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.
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 targeted 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 amplificons 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.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_356delCGCGCCT/p.Ser117*) and two missense variants (c.1510A>T/p.Pro504Leu and c.794G>T/p.Arg265Leu; Table 1, Table 2, Figure 1, Figure 2). Three variants were identified in four probands with high myopia, including one frameshift variant (c.350_356delCGCGCCT/p.Ser117*) and two missense variants (c.1510A>T/p.Pro504Leu and c.794G>T/p.Arg265Leu; Table 1, Table 2, Figure 1, Figure 2). Three variants were identified in four probands with high myopia, including one frameshift variant (c.350_356delCGCGCCT/p.Ser117*) and two missense variants (c.1510A>T/p.Pro504Leu and c.794G>T/p.Arg265Leu; Table 1, Table 2, Figure 1, Figure 2). 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.

| Variants | Position at chr2 (hg19) | Change | NM_003468 | NM_003468.3 | REVEL | CADD | SIFT | Polyphen-2 | PROVEAN | GnomAD | ACMG classification [21] | Family |
|----------|-------------------------|--------|------------|-------------|--------|------|------|------------|---------|--------|----------------|---------|
| 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.Ser477Alafs*130 | / | / | / | / | / | 0 | p=PVS1,PS4, PM2,PP1,PP4 | 9574 |
| 3        | 2.1E+08                  | c.1403_1406dupACCT | p.Tyr470Profs*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.
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 |
|-----------|-------------------|--------|--------|--------------------|----------------|--------------|--------------|--------------------------|--------|--------------|
| 9574-I:1  | c.1428delG        | p.Ser477Alafs*130 | M      | 32                 | No              | LP;1.2       | No;No        | NA                       | CC;ODH | Coloboma     |
| 9574-I:1  | c.1428delG        | p.Ser477Alafs*130 | M      | 1                  | Strabismus      | NA           | No;Yes        | NA                       | ICH;CC | Coloboma     |
| 9574-I:2  | c.1428delG        | p.Ser477Alafs*130 | F      | 3                  | photophobia     | NA           | Yes;Yes       | −1.25;+0.25              | CC;CC   | Coloboma     |
| 5485-I:1  | c.1403_1406dupACCT | p.Tyr470Profs*130 | M      | 52                 | No              | 1.5;1.5      | No;No         | (23.23;23.40)            | ODH,ICH;ODH,ICH | Coloboma-variant |
| 5485-II:1 | c.1403_1406dupACCT | p.Tyr470Profs*130 | M      | 26                 | NYS             | 0.08;0.05    | Yes;Yes       | −13.25;−8.50             | CC;CC   | coloboma     |
| 5485-II:2 | c.1403_1406dupACCT | p.Tyr470Profs*130 | F      | 11                 | NYS             | NA           | Yes;Yes       | −2.00;NA                 | CC;CC   | coloboma     |
| 12467-I:1 | c.1162G>A         | p.Gly388Ser     | M      | 23                 | Myopia          | 1.0;1.0      | No;No         | (26.23;26.28)            | ODH,ICH;ODH,ICH | Coloboma-variant, HM |
| 12467-II:1| c.1162G>A         | p.Gly388Ser     | F      | 0.8                | No, screening   | NA           | No;No         | NA                       | CC;CC   | coloboma     |
| 17413-I:2 | c.388C>A          | p.Arg130Ser     | F      | 28                 | No              | 1.2;1.2      | No;No         | NA                       | ODH;N   | Coloboma-variant |
| 17413-II:1| c.388C>A          | p.Arg130Ser     | F      | 2.5                | NYS             | NA           | Yes;Yes       | −3.75;−4.50              | CC;CC   | coloboma     |
| 18739-I:1 | c.1232A>G         | p.Tyr411Cys     | M      | 36                 | myopia          | 1.0;1.2      | No;No         | (25.60;25.20)            | ODH,ICH;ODH,ICH | Coloboma-variant |
| 18739-II:1| c.1232A>G         | p.Tyr411Cys     | M      | 4.8                | Poor vision     | 0.07;0.05    | No;No         | +6.75;+7.00              | N;ODH   | Coloboma-variant, PM |
| 13706-I:1 | c.794G>T          | p.Arg265L.Leu   | M      | 32                 | No              | 1.0;0.9      | No;No         | −3.00;−1.75              | ODH,ICH;ODH,ICH | Coloboma-variant |
| 13706-II:1| c.794G>T          | p.Arg265L.Leu   | M      | 5                  | NYS             | 0.05;0.15    | No;No         | −7.00;−6.25              | TF,FH;TF,FH | HM           |
| 9472-II:1 | c.1510A>T         | p.Met504L.eu    | M      | 48                 | Poor vision     | 0.1;FC       | No;No         | HM;HM                    | NA      | HM           |
| 1267-II:1 | c.1510A>T         | p.Met504L.eu    | F      | 19                 | Myopia          | 1.0;1.0      | No;No         | −8.50;−7.75              | NA      | NA           |
| 940-I:1   | c.350_356delCGCCGCT| p.Ser117*       | F      | 20                 | Myopia          | 1.2;1.0      | No;No         | −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.
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 (13706:1) had ICH and ODH. In addition, high myopia in both eyes was also present in a PPV carrier family member (12467:1) 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. LIKELY 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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