**RDH12 retinopathy: novel mutations and phenotypic description**

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**Purpose:** To identify patients with autosomal recessive retinal dystrophy caused by mutations in the gene, retinal dehydrogenase 12 (RDH12), and to report the associated phenotype.

**Methods:** After giving informed consent, all patients underwent full clinical evaluation. Patients were selected for mutation analysis based upon positive results from the Asper Ophthalmics Leber congenital amaurosis arrayed primer extension (APEX) microarray screening, linkage analysis, or their clinical phenotype. All coding exons of RDH12 were screened by direct Sanger sequencing. Potential variants were checked for segregation in the respective families and screened in controls, and their pathogenicity analyzed using in silico prediction programs.

**Results:** Screening of 389 probands by the APEX microarray and/or direct sequencing identified bi-allelic mutations in 29 families. Seventeen novel mutations were identified. The phenotype in these patients presented with a severe early-onset rod-cone dystrophy. Funduscopy showed severe generalized retinal pigment epithelial and retinal atrophy, which progressed to dense, widespread intraretinal pigment migration by adulthood. The macula showed severe atrophy, with pigmentation and yellowing, and corresponding loss of fundus autofluorescence. Optical coherence tomography revealed marked retinal thinning and excavation at the macula.

**Conclusions:** RDH12 mutations account for approximately 7% of disease in our cohort of patients diagnosed with Leber congenital amaurosis and early-onset retinal dystrophy. The clinical features of this disorder are highly characteristic and facilitate candidate gene screening. The term RDH12 retinopathy is proposed as a more accurate description.

Leber congenital amaurosis (LCA), first described by Theodor Leber in 1869 [1], is a heterogeneous autosomal recessive, generalized retinal dystrophy that presents at birth or soon after. The disorder is now recognized as the most severe form of a spectrum of early-onset retinal dystrophies (EORD), accounting for 3%–5% of childhood blindness in the developed world, with an estimated incidence of 2–3 per 100,000 live births [2]. Presentation is usually with reduced vision and nystagmus in early infancy. Undetectable or severely reduced rod and cone electroretinograms confirm the diagnosis [3,4]. The retinal appearance may initially be normal or show a variety of abnormalities, including white dots at the level of the retinal pigment epithelium (RPE), retinal pigment migration, retinal vascular attenuation, and macular atrophy.

To date, 14 causative genes, guanylate cyclase 2D (GUCY2D) [5], aryl hydrocarbon receptor interacting protein-like 1 (AIPL1) [6], retinal pigment epithelium-specific protein 65 (RPE65) [7], retinitis pigmentosa GTPase regulator interacting protein 1 (RPRGPI) [8], cone-rod homeobox-containing gene (CRX) [9], tubby like protein 1 (TULP1) [10], crumbs homolog-1 (CRB1) [11], retinol dehydrogenase 12 (RDH12) [12], centrosomal protein 290 kDa (CEP290) [13], lebercilin (LCA5) [14], spermatogenesis-associated protein 7 (SPATA7) [15], lecithin retinol acyltransferase (LRAT) [16], c-mer proto-oncogene tyrosine kinase (MERTK) [17], and IQ motif-containing protein 1 (IQCB1) [18], and one more locus, LCA9 [19]) have been identified. The RDH12 gene, consisting of seven coding exons was identified due to sequence homology to RD11 (originally named PSDR1) [20], and mapped to chromosome 14q23.3 [20,21]. RDH12 mapped to the same region of chromosome 14 as two loci for LCA known as LCA3/LCA13 [22]. In 2004, the first mutations in families mapped to LCA13, were identified [12].

RDH12 expression is highest in the retina, where it localizes to the inner segments of rod and cone photoreceptors [21,23]. The protein sequence places it in the short chain dehydrogenase/reductase family. It was thought to be responsible for the conversion of vitamin A (all-trans retinal) to 11-cis retinal during the regeneration of cone visual pigments. But in the murine model, disruption of RDH12 neither causes a retinal dystrophy nor affects the levels of all-trans and 11-cis retinoids [23]. It has been proposed that RDH12 functions to protect the retina from excessive all-trans...
retinal accumulation in continuous illumination [24,25]. There is some evidence, at least in the mouse retina, that RDH12 may be involved in detoxifying 4-hydroxynonenal in photoreceptor cells [26].

RDH12 mutations have been associated with LCA [27, 28], EORD [12], and with one family of autosomal-dominant retinitis pigmentosa [29]. Published phenotypic data suggests that visual symptoms first develop in early childhood. There is subsequent disease progression with extensive photoreceptor cell loss by adulthood [12,30-32]. Fundus examination at that stage shows a severe pigmentary retinopathy, with macular atrophy and vascular attenuation [12,30-33]. Electroretinographic findings reveal severe generalized loss of rod and cone photoreceptor function.

Here, we report 17 novel mutations in RDH12. To the best of our knowledge, this is the first study associating the clinical presentation with casual mutations in RDH12 in a large cohort.

METHODS

Patient selection: Patients with nonsyndromic autosomal recessive LCA or EORD were ascertained from the medical retina clinics of Moorfields Eye Hospital, London. All patients involved in this study provided written consent as part of a research project approved by the local research ethics committee. All investigations were conducted in accordance with the principles of the Declaration of Helsinki.

Clinical evaluation: All patients underwent age-appropriate assessment of visual acuity on a LogMAR scale and funduscopy. Retinal imaging, including color fundus photography (Topcon TRC 501A retinal camera; Topcon Corporation, Tokyo, Japan), high-resolution spectral domain optical coherence tomography (SD-OCT; Spectralis spectral domain OCT scanner; Heidelberg Engineering, Heidelberg, Germany) or time-domain OCT (TD-OCT; Stratusocut Model 3000 Scanner; Zeiss Humphrey Instruments, Dublin, CA), and retinal autofluorescence (AF) imaging using a confocal scanning laser ophthalmoscope (Zeiss Prototype; Carl Zeiss, Oberkochen, Germany) was performed where nystagmus did not preclude image acquisition and in those who were old enough to cooperate. Electrophysiology had often been previously performed elsewhere, but in those patients who had not undergone previous testing, full field electroretinography and pattern electroretinography were performed. In adults and older children, these were performed using gold foil recording electrodes according to International Society for Clinical Electrophysiology of Vision (ISCEV) standards [34,35]. A modified protocol using orbital surface electrodes was used was used in infants and younger children, as previously described [34-38].

DNA collection: Blood samples were collected in EDTA tubes. DNA was extracted using a Nucleon Genomic DNA extraction kit (BACC2; Tepnel Life Sciences, UK) or a Puregene kit (Invitrogen, Glasgow, UK) following the manufacturer’s instructions.

Apex chip: Genomic DNA from 389 unrelated affected patients were sent to Asper Ophthalmics (Tartu, Estonia) for analysis using the LCA APEX chip, as described previously [39,40]. Samples in which mutations were identified in other LCA genes were excluded from further study. Much of this work has been published elsewhere [39,41-44].

Autozygosity scan: A full genome-wide autozygosity scan was performed using all available members in families 9, 10, and 12. Samples were analyzed using the Affymetrix Gene Chip Human Mapping 50K Xba1 array following the manufacturer’s instructions (Affymetrix, Santa Clara, CA). Detailed methodology for genotyping using the GeneChip array has been previously described [45]. Genotypes for single nucleotide polymorphisms (SNPs) were called by the GeneChip DNA Analysis Software (GDAS v3.0; Affymetrix). A macro was written in Visual Basic within the

| Exon | Primer | PCR annealing (°C) | Size of fragment (bp) |
|------|--------|-------------------|----------------------|
| Exon 1F | TTTCCCCACATTCTTTTGGCC | 54 | 517 |
| Exon 1R | TCCACCATGGATCCACAACCC | 54 | 306 |
| Exon 2F | TAACGATCTTTAGTGAGCTCG | 54 | 405 |
| Exon 2R | TCCTGAATTTCTGATTCAGACC | 54 | 459 |
| Exon 3F | TCACTCTACGGTTGAAGGATGG | 54 | 460 |
| Exon 3R | TGAGCAAGAACCATTAGAG | 54 | 434 |
| Exon 4F | TGACACGTGCATGGTGCACCC | 54 | 884 |
| Exon 4R | TGATTTCTTTTGATCCAGGC | 54 | 2707 |
Nine of 28 mutations identified in this study were located in exon 5 (Figure 1). All 17 novel mutations were absent in 100 ECACC controls or in 50 Asian controls. Where DNA samples from parents and unaffected siblings were available, further analysis demonstrated that the disease segregated with the mutations.

Analysis of all identified missense mutations using in silico methods are shown in Table 3. All three programs identified the p.C70Y, p.R169Q, p.R169W, p.Y200C, and p.R239W mutations as being intolerant or damaging to the protein. For all of the missense mutations, at least one of the programs considered the protein change to be significant.

In total, 28 different alleles in 29 families from various ethnic origins were identified (Table 2). Twelve families were consanguineous, and they harbored homozygous mutations. Two other families also had homozygous mutations, even though they did not report consanguinity. The most common mutation identified was p.C201R (8/58 alleles, 14%). Overall, missense mutations were the most prevalent mutation identified, affecting 38/58 alleles (65%). Nonsense mutations accounted for 8/58 (14%), and frameshift mutations affected 10/58 alleles (17%). The remainder of mutations consisted of a deletion of a codon (2%), and a splice site mutation (2%). Only one coding SNP was identified, rs17852293 (c.482G>A, p.R161Q), located in exon 5.

Clinical phenotype: Appendix 1 summarizes the clinical features of the 32 patients. Twenty-one patients (66%) presented with reduced vision. Nyctalopia (6/32) and visual field constriction (7/32) were predominant features. Twenty-nine patients reported loss of vision that was slowly progressive by age five years. Interestingly, 11 patients reported that their vision dramatically deteriorated further and were able to specify the age at which this had occurred, a median age of 26 years. Fundus examination in adults and older children revealed characteristic dense intraretinal pigment migration throughout the retina that typically approached the macula from the equator in a concentric manner, with severe RPE atrophy and arteriolar attenuation (Figure 2A). The pigmentation showed "para-arteriolar sparing" in seven patients (Figure 2B). In the younger patients (6/32, age range 5–18 years), widespread RPE atrophy was the predominant feature, with pigmentation migration, when present, being confined to the retinal periphery (Figure 2C). Macular atrophy was present in all cases and was associated with striking yellow deposits in 18 patients (56%; Figure 2D). AF imaging in 10 of 13 patients failed to detect any macular AF (Figure 2E), corresponding to the severe macular atrophy. The youngest patients to undergo AF imaging had overall reduced levels of macular AF but also had a hyperautofluorescent signal at the fovea (families 6, 11, and 17; age range of 5–11 years).
Table showing the results of the mutational analysis in our cohort. Mutation type; Hom – homozygous mutation, Het – heterozygous mutation. Ethnic origin key - BC – British Caucasian, OC – Other Caucasian, GM – Gujarati muslim, GH - Gujarati Hindu, A – Afghanistan, P- Pakistani, I – Indian, B – Bangladeshi, KI-Kurdistani Iraqi, SA - Saudi Arabian DP - 1/2 Portuguese 1/2 Dominican Republic

| Family | Method of identification | Ethnic origin | Consang | Mutation Type | Mutations | Reference |
|--------|--------------------------|---------------|---------|---------------|-----------|-----------|
| 1      | Asper                    | BC            | No      | Het           | c.295C>A, p.L99I; c.883C>T, p.R295X | [32]      |
| 2      | Asper                    | GM            | Yes     | Hom           | c.601T>C, p.C201R | [33]      |
| 3      | Asper                    | BC            | No      | Het           | c.715C>T, p.R233X, c.806_810del5bp, p.A269AfsX1 | [32]; [12] |
| 4      | Asper                    | BC            | No      | Het           | c.700G>C, p.R233L, c.806_810del5bp, p.A269AfsX1 | [32]; [12] |
| 5      | Asper                    | BC            | No      | Het           | c.316 C>T, p.R106X, c.806_810del5bp, p.A269AfsX1 | Novel to this study [12]; |
| 6      | Asper                    | BC            | No      | Het           | c.451C>G, p.H151D, c.806_810del5bp, p.A269AfsX1 | Novel to this study [32]; [12] |
| 7      | Asper                    | BC            | No      | Hom           | c.146C>A, p.T49K | Novel to this study |
| 8      | Asper                    | B             | Yes     | Hom           | c.193C>T, p.R65X | [31]      |
| 9      | Asper                    | OC            | No      | Het           | c.506G>A, p.R169Q, c.57_60del, p.P20del | Novel to this study |
| 10     | Asper                    | BC            | No      | Het           | c.209G>A, p.C70Y, c.806_810del5bp, p.A269AfsX1 | Novel to this study [12]; |
| 11     | Asper                    | BC            | No      | Het           | c.144 C>T, p.R62X, c.806_810del5bp, p.A269AfsX1 | [12]      |
| 12     | Affymetrix               | KI            | Yes     | Hom           | c.599A>G, p.Y200C | Novel to this study |
| 13     | Affymetrix               | BC            | Yes     | Hom           | c.454T>A, p.F152I | Novel to this study |
| 14     | Affymetrix/Phenotype     | I             | No      | Het           | c.250C>T, p.R84X, c.381delA, p.G127GfsX1 | Novel to this study |
| 15     | Direct Seq               | A             | Yes     | Hom           | c.609C>A, p.S203R | Novel to this study |
| 16     | Direct Seq               | P             | Yes     | Hom           | c.506G>A, p.R169Q | Novel to this study |
| 17     | Direct Seq               | BC            | No      | Het           | c.505C>T, p.R169W, c.525C>T, p.S175L | Novel to this study [54]; |
| 18     | Direct seq               | PD            | No      | Het           | c.448+1G>A, c.698insGT, p.V233VfsX45 | Novel to this study |
| 19     | Phenotype                | P             | Yes     | Hom           | c.619A>G, p.N207D | Novel to this study |
| 20     | Phenotype                | GM            | Yes     | Hom           | c.601T>C, p.C201R | [33]      |
| 21     | Phenotype                | P             | Yes     | Hom           | c.506G>A, p.R169Q | Novel to this study |
| 22     | Phenotype                | GM            | Yes     | Hom           | c.601T>C, p.C201R | [33]      |
| 23     | Phenotype                | GH            | Unknown | Hom           | c.146C>T, p.T49M | [12]      |
| 24     | Phenotype                | SA            | Yes     | Hom           | c.609C>A, p.S203R | Novel to this study |
| 25     | Phenotype                | KI            | No      | Hom           | c.379G>T, p.G127X | [31]      |
| 26     | Phenotype                | GM            | Yes     | Hom           | c.506G>A, p.R169Q | [33]      |
| 27     | Phenotype                | OC            | No      | Het           | c.481C>T, p.R161W, c.714insC, p.V238VfsX34 | Novel to this study |
| 28     | Phenotype                | OC            | Yes     | Hom           | c.609C>A, p.S203R | Novel to this study |
| 29     | Phenotype                | OC            | No      | Het           | c.481C>T, p.R161W, c.806_810del5bp, p.A269AfsX1 | Novel to this study [12]; |

Table 2. Results of RDH12 mutational analysis.
Ten of 13 patients underwent either Stratus OCT (6/13) or SD-OCT (7/13) imaging, which showed marked macular thinning (Figure 2F). The respective average adult foveal thicknesses observed with TD-OCT and SD-OCT were 133 µm and 56 µm (normal adult mean values: 144 µm and 228 µm [49]). In the adults who underwent SD-OCT imaging, there was marked macular excavation, severe retinal thinning, and loss of the laminar architecture (6/7 patients; Figure 2F). OCT imaging of the three youngest patients, in whom the macula was better preserved on funduscopy, demonstrated a mean foveal thickness of 167 µm (TD-OCT, families 6 and 11) and 114 µm (SD-OCT, family 17), with some preservation of the laminar architecture.

Electroretinography was performed at our institution on nine patients (age range of 2–22 years). This showed undetectable or severely attenuated rod and cone responses, demonstrating severe generalized retinal dysfunction from a very young age. This included five of the seven children below age 16 who otherwise had relatively preserved visual acuities.

**DISCUSSION**

This report on the mutational analysis and detailed description of the phenotype in a cohort of 32 patients with *RDH12* mutations represents the largest such series to be studied to date. Seventeen novel mutations are described.

The majority of the variants identified were missense mutations, with only one SNP found. Several mutations occurred more than once in the present cohort. The most common mutation, occurring in 14% of alleles, was p.C201R, which was found to be homozygous in all patients of Gujurati Indian descent. This mutation has been previously reported in one patient of Indian ancestry [33] and may represent a founder mutation in this population. The p.A269AfsX1 mutation (identifed in 12% of alleles) was found in the compound heterozygous state with another mutation in patients who were all of British Caucasian descent. This mutation was originally described in a German male in the homozygous state [12], making this a northern European mutation. Exon 5 appears to be a mutational hotspot with 9/28 mutations located in it. Therefore, screening of exon 5 in a large cohort of patients could be a first step in the identification of *RDH12* mutations. The novel variant p.R161W affects the same codon as the only SNP seen in the screening of this cohort, rs17852293 (p.R161Q). In silico analysis of this variant was inconclusive, but it has been considered in this paper as a potential disease variant due to its being found in the compound heterozygous state with a frameshift mutation in families 27 and 29.

The characteristic phenotype associated with *RDH12* retinopathy comprises early-onset visual loss between birth and 5 years of age (78% in the present cohort). The visual loss was progressive, leading to severe visual loss in adulthood. The subjective symptoms of nyctalopia and visual field constriction were not frequently reported at the time of...
A recent study of the rhodopsin knockout (rho^{−/−}) mouse, a murine model of human retinitis pigmentosa, demonstrated that the migration of RPE cells along blood vessels within the inner retina is triggered by the close approximation and direct contact of the inner retinal vessels with the RPE [50]. This is a consequence of the loss of photoreceptor cells and subsequent reduction of retinal thickness, which causes an approximation of the inner retinal layers with the RPE. Subsequent bone spicule pigmentation occurs as pigmented cell clusters form over most of the retinal capillaries except for the large surface vessels. This may explain the distribution of the intraretinal pigment in RDH12 retinopathy, and possibly the observation of para-arteriolar sparing, which is also a feature in CRB1 disease [11]. The severe macular atrophy in the RDH12 phenotype is also consistent with the increased susceptibility at the macula to light-induced photoreceptor apoptosis that has been observed in RDH12 knockout mice [24], supporting evidence for the unique role of this protein in the photoreceptor inner segment as a retinoid regulator. The disease mechanism is also not solely dependent upon loss of enzymatic function. It has been shown that some missense mutations in RDH12 retain enzymatic function but are believed to undergo accelerated degradation [51].

RDH12 mutations account for 7% of disease in the cohort of patients with LCA or EORD at this institution, similar to the frequency of CRB1 mutations in the same group of patients [52]. This is higher than the previously published 2.7% [53]. This higher incidence and the number of novel changes may reflect the use of the APEX microarray to identify known and novel changes and the use of detailed phenotypic data. Currently there is no treatment for RDH12 associated disease. However, would a future treatment become available, the optimum time

| Mutation | Exon | SIFT Prediction | Polyphen-2 Prediction | pMUT NN output | Reliability | Prediction |
|----------|------|-----------------|----------------------|---------------|-------------|------------|
| p.T49M   | 2    | Intolerant      | PRD 0.951            | 0.4152        | 1           | Neutral    |
| p.T49K*  | 2    | Intolerant 0.01 | POS 0.888            | 0.6188        | 2           | Pathological |
| p.L91Q*  | 3    | Intolerant      | PRD 0.998            | 0.9223        | 8           | Pathological |
| p.H151D  | 5    | Intolerant      | PRD 0.992            | 0.3323        | 3           | Neutral    |
| p.F152I* | 5    | Intolerant      | PRD 0.968            | 0.2127        | 5           | Neutral    |
| p.R161Q  | 5    | Tolerant 0.38   | Benign 0.018         | 0.513         | 0           | Pathological |
| p.R161W* | 5    | Tolerant 0.18   | POS 0.798            | 0.7723        | 5           | Pathological |
| p.R169Q* | 5    | Intolerant      | PRD 0.997            | 0.5161        | 0           | Pathological |
| p.R169W* | 5    | Intolerant      | PRD 0.999            | 0.8159        | 6           | Pathological |
| p.S175L  | 5    | Intolerant      | PRD 0.997            | 0.2495        | 5           | Neutral    |
| p.Y200C  | 5    | Intolerant      | PRD 0.998            | 0.5467        | 0           | Pathological |
| p.C201R  | 5    | Tolerant 0.1    | POS 0.769            | 0.5209        | 0           | Pathological |
| p.S203R* | 5    | Intolerant      | PRD 0.998            | 0.3381        | 3           | Neutral    |
| p.N207D  | 5    | Intolerant      | PRD 0.994            | 0.1661        | 6           | Neutral    |
| p.Y233L  | 6    | Intolerant      | PRD 0.931            | 0.1899        | 6           | Neutral    |
| p.R239W  | 6    | Intolerant      | PRD 0.998            | 0.9122        | 8           | Pathological |

Changes highlighted by an asterisk are novel missense mutations identified in this study. SIFT results are reported to be tolerant if tolerance index ≥0.05 or intolerant if tolerance index <0.05. Polyphen-2 appraises mutations qualitatively as Benign, Possibly Damaging (POS) or Probably damaging (PRD) based on the model's false positive rate. pMUT is based on the use of different kinds of sequence information to label mutations, and neural networks to process this information NN=neural network values from 0 to 1. >0.5 is predicted as a disease associated mutation. Reliability=values 0–9. >5 is the best prediction.

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of intervention should be at a young age, before the onset of severe retinal pigmentation.

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Appendix 1. Clinical features of patients identified with *RDH12* mutations.

To access the data, click or select the words “Appendix 1.” This will initiate the download of a compressed (pdf) archive that contains the file. Abbreviations: CF represents counting fingers; DS represents diopter sphere; EE represents either eye; F represents female; HM represents hand movements; LE represents left eye; M represents Male, N/A represents not available; PCIOL represents posterior chamber intra ocular lens; PL represents perception of light; PSCLO represents posterior subcapsular lens opacification; RE represents right eye; VF represents visual field.