Detailed Clinical Characterisation, Unique Features, and Natural History of Autosomal Recessive *RDH12*-Associated Retinal Degeneration

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Synopsis

This retrospective chart review of 57 subjects with *RDH12*-associated retinal degeneration provides a comprehensive description of the timeline of vision loss and highlights a unique fundus signature that strongly suggests the genetic diagnosis.

Abstract

Background. Defects in retinol dehydrogenase 12 (*RDH12*) account for 3.4-10.5% of Leber congenital amaurosis (LCA) 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 of individuals with retinal degeneration and 2 likely disease-causing variants in *RDH12*.

Results. Fifty-seven subjects were enrolled from 9 countries. Thirty-three subjects had clinical records available from childhood. The data revealed a severe early-onset retinal degeneration, 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. Widefield imaging in 23 subjects revealed a unique, variegated watercolor-like pattern of atrophy in 13 subjects, and sparing of the peri-papillary 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 to 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 *RPE65* is the first genetic disorder to be treated with an FDA-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, while 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 photo-stimulation[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 etiologies have overlapping or even identical phenotypes, and any unique or pathognomonic features that can distinguish between etiologies 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 2 compound heterozygous likely disease-causing variants in *RDH12* were evaluated at the University of Michigan Kellogg Eye Center (2 subjects; 1 adult, 1 child), Moorfields Eye Hospital (27 subjects; 21 adults, 6 children), the Oregon Health Science University (OHSU) Casey Eye Institute (9 subjects; 8 adults, 1 child), and a recruitment letter sent through other clinicians (6 subjects; 1 adult, 5 children) and the RDH12 Fund for Sight (12 subjects; all children). Two additional subjects (1 adult, 1 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 2 out of 3 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: notes, genetic testing reports, and imaging, including visual fields, optical coherence tomography (OCT), color fundus photography, and fundus autofluorescence. Snellen visual acuity (VA) was converted to LogMAR. For these purposes, count finger vision was converted to a LogMAR of 2, hand motions 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 visual acuity scatter plot in **Figure 1**. Available Goldmann visual field (GVF) images were scanned, and the area of each isopter was measured using Adobe photoshop, subtracting the area of any included scotomas. For Octopus visual fields, 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.

RT-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 polymerase chain reaction (PCR) was run the same day in triplicate using Taqman probes with conjugated FAM for *RDH12* amplification and conjugated VIC for *PGK1* amplification (Applied Biosystems). Taqman probes were designed by Thermo Fisher Scientific, assay ID 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 normalized to *PGK1*.

Results

Subjects

Fifty-seven subjects from 50 families with retinal degeneration and two likely disease-causing variants in *RDH12* were enrolled, including 26 from the United States, 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 age 18) 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. Thirty-three subjects had documentation of subject- or family-reported presenting signs. The most commonly reported presenting signs were nystagmus in 8 subjects (24%), uncorrectable central vision loss in 7 subjects (21%), not reaching or difficulty finding dropped objects in 6 subjects (18%), and nyctalopia in 5 subjects (15%). Other presentations included toddlers who were overly cautious when learning to walk or seemed clumsy, who didn't 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**). Twenty-eight 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 (**Table S1**).

Visual Acuity

Visual acuity ranged from 20/30 to no perception of light (NPL). Visual acuity was variable in early childhood, with vision of 20/200 occurring as early as 2 years of age in one subject, and count fingers (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 under (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 (**Figure 1**). After the age of 10, progressive VA decline was common. However, out of 38 individuals older than 10 years, 6 subjects (16%) had documented 20/60 (LogMAR 0.5) or better vision in at least one eye, including 3 out of 31 subjects past the age of 20 (10%), with one mildly affected outlier retaining 20/100 vision at age 68.

Longitudinal data from 8 subjects that included assessments during adolescence confirmed that there was significant VA decline between the ages of 10 and 20 (**Figure 1**). The exceptions were subject 2, who already had CF vision in each eye by age 10, and subject 3, who maintained relatively stable VA until the age of 15, which is the latest data point. In subjects 1 and 4, VA was relatively stable until after age 12. 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 was available for 14 subjects. Using the most recent refraction for each subject, there were 6 subjects with mild hyperopia, ranging in age from 2 to 8 years, 6 subjects with moderate hyperopia, ranging in age from 7 to 11 years, and 2 subjects with high hyperopia, ages 3 and 5.

Visual Field and Electroretinography

Visual field (VF) constriction was a universal finding, and central or paracentral scotomas were also seen in some subjects. Visual field images were available for 16 subjects, ranging in age from 6 to 68, including 12 Goldmann visual fields and 4 Octopus visual fields, 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, and was severely diminished in subjects 10 and older, other than 2 outliers, ages 31 and 68. 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 visual field after the age of 20 in Figure 2 also had relative preservation of visual acuity in Figure 1, ranging from LogMAR 0.3 to 0.7.

Full-field Electroretinography (ERG) data was 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 watercolor-like pattern, which in most cases corresponded to the retinal vasculature. This pattern was visualized both clinically and on color fundus photography, and was further emphasized on fundus autofluorescence (FAF) (**Figure 3C and 3D**). 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 and 3B**). In a 13-year-old subject with more advanced disease the watercolor pattern extended into the periphery, with some areas of atrophy extending along the retinal vasculature (**Figure 3C and 3D**). In a 41-year-old with end-stage disease, there was widespread atrophy with variegated edges in the far periphery, demonstrating how the watercolor fundus progresses from the posterior pole outward (**Figure 3E and 3F**).

Out of 23 subjects with available FAF images, the watercolor pattern was seen in 13 individuals in at least some areas (**Figure S1**). In addition, 18 out of 23 had peri-papillary 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 watercolor pattern demarcated the borders of outer retinal atrophy (**Figure 3G and 3H**). The area of yellow atrophy seen in color fundus images corresponded with loss of ONL, ellipsoid zone, and disruption of the retinal pigment epithelium (RPE) as revealed by OCT 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), 7 subjects (12 eyes) had partially preserved ellipsoid zone in the macula (ages 3-22), and 3 subjects (6 eyes) had ellipsoid

zone at the fovea (ages 3-15). Twenty-four subjects (42 eyes) had partial preservation of ONL in the macula (ages 3-44), and 8 subjects (14 eyes) had preservation of ONL at the fovea (ages 3-15). There was no one over the age of 15 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 with mild nystagmus, but remained visually asymptomatic until the age of 5 or 6, when he began having mild night blindness and reduced peripheral vision. Genetic testing at the age of 6 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 blood, blood samples were collected and RNA isolated to assess *RDH12* transcript levels. The Ser13X variant is classified as likely pathogenic 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 to 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, visual field loss was more variable, but also showed a decline after age 10 for the smallest isopter. The data suggest that although some individuals have severe vision loss in early childhood (28% based on visual acuity) 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 watercolor-like appearance that is not found in other IRDs. The watercolor 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. Peri-papillary sparing was best visualized on fundus autofluorescence 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 summarized in **Table 2**.

The most mildly affected subject in our cohort initially presented at age 11 with uncorrectable reduced visual acuity, and she was most recently seen at age 70 with VA of 20/125 in each eye and mild to moderate visual field 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 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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Contributorship: ATF participated in study design, subject enrollment, data collection, data analysis, manuscript preparation and editing. ZB participated in data collection and manuscript editing. KHB participated in study design, subject enrollment, 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 enrollment, 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 enrollment, 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 enrollment, data collection, and manuscript editing.

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Variant		Alleles	Subjects	Poly-phen	Pro-vean	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[13,17,48]	p. Gly127X	2	1				
c. 883 C>T[13,34,45,48]	p.Arg295X	5	5				
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[13,45]	p. Arg239Argfs	3	2				
c.806_810delCCCTG[13,17,48]	p.Ala269Glyfs*2	22	18				<5% normal reductase activity[31]
Splice							
c. 448+1 G>A[13]		1	1				

Table 1. Cohort *RDH12* variants. Alleles shows number of alleles in the cohort (out of 114). Subjects shows number of subjects in the cohort (out of 57). Variants were analyzed in Polyphen (Prob D= probably damaging, Poss D= possibly damaging, B= benign), Provean (Del=deleterious, Neu= neutral), and SIFT (Dam= damaging, Tol= tolerated).

Category	Most common findings	Percent of Subjects
Presenting sign	Nystagmus Uncorrectable central vision Difficulty finding objects	(8/33) 24% (7/33) 21% (6/33) 18%
OCT	Outer retinal atrophy in macula	(35/35) 100%
Fundus Photo	Macular atrophy (including staphyloma in late stages) Variegated watercolor fundus	(24/24) 100% (15/22) 68%
Fundus Autofluorescence	Macular atrophy Watercolor fundus Peri-papillary sparing	(23/23) 100% (13/23) 57% (18/23) 78%

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