, Tspan12 was verified to cause vascular defect and affect neural cells through association with Norrin/β-catenin but not Wnt/β-catenin signaling. Formation of microaneurisms, aberrant fenestration, and delayed hyaloid vessel regression was reported in Tspan12 knockout mice [11].

In the Norrin/β-catenin pathway, Norrin (coded by NDP) worked as a ligand, while Frizzled-4 (FZD4) acted as the receptor of Norrin, in concert with low-density lipoprotein receptor-related protein-5 (LRP5) as coreceptor. Norrin binds to FZD4 and its coreceptor LRP5, forming a ternary complex. Together with the auxiliary component tetraspanin-12 (TSPAN12), this complex initiates downstream β-catenin signaling. Specifically, FZD4-bound Dishevelled and phosphorylated LRP5 recruited Axin to the plasma membrane, resulting in the suppression of β-catenin phosphorylation/degradation. The cytoplasmic levels of β-catenin consequently increased. Subsequently, β-catenin was translocated to the nucleus where it interacts with the T-cell factor/lymphoid enhancing factor, family of transcription factors, to initiate RNA transcription and elongation, as shown in Figure 1 [17–19]. This signaling pathway shared many similarities with the canonical Wnt/β-catenin pathway except that Norrin substituted Wnt as the ligand and traspan-12 had been linked to the Norrin/β-catenin signaling pathway. Norrin/Frizzled-4 signaling plays an important role in retinal vascular growth, remodeling, and maintenance [20].

Prior to this review, a great many mutations in NDP, FZD4, LRP5, and TSPAN12 had been reported by different study groups from different countries as disease-causing mutation of FEVR. Although most of the mutations were documented for once by one study group, some mutations seemed to be more common than others. Here, we presented the comprehensive list of currently known mutations in NDP, FZD4, LRP5, and TSPAN12 associated with FEVR and discussed their coding consequences. This aims in facilitating the construction of a complete spectrum of mutations that occur in the above four genes. We discuss about each gene mutation individually and then highlight how they disturb the protein interactions.

2. Materials and Methods

The current review article aimed to analyze the studies on FEVR caused by NDP, FZD4, LRP5, and TSPAN12 gene mutations to find the spectrum of these four genes. For this review study, an extensive search in PubMed and Web of Science up to December 30, 2017, was conducted independently by two individuals (Tong and Zhu) using the following search terms: “Familial exudative vitreoretinopathy” and “mutation”. To avoid losing relevant information, no limitations were set in the search. Furthermore, the related studies and the references of literatures were manually screened for additional potential eligible studies.

Mutations in NDP can result in Norrie disease and X-linked exudative vitreoretinopathy. Some earlier reports investigated Norrie disease (ND) and FEVR together. In addition, loss-of-function mutations in the LRP5 gene either cause osteoporosis pseudoglioma syndrome (OPPG) or FEVR depending on the functional severity of mutation. These distinct clinical entities share some common pathological features such as abnormal retinal blood vessel growth that may result in retinal detachment. So, we read the relevant articles of the candidates carefully to make sure the probands on whom the mutations were found were definitely diagnosed as FEVR. Then, we recorded the mutations related to FEVR and excluded those caused ND and OPPG. A total of 433 potentially relevant articles were identified, but only 41 studies involving FEVR patients caused by NDP, FZD4, LRP5, and TSPAN12 gene mutations were included in this review.

3. Results

3.1. NDP Mutations and Norrin Structure. The NDP gene locus mapped to chromosome Xp11.4 and comprised three exons. However, the first exon corresponds to the untranslated region of the gene that has regulatory functions, and only exons 2 and 3 of encode a secreted protein of 133 amino acids called Norrin or Norrie disease protein. Norrin consists of two major parts: a signal peptide at the amino-terminus of the protein that directs its localization and a region containing a typical motif of six cystines forming a cystine-knot. The cystine-knot motif is highly conserved in many growth factors as transforming growth factor-β, human chorionic gonadotropin, nerve growth factor, and platelet derived growth factor [21]. Cystine residues and their disulfide bonds in the cystine-knot play important structural and functional roles. Among 10 Frizzled family members, Norrin specifically binds to the transmembrane FZD4 with high affinity, forming a Norrin/FZD4 complex with LRP5 and TSPAN12 coreceptors to activate the Norrin/β-catenin signaling pathway [22]. Norrin was also reported to play a major role in controlling retinal vascular growth and architecture both in the developing eye and in adult vasculature.

Twenty-six nucleotide variants have been identified for NDP in patients with FEVR. These include 21 missense changes, 4 deletions, and 1 insertion resulting frame shift [2, 23–31] (Table 1 and Figure 2). Most of the mutations were found in single or only a few patients, while several mutations are generally more common. By far, the most prevalent mutation was c.362G>A (p.R121Q), distributed in Spanish, Mexican, Indian, Chinese, and Italian. It is noteworthy that although probands containing c.11_12delAT (p.H4fsX21), c.170C>G (p.S57X), and c.310A>C (p.K104Q) were definitely diagnosed as FEVR following explicit criteria, these three mutations were also reported to cause Norrie disease by other researches [28, 32, 33]. The ocular features and retinal changes observed in Norrie disease are similar to those observed in cases of FEVR. Not all the Norrie disease patients have mental retardation and develop a progressive sensorineural hearing loss; it is really difficult to distinguish Norrie disease from FEVR.
It was demonstrated from the three-dimensional structure of Norrin that two-monomer Norrins formed a homodimer in the crystal. The Norrin monomer contained exclusive β strands with two β-hairpins on one side and one β-hairpin on the other side. Crystal structures of Norrin in complex with the extracellular domain of FZD4 showed that two β-hairpins in Norrin (β1-β2 and β5-β6) interacted with three loops in FZD4 cystine-rich domain (FZD4-CRD) [38, 39]. There were 19 mutations located in domains from C39 to C65 and C96 to C126, which covered two β-hairpins (β1-β2 and β5-β6) and loops between them, namely, 73% of the mutations (19/26) concentrated in the interacting domains with FZD4-CRD. Specifically, 9 mutations were located in the Norrin dimer interface which was formed from β2 and β4 sheets of one monomer and β2' of another monomer (Table 1). Three mutations were reported from the cystine-knot motif, one of which (C65W) obviously impaired intermolecular disulfide bond-forming. Five mutations disturbed the hydrogen bonds or hydrophobic contacts between Norrin and FZD4 CRD in the Norrin-FZD4 CRD interface [38, 40]. Four mutations clustered on the edge of the Norrin molecule in the β1-β2 and β3-β4 loop regions were inferred as LRP5 binding sites because they did not affect Fz4 binding yet reduced the ability of Norrin to activate the TCF reporter [39]. The residues in the interaction interface are well defined and overlap with disease-associated mutations in NDP. The level of signaling activity of K104Q, R121Q, and L124F was between 20% and 80% of the wide-type Norrin, suggesting that even a modest decrement in Norrin/Fz4 signaling may have a significant phenotypic effect in humans [14, 41]. It is of no surprise that the mutations located in β1-β2 and β5-β6 obstructed the formation of two β-hairpins and the interactions between Norrin and FZD4.

3.2. FZD4 Mutations and FZD4-CRD Structure. The FZD4 gene is located on chromosome 11q14.2, and its mRNA consists of two exons coding for 537 amino acid protein called FZD4 or Frizzled-4 protein. FZD4 acted as the receptor for Wnt and Norrin along with LRP5, which has a pivotal role in various cellular processes including cell fate determination, control of cell polarity, and malignant transformation. The FZD4 contains a ∼120-residue N-terminal extracellular cystine-rich domain(CRD), seven helix transmembrane domains, three extracellular and three intracellular loops, and a C terminal cytoplasmic domain [42, 43]. The cystine-rich domain is indispensable to Wnts or Norrin and is conserved among Frizzled family members [22, 39]. The FZD4 carboxyl cytoplasmic region contains juxtamembrane KTXXXW motif which is responsible for association with Dishevelled to activate downstream signaling [44, 45].

In this update, we summarized a total of 121 mutations already reported in patients with FEVR in the literatures consisting of 70 missense mutations, 19 nonsense mutations, and 30 insertions or deletions that lead to either frame shifts or in-frame deletions; a single base change resulted in 2 amino acids extension and a whole-gene deletion [7, 24, 28, 29, 46–71] (Table 2 and Figure 3). No splice mutations have been reported for FZD4, and the mutations seem to cluster in two specific “hotspots”. Although the mutations span in whole FZD4 gene, 49% (59 of 121 mutations) and 13% (16 of 121 mutations) of them have a
Table 1: Spectrum of NDP gene mutations among patients with familial exudative vitreoretinopathy.

| Studies                      | No. of patients | No. of mutations | DNA variant       | Coding effect | Location of the amino residue | Mutant phenotypes                                                                 | Country of origin |
|------------------------------|-----------------|------------------|-------------------|---------------|-------------------------------|--------------------------------------------------------------------------------|------------------|
| Chen et al. [2]              | 30              | 1                | c.370C>T          | p.L124F       | Norrin dimer interface        | Retina detached                                                                    | UK               |
| Riveiro-Alvarez et al. [30]  | 45              | 1                | c.362G>A          | p.R121Q       | Norrin dimer interface        | Congenital blindness, phthisis bulbi                                              | Spain            |
| Dickinson et al. [23]        | 13              | 1                | c.307C>G          | p.L103V       | Norrin-FZD4 interface         | Not mentioned                                                                      | Australia        |
|                            |                 |                  | c.53T>A           | p.I118K       | Signal domain                 |                                                                                   |                  |
| Hiroyuki et al. [34]         | 62              | 3                | c.162G>C          | p.K54N        | Deductive Norrin-LRP5 interface | Retinal detachment and macular traction with temporal avascularization          | Japan            |
|                            |                 |                  | c.344G>T          | p.R115L       | Deductive Norrin-LRP5 interface | Retinal detachment                                                                 |                  |
| Pelcastre et al. [35]        | 127             | 3                | c.361C>T          | p.R121W       | Norrin dimer interface        | On-perfusion in peripheral retina                                                 | Mexico           |
|                            |                 |                  | c.362G>A          | p.R121Q       | Norrin dimer interface        | Retinal detachment                                                                 |                  |
|                            |                 |                  | c.11_12delAT      | p.H4RfsX21    | Signal domain                 | Bilateral total retinal detachment                                                |                  |
|                            |                 |                  | c.69delC          | p.D23EfsX9    | Signal domain                 | Pigmentation and vitreoretinal traction                                           |                  |
|                            |                 |                  | c.142_145delATCA  | p.I48VfsX55   | Premature termination         | Bilateral leukocoria and total retinal detachment                                 |                  |
| Musada et al. [36]          | 110             | 8                | c.170C>G          | p.S57X        | Norrin-FZD4 interface         | Retinal detachments and retrolental membranes                                    | India            |
|                            |                 |                  | c.338G>A          | p.G113D       | Near deductive Norrin-LRP5 interface | Avascular peripheral retina, straightening of the blood vessels, and dye leakage |                  |
|                            |                 |                  | c.362G>A          | p.R121Q       | Norrin dimer interface        | Retinal detachments with retrolental membranes                                   |                  |
| Liu Y. L. et al. [37]        | 40              | 1                | c.310A>C          | p.K104Q       | Norrin-FZD4 interface         | Bilateral total retinal detachment                                                | China            |
|                            |                 |                  | c.196G>A          | p.E66K        | Cystine-knot motif            | Weak eyesight, retinal vascular abnormalities                                     |                  |
|                            |                 |                  | c.203A>C          | p.H68P        | Cystine-knot motif            | Macular dragging                                                                  |                  |
|                            |                 |                  | c.281A>T          | p.H94L        | Norrin dimer interface        | Peripheral avascular zone and retinal exudates                                    |                  |
|                            |                 |                  | c.362G>A          | p.R121Q       | Norrin dimer interface        | Retinal fold, retinal detachment                                                  |                  |
|                            |                 |                  | c.334delG         | p.G113AfsX149 | Premature termination         | Bilateral tractional retinal detachment                                           |                  |
| Tang et al. [31]            | 100             | 5                | c.362G>A          | p.R121Q       | Norrin dimer interface        | Macula-involving retinal detachment                                               | China            |
|                            |                 |                  | c.313G>C          | p.A105F       | Norrin-FZD4 interface         | Complete retinal detachment                                                       |                  |
|                            |                 |                  | c.127C>A          | p.H43N        | Norrin-FZD4 interface         | Complete retinal detachment                                                       |                  |
| Iarossi et al. [24]         | 8               | 2                | c.362G>A          | p.R121Q       | Norrin dimer interface        | Predominantly involving retinal detachment                                         | Italian          |
|                            |                 |                  | c.313G>C          | p.A105F       | Norrin-FZD4 interface         | Complete retinal detachment                                                       |                  |
| Rao et al. [29]             | 31              | 3                | c.52_53ins32bp    | p.S29fs       | Premature termination         | Complete retinal detachment                                                       | China            |
|                            |                 |                  | c.195C>G          | p.C65W        | Cystine-knot motif, form disulfide bond with C126                               |                  |
tendency to bunch in the N terminal extracellular domain and C terminal intracellular domain, respectively.

The 120-residue N-terminal extracellular cystine-rich domain (CRD) domain, connected to the first transmembrane helix by a 50-amino-acid linker, was crucial to ligand recognition. In the CRD domain, mutations at C45, M105, and M157 were three most frequently reported mutations, for 4, 9, and 4 times by different studies, respectively. One of these mutations, C45Y, was found to disrupt protein folding, resulting in FZD4 being stuck in the cytoplasm with no membrane location [71]. It was supposed that the disulfide bond between Cys45 and Cys106 was imperative to protein transportation and functional activity. It was also visible from the crystal structure of FZD4-CRD that five disulfide bridges (Cys45–Cys106, Cys53–Cys99, Cys90–Cys128, Cys117–Cys158, and Cys121–Cys145) stabilized the α helices [38].

Two crystal structures of Norrin/FZD4-CRD complex and a FZD4 transmembrane domain had been registered in the Protein Data Bank [38, 40, 70]. The structures showed that one FZD4-CRD coupled a Norrin monomer with no interactions between the two FZD4-CRDs. Three loops between α helices were responsible for binding to the β-hairpins in Norrin [38]. The C-terminal tail of FZD4-CRD also made contribution to Norrin recognition. Residues V45, M59, L61, and L124 of Norrin and F96, M105, I110, M157, and M159 FZD4-CRD constituted a hydrophobic core at the binding interface [40]. Based on this, it is speculated that FEVR-related mutations at M105 and M157 may interrupt the binding of Norrin to FZD4. Biophysical analysis of Norrin and FZD4 demonstrated that the linker region of FZD4 contributes to a high-affinity interaction with Norrin and signaling [71]. Mutation C181Y in this domain not only destroyed the disulfide bond but also interrupted the binding
Table 2: Spectrum of FZD4 gene mutations among patients with familial exudative vitreoretinopathy.

| Studies               | No. of patients | No. of mutations | DNA variant | Coding effect | Location of the amino residue | Mutant phenotype                                      | Country of origin |
|-----------------------|-----------------|------------------|-------------|---------------|-----------------------------|--------------------------------------------------------|------------------|
| Zhang et al. [65]     | 49              | 5                | c.134G>A    | p.C45Y        | CRD domain, no plasma membrane localization, failed to mediate Norrin induction of these β-catenin target genes | Not mentioned                                          | China and USA    |
|                       |                 |                  | c.173A>G    | p.Y58C        | CRD domain, failed to bind Norrin, failed to mediate Norrin induction of these β-catenin target genes | Not mentioned                                          |                  |
|                       | 610T>C          | p.C204R          | C-terminal intracellular domain, failed to mediate Norrin induction of these β-catenin target genes | Not mentioned                                          |                  |
|                       |                 | p.W226X          | Transmembrane 1, failed to mediate Norrin induction of these β-catenin target genes | Not mentioned                                          |                  |
|                       |                 | p.W496X          | C-terminal intracellular domain, failed to mediate Norrin induction of these β-catenin target genes | Not mentioned                                          |                  |
| Drenser et al. [48]   | 123             | 5                | c.97C>T     | p.P33S        | Signal sequence             | 2-stage FEVR, rhegmatogenous retinal detachment         | USA              |
|                       |                 |                  | c.347T>C    | p.C117R       | CRD domain, conserved cystine residue | 2-stage FEVR, rhegmatogenous retinal detachment         |                  |
|                       |                 |                  | c.502C>T    | p.P168S       | CRD domain                  | 4B stage FEVR                                          |                  |
|                       |                 |                  | c.542G>A    | p.C181Y       | CRD domain, conserved cystine residue | 4B stage FEVR                                          |                  |
|                       |                 |                  | c.1513C>T   | p.Q505X       | Immediately downstream from KTxxxW motif | Bilateral retinal folds                               | Japan            |
| Qin et al. [56]       | 56              | 2                | c.1005G>C   | p.W335C       | Highly conserved across all members of the FZD family | Bilateral retinal folds                               |                  |
|                       |                 |                  | c.1024A>G   | p.M342V       | Intracellular loop 2, function not shown | Bilateral dragged disc                                 |                  |
| Robitaille et al. [7] | 27              | 2                | c.1479_1484del | p.M493_W494del | Failed to activate calcium/calmodulin-dependent protein kinase II and protein kinase C | Bilateral retinal detachment                           | Canada           |
|                       |                 |                  | c.1501_1502delCT | p.L501fsX333  | No membrane accumulation, failed to activate calcium/calmodulin-dependent protein kinase II and protein kinase C | Not mentioned                                          |                  |
| Kondo et al. [51]     | 24              | 4                | c.313A>G    | p.M105V       | CRD domain                  | Bilateral vitreous opacity, retinal eddutes, macular ectopia, falciform retinal fold | Japan            |
|                       |                 |                  | c.957G>A    | p.W319X       | Transmembrane domain        | Falciform retinal fold, chronic retinal detachment     |                  |
|                       |                 |                  | c.1250G>A   | p.R417Q       | Intracellular loop 3        | Falciform retinal fold, posterior synechiae, chronic retinal detachment |                  |
|                       |                 |                  | c.1463G>A   | p.G488D       | Transmembrane domain        | Falciform retinal folds                                 |                  |
| Studies                | No. of patients | No. of mutations | DNA variant       | Coding effect                          | Location of the amino residue                      | Mutant phenotype                                      | Country of origin |
|-----------------------|-----------------|------------------|-------------------|----------------------------------------|---------------------------------------------------|------------------------------------------------------|------------------|
| Dailey et al. [47]    | 421             | 11               | c.40 Del/inser    | Unknown                                | Not mentioned                                      | Not mentioned                                        | USA              |
|                       |                 |                  | c.97C>T           | p.P33S                                 | Signal sequence, reduced Wnt reporter activity     | Not mentioned                                        |                  |
|                       |                 |                  | c.151T>A          | p.S51T                                 | CRD domain                                         | Not mentioned                                        |                  |
|                       |                 |                  | c.169G>T          | p.G57C                                 | CRD domain                                         | Not mentioned                                        |                  |
|                       |                 |                  | c.349T>C          | p.C177R                                | CRD domain                                         | Not mentioned                                        |                  |
|                       |                 |                  | c.502C>T          | p.P168S                                | CRD domain, reduced Wnt reporter activity          | Not mentioned                                        |                  |
|                       |                 |                  | c.542G>A          | p.C181Y                                | CRD domain                                         | Not mentioned                                        |                  |
|                       |                 |                  | c.758G>A          | p.R253H                                | Transmembrane domain                               | Not mentioned                                        |                  |
|                       |                 |                  | c.1074A>C         | p.K386N                                | Transmembrane domain                               | Not mentioned                                        |                  |
|                       |                 |                  | c.1513C>T         | p.Q505X                                | Immediately downstream from KTxxW motif           | Not mentioned                                        |                  |
|                       |                 |                  | c.1589G>A         | p.G530E                                | C-terminal                                         | Not mentioned                                        |                  |
| Fei et al. [49]       | 61              | 3                | c.C205T           | p.H69Y                                 | CRD domain                                         | Peripheral avascular zone, dragged disc               | China            |
|                       |                 |                  | c.G400T           | p.E134X                                | CRD domain, failed to activate β-catenin reporter  | Not mentioned                                        |                  |
|                       |                 |                  | c.1506delAC       | p.T503fs                               | Failed to activate β-catenin reporter              | Increased branching of peripheral vessels, retinal detachment, Avascular zone, Retrolenticular fibrotic mass, neovascularization of optic disc |                  |
|                       |                 |                  | c.313A>G          | p.M105V                                | CRD domain                                         | Temporal dragging of optic disc, peripheral fibrous proliferation |                  |
|                       |                 |                  | c.631T>C          | p.Y211H                                | Linker upstream of transmembrane 1                | Straightening of temporal arcades, temporal dragging of optic disc, peripheral fibrous proliferation |                  |
|                       |                 |                  | c.1282-1285delGACA| p.D428fsX2                            | Intracellular loop 3                               | Temporal dragging of optic disc, peripheral fibrous proliferation |                  |
| Yang et al. [69]      | 56              | 5                | c.1482G>A         | p.W494X                                | Transmembrane domain                               | Retrolenticular fibrotic mass, lens dislocation, brushlike peripheral, avascular zone, neovascularization, peripheral fibrous proliferation | China            |
|                       |                 |                  | c.1513C>T         | p.Q505X                                | Immediately downstream from KTxxW motif           | Temporal dragging of optic disc, falciform retinal fold, branching of peripheral vessels, avascular zone, peripheral exudates |                  |
|                       |                 |                  | c.97C>T           | p.P33S                                 | Signal sequence, reduced Wnt reporter activity     | Peripheral lattice degeneration, atrophic holes, macular ectopia, bilateral peripheral avascular zone |                  |
| Nallathambi et al.    | 75              | 3                | c.244_251del8ins27 | p.F82fsX135                           | CRD domain                                         | Macular ectopia, terminal branching, peripheral avascular zone | India            |
|                       |                 |                  | c.610T>C          | p.C204R                                | CRD domain                                         | Temporal peripheral avascular zone, terminal branching, tractional retinal detachment |                  |
| Studies          | No. of patients | No. of mutations | DNA variant                       | Coding effect | Location of the amino residue | Mutant phenotype                      | Country of origin |
|-----------------|-----------------|------------------|-----------------------------------|---------------|-------------------------------|---------------------------------------|------------------|
| Seo et al. [59] | 51              | 9                | c.160C>T                          | p.Q54X        | CRD domain                     | 1B stage FEVR                         | Korea            |
|                 |                 |                  | c.313A>G                          | p.M105V       | CRD domain                     | 1B, 1A stage FEVR                     |                  |
|                 |                 |                  | c.456C>G                          | p.N152K       | CRD domain                     | 1B, 2B stage FEVR                     |                  |
|                 |                 |                  | c.470T>C                          | p.M157T       | CRD domain                     | 1B stage FEVR                         |                  |
| c.539_540delAG  |                 |                  | p.E180VfsX9                       | Linker upstream of transmembrane 1 | Intracellular loop 3 | 1B, 3A stage FEVR | 
| c.676T>A        |                 |                  | p.W226R                           | Transmembrane domain | Intracellular loop 3 | 1A stage FEVR | 
| c.120_1211delTT |                 |                  | p.L404VfsX54                      | Intracellular loop 3 | No protein                  | 2B stage FEVR | 
| c.1282_1285delGACA |            |                  | p.D428SfsX2                       | No protein     | No protein                  | No protein     | 
| Whole gene deletion |            |                  |                                   |                |                               |                        |                  |
| Musada et al. [66] | 110          | 7                | c.313A>G                          | p.M105V       | CRD domain                     | Diagnosed with FEVR, symptoms not mentioned | Indian       |
|                 |                 |                  | c.341T>G                          | p.I114S       | CRD domain                     | Diagnosed with FEVR, symptoms not mentioned |                  |
|                 |                 |                  | c.470T>C                          | p.M157T       | CRD domain                     | Diagnosed with FEVR, symptoms not mentioned |                  |
| c.1282_1285delGACA |            |                  | p.D428SfsX2                       | Intracellular loop 3 | Diagnosed with FEVR, symptoms not mentioned | 
| c.1286_1290delAGTTA |           |                  | p.K429RfsX28                      | Intracellular loop 3 | Diagnosed with FEVR, symptoms not mentioned | 
| c.1395_1396insT |                 |                  | p.R466SfsX6                       | Extracellular loop | Diagnosed with FEVR, symptoms not mentioned | 
| c.1613A>C       |                 |                  | p.S538SfsX2                       | C-terminus     | Diagnosed with FEVR, symptoms not mentioned | 
| Jia et al. [50] | 48              | 12               | c.39-49delCCCCGCGGGGGG            | p.P14fsX57    | Signal sequence, Truncated protein | Avascular retina, dragged macula         | China          |
|                 |                 |                  | c.65G>A                           | p.G22E        | Signal sequence, loss of activity | Nystagmus, retrolental fibroplasia, retinal detachment |                  |
|                 |                 |                  | c.205C>T                          | p.H69Y        | CRD domain, loss of activity   | Avascular retina, fibrous proliferation, and dragged macula |                  |
|                 |                 |                  | c.313A>G                          | p.M105V       | CRD domain, loss of activity   | Retinal vascular tortuosity, exudates, and avascularization |                  |
|                 |                 |                  | c.538G>A                          | p.E180K       | CRD domain, loss of activity   | Not mentioned                          |                  |
|                 |                 |                  | c.710C>G                          | p.T237R       | Linker upstream of transmembrane 1, loss of activity | Preterinal fibrosis, peripheral nonperfusion |                  |
| Peachey et al. [70] | 1              | 1                | c.1026A>G                         | p.M342V       | Intracellular loop 2           | Straightening of the retinal vessels, peripheral avascular areas | Japanese     |
|                 |                 |                  | c.1026A>G                         | p.M342V       | Intracellular loop 2           | Straightening of the retinal vessels, peripheral avascular areas | Japanese     |
Table 2: Continued.

| Studies            | No. of patients | No. of mutations | DNA variant | Coding effect | Location of the amino residue | Mutant phenotype                                                                 | Country of origin |
|--------------------|-----------------|------------------|-------------|---------------|-----------------------------|--------------------------------------------------------------------------------|------------------|
| Tang et al. [60]   | 100             | 14               | c.107G>A    | p.G36D        | Signal sequence             | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.133T>C    | p.C45R        | CRD domain                 | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.133T>A    | p.G45S        | CRD domain                 | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.134G>A    | p.C45Y        | CRD domain                 | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.158G>C    | p.G53S        | CRD domain                 | Macular dragging, Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.223G>A    | p.A75T        | CRD domain                 | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.268T>C    | p.C90R        | CRD domain                 | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.313A>G    | p.M105V       | CRD domain                 | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.957G>A    | p.W319X       | Transmembrane domain       | Avascular zone, increasing of peripheral vessels                                  | China            |
|                    |                 |                  | c.975_978delCCT | p.T326fsX356 | Transmembrane domain       | Neovascularization, increasing of peripheral vessels, SV                        | China            |
|                    |                 |                  | c.1034_1054delTTTATTTCCACATTGCAGCCT | p.S345_A351del | Intracellular loop 2       | Avascular zone, increasing of peripheral vessels, straightening of vessels       | China            |
|                    |                 |                  | c.1282_1285delGACA | p.D428fsX2  | Intracellular loop 3       | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels | China            |
|                    |                 |                  | c.1475delG  | p.G492fsX312  | Intracellular loop 3       | Neovascularization, increasing of peripheral vessels, straightening of vessels, vessel exudates | China            |
|                    |                 |                  | c.1498delA  | p.T500fsX312  | Truncated protein          | Not mentioned Avascular zone, increasing of peripheral vessels, straightening of vessels, vessel exudates | China            |
|                    |                 |                  | c.118G>C    | p.E40Q        | Signal sequence            | Not mentioned Development of posterior retina, ectopia of the macula, stretched retinal vessels, retinal detachment | Japan            |
|                    |                 |                  | c.611G>A    | p.C204Y       | CRD domain                 | Few abnormal temporal retinal branches, avascular peripheral fundus               | Japan            |
|                    |                 |                  | c.856G>T    | p.E286X       | Extracellular loop         | Macular ectopia, Haemorrhagic and exudative areas present in the retina          | Japan            |
|                    |                 |                  | c.1282_1285del | p.D428fsX2  | Intracellular loop 3       | Macular ectopia and peripheral, retinal detachment                             | Japan            |
|                    |                 |                  | c.1573G>C   | p.G525R       | C-terminus                 | Retrolental fibroplasia, falkiform retinal fold                                 | Japan            |
| Nikopoulos et al. [68] | 16              | 5                |             |               |                             |                                                                                | Netherlands      |
|                    |                 |                  |             |               |                             |                                                                                |                  |
| Kondo et al. [25]  | 1               | 1                | c.1250G>A   | p.R417Q       | Intracellular loop 3       | Retrolental fibroplasia, falkiform retinal fold                                 | Japanese         |
| Studies                  | No. of patients | No. of mutations | DNA variant |
|-------------------------|-----------------|------------------|-------------|
|                          |                 |                  | c.107G>A    | p.G36D | Signal sequence | Unable to obtain detailed clinical notes |
| Toomes et al. [69]      | 40              | 8                | c.314T>C    | p.M105T | CRD domain | Macula-off rhegmatogenous retinal detachment, inadequate vascularization |
| c.469A>G                |                 |                  | p.M157V     | CRD domain | Macular folds and retinal detachments |
| c.957delK               |                 |                  | p.W3196X323 | Transmembrane domain | Peripheral retinal fold |
| c.1490C>T              |                 |                  | p.5497T     | C-terminus | Disc-dragging |
| c.1088delA              |                 |                  | p.T5006X512 | KTxxxW domain | Small myopic optic disc, diffuse nonspecific pigmentary changes |
| c.1501_1502delCT        |                 |                  | p.L5016X533 | KTxxxW domain | Bilateral cicatrizied tractional retinal detachments |
| c.1513C>T              |                 |                  | p.Q505X     | Immediately downstream from KTxxxW motif | Temporal sector of retina with deficient vascularization |
| Robitaille et al. [57]  | 68              | 11               | c.316T>C    | p.C106G | CRD domain | Dragging of the retina, macular fold |
| c.470T>A                |                 |                  | p.M157K     | CRD domain | Peripheral pigmenitary, total retinal detachment, nonperfusion with leukocoria |
| c.633delC               |                 |                  | p.Y2116X    | Linker upstream of transmembrane 1 | Haemangiomatous lesion with exudation and peripheral avascular retina |
| c.1282_1285del         |                 |                  | p.D4286X2   | Intracellular loop 3 | Left macula dragged |
| c.1463G>T              |                 |                  | p.G488V     | Transmembrane domain | Bilateral dragging of the macula with peripherally straightened, avascular retina |
| c.1508insC              |                 |                  | p.T5036X31  | KTxxxW motif | Bilateral dragging of the macula, retina detachment |
| c.313A>G                |                 |                  | p.M105V     | CRD domain | Large elevated tight fold, large falciform fold |
| c.678G>A                |                 |                  | p.W226X     | Linker upstream of transmembrane 1 | Tractional retinal detachment |
| c.1448G>A               |                 |                  | p.W496X     | C-terminal intracellular domain | Not mentioned |
| c.1479_1484del         |                 |                  | p.M493_W494del | Transmembrane domain | Not mentioned |
| c.341T>C               |                 |                  | p.1114T     | CRD domain | Not mentioned |
| Studies                        | No. of patients | No. of mutations | DNA variant          | Coding effect | Location of the amino residue | Mutant phenotype                                                                 | Country of origin |
|-------------------------------|-----------------|------------------|----------------------|---------------|-------------------------------|--------------------------------------------------------------------------------|------------------|
| Robitaille et al. [67]        | 5               | 2                | c.1479_1484del       | p.M493_W494del| Transmembrane domain          | Absence of retinal vasculature, hypoplastic iris with posterior synechiae       | Canadian         |
|                              |                 |                  | c.341T>C             | p.I114T       | CRD domain                     | Falciform retinal folds, small atrophic retinal hole                           |                  |
| Boonstra et al. [46]          | 83              | 4                | c.668T>A             | p.M223K       | Linker upstream of transmembrane 1 | Diagnosed with FEVR, symptoms not mentioned                                 | Netherlands      |
|                              |                 |                  | c.957G>A             | p.W319X       | Transmembrane domain          | Diagnosed with FEVR, symptoms not mentioned                                 |                  |
|                              |                 |                  | c.1333A>C            | p.T445P       | Transmembrane domain          | Diagnosed with FEVR, symptoms not mentioned                                 |                  |
|                              |                 |                  | c.1448G>A            | p.W496X       | C-terminus, truncated protein  | Diagnosed with FEVR, symptoms not mentioned                                 |                  |
| Iarossi et al. [24]           | 8               | 3                | c.277C>T             | p.Q93X        | CRD domain                     | Large avascular area, falciform retinal fold                                 | Italian          |
|                              |                 |                  | c.542G>A             | p.C181Y       | CRD domain                     | Stage 3 and stage 2 FEVR                                                      |                  |
|                              |                 |                  | c.61G>T              | p.C204F       | CRD domain                     | Stage 4A FEVR                                                                |                  |
| Rao et al. [29]               | 31              | 2                | c.1282_1285delGACA   | p.D428SfsX2   | Intracellular loop 3           | Complete retinal detachment                                                  | China            |
|                              |                 |                  | c.227delA            | p.E76fs       | CRD domain                     | Falciform retinal detachment                                                 |                  |
| Murken et al. [53]            | 1               | 1                | c.1474delG           | p.G492fsX     | Intracellular loop 3           | Peripheral avascular zone and macular dragging                               | Mexico           |
| Schatz and Khan [58]          | 3               | 1                | c.349T>C             | p.C117R       | CRD domain, forms a disulfide bond with Cys158 | Mild temporal avascularity, mild peripheral temporal avascularity | Sweden           |
of Norrin. The FZD4 transmembrane domain structure showed mutations in key positions (M309L, C450I, C507F, and S508Y) of the ΔCRD-FZD4 structure which led to aberrant downstream signaling. However, no disease-causing mutation had been reported in abovementioned four amino residues.

The FZD4-mediated membrane recruitment of the cytoplasmic effector Dishevelled is a critical step in Wnt/β-catenin signaling. Considerable domains on FZD4 were identified as critical sites for recruitment of Dishevelled. A conserved motif (KTxxxW) located two amino acids after the seventh transmembrane domain was firstly verified to be crucial for membrane relocalization and phosphorylation of Dishevelled [44, 45]. The interaction between FZD4 and Dishevelled was further found to be pH- and charge-dependent [72]. Several amino residuals in intracellular loops 1, 2, and 3 and the flanking region near to intracellular loop 3 were also important for the intracellular location of Dishevelled while the mutant impaired the binding of Dishevelled [73–77]. Research based on FZD6 also showed that the linker domain, especially some conserved cystines, between the CRD domain and seven transmembrane core was imperative for Dishevelled recruitment [78]. One potential mechanism for FZD4 activation would be a Wnt/Norrin-induced movement of the seventh transmembrane domain to expose the key FZD4-Dishevelled interaction site [79]. Although 21% (26 of 121 mutations) of the mutations aggregated in the third intracellular loop and C terminal intracellular domain, it was not clear how the mutations affect the interaction between FZD4 and Dishevelled.

3.3. LRP5 Mutations and LRP5/LRP6 Structure. LRP5 gene, localized on human chromosome 11q13.2, consists of 23 exons and encodes 1615 amino acid single-pass transmembrane protein. LRP5 is a member of the low-density lipoprotein receptor family and belongs to a subfamily consisting of its mammalian homolog LRPLP and the Drosophila protein arrow. LRP5 and LRP6 share 73% identity in their extracellular domains. The LRP5/6 protein contains three domains including an extracellular domain, one transmembrane domain, and a cytoplasmic domain. The LRP5/6 ectodomain contains four β-propeller motifs (composed of six YWTD repeats) at the amino terminal end that alternate with four epidermal growth factor- (EGF-) like repeats (YWTD-EGF domain). These are followed by three low-density-lipoprotein receptor-like ligand-binding domains. LRP5 can act synergistically with FZD4 or other members of the Frizzled family to bind Wnts or Norrin, forming a functional ligand-receptor complex that triggers canonical Wnt/β-catenin or the Norrin/β-catenin signaling pathway and induce the transcription of target genes subsequently [80, 81].

Thus far, 58 causative mutations identified in patients with FEVR have been reported for LRP5, of which 46 mutations are missense changes, 6 frame shift mutations resulted by deletions, insertion, and duplication, 2 introduce premature stop codons, and 4 changes affect splicing [28, 29, 31, 46, 56, 59, 69, 69, 82–85] (Table 3 and Figure 4).

Mutations located in first, second, and third YWTD-EGF domain accounted for 12% (7 of 58 mutations), 38% (22 of 58 mutations), and 17% (10 of 58 mutations) of all the mutations, respectively. Thus, it can be seen causative mutations have a trend of clustering in the second YWTD-EGF domain since this segment is composed of only about 300 amino acids, accounting for less than 20% of whole LRP5 protein. Five of the included mutations (c.1828G>A, c.731C>G, c.1042C>T, c.1058G>A, and c.1481G>A) were also reported as causative mutation for OPPG [86], which was characterized as blindness and decreased bone density. But FEVR and OPPG were two different diseases because of the distinct pathogenesis of visual loss. OPPG patients often presented with blindness in the neonatal period and the symptoms initiated during early childhood. Inconformity of these results may was due to omission of bone density and definite pathogenesis of visual loss.

In the crystal of the first two YWTD-EGF structure of LRPLP, each of the two EGF domains packs tightly against the bottom surface of the preceding YWTD-EGF domains [87]. Extensive interface interactions was observed between the first β-propellers and second β-propellers, and the first EGF domain also interacts with the second β-propellers, which was critical to maintain the stability and orientation of LRPLP’s first two YWTD-EGF domains.

Early studies revealed that the interaction of LRPLP with Wnt-Fzd4 was mediated by the first two propeller domains [88], while other researchers pointed out that a single LRPLP might engage two different Wnt proteins simultaneously. LRPLP/6 binds to different Wnts via different regions or multiple domains together [89]. The four β-propeller domains in LRPLP/6 share a relatively low identity among them, indicating the functional differences among these YWTD-propellers. Ke et al. demonstrated that Norrin interacted with β-propeller domain 1 (BP1) and β-propeller domain 2 (BP2) but not BP3–4 of LRPLP. However, the binding sites of Norrin with LRPLP remain unclear. From these two perspectives, the mutations accumulated in the second YWTD-EGF domain may destroy the stable structure of first two β-propellers or interrupted their interaction with Norrin or Fzd4.

3.4. TSPAN12 Gene, Protein, and Spectrum. The TSPAN12 gene is located on chromosome 7q31 and encodes for a 305 amino acid transmembrane protein. TSPAN12 is a member of the tetraspanin family that shares certain specific structural features that distinguishes them from other proteins that pass the membrane four times. Both the N and C terminals of TSPAN12 were inside the cell membrane, and it has an unusually long C-terminal intracellular tail of approximately 60 amino acids. It contains four transmembrane domains connected by two extracellular loops (ECL-1 and ECL-2) and an intracellular loop. The ECL-1 is smaller compared to the ECL-2.

TSPAN12 was discovered to associate selectively with Norrin/β-catenin signaling but not with Wnt/β-catenin signaling. It acted as the fourth important component of Norrin/FZD4/LRP5 complex. Signaling reduction could be
| Studies            | No. of patients | No. of mutations | DNA variant | Coding effect               | Location of the amino residue | Mutant phenotype                                                                 | Country of origin |
|--------------------|-----------------|------------------|-------------|-----------------------------|------------------------------|---------------------------------------------------------------------------------|------------------|
| Toomes et al. [68] | 32              | 6                | c.518C>T    | p.T173M                     | First β-propeller motif       | Abnormal retinal vasculature and retinal fold                                    | USA              |
|                    |                 |                  | c.3502T>C   | p.Y1168H                    | Low-density-lipoprotein receptor-like ligand binding domains | Total retinal detachment and retinoschisis                                     |                  |
|                    |                 |                  | c.3840delA  | p.R1270fsX1438              | Premature termination         | Not mentioned                                                                    |                  |
|                    |                 |                  | c.4081T>G   | p.C1361G                    | Low-density-lipoprotein receptor-like ligand binding domains | Classic features of FEVR                                                 |                  |
|                    |                 |                  | c.4119_4120insC | p.K1374fsX1549             | Premature termination         | Not mentioned                                                                    |                  |
|                    |                 |                  | c.4488 + 2T>G | Splice-donor mutation       | Premature termination         | Undetermined                                                                    |                  |
| Qin et al. [56]    | 56              | 9                | c.433C>T    | p.L145F                     | First β-propeller motif       | Bilateral retrolental fibroplasias and total retinal detachment                 | Japan            |
|                    |                 |                  | c.803_812del | p.G268fsX272                | Premature termination         | Bilateral dragged macula                                                        |                  |
|                    |                 |                  | c.1330C>T   | p.R444C                     | Second β-propeller motif      | Severe falciform retinal fold                                                   |                  |
|                    |                 |                  | c.1564G>A   | p.A522T                     | Second β-propeller motif      | Tractional retinal detachment, severe macular ectopia along with peripheral fibrovascular mass |                  |
|                    |                 |                  | c.1604C>T   | p.T535M                     | Second β-propeller motif      | Bilateral retinal folds followed by total retinal detachment                    |                  |
|                    |                 |                  | c.1828G>A   | p.G610R                     | Second epidermal growth-like factor | Bilateral dragged macula                                                        |                  |
|                    |                 |                  | c.1850T>G   | p.F617C                     | Second epidermal growth-like factor | Bilateral retinal folds followed by total retinal detachment                    |                  |
|                    |                 |                  | c.2392A>G   | p.T798A                     | Third β-propeller motif       | Bilateral peripheral avascular retinas                                           |                  |
|                    |                 |                  | c.3361A>G   | p.N1121D                    | Fourth β-propeller motif      | Unilateral falciform retinal fold with bilateral retinal avascularization       |                  |
| Boonstra et al. [46]| 83              | 2                | c.1532A>C   | p.D511A                     | Second β-propeller motif      | Diagnosed with FEVR, symptoms not mentioned                                     | Netherlands      |
|                    |                 |                  | c.2413C>T   | p.R805W                     | Third β-propeller motif       | Diagnosed with FEVR, symptoms not mentioned                                     |                  |
| Nikopoulos et al. [28] | 16            | 4                | c.1321G>A   | p.E441K                     | Second β-propeller motif      | Not mentioned                                                                    | Netherlands      |
|                    |                 |                  | c.2978G>A   | p.W993X                     | EGF-like domain following the third “β-propeller” module                       | Not mentioned                                                                    |                  |
|                    |                 |                  | c.3758G>T   | p.C1253F                    | EGF-like domain following the third “β-propeller” module                       | Not mentioned                                                                    |                  |
|                    |                 |                  | c.4489-1G>A | Splice defect               | Not applied                   | Not mentioned                                                                    |                  |

Table 3: Spectrum of LRP5 gene mutations among patients with familial exudative vitreoretinopathy.
| Studies            | No. of patients | No. of mutations | DNA variant | Coding effect | Location of the amino residue | Mutant phenotype                                                                 | Country of origin |
|-------------------|-----------------|------------------|-------------|---------------|--------------------------------|----------------------------------------------------------------------------------|------------------|
| Yang et al. [69]  | 49              | 6                | c.891-892delTC | p.R298LfsX2   | Premature termination          | Retrolenticular fibrotic mass, retinal detachment, microcornea, flat anterior chamber | China            |
|                   |                 |                  | c.2484C>G    | p.I828M       | Third β-propeller motif        | Retrolenticular fibrotic mass, stretched ciliary process                         |                  |
|                   |                 |                  | c.2626G>A    | p.G876S       | Third epidermal growth like factor | Retrolenticular fibrotic mass, stretched ciliary process                         |                  |
|                   |                 |                  | c.3361A>G    | p.N1121D      | Fourth β-propeller motif       | Temporal dragging of optic disc, retrolenticular fibrotic mass                   |                  |
|                   |                 |                  | c.4025G>A    | p.R1342Q      | Low-density-lipoprotein receptor-like ligand binding domains                     | Microcornea, retrolenticular fibrotic mass, avascular zone                       |                  |
|                   |                 |                  | c.4087G>A    | p.D1363N      | Low-density-lipoprotein receptor-like ligand binding domains                     | Increased branching of peripheral vessels, retrolenticular fibrotic mass         |                  |
| Fei et al. [82]   | 2               | 2                | c.1264G>A    | p.A422T       | Second β-propeller motif        | Not mentioned                                                                   | China            |
|                   |                 |                  | c.1619T>C    | p.L540P       | Second epidermal growth like factor                                           | Not mentioned                                                                   |                  |
| Seo et al. [59]   | 51              | 4                | c.731C>G     | p.T244R       | First β-propeller motif         | 3A/2B stage FEVR                                                                | Korea            |
|                   |                 |                  | c.1330C>T    | p.R444C       | Second β-propeller motif        | 2A stage FEVR                                                                  |                  |
|                   |                 |                  | c.1833dupG   | p.C612VfsX25  | Premature termination           | 1B/4A stage FEVR                                                               |                  |
|                   |                 |                  | c.4098G>C    | p.D1366E      | Low-density-lipoprotein receptor-like ligand binding domains                     | 3B stage FEVR                                                                  |                  |
| Zhang et al. [85] | 4               | 4                | c.C1042T     | p.R348W       | First epidermal growth-like factor                                           | Not mentioned                                                                   | China            |
|                   |                 |                  | c.G1141A     | p.D381N       | Second β-propeller motif        | Not mentioned                                                                   |                  |
|                   |                 |                  | c.C1870T     | p.R624W       | Second epidermal growth-like factor                                           | Not mentioned                                                                   |                  |
|                   |                 |                  | c.A4550G     | p.Y1517C      | Cytoplasmic tail                                                             | Not mentioned                                                                   |                  |
| Studies            | No. of patients | No. of mutations | DNA variant | Coding effect Comparison | Location of the amino residue | Mutant phenotype Description                                                                 | Country of origin |
|--------------------|----------------|------------------|-------------|---------------------------|-------------------------------|---------------------------------------------------------------------------------------------|------------------|
| Tang et al. [31]   | 100            | 10               | c.1058G>A   | p.R353Q                   | First epidermal growth-like factor | Lateral retrolenticular fibrotic mass and total retinal detachment                           | China            |
|                    |                |                  | c.1183C>T   | p.R395W                   | Second β-propeller motif       | Falciform retinal fold                                                                   |                  |
|                    |                |                  | c.1318A>T   | p.I440F                   | Second β-propeller motif       | Retinal fold                                                                           |                  |
|                    |                |                  | c.1582G>A   | p.E528K                   | Second β-propeller motif       | Peripheral vascular deficiencies                                                      |                  |
|                    |                |                  | c.1942G>A   | p.V648I                   | Second epidermal growth-like factor | Rhegmatogenous retinal detachment                                                     |                  |
|                    |                |                  | c.2738G>T   | p.C913F                   | Third epidermal growth-like factor | Retinal fold and macular dragging                                                       |                  |
|                    |                |                  | c.4087G>C   | p.D1363H                  | Low-density-lipoprotein receptor-like ligand binding domains | Falciform retinal fold                                                                  |                  |
|                    |                |                  | c.4733C>T   | p.T1578M                  | Cytoplasmic tail                | Retinal fold and macular dragging                                                       |                  |
|                    |                |                  | c.92-2A>C   | Lesion mutation           | Premature termination           | Falciform retinal fold                                                                  |                  |
|                    |                |                  | c.4488 + 2T>G| Lesion mutation           | Premature termination           | Falciform retinal fold                                                                  |                  |
| Rao et al. [29]    | 31             | 5                | c.4205G>A   | p.G1402D                  | Transmembrane domain           | Falciform fold                                                                           | China            |
|                    |                |                  | c.2237G>C   | p.R746P                   | Third β-propeller motif         | Peripheral avascular zone                                                              |                  |
|                    |                |                  | c.2618A>T   | p.K873M                   | Third β-propeller motif         | Peripheral avascular zone                                                              |                  |
|                    |                |                  | c.1384C>T   | p.R462X                   | Second β-propeller motif        | Complete retinal detachment                                                             |                  |
|                    |                |                  | c.2817_2827+1del12bp| p.N940fs              | Premature termination           | Complete retinal detachment                                                             |                  |
| Liu et al. [83]    | 10             | 5                | c.542T>G    | p.M181R                   | First β-propeller motif         | Diagnosed with FEVR, symptoms not mentioned                                             | China            |
|                    |                |                  | c.1197G>T   | p.R399S                   | Second β-propeller motif        | Diagnosed with FEVR, symptoms not mentioned                                             |                  |
|                    |                |                  | c.1481G>A   | p.R494Q                   | Second β-propeller motif        | Diagnosed with FEVR, symptoms not mentioned                                             |                  |
|                    |                |                  | c.1507G>A   | p.G503R                   | Second β-propeller motif        | Diagnosed with FEVR, symptoms not mentioned                                             |                  |
|                    |                |                  | c.2626G>A   | p.G876S                   | Third epidermal growth-like factor | Diagnosed with FEVR, symptoms not mentioned                                             |                  |
| Peftianaki et al. [84] | 1             | 1                | c.2234C>T   | p.A745V                   | Third β-propeller motif         | Extensive exudative retinopathy and shallow retinal detachment                          | USA              |
rescued by TSPAN12 overexpression although direct binding with Norrin and FZD4 was not detected. However, another study reported that TSPAN12 interacted with Norrin and FZD4 via its extracellular loops and enhanced the FZD4 ligand selectivity for NDP [90]. Thus, TSPAN12 was postulated to elicit physiological levels of signaling that

Figure 3: Schematic diagram of the Frizzled-4 protein shows the locations of the mutations. A whole gene deletion and a deletion/insertion (c.40 del/inser) with unknown protein change are not shown. Superscript number means the reported times of the same or different mutations at a certain site. The color of the mutations which were reported more than one time was recolored as orange. The opacity varied with the reported frequency of the mutations.
| Studies           | No. of patients | No. of mutations | DNA variant | Coding effect   | Location of the amino residue | Mutant phenotypes                                      | Country of origin |
|------------------|-----------------|------------------|-------------|-----------------|-------------------------------|--------------------------------------------------------|-------------------|
| Savarese et al.  | 1               | 1                | c.668T>C    | p.L223P         | Transmembrane domain          | No sign of neovascularization                         | Pakistan          |
|                  |                 |                  | c.67-1G>C   | p.L23GfsX66     | Transmembrane domain, premature termination | Bilateral retinal folds                               |                   |
|                  |                 |                  | c.146C>T    | p.T49M          | First extracellular loop       | Bilateral congenital cataract, large retinal fold     |                   |
|                  |                 |                  | c.285 + 1g>a| p.R50DfsX12     | Premature termination          | Bilateral congenital cataract, large retinal fold     | Mexican and Pakistan                                   |
|                  |                 |                  | c.413A>G    | p.Y138C         | Second extracellular loop      | Peripheral retina avascularity                        |                   |
|                  |                 |                  | c.668T>C    | p.L223P         | Transmembrane domain          | Bilateral retinal folds, funnel retinal detachments  |                   |
| Poulter et al.   | 58              | 5                | c.67T>G     | p.L23X          | Transmembrane domain          | Bilateral retinal folds and unilateral, persistent   | USA, UK, Britain, |
| [93]             |                 |                  | c.149 + 3a>g| Splice-site mutation | Premature termination     | hyperplastic primary vitreous                        | Japan, Australia  |
|                  |                 |                  | c.218_219insGCTGTTT | p.F73LfsX119 | Transmembrane domain          | Macula ectopia, with a large retinal fold            |                   |
|                  |                 |                  | c.302T>A    | p.L101H         | Premature termination          | Lassic signs of FEVR                                 |                   |
|                  |                 |                  | c.629T>G    | p.M210R         | Bilateral macular traction     | Bilateral temporal retinal avascularity               |                   |
|                  |                 |                  | c.709G>C    | p.A237P         | Transmembrane domain          | Avascular peripheral retina                          | Netherlands       |
|                  |                 |                  | c.562G>C    | p.G188R         | Second extracellular loop      | Avascular peripheral retina                          |                   |
| Nikopoulos et al. | 43              | 2                | c.146C>T    | p.T49M          | First extracellular loop, conserved residue | Falciform retinal folds                              |                   |
| [68]             |                 |                  | c.313T>C    | p.C105R         | Transmembrane domain, conserved residue | Midperipheral retina, an avascular zone on the        | China             |
|                  |                 |                  | c.601delC   | p.L201FfsX14    | Conserved residue              | Inferotemporal dragging of the optic disc and macula  |                   |
| Yang et al.      | 49              | 3                | c.542G>T    | p.C181F         | Second extracellular loop, form disulfide bonds | Bilateral visual impairment, various ocular           | Israel            |
| [96]             |                 |                  |             |                 |                               | abnormalities                                        |                   |
| Gal et al.       | 64              | 1                |             |                 |                               |                                                        |                   |
| [91]             |                 |                  |             |                 |                               |                                                        |                   |
was required for normal retinal angiogenesis by promoting FZD4 multimerization cooperated with Norrin and facilitating selective ligand recognition [11].

We summarized 40 currently known mutations in TSPAN12 identified in patients affected with FEVR and discussed their coding consequences [6, 24, 29, 31, 54, 58, 59, 68, 83, 91–96] (Table 4 and Figure 5). All types of mutations were identified, including 22 missense mutations, 4 nonsense mutations, 9 splice-site mutations, 3 deletions, and 2 insertions. Mutations at residues T49, L140, C189, and L233 were reported more than one time. It was reported that L233P strongly impaired the TSPAN12 activity, while T49M mildly impaired the activity. Unfortunately, the authors did not investigate the signaling defect strength of L140X and C189Y/R. In all of the

| Studies                  | No. of patients | No. of mutations | DNA variant | Coding effect | Location of the amino residue | Mutant phenotypes                                                                 | Country of origin |
|-------------------------|----------------|------------------|-------------|---------------|-------------------------------|----------------------------------------------------------------------------------|------------------|
| Xu et al. [95]          | 85             | 3                | c.177delC   | p.Y59fsX67    | Premature termination          | Falciorm retinal folds                                                           | China            |
|                         |                |                  | c.C254T     | p.T85M        | Intracellular loop             | Pigment deposit, dragged disc                                                    |                  |
|                         |                |                  | c.566G>A    | p.C189Y       | Second extracellular loop, form disulfide bonds | Bilateral retinal folds                                                          |                  |
| Kondo et al. [92]       | 90             | 2                | c.419T>A    | p.L140X       | Second extracellular loop      | Abnormal retinal vessels with vitreous degeneration                              | Japan            |
|                         |                |                  | c.734T>C    | p.L245P       | C-terminal cytoplasmic tail    | Retinal fold resulting                                                          |                  |
| Seo et al. [59]         | 51             | 1                | c.56T>G     | p.L19R        | Transmembrane domain           | 3A stage FEVR                                                                     | Korea            |
| Ganeswara               |                |                  | c.125T>C    | p.V42A        | First extracellular loop       | Diagnosed with FEVR, symptoms not mentioned                                      | India            |
| Rao Musada et al. [2016]| 110            | 3                | c.334G>A    | p.V112I       | Second extracellular loop      | Diagnosed with FEVR, symptoms not mentioned                                      |                  |
|                         |                |                  | c.479G>A    | p.C160Y       | Second extracellular loop      | Diagnosed with FEVR, symptoms not mentioned                                      |                  |
| Tang et al. [31]        | 100            | 8                | c.655delC   | p.Q219NfsX5   | Premature termination          | Diagnosed with FEVR, symptoms not mentioned                                      | China            |
|                         |                |                  | c.916-918+3delTAAAAA | p.*306Exe+35 | Elongated protein              | Diagnosed with FEVR, symptoms not mentioned                                      |                  |
|                         |                |                  | c.150-1G>A  | Splice acceptor mutations | Not applied                    | Diagnosed with FEVR, symptoms not mentioned                                      |                  |
|                         |                |                  | c.285+1G>A  | Splice acceptor mutations | Not applied                    | Diagnosed with FEVR, symptoms not mentioned                                      |                  |
|                         |                |                  | c.469-1G>A  | Splice acceptor mutations | Not applied                    | Diagnosed with FEVR, symptoms not mentioned                                      |                  |
| Iarossi et al. [24]     | 8              | 1                | c.67-2A>G   | Defective splicing           | Not applied                    | Falciorm retinal fold                                                            | Italia           |
| Rao et al. [29]         | 31             |                  | c.345T>G    | p.Y115X        | Second extracellular loop      | Falciorm folds, complete retinal detachment                                      | China            |
| Liu et al. [83]         | 10             |                  | c.566G>A    | p.C189Y       | Second extracellular loop      | Falciorm folds, complete retinal detachment                                      | China            |
| Schatz and Khan [58]    | 3              |                  | c.565T>C    | p.C189R       | Second extracellular loop, affects cystine residues forming | Falciorm folds, complete retinal detachment                                      | Sweden           |

Table 4: Continued.
mutations, 38% (15 in 40 mutations) of them were located in the ECL-2 domain. These mutations were highly consistent with the biochemical results. TSPAN12 is anchored to the Norrin receptor complex via an interaction of the LEL with FZD4. The ECL-2 domain of TSPAN12 is essential for enhancing Norrin-induced FZD4 signaling. TSPAN12 can also alleviate the defects of FZD4 M105V, a mutation that destabilizes the NDP/FZD4 interaction [90].

Figure 4: Schematic representation of LRP5 protein shows the location of the mutations within the protein domains. Four splice site mutations are not shown. Superscript number means the reported times of the same or different mutations at a certain site. The color of the mutations which were reported more than one time was recolored as orange. The opacity varied with the reported frequency of the mutations.
4. Discussion

FEVR causing \textit{NDP}, \textit{FZD4}, \textit{LRP5}, and \textit{TSPAN12} mutations was reported from 15 countries including USA, UK, China, Spain, India, Australia, Mexico, Japan, Netherlands, Italy, Canada, Korea, Sweden, Pakistan, and Israel. Top three countries with the largest number of reported mutations about \textit{NDP}, \textit{FZD4}, \textit{LRP5}, and \textit{TSPAN12} genes were China, Netherlands, and Japan. The number of reported mutations did not completely match the population, since the three most populous countries were China, India, and USA. One of the major reasons contributing to this phenomenon might be the number of research groups was more in China, Netherlands, and Japan than that in other regions. Although most of the mutations were reported by only one study just once, some specific mutations were more common than others. For example, mutations of \textit{NDP} at c.362G (p.R121) was independently reported by 5 different studies and distributed in Spanish, Indian, Mexican, Chinese, and Italian.

\textit{FZD4} c. 313A>G (p.M105V) was reported for 8 times by 8 different research groups. Thus, it is significant to investigate the structure and function changes of the coding protein which resulted by the widely reported mutations.

Although the mutations scattered widely through the whole genes, they have an inclination to distribute in certain areas. From the point of view of the coding proteins, the mutations concentrated at the N-terminal and C-terminal domains of Norrin. There were 19 mutations located in domains from C39 to C65 and C96 to C126, which covered the two \(\beta\)-hairpins (\(\beta_1\)-\(\beta_2\) and \(\beta_5\)-\(\beta_6\)) and loops between and was crucial for binding with \textit{FZD4-CRD}, namely, 73% of the mutations (19/26) concentrated in the interacting domains with \textit{FZD4-CRD}. In terms of \textit{FZD4}, 49% (59 of 121 mutations) of the mutations were positioned in the extracellular domain, which played a significant role in ligand recognition, while 13% (16 of 121 mutations) of the mutations were positioned in the intracellular domain which recruited Dishevelled to activate downstream signaling. The sum of

\[\begin{align*}
&L_{19}, R_{23}, 2, 237, 245, L_{223}, P \quad L_{237}, P, L_{245}, P, H_{2N}, C_{YM}, 1 M_{1T}, 115 \quad Y_{115^*} \quad 147 \quad T_{147} Y_{6^* 12} \quad C_{140} Y, C_{160} Y, C_{199} F, C_{189} Y_{2^*} R, G_{190} R, L_{201} Y, X_{140} 2, L_{201}, F_{140} \quad M_{210}, R, L_{211}, X_{2^*} 14, 2, 237, 245, \quad L_{223}, P, L_{237}, P, L_{245}, P, H_{2N}, C_{YM}, 1 M_{1T}, 115 \quad Y_{115^*} \quad 147 \quad T_{147} Y_{6^* 12} \quad C_{140} Y, C_{160} Y, C_{199} F, C_{189} Y_{2^*} R, G_{190} R, L_{201} Y, X_{140} 2, L_{201}, F_{140} \quad M_{210}, R, L_{211}, X_{2^*} 14, 2, 237, 245, \quad \end{align*}\]
mutations from the two domains accounted for 61% of total reported mutations. The tendency of mutations accumulating in certain domains was more obvious in regard to LRP5 protein. More than a third of reported mutations (38%, 22/58) were found from the second YWTD-type β-propeller domain and EGF domain, which were comprised of approximately 300 amino acids, accounting for less than 20% of whole LRP5 protein. But whether the second YWTD-EGF domains interacted with Norrin and FZD4 directly or not remained unknown. As far as TSPAN12 was concerned, it seemed that the mutations were intensively located in the ECL-2 domain (38%, 15/40). A recent study revealed that the large extracellular loop of TSPAN12 is located in the ECL-2 domain (38%, 15/40). A recent study revealed that the large extracellular loop of TSPAN12 is required for enhancing Norrin-induced FZD4 signaling. In conclusion, the “hotspots” where mutations clustered were highly consistent with the domains participating protein interactions.

Overall, mutations in NDP, FZD4, LRP5, and TSPAN12 genes explained up to ~50% of all FEVR cases worldwide [97]. Besides the four genes we reviewed in this review, ZNF408, KIF11, RCBTB1, CTNNB1, and JAG1 were also reported to be the disease-causing genes of FEVR. The proteins encoded by NDP, FZD4, LRP5, TSPAN12, and CTNNB1 genes participate in the Norrin/β-catenin pathway, the signaling which is critical for retinal angiogenesis by controlling retinal vascular growth and architecture. The connection of proteins coded by ZNF408, KIF11, and RCBTB1 genes with the Norrin/β-catenin pathway was still unclear. A comprehensive spectrum covering other four causative genes (ZNF408, KIF11, RCBTB1, and CTNNB1) and further investigation on the biochemical functions of their coding proteins will undoubtedly facilitate thorough understanding of the pathogenic mechanism of FEVR.

Pathogenic mutations in NDP and FZD4 lead to a number of retina-related diseases including FEVR, Norrie disease, persistent hyperplastic primary vitreous, advanced stage of retinopathy of prematurity, and Coats disease. These diseases can be diagnosed according to their unique symptoms which can be distinguished from FEVR [98]. The common characteristic of these NDP and FZD4 related diseases was defects in the vascularization of the retina. Further study on the role of the Norrin/β-catenin pathway in the retinal vascular may promote the understanding of the mechanism of the pathogenic mutations [12]. Furthermore, other sprouting angiogenesis associated components will in some way help provide in-depth insight about these retina-related diseases.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this article.

**Authors’ Contributions**

HX and YT contributed equally to this work. HX and MP conceived and designed the review. HZ and YT performed the literature search and data collection. HX and YT wrote the paper. HX and MP critically revised the manuscript for important intellectual content.

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