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Familial Exudative Vitreoretinopathy With and Without Pathogenic Variants of Norrin/β-Catenin Signaling Genes

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Purpose: To determine the clinical characteristics of familial exudative vitreoretinopathy (FEVR) associated with or without pathogenic variants of the Norrin/β-catenin genes.

Design: This was a multicenter, cross-sectional, observational, and genetic study.

Subjects: Two-hundred eighty-one probands with FEVR were studied.

Methods: Whole-exome sequence and/or Sanger sequence was performed for the Norrin/β-catenin genes, the *FZD4*, *LRP5*, *TSPAN12*, and *NDP* genes on blood collected from the probands. The clinical symptoms of the probands with or without the pathogenic variants were assessed as well as differences in the inter Norrin/β-catenin genes.

Main Outcome Measures: The phenotype associated with or without pathogenic variants of the Norrin/β-catenin genes.

Results: One-hundred eight probands (38.4%) had 88 different pathogenic or likely pathogenic variants in the genes: 24 with the *FZD4*, 42 with the *LRP5*, 10 with the *TSPAN12*, and 12 with the *NDP* gene. Compared with the 173 probands without pathogenic variants, the 108 variant-positive probands had characteristics of familial predisposition (63.9% vs. 37.6%, $P < 0.0001$), progression during infancy (75.0% vs. 53.8%, $P = 0.0004$), asymmetrical severity between the 2 eyes (50.0% vs. 37.6%, $P = 0.0472$), and nonsyndromic characteristics (10.2% vs. 17.3%, $P = 0.1185$). The most frequent stage at which the more severe eye conditions was present was at stage 4 in both groups (40.7% vs. 34.7%). However, the advanced stages of 3 to 5 in the more severe eye were found more frequently in probands with variants than in those without variants (83.3% vs. 58.4%, $P < 0.0001$). Patients with rhegmatogenous retinal detachments progressed from stage 1 or 2 were found less frequently in the variant-positive probands (8.3% vs. 17.3%, $P = 0.0346$). Nine probands with *NDP* variants had features different from probands with typical Norrin/β-catenin gene variants including the sporadic, symmetrical, and systemic characteristics consistent with Norrie disease.

Conclusions: The results showed that the clinical characteristics of FEVR of patients with variants in the Norrin/β-catenin genes are different from those with other etiologies. We recommend that clinicians who diagnose a child with FEVR perform genetic testing so that the parents can be informed on the prognosis of the vision and general health in the child.

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Familial exudative vitreoretinopathy (FEVR, MIM#133780, #305390, #601813, #613310) is a hereditary vitreoretinal disorder that was first reported by Criswick and Schepens in 1969.[1] Familial exudative vitreoretinopathy is characterized by a defective vascular development in the peripheral retina. The affected patients are at risk of developing retinal detachments (RDs) and blindness due to secondary retinal ischemia resulting from the deficient blood supply to the retina. The expressivity of FEVR varies among patients from the same family or even between the 2 eyes of 1 patient. The clinical presentation varies widely ranging from asymptomatic peripheral vascular changes to total RD.

Familial exudative vitreoretinopathy is genetically heterogeneous, and the inheritance pattern is diverse. Autosomal dominant (AD), autosomal recessive (AR), and X-linked modes of inheritance are known to occur with AD the most common.[2] Several genes are known to be causative of FEVR. Genes of the Norrin/β-catenin signaling pathway consisting of the *FZD4*, *LRP5*, *TSPAN12*, and *NDP* genes encode proteins of a ligand-receptor complex that are

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expressed in the retinal vascular endothelial cells.[3–6] These genes represent distinct variations of Wnt/β-catenin signaling, and they play a role in the development of the retinal vasculature.[7,8] Mutations in these genes account for approximately 50% of all FEVR patients.[2]

Although FEVR has been thought to be a nonsyndromic disorder, more severe loss-of-function mutations of the same Norrin/β-catenin genes can cause syndromic disorders with severe vitreoretinopathy. Norrie disease (ND, MIM #310600) is caused by mutations in the *NDP* gene, and it is associated with mental retardation and hearing loss.[9] The osteoporosis-pseudoglioma syndrome (OPPG, MIM #259770) is caused by mutations in the *LRP5* gene, and it is associated with spontaneous skeletal fractures due to the osteoporosis.[10] Moreover, variants in the *KIF11* and *CTNNB1* genes are known to be associated with a FEVR-like phenotype. Because patients with variants in these genes are associated with microcephaly and other systemic symptoms and often with de novo mutations, they appear to be different from those with mutations of the Norrin/β-catenin signaling genes.[11,12]

Several genes have been recently reported to be associated with FEVR including the *ZNF408*, *RCBTB1*, *ILK*, *DLG1*, *JAG1*, *CTNNA1*, *CTNND1* and *LRP6* genes.[13–20] However, a link between these genes and the FEVR phenotype is still provisional, and some of them may be unrelated to FEVR according to the Online Mendelian Inheritance in Man database (OMIM, https://www.omim.org/, assessed October 23, 2023).

Thus, FEVR and the genes associated with it are yet to be definitively determined and need to be precisely categorized. To the best of our knowledge, the results of studies contrasting the FEVR phenotype between those caused by mutations of the Norrin/β-catenin genes and those by other etiologies have not been reported.

Thus, the purpose of this study was to determine the clinical characteristics of probands with pathogenic variants of the Norrin/β-catenin genes in a Japanese cohort with FEVR.

Methods

This was a multicenter retrospective case series study. The procedures used conformed to the tenets of the Declaration of Helsinki, and they were approved by the Ethics Committee of the University of Occupational and Environmental Health, Japan (Project code 20-148), Kindai University (22-132), the Jikei University School of Medicine (24-231 6997), and the National Center for Child Health and Development (518). Patients who were examined between 2010 and 2023 in the 4 hospitals were studied. A signed informed consent was obtained from all of the patients or their parents for the initial examinations and for the use of the findings in future scientific publications. The parents were assured that all personal information would be anonymized.

Patients from Fukuoka University whose findings were presented in our earlier studies were included and re-evaluated by performing whole-exome sequencing (WES) for their DNA samples after approval of the Ethics Committee of Fukuoka University (U21-04-015).[21–24]

All of the patients were Japanese and were born at full term with normal weight and without a history of either prematurity or oxygen-supplementation. The diagnosis of FEVR was based on the presence of at least one of the typical clinical signs, which is peripheral retinal avascularization with abnormal retinal vascular formation, retinal exudates, retinal neovascularization, peripheral fibrovasuclar mass, macular ectopia, retinal folds, retinal detachment, or vitreous hemorrhages.

The ocular examinations included measurements of the refractive error, best-corrected visual acuity, and intraocular pressure. In addition, slit-lamp biomicroscopy, ophthalmoscopy, ultrasonography, and optical coherence tomography (DRI OCT Triton, Topcon, Tokyo, Japan) were performed. Fluorescein angiography was performed with an ultra-widefield fundus camera (Optos 200Tx, Optos PLC, Dunfermline, Scotland, UK) and/or the RetCam3 (Clarity, Pleasanton, CA, USA).

The severity of FEVR was based on the Pedergust and Trese[25] report as follows: stage 1, avascular peripheral retina; stage 2, retinal neovascularization; stage 3, extramacular RD; stage 4, RD involving the macula; and stage 5, total RD. In addition, eyes with a rhegmatogeous retinal detachment (RRD) associated with less severe retinopathy of stages 1 or 2 were classified as "RRD." Eyes with preexisting stage 3 or more advanced retinopathy that progressed to RRD were categorized as their original stage.

Laboratory Studies

The reference sequences of the *FZD4* (NM_012193.4), *LRP5* (NM_002335.4), *TSPAN12* (NM_012338.4), and *NDP* (NM_000266.4) genes were used with a variation number based on its cDNA sequence with +1 corresponding to the first nucleotide of the initiation codon (ATG). DNA samples were extracted from peripheral blood using a DNA extraction kit (QiaAmp, Qiagen, Chatsworth, CA). The samples from the probands were screened by Sanger sequencing and/or WES for the coding sequences of these genes. A detailed explanation of the sequencing procedures has been presented.[21–24,26] In brief, polymerase chain reaction followed by Sanger sequencing was performed on the coding exons of these genes. For WES, the SureSelect human all exons V4, V5, or V6 (Agilent, Santa Clara, CA, USA) were used for the clonal clustering of a recorded DNA library. A genome coordinate of GRCh37 was used for the sequence mapping. The genotype of the family members was determined by Sanger sequencing if the probands had significant variants and their DNA were available. The samples from 49 probands analyzed by Sanger sequence in our earlier studies were re-examined by WES.[21–24]

Assessment of Pathogenicity

A search was made for the allele frequency of the variants using a global population database of the Genome Aggregation Database (gnomAD) and local databases of the Japanese population (Human Genetic Variation Database, HGVD; and the Tohoku Medical Megabank Organization database, Tommo3).[27–29] Common variants with minor allele frequency of >0.01 in at least one of the 3 databases were excluded. Conservation of the amino acid residues among humans and other species, for example, rhesus monkey, mice, elephant, chicken, zebrafish, and frog, was assessed by the UCSC Genome Browser.[30] The functional domains of each protein were annotated from the FEATURES of the NCBI Reference Sequence (NP_036325.2, NP_002326.2, NP_036470.1, and CAA46713.1).[31] The variants listed in the human gene mutation database (HGMD, 2023.2 version, https://portal.biobase-international.com/hgmd/pro/star/php) were determined to be known pathogenic variants.

Based on the pathogenic significance and the presence or absence of segregation within the family, the variants were

Table 5. Demographic Characteristics Between Probands With or Without Pathogenic Variants of the Norrin/β-Catenin Genes

	Probands With Variants in the Norrin/β-Catenin Genes (n = 108)	Probands Without Variants in the Norrin/β-Catenin Gene (n = 173)	*P*
Male	66 (61.1%)	113 (65.3%)	
Female	42 (38.9%)	60 (34.7%)	0.5243
Familial	69 (63.9%)	65 (37.6%)	
Sporadic	39 (36.1%)	108 (62.4%)	<0.0001
Infantile case	81 (75.0%)	93 (53.8%)	
Juvenile or adult case	27 (25.0%)	80 (46.2%)	0.0004
Syndromic	11 (10.2%)	30 (17.3%)	
Nonsyndromic	97 (89.8%)	143 (82.7%)	0.1185
Symmetry*	54 (50.0%)	108 (62.4%)	
Asymmetry*	54 (50.0%)	65 (37.6%)	0.0472
Stage of more severe eyes			
Stage 1	6 (5.6%)	32 (18.5%)	0.0020
Stage 2	3 (2.8%)	10 (5.8%)	0.3821†
Stage 3	21 (19.4%)	18 (10.4%)	0.0499†
Stage 4	44 (40.7%)	60 (34.7%)	0.3128†
Stage 5	25 (23.1%)	23 (13.3%)	0.0356†
Stage R	9 (8.3%)	30 (17.3%)	0.0346†
Stage 3/4/5	90 (83.3%)	101 (58.4%)	
Stage 1/2/R	18 (16.7%)	72 (41.6%)	<0.0001
Stage of all eyes			
Stage 3/4/5	153 (70.8%)	150 (43.4%)	
Stage 0/1/2/R	63 (29.2%)	196 (56.7%)	<0.0001

R = rhegmatogenous retinal detachment from stage 1 or 2.
*R was assigned to the original stages 1 and 2.
†A result from a 2 × 2 comparison between the target stage and other stages.

determined to be pathogenic or likely pathogenic-based on the standard and guidelines of the American College of Medical Genetics and Genomics.[32] A rule of PP3 (multiple lines of supporting computational evidence) was applied if the variants were predicted to be deleterious in 3 or more of the 5 in-silico programs (GERP++, SIFT, M-CAP, REVEL, and Polyphen-2, Tables S1–S4).[33–37] In addition, the CADD program was also tested for reference purposes, although no threshold score to be deleterious is proposed for the program.[38] Variants of unknown significance (VUS) were not included in this study. A rule of PP2 (missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of the disease) was applied to the 4 genes in which the number of pathogenic missense variants out of non-VUS missense variants were more than a threshold of 80.8% based on the VarSome (https://varsome.com; October 12, 2023 version, 72/73 = 98.6% for *NDP*, 75/79 = 94.9% for *FZD4*, 189/202 = 93.6% for *LRP5*, and 32/36 = 88.9% for *TSPAN12*).[39]

Statistical Analyses

Statistical analyses were performed with the Prism 9 software (version 9.5.1; GraphPad Software, Boston, MA). The Fisher exact test for 2 × 2 contingency tables or chi-square test for other contingency tables was used to determine the significance of categorized data. For testing differences between 4 groups of genes, due to the small sample size, post-hoc tests were not performed. A *P* value <0.05 was taken to be statistically significant.

Results

This study included 281 probands with 179 male probands and 102 female probands (Table 5). One-hundred seventy-four probands were infantile cases that had been diagnosed at ≤5 years of age with congenital falciform retinal fold or more severe retinopathy in at least 1 eye. The remaining 107 probands were classified as juvenile or adult patients. Forty-one probands had extraocular symptoms, and 240 probands were non-syndromic cases. One hundred thirty-four were familial, and 147 were sporadic cases.

Of the 281 probands with FEVR, 108 (38.4%) had 88 different pathogenic or likely pathogenic variants in the *FZD4*, *LRP5*, *TSPAN12*, and *NDP* genes (Tables S1–S4 and Tables 5–10).

Clinical Differences Between Probands With and Without Variants in the Norrin/β-Catenin Signaling Pathway Genes

Of the 108 probands, 66 were male probands (61.1%), and 42 (38.9%) were female probands (Table 5). The difference in the predisposition of male probands in cases with and without the variants was not significant. Sixty-nine variant-positive probands (63.9%) had familial FEVR, and the remaining 39 (36.1%) had sporadic FEVR. The frequency of the familial case was significantly higher in the probands with variants than those without variants (63.9% vs. 37.6%; $P < 0.0001$). Eighty-one probands (75.0%) were infantile cases, and 27 (25.0%) were juvenile or adult cases. The proportion of infantile cases was significantly higher in the probands with variants than those without variants (75.0% vs. 53.8%, $P = 0.0004$).

Eleven (10.2%) of the variant-positive probands had systemic symptoms and developed cognitive abnormalities

Table 6. Clinical Characteristics and Genotype of Probands With FEVR Carrying Pathogenic *FZD4* Variants

ID	Age	Sex	Stage RE/LE	Familial/ Sporadic	Genotype		Segregation[†]		Variant Earlier Report	Comment
					Allele 1	*Allele 2*	*Father (Phenotype)*	*Mother (Phenotype)*		
1	0	F	5/1	Familial	c.9G>A (p.W3*)	Wt	U	U	No	Sibling affected
2	0	F	3/3	Familial	c.80dupT(p.L27Ffs*103)	Wt	p.L27Ffs*103 (A)	Wt (N)	No	
3	0	F	4/1	Familial	c.173A>C (p.Y58S)	wt	U	Wt (N)	No	
4	14	F	5/3	Familial	c.173A>C (p.Y58S)	Wt	Wt (N)	p.Y58S (A)	No	
5	6	M	1/1	Familial	c.265G>T (p.G89C)	Wt	U	U	No	Sibling affected
6	0	M	4/3	Familial	c.313A>G (p.M105V)	Wt	p.M105V (A)	Wt (N)	[21]	Included in our earlier report[21]
7	0	M	3/4	Familial	c.313A>G (p.M105V)	Wt	p.M105V (A)	Wt (N)	[21]	
8	3	M	4/4	Familial	c.313A>G (p.M105V)	Wt	Wt (N)	p.M105V (A)	[21]	
9	0	M	3/3	Familial	c.313A>G (p.M105V)	Wt	p.M105V (A)	Wt (N)	[21]	
10	0	M	4/1	Familial	c.313A>G (p.M105V)	Wt	p.M105V (A)	Wt (U)	[21]	Sibling affected
11	2	M	3/1	Sporadic	c.326_328del (p.K109del)	Wt	U	U	No	
12	30	F	3/3	Sporadic	c.341T>C (p.I114T)	Wt	U	U	[58]	
13	3	F	3/3	Familial	c.380G>A (p.R127H)	Wt	p.R127H (A)	Wt (A)	[59]	
14	2	M	4/1	Familial	c.430A>C (p.N144H)	Wt	U (A)	U	No	
15	5	M	1/4	Familial	c.836_942del (p.R279Sfs*24)	Wt	U	U (A)	No	
16	0	M	4/3	Familial	c.845G>A (p.C282Y)	Wt	p.C282Y (A)	Wt (N)	No	
17	0	F	4/4	Sporadic	c.957G>A (p.W319*)	Wt	Wt (N)	Wt (N)	[21]	Included in our earlier report[21]: *de novo*
18	0	M	4/4	Familial	c.1005G>C (p.W335C)	Wt	Wt (A)	p.W335C (A)	[21]	Included in our earlier report[22]
19	0	F	4/1	Familial	c.1005G>C (p.W335C)	Wt	Wt (U)	p.W335C (A)	[22]	Included in our earlier report[22]
20	9	F	2/2	Familial	c.1024A>G (p.M342V)	Wt	Wt (A)	p.M342V (A)	[60]	Included in our earlier report[41]
21	8	F	3/3	Sporadic	c.1024A>G (p.M342V)	Wt	U	U	[60]	Included in our earlier report[22]
22	2	F	3/3	Sporadic	c.1024A>G (p.M342V)	Wt	Wt (U)	p.M342V (U)	[60]	
23	0	F	4/1	Sporadic	c.1024A>G (p.M342V)	Wt	U	U	[60]	
24	8	F	R/1	Familial	c.1024A>G (p.M342V)	Wt	Wt (N)	Wt (N)	[60]	*de novo*, sibling affected
25	6	M	1/R	Familial	c.1024A>G (p.M342V)	Wt	p.M342V (A)	Wt (N)	[60]	
26	0	F	1/3	Sporadic	c.1024A>G (p.M342V)	Wt	p.M342V (A)	Wt (N)	[60]	
27	0	F	1/4	Familial	c.1159delC (p.L387Sfs*44)	Wt	p.L387Sfs*44 (A)	Wt (N)	No	
28	5	M	1/1	Familial	c.1159delC (p.L387Sfs*44)	Wt	p.L387Sfs*44 (U)	Wt (N)	No	
29	0	M	4/4	Familial	c.1159delC (p.L387Sfs*44)	Wt	Wt (N)	p.L387Sfs*44 (A)	No	
30	11	F	4/2	Familial	c.1250G>A (p.R417Q)	Wt	Wt (N)	p.R417Q (A)	[21]	Included in our earlier report[21]
31	13	M	1/1	Familial	c.1250G>A (p.R417Q)	Wt	p.R417Q (A)	Wt (N)	[21]	Sibling affected
32	0	F	5/4	Sporadic	c.1250G>A (p.R417Q)	c.1250G>A (p.R417Q)	p.R417Q (A)	p.R417Q (A)	[21]	Included in our earlier report[61]
33	0	M	4/5	Familial	c.1282_1285del (p.D428Sfs*2)	Wt	Wt (N)	p.D428Sfs*2 (A)	[62]	Sibling affected
34	39	F	3/3	Familial	c.1282_1285del (p.D428Sfs*2)	c.205C>T (p.H69Y)	p.D428Sfs*2 (A)	Wt (A)	[62]	
35	18	M	1/R	Familial	c.1400A>G (p.Y467C)	Wt	U	U (A)	No	
36	0	F	4/1	Familial	c.1423G>C (p.A475P)	Wt	p.A475P (A)	Wt (N)	[41]	Microcephaly, mental retardation
37	0	M	4/4	Familial	c.1463G>A (p.G488D)	c.205C>T (p.H69Y)	Wt (N)	p.G488D (A)	[21]	Included in our earlier report[21]
38	14	M	R/1	Familial	c.1488G>C (p.W496C)	Wt	Wt (N)	p.W496C (A)	No	
39	4	M	2/1	Familial	c.1511G>A (p.W504*)	Wt	U	U	No	Sibling affected

A = affected phenotype; F = female; FEVR = familial exudative vitreoretinopathy; LE = left eye; M = male; N = normal phenotype; RE = right eye; U = undetermined genotype and/or phenotype; wt = wild type.

Underlined common variant, c.205C>T (p.H69Y) is not included in the analysis.

[†]All variants found as heterozygous in the parent(s).

Table 7. Clinical Characteristics and Genotype of Probands With FEVR Carrying Pathogenic *LRP5* Variants

ID	Age	Sex	Stage RE/LE	Familial/ Sporadic	Genotype		Segregation†		Variant Earlier Report	Comment
					Allele 1	*Allele 2*	*Father (Phenotype)*	*Mother (Phenotype)*		
40	19	M	1/R	Familial	c.362A>G (p.K121R)	Wt	Wt (N)	p.K121R (A)	No	Sibling affected
41	0	M	4/4	Familial	c.433C>T (p.L145F)	Wt	Wt (N)	p.L145F (A)	[22]	Mental retardation, included in our earlier report,[22] sibling affected
42	9	F	4/1	Sporadic	c.433C>T (p.L145F)	FZD4:p.H69Y	U	p.L145F (N)	[22]	
43	1	M	0/5	Sporadic	c.433C>T (p.L145F); FZD4:p.H69Y	Wt	Wt (N)	p.L145F (N)	[22]	
44	0	M	5/1	Sporadic	c.556C>T (p.R186W)	Wt	p.R186W (N)	Wt (N)	No	
45	10	F	4/3	Sporadic	c.871C>T (p.R291W)	Wt	U	U	[46]	
46	2	F	4/4	Familial	c.1145C>T (p.P382L)	Wt	p.P382L (A)	Wt (N)	[63]	Reported as OPPG
47	1	M	3/1	Familial	c.1145C>T (p.P382L)	Wt	U (A)	U	[63]	
48	0	M	4/5	Familial	c.1282C>T (p.R428*)	Wt	Wt (N)	p.R428* (A)	[10]	Sibling affected
49	0	M	4/4	Familial	c.1321G>A (p.E441K)	Wt	p.E441K (A)	Wt (N)	[62]	Paternal grandfather affected
50	35	F	4/3	Familial	c.1564G>A (p.A522T)	Wt	U	U	[22]	Included in our earlier report,[22] sibling affected
51	0	M	5/1	Sporadic	c.1994A>G (p.N665S)	Wt	U	U	No	
52	2	F	3/3	Sporadic	c.2254C>T (p.R752W)	Wt	p.R752W (N)	Wt (N)	[64]	Reported as OPPG
53	21	M	R/1	Sporadic	c.2392A>G (p.T798A)	Wt	U	U	[22]	
54	29	M	1/1	Familial	c.2392A>G (p.T798A)	Wt	Wt (N)	p.T798A (A)	[22]	Included in our earlier report[22]
55	4	F	4/3	Sporadic	c.2973C>G (p.I991M)	Wt	U	U	No	
56	2	F	1/1	Familial	c.2973C>G (p.I991M)	Wt	U (A)	U	No	
57	12	F	4/4	Familial	c.3232C>T (p.R1078*)	Wt	Wt (N)	p.R1078* (A)	[65]	Reported as OPPG
58	19	F	R/1	Sporadic	c.3361A>G (p.N1121D)	Wt	U	U	[22]	
59	0	F	3/3	Familial	c.4454_4465del (p.S1485_S1488del)	Wt	p.S1485_S1488del (A)	Wt (N)	No	
60	0	M	4/4	Familial	c.4001-1G>C	Wt	U	U	No	
61	5	M	0/3	Familial	c.4042T>C (p.C1348R)	c.4619C>T (p.T1540M)	p.C1348R (A)	Wt (N)	No	
62	0	F	3/4	Familial	c.4148A>C (p.H1383P)	Wt	Wt (N)	p.H1383P (A)	[61]	Reported as retinopathy of prematurity
63	30	M	5/4	Sporadic	c.4488G>A (p.P1496=)	Wt	U	U	No	
64	0	F	2/1	Familial	c.4643G>T (p.C1548F)	Wt	p.C1548F (A)	Wt (N)	[66]	
65	0	M	4/4	Sporadic	c.121C>T (p.R41W)	c.1145C>T (p.P382L)	p.P382L (N)	p.R41W (N)	Ref. 67 for p.R41W, Ref. 63 for p.P382L	Reported as retinal disease,
66	0	F	5/4	Sporadic	c.362A>G (p.K121R)	p c.3877G>A (p.E1293K)	p.K121R (N)	p.E1293K (N)	No	
67	0	M	5/4	Sporadic	c.362A>G (p.K121R)	c.1412+1G>A	p.K121R (N)	c.1412+1G>A (N)	No	
68	30	F	4/4	Familial	c.433C>T (p.L145F)	c.1270G>A (p.D424N)	U	U	Ref. 22 for p.L145F, Ref. 43 for p.D424N	
69	11	F	3/3	Sporadic	c.803_812del (p.G269Rfs*4)	c.1828G>A (p.G610R)	p.G269Rfs*4 (N)	p.G610R (N)	[22]	Included in our earlier report[22]
70	0	M	5/5	Familial	c.961T>C (p.C321R)	c.2227G>A (p.E743K)	p.E743K (N)	p.C321R (A)	No	
71	2	M	4/4	Sporadic	c.1021G>A (p.E341K)	c.4835C>A (p.T1612K)	U	U	No	

(*Continued*)

Table 7. (Continued.)

ID	Age	Sex	Stage RE/LE	Familial/ Sporadic	Genotype		Segregation†		Variant Earlier Report	Comment
					Allele 1	*Allele 2*	*Father (Phenotype)*	*Mother (Phenotype)*		
72	0	M	5/4	Sporadic	c.1333C>T (p.L445F)	c.3280G>A (p.E1094K)	p.L445F (N)	p.E1094K (N)	Ref. 68 for p.L445F, Ref. 69 for p.E1094K as OPPG	
73	5	M	3/3	Familial	c.1433G>A (p.W478*)	c.1888G>A (p.G630S)	U	U (A)	Ref. 46 for p.W478*, No for p.G630S	
74	2	M	4/4	Sporadic	c.1604C>T (p.T535M)	c.1850T>G (p.F617C)	p.T535M (N)	p.F617C (N)	Ref. 22 for both	Diagnosis of OPPG, included in our earlier report[22]
75	0	F	2/3	Familial	c.1873T>C (p.C625R)	c.3569G>A (p.R1190H)	p.C625R (A)	p.R1190H (A)	No	Sibling affected
76	6	F	4/1	Familial	c.2783G>A (p.C928Y)	c.3361A>G (p.N1121D)	p.N1121D (N)	p.C928Y (N)	No for p.C928Y, Ref. 22 for p.N1121D	Sibling affected
77	0	F	4/3	Sporadic	c.4042T>C (p.C1348R)	c.4457C>A (p.S1486*)	p.C1348R (N)	p.S1486* (N)	No for both	

A = affected phenotype; F = female; FEVR = familial exudative vitreoretinopathy; M = male; N = normal phenotype; OPPG = osteoporosis-pseudoglioma syndrome; U = undetermined genotype and/or phenotype; wt = wild type.
Underlined common variants, c.4619C>T (p.T1540M) and p.H69Y in *FZD4* is not included in the analysis.
†All variants found as heterozygous in the parent(s).

later. Syndromic patients were found less frequently in the probands with variants than those without variants, but this difference was not significant (10.2% vs. 17.3%, $P = 0.1185$). Nine male patients with variants of *NDP* had bilateral congenital retinal detachments since infancy and later had a wide range in the degree of mental retardation. A diagnosis of ND was made (Table 9). One *FZD4*-positive proband, patient 36, had microcephaly and mental retardation. Two *LRP5*-positive probands developed systemic symptoms: Patient 74 had a lumbar compression fracture and subsequent multiple bone fractures in adolescence leading to a diagnosis of OPPG (Table 7),[22] and patient 41 had mental retardation only.

Asymmetry was found more frequently in the probands with variants than those without variants when RRD was assigned to the original stage 1 or 2 (50.0% vs. 37.6%, $P < 0.0472$, Table 5). The most frequent stage with more severe eyes was stage 4 in both groups (40.7% vs. 34.7%). However, the advanced stages of 3 to 5 in the more severe eyes were more frequently found in the probands with variants than those without variants (83.3% vs. 58.4%, $P < 0.0001$, Table 5). For all 562 eyes, when RRD was assigned to the original stage 1 or 2, eyes with the advanced stages were also more frequently found in the probands with variants than those without variants (70.8% vs. 43.4%, $P < 0.0001$, Table 5 and Table S11). Patients with RRDs who progressed from stage 1 or 2 were found less frequently in the variant-positive probands (8.3% vs. 17.3%, $P = 0.0346$).

Overview of Identified Variants

Of the 88 variants found, there were 24 *FZD4* variants, 42 *LRP5* variants, 10 *TSPAN12* variants, and 12 *NDP* variants (Tables S1–S4). Forty-three were novel variants, and 45 were known variants that included 24 variants found in our earlier studies.[21–24] Thirty-six of the variants were reported to have the phenotype of FEVR, 5 were ND, 2 were OPPG, and 2 were retinopathy of prematurity, a phenotype mimicking a nongenetic disorder. Of the 88 variants, 24 were truncation variants, which are nonsense, frameshift, or splicing variants, 60 were missense variants, and 4 were in-frame deletion/insertion variants. All missense variants were found to be conserved amino acids among the tested species, and 51 (85.0%) were in the conserved domains. Fifty-seven (95.0%) missense variants were predicted to be deleterious in more than 3 programs of the 5 in silico programs (Tables S1–S4). The remaining 2 variants were synonymous variants located in the exonic splicing consensus sites considered to cause splicing errors.[40]

Two reported probands, patient 105 with *LRP5*:p.N1121D and patient 108 with variant *NDP*: p.I18K,[22,23] were digenic with the newly identified partner variants *FZD4*:p.W226C and *TSPAN12*:p.A94=, respectively (Table 10).

All variants were rare variants with an allele frequency of <0.0005 or were not found in all examined databases (Tables S1–S4). Seventy-one variants (80.7%) were found only once in a family, and 17 variants (19.3%) were found in multiple families. p.M342V of the *FZD4* gene was found the most frequently (n = 7), followed by p.L140* in the *TSPAN12* gene (n = 6).

Table 8. Clinical Characteristics and Genotype of Probands With FEVR Carrying Pathogenic *TSPAN12* Variants

ID	Age	Sex	Stage RE/LE	Familial/Sporadic	Genotype		Segregation†		Earlier Report	Comment
					Allele 1	*Allele 2*	*Father (Phenotype)*	*Mother (Phenotype)*		
78	22	F	1/1	Familial	c.232G>A (p.G78R)	Wt	U	U	[48]	Sibling affected
79	1	M	4/3	Familial	c.338G>A (p.W113*)	Wt	p.W113* (A)	Wt (N)	No	
80	0	M	1/4	Familial	c.380_385dup (p.D127_M128dup)	Wt	p.D127_M128dup (A)	Wt (N)	No	
81	12	M	1/R	Familial	c.402G>C (p.R134S)	Wt	Wt (N)	p.R134S (A)	[24]	Included in our earlier report[24]
82	0	F	3/3	Familial	c.419T>A (p.L140*)	Wt	p.L140* (A)	Wt (N)	[24]	Included in our earlier report[24]
83	0	M	4/3	Sporadic	c.419T>A (p.L140*)	Wt	U	Wt (N)	[24]	Included in our earlier report[24]
84	0	M	3/3	Familial	c.419T>A (p.L140*)	Wt	Wt (N)	p.L140* (A)	[24]	
85	8	M	R/1	Familial	c.419T>A (p.L140*)	Wt	Wt (N)	p.L140* (A)	[24]	
86	19	M	4/1	Familial	c.419T>A (p.L140*)	Wt	U	p.L140* (A)	[24]	
87	20	F	4/3	Familial	c.419T>A (p.L140*)	Wt	Wt (N)	p.L140* (A)	[24]	
88	1	M	4/1	Familial	c.644delG (p.R215Kfs*9)	Wt	p.R215Kfs*9 (A)	Wt (N)	No	
89	0	M	1/1	Familial	c.734T>C (p.L245P)	Wt	Wt (N)	p.L245P (A)	[24]	Included in our earlier report,[24] sibling affected
90	0	F	5/4	Sporadic	c.738G>A (p.W246*)	Wt	Wt (N)	p.W246* (A)	[48]	

A = affected phenotype; F = female; FEVR = familial exudative vitreoretinopathy; M = male; N = normal phenotype; U = undetermined genotype and/or phenotype; wt = wild type.
†All variants found as heterozygous in the parent(s).

Table 9. Clinical Characteristics and Genotype of Probands With FEVR Carrying Pathogenic *NDP* Variants

ID	Age	Sex	Stage RE/LE	Familial/Sporadic	Genotype		Segregation† Mother (Phenotype)	Earlier Report	Comment
					Allele 1	*Allele 2*			
91	0	M	5/5	Familial	c.11_12del (p.H4Rfs*21)	-	p.H4Rfs*21 (N)	Ref. 62 reported as ND	Diagnosis of ND, sibling affected
92	0	M	5/4	Familial	c.88_104del (p.F30Pfs*21)	-	p.F30Pfs*21 (N)	No	Diagnosis of ND
93	3	M	4/1	Sporadic	c.112C>T (p.R38C)	-	p.R38C (N)	Ref. 70 reported as ND	Diagnosis of ND
94	7	M	3/3	Sporadic	c.162G>C (p.K54N)	-	p.K54N (N)	[71]	Included in our earlier report[23]
95	3	M	3/3	Familial	c.162G>C (p.K54N)	-	p.K54N (A)	[71]	Included in our earlier report[23]
96	1	M	5/5	Familial	c.175-1G>A	-	c.175-1G>A (A)	Ref. 23 reported as ND	Diagnosis of ND, included in our earlier report[24]
97	0	M	5/5	Sporadic	c.194G>A (p.C65Y)	-	p.C65Y (N)	Ref. 72 reported as ND	Diagnosis of ND
98	0	M	5/5	Sporadic	c.290G>C (p.R97P)	-	p.R97P (N)	Ref. 73 reported as ND	Diagnosis of ND, included in our earlier report[23]
99	0	M	5/5	Sporadic	c.295_300del (p.Q99_T100del)	-	p.Q99_T100del (N)	No	Diagnosis of ND
100	0	M	5/5	Sporadic	c.334_340del (p.G112Cfs*148)	-	U	No	Diagnosis of ND
101	11	M	3/3	Sporadic	c.344G>T (p.R115L)	-	p.R115L (N)	[23]	Included in our earlier report[23]
102‡	21	M	4/3	Familial	c.344G>T (p.R115L)	-	p.R115L (N)	[23]	
103	0	M	5/5	Sporadic	c.376T>G (p.C126G)	-	p.C126G	No	Diagnosis of ND

A = affected phenotype; F = female; FEVR = familial exudative vitreoretinopathy; M = male; N = normal phenotype; ND = Norrie disease; U = undetermined genotype and/or phenotype; wt = wild type.
†All variants found as heterozygous in the parent.
‡The patient additionally had *LRP5*:p.T1540M.

Table 10. Clinical Features and Genotype of Probands With FEVR Carrying Digenic Variants

ID	Age	Sex	Stage RE/LE	Familial/ Sporadic	Genotype		Segregation*		Earlier Report	Comment
					Allele 1	*Allele 2*	*Father (Phenotype)*	*Mother (Phenotype)*		
104	0	F	4/3	Sporadic	*FZD4*: c.173A>G (p.Y58C)	*LRP5*: c.1985C>T (p.T662I)	p.T662I (N)	p.Y58C (N)	Ref. 74 for p.Y58C, No for p.T662I	
105	9	M	4/3	Familial	*FZD4*: c.678G>T (p.W226C)	*LRP5*: c.3361A>G (p.N1121D)	p.N1121D (A)	p.W226C (N)	No for p.W226C, Ref. 22 for p.N1121D	Included in our earlier report[22]
106	14	M	5/4	Familial	[*FZD4*:p.R417Q;*LRP5*:p.R444C]	Wt	[p.R417Q; p.R444C] (A)	Wt (N)	[22]	Included in our earlier report[22]
107	9	M	4/1	Sporadic	[*LRP5*: c.3361A>G (p.N1121D); *TSPAN12*: c.194C>T (p.P65L)]: pa-N	Wt	[p.N1121D; p.P65L] (N)	Wt (N)	Ref. 22 for p.N1121D, Ref. 75 for p.P65L	
108	0	M	5/1	Sporadic	*NDP*: c.53T>A (p.I18K)	*TSPAN12*: c.282A>G (p.A94=)	p.A94= (N)	p.I18K (N)	Ref. 23 for p.I18K, No for p.A94=	Included in our earlier report[23]

A = affected phenotype; F = female; FEVR = familial exudative vitreoretinopathy; LE = left eye; M = male; N = normal phenotype; RE = right eye; U = undetermined genotype and/or phenotype; wt = wild type.
*All variants found as heterozygous in the parent(s).

Characteristics of Proband by Gene

Of the 108 probands, 39 (36.1%) had *FZD4* variants, 38 (35.2%) had *LRP5* variants, 13 (12.0%) had *TSPAN12* probands, 13 (12.0%) had *NDP* variants, and 5 (4.6%) had digenic variants (Tables 6–10, and Table 12). Of the 5 digenic probands, 3 cases were *trans* with transmission from the parents, and 2 cases with *cis* transmission.

The highest percentage of familial cases was found in the *TSPAN12*-positive probands at 84.6% (n = 11), followed by *FZD4* at 79.5% (n = 31), and *LRP5* at 52.6% (n = 20, Tables 12, S13 and S14). The *NDP*-positive and digenic probands had a lower familial rate of 38.5% (n = 5) and 40.0% (n = 2), respectively. When the *LRP5*-positive probands were separated into monoallelic (AD-*LRP5*) and biallelic (AR-*LRP5*) cases, familial predisposition was found more frequently in probands with variants in the *FZD4* and *TSPAN12* genes, and in the AD-*LRP5* than in the *NDP*, digenic, and AR-*LRP5* genes (74.0% vs. 38.7%, $P = 0.0008$, Table S15).

The asymmetry rate was highest in the digenic probands (100%, n = 5, Table S14). On the other hand, *NDP*-positive probands had the lowest asymmetry rates as 23.1%. Stage 4 was the most frequent stage at which more severe eye changes were detected in the probands with variants in the *FZD4* (46.2%), *LRP5* (42.1%), *TSPAN12* (38.5%), and digenic (60.0%) genes (Table 12). In the *NDP*-positive probands, stage 5 was the most prevalent at 61.5%, and all were diagnosed with ND. For the remaining 5 *NDP*-positive probands, stage 3 was the most prevalent at 60.0% (n = 3). Eyes at the advanced stages were more frequently found in patients with AR-*LRP5* than in AD-*LRP5* variants, although it was not statistically significant (100% vs. 76.0%, $P = 0.0764$, Table S14). Nine patients with RRD carried variants of *FZD4* (n = 4), *LRP5* (n = 3), and *TSPAN12* (n = 2, Table 12).

Common Variants

In addition to the main variants, we found 2 exceptional missense variants with a minor allele frequencies of ~0.01 in the local population databases: *FZD4*:p.H69Y and *LRP5*:p.T1540M (Table S16). The probands with these variants had findings favoring a pathogenic judgement as located in the functional domains, supporting functional assays and computational analyses, and/or high prevalence among FEVR patients. In the variant-positive group, 5 probands carried one of these variants in the compound heterozygous status (Tables 6, 7 and 9). In the variant-negative group, there were 18 probands who had *FZD4*:p.H69Y and/or *LRP5*:p.T1540M.

Variants of Unknown Significance

One VUS c.58G>A (p.G20R) in the *NDP* gene was detected in patient 46 (Table 7). In addition, 3 VUS, c.4124C>T (p.P1375L) and c.4354G>A (p.A1452T) in the *LRP5* gene and c.154G>C (p.E52Q) in the *TSPAN12* gene were detected in the Norrin/β-catenin signaling pathway genes-negative probands. The family with p.E52Q was reported earlier.[24]

Table 12. Genetic and Clinical Characteristics of the 108 Variant-Positive Probands With FEVR

	FZD4 n = 39 (36.1%)	*LRP5* n = 38 (35.2%)	*TSPAN12* n = 13 (27.8%)	*NDP* n = 13 (27.8%)	Digenic n = 5 (4.6%)	Total n = 108 (100%)
Male	20 (51.3%)	20 (52.6%)	9 (69.2%)	13 (100.0%)	4 (80.0%)	66 (61.1%)
Female	19 (48.7%)	18 (47.4%)	4 (30.8%)	0 (0.0%)	1 (20.0%)	42 (38.9%)
Familial	31 (79.5%)	20 (52.6%)	11 (84.6%)	5 (38.5%)	2 (40.0%)	69 (63.9%)
Sporadic	8 (20.5%)	18 (47.4%)	2 (15.4%)	8 (61.5%)	3 (60.0%)	39 (36.1%)
Infantile case	29 (74.4%)	27 (71.1%)	9 (69.2%)	11 (84.6%)	5 (100.0%)	81 (75.0%)
Juvenile or adult case	10 (25.6%)	11 (28.9%)	4 (30.8%)	2 (15.4%)	0 (0.0%)	27 (25.0%)
Syndromic	1 (2.6%)	2 (5.3%)	0 (0.0%)	8 (61.5%)	0 (0.0%)	11 (10.2%)
Nonsyndromic	38 (97.4%)	36 (94.7%)	13 (100%)	5 (38.5%)	5 (100.0%)	97 (89.8%)
Symmetry*	19 (48.7%)	19 (50.0%)	6 (46.2%)	10 (76.9%)	0 (0.0%)	54 (50.0%)
Asymmetry*	20 (51.3%)	19 (50.0%)	7 (53.8%)	3 (23.1%)	5 (100.0%)	54 (50.0%)
Stage of more severe eye						
Stage 1	2 (5.1%)	2 (5.3%)	2 (15.4%)	0 (0%)	0 (0%)	6 (5.6%)
Stage 2	2 (5.1%)	1 (2.6%)	0 (0%)	0 (0%)	0 (0%)	3 (2.8%)
Stage 3	9 (23.1%)	7 (18.4%)	2 (15.4%)	3 (23.1%)	0 (0%)	21 (19.4%)
Stage 4	18 (46.2%)	16 (42.1%)	5 (38.5%)	2 (15.4%)	3 (60.0%)	44 (40.7%)
Stage 5	4 (10.3%)	9 (23.7%)	2 (15.4%)	8 (61.5%)	2 (40.0%)	25 (23.1%)
Stage R	4 (10.3)	3 (7.9%)	2 (15.4%)	0 (0%)	0 (0%)	9 (8.3%)

FEVR = familial exudative vitreoretinopathy; R = rhegmatogenous retinal detachment.
*R was assigned to the original stage.

Discussion

Our results showed that 38.4% of the probands had pathogenic or likely pathogenic variants in the genes of the Norrin/β-catenin signaling pathway. The variant-positive probands had more familial predisposition, more infantile cases, fewer syndromic cases, and more frequent advanced cases than probands who did not have variants in the Norrin/β-catenin signaling genes.

The etiologies of the FEVR phenotypes in the variant-negative probands were varied, and the exact cause was not determined. They included 12 patients with 11 pathogenic variants in the *KIF11* gene, 3 patients 3 with pathogenic variants in the *CTNNB1* gene, and 3 patients with a pathogenic variant in the *ATOH7* gene. Details of the phenotypes have been described elsewhere.[41,42] All patients with variants in the *KIF11* or *CTNNB1* genes had microcephaly and were often found to be de novo, consistent with previous reports.[11,12] All patients with the mutant *ATOH7* gene were sporadic cases associated with optic nerve hypoplasia.[42] In contrast, the Norrin/β-catenin gene variant-positive probands were often familial and had nonsyndromic features except for the ND patients.

Among the variant-negative probands, 10 patients had 11 heterozygous rare VUS in either genes *ZNF408* (2), *JAG1* and *DLG1* (1), *ILK* (1), *CTNNA1* (1), *CTNND1* (2), or *LRP6* (3). However, none of the variants was confirmed to segregate with the disease or to show a consistent phenotypic specificity, that is, the presence or absence of syndromic features. Notably, for 1 variant, p.S126N in the *ZNF408* gene that had been included in our earlier study,[18] an identical variant was also found in a patient without FEVR. So far, we remain cautious about whether these variants in the genes are linked with FEVR phenotype.

We had 1 interesting case: Patient 36 with a paternal *FZD4* variant later turned out to have a de novo variant in the *CTNNB1* gene (manuscript in preparation). This suggested that the *FZD4* variant was not involved in the systemic symptoms.

According to the results of previous studies on a large number of FEVR families, 28% to 67% (median of 46%) of the genes were identified.[43–49] Variants in both the *FZD4* and *LRP5* genes were found more frequently in proximity to each other. These studies showed consistent properties with those in this study. We found that a bi-allelic inheritance pattern was relatively common for the *LRP5* gene but not for the other genes in which digenic FEVR was observed. The genetic background was complicated in some pedigrees, and they were then classified as sporadic cases.

When examining the differences in the phenotypes by the genes, patients with variants in the *FZD4*, *TSPAN12*, and AD-associated *LRP5* genes tended to have less severe retinal changes with familial predisposition. In contrast, patients with variants in the *NDP* and AR-associated *LRP5* genes had more advanced retinal stages, and they tended to be found as sporadic cases. AR-*LRP5* was associated with more severe retinal phenotypes as reported earlier.[50] However, a clear spectrum has to be established because some *LRP5* variants were reported to be either AR-FEVR or AD-FEVR.[2,50]

We found that unilateral or bilateral stage 4 cases represented by congenital retinal folds were the most common phenotype of Norrin/β-catenin-related FEVR. With respect to the retinal and systemic phenotypes, ND was exceptional and should be considered to be distinct from common FEVR. Norrie disease is likely caused by specific *NDP* variants, that is, those with a truncation of the gene that abolish gene expression, or by variants with a gain or loss of cysteine leading to conformational deficits of the protein.[9]

Thus, an earlier genetic diagnosis can be helpful and would facilitate earlier rehabilitation of the systemic problems. In contrast, distinguishing AR-FEVR caused by *LRP5* variants from OPPG appears to be difficult. Patients with OPPG have a wider range of retinal severity, and no clear spectrum of the *LRP5* gene has been established.[10]

Our study confirms that RRD is one of the major phenotypes of FEVR in the Asian populations.[51–53] Huang et al[54] reported that 38% (3/8) of RRD families had *LRP5* or *FZD4* variants. Our cohort included 38 RRD cases and the variant identification was 23.7%. In Asians, the RRD was associated with relatively good vision because the eyes tended to lack fibrovascular proliferation, they occurred later in childhood or early adulthood and did not have a macular detachment.[51,53] On the other hand, eyes at advanced stages had retinal tears and may require vitrectomy but with unfavorable outcomes.[55] Thus, eyes at stage 1 or 2 associated with RRD cannot be classified by the Pendergast classification accurately.[25] A description such as "stage 1 + RRD" is recommended.

It is still being debated whether the common variants have a pathogenic effect as a genetic modifier.[2] We found 2 common variants with pathogenic properties. Similar variants, p.P33S and p.P168S in the *FZD4* gene, were suggested to be associated with FEVR and other diseases including retinopathy of prematurity.[56] These variants may contribute to the greater diversity not only in the retinal severity but also in the occurrence of sporadic cases.[2]

This study has several limitations. We did not assess other types of FEVR-causing genes. A diagnosis of familial or sporadic FEVR was not conclusive because the family members did not always receive diagnostic examinations such as fluorescein angiography.[57] It remains possible that a diagnosis of syndromic FEVR was missed in patients with a limited period of follow-up and milder symptoms. The application of the American College of Medical Genetics and Genomics criteria was less stringent for PP2 and PP3. The pathogenicity of the 2 synonymous splicing variants have not been evident by experimental assays.

In conclusion, we have presented the first report of a comprehensive genetic study of the Norrin/β-catenin genes in a Japanese cohort with FEVR. Gene-specific clinical predisposition possibly exists in FEVR. The contrasted clinical features in the Norrin/β-catenin genes can contribute to build the genotype-phenotype relationship from different etiologies. We recommend that clinicians who diagnose a child with FEVR should perform genetic testing so that the parents can be informed on the prognosis of the vision and general health in the child.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used GPT-3.5 in order to improve language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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HUMAN SUBJECTS: Human subjects were included in this study. The procedures used conformed to the tenets of the Declaration of Helsinki, and they were approved by the Ethics Committee of the University of Occupational and Environmental Health, Japan (Project code 20-148), Kindai University (22-132), the Jikei University School of Medicine (24-231 6997), and the National Center for Child Health and Development (518). A signed informed consent was obtained from all of the patients or their parents for the initial examinations and for the use of the findings in future scientific publications.

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Abbreviations and Acronyms:
AD = autosomal dominant; **AR** = autosomal recessive; **FEVR** = familial exudative vitreoretinopathy; **ND** = Norrie disease; **OPPG** = osteoporosis-pseudoglioma syndrome; **RD** = retinal detachment; **RRD** = rhegmatogeous retinal detachment; **VUS** = variants of unknown significance; **WES** = whole exome sequencing.

Keywords:
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