• Basic Research •

De novo variant in *GUCY2D* gene causing atypical cone-rod dystrophy in a consanguineous family and literature review

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Abstract

● **AIM:** To analyze the pathogenicity and clinical features of patients in a consanguineous cone-rod dystrophy (CRD) family due to heterozygous variants in the *GUCY2D* gene.

● **METHODS:** Whole exome sequencing was used to screen for pathogenic genes and candidate pathogenic variants were obtained by bioinformatics analysis. Sanger sequencing was used for validation and familial co-segregation analysis to determine pathogenic variants. Pymol software was applied to produce a 3D structure image of the protein to analyze the structural and functional alterations of the protein. The pathogenicity of genetic variants was evaluated according to ACMG guidelines.

● **RESULTS:** The chief clinical symptoms of this proband included obvious visual impairment, protanopia and deuteranopia, peripheral punctate pigment, arteriolar attenuation, structural and functional abnormalities revealed by optical coherence tomography (OCT) and electroretinography (ERG) including thinning of the outer retinal layer, a discontinuous external limiting membrane (ELM) and ellipsoid zone (EZ), granular hyperreflective projections between the retinal pigment epithelium and the interdigitation zone, severe attenuation of photopic responses with mild reduced scotopic responses. Whole-exome sequencing revealed that the proband carried a heterozygous variant of the *GUCY2D* gene: c.2512C>T: p.Arg838Cys. Three-dimensional molecular structure analysis of the protein revealed that amino acid 838 was mutated from polar positively charged arginine to polar uncharged cysteine, and the spatial structure of the protein changed greatly. Sanger sequencing co-segregation analysis confirmed that such a variant was detected in neither the phenotypically normal parents nor the daughter of the proband, which was presumed to be a *de novo* one. The variant was determined to be pathogenic according to ACMG guidelines. The heterozygous variant at the same site was detected in the abnormal proband's son with moderate attenuation of photopic electroretinographic responses and normal scotopic electroretinographic responses, supporting autosomal dominant inheritance.

● **CONCLUSION:** The *de novo* variant causing atypical autosomal dominant CRD is identified in a Chinese consanguineous family and this variant passes through this family in an autosomal dominant mode of inheritance, revealing the complex diversity and unpredictability of the inheritance mode for common single-gene genetic disease.

● **KEYWORDS:** cone-rod dystrophy; *GUCY2D* gene; genetic variants; autosomal dominant

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INTRODUCTION

Cone-rod dystrophy (CRD, OMIM#120970) is a group of highly genetical and clinically heterogeneous retinal

disorders that is a common cause of visual impairment in children and adults worldwide[1], with a prevalence of 1/100 000 to 1/20 000[2]. As against retinitis pigmentosa (RP), CRD is characterized by the loss of cone photoreceptors first, followed by rod involvement[3]. RP begins with degeneration of the peripheral retinal rod cells, and as the disease progresses, there is loss of cone photoreceptors[4]. However, in advanced stages, it is difficult to differentiate between the two based on a fundus examination alone. In patients with CRD, the loss of cone photoreceptors leads to early vision loss, varying degrees of color vision abnormalities and photophobia. The central scotoma may also be observed in visual field testing, after which loss of rod photoreceptors leads to nyctalopia and progressive loss of peripheral visual field[5]. CRD usually occurs in childhood or early adulthood, and funduscopic examination often reveals focal macular retinal pigment epithelial atrophy or bulls-eye maculopathy, as well as temporal optic disc pallor at an early age, whereas peripheral retinal pigment epithelial atrophy, pigmentation, and arteries attenuation may be present in advanced stage[6]. As examined by full-field electroretinography (ERG), CRD is characterized by a reduced response of both cone and rod cells; however, cone cells are more severely affected than rod specific ERG components[7]. Typical RP is mainly manifested as early reduction of night vision and loss of peripheral visual field, whereas CRD presents with impaired central vision in the early stage of the disease; therefore, CRD patients are more severely affected than RP patients, and the impact on the patient's ability to lead an autonomous life is much greater than that of RP patients.

A total of 37 genes associated with CRD have been identified to date (RetNet:https://sph.uth.edu/Retnet/sum-dis.htm), of which the most common pathogenic genes are *ABCA4*, *CRX*, *GUCY2D*, and *RPGR*[8], and their modes of inheritance are mainly autosomal dominant, autosomal recessive, and sex-linked. Mutations in the *GUCY2D* gene account for more than 40% of dominantly inherited CRD cases[9]. The *GUCY2D* gene is localized in the chromosome 17p13.1 region, which is 16 Kb long and consists of 20 exons. It is expressed mainly in photoreceptor cells, and the encoded protein is a photoreceptor specific outer segment guanylate cyclase responsible for the conversion of GTP into cGMP[10]. Mutations in the *GUCY2D* gene prevent the cGMP level in photoreceptor cells from recovering after stimulation, leaving the photoreceptor cells chronically polarized. This results in autosomal dominant or recessive CRD type 6 (OMIM#601777), autosomal dominant central reticular choroidal dystrophy type 1 (OMIM#215500), or autosomal recessive Leber congenital amaurosis (LCA) type 1 (OMIM#204000) or autosomal recessive congenital stationary night blindness type 1I (OMIM#618555)[11]. Over 140 pathogenic variants have been identified so far in *GUCY2D*, 88% of which cause autosomal recessive LCA while heterozygous missense variants cause autosomal dominant cone-rod dystrophy (adCRD)[12].

Due to high heritability and clinical heterogeneity, CRD can exhibit different clinical manifestations and signs at different stages of the disease, and there is phenotypic overlap with other types of inherited retinal diseases. Therefore, early and accurate diagnosis remain a very difficult challenge for ophthalmologists. Genetic screening combined with clinical phenotyping can improve the efficiency and accuracy of clinical diagnosis and genetic counseling of CRD patients. Here, we report a consanguineous family with atypical CRD caused by a *de novo* heterozygous missense variant c.2512C>T: p.Arg838Cys in the *GUCY2D* gene and this variant passed through this family in an autosomal dominant mode of inheritance.

PARTICIPANTS AND METHODS

Ethical Approval The study project was approved by the Ethics Committee of the Gansu Aier Ophthalmology and Optometry Hospital (Approval No.GSAIER2023IRB03) and in strict compliance with the Declaration of Helsinki. Guardians of children were informed and signed written informed consent.

Methods

Clinical data collection A CRD family was collected from Gansu Aier Ophthalmology and Optometry Hospital. Family history, obsterical history, and systemic disease history were analyzed in detail, and a family pedigree was drawn. The patient and family members received comprehensive ophthalmic examinations including uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA), slit-lamp with a lens, color fundus photography (CFP, TRC-NW300, TOPCON, Japan), panoramic ophthalmoscope [Daytona (P200T), UK], optical coherence tomography (OCT, HD-OCT4000, Carl Zeiss Meditec, USA), pattern visual evoked potentials (PVEP), and full-field electroretinogram [ffERG, LCK TECHNOLOGIES (RETeval), USA].

The diagnosis of CRD is established by referring to the criteria as specified in Xun-Lun Sheng's "Foundation and Clinic of Hereditary Eye Diseases"[13]: 1) an early loss of visual acuity and color discrimination from adolescence or in early adult life, followed by nyctalopia and night blindness; 2) visual field: the central scotoma appears early, and then progressive peripheral field loss; 3) fundus: the retina may appear normal or only temporal pallor of the optic disc in the early stage, and varying degrees of pigmentary disturbance or atrophy may be evident in later stages. OCT can detect early structural abnormalities including thinning of the outer retinal layer

Table 1 DNA sequencing parameters for the proband and family members

Item	Numerical value
Raw sequencing data size (Mb)	9888.97
Total number of reads sequenced (entries)	65926442
Average sequencing depth of the target region (×)	127.40
Average sequencing depth of the target region ≥1× coverage (%)	99.25
Average sequencing depth of the target region ≥10× coverage (%)	99.02
Average sequencing depth of the target region ≥30× coverage (%)	98.5
20% × average coverage depth (%)	98.68
Average sequencing depth of the mitochondrial genome (%)	3270.21
Q30 yield (%)	94.37

and a discourteous ellipsoid zone (EZ); 4) ERG: early loss of photopic (cone) responses and later progressive loss of scotopic (rod) responses.

Genome-wide exome sequencing Genomic DNA is extracted from the subject's sample to construct a genomic library. Target gene exons and adjacent splice sites (about 20 bp), as well as the full length of the mitochondrial genome, were captured by probe hybridization and enriched. The enriched genes were subjected to quality control and sequenced using a high-throughput sequencer. Table 1 was the target capture sequencing parameters from the subject's sample.

Interpretation of Variant Pathogenicity The rules for the interpretation of sequence variant data refer to the standards and guidelines for the classification of genetic variation issued by the American College of Medical Genetics and Genomics (ACMG), as well as a series of general recommendations and rules successively issued by the ClinGen Sequence Variant Interpretation (SVI) Expert Panel. It is recommended in the Guidelines that sequence variants are categorized into five levels based on the types of genomic sequence variants, database information, *etc*., namely pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. Each type of evidence was analyzed individually subject to the principle of cumulative effect of evidence for the pathogenicity of sequence variants, and finally the individual criteria were combined according to the scoring rules in the Guidelines to select a classification from the 5-level system.

Literature Review A search was conducted on PubMed using "cone-rod dystrophy, CRD", "*GUCY2D* gene", "Arg838", "mutation" as the terms to search the relevant literature to analyze the relationship between *GUCY2D* gene Arg838 and clinical phenotypes of autosomal dominant CRD as shown in Table 2.

RESULTS

Clinical Characterisation Proband (II-1) from a consanguineous family, 38-year-old female, presented with poor visual acuity in the left eye since childhood. BCVA: 0.3 [-5.25 diopter sphere (DS)/-0.75 diopter cylinder (DC)×10°] in the right eye, 0.05 (-8.00 DS/ -0.50 DC×135°) in the left eye. Chromoptometry suggested protanopia and deuteranopia, and no obvious abnormality was found in both anterior segments. In the right eye, the optic disc was light red in color, around which peripapillary atrophy was visible, the blood vessels were slender, no pigmentation was not found in the peripheral retina, and the light reflex disappeared in the fovea centralis. In the left eye, the optic disc was light red in color, around which patchy peripapillary atrophy was visible (more obvious than that in the right eye), the retina showed the presence of leopard spot changes, punctate pigmentation was visible in the peripheral retina, the blood vessels were slender and tortuous, and the light reflex disappeared in the fovea centralis (Figure 1A, II-1). OCT revealed abnormalities including thinning of the outer retinal layer, a discontinuous external limiting membrane (ELM) and EZ, granular hyperreflective projections between the retinal pigment epithelium and the interdigitation zone, and the thickness of the choroid became thinner (Figure 1C, II-1). Fundus autofluorescence showed the patchy hyperfluorescent focus visible in the macular region of right eye, in which the punctate hypofluorescence was seen. The hypofluorescence of about 1 papillary diameter (PD) in size was observed in the macular region of left eye, with the hyperfluorescence surrounding the center and periphery (Figure 1B, II-1). ERG examination showed that there was a severely decreased amplitude of photopic 3.0 ERG a- and b-waves in both eyes; photopic 3.0 flicker decreased moderately in both eyes; and a mildly decreased amplitude of scotopic 3.0 a-wave in the left eye (Table 3).

The proband's son (III-2), 11-year-old male, BCVA: 0.9+ (-4.50 DS/-1.75 DC×180°) in the right eye, 0.9+ (-2.75 DS/ -2.00 DC ×170°) in the left eye. The color vision was normal in both eyes, and no significant abnormalities were found in the examination of both anterior segments and fundus (Figure 1A–1C, III-2). ERG examination showed that there was a moderately decreased amplitude of photopic 3.0 ERG b-wave in the right eye and severely decreased amplitude of a-wave in the left eye; photopic 3.0 flicker decreased moderately in both eyes; and scotopic 0.01 ERG and scotopic 3.0 ERG were

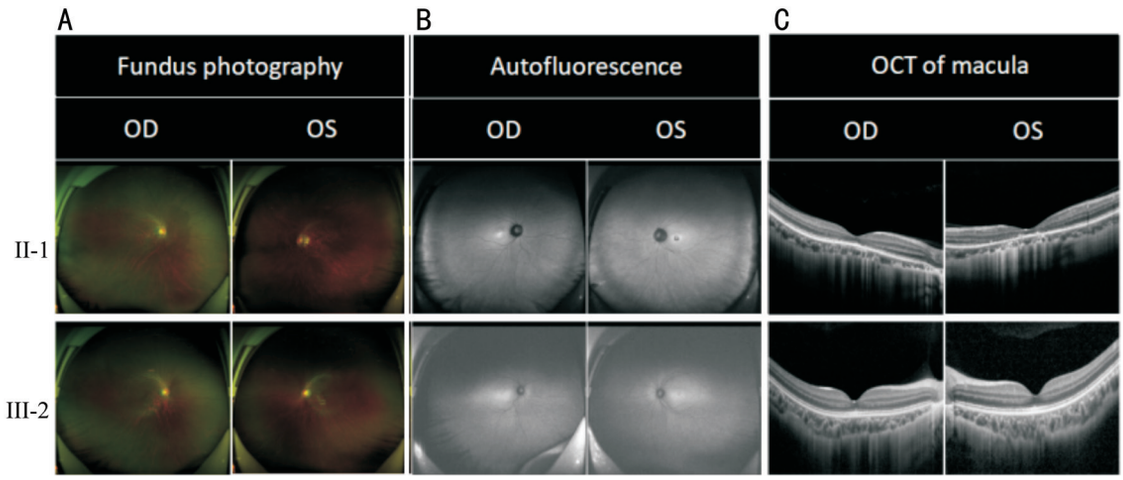

Figure 1 Ophthalmologic examination of proband (II-1) and son (III-2) A: Fundus photography: II-1 showing leopard spot changes, arteriolar attenuation, peripapillary atrophy, punctate pigmentation. III-2 was normal; B: Autofluorescence: II-1 demonstrating patchy hyperfluorescent in the macular region with the punctate hypofluorescence in right eye and central hypofluorescence surrounding with the hyperfluorescence (1/10 PD in size) in left eye; C: Optical coherence tomography: II-1 indicates normal structure in the right eye, and thinning of the outer retinal layer, a discontinuous external limiting membrane and ellipsoid zone, granular hyperreflective projections between the retinal pigment epithelium and the interdigitation zone in left eye. III-2 having normal structure in both eyes. OD: Right eye; OS: Left eye.

Table 2 Pathogenic variation and associated clinical phenotype of the *GUCY2D* gene at Arg838

Nucleotide change	Protein change	Mutation type	Clinical phenotype				Literature
			Photophobia	Color vision deficiency	Fundus	Electroretinography	
c.2512C>T	p.Arg838Cys	Missense	+	+	Macular atrophy, spotty pigmentation observed in the peripheral retina	Severe cone dysfunction in both eyes, and a mild decrease in rod function in the left eye	This study
c.2512C>A	p.Arg838Ser	Missense	+	+	Central macular atrophy	Loss of cone function	Payne[14]
c.2513G>C	p.Arg838His	Missense					
c.2513G>C	p.Arg838Pro	Missense	+	-	Macular degeneration, salt-and-pepper fundus appearance at the posterior pole	Loss of cone function	Garcia-Hoyos[15]
c.2512C >T	p.Arg838Cys	Missense	+	-	Bulls-eye maculopathy early in the disease, with later peripheral retinal involvement	Loss of cone function, with progressive abnormality of rod responses later	Kelsell[16]

normal in both eyes (Table 3). Eye examinations of other subjects in this family showed no abnormalities other than refractive errors.

Genetic Testing and Pathogenicity Analysis Genetic variant analysis was performed on the whole exome and adjacent splice region of the proband, and it was found that the proband carried a heterozygous variant on the *GUCY2D* gene: C.2512C>T: p.Arg838Cys, which is a substitution of base 2512 of the cDNA from C to T, resulting in a change of the codon at position 838 from encoding arginine to encoding cysteine. First-generation sequencing validation showed that the above variants were true and reliable (Figure 2A, 2B). Such variant was not detected in both parents of the proband and was presumed to be the *de novo* one or the presence of germline mosaicism in one of the parents accordingly. Such variant was not detected in the husband and daughter of the proband while the heterozygous variant was detected in the son. This variant has been reported in the literature[14-16] and is classified as pathogenic with reference to the relevant guidelines as specified by the ACMG based on the following. It has been reported that this variant altered the sensitivity of the protein to Ca^{2+} inhibition as pathogenic strong (PS3). It was *de novo* in patients as pathogenic strong (PS2). It has been reported that this variant was detected as pathogenic strong (PS4) in CRD patients. Such variant, a rare one, had a frequency of 0 in the gnomAD database as pathogenic moderate (PM2). At the same amino acid site of such variant, there was another pathogenic variant p.Arg838His, which was pathogenic moderate (PM5). It was predicted by six bioinformatics prediction software that the variant would have harmful effects on the genes or gene products as pathogenic supporting (PP3; Table 4). The clinical phenotype of the variant carrier was highly consistent with the disease caused by the abnormal *GUCY2D* gene as pathogenic supporting (PP4). This variant site was highly conserved across species (Figure 2C). The Swiss model was applied to construct the wild-type and mutant-type models

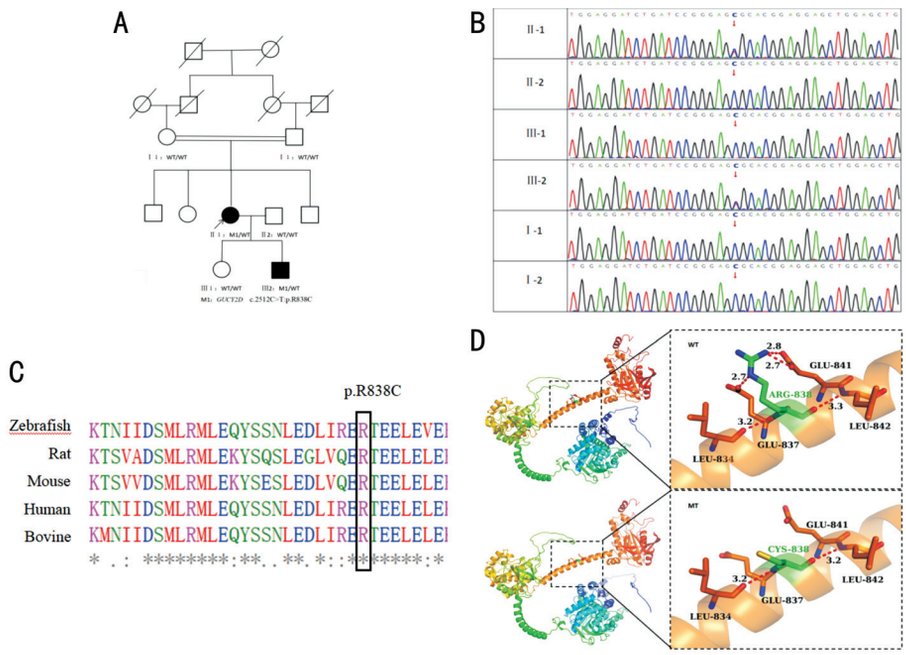

Figure 2 Genetic testing and pathogenicity analysis of the proband A: Pedigree of the family: The *GUCY2D* gene variant c.2512C>T: p.Arg838Cys is co-segregated between patient and normal phenotypic members in the family; B: Sanger sequencing diagram: Such a variant was not detected in both parents of the proband and was presumed to be the de novo one or the presence of germline mosaicism in one of the parents accordingly. Such a variant was detected in the proband's son (III-2); C: Conservation analysis: The *GUCY2D* gene variant c.2512C>T was highly conserved across species; D: Wild-type and mutant-type structure modeling of *GUCY2D* p.Arg838Cys. Analysis of 3D image of the protein structure revealed that amino acid No.838 changed from polar positively charged arginine mutated to polar uncharged cysteine, resulting in significant changes in the spatial structure of proteins.

Table 3 Full-field electroretinogram findings in both eyes of the proband (II-1) and son (III-2)

Electroretinogram	II-1		III-2	
	Right eye	Left eye	Right eye	Left eye
Scotopic 0.01 (μV)	b-wave 266.7	b-wave 287.8	b-wave 279.5	b-wave 290.7
Scotopic 3.0 (μV)	a-wave 232.4	a-wave 224.3	a-wave 295.0	a-wave 309.2
	b-wave 542.2	b-wave 556.8	b-wave 518.7	b-wave 508.3
Photopic 3.0 (μV)	a-wave 18.4	a-wave 5.3	a-wave 31.4	a-wave 3.0
	b-wave 36.6	b-wave 58.4	b-wave 85.7	b-wave 148.3
Photopic 3.0 flicker (μV)	N1-P1 47.4	N1-P1 61.3	N1-P1 43.5	N1-P1 55.7

of *GUCY2D* p.Arg838Cys, and analysis of 3D images of the protein structure made by Pymol revealed that the wild-type protein at position 838 was polar positively charged arginine. The mutation resulted in the substitution of polar positively charged arginine at position 838 for small and polar uncharged cysteine (Figure 2D). Therefore, the variant was classified as pathogenic (PS3+PS2+PS4+PM2+PM5+PP3+PP4) as assessed by the standards and guidelines for the interpretation of sequence variants. Copy number variation (CNV) analysis of the whole exome and adjacent splice regions of the subject did not reveal pathogenic/likely pathogenic CNVs that were highly correlated with the clinical phenotype of the subject.

Literature Review As reported by previous, exon 13 of most autosomal dominant CRDs associated with *GUCY2D* carries a pathogenic missense allele, which mainly affects codon 838. Table 2 showed comparison between Arg838-CRD pathogenic variant and related clinical phenotypes and the phenotypes in this study.

Table 4 The effects of *GUCY2D* c.2512C>T variant on its protein function predicted by different analyzing software

Software	Score	Predicted signal
Polyphen2_HDIV	D	Damaging
SIFT	D	Deleterious
VEST4	0.944	Deleterious
Mutation Taster	D	Disease causing
DANN_score	0.999	Deleterious
MVP_score	0.967	Deleterious

DISCUSSION

In this study, we analyzed the clinical phenotypes and genotypes of two patients with CRD in a Chinese consanguineous family caused by *GUCY2D* gene mutations.

CRD can be divided into two stages in the progression of the disease[13]. The main symptom in the first stage is vision loss with no significant improvement in corrected visual acuity, which usually occurs in childhood (under 10 years old). Patients often have paracentral fixation, which projects light onto the paracentral fovea area where retinal damage is less severe. There are severe photophobia and varying degrees of dischromatopsy. Funduscopic examination shows macular pigmentation, varying degrees of retinal atrophy, and normal or mildly arteriolar attenuation. Optic discs are pale in color, especially in the area of nerve fiber distribution in the papillomacular bundle. Perimetry indicates the central scotoma and normal peripheral visual field. The ERG shows a decrease in both cone and rod responses, and the decrease in cone is more pronounced than that in rod. In the second stage, nyctalopia occurs and the peripheral visual field deficit worsens, so the patients have nocturnal autonomous movement disorder (difficulty) and continued loss of central vision, often accompanied by nystagmus. In this study, the proband whose parents were consanguineous had poor visual acuity in the left eye since childhood. BCVA was 0.3 in the right eye and 0.05 in the left eye, and both eyes were protanopia and deuteranopia, and atrophic lesions were visible in the macular area of the fundus. ERG showed a decrease in both cone and rod responses, and the decrease in cone is more pronounced than that in rod. The proband had no nyctalopia for the time being and still retained part of the peripheral retinal function, and thus was in the first stage of CRD. The proband's son had normal color vision in both eyes, BCVA of 1.0, clear optic disc boundary, light red color, and no obvious abnormalities in the macula and retina. There was a moderate or severe decrease in photopic 3.0 ERG and photopic 3.0 flicker in both eyes, but the scotopic adaptation was in a normal state, suggesting a diagnosis of early stage CRD subject to moderate decrease in cone function and normal rod function in both eyes. Many young patients diagnosed with cone dystrophy (COD) are actually in the early stages of CRD. Clinically, COD is generally diagnosed in children and young patients without careful analysis of the complete clinical phenotype of family members, and the middle-aged and elderly are prone to be diagnosed with RP.

GUCY2D gene encodes retinal guanylate cyclase 1 (RetGC1), an enzyme predominantly expressed in the photoreceptor outer segments of the cones[17]. RetGC1 plays a key role in phototransduction by restoring cytoplasmic cGMP levels, allowing the inward flow of intracellular Ca^{2+} and restoring the cellular basal state[18]. *GUCY2D* variants can lead to two major retinal disorders, CRD and LCA. Most patients with autosomal dominant CRD associated with *GUCY2D* develop the disease before puberty, and exon 13 carries the pathogenic missense allele, which predominantly affects codon 838[19-20]. This dominant variant reduces Ca^{2+} levels and decreases its sensitivity, thereby forcing the Na^{+}/Ca^{2+} channel to open and affecting the function of obtaining proteins[21]. The missense variants c.2512C>A (p.Arg838Ser) and c.2513G>A (p.Arg838His) reported by Payne *et al*[14], the missense variant c.2513G>C (p.Arg838Pro) reported by Garcia-Hoyos *et al*[15], and the missense variant c.2512C>T (p.Arg838Cys) reported by Kelsell *et al*[16] are located at codon 838, which changes codon 838 from encoding arginine to encoding other kinds of amino acids, resulting in *GUCY2D*-associated autosomal dominant CRD. Rodilla *et al*[22], in 2023, conducted a retrospective study on the genotypes and clinical phenotypes of *GUCY2D*-associated retinopathies: CRD reached 66.7% in 47 patients with retinal dystrophy. A total of 23 *GUCY2D* variants were identified, and biallelic variants accounted for 28%. The majority of patients carry dominant alleles associated with CRD. The most common variant was c.2513G>A (p.Arg838His), which accounted for 30% of all *GUCY2D* families. Therefore, it can be said that the pathogenic changes of Arg838 residue are the main cause of *GUCY2D*-associated retinopathy. However, a multicellular retrospective study by Neubauer *et al*[23] reported that p.Arg838Cys was the first variant of *GUCY2D*-associated autosomal dominant CRD, while p.Arg838His was only the second variant. *GUCY2D* variants can also lead to LCA, which are characterized by earlier and more severe visual impairment, mostly showing nystagmus, photophobia, and hyperopia. Patients with *GUCY2D*-associated LCA have relatively stable photoreceptor number and function, with only a slight reduction or even no progression in the foveal outer nuclear layer and EZ over an average of 5y of follow-up[24-25]. CRD, on the other hand, is primarily characterized by atrophic maculopathy, which exhibits progressive impairment of photoreceptor function. In this study, a heterozygous variant of *GUCY2D* gene was found in this family: c.2512C>T: p.Arg838Cys, which affects codon 838. Although the variant site has been reported, the changes in the spatial structure of the mutant protein have not been further studied. We constructed wild-type and mutant-type models of p.Arg838Cys and analyzed the 3D structure images of the protein (Figure 2D). It was found that the wild-type protein at position 838 was polar and positively charged arginine, and the backbone N atom formed a hydrogen bond with the uncharged and non-polar leucine O atom at position 834 at a hydrogen bonding distance of 3.2 Å. The backbone O atom formed a hydrogen bond with the non-polar and uncharged leucine backbone N atom at position 842 at a hydrogen bonding distance of 3.3 Å. The guanidine group of the side chain formed hydrogen bonds with the polar negatively charged glutamate side chains at positions 837

and 841 at distances of 2.7, 2.7, and 2.8 Å, respectively. The mutation of p.Arg838Cys resulted in the substitution of the polar positively charged arginine at position 838 by the small and polar-uncharged cysteine, the backbone N atom formed a hydrogen bond with the uncharged and non-polar leucine O atom at position 834 at a hydrogen bonding distance of 3.2 Å. The backbone O atom formed a hydrogen bond with the non-polar and uncharged leucine backbone N atom at position 842 at a hydrogen bonding distance of 3.2 Å. The side chain hydrogen bonding interactions were lost, and these changes in amino acid interactions resulted in the change of structure and function of mutant protein.

Consanguineous marriage increases the inbreeding coefficient, and the offspring have a large amount of genetic material from the same ancestor, which makes the offspring more likely to suffer from recessive genetic disorders[26]. Thus, in genetic studies, consanguineous families and patients with this particular genomic structure in their family are a high-quality resource for studying the pathogenic genes of recessively inherited diseases. In this study, the parents of the proband were consanguineous, but no abnormal variants were passed on to the proband, and the variants carried by the proband were *de novo* heterozygous ones. It should be noted that when a *de novo* variant is considered as pathogenic strong, the patient's family history needs to be consistent with the *de novo* variant characteristics. For example, the parents of a patient with a dominant genetic disorder neither had the disease nor carried the variant, but two children with the same clinical phenotype were born in succession and carried the pathogenic variant of the same gene, which did not conform to the inheritance mode of autosomal dominant and *de novo* variant, and it was thus presumed that the source of genetic variant in the family might be due to the parents being carriers of germline mosaicism[27]. However, the presence of germline mosaicism is not evident in one-child families, and such a variant is usually considered a *de novo* one, but the possibility of germline mosaicism should be taken into account in genetic counseling. In this study, the proband's parents were not detected with this variant and had a normal clinical phenotype, while her brothers and sister did not have the same clinical phenotype, which was consistent with the *de novo* mutation. The proband's son was detected with a heterozygous variant at the same site. ERG suggested a moderate decrease in cone function in both eyes, which was consistent with early CRD clinical features. Therefore, the variant carried by the proband was a *de novo* heterozygous one, and the family started a new genetic process in an autosomal dominant manner from the proband. Genetic screening for common recessive single-gene genetic diseases may not provide guidance for the parents of patients with *de novo* gene variants before pregnancy[28]. Therefore, it is recommended that they have genetic screening in an early pregnancy or seek assistance from third-generation assisted reproductive techniques to minimize affected children's birth.

As for inherited retinal disorders (IRDs), the same genetic mutation can have different phenotypes, and intrafamilial clinical variability is likewise common, which may be related to genetic modification of common pathogenic alleles or the coexistence of pathogenic mutations segregated from more than 1 gene in the same pedigree. It may also be related to different phenotypes caused by different alleles in the same gene[29]. In IRDs, intrafamilial expression is associated with allelic differences and modifications. In this study, both the proband and the son carried the pathogenic mutation at the same site, and the son's pathogenic alleles were inherited mainly from the affected mother with *GUCY2D*-related CRD phenotype, but their clinical phenotypes were significantly different. The proband had early central vision loss, dischromatopsy, and macular choroidal atrophy. The ERG showed significant cone-rod dysfunction, and the son had no other abnormal signs except cone dysfunction. On one hand, it is considered that the proband's son is still young and some of the CRD phenotypes have not yet manifested or are in the insidious stage of the disease. On the other hand, it is considered to be related to genetic modifications and environmental factors between individuals, which produce different degrees of penetrance, just as some phenotypic differences among individuals with the same genetic background can be explained by epigenetics[30]. This also illustrates the complexity of the phenotypes associated with *GUCY2D* and highlights the need for comprehensive molecular testing and deeper phenotypic analysis when inconsistent retinal manifestations occur in the same family, and later on, the follow-up observations of the proband and family members will be intensified.

In conclusion, the major genetic effect of consanguineous marriage is to increase the incidence of autosomal recessive disorders in the population. In this study, a *de novo* variant c.2512C>T: p.Arg838Cys in *GUCY2D* gene was identified in the proband of a Chinese consanguineous family and this variant was found to transmit in the family in an autosomal dominant manner (starting a new genetic process in an autosomal dominant manner), but with reduced penetrance and variable expressivity, revealing the complex diversity and unpredictability of the inheritance mode for common single-gene genetic disease. The results of the study enriched the spectrum pathogenic genetic variants and phenotypic spectrum of *GUCY2D*-CRD.

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