Clinical and Genetic Analysis of *RDH12*-Associated Retinopathy in 27 Chinese Families: A Hypomorphic Allele Leads to Cone-Rod Dystrophy

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Purpose. The purpose of this study was to elucidate the genetic basis of 2 distinct phenotypes associated with biallelic variants in *RDH12*.

Methods. Patients with biallelic variants in *RDH12* were recruited from our genetic eye clinic. Ocular phenotypes were evaluated. Genotype-phenotype correlations were further clarified using in-house and existing databases.

Results. In total, 22 biallelic *RDH12* variants, including 5 novel variants, were identified in 29 patients from 27 families. Two distinct phenotypes were observed: early-onset and generalized retinal dystrophy with severe impairment of rods and cones in 24 patients (82.8%, 24/29), and late-onset cone-rod dystrophy (CORD) with central macular atrophy in 5 patients from 5 unrelated families (17.2%, 5/29), in which a hypomorphic allele (c.806C>G/p.Ala269Gly) was shared by all 5 patients. During follow-up, patients with late-onset CORD were relatively stable and did not progress to the severe form, which was considered to be an independent manifestation of *RDH12*-associated retinopathy caused by specific genotypes.

Conclusions. The hypomorphic allele is responsible for the unique late-onset CORD in 5 families with recessive *RDH12*-associated retinopathy, in contrast to the well-known severe and generalized retinopathy. Determining the therapeutic value of interventions may depend on understanding the molecular mechanisms underlying manifestation of this hypomorphic variant only in the central macular region, with relative preservation of the peripheral retina.

Keywords: *RDH12*, Ala269Gly, hypomorphic, cone-rod dystrophy (CORD), retinopathy

The *RDH12* gene (OMIM: 608830), located on chromosome 14q24.1, is expressed in the inner segments of photoreceptors. Retinol dehydrogenase 12, a 316-amino-acid protein encoded by *RDH12*, is a critical nicotinamide adenine dinucleotide phosphate (NADPH)-dependent retinal reductase in the visual cycle[1,2] and plays a role in the detoxification of lipid peroxidation products.[3] Owing to its small size and function as an enzyme in the visual cycle, *RDH12* has the potential to be harnessed in gene therapy. In this regard, gene therapy for another gene encoding another enzyme in visual cycle, *RPE65*, has been approved by the US Food and Drug Administration.[4]

Variability in *RDH12*-associated recessive phenotypes has been reported, ranging from severe forms, such as Leber congenital amaurosis (LCA) and early-onset severe retinal dystrophy (EOSRD) to relatively restricted lesions, including cone-rod dystrophy (CORD) and macular dystrophy (MD). The classical phenotype of recessive *RDH12*-associated retinopathy typically manifests as early-onset visual impairment and widespread retinal degeneration, with macular-specific involvement.[5–12] Milder presentations are observed in a small proportion of patients. However, the underlying genotype-phenotype correlation for variable phenotypes and mechanisms underscoring milder phenotypes remain unknown. Furthermore, heterozygous truncation variants in *RDH12* have been reported to cause autosomal dominant retinitis pigmentosa (adRP) in a small subset of patients.[13–15] Investigating the natural history and underlying genotype-phenotype correlations of *RDH12* may facilitate identification of therapeutic windows of opportunity.

In this study, 29 patients from 27 unrelated families with biallelic *RDH12* variants were selected from our in-house dataset of various eye conditions, which included 3 reported families, for comprehensive analysis.[16–18] The clinical data and potential genotype-phenotype correlations of these patients were systematically evaluated. In particular, 5 unrelated patients harbored the same hypomorphic allele, c.806C>G/p.(Ala269Gly), presented with a unique form of *RDH12*-associated CORD. The observation that a hypomorphic variant contributed to a relatively mild

phenotype provides underlying clues regarding the genotype-phenotype correlation of *RDH12* and sheds light on the clinical interpretation of *RDH12*-associated retinopathy.

Methods

Subjects

This retrospective case series study was approved by the institutional review board of Zhongshan Ophthalmic Center. All probands with various genetic eye conditions and their available family members were enrolled in our ongoing study at the Genetic Eye Clinic, Zhongshan Ophthalmic Center, Guangzhou, China. After obtaining signed informed consent conforming to the tenets of the Declaration of Helsinki from participants or their guardians, clinical data and peripheral venous blood of participants were collected. Genomic DNA was extracted from the leukocytes of venous blood according to the method described in our previous study.[19]

Variant Detection and Identification

Variants of *RDH12* were selected from an in-house exome sequencing dataset, including targeted exome sequencing (TES) and whole-exome sequencing (WES), of patients with various eye diseases and healthy controls. The procedures for performing TES, WES, and multistep bioinformatic analysis of detected variants were the same as the method described in our previous studies.[20–22] Allele frequencies of all identified variants in *RDH12* were annotated using the Genome Aggregation Database (gnomAD; https://gnomad.broadinstitute.org/). The possible impact of missense variants was predicted using five in silico tools: Rare Exome Variant Ensemble Learner (REVEL; https://sites.google.com/site/revelgenomics/),[23] Combined Annotation Dependent Depletion (CADD; https://cadd.gs.washington.edu/),[24] Sorting Intolerant Form Tolerant (SIFT; https://sift.jcvi.org/),[25] Polymorphism Phenotyping version 2 (PolyPhen-2; https://genetics.bwh.harvard.edu/pph2/),[26] and Protein Variation Effect Analyzer (PROVEAN; https://provean.jcvi.org/).[27] The potential impact of splicing changes was predicted using the Human Splicing Finder system (HSF; https://www.genomnis.com/access-hsf).[28] All potential pathogenic variants (including heterozygous truncation variants that may be associated with *RDH12*-related adRP) were confirmed by Sanger sequencing and co-segregation analysis of available family members.

Clinical Assessment

Patients harboring potential biallelic pathogenic variants or heterozygous truncation variants and presenting with *RDH12*-associated retinopathy were further clinically assessed. The available clinical data for these patients, including sex, age at onset, age at examination, symptoms, family history, and clinical diagnosis, were comprehensively reviewed and recorded. Detailed ophthalmic examinations of the patients were conducted at baseline and each follow-up visit. These examinations included: (1) best-corrected visual acuity (BCVA); (2) direct ophthalmoscopy (by a senior ophthalmologist, author Q.Z.); (3) fundus photography and fundus autofluorescence (FAF; Canon, Japan); (4) wild-field scanning laser ophthalmoscope and wild-field fundus autofluorescence (Optos, UK); (5) optical coherence tomography (OCT; SVision Research, China, or Carl Zeiss Meditec, USA, or Heidelberg Engineering, Germany); (6) fundus fluorescein angiography (FFA; Heidelberg Engineering, Germany); and (7) full-field electroretinography (ERG; according to International Society for Clinical Electrophysiology of Vision [ISCEV] Standards).[29]

Literature Review of *RDH12* Variants

The keyword "RDH12" was searched in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) until May 1, 2022. Variants of *RDH12* in the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/ac/validate.php) were reviewed in March 2021. All publications in English related to *RDH12* were collected, and duplicates were excluded. The allele number and frequency, mutation type, and associated phenotypes of these published variants were summarized.

Structure of Proteins

The SWISS-MODEL online server (https://swissmodel.expasy.org)[30] was used to generate 3D models of wild-type (Swiss-Prot accession Q96NR8) and reported adRP-related *RDH12* mutant proteins. Three mutant proteins of three C-terminal disease-free heterozygous frameshift variants were generated as controls. Proteins were visualized using PyMOL software (https://pymol.org/2/), and the 3D structures of the wild-type and mutant proteins were compared.

Statistical Analysis

Statistical analyses were performed using the SPSS Statistics (version 25.0; IBM Corp., Armonk, NY, USA). BCVA is described in decimal units. The age of onset and age of examination are presented as median (years). Differences between 2 distinct *RDH12*-associated phenotypes were compared using the Mann-Whitney *U*-test. Statistical significance was defined as $P < 0.05$

Results

Identification of *RDH12* Variants

In our cohort, biallelic variants in *RDH12* were detected in 27 families (Fig. 1), of which 24 were newly recruited and 3 were reported.[16–18] A total of 22 variants in *RDH12* were identified, including 15 missense variants, 4 nonsense variants, and 3 frameshift variants. Of the variants, 5 were novel, namely, c.162_163dup/p.(Thr55Argfs*21), c.179C>A/p.(Ala60Asp), c.442C>A/p.(His148Asn), c.447_448del/p.(Gly150Profs*22), and c.787_788dup/p.(Gln263Hisfs*16; Fig. 2A, Table). The c.437T>A/p.(Val146Asp) variant was the most frequent variant in our cohort, with an allele frequency of 20.4% (11/54). This was followed by the c.146C>T/p.(Thr49Met), c.505C>G/p.(Arg169Gly), c.524C>T/p.(Ser175Leu), and c.806C>G/p.(Ala269Gly), with an allele frequency of 9.3% (5/54). The top 5 common variants accounted for approximately 77.8% (21/27) of the families with recessive *RDH12*-associated retinopathy. The most common genotype was biallelic missense variants, which accounted for 74.1% (20/27) of families (Fig. 2C). The other two genotypes, missense variant in trans with truncation variant and biallelic truncation variants, accounted for 22.2% (6/27) and 3.7% (1/27) of cases, respectively (see Fig. 2C).

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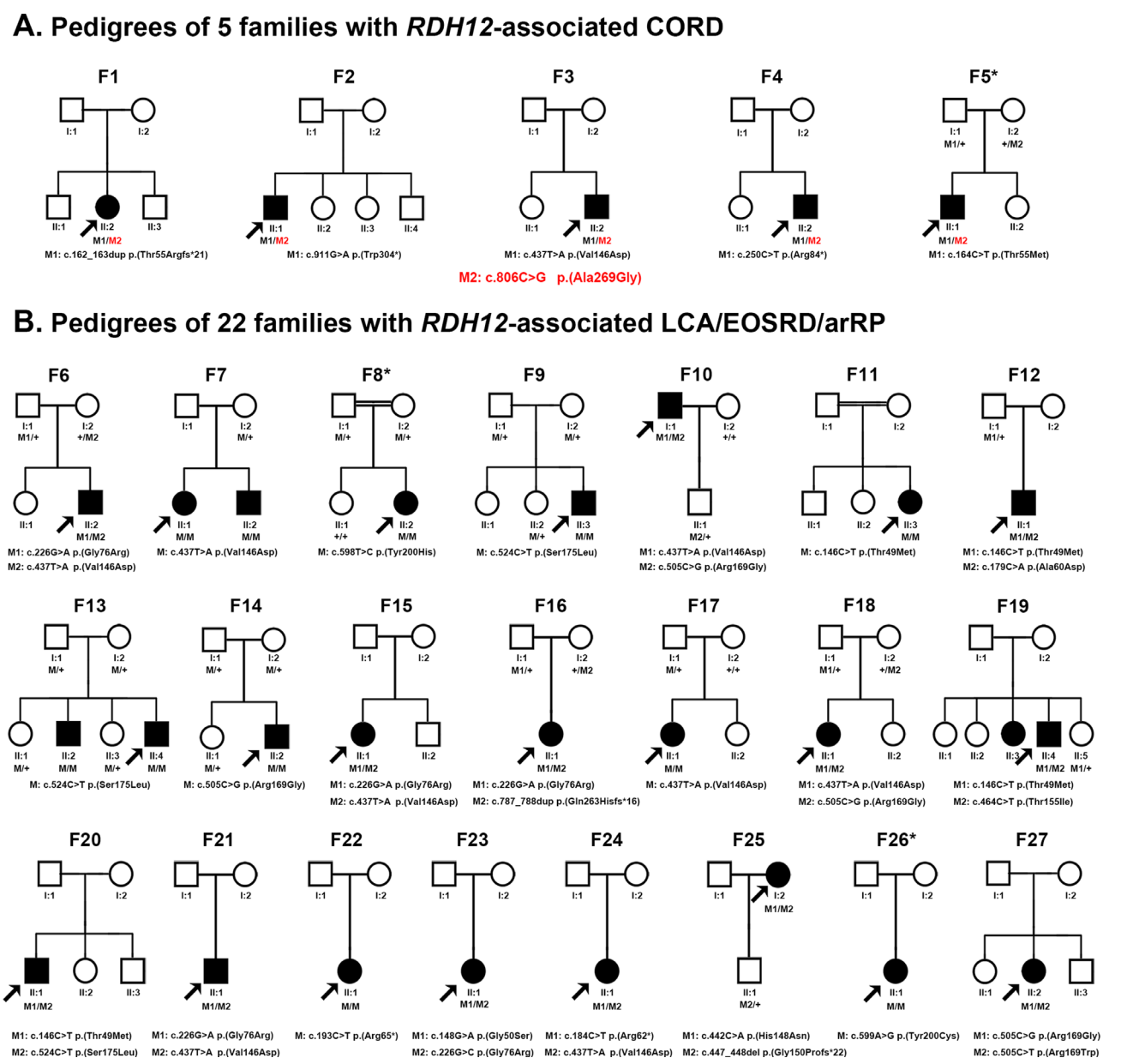

Figure 1. The pedigrees of 27 families with biallelic variants in *RDH12*. (**A**) Pedigrees of five families with *RDH12*-associated CORD. (**B**) Pedigrees of 22 families with *RDH12*-associated LCA/EOSRD/arRP. The asterisk indicates that these families have been reported in our previous studies. CORD, cone-rod dystrophy, LCA, Leber congenital amaurosis; EOSRD, early-onset severe retinal dystrophy; arRP, autosomal recessive retinitis pigmentosa.

All missense variants were predicted to be damaged based on at least three online in silico tools (see Table). The allele frequencies of these variants in the gnomAD database ranged from 0 to 71/270908 (see Table). Notably, all 5 families harboring the p.(Ala269Gly) variant exhibited mild *RDH12*-associated retinopathy (see Fig. 1A). The p.(Ala269Gly) variant was a hypomorphic variant with a high allele frequency in the gnomAD database (0.03%, 71/270908), especially in East Asia (0.4%, 71/19766). The mild form of *RDH12*-associated retinopathy was strongly associated with this hypomorphic allele, regardless of the presence of a potential pathogenic missense variant or deleterious truncation variant in the other allele in trans (Fig. 2D).

Phenotypic Spectrum of *RDH12*-Associated Retinopathy

Among the 29 patients with biallelic *RDH12* variants, 24 patients from 22 families were diagnosed with severe retinopathy, including 18 families with arRP, 3 families with LCA, and one family with EOSRD (see Fig. 2C), in which both cones and rods were severely impaired simultaneously. However, no heterozygous variants associated with adRP were identified in our cohort. Of note, five patients from five families harboring the same hypomorphic p.(Ala269Gly) variant presented with central macular lesions and were diagnosed with CORD.

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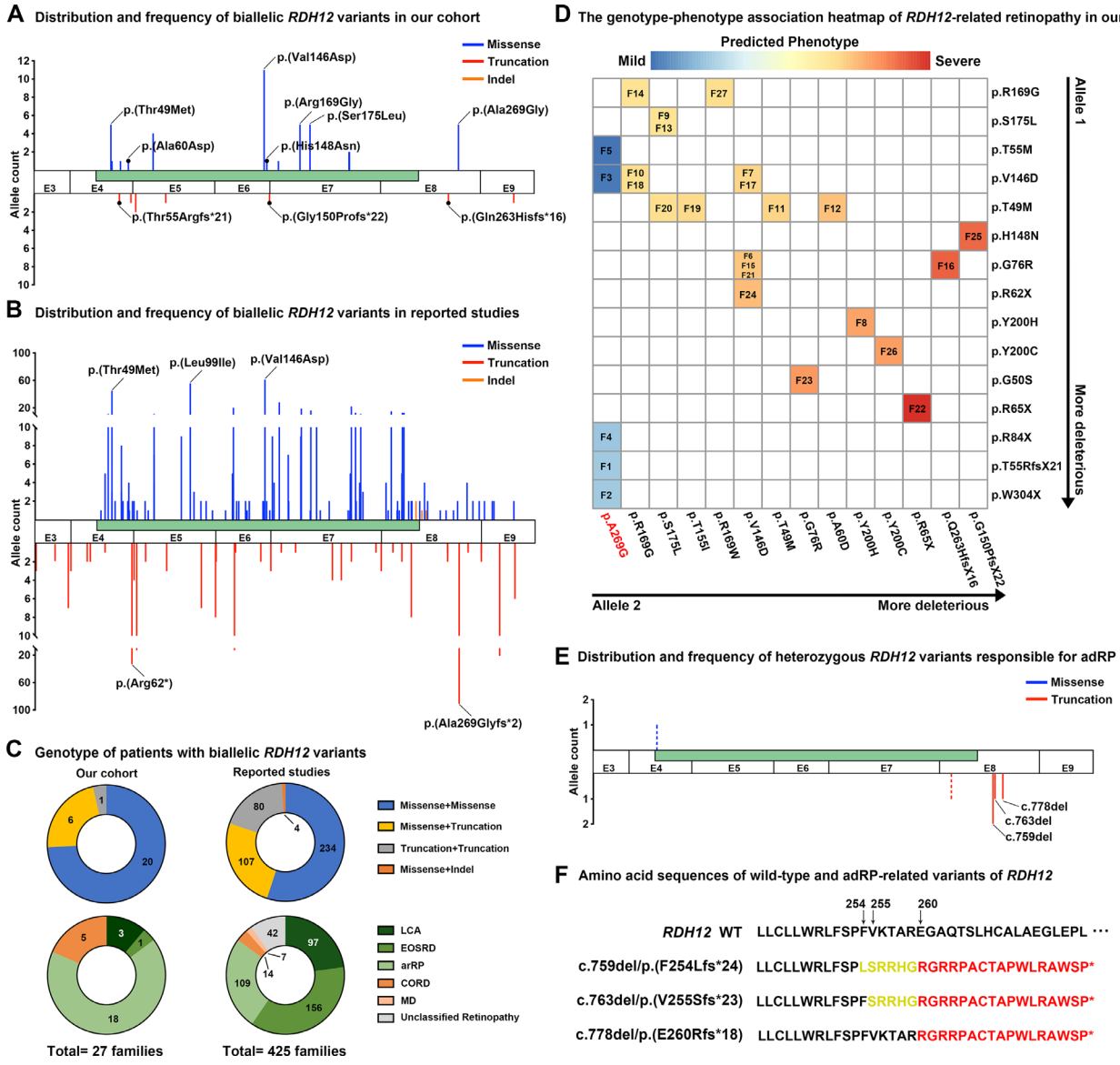

Figure 2. *RDH12* variants were identified in our cohort and reported studies. (**A**) Distribution and frequency of biallelic *RDH12* variants in our cohort. The *black solid circle* indicates that these variants were novels identified in this study. The *blue, red, and orange lines* represent missense, truncation, and indel variants, respectively. E3 to E9 indicates exons 3 to 9 in the *RDH12* coding region. (**B**) Distribution and frequency of biallelic *RDH12* variants in reported studies. The *blue, red, and orange lines* represent missense, truncation, and indel variants, respectively. E3 to E9 indicates exons 3 to 9 in the *RDH12* coding region. (**C**) Genotype and phenotype of patients with biallelic *RDH12* variants in our cohort and reported studies. EOSRD, early-onset severe retinal dystrophy; arRP, autosomal recessive retinitis pigmentosa; LCA, Leber congenital amaurosis; CORD, cone-rod dystrophy; MD, macular dystrophy. (**D**) Heatmap was based on the ordering of the deleteriousness of each allele from low to high. The color of each cell, from cool to warm, indicates that the prediction of phenotype based on the predicted pathogenicity of biallelic alleles from mild to severe. This heatmap was primarily used to demonstrate that the hypomorphic variant was associated with mild forms of *RDH12*-related retinopathy. However, for the remaining families with severe forms of *RDH12*-associated retinopathy, phenotypic severity was difficult to reconcile with the prediction heatmap. (**E**) Distribution and frequency of heterozygous *RDH12* variants in reported studies. The *blue and red lines* represent missense and truncation variants, respectively. E3 to E9 indicates exons 3 to 9 in the *RDH12* coding region. (**F**) Amino acid sequence of wild-type and adRP-related proteins of *RDH12*.

The 5 patients with *RDH12*-associated CORD had an age of onset after 10 years old (median = 23 years old), which was significantly later than that of patients with *RDH12*-associated LCA/EOSRD/arRP ($P = 1.5 \times 10^{-3}$; Supplementary Fig. S1C). One patient (F3-II:2) did not present with impaired vision until the age of 29 years. BCVA at the first examination ranged from 0.15 to 0.6, and 4 of the 5 patients had a BCVA better than 0.3 (see Supplementary Fig. S1A). The fundus of these *RDH12*-associated patients with CORD showed unique central macular atrophy. On posterior color fundus photography, the atrophy of the parafovea region seemed to be relatively more severe than the fovea, although OCT revealed marked atrophy of the outer retinal layer in both the fovea and parafovea regions, which was predominantly by the obvious decline in the outer nuclear layer thickness and loss of the external limiting membrane and ellipsoid zone (Fig. 3). FAF exhibited a hypo-autofluorescence

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Table. Variants of *RDH12* (NM_152443.2) Identified in Our Cohort

No.	Exon	Nucleotide Change	Effect	Allele No.	REVEL	CADD	SIFT	PolyPhen-2	PROVEAN	GnomAD (v2.1.1)		HGMD	First Report
										ALL	EA		
1	4	c.146C>T	p.(Thr49Met)	5	0.843	28.2	D(0)	D(0.976)	D(−5.41)	5/282820	2/19948	DM	Janecke et al., 2004
2	4	c.148G>A	p.(Gly50Ser)	1	0.968	28.5	D(0)	D(1)	D(−5.59)	/	/	DM?	Gao et al., 2019
3	4	c.162_163dup	p.(Thr55Argfs*21)	1	/	/	/	/	/	/	/	/	Novel
4	4	c.164C>T	p.(Thr55Met)	1	0.896	28.5	D(0)	D(1)	D(−5.57)	6/251482	2/18392	DM	Thompson et al., 2005
5	4	c.179C>A	p.(Ala60Asp)	1	0.907	27.8	D(0.001)	D(1)	D(−5.39)	/	/	/	Novel
6	4	c.184C>T	p.(Arg62*)	1	/	35	/	/	/	16/282744	/	DM	Janecke et al. 2004
7	5	c.193C>T	p.(Arg65*)	2	/	37	/	/	/	4/251488	/	DM	Thompson et al., 2005
8	5	c.226G>A	p.(Gly76Arg)	4	0.778	28.4	D(0.006)	D(0.993)	D(−3.33)	1/251494	/	DM	Wang et al., 2015
9	5	c.226G>C	p.(Gly76Arg)	1	0.778	27.8	D(0.006)	D(0.993)	D(−3.33)	/	/	DM	Aldahmesh et al., 2009
10	5	c.250C>T	p.(Arg84*)	1	/	39	/	/	/	1/251492	/	DM	Mackay et al., 2011
11	6	c.437T>A	p.(Val146Asp)	11	0.947	27	D(0)	D(0.994)	D(−6.16)	3/251474	3/18394	DM	Fu et al., 2013
12	6	c.442C>A	p.(His148Asn)	1	0.764	26.7	D(0.001)	D(0.974)	D(−6.76)	1/251464	/	/	Novel
13	6	c.447_448del	p.(Gly150Profs*22)	1	/	/	/	/	/	/	/	/	Novel
14	7	c.464C>T	p.(Thr155Ile)	1	0.898	27.1	D(0.007)	D(1)	D(−5.62)	6/282750	/	DM	Thompson et al., 2005
15	7	c.505C>G	p.(Arg169Gly)	5	0.922	25.5	D(0.001)	D(1)	D(−6.99)	4/251474	4/18394	DM	Wang et al., 2015
16	7	c.505C>T	p.(Arg169Trp)	1	0.921	25.9	D(0)	D(1)	D(−7.98)	2/251474	/	DM	Mackay et al., 2011
17	7	c.524C>T	p.(Ser175Leu)	5	0.968	25.5	D(0)	D(0.999)	D(−5.94)	6/282866	/	DM	Coppieters et al., 2010
18	7	c.598T>C	p.(Tyr200His)	2	0.986	29.3	D(0)	D(0.999)	D(−4.96)	/	/	DM	Xu et al., 2014
19	7	c.599A>G	p.(Tyr200Cys)	2	0.974	29.8	D(0)	D(0.999)	D(−8.9)	1/31408	/	DM	Stone et al., 2007
20	8	c.787_788dup	p.(Gln263Hisfs*16)	1	/	/	/	/	/	1/240006	1/18094	/	Novel
21	8	c.806C>G	p.(Ala269Gly)	5	0.667	28.7	D(0.002)	D(0.998)	D(−3.83)	71/270908	71/19766	DM	Huang et al., 2016
22	9	c.911G>A	p.(Trp304*)	1	/	42	/	/	/	/	/	DM	Sodi et al., 2010

Notes: D, damaging; DM, disease-causing mutations; Int, intron. REVEL, Rare Exome Variant Ensemble Learner (https://sites.google.com/site/revelgenomics/); CADD, Combined Annotation-Dependent Depletion (https://cadd.gs.washington.edu/); SIFT, Sorting Tolerant From Intolerant (http://sift.jcvi.org/); PolyPhen-2, Polymorphism Phenotyping version 2 (http://genetics.bwh.harvard.edu/pph2/); PROVEAN, Protein Variation Effect Analyzer (http://provean.jcvi.org/); HGMD, Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/ac/validate.php).

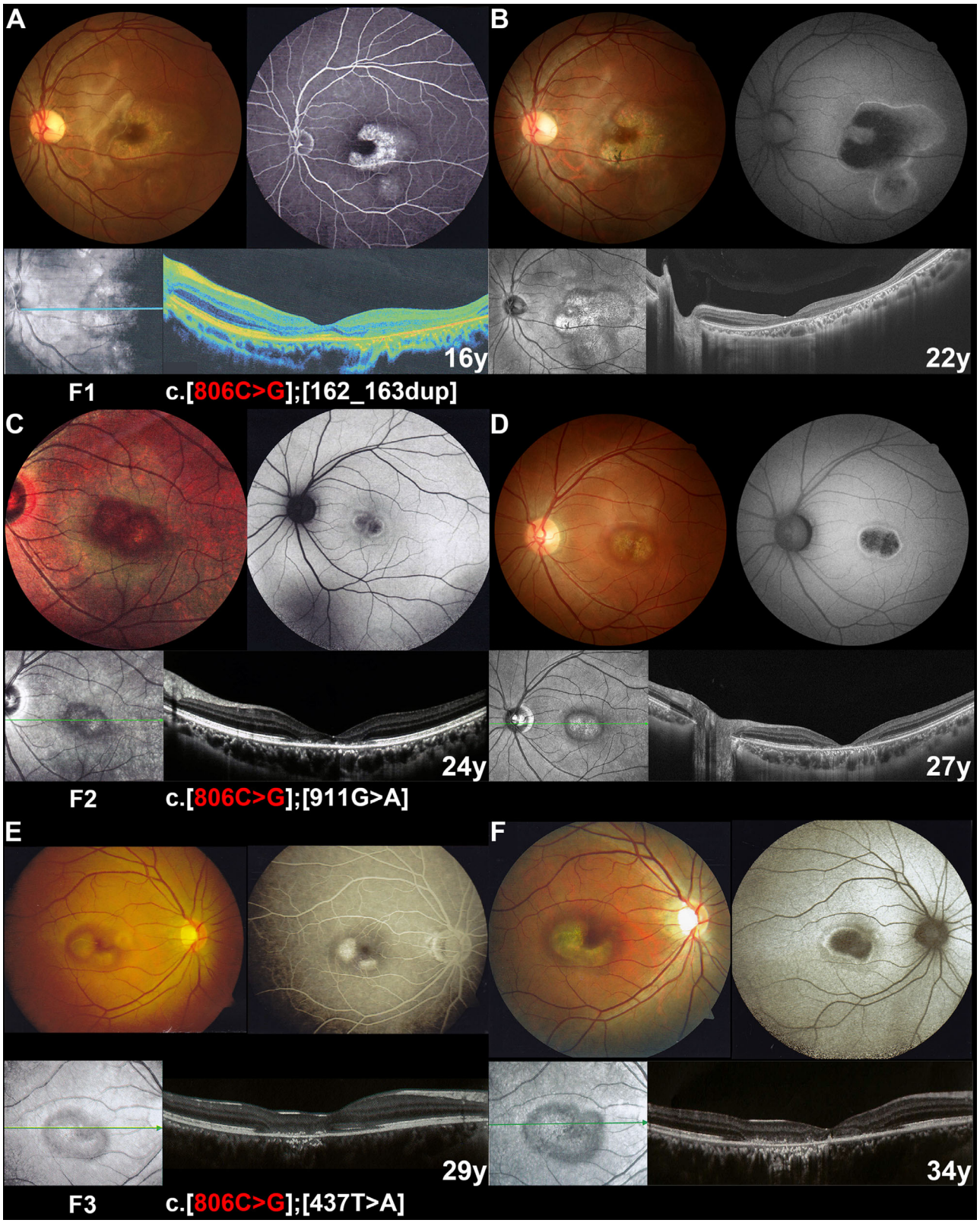

Figure 3. Follow-up assessments of patients with *RDH12*-associated CORD in our cohort. Panels (**A**), (**C**), and (**E**) depict multimodal fundus examination at first examination. Panels (**B**), (**D**), and (**F**) depict multimodal fundus examination at the last follow-up. The fundus presents with central macular atrophy and is characterized by hypo-autofluorescence with a hyper-autofluorescence border on fundus autofluorescence. Optical coherence tomography revealed marked atrophy of the outer retinal layer in the macular region, with evident decline of the outer nuclear layer thickness and loss of the external limiting membrane and ellipsoid zone. Follow-up of these patients for 3 to 6 years revealed that the region of macular atrophy expanded slightly and gradually merged into a patch.

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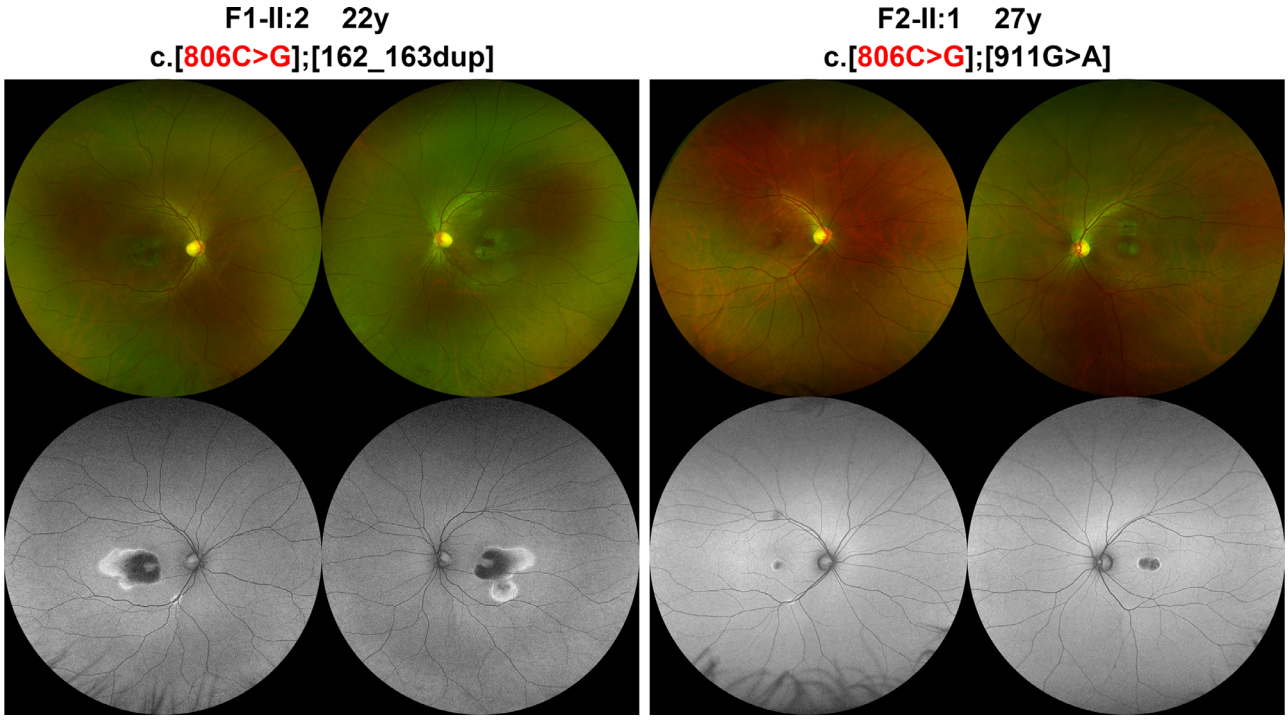

Figure 4. Wide-field fundus photographs and fundus autofluorescence of patients with *RDH12*-associated CORD only with macular atrophy and without peripheral retinal involvement.

region surrounded by a hyper-autofluorescence border (see Figs. 3B, 3C, 3D, 3F). FFA exhibited hyper-fluorescence, suggesting underlying damage to the retinal pigment epithelium (see Figs. 3A, 3E). The central macular lesion was slightly expanded (see Fig. 3B), and central macular atrophy gradually merged into a patch (see Figs. 3D, 3F) in the follow-up observations of the 5 patients over 3 to 6 years. No peripheral retinal involvement was observed in the late stage in patients at follow-up (Fig. 4). Four of the five patients underwent ERG examination (including one reported patient, whose ERG has been reported in our published research[16] and is not presented here), which demonstrated normal rod responses and a mild-to-moderate reduction in cone responses (Supplementary Fig. S2A).

The age of onset of the 24 patients with *RDH12*-associated LCA/EOSRD/arRP ranged from less than 1 year old to 24 years old (median = 3 years old). Of all the patients, 66.7% (16/24) were diagnosed before 3 years of age (see Supplementary Fig. S1C). Patients with *RDH12*-associated LCA/EOSRD/arRP were examined at a median age of approximately 16 years, when almost all patients presented with widespread retinal involvement. This was earlier than that in patients with *RDH12*-associated CORD, who were examined at a median age of approximately 24 years of age and did not present with peripheral retinal involvement (see Supplementary Fig. S1D). BCVA at the first examination ranged from hand motion to 0.6, and 90.9% (20/22) of patients had a BCVA of no more than 0.3. Of all the patients, 40.9% (9/22) had a BCVA of less than 0.1 (Supplementary Fig. S1). All 24 patients presented with widespread tapetoretinal degeneration and typical bone-spicule pigmentation in the fundus (Fig. 5). In particular, characteristic *RDH12*-associated macular atrophy or macular excavation was observed in 83.3% (20/24) of patients. Of these 24 patients, 11 underwent the ERG examination and exhibited severely reduced or undetectable responses in both cones and rods (see Supplementary Fig. S2B).

Review of *RDH12* Variants and Related Phenotypes

A total of 142 variants were reported to cause recessive *RDH12*-associated retinopathy in 425 families (Supplementary Table S1), including 100 missense variants (70.4%), 15 frameshift variants (10.6%), 14 nonsense variants (9.9%), 10 splicing variants (7.0%), and 3 in-frame deletion variants (2.1%). The most common variant was c.806_810del/p.(Ala269Glyfs*2), with an allele frequency of 10.7% (91/851), which was not identified in East Asian patients and was absent in the East Asian general populations (see Fig. 2B, Supplementary Table S1). The following common variants were identified: c.437T>A/p.(Val146Asp), c.295C>A/p.(Leu99Ile), c.146C>T/p.(Thr49Met), and c.184C>T/p.(Arg62*), with allele frequencies of 7.2% (61/851), 6.6% (56/851), 5.3% (45/851), and 3.9% (33/851), respectively (see Fig. 2B, Supplementary Table S1). The top 5 common variants accounted for approximately 52.7% (224/425) of the families. The most common genotype was biallelic missense variants, which accounted for 55.1% (234/425) of the families (see Fig. 2C). Other genotypes, including missense variant in trans with truncation variant, biallelic truncation variants, and missense variant in trans with in-frame deletion variant, accounted for 25.2% (107/425), 18.8% (80/425), and 0.9% (4/425), respectively (see Fig. 2C). Of the 425 families with recessive *RDH12*-associated retinopathy, 156 were diagnosed with EOSRD, 109 with arRP, 97 with LCA, 14 with CORD, 7 families with MD, and 42 with unclassified retinopathy (see Fig 2C).

The age of onset was available for 206 patients with *RDH12*-associated LCA/EOSRD/arRP and ranged from

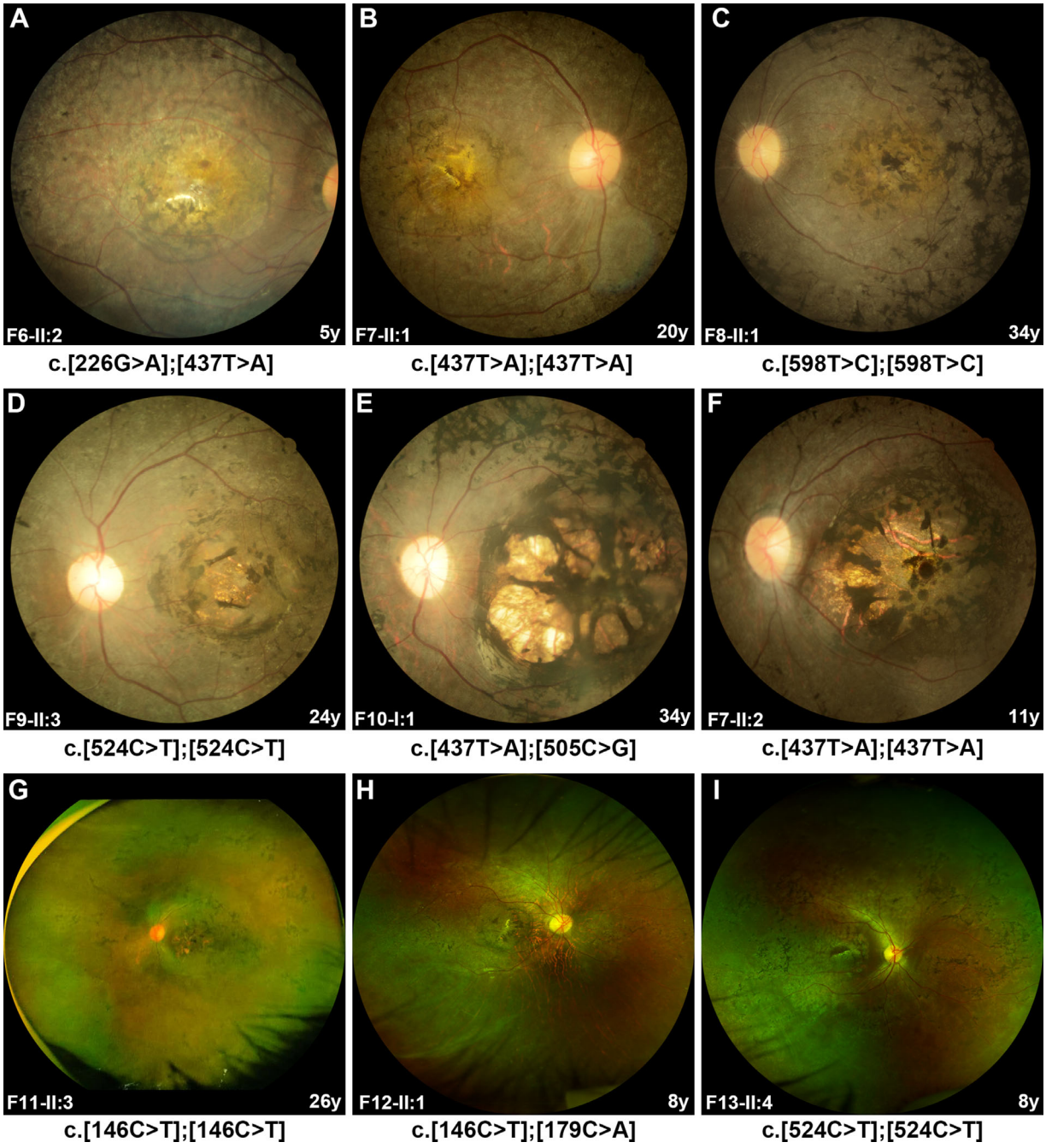

Figure 5. Fundus changes in patients with *RDH12*-associated severe retinopathy in our cohort. The fundus exhibits widespread tapetoretinal degeneration with bone-spicule pigmentation and macular atrophy or macular excavation.

2 months to 26 years (median = 3 years old), which was consistent with our cohort (see Supplementary Fig. S1C). Of these patients, 56.8% (117/206) were ≤3 years old, and only 9.2% (19/206) were >10 years old. BCVA data were available for 242 patients, whose BCVA at first examination ranged from no light perception to 0.8. Of all the patients, 88.4% (214/242) had a BCVA of no more than 0.3, and 57.4% (139/242) had a BCVA of less than 0.1 (see Supplementary Fig. S1B).

Up to now, 14 families with available fundus photographs for review have been reported to have *RDH12* biallelic variants and presented with a mild form of retinopathy (6 with MD, 4 with CORD , 2 with macula-predominant retinopathy, and 2 with rod-cone dystrophy cases)[8,31–33] (Supplementary Table S2). Specifically, 20 different variants were identified in these families, including 16 missense variants, one frameshift variant, one nonsense variant, one splicing variant, and one in-frame deletion variant (see Supplementary Table S2). Although these families had similar phenotypes, no variant was responsible for all the families, such as the p.(Ala269Gly) variant in our cohort. The median age at onset in these patients was 7 years, which was significantly earlier than that in our cohort ($P = 0.01$; see Supplementary Fig. S1C). Of all the patients, 62.5% (10/16) had a BCVA of no more than 0.3. Although the fundus changes in these 14 families were characterized by macular-predominant

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retinal degeneration, their macular lesions were relatively more advanced than those in the 5 patients with CORD in our cohort.

To date, four families of *RDH12*-associated adRP have been reported.[13–15] Although the exact age of onset of these patients with *RDH12*-associated adRP was not available for statistical analysis, some cases were reported to occur in early-to-mid teenage years,[15] which was significantly later than that of patients with recessive *RDH12*-associated retinopathy in published literature (median age of 3 years old for patients with LCA/EOSRD/arRP, and median age of 7 years old for patients with CORD/MD). A later age of examination was noticed in patients with *RDH12*-associated adRP (see Supplementary Fig. S1D). Among patients with adRP with accessible clinical data, none had a BCVA <0.6, which was significantly better than that of patients with recessive *RDH12*-associated retinopathy (see Supplementary Fig. S1B).

Single Heterozygous Variants of *RDH12*

Five heterozygous variants of *RDH12* have been reported in patients with adRP (see Fig. 2E, Supplementary Table S1). The c.778del/p.(Glu260Argfs*18) variant in *RDH12*, the first variant reported to cause *RDH12*-associated adRP, was identified in a large 6-generation family.[13] In addition, the c.759del/p.(Phe254Leufs*24)[14] and c.763del/p.(Val255Serfs*23)[15] variants co-segregated with the disease in two and one adRP families, respectively. These three adRP-related variants were located downstream of the domain on the penultimate exon of *RDH12* (see Fig. 2E). Another heterozygous c.680_684delinsT/p.(Ala227Valfs*50) variant, was detected in a singleton patient with retinitis pigmentosa (RP).[34] However, this variant in trans with the most common p.(Ala269Glyfs*2) variant was identified in patients with recessive *RDH12*-associated retinopathy.[9] A missense variant, c.121G>T/p.(Val41Leu), which was predicted as benign by 2 prediction tools, was detected in a singleton patient with RP without co-segregation analysis.[35] Therefore, more evidence is required for the two variants p.(Val41Leu) and p.(Ala227Valfs*50).

The effects of three adRP-related heterozygous variants (c.759del,[14] c.763del,[15] and c.778del[13]) on amino acid sequences were analyzed. Of note, although the amino acid sequences at the C-terminal of the three mutant proteins were different, they all produced an identical terminal peptide (RGRRPACTAPWLRAWSP; see Fig. 2F). Furthermore, wild-type and mutant *RDH12* protein structures were simulated using the SWISS-MODEL online server and PyMOL software. The three variants had the same secondary structure of the mutant protein, which had lost the C-terminal loop and helix structure compared to the wild-type protein (see Supplementary Fig. S3). Furthermore, highly similar helical structures were formed at the C-terminal of the three mutant proteins (see Supplementary Fig. S3).

Discussion

This study systematically analyzed the genotypic and phenotypic spectra of *RDH12* based on our in-house dataset and a comprehensive literature review. In total, 22 variants, including 5 novels, were identified in 29 patients from 27 families with a biallelic status. The most frequent variant was p.(Val146Asp), which was distinct to the most common variant, p.(Ala269Glyfs*2), in other ethnic groups (see Supplementary Table S1). The phenotypic spectrum of *RDH12* was categorized into two distinct forms: severe *RDH12*-associated LCA/EOSRD/arRP and mild CORD, which is characterized by central macular atrophy. During follow-up, the CORD phenotype did not progress to the severe forms and was considered to be an independent manifestation of *RDH12*-associated retinopathy caused by specific genotypes. The hypomorphic c.806C>G/p.(Ala269Gly) allele, which has a high population frequency particularly in East Asia (0.4%, 71/19766, based on gnomAD), was responsible for the unique CORD in 5 unrelated families. These findings provide novel insight into molecular mechanisms by which this hypomorphic variant results in a mild phenotype.

Since confirmation of *RDH12* as the causal gene of LCA13 (OMIM 612712),[5] its genotypic and phenotypic spectra have continuously expanded. To date, 147 variants in *RDH12* have been identified as biallelic, underscoring a different spectrum of *RDH12* among different ethnic backgrounds. The most common variant, p.(Ala269Glyfs*2), accounted for approximately 16.4% (74/452, including 425 families and 27 families in our cohort) of the families with recessive *RDH12*-associated retinopathy (see Supplementary Table S1). However, to date, this variant has not been reported in Chinese or East Asian populations, which is supported by several studies on East Asian populations,[8,36–39] including this study. Consistent with this, in gnomAD, 43 allele counts of this variant were detected only in non-East Asian populations. The most common variant in our cohort was p.(Val146Asp), which was the same variant observed in another Chinese cohort and was only identified in Chinese patients based on a comprehensive literature review (see Supplementary Table S1). Similarly, in gnomAD, all alleles of this variant have been detected in East Asia. Therefore, summarizing and identifying ethnicity-specific variants may facilitate interpretation of the variants detected in different populations and provide more accurate genetic counseling for patients.

Recessive *RDH12*-associated retinopathy has a broad spectrum of phenotypes, including the most frequent severe forms of retinopathy (LCA, EOSRD, and arRP) and mild forms of retinopathy (CORD and MD). Follow-up assessments of patients with mild retinopathy revealed relatively stable and slower progression, which confirmed that this condition was not an early stage of severe forms. Specific genotypes may underpin *RDH12*-associated CORD. Specifically, the hypomorphic variant c.806C>G/p.(Ala269Gly) may be responsible for this unique *RDH12*-associated CORD. The mechanism underlying the contribution of this variant to CORD may be explained by the following observations. Most individuals with heterozygous *RDH12* variants were disease-free, indicating that a single allele was sufficient to maintain the activity of retinol dehydrogenase, except for patients with *RDH12*-associated adRP. In individuals carrying one hypomorphic allele, such as the c.806C>G/p.(Ala269Gly) variant, regardless of the deleteriousness of other alleles, the retinol dehydrogenase activity was partially maintained by the hypomorphic allele, resulting in mild retinopathy. Similarly, the c.701G>A/p.(Arg234His) variant reportedly causing *RDH12*-associated CORD/MD[31–33] was predicted to be benign by 3 in silico prediction tools, with an allele frequency of 25 of 280120 in gnomAD. In vitro functional

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assays revealed that the c.701G>A/p.(Arg234His) variant retained 44% of wild-type enzyme activity.[40]

Genotype-phenotype association analysis of Mendelian diseases remains a great challenge in clinical practice. The genotype-phenotype association heatmap was based on the ordering of the deleteriousness of each variant, which theoretically predicted the phenotype severity of patients with different genotypes. In this study, it was found that the hypomorphic variant was strongly associated with mild forms of *RDH12*-associated retinopathy, which was closely predicted by the heatmap (see Fig. 2D). However, for the remaining families with severe forms of *RDH12*-associated retinopathy, phenotypic severity was difficult to reconcile with the prediction heatmap, which only provided certain reference values. Patient's phenotypes are determined by various factors, including genetic background and environmental factors. In our cohort, approximately 89.5% (17/19) of patients with arRP had age of onset within 10 years old. Moreover, the BCVA, fundus changes, and ERG of the 3 groups of patients (LCA, EOSRD, and arRP) were very similar. Therefore, although there were slightly differences existed between patients with *RDH12*-associated severe retinopathy, but it was difficult to identify clear phenotypic boundaries between them. Additionally, several compound heterozygous missense variants (p.Tyr200) were predicted to be associated with severe disease by the heatmap because these missense variants are located at key active sites (positions at 200-204 amino acids),[14,41] disruption of which may result in loss of retinol dehydrogenase activity. Therefore, assessment of the deleteriousness of biallelic variants in *RDH12* may help to predict the severity of recessive *RDH12*-associated retinopathy and will be valuable for clinical genetic interpretation.

Reports suggest that a single heterozygous variant of the autosomal recessive genes may cause ad-related retinopathy, such as, the heterozygous p.(Arg373Cys) variant in *PROM1*,[42–44] heterozygous p.(Asp477Gly) variant in *RPE65*,[45–47] and heterozygous substitution of p.Arg838 in *GUCY2D*.[48–51] Similarly, heterozygous frameshift variants of *RDH12* have been identified in patients with adRP.[13–15] Analysis of these adRP-related heterozygous variants in *RDH12* revealed that these variants produced identical terminal peptides and formed highly similar helical structures at the C-terminal of the mutant proteins (see Figs. 2E, 2F, Supplementary Fig. S3). It is speculated that heterozygous truncation variants located in the penultimate exon may escape nonsense-mediated mRNA decay (NMD),[52] produce aberrant proteins carrying toxic peptides (RGRRPACTAPWLRAWSP) at the C-terminal, and interrupt the function of wild-type proteins via dominant-negative effects. Theoretically, other truncating variants located downstream of this adRP-related *RDH12* region would also escape NMD, such as c.784del/p.(Ala262Argfs*16), c.784dup/p.(Ala262Glyfs*11), c.806_810del/p.(Ala269Glyfs*2), c.823G>T/p.(Glu275*), c.883C>T/p.(Arg295*), and c.912G>A/p.(Trp304*). However, these variants have not been causally linked to *RDH12*-associated adRP. The most likely explanation is that these variants can indeed escape NMD, but produce aberrant proteins that do not carry toxic peptides at the C-terminal and lake dominant-negative effects. Therefore, a toxic peptide of 17 amino acids starting from p.260 (RGRRPACTAPWLRAWSP) produced by heterozygous frameshift variants may be critical for *RDH12*-associated adRP. This hypothesis is similar to that proposed by Muthiah MN, et al.[15] Therefore, attention should be paid to these detected frameshift variants of *RDH12* in a heterozygous status located downstream of the domain.

In summary, this study systematically analyzed the genotypic and phenotypic spectra of *RDH12* based on an in-house dataset and literature review. An ethnic-specific spectrum of *RDH12* variants was identified, which facilitated the interpretation of variants in different populations. Two distinct forms of *RDH12*-associated retinopathy were revealed to be caused by different genetic bases. The hypomorphic variant c.806C>G/p.(Ala269Gly) is responsible for a unique *RDH12*-associated CORD characterized by central macular atrophy. The generation of a specific amino acid sequence (RGRRPACTAPWLRAWSP) at the C-terminal of the mutant protein may be a crucial reason for *RDH12*-associated adRP caused by heterozygous frameshift variants. The findings of this study illustrate the importance of assessing genetic variants and associated phenotypes in diverse populations and provide new insights for the genetic counseling of patients harboring *RDH12* variants.

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