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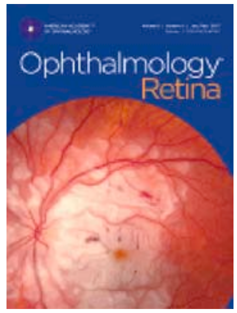

The Natural History of Leber Congenital Amaurosis and Cone-Rod Dystrophy Associated with Variants in the *GUCY2D* Gene

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The Natural History of Leber Congenital Amaurosis and Cone-Rod Dystrophy Associated with Variants in the *GUCY2D* Gene

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This article contains additional online-only material. The following should appear online-only: Supplemental Figures 1, 2 and Supplemental Tables 1, 2, 3, 4.

Abstract

Objective

To describe the spectrum of Leber congenital amaurosis (LCA) and cone-rod dystrophy (CORD) associated with the *GUCY2D* gene, and to identify potential clinical endpoints and optimal patient selection for future therapeutic trials.

Design

International multicenter retrospective cohort study.

Subjects

82 patients with *GUCY2D*-associated CORD and LCA from 54 molecularly confirmed families.

Methods

Data were gathered by reviewing medical records for medical history, symptoms, best-corrected visual acuity (BCVA), ophthalmoscopy, visual fields, full-field electroretinography and retinal imaging (fundus photography, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence).

Main Outcomes Measures

Age of onset, annual decline of visual acuity, estimated visual impairment per age, genotype-phenotype correlations, anatomic characteristics on funduscopy, and multimodal imaging.

Results

Fourteen patients with autosomal recessive LCA and 68 with autosomal dominant CORD were included. The median follow-up time was 5.2 years (interquartile range (IQR), 2.6-8.8) for LCA, and 7.2 years (IQR, 2.2-14.2) for CORD. Generally, LCA presented in the first year of life. The BCVA in LCA ranged from no light perception to 1.00 logMAR, and remained relatively stable during follow-up. Imaging for LCA was limited, but showed little to no

structural degeneration. In CORD, progressive vision loss started around the second decade of life. The annual decline rate of visual acuity was 0.022 logMAR ($P < 0.001$), which did not differ between the c.2513G>A and the c.2512C>T *GUCY2D* variant ($P = 0.798$). At the age of 40 years the probability of being blind or severely visually impaired was 32%. The integrity of the ellipsoid zone (EZ) and external limiting membrane (ELM) on SD-OCT were correlated significantly with BCVA (Spearman's $\rho = 0.744$, $P = 0.001$ and $\rho = 0.712$, $P < 0.001$, respectively) in CORD.

Conclusion

LCA due to variants in *GUCY2D* results in severe congenital visual impairment with relatively intact macular anatomy on funduscopy and available imaging, suggesting a long preservation of photoreceptors. Despite large variability, *GUCY2D*-associated CORD generally presented during adolescence with a progressive loss of vision and culminated in severe visual impairment during mid to late-adulthood. The integrity of the ELM and EZ may be suitable structural endpoints for therapeutic studies in *GUCY2D*-associated CORD.

INTRODUCTION

Leber congenital amaurosis (LCA) and Cone-rod dystrophy (CORD) are relatively common inherited retinal dystrophies (IRDs), which frequently lead to significant visual impairment in young patients.[1-3] Typically, LCA causes severe congenital visual impairment or blindness with absent to minimal residual electroretinography (ERG) responses, often in combination with nystagmus, photophobia, and eye poking.[2,4] Patients with CORD tend to present with mild to moderate loss of visual acuity and/or color vision disturbance within the first decades of life, which generally progresses to severe visual impairment or even legal blindness during adulthood.[5,6] One of the most common genes causing these conditions is the *GUCY2D* gene, which has been found to be responsible for up to 20% of all LCA, and up to 25% of all autosomal dominant CORD cases.[3,4]

The *GUCY2D* gene encodes the enzyme retinal guanylate cyclase 1, which plays a crucial role in photoreceptors recovery to the dark-adapted state by regulating intracellular Ca^{2+} concentration.[7] Currently, there is no treatment available for patients with IRDs associated with *GUCY2D*. However, for *GUCY2D*-associted LCA improvements in electrophysiology and visual function were seen after experimental gene replacement therapy in animal and preliminary human phase I/II studies.[8,9]

Interestingly, the great majority of patients with CORD associated with *GUCY2D* have disease-causing variants in codon 838, out of which up to 87% are the two most prevalent variants described in the current study.[10] The high prevalence of these variants and their proximity to each other make them attractive potential targets for gene editing approaches. In animal models CRISPR/Cas9 was successfully used to knockout *GUCY2D* expression, which may be a viable first step towards a therapeutic approach for *GUCY2D*-associated CORD.[11]

To select the most appropriate design for human gene therapy trials, as well as the most suitable candidates and clinical endpoints for such trials, a thorough understanding of IRDs associated with *GUCY2D* is essential. In addition, patients and clinicians will benefit from such insights to enable more accurate clinical and prognostic information. Therefore, this international multicenter retrospective study aims to deliver a detailed description of the natural course, phenotype and genotype of *GUCY2D*-associated LCA and CORD.

METHODS

Study population and data collection

Patients were collected from the Delleman archive, a large database for inherited eye diseases at the Amsterdam University Medical Centers (Amsterdam, the Netherlands), and various Dutch expertise centers within the RD5000 consortium, a national consortium for IRDs.[12] Data from additional patients were included from the University Hospital Ghent (Ghent, Belgium), as well as 4 academic tertiary referral centers in the United Kingdom (Moorfields Eye Hospital, St James`s University Hospital Leeds, Oxford Eye Hospital, University Hospital Southampton). For inclusion, patients needed either a molecularly confirmed diagnosis, or a typical clinical presentation and an affected first-degree family member with confirmed disease-causing *GUCY2D* variants. This study was approved by the ethics committee of the Erasmus Medical Center and adhered to the tenets of the Declaration of Helsinki. Eighteen CORD patients from 4 families have been described earlier, but substantial new follow-up data are described in this article.[13-15] Additionally, for 13 of these patients from a large Dutch family the *GUCY2D* gene had not been identified as disease-causing gene in the previous publication from 1992.[15]

Data collection was performed by standardized review of medical records for medical history, age at onset, symptoms, family history, best-corrected visual acuity (BCVA), refractive error, dilated fundus examination, full-field ERG, Goldmann visual field testing, color vision testing, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence (FAF) images, and color fundus photography. Measurements of retinal thickness and evaluation of layer integrity on SD-OCT were performed within a radius of 500µm around the foveal center in the Heidelberg Eye Explorer (Heidelberg Engineering, Heidelberg, Germany). The integrity of EZ and ELM were graded as either intact, disrupted or absent by two independent graders (L.C.H., H.A.). The graders were also masked for other image modalities. Weighted Cohen`s kappa analyses was performed and showed a high inter-rater reliability for the integrity of EZ ($\kappa = 0.892 \pm 0.03$, $P < 0.001$) as well as for ELM integrity ($\kappa = 0.918 \pm 0.02$, $P < 0.001$). For the 12 cases in which the graders disagreed, a trained expert referee (C.J.F.B.) decided on the subgroup. The autofluorescence patterns on FAF images were described for each patient by a retinal specialist (C.J.F.B.) and the frequencies for each pattern were subsequently reported.

Statistical analysis

For statistical analysis, SPSS version 23.0 (IBM, Corp, Armonk, NY) and the R software environment were used.[16] Categorical data were expressed in proportions. Continuous data were reported in either mean with standard deviation or median with interquartile ranges (IQR). A multistate model was used to estimate the disease progression based on categories of visual impairment, with the R-package msm. The following states were used as defined by World Health Organization based on the better-seeing eye: mild visual impairment ($20/67 \leq BCVA < 20/40$), moderate visual impairment ($20/200 \leq BCVA < 20/67$), severe visual impairment ($20/400 \leq BCVA < 20/200$), and blindness ($BCVA < 20/400$).[17] Linear mixed models were applied to analyze the annual decline rate of BCVA using data of both eyes. To account for inter-eye correlation a random intercept was added to the model.[18] For this analysis, the BCVA values were converted to logarithm of the minimum angle of resolution (logMAR). For hand movement a value of 2.7, for light perception 2.8, and for no light perception 2.9 logMAR was used. Asymmetry of BCVA was defined as minimum difference of 0.3 logMAR between the right and left eye at two consecutive visits.[19]

RESULTS

Participants

In total, 82 patients from 54 families were included in this study. Fourteen (17.1%) were diagnosed with autosomal recessive LCA and 68 (82.9%) with autosomal dominant CORD. For 65 out of 82 (79.3%) patients, follow-up data were available with a median follow-up time of 5.2 year (IQR: 1.2-10.8) and a mean number of visits of 3.0 (IQR: 2.0-6.5). The clinical characteristics of the cohort per group are presented in Table 1.

Disease onset, reported symptoms and visual acuity

The median age at onset for LCA was 0.3 years with a range of 0.0-2.2 years, and patients presented with congenital severe visual impairment. Nystagmus was reported in 11/14 (78.6%) patients, photophobia in 6/14 (42.9%), eye poking in 5/14 (35.7%), and nyctalopia in 1/14 (7.1%). Visual acuity data were available for 12/14 (85.7%) patients and ranged from no light perception to 1.0 logMAR (20/200 Snellen equivalent). The visual acuity did not change significantly during follow-up ($P = 0.811$).

In CORD, the reported median age at onset was 14.6 years (IQR: 10.0-26.5 years). Patients presented with mild central vision loss and/or color vision disturbances (Table 1). In one Dutch family, extensive color vision testing in 3 young asymptomatic family members revealed a tritan color vision disturbance as first symptom, and loss of visual acuity only started to develop at later age. The BCVA declined with a rate of 0.022 logMAR annually in CORD patients ($P < 0.001$) based on data of both eyes (Fig 1). The probabilities of being in different states of visual impairment based on the better-seeing eye per age are illustrated in

Figure 1 and Supplemental Table 1 (available at www.ophthalmologyretina.org). The BCVA between eyes was strongly correlated (Spearman`s $\rho = 0.897$, $P < 0.001$). Asymmetry in BCVA between eyes was seen in 1/14 (7.1%) LCA patients and 13/67 (19.4%) CORD patients.

Ophthalmic findings, funduscopy and multimodal imaging

In 3/14 (21.4%) of LCA patients, an abnormal foveal reflex was described in funduscopic reports. Two (14.3%) LCA patients, were reported to have peripheral mottled RPE pigmentation and 1/14 (7.1%) patient with RPE atrophy in the peripheral retinal. Bilateral optic disc pallor was found in 1/14 (7.1%) patient. SD-OCT and FAF imaging was available for 8 out 14 (57.1%) LCA subjects, but the imaging quality was variable due to nystagmus, and only 3/14 (21.4%) had macular scans of sufficient quality. These 3 SD-OCT scans showed a remarkably intact retinal structure and photoreceptor layers in the peripheral and central retina (Fig 2). On FAF imaging in 3/8 (37.5%) LCA patients, central autofluorescence was slightly increased, resulting in a lack of normal foveal hypoautofluorescence (Fig 2F). Three LCA patients (21.4%) had complications likely caused by eye poking, which included bilateral keratoconus, disrupted Descemet’s membranes, hydrops, leucoma, corneal scars, and cortical cataract. The cortical cataract developed at the age of 6 in the right eye and the patient underwent uncomplicated cataract surgery with intraocular lens insertion one year later. The BCVA before and after surgery was light perception.

The most prominent funduscopic findings described in CORD patients included macular RPE atrophy in 16/65 (24.6%), macular RPE alteration in 18/65 (27.7%), macular mottling in 20/65 (30.8%), and bull’s-eye maculopathy in 6/65 (9.2%) patients, based on the descriptions in the case notes. In the peripheral retina, RPE atrophy was reported in 1/65 patients (1.5%), RPE alterations in 4/65 patients (6.2%) and lattice-like lesions in 1/65 patients (1.5%). Attenuated retinal blood vessels were recorded in 10/65 (15.4%) and cataract in 8/65 CORD patients (12.3%). Myopia was found in 24/35 (68.6%) patients and high myopia (more than -6 diopters) in 10/35 (28.5%) patients. Patients with high myopia did not show a more severe annual decrease of BCVA compared to the rest of the cohort ($p = 0.738$).

SD-OCT scans were available for 46/68 (67.6%) and FAF images for 45/68 (66.2%) CORD patients. The imaging showed increased central autofluorescence in early stages, which progressed towards macular atrophy and central absence of autofluorescence (Fig 3). All patient had FAF abnormalities. The frequency of FAF patterns found in this cohort together with integrity of EZ and ELM on SD-OCT are displayed in Table 2. One patient had drusenoid hyperautofluorescent dots on FAF (Fig 4D-H). Disruption as well as absence of the foveal EZ and ELM layer correlated strongly with a decrease in BCVA (Spearman`s $\rho = 0.744$, $P < 0.001$ and, Spearman`s $\rho = 0.712$, $P < 0.001$, respectively). The median central retinal thickness (CRT) was 142.0 µm (IQR: 119.5–189.0 µm) and annually decreased by 2.7 µm ($P < 0.001$). The CRT correlated strongly between eyes (Spearman`s $\rho = 0.933$, $P < 0.001$), but only showed a moderate negative correlation with BCVA (Spearman`s $\rho = -0.551$, $P < 0.001$). Correlation plots regarding the CRT and integrity of the EZ and ELM are displayed in Supplemental Figure 1 (available at www.ophthalmologyretina.org). In 8/46 patients (17.4%), an hyporeflective space (optical gap) beneath the macula was seen on SD-OCT at a median age of 49.0 years (range: 21.2-68.0 years; Fig 3D).

Full-field electroretinography and visual fields

ERG findings were recorded in 5/14 (35.7%) patients with LCA, at a median age of 1.4 years (range: 0.2-1.8 years), of whom all had fully extinguished scotopic and photopic responses (Table 1).

Full-field ERG examination data were available for 35/68 (51.5%) CORD patients. Most commonly, an isolated cone dysfunction was seen in 17/35 (48.6%) patients, at a median age of 33.3 years (IQR: 30.3-38.3 years), and a cone-rod pattern in 14/35 (40.0%) patients at a median age of 35.7 years (IQR: 30.2-46.9 years). Four patients (11.4%) had a normal ERG at a median age of 19 years (range: 5-34 years). Goldmann visual fields were available for 12/68 (16.6%) CORD patients. In 9/12 patients (75.0%), the Goldmann visual field showed a central scotoma, in 2/12 (16.6%) a ring scotoma, and no visual field defects in 1/12 (8.3%) patients.

Genetic characteristics and genotype-phenotype correlations

In the LCA patients, a total of 14 different *GUCY2D* variants were identified. Ten (71.4%) of these variants were missense mutations, 3 (31.4%) were nonsense mutations, and 1 (7.1%) was a frameshift mutation (Supplemental Table 2 and 3, available at www.ophthalmologyretina.org). Two of the LCA patients were siblings (patient 27 and 28), while the remaining 12 patients were isolated cases. The most common variant in these patients was c.1694T>C (p.(Phe565Ser)), which was found in 4 patients, with 3 being homozygous and 1 patient being compound heterozygous. Four patients were compound heterozygous for c.2302C>T (p.(Arg768Trp)) with different variants on the other allele. The two siblings mentioned before both had a homozygous c.2773G>T (p.(Glu925*)) variant, which has not been reported before. Other novel variants in this cohort were the c.518dup (p.(Tyr173*)), c.2861_2862del (p.(Leu954fs*16)), and c.2939A>C (p.(His980Pro)) variants. The c.2620G>A (p.(Glu874Lys)) found in patient 12 has been reported earlier reported in a case of autosomal recessive CORD, but to our knowledge not in LCA yet.[20]

In CORD, 3 different variants were found, all of which involved codon 838 (Supplemental Table 3 and 4, available at www.ophthalmologyretina.org). The most common variant was c.2513G>A (p.(Arg838His)), which was found in 37/68 (54.4%) patients from 16 different families. This included one large multigenerational family of 15 affected members from the Netherlands. The c.2512C>T (p.(Arg838Cys)) variant was present in 30/68 (44.1%) patients from 24 families. The aforementioned variants were both missense variants. One CORD patient (1.5%) had the complex (p.(Glu837_Arg838delinsAspSer)) variant and had an atypical phenotype with extensive atrophy in the entire inferior pole (Fig 4A-C). The BCVA at baseline and the rate of BCVA decline did not differ between patients with the c.2513G>A and the c.2512C>T variant (P = 0.625 and P =0.748, respectively; see Supplemental Fig 2, available at www.ophthalmologyretina.org).

DISCUSSION

This large multicenter retrospective cohort study describes the spectrum of phenotypes, genotypes and natural course of 14 autosomal recessive LCA and 68 autosomal dominant CORD patients from 54 presumably unrelated families with disease causing variants in the *GUCY2D* gene.

The LCA patients in this cohort showed a severe congenital visual impairment, often in combination with nystagmus and photophobia. This presentation has also been observed in LCA associated with other genes.[2] However, in contrast to many other LCA-associated genes, the visual acuity, albeit often severely reduced, appeared to remain relatively stable during follow-up in this cohort of *GUCY2D*-associated LCA patients, and only subtle retinal changes were reported on funduscopy. In the 3 LCA patients with SD-OCT imaging of sufficient quality, a relatively intact retinal structure was observed. This is in accordance with earlier studies on *GUCY2D*-associated LCA, in which slight changes of visual acuity and retinal structure were mostly described in patients above 47 years of age.[4] However, other studies have shown possible slow decrease of foveal outer nuclear layer thickness and decrease of EZ reflectivity in *GUCY2D*-associated LCA.[21,22] The description of patients of older age and with longer follow-up periods in future studies may be informative to better determine the long-term functional as well as structural natural course of LCA associated with *GUCY2D*.

The study by Bouzia et al. also found 6 relatively milder LCA cases, with two patients even having a visual acuity of 20/60.[4] This was hypothesized to be due to the presence of at least one missense variant in *GUCY2D* exon 2 in these patients, which presumably causes a less severe phenotype.[4] In our study on the contrary, visual acuity ranged from no light perception to only 20/200, and the only included patient with a missense variant in exon 2 (c.587A>T (p.(Glu196Val)) had a BCVA of 20/400 at an age of 24 years.

In CORD patients of this cohort, disease onset occurred in the second decade of life, and showed a gradual decline in visual acuity of 0.022 logMAR per year. At 56 years, there was a 50% estimated probability of having severe visual impairment or being blind based on the better-seeing eye. Funduscopic reports and imaging in CORD showed a progressive development of macular atrophy with increasing age, which can also involve the peripapillary area. The majority of *GUCY2D*-associated CORD patients were myopic; some had severe myopia, which is in accordance with earlier studies.[5,6,10]

Eight CORD patients had a hyporeflective space (optical gap) beneath the macula on SD-OCT (Fig 3J) mimicking subretinal fluid. However, this phenomena has been described previously in IRDs, and is hypothesized to not represent primary leakage of fluid, but rather an empty space, as a result of outer photoreceptor tissue loss.[23]

The chorioretinal atrophy in advanced cases of *GUCY2D*-associated CORD can also resemble end-stage central areolar choroidal dystrophy associated with the *PRPH2* gene, or geographic atrophy found in end-stage atrophic age-related macular degeneration.[24-26] The resemblance with AMD has also been described in other IRDs, but the absence of drusen and in most cases an early onset around the second decade of life in *GUCY2D*-associated CORD is helpful in differentiating the condition from AMD.[26] However, one patient displayed drusenoid hyperautofluorescent dots on FAF and later developed atrophy (Fig. 3D-H), which could complicate differentiation.

It is noteworthy that 3 patients, who were screened for CORD at young age due to known affected family members, had an isolated tritan color vision disturbance, without other clear abnormalities or symptoms.[15] This could indicate that the S-cones, which are sensitive to blue light, may be affected first in *GUCY2D*-associated CORD, and tritan color vision defects may be the earliest measurable symptoms. This finding has not only been described earlier in young *GUCY2D*-associated CORD patients, but has also been found in CORD associated with pathologic variants in the *IRXB* gene cluster.[27,28] The early S-cone dysfunction may be due to the fact that these cones are more vulnerable to light-induced cell damage, which may be exacerbated by an underlying genetic condition.[29] Another explanation could be that the area around the foveal center, which contains the majority of S-cones, is affected first in some IRDs.

The c.2513G>A (p.(Arg838His)) and c.2512C>T (p.(Arg838Cys)) variants have been by far the most common in *GUCY2D*-associated CORD in the current cohort, which is similar to earlier studies describing 87% of CORD cases to be associated with these variants.[10,30] A literature review of 132 *GUCY2D*-associated CORD patients reported that the c.2513G>A (p.(Arg838His)) variant may be associated with a more severe phenotype compared to the c.2512C>T (p.(Arg838Cys)) variant, which was not the case in this cohort of 68 patients.[10] Considerable phenotypic variability was observed in this study between subjects, even within families carrying the same *GUCY2D* variant. For example, in the earlier described large family with the c.2513G>A (p.(Arg838His) variant one patient had a BCVA of 20/33 and another a BCVA of 20/285 both at an age of 35 years. This high degree of interindividual variability, even within families, is well-known in other autosomal-dominantly IRDs such as those associated with the *BEST1* and *PRPH2* genes.[31,32] The large phenotypic variability in autosomal dominant diseases has been attributed to many factors such as genetic modifiers, allelic variation, variable gene expression or environmental factors.[33-36] Incomplete penetrance and variable expressivity has also been documented in other *GUCY2D*-associated CORD cohorts, but no clear underlying mechanism or combination of factors has been discovered yet.[13] We observed a high degree of symmetry in BCVA between eyes in LCA as well as CORD patients, which would support the use of the fellow-eye as an untreated control eye in future trials. Furthermore, our data suggest that there may be a relatively large window of opportunity for gene therapy in *GUCY2D*-LCA, since visual acuity and photoreceptor layers on the available imaging seemed to remain relatively stable during follow-up. In *GUCY2D*-associated CORD severe visual impairment generally started to occur around the fourth decade of life, which suggests that the optimal window of opportunity for future treatments may lay within the first three to four decades of life. However, some CORD patients above this age showed relatively intact photoreceptor layers and visual acuity, which would suggest that older individuals may also be eligible for future therapeutic intervention. An increasingly compromised integrity of the EZ and ELM layers, as well as a decrease in CRT, correlated significantly with a decrease in visual acuity, which has also been found previously for several other retinal dystrophies.[37,38] This may imply that these structural parameters may be suitable criteria for treatment candidate selection, and may serve as (surrogate) endpoints in future therapeutic trials. However, prospective natural history study may be necessary to validate and possibly further develop these endpoints by possibly also including quantitative measurement of changes in these layers. The retrospective nature of this study is associated with certain inherent limitations, such as variability in available data and in intervals between visits. Especially for the LCA patients qualitative sufficient imaging as well as long-term follow-up was limited, and future studies with more long-term follow-up may be needed to better understand the spectrum and natural course of *GUCY2D*-LCA. Furthermore, similar to earlier cohorts only a few different variants have been described for CORD patients in the current study, which may not represent the entire phenotypic spectrum for *GUCY2D*-associated CORD.[10,30,39] Nonetheless, we have described the currently largest cohort of *GUCY2D*-associated retinal dystrophies and included longitudinal data contrary to most earlier studies. Studies as the current one are not only important to facilitate clinical counseling, but can also aid in choosing the optimal design for future therapeutic as well as prospective natural history studies.

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Legends Figures

Figure 1. Evolution of visual acuity in autosomal dominant cone-rod dystrophy associated with *GUCY2D*. **A,** Plot of linear mixed effects model using the cross-sectional and longitudinal best-corrected visual acuity (BCVA) measurements of the both eyes to predict the average evolution of BCVA with increasing age. In the entire population of *GUCY2D*-CORD, a significant annual decline of 0.022 logMAR ($P < 0.001$) was found. **B**, Graph displaying the disease progression estimated by a multistate model in the better-seeing eye. The curves represent the probabilities of having mild visual impairment (20/67 ≤ BCVA < 20/40), moderate visual impairment (20/200 ≤ BCVA < 20/67), severe visual impairment (20/400 ≤ BCVA < 20/200), and blindness (BCVA <20/400). The distances between the lines indicate the probability of being in a certain category of visual impairment at each age. The distance between: 1) the 0% mark and the green line represents the probability of having no significant visual impairment; 2) between the green and blue line represents the probability of having mild visual impairment; 3) between the blue and yellow line represents the probability of having moderate visual impairment; 4) between the yellow and red line represents severe visual impairment; 5) between the red line and 100% mark represents the probability of being blind, for each individual age. At an age of 40 years the multistate model estimated that *GUCY2D*-CORD patients have a probability of 21.8% to have no significant visual impairment, 5.5% to have mild visual impairment, 41.1% to have moderate visual impairment, 12.4% to have severe visual impairment, and 19.2% to be blind (for further specification of all probabilities at different ages see Supplemental Table 1, available at www.ophthalmologyretina.org). BCVA, best corrected visual acuity; FC, finger counting; HM, hand movements; logMAR, logarithm of the minimum angle of resolution.

Figure 2. Findings on imaging in two patients with *GUCY2D*-associated Leber congenital amaurosis. **A, B, C, D,** Fundus photograph, fundus autofluorescence, near-infrared image and spectral-domain optical coherence tomography (SD-OCT) scan of a 10-year-old patient with heterozygous missense variants in *GUCY2D* (c.2620G>A; p.(Glu874Lys) and c.2939A>C; p.(His980Pro)). On color fundus photography (**A**), mild foveal RPE alterations and on SD-OCT (**D)** an intact retinal anatomy was seen. On fundus autofluorescence (**B**), hyperautofluorescent changes in the fovea were seen, surrounded by a slightly hypoautofluorescent ring with hyperautofluorescent borders. The best-corrected visual acuity (BCVA) was 20/400 in both eyes. **E, F, G, H** Fundus photograph, fundus autofluorescence, near-infrared image and SD-OCT scan of a 28-year-old patient (homozygous c.1694T>C; p.(Phe565Ser) *GUCY2D* variants) with nearly normal appearance on imaging. The BCVA was light perception in both eyes.

Figure 3. Spectrum of findings on imaging in patients with *GUCY2D*-associated cone-rod dystrophy (CORD). **A,** Normal fundus autofluorescence image of a 30-year-old healthy male as reference showing a typical central hypoautofluorescence in the macular area. **B, C,** Fundus autofluorescence image and spectral-domain optical coherence tomography (SD-OCT) scan of a 17-year-old-patient (c.2513C>T; p.(Arg838Cys) variant in *GUCY2D*). On fundus autofluorescence (**B**), an absence of normal central hypoautofluorescence can be seen compared to the healthy reference (**A**). SD-OCT (**C**) showed a continuous external limiting membrane (ELM) and ellipsoid zone (EZ), with possibly mild irregularity of the subfoveal EZ. The visual acuity was 20/33 in the right and 20/32 in the left eye. **D, E, F,** Fundus photograph, fundus autofluorescence image and SD-OCT scan of the left eye of a 14-year-old patient (c.2513C>T; p.(Arg838Cys) variant in *GUCY2D*). The fundus photograph was normal, but an absence of foveal hypoautofluorescence was observed (**E**). On the SD-OCT scan (**F**) the central EZ layer seemed slightly thickened. Visual acuity was 20/80 in both eyes. **G, H, I, J** Fundus photograph, fundus autofluorescence image and SD-OCT scan of the left eye of a 26-year-old patient (c.2513C>T; p.(Arg838Cys) variant in *GUCY2D*) with a relatively normal presentation on the color fundus photograph and fundus autofluorescence image. On SD-OCT (**I**) at the age of 26, slight focal disruption of the EZ was present, with an ELM in the macular area that was largely intact and a best-corrected visual acuity (BCVA) of 20/50. Interestingly, one year later this patient developed bilateral, hyporeflective spaces (optical gap) in the central maculae, mimicking subretinal fluid accumulation (**J**). The BCVA dropped to 20/100 in the left eye at this last visit. **K, L, M,** Color fundus photograph, fundus autofluorescence and SD-OCT image of a 28-year-old patient (c.2512C>T; p.(Arg838Cys) variant in *GUCY2D*) showing macular atrophy. The color fundus photograph (**K**) showed atrophic macular mottling, fundus autofluorescence (**L**) showed a small hyperautofluorescent center surrounded by a hypoautofluorescent ring with a hyperautofluorescent border. SD-OCT

(**M**) revealed macular thinning with an absent central EZ and ELM and attenuated outer nuclear layer (BCVA in both eyes: 20/400). **N, O, P,** Color fundus photograph, fundus autofluorescence and SD-OCT image of the right eye of a 52-year-old patient (c.2513G>A; p.(Arg838His) *GUCY2D* variant) with marked atrophy of the central macula. Fundus photography shows central chorioretinal atrophy (**N**). This area corresponds to an absence of autofluorescence (**O**), and a profound atrophy of the central retina on SD-OCT (**P**). **Q, R, S, T** Color fundus photograph, fundus autofluorescence and SD-OCT of a 46-year-old patient (c.2512C>T; p.(Arg838Cys) *GUCY2D* variant) with extensive macular, parapapillary and also peripheral atrophy. An absence of autofluorescence can be seen in a relatively large central area and around the optic disc, surrounded by a hyperautofluorescent border (**R**). Thinning of the retina around the optic disc can also be seen on SD-OCT (**S, T**). The BCVA of this eye was counting fingers in both eyes.

Figure 4. Atypical imaging findings in cone-rod dystrophy associated with GUCY2D **A, B, C** Fundus autofluorescence, near-infrared image, and SD-OCT scan of a patient (c.2511_2512delinsCA (p.(Glu837_Arg838delinsAspSer) complex variant) with an atypical *GUCY2D*-CORD phenotype with extensive atrophic changes in the entire inferior pole. The visual acuity was 1.8 logMAR. **D,** Fundus autofluorescence image of a patient (c.2513G>A p.(Arg838His) *GUCY2D* variant) with drusenoid hyperautofluorescent dots and an optical gap on SD-OCT (**F**). During follow-up the patient developed macular atrophy (**F, G, H**).

Table 1. Clinical Characteristics of Patients with *GUCY2D*-Associated Leber Congenital Amaurosis and Cone-Rod Dystrophy

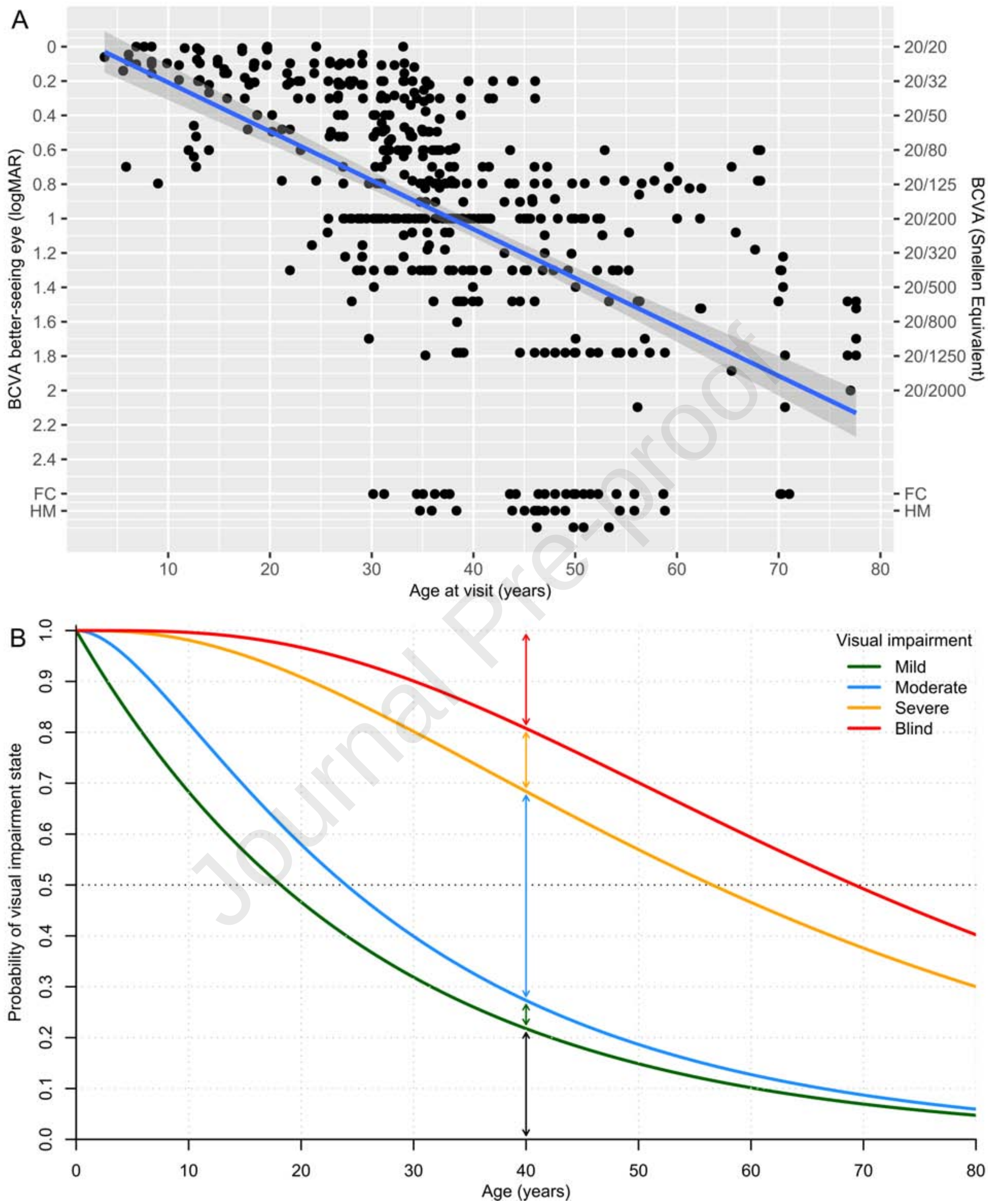
Characteristics	LCA (n = 14)	CORD (n = 68)
Gender		
Male, n (%)	6/14 (43%)	32/68 (47%)
Female, n (%)	8/14 (57%)	36/68 (53%)
Age at onset, n (%)[b]	14/14 (100%)	49/68 (72%)
Median (IQR), years	0.3 (0.0-0.6)	14.7 (10.0-26.5)
Range, years	0.0-2.2	2.0-40.0
Age at last examination		
Mean ± SD, years	13.2 ± 11.3	42.5 ± 16.3
Median (IQR), years	9.9 (7.1-16.4)	40.5 (32.1-54.0)
Range, years	0.6-35.6	9.9-78.4
Number of visits		
Mean ± SD	4.2 ± 2.0	5.3 ± 4.0
Median (IQR)	3.0 (3.0-5.0)	4.0 (3.0-7.0)
Range	2.0-8.0	2.0-21.0
Follow-up time, n (%)[b]	12/14 (86%)	53/68 (78%)
Median follow-up time (IQR), years	5.2 (2.6-8.8)	7.2 (2.2-14.2)
Range, years	0.2-15.9	0.3-77.7
Spherical equivalent refraction, n (%)[b]	6/14 (43%)	35/68 (52%)
Median (IQR), D	2.3 (2.0 to 4.9)	-4.1 (-6.6 to -0.6)
Range, D	1.4 to 9.0	-18.8 to 2.6
High myopia (<-6), n (%)	0/13 (0%)	10/35 (29%)
Moderate myopia (-3D > SER ≥ -6D), n (%)	0/13 (0%)	8/35 (23%)
Mild myopia (-0.75D > SER ≥ -3D), n (%)	0/13 (0%)	6/35 (17%)
SER ≥ -0.75, n (%)	6/6 (100%)	11/35 (31%)
Last available BCVA better-seeing eye, n (%)[b]	12/14 (86%)	67/68 (99%)
Median BCVA, logMAR (IQR)	2.8 (1.4-2.8)	0.9 (0.26-1.3)
Median BCVA, Snellen equivalent (IQR)	LP (LP to 20/500)	20/150 (20/400-20/36)
Last available BCVA worse-seeing eye, n (%)[b]	12/14 (86%)	67/68 (99%)
Median BCVA, logMAR (IQR)	2.8 (2.6-2.8)	1.0 (0.5-1.6)
Median BCVA, Snellen equivalent (IQR)	LP (LP to CF)	20/200 (20/660-20/67)
Recorded symptoms[a], n (%)[b]	14/14 (100%)	65/68 (96%)
Nystagmus	11/14 (79%)	0/65 (0%)
Photophobia	6/14 (43%)	24/65 (37%)
Nyctalopia	1/14 (7%)	1/65 (2%)
Color vision disturbance	1/14 (7%)	14/65 (22%)
Full-field electroretinogram pattern, n (%)[b]	5/14 (36%)	35/68 (52%)
Scotopic and photopic extinguished	5/5 (100%)	0/35 (0%)
Cone isolated	0/5 (0%)	17/35 (49%)
Cone-rod pattern	0/5 (0%)	14/35 (40%)
Normal	0/5 (0%)	4/35 (11%)

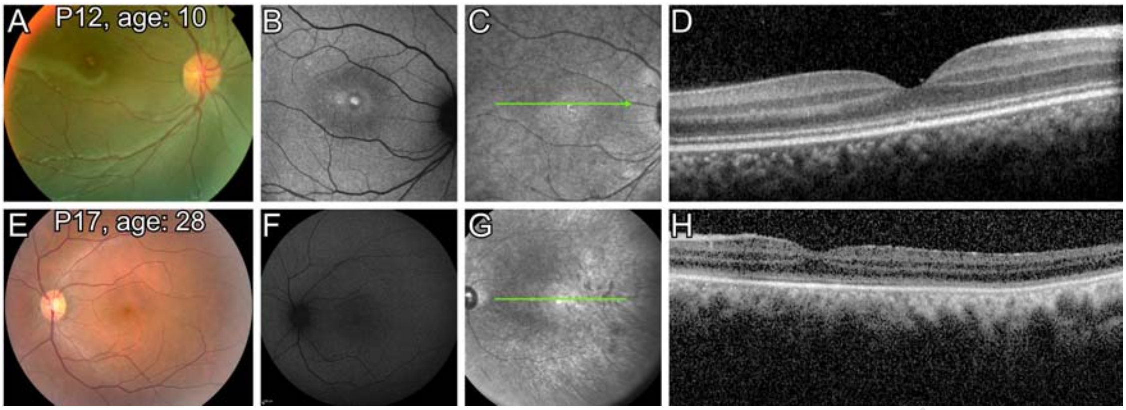
[a] Patients could present multiple different symptoms simultaneously; [b] availability of data. BCVA, best-corrected visual acuity; D, diopters; CF, counting fingers; LP, light perception; IQR, interquartile range; logMAR, logarithm of the minimum angle of resolution; RPE, retinal pigment epithelium; SD, standard deviation; SER, spherical equivalent refraction.

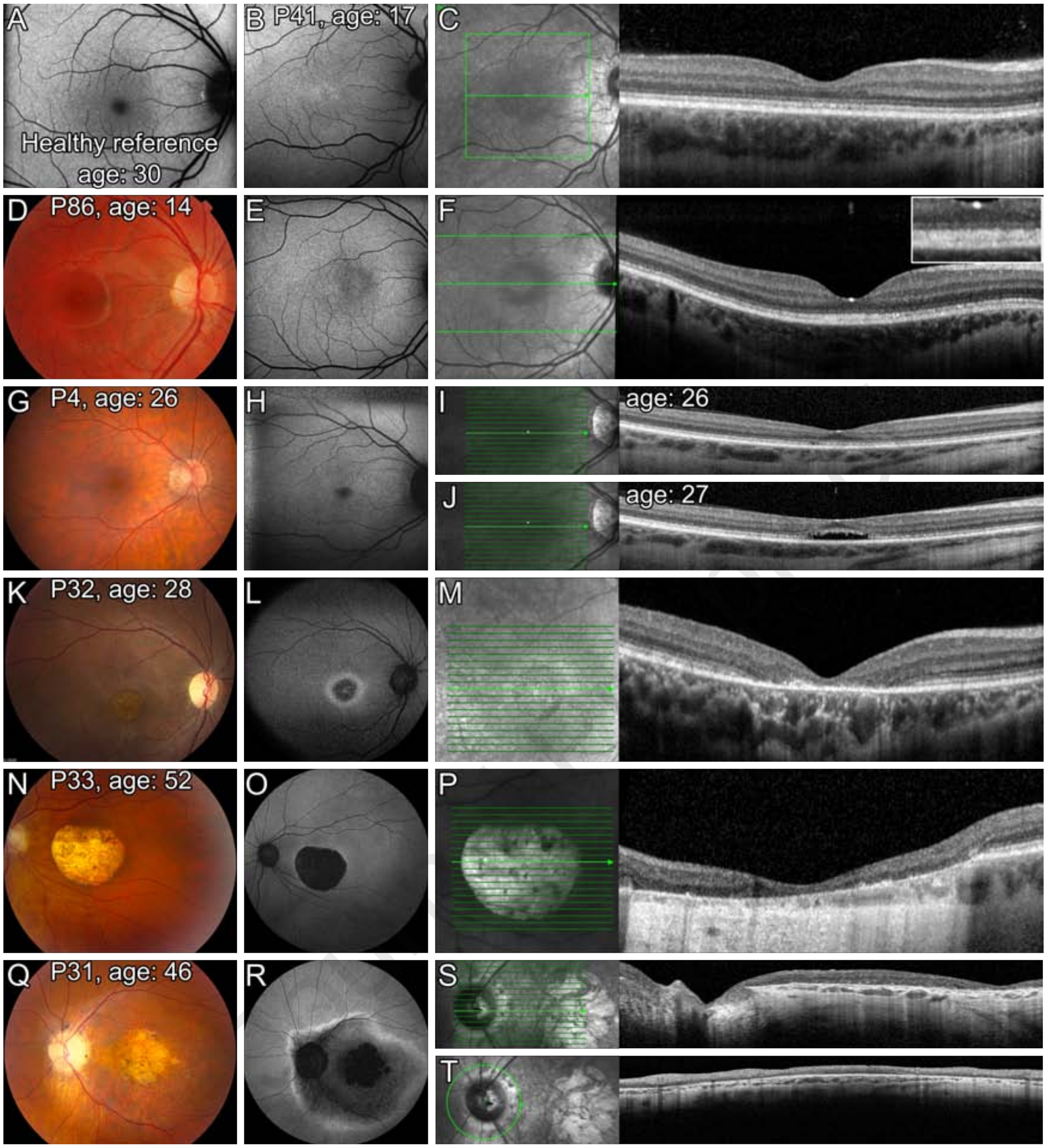
Table 2. Imaging Findings in Patients with GUCY2D-Associated Cone-Rod Dystrophy and Leber Congenital Amaurosis at first visit

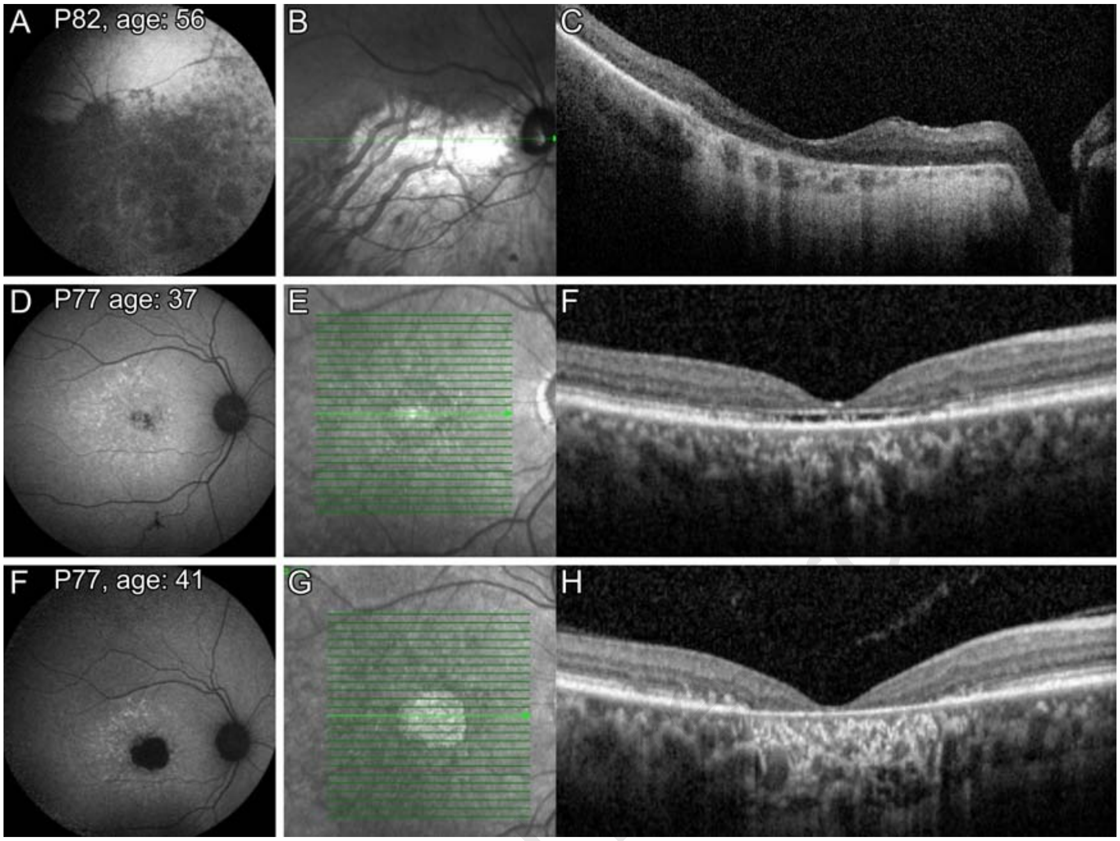
	Cone-rod Dystrophy	**n (%)**	**Age (years)**
FAF	Increased foveal AF or hyper-AF	16/45 (36%)	5.5-67.9
	Increased foveal AF or hyper-AF with hyper-AF borders	4/45 (9%)	12.9-56.5
	Central granular AF with hyper-AF borders	13/45 (29%)	33.2-65.4
	Central AF absence with hyper-AF borders	6/45 (13%)	36.2-49.6
	Central AF absence without hyper-AF borders	4/45 (9%)	36.2-49.6
	Other FAF pattern	2/45 (4%)	35.7- 54.2
	Structural Integrity on SD-OCT		
EZ	Intact	9/47 (19%)	5.6-37.0
	Disrupted	9/47 (19%)	12.5-68.0
	Absent	29/47 (62%)	27.4-70.2
ELM	Intact	18/46 (39%)	5.6-68.0
	Disrupted	4/46 (9%)	12.8-37.0
	Absent	24/46 (52%)	27.4-70.2
	Leber Congenital Amaurosis	**n (%)**	**Age (years)**
	Normal FAF pattern	1/5 (20%)	15.5
	Increased foveal AF or hyper-AF	2/5 (40%)	9.8-28.9
	Foveal hyper-AF with hyper-AF borders	1/5 (20%)	10.3
	Hyper-AF ring only	1/5 (20%)	28.1
	Structural Integrity on SD-OCT		
EZ/ELM	Intact	3/3 (100%)	12.0-28.0

AF, autofluorescence; ELM, external limiting membrane; EZ, ellipsoid zone; FAF, fundus autofluorescence; SD-OCT, spectral-domain optical coherence tomography.


A
BCVA better-seeing eye (logMAR)
BCVA (Snellen Equivalent)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
2.2
2.4
FC
HM
20/20
20/32
20/50
20/80
20/125
20/200
20/320
20/500
20/800
20/1250
20/2000
FC
HM
10
20
30
40
50
60
70
80
Age at visit (years)
B
Probability of visual impairment state
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
10
20
30
40
50
60
70
80
Age (years)
Visual impairment
Mild
Moderate
Severe
Blind


A
P12, age: 10
B
C
D
E
P17, age: 28
F
G
H


A
Healthy reference
age: 30
B P41, age: 17
C
D P86, age: 14
E
F
G P4, age: 26
H
I
age: 26
J
age: 27
K P32, age: 28
L
M
N P33, age: 52
O
P
Q P31, age: 46
R
S
T


A P82, age: 56
B
C
D P77 age: 37
E
F
F P77, age: 41
G
H

Précis

Leber congenital amaurosis and cone-rod dystrophy associated with the *GUCY2D* gene may be promising candidates for future gene therapy due to their natural history and genetic properties.