• Clinical Research •

Gene-related retinal detachment in a young Chinese cohort: ACMG/AMP applicability and VUS analysis

Wen-Jia Yan, Shu-Wen Xing, Xiao-Yan Li, Xiao-Ling Luo, Yang-Ming Xu, Yao Ni, Jing-Jing Chen

State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou 510060, Guangdong Province, China

Co-first Authors: Wen-Jia Yan and Shu-Wen Xing

Correspondence to: Jing-Jing Chen and Yao Ni. State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangdong Provincial Key Laboratory of Ophthalmology and Visual Science, Guangdong Provincial Clinical Research Center for Ocular Diseases, Guangzhou 510060, Guangdong Province, China. chenjingjing@gzzoc.com; niyao_dy@163.com

Received: 2026-03-17 Accepted: 2026-05-12

Abstract

● **AIM:** To investigate the genetic mutation profiles of gene-related retinal detachment (RD) and evaluate the utility of The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) pathogenicity classification system with emphasis on variants of uncertain significance (VUS) in a young Chinese cohort.

● **METHODS:** A consecutive cohort of 28 patients under 30y with RD and clinical features suggesting genetic etiology was enrolled between September 2024 and August 2025 at Zhongshan Ophthalmic Center. All patients underwent comprehensive ophthalmic examinations, genetic testing, and surgical repair. Genetic variants were interpreted *via* the ACMG/AMP criteria.

● **RESULTS:** The cohort consisted of 21 males and 7 females, with a mean age of 15.1±6.71y (ranged: 4–29). The predominant etiologies were Stickler syndrome (10/28, 35.7%), familial exudative vitreoretinopathy (FEVR; 6/28, 21.4%), and Marfan syndrome (4/28, 14.3%). A total of 30 disease-associated variants were identified, among which 60.0% (18/30) were classified as pathogenic/likely pathogenic (P/LP) and 40.0% (12/30) as VUS per ACMG/AMP criteria. The primary factors contributing to VUS classification included high population allele frequency (33.3%), variant novelty (33.3%), and discrepant *in silico* predictions (25.0%). Patients with P/LP variants exhibited a significantly higher prevalence of high myopia (<-6 D; 93.8% *vs* 50.0%, *P*=0.027) and tessellated fundus (87.5% *vs* 50.0%, *P*=0.044) compared to those with VUS/not available (NA) variants.

● **CONCLUSION:** Stickler syndrome, FEVR, and Marfan syndrome are the leading causes of gene-related RD in the Chinese Han cohort. A high VUS rate (40.0%) poses diagnostic challenges, primarily due to population-specific frequency differences, novel variants, and insufficient functional evidence. By integrating clinical history, phenotypic manifestations, and family history, a clear diagnosis can be established in 66.7% of VUS cases. Ethnically tailored genomic databases and expanded multicenter cohorts are needed to improve VUS resolution and enhance the clinical utility of genetic testing in young RD individuals.

● **KEYWORDS:** gene-related retinal detachment; genetics; variants of uncertain significance

DOI:10.18240/ijo.2026.07.13

Citation: Yan WJ, Xing SW, Li XY, Luo XL, Xu YM, Ni Y, Chen JJ. Gene-related retinal detachment in a young Chinese cohort: ACMG/AMP applicability and VUS analysis. *Int J Ophthalmol* 2026;19(7):1325-1334

INTRODUCTION

Retinal detachment (RD) poses a significant threat to eye health across all ages. The etiology, clinical features, and risk factors of RD in children and adolescents differs from those in adults[1]. According to previous research, hereditary diseases play important roles in the occurrence of RD among children and adolescents, accounting for 36%–50% of all cases[2-3].

Genetic testing has emerged as a key diagnostic tool for identifying the underlying causes and assists in the management of patients. The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) classification system is widely applied to evaluate the pathogenicity of detected genetic variants,

providing a standardized framework for interpretation[4]. Nevertheless, several challenges remain. While the ClinVar functions as a large-scale variant classification resource, a substantial proportion (36%) of its over 2 million entries remain variants of uncertain significance (VUSs), with an additional 5% showing conflicting classifications[5-6]. This predominance of VUSs poses notable challenges to accurate variant interpretation, limiting the diagnostic utility for patients undergoing genetic evaluation. Moreover, given the significant genetic and phenotypic diversity among different ethnic groups[7], ethnicity-specific evaluation of the existing interpretation criteria may be necessary.

Using gene-related RD as a model, this study aims to explore clinical characteristics and genetic mutation profiles in Chinese young RD patients, evaluate the utility of the ACMG classification system, and discuss its implications for personalized management, along with an analysis of the potential etiologies underlying VUSs.

PARTICIPANTS AND METHODS

Ethical Approval The study was approved by the Ethics Committee of Zhongshan Ophthalmic Center (Approval No.2023KYPJ317). Participants gave informed consent to participate in the study before taking part. All procedures adhered to the Declaration of Helsinki.

Study Design This was a consecutive cohort of young individuals who underwent primary surgical repair for RD by a single ophthalmologist from September 1, 2024, to August 31, 2025, at the Pediatric Retinal Department of Zhongshan Ophthalmic Center, Sun Yat-sen University. Inclusion criteria were patients aged under 30y diagnosed with RD and clinical features suggestive of underlying genetic etiology, including extensive retinal degeneration, abnormal vitreous liquefaction, syneresis, and membranes, abnormalities of retinal vessels, retinal pigmentary abnormalities, early-onset high myopia (eoHM), color vision deficiency, visual acuity refractory to optical correction, or other ocular developmental anomalies. Exclusion criteria were patients with a history of ocular trauma, prior retinal surgery, history of retinopathy of prematurity, or complicated with other non-hereditary ocular diseases that may lead to RD.

Patients underwent comprehensive bilateral ophthalmic examinations, including refractive status, best-corrected visual acuity (BCVA), non-contact tonometry (NCT), slit-lamp biomicroscopy, scanning laser ophthalmoscopy (SLO), fundus fluorescein angiography (FFA), and optical coherence tomography (OCT). Systemic conditions and family history were also recorded.

Genetic Testing and Variant Interpretation Whole-exome sequencing (WES) was performed on peripheral blood samples obtained from the proband and parents. Library preparation and targeted capture were conducted using standard commercial kits, followed by sequencing on an MGISEQ-2000RS platform (MGI). After base calling, reads were aligned to the human reference genome (hg19) using Burrows-Wheeler Aligner-Maximal Exact Match (BWA-MEM). Variant calling for single-nucleotide variants and indels was performed using the Sentieon implementation of the Genome Analysis Toolkit (GATK) pipeline, with quality recalibration applied *via* Variant Quality Score Recalibration (VQSR).

Variants were annotated and filtered using VCFANNO in a stepwise manner: 1) database annotation: variants were cross-referenced with HGMD, ClinVar, and OMIM to identify known pathogenic/likely pathogenic (P/LP) changes. Population frequencies were derived from GnomAD (East Asian); variants with minor allele frequency ≥1% were classified as common and excluded; 2) Functional prediction: SpliceAI was used to prioritize splice-site variants. Protein-truncating variants (nonsense, frameshift, canonical splice-site) were retained. Missense variants were prioritized using a REVEL score >0.6 as the key computational criterion[8]. REVEL is an ensemble predictor that integrates outputs from 13 established tools (*e.g.*, SIFT, PolyPhen-2, MutationTaster) and conservation metrics. It was trained on independently curated variant sets, providing a robust score (0–1) where higher values indicate increased pathogenic likelihood; 3) Filtering: Common variants were removed first. Remaining variants were prioritized if they were protein-truncating, affected splice sites, or were annotated as P/LP in clinical databases.

In all families, co-segregation analysis of candidate variants was performed in available family members through Sanger sequencing. Variants were interpreted according to ACMG/AMP guidelines[9], and a simplified workflow is shown in Figure 1. Primary findings included pathogenic, likely pathogenic, and VUS variants in genes related to the patient's phenotype. Secondary findings, reported for clinical reference, included benign/likely benign variants, single pathogenic alleles in recessive or X-linked genes (in females), rare VUS, and incidental findings in genes listed in the ACMG SF v3.2[10].

The final diagnosis was established through a comprehensive assessment integrating ophthalmic, systemic evaluations, and genetic findings. All patients included received pars plana vitrectomy (PPV) and/or scleral buckling (SB) by one experienced surgeon. The surgical strategy followed protocols similar to those outlined in the Primary Retinal Detachment Outcomes Study Report[11-12]. We summarized the clinical features and genetic profiles of gene-related RD in Chinese adolescents and analyzed the potential causes of the VUSs. The Kruskal-Wallis test was used to compare data between groups. The χ^2 test or Fisher's exact test was applied to the

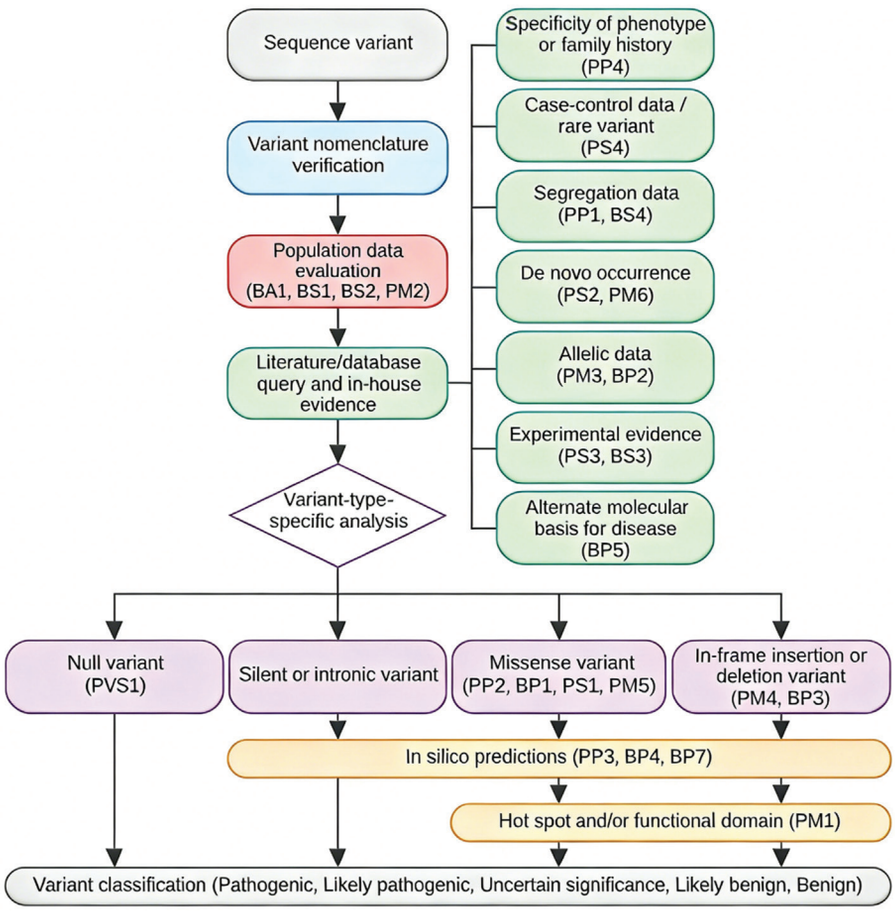

Figure 1 Strategic planning workflow for interpretation of sequence variants Pathogenic criterion (BA, BS, BP, PM, PP, PS, PVS1)[4]. BA: Benign stand-alone; BS: Benign strong; BP: Benign supporting; PM: Pathogenic moderate; PP: Pathogenic supporting; PS: Pathogenic strong; PVS1: Pathogenic very strong 1.

classified variables in different genotype groups. A statistically significant difference was considered when $P<0.05$. The data analysis was performed using SPSS v25.0 (SPSS/IBM, Chicago, IL, USA).

RESULTS

Demographic Characteristics A total of 28 patients were enrolled, comprising 21 males and 7 females, with a mean age of 15.1±6.71y (range: 4–29). The cohort included 10 cases of Stickler syndrome, 6 cases of familial exudative vitreoretinopathy (FEVR), 4 cases of Marfan syndrome, 2 cases of eoHM, and 6 cases that were categorized as "Others" [one case each of Knobloch syndrome, high myopia with cataract and vitreoretinal degeneration (MCVD; OMIM 614292), osteogenesis imperfecta type I, Alport syndrome, neurofibromatosis type I, and Wagner syndrome type I]. The demographic data are summarized in Table 1.

Subgroup analyses were performed based on diagnoses. The age at onset was significantly higher in patients with Marfan syndrome compared to those with eoHM (21.3±2.63 *vs* 5.5±2.12y, $P=0.021$). No other significant inter-subgroup differences in age were observed. In all diagnostic subgroups, males predominated among the cohort, with an overall male-to-female ratio of approximately 3:1. Bilateral RD at presentation was observed in 28.6% of patients overall. While this presentation appeared more common in the Marfan syndrome subgroup, the intergroup comparison showed no statistically significant difference. Positive family history was frequent in patients with FEVR and Marfan syndrome (66.7% and 75.0%, respectively). A high prevalence of systemic comorbidities was identified in patients with Stickler syndrome and Marfan syndrome (70.0% and 100%, respectively).

The overall average spherical equivalent of the 28 patients was -7.38±4.99 D, ranging from -17.5 to +1.75 D. Subgroup analysis showed that Stickler syndrome group, Marfan syndrome group, and eoHM group had average high myopia (mean -9.79±4.87 D, -6.91±5.72 D, and -7.38±0.18 D, respectively), followed by FEVR group with an average spherical equivalent of -4.58±5.23 D. No significant inter-subgroup differences in spherical equivalent were noted. See Table 1 and Figure 2 for details.

Genetic Results A total of 30 disease-associated variants were identified among the 28 patients. Genetically, 25 patients were diagnosed with autosomal dominant disorders, and 3 with autosomal recessive disorders (including one case each

Table 1 Demographic information of 28 patients with gene-related retinal detachment

Characteristics	Overall (*n*=28)	Stickler (*n*=10)	FEVR (*n*=6)	Marfan (*n*=4)	eoHM (*n*=2)	Others (*n*=6)
Age, y						
Mean±SD	15.1±6.71	15.1±7.37	17.2±5.64	21.3±2.63	5.5±2.12	12.2±5.12
Range	4–29	6–29	8–23	15–29	4–7	6–19
Median	15	12.5	19	20.5	5.5	11.5
Gender, *n* (%)						
Male	21 (75.0)	8 (80.0)	4 (66.7)	3 (75.0)	1 (50.0)	5 (83.3)
Female	7 (25.0)	2 (20.0)	2 (33.3)	1 (25.0)	1 (50.0)	1 (16.7)
Laterality, *n* (%)						
Unilateral	20 (71.4)	7 (70.0)	5 (83.3)	2 (50.0)	1 (50.0)	4 (66.7)
Bilateral	8 (28.6)	3 (30.0)	1 (16.7)	2 (50.0)	1 (50.0)	2 (33.3)
Family history, *n* (%)						
Positive	9 (32.1)	1 (10.0)	4 (66.7)	3 (75.0)	0	1 (16.7)
Negative	19 (67.9)	9 (90.0)	2 (33.3)	1 (25.0)	2 (100)	5 (83.3)
Spherical equivalent, D						
Mean±SD	-7.38±4.99	-9.79±4.87	-4.58±5.23	-6.91±5.72	-7.38±0.18	-6.46±4.76
Minimum	-17.5	-17.5	-14.5	-14.0	-7.50	-12.0
Maximum	+1.75	-5.00	-0.25	-0.50	-7.25	+1.75
Systemic comorbidities, *n* (%)						
Positive	16 (57.1)	7 (70.0)	1 (16.7)	4 (100)	0	4 (66.7)
Negative	12 (42.9)	3 (30.0)	5 (83.3)	0	2 (100)	2 (33.3)

SD: Standard deviation; D: Diopter; FEVR: Familial exudative vitreoretinopathy; eoHM: Early-onset High Myopia; Others include: one case each of Knobloch syndrome, MCVD (high myopia with cataract and vitreoretinal degeneration, OMIM 614292), osteogenesis imperfecta I, Alport syndrome, neurofibromatosis I, and Wagner syndrome I.

of compound heterozygous eoHM, compound heterozygous Knobloch syndrome, and homozygous MCVD). Functionally, the majority of variants (18/30, 60.0%) resided in genes associated with connective tissue abnormalities (*e.g.*, *COL1A1*, *COL2A1*, *COL11A1*, *COL4A3*, *COL18A1*, *FBN1*, *VCAN*), while 6 (6/30, 20.0%) were in genes linked to retinal vascular development (*e.g.*, *LRP5*, *TSPAN12*). All variant details are summarized in Table 2.

According to the ACMG classification system, 60.0% (18/30) of the variants were classified as P/LP, and 40.0% (12/30) as VUS. Specifically, variants associated with Stickler syndrome showed a higher detection rate of P/LP variants (8/10, 80%), with the majority attributable to established pathogenic variants in *COL2A1* (6/6, 100%). In contrast, patients with FEVR had a higher proportion of VUSs (4/6, 66.7%). For Marfan syndrome, eoHM, and other sporadic cases, the proportion of variants classified as P/LP according to ACMG criteria was approximately half. See Table 2 and Figure 3 for details.

Analysis of the Variants of Uncertain Significance The 12 VUSs were analyzed separately. Based on the ACMG/AMP framework, evidence from 5 categories-population data, *in silico* prediction, variant-specific characteristics, literature reports, and segregation analysis-was evaluated to assign each variant a pathogenic criterion leaning toward either pathogenic (P) or benign (B). Variants were classified as novel if they lacked documented evidence in both the published literature and the ClinVar database. Among the 12 VUS cases, after incorporating family history and co-segregation data, a clearer clinical diagnosis could be established in 8 cases (66.7%). These cases had either a first-degree relative with a similar RD phenotype carrying the same VUS, or a family pedigree supporting a clear inheritance pattern (*e.g.*, autosomal dominant). In contrast, the remaining 4 VUS cases (33.3%) lacked informative family history but still presented with typical clinical phenotypes consistent with the suspected genetic disorder. Therefore, the presence or absence of family history and co-segregation information was the key factor distinguishing VUS cases with a more definitive diagnosis from those without.

Table 3 summarizes the causes of ambiguity for the 12 VUSs: a high population frequency or allele count (33.3%, 4/12), the novelty of the variant (33.3%, 4/12), and discrepant *in silico* predictions (25.0%, 3/12) were the primary reasons. In one remaining case (RD24), the interpretation was complicated by a synonymous variant at the identical amino acid position that is listed as likely benign.

Comparative Analysis of Management and Risk Factors Between Patient Subgroups with Pathogenic/Likely Pathogenic *vs* VUS/Not Available Variants The 28 patients were categorized into two subgroups based on variant

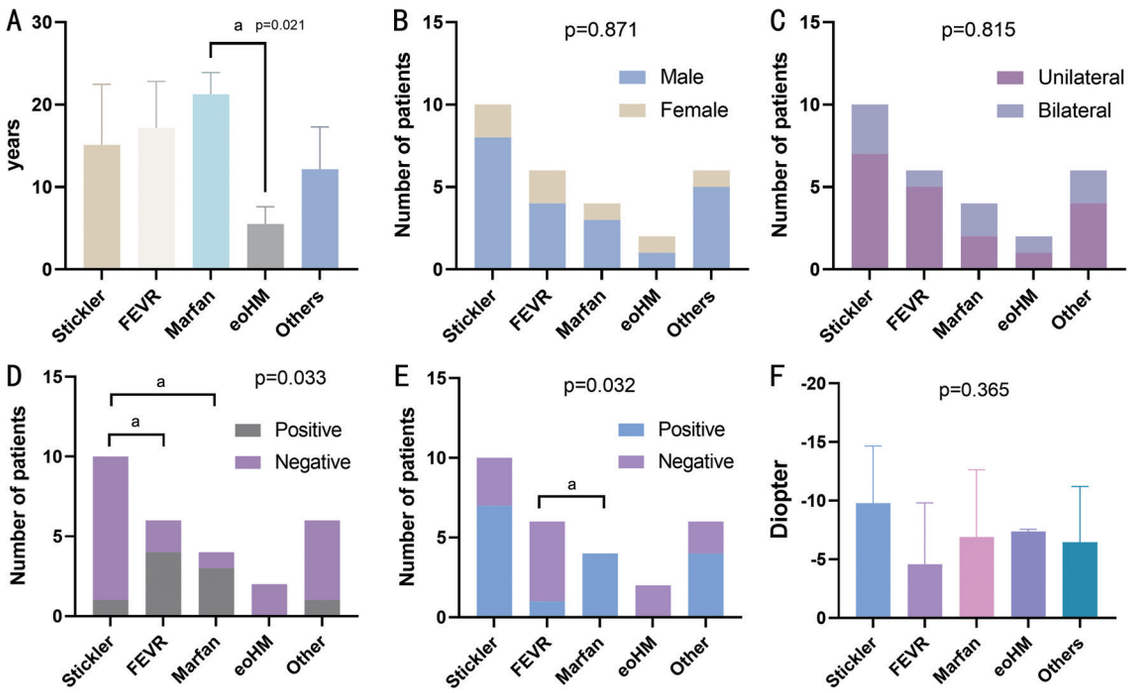

Figure 2 Demographic information of 28 patients (30 variants) with gene-related retinal detachment Statistics: Two-sided Fisher's exact test with Bonferroni correction. [a]*P*<0.05. A: Comparative age distribution of the five disease subgroups; B: Comparative gender distribution of the five disease subgroups; C: Comparative laterality distribution of the five disease subgroups; D: Family history of the five disease subgroups; E: Systemic comorbidities of the five disease subgroups; F: Spherical equivalent of the five disease subgroups. FEVR: Familial exudative vitreoretinopathy; eoHM: Early-onset high myopia.

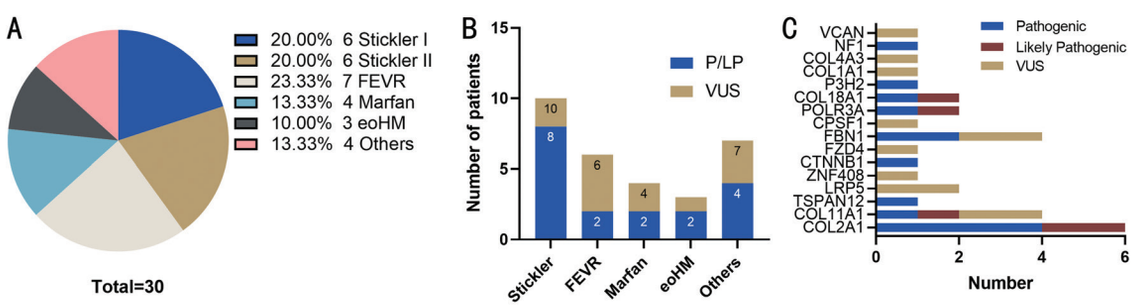

Figure 3 Genetic and pathogenicity characterization of variants in young Chinese retinal detachment cohort A: Diagnosis-based distribution of variants in young Chinese RD patients; B: ACMG pathogenicity distribution stratified by diagnosis; C: ACMG pathogenicity distribution stratified by genotype. RD: Retinal detachment; FEVR: Familial exudative vitreoretinopathy; eoHM: Early-onset high myopia; Others: One case each of Knobloch syndrome, MCVD (characterized by high myopia with cataract and vitreoretinal degeneration, OMIM 614292), osteogenesis imperfecta type I, Alport syndrome, neurofibromatosis type I, and Wagner syndrome type I; P/LP: Pathogenic/likely pathogenic; VUS: Variants of uncertain significance; ACMG: The American College of Medical Genetics and Genomics.

pathogenicity: 1) variants with high disease-causing potential (P/LP); 2) variants undetermined by ACMG guidelines [(VUS/not available (NA)]. Therapeutic interventions and ocular risk factors between groups are presented in Table 4. While no statistically significant difference was noted in the management strategies between the two subgroups (*P*=0.121), a higher proportion of patients in the P/LP subgroup underwent PPV with or without SB (75.0%, 12/16) compared to those in the VUS/NA group (41.7%, 5/12).

All patients in the P/LP subgroup had myopia (100%), with the vast majority (93.8%, 15/16) presenting with high myopia (<-6 D). In contrast, 50.0% (6/12) of patients in the VUS/NA group had high myopia, and this difference in refractive status distribution was statistically significant (*P*=0.027). Furthermore, tessellated fundus was significantly more prevalent in the P/LP subgroup (87.5% *vs* 50.0%, *P*=0.044). The prevalence of vascular abnormality showed a trend toward being higher in the VUS/NA group (41.7% *vs* 6.3%, *P*=0.057). No significant differences were found between subgroups for other retinal features, including extensive retinal degeneration, vitreous proliferation with traction, and retinal dialysis (all *P*>0.05).

Table 2 Variants identified in 28 young chinese retinal detachment patients

ID	Gene	Diagnosis	Inheritance pattern	Zygosity	Nucleotide	Protein change	GnomA (East Asian)	REVEL	ACMG pathogenicity	Pathogenic criterion[4]	ClinVar	Source
RD1	*COL2A1*	Stickler I	AD	Het	c.2353C>T	p.Arg785Ter	Absent	NA	Pathogenic	PVS1+PS4+PM2	Pathogenic	PMID: 10729292
RD3	*COL2A1*	Stickler I	AD	Het	c.1221+1G>A	Aberrant splicing	Absent	NA	Pathogenic	PVS1+PM2+PP5	Likely pathogenic	PMID: 20179744
RD9	*COL2A1*	Stickler I	AD	Het	c.2710C>T	p.Arg904Cys	Absent	0.81	Likely pathogenic	PM2+PP3+PP4+PS4+PM6	Pathogenic	PMID: 2801869
RD11	*COL2A1*	Stickler I	AD	Het	c.3106C>T	p.Arg1036Ter	0.0000007	0.96	Pathogenic	PVS1+PM2+PP5	Pathogenic	PMID: 32039712
RD18	*COL2A1*	Stickler I	AD	Het	c.1693C>T	p.Arg565Cys	0.0000007	0.69	Likely pathogenic	PS1+PM2+PP3	Likely pathogenic	PMID: 11007540
RD8	*COL2A1*	Stickler I	AD	Het	c.2619delG	p.Pro874LeufsTer7	Absent	NA	Pathogenic	PVS1+PM2	NA	Novel
RD27	*COL11A1*[a]	Stickler II	AD	Het	c.820G>T	p.Ala274Ser	0.0000007	0.63	VUS	PM2	NA	Novel
RD28	*COL11A1*[a]	Stickler II	AD	Het	c.968G>A	p.Arg323Lys	0.000001	0.25	VUS	PP1+PM2+BP4	NA	Novel
RD5	*COL11A1*	Stickler II	AD	Het	c.1972G>C	p.Gly658Arg	Absent	0.98	Likely pathogenic	PM2+PM5+PP1+PP3	NA	Novel
RD15	*COL11A1*	Stickler II	AD	Het	c.3168+1G>T	Aberrant splicing	Absent	NA	Pathogenic	PVS1+PM2+PP5+PP1	Pathogenic	PMID: 28315471
RD19	*TSPAN12*	FEVR	AD	Het	c.438dupT	p.Thr147TyrfsTer12	Absent	NA	Pathogenic	PVS1+PM2	NA	Novel
RD16	*LRP5*[a]	FEVR	AD	Het	c.3361A>G	p.Asn1121Asp	0.00022	0.61	VUS	PP1+PP2+BP4+BS1	Conflicting	PMID: 35754005
RD25	*LRP5*[a]	FEVR	AD	Het	c.518C>T	p.Thr173Met	0.0002	0.61	VUS	PP1+PM1+PP2+PP3+BS1	Conflicting	PMID: 15024691
RD20	*ZNF408*[a]	FEVR	AD	Het	c.827A>C	p.Gln276Pro	Absent	0.027	VUS	PP1+PM2+BP4	NA	Novel
RD23	*CTNNB1*	FEVR	AD	Het	c.533delT	p.Ser179LeufsTer30	Absent	NA	Pathogenic	PVS1+PM2	NA	Novel
RD21	*FZD4*[a]	FEVR	AD	Het	c.1541C>T	p.Ser514Phe	Absent	0.9	VUS	PM2	Uncertain significance	Novel
RD7	*FBN1*	Marfan syndrome	AD	Het	c.1421G>T	p.Cys474Phe	Absent	0.92	Pathogenic	PM1+PM2+PM5+PP2+PP3+PP5	Pathogenic	PMID: 10721679
RD10	*FBN1*	Marfan syndrome	AD	Het	c.184C>T	p.Arg62Cys	0.0000014	0.73	Pathogenic	PM1+PM2+PP1+PP2+PP3+PP5	Pathogenic	PMID: 16765689
RD24	*FBN1*[a]	Marfan syndrome	AD	Het	c.2269G>C	p.Asp757His	Absent	0.77	VUS	PM1+PM2+PP1+PP2+PP3	Uncertain significance	PMID: 27229674
RD22	*FBN1*[a]	Marfan syndrome	AD	Het	c.8177G>A	p.Arg2726Gln	0.00014	0.39	VUS	PP1+PP2+BP4	Conflicting	PMID: 23579484
RD26	*CPSF1*[a]	eoHM	AD	Het	c.3096+5G>A	Aberrant splicing	0.00009	NA	VUS	BS1+BS2	Uncertain significance	Novel
RD2	*POLR3A*	eoHM	AR	Comp Het	c.2005C>T	p.Arg669X	0.000007	NA	Pathogenic	PVS1+PM2+PP5	Likely pathogenic	PMID: 30414627
					c.1142C>T	p.Ala381Val	Absent	0.62	Likely pathogenic	PM2+PP3	NA	Novel
RD4	*COL18A1*	Knobloch syndrome	AR	Comp Het	c.1791delT	p.Pro600LeufsTer124	0.0000008	NA	Pathogenic	PVS1+PM2	NA	Novel
					c.3598_3613del	p.Phe1205CysfsTer41	Absent	NA	Likely pathogenic	PVS1	NA	Novel
RD6	*P3H2*	MCVD	AR	Hom	c.1684C>T	p.Arg562Ter	Absent	NA	Pathogenic	PVS1+PM2+PP5	Pathogenic	Novel
RD12	*COL1A1*[a]	OI type I	AD	Het	c.3766G>A	p.Ala1256Thr	0.00144	0.91	VUS	PP2+PP3+BS1	Conflicting	PMID: 21667357
RD13	*COL4A3*[a]	Alport Syndrome	AD	Het	c.2900G>A	p.Gly967Asp	Absent	0.97	VUS	PM2+PP3	NA	Novel
RD14	*NF1*	Neurofibromatosis I	AD	Het	c.7227del	p.Va12410SerfsTer8	Absent	NA	Pathogenic	PVS1+PM2+PP5	Pathogenic	Novel
RD17	*VCAN*[a]	Wagner I	AD	Het	c.101G>A	p.Gly34Asp	0.0000007	0.58	VUS	PP1+PM2+PP3	Uncertain significance	Novel

[a]Secondary findings. Pathogenic criterion[4]. ACMG: The American College of Medical Genetics and Genomics; FEVR: Familial exudative vitreoretinopathy; eoHM: Early-onset high myopia; MCVD: High myopia with cataract and vitreoretinal degeneration (OMIM: 614292); OI: Osteogenesis imperfecta; AD: Autosomal dominant; AR: Autosomal recessive; Het: Heterozygous; Hom: Homozygous; Comp Het: Compound heterozygous; NA: Not available; VUS: Variant of unknown significance; BS: Benign strong; BP: Benign supporting; PM: Pathogenic moderate; PP: Pathogenic supporting; PS: Pathogenic strong; PVS1: Pathogenic very strong 1.

Table 3 Potential causes of the variants of uncertain significance

ID	Gene	Nucleotide	Pathogenic criterion[4]	Population data	*In silico* prediction	Variant-specific characteristics	Literature reports	Segregation analysis	Potential causes for VUS
RD27	*COL11A1*[a]	c.820G>T	PM2	P (PM2)	P	NA	Novel	NA	Novel, lacking evidence
RD28	*COL11A1*[a]	c.968G>A	PP1+PM2+BP4	P (PM2)	B (BP4)	B (BP4)	Novel	P (PP1)	Benign prediction
RD16	*LRP5*[a]	c.3361A>G	PP1+PP2+BP4+BS1	B (BS1)	P	P (PP2)	PMID:35754005	P (PP1)	High population frequency
RD25	*LRP5*[a]	c.518C>T	PP1+PM1+PP2+PP3+BS1	B (BS1)	P	P (PM1+PP2+PP3)	PMID:15024691	P (PP1)	High population frequency
RD20	*ZNF408*[a]	c.827A>C	PP1+PM2+BP4	P (PM2)	B (BP4)	B (BP4)	Novel	P (PP1)	Benign prediction
RD21	*FZD4*[a]	c.1541C>T	PP1+PM2	P (PM2)	P	NA	Novel	P (PP1)	Novel, Lacking evidence
RD24	*FBN1*[a]	c.2269G>C	PM1+PM2+PP1+PP2+PP3	P (PM2)	P	P (PP2+PP3+PM1)	PMID:27229674	P (PP1)	Benign at same codon[b]
RD22	*FBN1*[a]	c.8177G>A	PP1+PP2+BP4	0.00014	B (BP4)	P (PP2)	PMID:23579484	P (PP1)	Benign prediction
RD26	*CPSF1*[a]	c.3096+5G>A	BS1+BS2	B (BS1+BS2)	NA	NA	Novel	NA	High population frequency
RD12	*COL1A1*[a]	c.3766G>A	PP2+PP3+BS2	B (BS2)	P	P (PP2+PP3)	PMID:21667357	NA	High population frequency
RD13	*COL4A3*[a]	c.2900G>A	PM2+PP3	P (PM2)	P	P (PP3)	Novel	NA	Novel, lacking evidence
RD17	*VCAN*[a]	c.101G>A	PP1+PM2+PP3	P (PM2)	P	P (PP3)	Novel	P (PP1)	Novel, lacking evidence

[a]Secondary findings, [b]Synonymous variant affecting the same amino acid position has been classified as likely benign. Pathogenic criterion[4]. BS: Benign strong; BP: Benign supporting; PM: Pathogenic moderate; PP: Pathogenic supporting; RD: Retinal detachment; P: Pathogenic; B: Benign; NA: Not available; VUS: Variants of uncertain significance.

Table 4 Management and risk factors between patient subgroups with pathogenic/likely pathogenic *vs* VUS/NA variants *n* (%)

Clinical characteristics	*n*=28	Subgroup analysis		*P*
		Pathogenic/likely pathogenic (*n*=16)	VUS/NA (*n*=12)	
Management				0.121
PPV with or without SB	17	12 (75.0)	5 (41.7)	
SB	11	4 (25.0)	7 (58.3)	
Refractive status				0.027
No myopia	2	0	2 (16.7)	
Mild to moderate myopia (>-6 diopters)	5	1 (6.3)	4 (33.3)	
High myopia (<-6 diopters)	21	15 (93.8)	6 (50.0)	
Tessellated fundus	20	14 (87.5)	6 (50.0)	0.044
Extensive retinal degeneration	10	7 (43.8)	3 (25.0)	0.434
Vascular abnormality	6	1 (6.3)	5 (41.7)	0.057
Vitreous proliferation and retinal traction	11	4 (25.0)	7 (58.3)	0.121
Retinal dialysis	3	3 (18.8)	0	0.255

Statistic: Fisher's exact test with Bonferroni correction. PPV: Pars plana vitrectomy; SB: Scleral buckling; VUS: Variant of unknown significance; NA: Not available.

DISCUSSION

In this young RD cohort, we preliminarily delineated the etiological spectrum, age at onset, and sex distribution of Gene-related RD, and analyzed the potential etiologies underlying VUSs. We observed that Stickler syndrome (10/28, 35.7%), FEVR (6/28, 21.4%), and Marfan syndrome (4/28, 14.3%) were identified as the predominant causes, with eoHM (2/28, 7.1%) also being relatively common. Sporadic conditions such as Knobloch syndrome, MCVD, osteogenesis imperfecta type I, Alport syndrome, and Wagner syndrome were also implicated in RD. The overall male-to-female ratio was 3:1, consistent with previous studies reporting a male-to-female ratio of approximately 2:1 in RD cohorts[13]. This sex imbalance may be attributed to longer axial length, a wider posterior vitreous base in males, and early posterior vitreous detachment (PVD) among males[14-16].

Etiologically, 60.0% of variants in this cohort were in connective tissue-related genes (*COL1A1*, *COL2A1*, *FBN1*, *etc*.)[17], and 20.0% in retinal vascular development genes (*LRP5*, *TSPAN12*)[18]. As is well established, the principal ocular risk factors for RD, namely axial myopia, vitreous abnormalities, and peripheral retinal degeneration[19], converge in distinct yet overlapping ways across different genetic etiologies. In connective tissue disorders such as Stickler syndrome, the pathophysiology is prominently driven by axial myopia, inherited vitreous abnormalities, retinal degeneration, and early PVD[20]. *FBN1* variants dysregulate transforming growth factor beta (TGF-β) homeostasis, enhancing retinal stiffness and tear susceptibility[21]. In these patients, a retinal dialysis was a frequent finding, and a high rate of bilateral involvement was also observed[22-24]. In contrast, in retinal vascular developmental diseases like FEVR, the primary

predisposing factor is peripheral retinal avascularity[18,25]. The failure of peripheral retinal vascularization results in ischemic, thin, and fragile retina, which is intrinsically prone to developing atrophic holes[26]. Nevertheless, myopia and vitreous traction are also frequently present in FEVR patients, contributing additively to the overall RD risk. Therefore, while the initiating molecular defects differ, these genetically distinct disorders share a final common pathway to RD that involves these classic ocular risk factors.

Genetically, 40% of detected variants were classified as VUS *via* ACMG criteria. This high VUS rate aligns with broader genomic medicine challenges: Fowler and Rehm[27] noted that VUS account for 36% of variants in ClinVar, reflecting the difficulty in interpreting rare or understudied variants. Per ACMG guidelines, VUS lack sufficient evidence for pathogenicity, but their clinical relevance cannot be dismissed. In our cohort, patients with Stickler syndrome exhibited the lowest rate of VUS (2/10, both in the *COL11A1* gene). This may be attributed to the relatively high disease awareness and the more comprehensive research data available for Stickler syndrome. In contrast, patients with FEVR showed the highest VUS rate (4/6), a finding consistent with recent studies on the spectrum of FEVR-associated genes. In a recent study involving 54 FEVR-RD patients who all received sequencing, a pathogenic mutation was identified in only 38.9% (21/54) of cases[28]. The study also reported a high proportion (14/22) of novel variants in detected genes. In our cohort, the majority of VUSs (10/12, 83.3%) had at least two evidence codes supporting pathogenicity, such as PP2 and PM2. These patients demonstrated well-documented, classic clinical presentations and family histories consistent with the detected monogenic disorder diagnosis. This apparent discrepancy mainly arises from novel variants (4/12) or lacking evidence in certain populations (4/12) or lacking functional data (3/12), thus limiting available evidence for pathogenicity classification, leading to the VUS classification. A simplistic exclusion of pathogenicity based solely on the "VUS" label is inappropriate and may lead to missed diagnosis of potential hereditary etiologies or improper clinical decision-making[29].

For the clinical evaluation and management of patients with VUS or NA, a comprehensive assessment integrating multiple dimensions is essential. First, detailed family history inquiry is mandatory, as our study found that positive family history was common in patients with hereditary RD for VUS cases (8/12, 66.7%), a positive family history of RD or related ocular/systemic abnormalities may imply an underlying genetic cause that has not yet been identified. Second, a thorough ocular examination of the contralateral eye is necessary, as genetically associated RD often has bilateral ocular manifestations. Third, systemic evaluation should not be neglected; our study showed that 100% of patients with Marfan syndrome and 70% of patients with Stickler syndrome had systemic complications, and for VUS/NA cases with typical ocular phenotypes, systemic examinations (*e.g.*, cardiovascular evaluation for Marfan syndrome, skeletal evaluation for Stickler syndrome) can assist in etiological inference and avoid missed diagnosis.

Some studies have attempted to reclassify VUS through various approaches[30]. Solaki *et al*[31], using a medium-throughput aequorin-based luminescence bioassay to assess the function of mutant *CNGA3* channels *via* quantifying calcium influx, reclassified the pathogenicity of *CNGA3* variants associated with achromatopsia based on ACMG/AMP criteria. Hu *et al*[32] integrated results from a validated homology-directed repair (HDR) functional assay into the ACMG/AMP framework to reclassify 133 VUSs in the DNA-binding domain of the *BRCA2* gene. These molecular, cellular, or biological experiments directly assess the functional effects of variants, providing empirical evidence for the classification of VUS. Another study investigated the distinct rates of VUS reclassification observed when subclassifying VUS by evidence level[33]. This study involved four clinical laboratories that have been subclassifying VUS to help prioritize investigation and guide reporting decisions. Each laboratory developed a distinct approach for how these subclasses are used in their laboratories and, in some cases, displayed on reports. The researchers examined the composition of each laboratory's VUS subclasses and the likelihood variants from each subclass were reclassified toward pathogenic or benign. They found that variants in the lowest subclass of VUS were never reclassified as likely pathogenic or pathogenic, whereas those in the highest subclass were much more likely to be reclassified as pathogenic or likely pathogenic. Given that forthcoming professional guidance in variant classification will advise the use of VUS subclasses, the experience from this study can inform future practices. However, they are associated with high costs and difficulties in extending to all genes. Therefore, integrating clinical data, aggregating variant results in databases, and improving population frequency and clinical phenotype data are of crucial importance.

Besides, we observed discrepancies in variant interpretation across different ethnic groups. A notable example is the *COL1A1* c.3766G>A variant. Its allele frequency is markedly higher in East Asian subsets (0.00144 in GnomAD Exome v4) than in the general population (0.00011), a discrepancy that confounds pathogenicity assessment. This may be attributable either to limitations in the representativeness of East Asian genomic databases or to true population-specific differences in gene expression or variant effect. This supports calls for ethnically tailored reference datasets and functional validation protocols to enhance classification accuracy in different ethnicities.

Our study has several notable constraints. First, the small sample size (28 patients and 30 detected variants) and single-center design may limit the generalizability of our findings. Second, the interpretation of VUS remains constrained by the limited availability of functional validation data. Third, our inclusion criteria requiring clinical features suggestive of a genetic etiology inherently select for a population with higher pretest probability of hereditary disease. This preselection likely overestimates the contribution of genetic factors to young RD compared to the general young RD population, and our findings should be interpreted with this bias in mind.

Nevertheless, by providing the first application-focused evaluation of the ACMG/AMP guidelines in Chinese gene-related RD patients, our work directly highlights critical gaps that future studies must address. To this end, we recommend two key initiatives: 1) expanding patient cohorts through multicenter studies across diverse regions of China to validate observed genotype-phenotype correlations; 2) developing ethnically matched genomic databases, particularly integrating comprehensive Han Chinese population data, to enhance the accuracy of ACMG/AMP classification and improve VUS resolution.

ACKNOWLEDGEMENTS

Foundation: Supported by the 2024 Modern Hospital Management Research Projects (No.28BC2024002-1).

Conflicts of Interest: Yan WJ, None; **Xing SW,** None; **Li XY,** None; **Luo XL,** None; **Xu YM,** None; **Ni Y,** None; **Chen JJ,** None.

REFERENCES

1 Nuzzi R, Lavia C, Spinetta R. Paediatric retinal detachment: a review. *Int J Ophthalmol* 2017;10(10):1592-1603.

2 Eissa MG, Macky TA, Mortada HA. Risk factors and prognosis of pediatric rhegmatogenous retinal detachment in Egypt: a university hospital based study. *Int J Ophthalmol* 2023;16(12):2034-2040.

3 Chen CL, Huang SJ, Sun LM, *et al*. Analysis of etiologic factors in pediatric rhegmatogenous retinal detachment with genetic testing. *Am J Ophthalmol* 2020;218:330-336.

4 Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015;17(5):405-424.

5 Kurtovic-Kozaric A, Delalic L, Mutapcic B, *et al*. Comprehensive evaluation of AlphaMissense predictions by evidence quantification for variants of uncertain significance. *Front Genet* 2024;15:1487608.

6 Rehm HL, Alaimo JT, Aradhya S, *et al*. The landscape of reported VUS in multi-gene panel and genomic testing: time for a change. *Genet Med* 2023;25(12):100947.

7 Landstrom AP, Chahal AA, Ackerman MJ, *et al*. Interpreting incidentally identified variants in genes associated with heritable cardiovascular disease: a scientific statement from the American heart association. *Circ Genom Precis Med* 2023;16(2):e000092.

8 Ioannidis NM, Rothstein JH, Pejaver V, *et al*. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet* 2016;99(4):877-885.

9 Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet* 2017;100(2):267-280.

10 Miller DT, Lee K, Abul-Husn NS, *et al*. ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* 2023;25(8):100866.

11 Ryan EH, Joseph DP, Ryan CM, *et al*. Primary retinal detachment outcomes study: methodology and overall outcomes—primary retinal detachment outcomes study report number 1. *Ophthalmol Retina* 2020;4(8):814-822.

12 Ryan EH, Ryan CM, Forbes NJ, *et al*. Primary retinal detachment outcomes study report number 2. *Ophthalmology* 2020;127(8):1077-1085.

13 Van de Put MAJ, Hooymans JMM, Los LI, *et al*. The incidence of rhegmatogenous retinal detachment in the Netherlands. *Ophthalmology* 2013;120(3):616-622.

14 Radeck V, Helbig H, Maerker D, *et al*. Rhegmatogenous retinal detachment repair—does age, sex, and lens status make a difference *Graefes Arch Clin Exp Ophthalmol* 2022;260(10):3197-3204.

15 Hussain SZM, Jeeva I, Rehman Siddiqui MA. Relationship of gender with laser retinopexy for retinal breaks. *Cureus* 2023;15(5):e39714.

16 Tillmann A, Ceklic L, Dysli C, *et al*. Gender differences in retinal diseases: a review. *Clin Exp Ophthalmol* 2024;52(3):317-333.

17 Malý P, Chrastinová L, Malý M, *et al*. Carotid artery dissection and connective tissue disorders: a review of known gene/protein mutation findings. *Mil Med Sci Lett* 2025;94(4):162-169.

18 Le V, Abdelmessih G, Dailey WA, *et al*. Mechanisms underlying rare inherited pediatric retinal vascular diseases: FEVR, norrie disease, persistent fetal vascular syndrome. *Cells* 2023;12(21):2579.

19 Schick T, Heimann H, Schaub F. Retinal detachment Part 1-epidemiology, risk factors, clinical characteristics, diagnostic approach. *Klin Monbl Augenheilkd* 2020;237(12):1479-1491.

20 Alsharif FR, Alwisi N, Irshaidat S, *et al*. Rhegmatogenous retinal detachment in stickler syndrome: a systematic review and Meta-analysis. *Ophthalmol Retina* 2026;10(4):420-441.

21 Coelho SG, Almeida AG. Marfan syndrome revisited: From genetics to the clinic. *Rev Port Cardiol (Engl Ed)* 2020;39(4):215-226.

22 Ripandelli G, Rossi T, Pesci FR, *et al*. The prophylaxis of fellow-eye retinal detachment in stickler syndrome: a retrospective series. *Retina* 2022;42(2):250-255.

23 AlEissa MM, Alhawsawi AA, Milibari D, *et al*. Genetics and clinical findings associated with early-onset myopia and retinal detachment in Saudi Arabia. *Genes (Basel)* 2025;16(7):848.

24 Stephenson KAJ, Dockery A, O'Keefe M, *et al*. A FBN1 variant manifesting as non-syndromic ectopia lentis with retinal detachment: clinical and genetic characteristics. *Eye (Lond)* 2020;34(4):690-694.

25 Chokchaitanasin R, Trerayapiwat K, Wuthisiri W, *et al*. Clinical

characteristics and long-term visual prognosis of familial exudative vitreoretinopathy. *Am J Ophthalmol* 2026;281:480-493.

26 Wang Y, Liu XY, Yan WJ, *et al*. Phenotyping and genotyping FEVR: molecular genetics, clinical and imaging features, and therapeutics. *Prog Retin Eye Res* 2025;108:101387.

27 Fowler DM, Rehm HL. Will variants of uncertain significance still exist in 2030? *Am J Hum Genet* 2024;111(1):5-10.

28 Chen CL, Wang ZR, Sun LM, *et al*. Next-generation sequencing in the familial exudative vitreoretinopathy-associated rhegmatogenous retinal detachment. *Invest Ophthalmol Vis Sci* 2019;60(7):2659-2666.

29 Chen E, Facio FM, Aradhya KW, *et al*. Rates and classification of variants of uncertain significance in hereditary disease genetic testing. *JAMA Netw Open* 2023;6(10):e2339571.

30 Walsh N, Cooper A, Dockery A, *et al*. Variant reclassification and clinical implications. *J Med Genet* 2024;61(3):207-211.

31 Solaki M, Wissinger B, Kohl S, *et al*. Functional evaluation allows ACMG/AMP-based re-classification of CNGA3 variants associated with Achromatopsia. *Genet Med* 2023;25(12):100979.

32 Hu CL, Susswein LR, Roberts ME, *et al*. Classification of *BRCA2* variants of uncertain significance (VUS) using an ACMG/AMP model incorporating a homology-directed repair (HDR) functional assay. *Clin Cancer Res* 2022;28(17):3742-3751.

33 Bennett G, Karbassi I, Chen WJ, *et al*. Distinct rates of VUS reclassification are observed when subclassifying VUS by evidence level. *Genet Med* 2025;27(6):101400.