Detailed clinical characterisation, unique features and natural history of autosomal recessive *RDH12*-associated retinal degeneration

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ABSTRACT

Background Defects in retinol dehydrogenase 12 (*RDH12*) account for 3.4%–10.5 % of Leber congenital amaurosis and early-onset severe retinal dystrophy (EOSRD) and are a potential target for gene therapy. Clinical trials in inherited retinal diseases have unique challenges, and natural history studies are critical to successful trial design. The purpose of this study was to characterise the natural history of *RDH12*-associated retinal degeneration.

Methods A retrospective chart review was performed in individuals with retinal degeneration and two likely disease-causing variants in *RDH12*.

Results 57 subjects were enrolled from nine countries. 33 subjects had clinical records available from childhood. The data revealed an EOSRD, with average age of onset of 4.1 years. Macular atrophy was a universal clinical finding in all subjects, as young as 2 years of age. Scotopic and photopic electroretinography (ERG) responses were markedly reduced in all subjects, and a non-recordable ERG was documented as young as 1 year of age. Assessment of visual acuity, visual field and optical coherence tomography revealed severe loss of function and structure in the majority of subjects after the age of 10 years. Widefield imaging in 23 subjects revealed a unique, variegated watercolour-like pattern of atrophy in 13 subjects and sparing of the peripapillary area in 18 subjects.

Conclusions This study includes the largest collection of phenotypic data from children with *RDH12*-associated EOSRD and provides a comprehensive description of the timeline of vision loss in this severe, early-onset condition. These findings will help identify patients with *RDH12*-associated retinal degeneration and will inform future design of therapeutic trials.

INTRODUCTION

Inherited retinal degenerations (IRDs) encompass a diverse group of blinding disorders, for nearly all of which there are no treatments. The relative accessibility of the retina compared with other tissues has made IRDs an early target of gene therapy. Leber congenital amaurosis (LCA) is the most severe form of IRD with 25 causative genes identified to date, and LCA2 caused by defects in retinoid isomerohydrolase RPE65 (*RPE65*) is the first genetic disorder to be treated with a Food and Drug Administration-approved gene therapy.[1–12] LCA13 due to recessive mutations in *RDH12* accounts for approximately 3.4%–10.5% of LCA and early-onset severe retinal dystrophy (EOSRD) and is particularly devastating due to early macular atrophy.[13–17] *RDH12* encodes retinol dehydrogenase 12, an enzyme expressed in photoreceptors that reduces all-*trans*-retinal to all-*trans*-retinol.[18] Following the success in gene supplementation therapy for another visual cycle enzyme, RPE65, *RDH12*-associated retinal degeneration is now also a potential target for gene therapy. Although the conversion of all-*trans*-retinal to all-*trans*-retinol is a critical step in the visual cycle, a number of studies have shown that this step is largely performed by RDH8 in photoreceptor outer segments, whereas RDH12 is located in the inner segment and reduces excess all-*trans* and 11-*cis* retinaldehydes that leak into the inner segment during periods of high photostimulation.[19–22] Thus, RDH12 is proposed to protect the photoreceptor inner segment from toxic buildup of multiple damaging aldehydes. Loss of this critical function is particularly detrimental to the macula early in life.[23] The natural history of *RDH12*-associated retinal degeneration requires detailed definition to aid the effective design and testing of treatment strategies.

Many genetic aetiologies have overlapping or even identical phenotypes, and any unique or pathognomonic features that can distinguish between aetiologies is helpful in directing genetic testing strategies, especially in areas where genetic testing is not widely available. After genotyping, one of the biggest challenges for developing therapies for IRDs is appropriate clinical trial design and determining optimal outcome measures, which may be different for distinct genotypes.[24] This retrospective natural history study reports unique phenotypic features that strongly suggest a genetic diagnosis of *RDH12*-associated retinal degeneration, and moreover, defines milestones in disease progression early in life when the retina may be most amenable to treatment.

METHODS

Subject ascertainment and genetic testing

Subjects with retinal degeneration and either a homozygous or two compound heterozygous likely

disease-causing variants in *RDH12* were evaluated at the University of Michigan Kellogg Eye Center (two subjects: one adult and one child), Moorfields Eye Hospital (27 subjects: 21 adults and 6 children), the Oregon Health Science University (OHSU) Casey Eye Institute (nine subjects: eight adults and one child), and a recruitment letter sent through other clinicians (six subjects: one adult and five children) and the RDH12 Fund for Sight (12 subjects: all children). Two additional subjects (one adult and one child) contacted us after learning of our work on *RDH12* by word of mouth or online. Variants were considered likely disease causing if they were nonsense, frameshift or canonical splice site variants, or if they were missense variants with either in vitro data showing reduced function or in silico analysis predicting reduced function in two out of three tools (Polyphen, Provean and SIFT).[25–27] Genetic testing was performed using a variety of strategies, including single-gene sequencing and next-generation sequencing gene panels. This study was performed in accordance with the Declaration of Helsinki. The research was approved by the Institutional Ethics Committee at Moorfields Eye Hospital, and the Institutional Review Boards at the University of Michigan and OHSU.

Clinical data

Clinical records were requested including the following: notes, genetic testing reports and imaging, including visual fields (VFs), optical coherence tomography (OCT), colour fundus photography and fundus autofluorescence (FAF). Snellen visual acuity (VA) was converted to logarithmic minimum angle of resolution (LogMAR). For these purposes, count finger (CF) vision was converted to a LogMAR of 2, hand motion vision was converted to a LogMAR of 3, light perception vision was converted to a LogMAR of 4 and no light perception vision was converted to a LogMAR of 5.[28] The earliest recorded VA for each eye, for each subject, was used for the VA scatter plot in figure 1. Available Goldmann VF (GVF) images were scanned, and the area of each isopter was measured using Adobe photoshop, subtracting the area of any included scotomas. For Octopus VFs, the area of each isopter was automatically calculated by the Octopus software. Fundus photos, autofluorescence images and OCT images were collected when available. Available images varied widely between subjects and were obtained with Zeiss, Heidelberg and Optos cameras. Due to the heterogeneity of image files, quantitative analysis was not possible and analysis was descriptive.

Reverse transcription-PCR

RNA was extracted from peripheral blood using PAXgene Blood RNA Kits (PreAnalytix). Coding DNA was made with 250 ng total RNA using SuperScript II reverse transcriptase (RT) (Invitrogen), and multiplexed RT-coupled PCR was run the same day in triplicate using Taqman probes with conjugated carboxyfluorescein (FAM) for *RDH12* amplification and conjugated 2'-chloro-7'phenyl-1,4-dichloro-6-carboxy-fluorescein (VIC) for *PGK1* amplification (Applied Biosystems). Taqman probes were designed by Thermo Fisher Scientific, assay ID

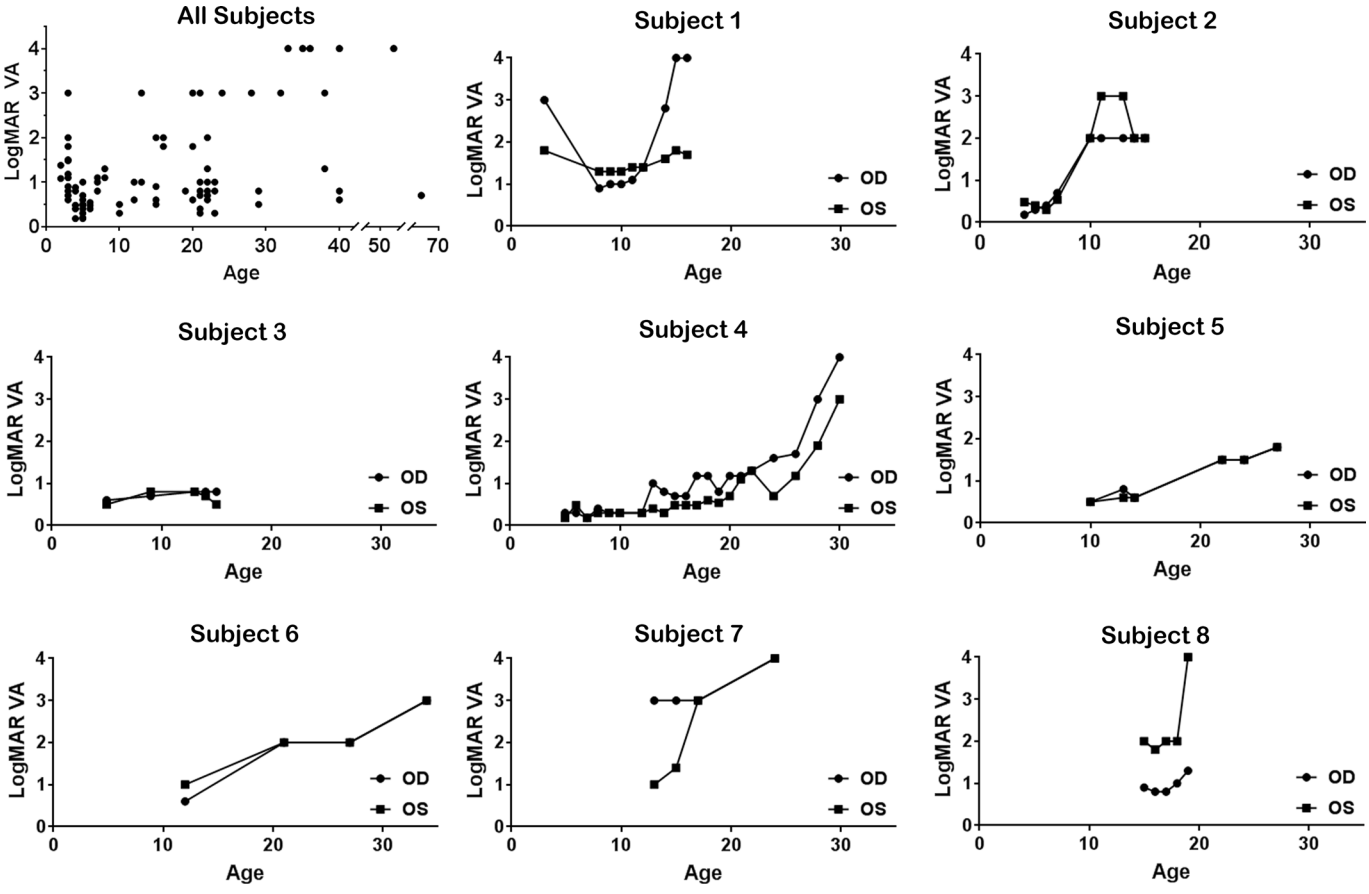

Figure 1 VA progression with age. A scatter plot is shown in the upper left with the first VA recorded for each eye for each patient. For conversion to LogMAR, count fingers=2, hand motions=3, perception of light=4, no perception of light=5. VA is variable in early childhood and severe vision loss is common after age 10 years, with few subjects retaining LogMAR 0.5 or better. Longitudinal VA during adolescence is shown for eight individual subjects. LOgMAR, logarithmic minimum angle of resolution; VA, visual acuity.

Hs00288401_m1 using Refseq NM_152443.2 for *RDH12* and assay ID Hs00943178_g1 using NM_000291.3 for *PGK1*. PCR reactions were run in the Biorad iCycler. Relative *RDH12* transcript levels were normalised to *PGK1*.

RESULTS

Subjects

In all, 57 subjects from 50 families with retinal degeneration and two likely disease-causing variants in *RDH12* were enrolled, including 26 from the USA, and 31 from other countries (Great Britain, India, Pakistan, Saudi Arabia, Bangladesh, Cyprus, China and Spain). The number of visits ranged from 1 to 21, with an average of 5.2. For all visits, subject ages ranged from 2 to 70 years. For 32 out of 57 subjects (56%), clinical data from childhood (before 18 years) was available, with age at first visit ranging from 2 to 16 years (average 6.0). Subject- or parent-reported age of onset ranged from infant (3 months) to 22 years (average 4.1 years, median 3 years), with the 22-year old being an outlier. In total, 33 subjects had documentation of subject- or family-reported presenting signs. The most commonly reported presenting signs were nystagmus in eight subjects (24%), uncorrectable central vision loss in seven subjects (21%), not reaching or difficulty finding dropped objects in six subjects (18%) and nyctalopia in five subjects (15%). Other presentations included toddlers who were overly cautious when learning to walk or seemed clumsy, who did not look at faces or make eye contact, and strabismus.

Sequence variants

A total of 42 likely disease-causing sequence variants were identified in the cohort, including 30 missense variants, 6 nonsense variants, 5 frameshift variants and 1 splice site variant (table 1). In all, 28 of the mutations have been previously reported. The most common mutation was a 5 bp deletion at codon 269. Eight of the variants had in vitro functional data to support pathogenicity.[16 29–32] A summary of genotype and phenotype for each subject is available in the supplemental material (online supplementary table S1).

Visual acuity

VA ranged from 20/30 to no perception of light. VA was variable in early childhood, with vision of 20/200 occurring as early as 2 years of age in one subject, and CF vision occurring as early as 3 years of age while other young children retained excellent VA (figure 1). Seven out of 25 subjects aged 10 years and below (28%) had a vision of 20/200 or worse in the better seeing eye. The variability in early childhood was likely due in part to differences in disease severity but also possibly due to suboptimal cooperation, a common confounder in young children. This was demonstrated by longitudinal data in subject 1, who showed marked improvement in measured VA in each eye between the ages of 3 and 8 years (figure 1). After the age of 10 years, progressive VA decline was common. However, out of 38 individuals older than 10 years, six subjects (16%) had documented 20/60 (LogMAR 0.5) or better vision in at least one eye, including 3 out of 31 subjects over the age of 20 years (10%), with one mildly affected outlier retaining 20/100 vision at the age 68 years.

Longitudinal data from eight subjects that included assessments during adolescence confirmed that there was significant VA decline between the ages of 10 and 20 years (figure 1). The exceptions were subject 2, who already had CF vision in each eye by age 10 years, and subject 3, who maintained relatively stable VA until the age of 15 years, which is the latest data point. In subjects 1 and 4, VA was relatively stable until after age 12 years. Subjects 5, 6, 7 and 8 have no clinical data from early childhood but showed rapid VA decline between the ages of 10 and 20.

Refraction data were available for 14 subjects. Using the most recent refraction for each subject, there were six subjects with mild hyperopia, ranging in age from 2 to 8 years, six subjects with moderate hyperopia, ranging in age from 7 to 11 years, and two subjects with high hyperopia, ages 3 and 5 years.

VF and ERG

VF constriction was a universal finding, and central or paracentral scotomas were also seen in some subjects. VF images were available for 16 subjects, ranging in age from 6 to 68 years, including 12 GVFs and 4 Octopus VFs, which have been shown to give comparable results.[33] As seen in figure 2, for the smallest and dimmest isopter (I4e), VF area was variable in subjects before the age of 10 years and was severely diminished in subjects 10 years and older, other than two outliers, ages 31 and 68 years. The trend disappeared with increasing target size, as the larger isopters had better VF preservation in most subjects. Of note, the 68-year old, with well-preserved VF for isopters I4e and III4e, is the previously discussed mildly affected outlier with 20/100 VA (figure 2). Furthermore, the other subjects with relative preservation of VF after the age of 20 years in figure 2 also had relative preservation of VA in figure 1, ranging from LogMAR 0.3 to 0.7.

Full-field electroretinography (ERG) data were available in 27 subjects and revealed markedly reduced rod and cone responses. A non-recordable ERG was reported in a subject as young as 1 year of age, and the oldest subject with recordable responses was 29 years old. This individual had exceptionally mild ERG changes and presentation, with age of onset at 22 years.

Retinal findings and imaging

Macular atrophy was a universal finding documented on examination in all subjects, even as young as 2 years of age. With disease progression, the area of atrophy extended peripherally in a unique variegated watercolour-like pattern, which in most cases corresponded to the retinal vasculature. This pattern was visualised both clinically and on colour fundus photography and was further emphasised on FAF (figure 3). In a 3-year-old subject with early disease, the atrophy was confined to the macula, and mild perivascular hyperautofluorescence was seen along the arcades on FAF (figure 3A,B). In a 13-year-old subject with more advanced disease, the watercolour pattern extended into the periphery, with some areas of atrophy extending along the retinal vasculature (figure 3C,D). In a 41-year-old with end-stage disease, there was widespread atrophy with variegated edges in the far periphery, demonstrating how the watercolour fundus progresses from the posterior pole outward (figure 3E,F).

Out of 23 subjects with available FAF images, the watercolour pattern was seen in 13 individuals in at least some areas (online supplementary figure S1). In addition, 18 out of 23 had peripapillary sparing on FAF (figure 3D). These features were less evident in end-stage disease with widespread atrophy, but common in all subjects with earlier disease and remaining areas of preserved retina.

OCT imaging demonstrated that the variegated watercolour pattern demarcated the borders of outer retinal atrophy (figure 3G,H). The area of yellow atrophy seen in colour fundus images corresponded with loss of outer nuclear layer (ONL), ellipsoid zone and disruption of the RPE as revealed by OCT

Table 1 Cohort *RDH12* variants

Variant		Alleles	Subjects	Polyphen	Provean	SIFT	Functional studies
Missense							
c.133 A>G	p.Thr45Ala	1	1	Prob D	Del	Dam	
c.139 G>A[32]	p.Ala47Thr	1	1	Prob D	Del	Dam	<10% normal reductase activity[32]
c.146 C>T[16 29 37–40]	p.Thr49Met	4	4	Prob D	Del	Dam	Reduced affinity for NADPH and increased proteosomal degredation[29 30 32]
c.146 C>A	p.Thr49Lys	1	1	Poss D	Del	Dam	
c.178G>A[41]	p.Ala60Thr	1	1	Prob D	Del	Dam	
c.185 G>T	p.Arg62Leu	1	1	Prob D	Del	Tol	
c.209 G>A[13]	p.Cys70Tyr	1	1	Prob D	Del	Dam	
c.226 G>A[42]	p.Gly76Arg	1	1	Prob D	Del	Dam	
c.295 C>A[13 14 17 23 34 38 43–45]	p.Leu99Ile	7	4	Prob D	Del	Dam	<10% normal reductase activity[32]
c.302 A>G	p.Asp101Gly	2	2	Prob D	Neu	Dam	
c.325 G>C	p.Ala109Pro	1	1	Prob D	Neu	Dam	
c.377 C>T[46 47]	p.Ala126Val	2	2	Prob D	Del	Dam	
c.377 C>A	p.Ala126Glu	2	2	Prob D	Del	Dam	
c.383 T>G	p.Val128Gly	1	1	Prob D	Del	Dam	
c.400 T>C	p.Ser134Pro	1	1	Prob D	Del	Tol	
c.451 C>G[13 17 48]	p.His151Asp	1	1	Prob D	Del	Dam	<10% normal reductase activity[32]
c.454 T>A[13]	p.Phe152Ile	2	1	Prob D	Del	Dam	
c.464 C>T[14 32 43 48]	p.Thr155Ile	2	1	Prob D	Del	Dam	<10% normal reductase activity[32]
c.481 C>T[13 38]	p.Arg161Trp	2	2	Prob D	Del	Dam	
c.506 G>A[13]	p.Arg169Gln	3	2	Prob D	Del	Dam	
c.601 T>C[13 31]	p.Cys201Arg	10	5	Poss D	Del	Tol	30% expression and <10% normal reductase activity[16 31]
c.609 C>A[13]	p.Ser203Arg	7	4	Prob D	Del	Dam	
c.619 A>G[13]	p.Asn207Asp	5	3	Poss D	Del	Dam	
c.671 C>T	p.Thr224Ile	1	1	Prob D	Del	Dam	
c.677 A>G[17 48]	p.Tyr226Cys	1	1	Prob D	Del	Dam	
c.697 G>C[13]	p.Val233Leu	1	1	Prob D	Del	Dam	
c.698 T>A	p.Val233Asp	2	2	Prob D	Del	Dam	
c.701 G>A[14 32 43 49]	p.Arg234His	1	1	B	Neu	Tol	44% normal reductase activity[32]
c.715 C>T[13 38]	p.Arg239Trp	1	1	Prob D	Del	Dam	<20% normal reductase activity[32]
c.910 T>C	p.Trp304Arg	1	1	Prob D	Del	Dam	
Nonsense							
c.38 C>A	p.Ser13X	2	1				
c.184 C>T[13 16 17 40 45]	p.Arg62X	3	3				
c.193 C>T[13 48]	p.Arg65X	2	1				
c.316 C>T[13]	p.Arg106X	1	1				
c.379 G>T[13 17 18]	p.Gly127X	2	1				
c.883 C>T[13 34 45 48]	p.Arg295X	5	5				

Continued

Table 1 Continued

Variant		Alleles	Subjects	Polyphen	Provean	SIFT	Functional studies
Frameshift							
c.57_60delTCCA[45]	p.Ala19Alafs	4	3				
c.680_684delinsT	p.Ala2 27Valfs*50	1	1				
c.698insGT[13]	p.Val233Valfs*46	1	1				
c.714_715insC[1345]	p.Arg239Argfs	3	2				
c.806_810delCCCTG[1317 48]	p.Ala269Glyfs*2	22	18				<5% normal reductase activity[31]
Splice							
c.448+1 G>A[13]		1	1				

Alleles show number of alleles in the cohort (out of 114). Subjects show number of subjects in the cohort (out of 57). Variants were analysed in Polyphen, Provean and SIFT.
B, benign; Dam, damaging; Del, deleterious; NADPH, nicotinamide adenine dinucleotide phosphate hydrogen; Neu, neutral; Poss D, possibly damaging; Prob D, probably damaging; Tol, tolerated.

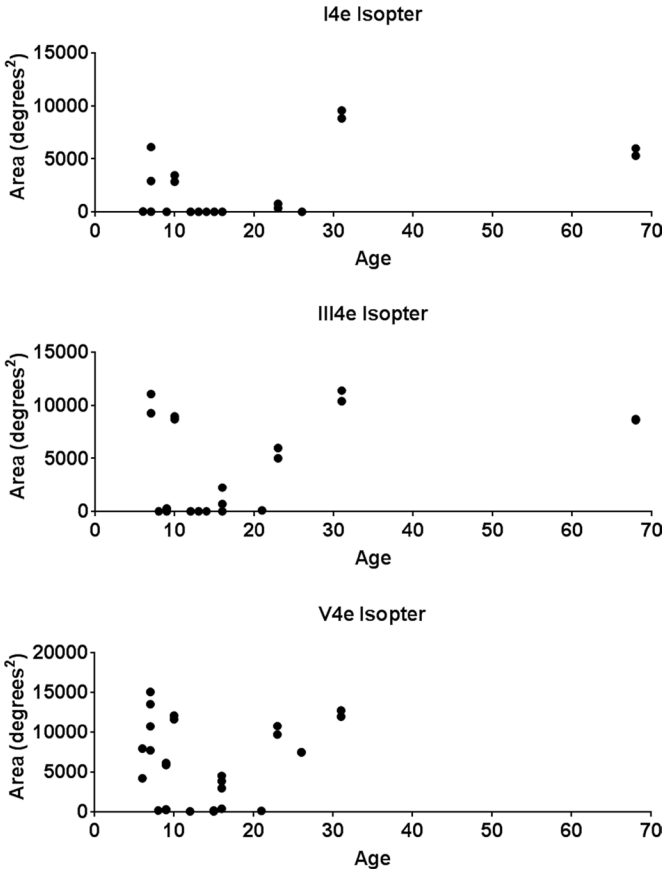

Figure 2 VF progression with age. A scatter plot is shown for calculated VF area for the I4e, III4e and V4e isopters for patients with available fields, one field per eye per patient. Severe VF loss was common after age 10 years, especially for the smallest isopter. VF, visual field.

imaging while the darker border corresponded to thinning of ONL and attenuation of the ellipsoid zone on OCT.

OCT images with horizontal cuts through the fovea were available from 36 subjects (67 eyes). Age at the time of OCT ranged from 3 to 58 years (average 28). Out of 36 subjects (67 eyes), seven subjects (12 eyes) had partially preserved ellipsoid zone in the macula (ages 3–22 years), and three subjects (six eyes) had ellipsoid zone at the fovea (ages 3–15 years). In all, 24 subjects (42 eyes) had partial preservation of ONL in the macula (ages 3–44 years), and eight subjects (14 eyes) had preservation of ONL at the fovea (ages 3–15 years). There was no one over the age of 15 years with preservation of either ellipsoid zone or ONL at the fovea, consistent with the VA findings that adolescence is a period of significant disease progression. In addition, advanced disease was associated with the development of posterior staphyloma. Out of 36 subjects (67 eyes), 16 subjects (31 eyes) demonstrated posterior staphyloma on OCT, ranging in age from 21 to 58.

RDH12 transcript levels can link genotype with phenotype

One subject presented at the age of 2 years with mild nystagmus, but remained visually asymptomatic until the age of 5 or 6 years, when he began having mild night blindness and reduced peripheral vision. Genetic testing at the age of 6 years revealed homozygous early nonsense mutations in *RDH12* (Ser13X). His VA has remained relatively well preserved to date (20/40 in each eye at 8 years of age). Because *RDH12* is expressed in peripheral

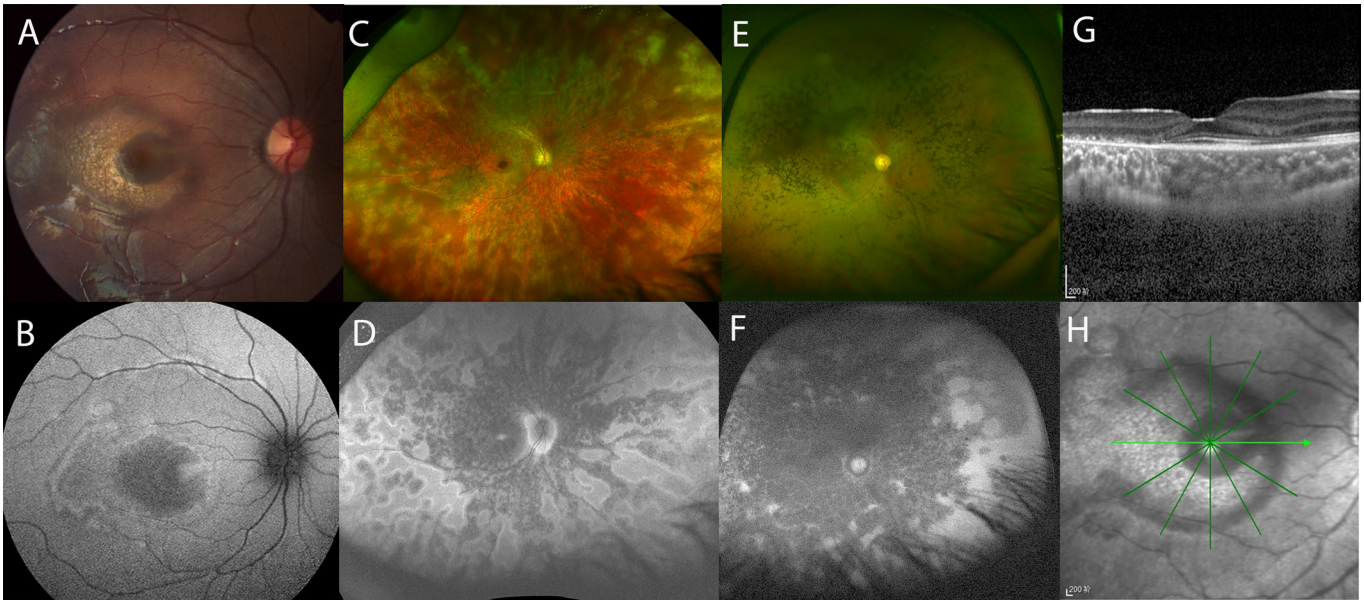

Figure 3 Distinct variegated pattern of atrophy on colour photos and FAF in three patients at different stages of disease. (A) 30° colour photo and (B) FAF of a 3-year-old subject. (C) Optos colour photo, (D) FAF of a 13-year-old subject, (E) Optos colour photo and (F) FAF of a 41-year-old subject, showing evolution of the variegated atrophy in the different stages of disease. (G) OCT and (H) corresponding red-free photo in the right eye of the 3-year-old subject from (A) and (B), showing the outer retinal atrophy corresponding with the borders of the variegated fundus pattern. FAF, fundus autofluorescence; OCT, optical coherence tomography.

blood, blood samples were collected and RNA isolated to assess *RDH12* transcript levels. The Ser13X variant is classified as likely pathogenical and expected to result in nonsense mediated decay and a null phenotype. Although *RDH12* expression in blood was variable in normal controls, the affected subject had consistently detectable *RDH12* transcript over 45% compared with controls. A downstream methionine at position 17 with codon ATG may serve as an alternative translation start site and account for the relatively mild phenotype in this individual.

DISCUSSION

This study includes the largest well-characterised cohort of subjects, and moreover the largest cohort of children, with *RDH12*-associated retinal degeneration, and therefore provides the most comprehensive description to date of the timeline of vision loss in this severe, early-onset condition. In early childhood, there is variable VA, which typically declines after the age of 10 years. Longitudinal VA data for several subjects confirmed that adolescence is a period of significant visual decline. OCT also demonstrated universal loss of the ellipsoid zone and ONL in the fovea during adolescence. Possibly not surprisingly, VF loss was more variable but also showed a decline after age 10 years for the smallest isopter. The data suggest that although some individuals have severe vision loss in early childhood (28% based on VA), others who retain useful vision until adolescence are at risk for significant progression before adulthood. Furthermore, there appeared to be a small subset of individuals (10%) who retained useful vision well into adulthood, thus increasing the potential therapeutic window. The youngest subject in our cohort with fundus imaging was 3 years old and showed macular atrophy with sparing of the fovea. Additional OCT studies in early childhood are needed to determine whether this is a common early phenotype, which would potentially allow early intervention to salvage the fovea. The strengths of these data include the large number of children and the availability of longitudinal data for some subjects. It is retrospective in nature, and thus the heterogeneity of available clinical data between subjects limits our ability to perform quantitative analyses.

As many inherited retinal diseases have significant overlap in phenotype, distinct fundus findings that point to a particular genetic diagnosis can be clinically useful. This study highlights a unique fundus signature in *RDH12*-associated retinal degeneration, namely a watercolour-like appearance that is not found in other IRDs. The watercolour fundus pattern outlines the border between preserved and degenerated retina, expands with progression of the disease and is less apparent in end-stage disease. In the majority of cases, the atrophy corresponded to retinal vasculature. Peripapillary sparing was best visualised on FAF and was common until late in disease, which has been previously described in *RDH12*-associated retinal degeneration and was first described in Stargardt disease.[34–36] This distinct appearance may help to identify individuals with this condition. The most common phenotypic features of *RDH12*-associated retinal degeneration are summarised in table 2.

One of the most mildly affected subjectsI in our cohort initially presented at the age of 11 years with uncorrectable reduced VA, and she was most recently seen at the age of 70 years with VA of 20/125 in each eye and mild to moderate VF constriction. Of note, her genotype is c.701 G>A (p. Arg234His) and c.806_810delCCCTG (p. Ala269Glyfs*2). While the latter variant results in a frameshift and is expected to act as a null allele, the Arg234His variant is predicted benign by Polyphen-2 and has previously been tested in vitro and retained 44% of normal enzyme activity.[32] The Arg234His variant was also previously reported in compound heterozygous form with N125K (which demonstrates <10% normal activity in vitro) in a 21-year-old subject with a relatively mild phenotype.[32] Thus, Arg234His likely acts as a hypomorphic allele and explains our subject's preserved visual function even in late adulthood. This also suggests that restoration of less

Table 2 Most common phenotypic features of *RDH12*-associated retinal degeneration

Category	Most common findings	Percent of subjects
Presenting sign	Nystagmus	(8/33) 24%
	Uncorrectable central vision	(7/33) 21%
	Difficulty finding objects	(6/33) 18%
OCT	Outer retinal atrophy in macula	(35/35) 100%
Fundus photo	Macular atrophy (including staphyloma in late stages)	(24/24) 100%
	Variegated watercolour fundus	(15/22) 68%
Fundus autofluorescence	Macular atrophy	(23/23) 100%
	Watercolour fundus	(13/23) 57%
	Peripapillary sparing	(18/23) 78%

The most common findings in each category are listed, along with the prevalence of the finding in our cohort (number of subjects with finding/number of subjects with available data).
OCT, optical coherence tomography.

than 50% RDH12 function may benefit patients. Other genotype–phenotype correlations may require the use of RT-PCR to evaluate transcript levels of *RDH12*, which is expressed in peripheral blood leukocytes. We have demonstrated this in a subject with a relatively mild phenotype and only mildly reduced transcript levels despite a homozygous early nonsense variant (p.Ser13X).

This study contributes to the current understanding of the natural history of *RDH12*-associated retinal degeneration and has identified a unique fundus signature that is strongly suggestive of the genetic diagnosis. These data highlight the window of opportunity and the need to target future therapeutic strategies towards young children in order to potentially preserve vision. It also demonstrates that adolescence may be a period of relatively rapid progression for many patients, which may allow demonstration of therapeutic efficacy over a relatively short time period in the setting of a clinical trial.

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Contributors AF participated in study design, subject enrolment, data collection, data analysis, manuscript preparation and editing. ZB participated in data collection and manuscript editing. KHB participated in study design, subject enrolment, manuscript preparation and editing. NK participated in data collection and manuscript editing. MEV participated in data collection and manuscript editing. KLF participated in data collection, data analysis and manuscript editing. NDP participated in data collection, data analysis and manuscript editing. KY participated in subject enrolment, data collection and manuscript editing. NWK participated in data collection and manuscript editing. JRH participated in data collection and manuscript editing. ARW participated in data collection and manuscript editing. MEP participated in subject enrolment, data collection and manuscript editing. RRA participated in study design and manuscript editing. DAT participated in study design and manuscript editing. MM participated in study design, subject enrolment, data collection and manuscript editing.

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Competing interests ATF reports a grant from the Vitreoretinal Surgery Foundation and a K12 grant from the National Institute of Health, during the conduct of the study; other from Ionis Pharamceuticals, outside the submitted work. RRA reports a patent on RDH12 gene therapy. DAT reports a grant from the Foundation Fighting Blindness, during the conduct of the study; In addition, DAT has a patent for viral vectors comprising RDH12 coding regions and methods of treating retinal dystrophies pending.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Additional deidentified clinical data for the subjects presented in this manuscript are available from the corresponding author (ahteich@umich.edu) for 3 years after publication. However, a data transfer agreement may be required per institutional regulations.

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