

# Confirming and expanding the phenotypes of *FZD5* variants: Coloboma, inferior chorioretinal hypoplasia, and high myopia

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**Purpose:** Two frameshift and two indel variants in *FZD5* have been reported to cause coloboma in two families with incomplete penetrance and in two isolated cases in previous studies, respectively. This study aims to confirm this association and expand related specific phenotypes based on the genotype-phenotype analysis of *FZD5* variants.

**Methods:** Variants in *FZD5* were collected from our in-house exome sequencing data of 5,845 probands with different eye conditions. Multistep bioinformatics analysis was used to classify the variants. Potential pathogenic variants and phenotypic variations were further evaluated based on family segregation and genotype-phenotype analysis.

**Results:** In total, 63 rare variants were detected in *FZD5*. Multistep bioinformatics and genotype-phenotype analyses suggested that eight rare heterozygous variants in nine families should be considered potential pathogenic variants: three novel frameshift variants (c.350\_356delCGCCGCT/p.Ser117\*, c.1403\_1406dupACCT/p.Tyr470Profs\*130, and c.1428delG/p.Ser477Alafs\*130) and five novel missense variants (c.388C>A/p.Arg130Ser, c.794G>T/p.Arg265Leu, c.1162G>A/p.Gly388Ser, c.1232A>G/p.Tyr411Cys, and c.1510A>T/p.Met504Leu). Among the nine families, carriers of these variants showed overlapping phenotypes, including typical uveal coloboma (12 eyes of seven patients from four families), inferior chorioretinal hypoplasia (ICH) or optic disc hypoplasia (ODH; 12 eyes of eight patients from six families), and high myopia (10 eyes of five patients from five families) within individual families or among different families.

**Conclusions:** The data presented in this study confirmed that variants in *FZD5*, not only frameshift variants but also missense variants, are a common cause of uveal coloboma. In addition, ICH, ODH, and high myopia may be variant phenotypes that are frequently associated with *FZD5* variants.

Ocular coloboma is a congenital disorder that involves different components of the eye, including the iris, ciliary body, choroid, retina, and optic nerve [1]. It arises from the failed closure of an embryonic fissure during eye development. The incidence of ocular coloboma ranges from 0.5 to 7.5 per 10,000 births [2], and ocular coloboma accounts for up to 10% of blindness cases among children [3] and 0.7%–1.9% of blindness cases among adults [4]. The occurrence of ocular coloboma can be isolated, but it is frequently accompanied by other developmental abnormalities of the eye, such as microphthalmos, anophthalmia, and microcornea [5]. In

rare cases, axial elongation associated with myopia is also present in patients with coloboma [6]. Genetic defects play an important role in the development of coloboma. Thus far, at least 78 genes and six loci have been reported to be associated with coloboma, as well as related syndromes [7]. However, in most cases of isolated coloboma, only a few pathogenic variants of genes could be confirmed in a small portion of families [7-10].

The frizzled class receptor 5 (*FZD5*, OMIM 601723), which is mapped to human chromosome 2q33.3 and consists of two exons, is a recently identified gene responsible for coloboma. *FZD5* encodes a receptor of the Wnt signaling pathway, which plays a specific role in early eye development in mice, zebrafish, and *Xenopus* [11-15]. A frameshift variant in *FZD5* was initially reported to result in uveal coloboma in a large family with incomplete penetrance [15]. Recently, one

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frameshift and two indel variants in *FZD5* were identified in a small family and in two isolated cases [16].

In this study, variants in *FZD5* were collected from exome sequencing data from 5,845 probands with different eye conditions. Multistep bioinformatics and genotype-phenotype analysis classified eight potential pathogenic variants (PPVs). Overlapping related phenotypes were observed in different eyes of the same patients or in different individuals within and among families, including uveal coloboma, inferior chorioretinal hypoplasia (ICH) or optic disc hypoplasia (ODH), and high myopia. This study not only confirmed the association of *FZD5* variants with uveal coloboma but also expanded the mutation spectrum and associated phenotypes.

## METHODS

*Probands and family members:* Probands with various eye conditions and their available family members were recruited from the Pediatric and Genetic Eye Clinic, Zhongshan Ophthalmic Center, Guangzhou, China. Written informed consent adhering to the tenets of the Declaration of Helsinki and conforming to the Guidance for Sample Collection for Human Genetic Diseases (863-Plan) of the Ministry of Public Health of China was obtained from participating individuals or their guardians. Peripheral venous blood and clinical data were collected from the participants, and genomic DNA was prepared from peripheral venous blood as previously described [17]. This study was approved by the Institutional Review Board of Zhongshan Ophthalmic Center.

*Mutation detection:* Exome sequencing was performed on genomic DNA samples from the participants, including whole exome sequencing (WES) on 5,307 probands and target exome sequencing (TES) on 538 probands. The procedures for WES and TES have been described in our previous studies [18,19]. Rare variants in *FZD5* were collected from the exome data of 5,845 probands with different eye conditions. Bioinformatic analysis was performed to evaluate the pathogenicity of *FZD5* as follows [20]: (1) variants in noncoding regions, synonymous variants without effects on the splicing site according to the Berkeley Drosophila Genome Project (BDGP), and variants in the patients with pathogenic variants in other genes were considered to be benign variants; (2) all missense variants were predicted by five computational tools, namely, Combined Annotation Dependent Depletion (CADD), Rare Exome Variant Ensemble Learner (REVEL), Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping version 2 (PolyPhen-2), and Protein Variation Effect Analyzer (PROVEAN); and (3) pathogenic evidence for each potentially pathogenic variant was defined according to the standards and guidelines of the American College of

Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) [21].

Validation of potentially pathogenic variants, as well as available segregation analysis were conducted by Sanger sequencing. Eight pairs of primers were designed to amplify the fragments covering the variant positions using primer3.0. The amplicons were sequenced using the BigDye Terminator cycle sequencing kit v3.1 on a 3500xL Dx Genetic Analyzer [22].

*Phenotypic characterization:* Routine clinical data, including visual acuity, refraction and axial length (AL), slit-lamp examination, ultrasound biomicroscopy (UBM), fundus photographs, optical coherent tomography (OCT), and ultrasonography, were obtained from probands and available family members with *FZD5* variants.

## RESULTS

*Variants detected in FZD5 from 5,845 probands:* Four polymorphisms in *FZD5* were initially excluded from further analysis, including chr2: g.208633627C>T in the 5'-untranslated region (5'-UTR), c.51A>G (p. Leu17Leu), c.647C>T (p.Pro216Leu), and c.1371C>T (p.Tyr457Tyr). In total, 63 rare variants in *FZD5*, all heterozygous, were detected in our in-house exome data from 5,845 probands with different eye conditions, including 20 missense, 20 in 3'-UTR, 13 synonymous, seven in 5'-UTR, and three frameshift variants. Synonymous variants and variants in the untranslated region were excluded for further analysis based on bioinformatics and previous evidence. For the remaining 23 variants affecting amino acid sequence, bioinformatics analysis, phenotype analysis, and segregation analysis suggested that eight of the 23 variants were PPVs (Table 1) and the other 15 were likely benign (Appendix 2). All the eight PPVs were novel and presented in nine families (Figure 1). Of the eight PPVs, four variants were identified in four probands with coloboma, including two frameshift variants (c.1428delG/p.Ser477Alafs\*130 and c.1403\_1406dupACCT/p.Tyr470Profs\*130) and two missense variants (c.388C>A/p.Arg130Ser and c.1162G>A/p.Gly388Ser; Table 1, Table 2, Figure 1, Figure 2). Three variants were identified in four probands with high myopia, including one frameshift variant (c.350\_356delCGCCGCT/p.Ser117\*) and two missense variants (c.1510A>T/p.Met504Leu and c.794G>T/p.Arg265Leu; Table 1, Table 2, Figure 1, Figure 3). The remaining missense variant (c.1232A>G/p.Tyr411Cys) was identified in a proband with posterior microphthalmia (Table 1, Table 2, Figure 1). All eight PPVs were confirmed by Sanger sequencing in the nine families and co-segregated with diseases among available family members with variable overlapping phenotypes

TABLE 1. RARE VARIANTS IN *FZD5* PREDICTED TO BE POTENTIALLY DAMAGING.

Position at chr2 (hg19)	Change	NM_003468	Effect	REVEL score	CADD score	SIFT score	Polyphen-2 score	PROVEAN pred	GnomAD Allele	ACMG classification [21]	Family ID
1	2.1E+08 c.1510A>T		p.Met504Leu	0.828	26.3	D (0.011)	PB (0.854)	D	1/143348	PP=PS4,PP2, PP3	1267, 9472
2	2.1E+08 c.1428delG		p.Ser477 Alafs*130	/	/	/	/	/	0	p=PVS1,PS4, PM2,PP1,PP4	9574
3	2.1E+08 c.1403_1406dupACCT		p.Tyr470 Profs*130	/	/	/	/	/	0	p=PVS1,PS4, PM2,PP1,PP4	5485
4	2.1E+08 c.1232A>G		p.Tyr411Cys	0.936	29.4	D (0.001)	PD (1.000)	D	1/143012	PP=PS4,PP2,PP3	18739
5	2.1E+08 c.1162G>A		p.Gly388Ser	0.972	29.9	D (0.000)	PD (1.000)	D	0	PP=PS4,PM2, PP1-PP4	12467
6	2.1E+08 c.794G>T		p.Arg265Leu	0.566	27.6	T (0.055)	PB (0.617)	D	0	PP=PS4,PM2, PP2	13706
7	2.1E+08 c.388C>A		p.Arg130Ser	0.594	24.3	T (0.275)	PB (0.835)	D	0	PP=PS4,PM2, PP4	17413
8	2.1E+08 c.350_356delCGCCGCT		p.Ser117*	/	/	/	/	/	0	p=PVS1,PS4, PM2	940

Note: In gnomAD, 5% of variants had REVEL or CADD scores greater than 0.856 or 29.1, while 75% had scores less than 0.613 or 25.1, respectively. None of the nine variants was present in HGMD, p = pathogenic, PP = possibly pathogenic, D = damaging, PD = probably damaging, PB = possibly damaging, T = tolerated. According to the ACMG criteria: PVS1 = predicted null variant in a gene where LOF is a known mechanism of disease; PS4 = prevalence in affected statistically increased over controls; PM2 = absent in population database, PP1 = cosegregation with diseases in multiple affected patients, PP2 = missense in gene with low rate of benign missense variants and pathogenic missense common; PP3 = multiple lines of computational evidence support a deleterious effect on the gene/gene product; PP4 = patient's phenotype or FH highly specific for gene.

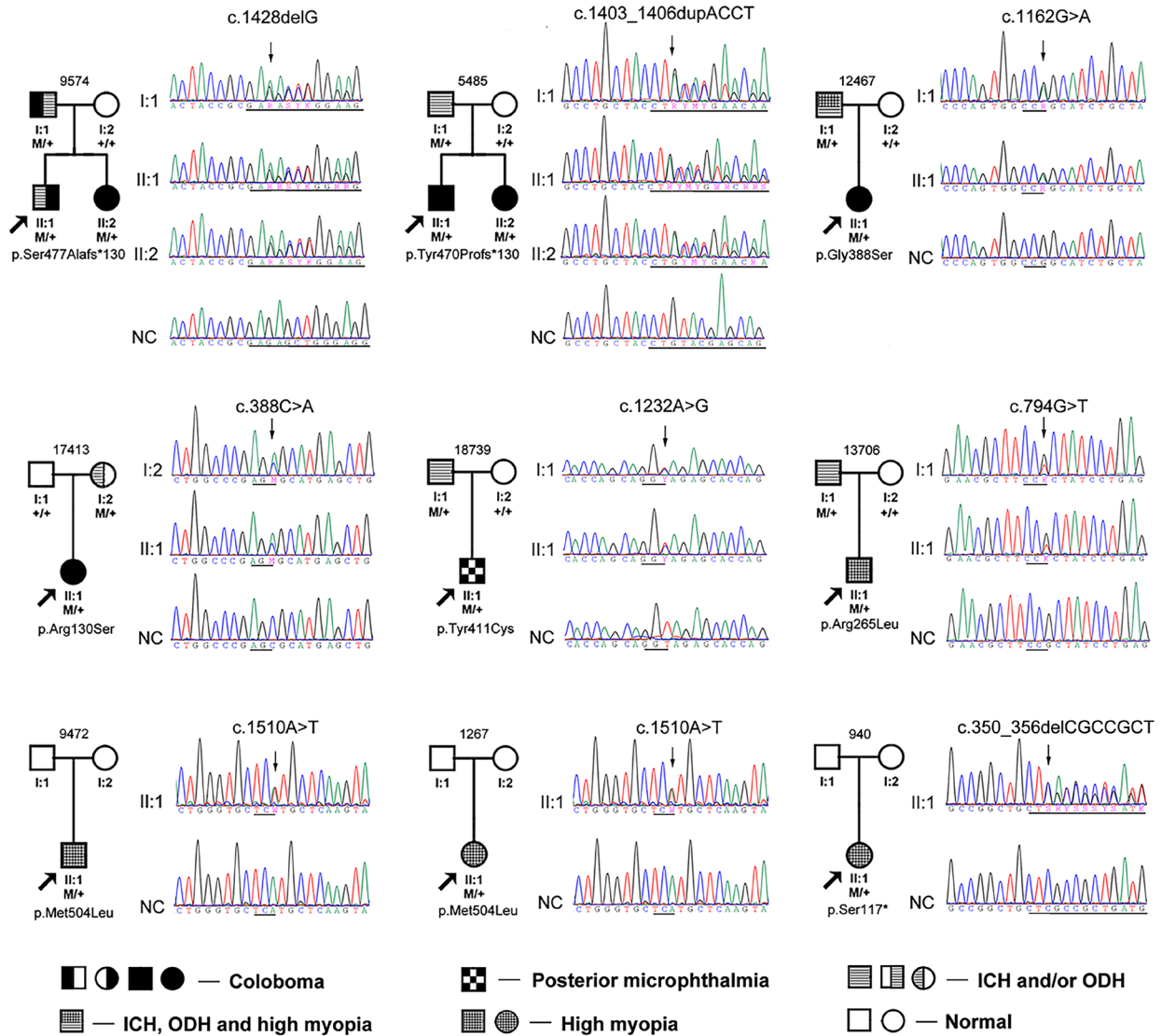


Figure 1. The pedigrees of nine families with different phenotypes and sequence chromatography of eight heterozygous variants identified in *FZD5*. In the pedigrees, M means mutation site, + means normal allele. The filled squares (male) and circles (female) represent affected individuals. The filled patterns were divided into five types: solid black pattern (ocular coloboma), striped pattern (ICH or ODH), checked pattern (high myopia), black and white checked pattern (posterior microphthalmia), and mixed pattern (high myopia, ICH and ODH). ICH, inferior chorioretinal hypoplasia; ODH, optic disc hypoplasia. The black arrows indicate probands.

and rarely incomplete penetrance (Figure 1). Furthermore, all eight variants were located in the two domains of *FZD5*, namely, the extracellular cysteine-rich Wnt-binding domain and the seven transmembrane Frizzled domains (NCBI database; Figure 4A), and all five missense variants involved residues at conserved positions among eight species (Figure 4B).

*Phenotypic expressivity: Coloboma, posterior microphthalmos, inferior chorioretinal hypoplasia, and high myopia:* In the nine families, PPVs of *FZD5* were detected in 17

individuals with variable overlapping phenotypes. The clinical features of 17 individuals from nine families with *FZD5* variants are summarized in Table 2. The phenotypes of these affected individuals were variable and included coloboma, ICH, ODH, high myopia, and posterior microphthalmos. The overlapping associated phenotypes were present in different individuals within and among families, as well as in different eyes of the same individual.

Typical uveal coloboma was present in 12 eyes of seven patients from four families (Table 2, Figure 1). Variable initial

**TABLE 2. CLINICAL INFORMATION OF THE PROBANDS AND AFFECTED SIBLINGS WITH FZD5 MUTATIONS IDENTIFIED IN THIS STUDY (ACCESSION NUMBER NM\_003468.3).**

Family ID	Nucleotide change	Effect	Gender	Age (year) at exam		Initial symptom	Visual acuity		Iris coloboma	Refraction (axial length)	Fundus		Clinic group
				exam	at		OD;OS	OD;OS			OD;OS	OD;OS	
9574-I:1	c.1428delG	p.Ser477 Alafs*130	M	32	No	No	LP;1.2	No;No	NA	NA	CC;ODH	Coloboma	
9574-II:1	c.1428delG	p.Ser477 Alafs*130	M	1	Strabismus	Strabismus	NA	No;Yes	NA	NA	ICH;CC	Coloboma	
9574-II:2	c.1428delG	p.Ser477 Alafs*130	F	3	photophobia	photophobia	NA	Yes;Yes	-1.25;+0.25	-1.25;+0.25	CC;CC	Coloboma	
5485-I:1	c.1403_1406dup ACCT	p.Tyr470 Profs*130	M	52	No	No	1.5;1.5	No;No	(23.23;23.40)	(23.23;23.40)	ODH,ICH; ODH,ICH	Coloboma-variant	
5485-II:1	c.1403_1406dup ACCT	p.Tyr470 Profs*130	M	26	NYS	NYS	0.08;0.05	Yes;Yes	-13.25;-8.50	-13.25;-8.50	CC;CC	coloboma	
5485-II:2	c.1403_1406dup ACCT	p.Tyr470 Profs*130	F	11	NYS	NYS	NA	Yes;Yes	-2.00;NA	-2.00;NA	CC;CC	coloboma	
12467-I:1	c.1162G>A	p.Gly388Ser	M	23	Myopia	Myopia	1.0;1.0	No;No	(26.23;26.28)	(26.23;26.28)	ODH,ICH; ODH,ICH	Coloboma-variant, HM	
12467-II:1	c.1162G>A	p.Gly388Ser	F	0.8	No, screening	No, screening	NA	No;No	NA	NA	CC;CC	coloboma	
17413-I:2	c.388C>A	p.Arg130Ser	F	28	No	No	1.2;1.2	No;No	NA	NA	ODH;N	Coloboma-variant	
17413-II:1	c.388C>A	p.Arg130Ser	F	2.5	NYS	NYS	NA	Yes;Yes	-3.75;-4.50	-3.75;-4.50	CC;CC	coloboma	
18739-I:1	c.1232A>G	p.Tyr411Cys	M	36	myopia	myopia	1.0;1.2	No;No	(25.60;25.20)	(25.60;25.20)	ODH, ICH;ICH	Coloboma-variant	
18739-II:1	c.1232A>G	p.Tyr411Cys	M	4.8	Poor vision	Poor vision	0.07;0.05	No;No	+6.75;+7.00	+6.75;+7.00	N;ODH	Coloboma-variant, PM	
13706-I:1	c.794G>T	p.Arg265Leu	M	32	No	No	1.0;0.9	No;No	-3.00;-1.75	-3.00;-1.75	ODH,ICH; ODH,ICH	Coloboma-variant	
13706-II:1	c.794G>T	p.Arg265Leu	M	5	NYS	NYS	0.05;0.15	No;No	-7.00;-6.25	-7.00;-6.25	TF,FH;TF,FH	HM	
9472-II:1	c.1510A>T	p.Met504Leu	M	48	Poor vision	Poor vision	0.1;FC	No;No	HM;HM	HM;HM	NA	HM#	
1267-II:1	c.1510A>T	p.Met504Leu	F	19	Myopia	Myopia	1.0;1.0	No;No	-8.50;-7.75	-8.50;-7.75	NA	HM	
940-I:1	c.350_356 delCGCCGCT	p.Ser117*	F	20	Myopia	Myopia	1.2;1.0	No;No	-7.50;-6.50	-7.50;-6.50	NA	HM	

Notes: M = male; F = female; NA = not available; YS = nystagmus; CC = coloboma; HM = high myopia; LP = light perception; FC = finger counting; ODH = optic disc hypoplasia; TF = tessellated fundus; ICH = inferior chorioretinal hypoplasia (inferior tessellated fundus); FH = foveal hypoplasia; PM = posterior microphthalmia; n = normal. # Both eyes had high myopia since childhood and had primary open-angle glaucoma and complicated cataracts at the time of sample collection at age 48 years.

symptoms were recorded in the seven patients, including nystagmus in three cases, photophobia in one patient, strabismus in one patient, and no symptoms in two patients, and the visual acuity of the seven patients ranged from light reception to 1.2 Snellen equivalent (Table 2). Of the seven patients, five showed bilateral coloboma (family 9574-II:2 in Figure 2C; family 5485-II:1 and II:2 in Figure 2D, E; family 12467-II:1 in Figure 2F and Figure 3H; family 17413-II:1 in Figure 2G), while the other two patients had uveal coloboma in one eye and ICH or ODH in the contralateral eye (family 9574-I:1

and II:1 in Table 2). Posterior staphyloma was observed under ultrasonography in three eyes from two patients with typical uveal coloboma (5485-II:2 and 17413-II:1) in Appendix 1. One patient (9574-I:1) showed uveal coloboma in the right eye (Figure 2A) and ODH in the left eye, while another patient (9574-II:1) showed uveal coloboma in the left eye (Figure 2B) and ICH in the right eye (Figure 3G). Moreover, clinical heterogeneity in different members with the PPV in the same family were also present in the four families with uveal coloboma. Bilateral uveal and iris coloboma were present in

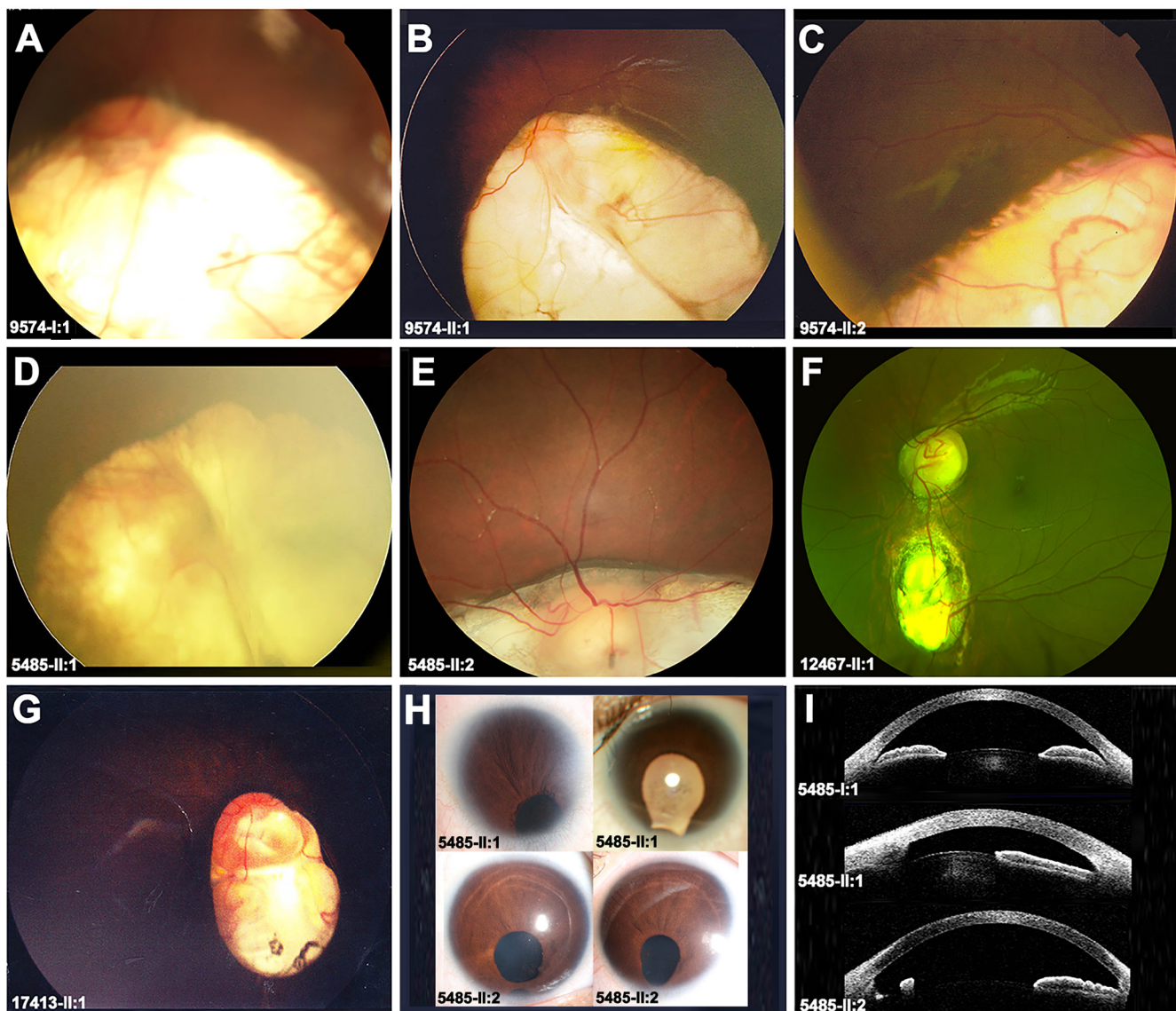


Figure 2. The coloboma changes of ophthalmic examination results in patients with *FZD5* variants. **A-G**: The fundus images demonstrated uveal coloboma in seven individuals (9574-I:1, 9574-II:1, 9574-II:2, 5485-I:1, 5485-II:2, 12467-II:1, 17413-II:1). **H**: The proband (5485-II:1) had microcornea with iris coloboma in both eyes and his sister (5485-II:2) had inferior iris coloboma in both eyes. **I**: The UBM result from family 5485 illustrated iris coloboma in the right eyes of 5485-II:1 and 5485-II:2, while the UBM graph of the right eye in the father from family 5485-I:1 was normal.

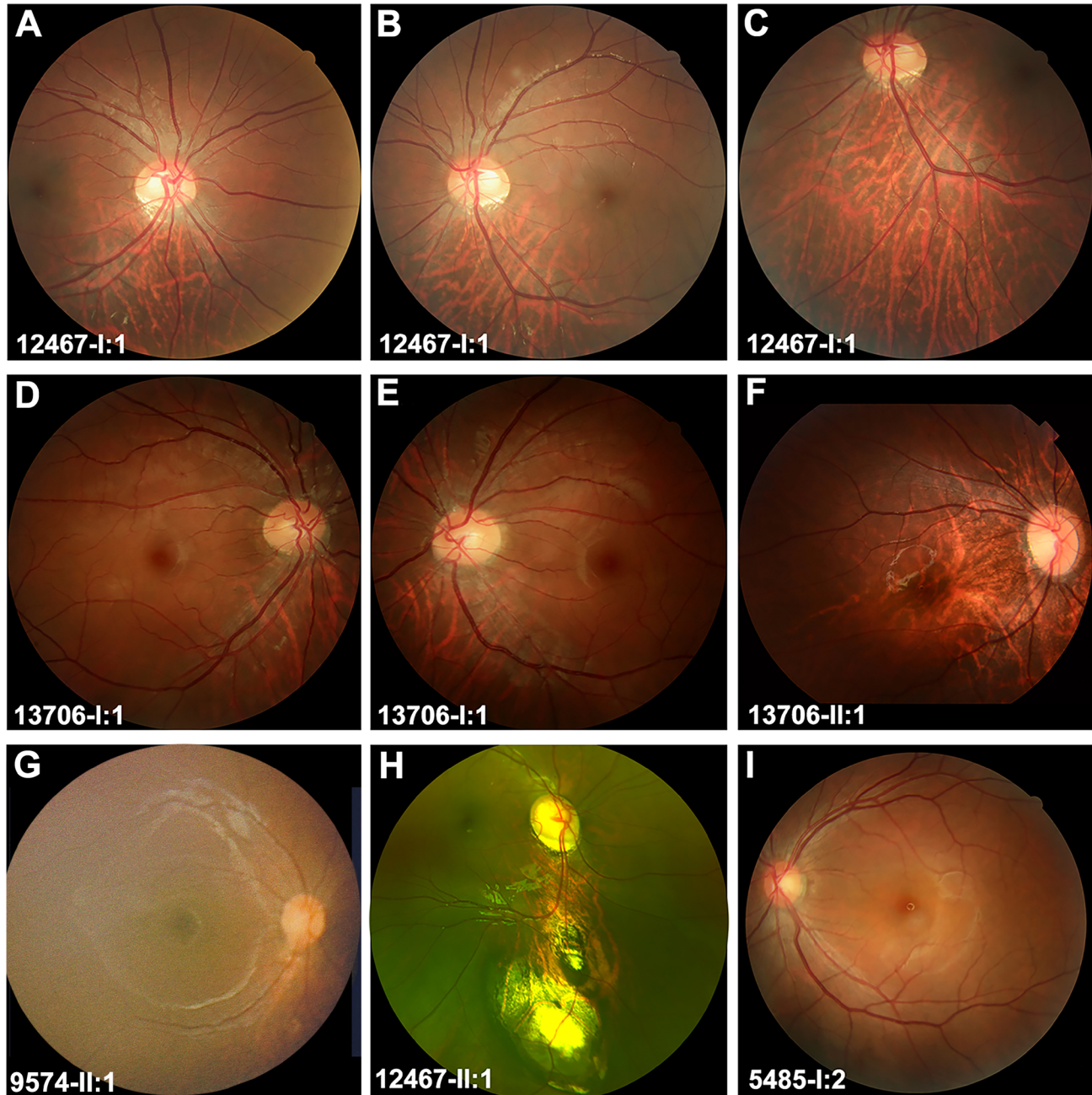


Figure 3. Representative fundus photographs from patients with *FZD5* variants. **A-E**: Inferior chorioretinal and optic disc hypoplasia presented in both eyes from two patients (12467-I:1 and 13706-I:1). **F**: The fundus photograph showed typical features of myopic fundus: tessellated retina and partial foveal atrophy in one patient (13706-II:1). **G**: Inferior chorioretinal hypoplasia was observed in the lower left area of the fundus in the right eye from patient 9574-II:1. **H**: Uveal coloboma and tessellated fundus, located between the coloboma and optic disc, were observed in patient 1267-II:1. **I**: A normal fundus photo in individual 5485-I:2.

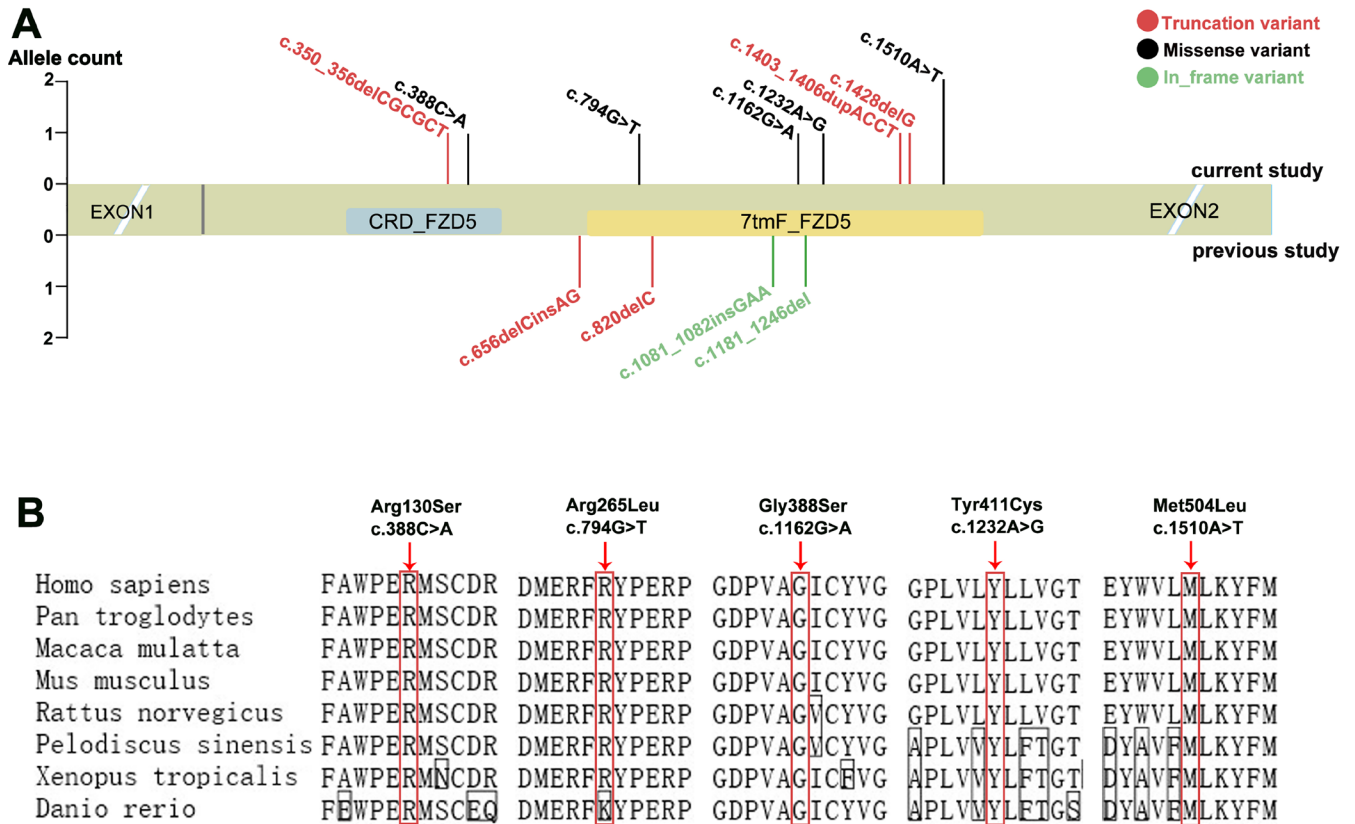


Figure 4. Distribution and conservation analysis of *FZD5* variants. **A**: The allele count and distribution of all *FZD5* variants in the mRNA sequence in the current study and previous studies (Ref. [NM\\_003468.3](#)). The light green rectangle represents the mRNA sequence of *FZD5*. The potential pathogenic variants in the current study are shown above the structure of the mRNA sequence, while variants in other previous studies are shown below the structure of the mRNA sequence. The red, black, and green variants represent truncation variants, missense variants, and in-frame variants, respectively. The blue area represents the extracellular cysteine-rich Wnt-binding domain, the yellow area represents seven transmembrane Frizzled domains, and the two blank areas before and after the sequence represent the 5'UTR and 3'UTR, respectively. The blank area between two slashes indicates the partial sequences of the 5'UTR and 3'UTR. **B**: The conservation analysis of five missense variants identified in our study. Sequence alignment of the sequences of *Homo sapiens* (humans) and seven other species, including *Pan troglodytes* (chimpanzee), *Macaca mulatta* (monkey), *Mus musculus* (house mouse), *Rattus norvegicus* (rat), *Pelodiscus sinensis* (Chinese soft-shelled turtle), *Xenopus tropicalis* (Western clawed frog), and *Danio rerio* (zebrafish). The five missense variants were located in the conserved region of the *FZD5* protein among eight species.

siblings (5485-II:1 and 5485-II:2; Figure 2D,E,H,I). However, the father (5485-I:1) with the same frameshift mutation had bilateral ODH and ICH. The same situation was also present in the other two families, 12467 and 17413, where both of the probands (12467-II:1 and 17413-II:1) from the two families had typical bilateral uveal coloboma. However, the father (12467-I:1) with the PPV in family 12467 showed bilateral high myopia, ODH, and ICH (Figure 3A-C), while the mother (17413-I:2) with the PPV in family 17413 showed unilateral ODH in the right eye but a normal-like fundus in the left eye. Therefore, these heterogeneous features in family members with PPVs, such as ODH and ICH, were likely to be variant phenotypes associated with uveal coloboma, which might be

considered a mild phenotype of uveal coloboma, as suggested before [23]. In addition to the seven eyes of five people from the four families mentioned above, ICH or ODH was also observed in five eyes of three other family members with PPVs in two other families (Figure 3D,E): the proband in family 18739 had posterior microphthalmia while the proband in family 13706 had high myopia (Figure 3F). Moreover, ICH was also observed in a patient with typical uveal coloboma (Figure 3H). In these families, ODH and ICH (inferior tessellated fundus) might be considered coloboma-variant.

In addition to four families with typical uveal coloboma and one family with posterior microphthalmia, PPVs in *FZD5*



were also identified in four additional families, where all probands had bilateral high myopia (families 13706, 9472, 1267, and 940; Table 2, Figure 1, Figure 3F). One PPV carrier from one family (13706-I:1) had ICH and ODH. In addition, high myopia in both eyes was also present in a PPV carrier family member (12467-I:1) where the proband had bilateral uveal coloboma.

Among the 34 eyes from 17 individuals with pathogenic variants of *FZD5* in our cohort, typical coloboma, coloboma-variant, and high myopia were present in 12, 12, and 10 eyes, respectively. Moreover, typical coloboma in eight eyes was the most common among 14 eyes of seven cases with frameshift variants, whereas coloboma-variant and high myopia were present in four and two eyes, respectively. For the 20 eyes of 10 individuals with missense variants, coloboma-variant (eight eyes) and high myopia (eight eyes) were more common than typical coloboma (four eyes).

## DISCUSSION

In this study, eight PPVs (three frameshift variants and five missense variants) were detected in probands from nine families. In total, these variants were present in 17 individuals from the nine families. Closely related but different phenotypes were observed in 17 individuals, and these phenotypes overlapped in different eyes of the same individual, as well as in different individuals within the same families or among different families, including uveal coloboma in 12 eyes of seven patients from four families; ICH or ODH in 12 eyes of eight patients from six families; high myopia in five patients, including one who also had ICH and ODH; and posterior microphthalmos in both eyes of one individual from one family. The following lines of evidence strongly suggest that variants in *FZD5* contribute to these phenotypes, including extremely rare PPVs in *FZD5* highly enriched in specific families, association with closely related phenotypes in 17 individuals with *FZD5* variants, cosegregation of the variants with the phenotypes in the nine families, and overlapping phenotypes within individual families.

Loss-of-function variants are highly rare in *FZD5* (pLoF = 0.98) based on the gnomAD database. In addition, missense variants predicted to be damaging by multiple online tools are also highly rare. The frameshift variants and PPVs described in current studies are exclusively present in the nine families described here. Such variants with damaging effects were not detected in families with other eye conditions. Based on the phenotype analysis, segregation analysis, and ACMG criteria (PVS1, PS4, PM2, PP1, and PP4) [21], two novel frameshift variants in *FZD5*, c.1428delG and c.1403\_1406dupACCT, were considered pathogenic variants

in two cases with uveal coloboma. Two frameshift variants in *FZD5* have been reported to cause coloboma in two families before [15,16]. The truncated *FZD5* protein, which lacks the seven transmembrane Frizzled domains, affects the activity of the Wnt signaling pathway, which was confirmed by functional analysis [15]. The frameshift variants in *FZD5* that were identified in the current study may have similar effects. Through bioinformatic analysis, genotype-phenotype analysis, and conservation analysis, five missense variants, including two missense variants c.1162G>A and c.388C>A in two cases with coloboma, two missense variants c.1510A>T and c.794G>T in three cases of high myopia, and one missense variant c.1232A>G in the case of posterior microphthalmia, were considered likely pathogenic variants in *FZD5* based on the ACMG criteria, although missense variants in *FZD5* have not been reported to cause any hereditary disease before. Interestingly, an apparent pathogenic variant, c.350\_356delCGCCGCT, was identified in a proband with bilateral high myopia, which not only supports the variable phenotypes observed in different family members but also indicates that high myopia is a closely related phenotype of *FZD5* variants.

The following points provide strong evidence to support the association of *FZD5* variants with the phenotype described above: 1) novel variants with significant damaging effects are highly rare in existing databases; 2) such PPVs are exclusively present in families with related phenotypes; 3) overlapping phenotypes associated with *FZD5* variants, including coloboma, ICH or ODH, and high myopia, are observed between different eyes of the same person, among different members within the same family, or among different members in different families; and 4) cosegregation of related phenotypes occurs in most families. Such variable phenotypes associated with *FZD5* variants have not been reported before, although incomplete penetrance was present in the largest family with the first coloboma-associated variant [15]. However, overlapping phenotypes between coloboma and high myopia or between coloboma and ICH have been described before in other genes. With an autosomal dominant inheritance of ocular coloboma due to *SOX2* and *PAX6*, family members with same variant exhibited myopia or ICH [23,24]. Furthermore, in cases with syndromic ocular coloboma due to variants in *SALL4* or *TFAP2A*, individuals carrying the same variant may showed coloboma in one eye and ODH in the other or myopia and ODH [25-27].

In conclusion, eight novel variants were confirmed in our study in nine families with various phenotypes, including coloboma, ICH, ODH, and high myopia. Our results provide additional evidence confirming that *FZD5* variants, including

frameshift and missense variants, can be the potential pathogenic cause of coloboma. In addition, overlapping phenotypes, including coloboma, ICH or ODH, and high myopia, exclusively occurred in individuals with *FZD5* variants. Therefore, *FZD5* variants may also lead to a coloboma-related phenotype, such as ICH, ODH, or high myopia.

#### APPENDIX 1. SUPPLEMENTARY FIGURE 1.

To access the data, click or select the words “[Appendix 1.](#)” The ultrasonography results from patients with potential pathogenic variants in *FZD5*. Ultrasonography revealed posterior staphyloma in both eyes from one patient (5485-II:2; **A-B**) and posterior staphyloma in the right eye from another patient (17413-II:1; **C-D**)

#### APPENDIX 2. LIKLEY BENIGN VARIANTS IN *FZD5* DETECTED IN OUR STUDY.

To access the data, click or select the words “[Appendix 2.](#)” Note: In gnomAD, 5% variants had REVEL or CADD scores greater than 0.856 or 29.1, while 75% had such scores less than 0.613 or 25.1. All the 15 variants were not present in HGMD, Abbreviations: B=benign; T=tolerated; N=neutral; PB=possibly damaging; D=damaging.

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