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Photoreceptor Layer Topography in Children with Leber Congenital Amaurosis Caused by *RPE65* Mutations

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Abstract

PURPOSE—To study the topography of photoreceptor loss early in the course of Leber congenital amaurosis (LCA) caused by *RPE65* mutations.

METHODS—Young patients with *RPE65*-LCA ($n = 9$; ages, 6–17 years) were studied with optical coherence tomography (OCT) in a wide region of central retina. Outer nuclear layer (ONL) thickness was mapped topographically and compared with that in normal subjects and in older patients with *RPE65*-LCA.

RESULTS—Photoreceptor layer topography was abnormal in all young patients with *RPE65*-LCA. Foveal and extrafoveal ONL was reduced in most patients. There were interindividual differences, with ONL thicknesses at most retinal locations ranging from near the detectability limit to a significant fraction of normal. These differences were not clearly related to age. In most patients, there was a thinner ONL inferior to the fovea compared with that in the superior retina. Summary maps obtained by aligning and averaging photoreceptor topography across all young patients showed a relative preservation of ONL in the superior-temporal and temporal pericentral retina. These retinal regions also showed the greatest magnitude of interindividual variation.

CONCLUSIONS—Photoreceptor loss in the foveal and extrafoveal retina was prominent, even in the youngest patients studied. Differences in the topography of residual photoreceptors in children with *RPE65*-LCA suggest that it may be advisable to use individualized ONL mapping to guide the location of sub-retinal injections for gene therapy and thereby maximize the potential for efficacy.

Human clinical trials of subretinal gene replacement therapy in Leber congenital amaurosis (LCA) due to mutations in the gene encoding RPE65 (retinal pigment epithelium-specific 65-kDa protein) are ongoing in different centers.^{1–4} Current trials are open-label uniocular single subretinal injections of adeno-associated viral vector altered to carry the human *RPE65* gene. Studies are being conducted first in small cohorts of adult subjects. In two recent reports of

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preliminary results in patients ranging in age from 17 to 26 years of age, the authors indicate an intention to advance to cohorts of children with *RPE65*-LCA^{5–7}

What do we know about the retinal disease in children with *RPE65*-LCA? Normal photoreceptor histology in models of *RPE65*-LCA in young mice and dogs (for example, Refs. 8⁹) could lead to an assumption that patients with *RPE65*-LCA may also have normal or near normal photoreceptor counts early in life. Human *RPE65*-LCA, however, is not only a biochemical dysfunction of the visual cycle but also a serious retinal degeneration that is present, even in the first decade of life.^{10–12} This important difference between young humans and young disease models of *RPE65*-LCA has therapeutic implications. Children with better-preserved photoreceptors and RPE cells, for example, should in theory have greater potential for therapeutic efficacy. Experimental support for this logical concept was provided in proof-of-concept studies that compared *Rpe65*^{−/−} mice at young and older ages.¹⁰ Mutant mice at later stages of disease (i.e., with considerable photoreceptor loss) showed less restoration of retinal function and retinoid biochemistry after subretinal gene replacement therapy than did younger mice.¹⁰

Cross-sectional retinal imaging with optical coherence tomography (OCT) provides the opportunity to determine non-invasively in humans the photoreceptor cell layer thickness in a wide expanse of central retina.¹⁰ We studied photoreceptor layer thickness in young patients with *RPE65*-LCA to inquire whether there were retinal locations with a higher likelihood of retained photoreceptors and thereby more suitable as loci for subretinal injections of vector-gene product. There was variation between subjects in the degree of photoreceptor layer integrity, but summary maps of photoreceptor loss revealed consistent patterns.

MATERIALS AND METHODS

Human Subjects

There were nine young patients with *RPE65* mutations (ages, 6–17 years), representing seven families (Table 1). Two patients with *RPE65*-LCA at ages 20 and 23 years were included for comparison with patterns of results found in younger patients. Normal subjects for optical coherence tomography (OCT) were also included. Informed consent or assent was obtained for the OCT studies in all subjects; procedures adhered to the Declaration of Helsinki and were approved by the institutional review board of the University of Pennsylvania.

Optical Coherence Tomography

Cross-sectional images of retina were obtained with OCT. The principles of the method and our recording and analysis techniques have been published.^{10,13–17} Other methods of quantitative OCT image analysis with mainly foveal longitudinal reflectivity profiles (LRPs) have also been reported.¹⁸ Data were acquired with an OCT3 instrument (Carl Zeiss Meditec, Inc., Dublin, CA) with a theoretical axial resolution in retinal tissue of ~8 μm. A subset of patients ($n = 3$; P4, P5, P10) had ultrahigh-speed and higher resolution (~6 μm) imaging with frequency domain OCT (RTVue-100; Optovue Inc., Fremont, CA). In addition to horizontal and vertical scans crossing the anatomic fovea, topographic analysis was performed in all patients. For this purpose, a rectangular region of the retina (18 × 12 mm) centered on the fovea was sampled with dense raster scans. With OCT3, coverage was obtained with parallel horizontal raster scans of 6-mm length, vertically separated by 0.3 mm.^{10,15} With frequency domain OCT, horizontal raster scans were also of 6-mm length, but a finer vertical separation of 0.06 mm¹⁷ was afforded by the ultrahigh scanning speed.

Postacquisition processing of OCT data was performed with custom programs (MatLab ver. 6.5; MathWorks, Natick, MA). Topographic analysis was performed after determining the

precise location and orientation of each scan relative to retinal features (such as blood vessel patterns, optic nerve head boundaries, and regions of RPE depigmentation) using the en face images of the fundus. Continuous segments of LRPs making up each OCT scan were allotted to regularly spaced bins ($0.3 \times 0.3 \text{ mm}^2$) in a rectangular coordinate system centered at the fovea. All available waveforms in each bin were aligned by using a dynamic cross-correlation algorithm. Automatic alignment was manually overridden when crossing structures that interrupted local lateral isotropy of signals. Aligned waveforms in each bin were averaged. Outer photoreceptor nuclear layer (ONL) thickness was defined on the averaged waveform as the major intraretinal signal trough delimited by the signal slope maxima. Missing data were interpolated bilinearly, thickness values were mapped to a pseudocolor scale, and locations of blood vessels and optic nerve head were overlaid for reference.^{10,13,16,19}

Individual ONL thickness maps of each patient were aligned to a mean normal thickness map ($n = 6$; age range, 21–41 years; mean age, 26, SD 7.8) by translating to match the location of the anatomic fovea and rotating ($< \pm 7^\circ$) to align the fovea-papillary axis. The topography of ONL thickness loss was estimated in each patient with the following formula: (normal mean ONL thickness – patient ONL thickness)/(normal mean ONL thickness). A 3×3 moving median smoothing filter was applied to the result. ONL loss maps were resectioned along vertical, horizontal, and diagonal meridians to clarify the relationship between individual maps and summaries. Summary maps of the mean ONL thickness, its variation, and the mean ONL loss were calculated for the young patients with *RPE65*-LCA. The mean and SD were determined for each sample; a 3×3 moving median smoothing filter was applied to the result.

RESULTS

Cross-sectional OCT images along the vertical meridian in a 15-year-old normal subject (Fig. 1A) and P4, a 10-year-old patient with *RPE65*-LCA (Fig. 1B; Table 1), are shown. Normal retina had a foveal depression surrounded by thicker retina and then decreasing thickness with distance from the fovea. There were layers of low reflectivity (inner nuclear layer; ONL) and intervening layers of higher reflectivity (inner plexiform layer, outer plexiform layer). Near the retinal surface there was a broader band of high reflectivity representing the nerve fiber layer. Deep in the retina, there was a multilayer complex composed of signals originating from the outer limiting membrane, the photoreceptor inner/outer segment interface, the RPE, and the anterior choroid.^{14,15} P4, at age 10, also showed a foveal depression with normal-appearing laminar architecture, but foveal ONL was reduced in thickness and there was thinned ONL extending both superiorly and inferiorly.

Maps of ONL thickness across a wide expanse of central retina are shown in normal subjects (Fig. 1C) and four patients of different ages (Figs. 1D–G). ONL thickness of the normal retina peaks centrally and declines with distance from the fovea; extrafoveal thinning occurs more gradually in the superior than inferior retina. P1, at age 6 years, has generally thinned ONL but better preserved ONL in a central island and a superior retinal region near the vascular arcades. There was a nearly undetectable ONL in an incomplete annulus surrounding the central island and extending inferiorly (Fig. 1D). P4, at age 10 years, showed a different pattern of retained ONL. The central island was greater in extent than in P1 but surrounding retina had little or no measurable ONL. The pattern in P4 cannot be interpreted as a more advanced stage of P1 despite the older age of P4. These patterns in young patients can be compared with those in older patients. For example, P10, at age 20 years, had a pattern of preserved ONL similar to that of P1 but the central island had less thickness (Fig. 1F). P11 at age 23 years showed a pattern of ONL preservation similar to that of P4 (Figs. 1F, 1G). Unexpectedly, P11, the older patient by 13 years, had more central ONL thickness than P4.

Photoreceptor topography in seven other young patients with *RPE65*-LCA are depicted (Fig. 2). P2 and P3, both 7-year-old patients with *RPE65*-LCA, had abnormally thinned ONL throughout the retinal region studied but differed in degree of abnormality (Figs. 2A, 2B). P2 retained a central island of ONL with parafoveal and perifoveal thinning; the superior perifovea retained more ONL than at comparable eccentricities inferiorly. There were scattered patches of detectable ONL, such as that superior to the optic nerve head (Fig. 2A). P3 had more retained ONL, especially in the superior and superior-temporal retina. Inferior to the fovea was a region of very thin ONL but further inferiorly, ONL became detectably thicker (Fig. 2B). P6 and P5, both age 12 years, retained greater ONL in superior and temporal retina than inferiorly. P6 had severely reduced foveal ONL, whereas P5 retained a more extensive, albeit abnormal, central island (Figs. 2C, 2D). P7 and P8, ages 13 and 14, respectively, had mainly central islands of detectable ONL. The map of P8 suggests the presence of ONL eccentric to the vascular arcades in all quadrants beyond the central region scanned. P9 at age 17 had a limited central island of ONL and scattered islands in the superior and supero-temporal retina outside of the arcades.

An average ONL thickness map from the sampled region of retina is shown for the cohort of nine young patients with *RPE65*-LCA (Fig. 2H). This summary map shows retained ONL centrally and into the supero-temporal retina. The variability of the measurements is also depicted (Fig. 2H, inset); darker grays correspond to regions of greater variability with higher values in the supero-temporal and temporal retina.

Photoreceptor loss maps for the nine young patients with *RPE65*-LCA were also generated based on comparison with normal mean ONL topography (Fig. 3A). Based on the summary map of ONL loss for this young age group, the superior-temporal and temporal regions appear least affected by photoreceptor degeneration. Individual data along four radii (S-T, T, S, I) show wide interindividual differences (Fig. 3B).

DISCUSSION

Photoreceptors are lost across a wide region of human central retina as early as the first decade of life in patients with *RPE65*-LCA. Differences in severity were evident between unrelated patients of the same age with different *RPE65* mutations. The basis for these differences is not currently known. The relation between in vitro analyses of isomerohydrolase activity^{20–22} and initial severity and rate of human *RPE65*-LCA retinal degeneration is unexplored. Also unknown are the effects of genetic background and environmental influences on disease severity.^{23,24}

How does the degree of disease severity in humans compare with the animal models of *Rpe65* deficiency? *Rpe65*-mutant mice show only minimal ONL thickness reduction in the first 3 to 4 months of life and *RPE65*-mutant dogs also have little or no loss of ONL during the first year of life.^{8,9,25–29} Based on an assumption of allometric scaling of rates of retinal degeneration across species, the first decade of human life would correspond to 4 weeks in the mouse's life and about 1 year in the dog.³⁰ Such interspecies comparisons suggest that human retinal degeneration is more aggressive than that in the animal models.

Topographical analysis of the *RPE65*-deficient human photoreceptor layer showed residual islands not only in the foveal region¹¹ but also in the extrafoveal retina. On average, the retinas of these young patients with *RPE65*-LCA had greater defects inferiorly than superiorly. Considering that the ratio of rods to cones outside of the fovea rises from 5:1 at 1 mm eccentricity to approximately 25:1 at 4 mm eccentricity,³¹ the observed ONL thinning in these patients is mainly attributable to rod photoreceptor loss. Greater preservation of structure superiorly than inferiorly could simply be because there were higher rod photoreceptor densities in the superior perifovea to begin with, as in the normal “rod hotspot.”³¹ It is notable

that there is some evidence in the *RPE65*-mutant canine of greater photoreceptor loss in the inferior than in the superior retina.^{25,32}

Despite major scientific progress in understanding molecular details of the retinoid cycle and the consequences of deficiency in isomerohydrolase (RPE65) activity,³³ the human disease sequence leading from *RPE65* mutation to retinal degeneration remains uncertain. Apoptotic cell death of rods in the Rpe65-deficient mouse has been postulated to be caused by continuous activation of the phototransduction cascade by the apoprotein opsin.^{34–37} Another theoretical contributor to retinal degeneration is RPE disease with secondary photoreceptor loss, such as could occur from continuous retinyl ester accumulation.^{9,25,27,38,39} It has been argued, however, that this does not have a significant role in the loss of rod cells.³⁴ From another viewpoint, why do some rod photoreceptors survive over long periods of chromophore deprivation due to RPE65 deficiency? Residual rod function in Rpe65-deficient mice has been shown to be supported by an alternate pathway that produces isorhodopsin,^{23,40,41} but it is unknown whether this pathway operates in human retina.

The spectrum of severity of disease expression within our study population leads to the recommendation that detailed cross-sectional retinal imaging and mapping be conducted in prospective candidates for clinical trials in *RPE65*-LCA, independent of age. Photoreceptor layer maps based on the optical scans, with superimposed retinal landmarks, should optimally be available to the retinal surgeon and guide the placement of the retinotomy site and induced retinal detachment. Treatment outcomes may then be interpreted in the context of the photoreceptor disease present at the time of intervention, and expectations of therapy can be realistic. If pretreatment ONL mapping in each patient is not feasible, then the average patterns we provide may be useful guides to select a preferred regional site for subretinal injection of gene vector.

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References

1. www.clinicaltrials.gov/ct2/results?term=NCT00481546
2. www.clinicaltrials.gov/ct2/results?term=NCT00643747
3. www.clinicaltrials.gov/ct2/results?term=NCT00516477
4. www.clinicaltrials.gov/ct2/results?term=NCT00422721
5. Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med* 2008;358(21):2231–2239. [PubMed: 18441371]
6. Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. *N Engl J Med* 2008;358(21):2240–2248. [PubMed: 18441370]
7. Miller JW. Preliminary results of gene therapy for retinal degeneration. *N Engl J Med* 2008;358(21):2282–2284. [PubMed: 18441372]
8. Redmond TM, Yu S, Lee E, et al. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. *Nat Genet* 1998;20(4):344–351. [PubMed: 9843205]
9. Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet* 2001;28(1):92–95. [PubMed: 11326284]
10. Jacobson SG, Aleman TS, Cideciyan AV, et al. Identifying photoreceptors in blind eyes caused by RPE65 mutations: prerequisite for human gene therapy success. *Proc Natl Acad Sci USA* 2005;102(17):6177–6182. [PubMed: 15837919]

11. Jacobson SG, Aleman TS, Cideciyan AV, et al. Human cone photoreceptor dependence on RPE65 isomerase. *Proc Natl Acad Sci USA* 2007;104(38):15123–15128. [PubMed: 17848510]
12. Porto FB, Perrault I, Hicks D, et al. Prenatal human ocular degeneration occurs in Leber's congenital amaurosis (LCA2). *J Gene Med* 2002;4(4):390–396. [PubMed: 12124981]
13. Jacobson SG, Cideciyan AV, Aleman TS, et al. RDH12 and RPE65, visual cycle genes causing Leber congenital amaurosis, differ in disease expression. *Invest Ophthalmol Vis Sci* 2007;48(1):332–338. [PubMed: 17197551]
14. Huang Y, Cideciyan AV, Papastergiou GI, et al. Relation of optical coherence tomography to microanatomy in normal and rd chickens. *Invest Ophthalmol Vis Sci* 1998;39(12):2405–2416. [PubMed: 9804149]
15. Jacobson SG, Cideciyan AV, Aleman TS, et al. Crumbs homolog 1 (CRB1) mutations result in a thick human retina with abnormal lamination. *Hum Mol Genet* 2003;12(9):1073–1078. [PubMed: 12700176]
16. Aleman TS, Cideciyan AV, Sumaroka A, et al. Inner retinal abnormalities in X-linked retinitis pigmentosa with RPGR mutations. *Invest Ophthalmol Vis Sci* 2007;48(10):4759–4765. [PubMed: 17898302]
17. Aleman TS, Cideciyan AV, Sumaroka A, et al. Retinal laminar architecture in human retinitis pigmentosa caused by rhodopsin gene mutations. *Invest Ophthalmol Vis Sci* 2008;49:1580–1590. [PubMed: 18385078]
18. Barthelmes D, Sutter FK, Kurz-Levin MM, et al. Quantitative analysis of OCT characteristics in patients with achromatopsia and blue-cone monochromatism. *Invest Ophthalmol Vis Sci* 2006;47(3):1161–1166. [PubMed: 16505054]
19. Jacobson SG, Cideciyan AV, Sumaroka A, et al. Remodeling of the human retina in choroideremia: rab escort protein 1 (REP-1) mutations. *Invest Ophthalmol Vis Sci* 2006;47(9):4113–4120. [PubMed: 16936131]
20. Redmond TM, Poliakov E, Yu S, Tsai JY, Lu Z, Gentleman S. Mutation in key residues of RPE65 abolishes its enzymatic role as isomerohydrolase in the visual cycle. *Proc Natl Acad Sci USA* 2005;102(38):13658–13663. [PubMed: 16150724]
21. Takahashi Y, Chen Y, Moiseyev G, Ma JX. Two point mutations of RPE65 from patients with retinal dystrophies decrease the stability of RPE65 protein and abolish its isomerohydrolase activity. *J Biol Chem* 2006;281(31):21820–21826. [PubMed: 16754667]
22. Chen Y, Moiseyev G, Takahashi Y, Ma JX. Impacts of two point mutations of RPE65 from Leber's congenital amaurosis on the stability, subcellular localization and isomerohydrolase activity of RPE65. *FEBS Lett* 2006;580(17):4200–4204. [PubMed: 16828753]
23. Fan J, Wu BX, Sarna T, Rohrer B, Redmond TM, Crouch RK. 9-cis Retinal increased in retina of RPE65 knockout mice with decrease in coat pigmentation. *Photochem Photobiol* 2006;82(6):1461–1467. [PubMed: 16553465]
24. Danciger M, Ogando D, Yang H, et al. Genetic modifiers of retinal degeneration in the rd3 mouse. *Invest Ophthalmol Vis Sci* 2008;49(7):2863–2869. [PubMed: 18344445]
25. Acland GM, Aguirre GD, Bennett J, et al. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. *Mol Ther* 2005;12(6):1072–1082. [PubMed: 16226919]
26. Rohrer B, Goletz P, Znoiko S, Ablonczy Z, Ma JX, Redmond TM, Crouch RK. Correlation of regenerable opsin with rod ERG signal in Rpe65^{-/-} mice during development and aging. *Invest Ophthalmol Vis Sci* 2003;44(1):310–315. [PubMed: 12506090]
27. Narfström K, Katz ML, Bragadottir R, et al. Functional and structural recovery of the retina after gene therapy in the RPE65 null mutation dog. *Invest Ophthalmol Vis Sci* 2003;44(4):1663–1672. [PubMed: 12657607]
28. Dejneka NS, Surace EM, Aleman TS, et al. In utero gene therapy rescues vision in a murine model of congenital blindness. *Mol Ther* 2004;9(2):182–188. [PubMed: 14759802]
29. Pang JJ, Chang B, Hawes NL, et al. Retinal degeneration 12 (rdl2): a new, spontaneously arising mouse model for human Leber congenital amaurosis (LCA). *Mol Vis* 2005;11:152–162. [PubMed: 15765048]

30. Wright AF, Jacobson SG, Cideciyan AV, et al. Lifespan and mitochondrial control of neurodegeneration. *Nat Genet* 2004;36(11):1153–1158. [PubMed: 15514669]
31. Curcio CA, Sloan KR, Kalina RE, Hendrickson AE. Human photoreceptor topography. *J Comp Neurol* 1990;292(4):497–523. [PubMed: 2324310]
32. Jacobson SG, Acland GM, Aguirre GD, et al. Safety of recombinant adeno-associated virus type 2-RPE65 vector delivered by ocular subretinal injection. *Mol Ther* 2006;13(6):1074–1084. [PubMed: 16644289]
33. Travis GH, Golczak M, Moise AR, Palczewski K. Diseases caused by defects in the visual cycle: retinoids as potential therapeutic agents. *Annu Rev Pharmacol Toxicol* 2007;47:469–512. [PubMed: 16968212]
34. Woodruff ML, Wang Z, Chung HY, Redmond TM, Fain GL, Lem J. Spontaneous activity of opsin apoprotein is a cause of Leber congenital amaurosis. *Nat Genet* 2003;35(2):158–164. [PubMed: 14517541]
35. Fan J, Woodruff ML, Cilluffo MC, Crouch RK, Fain GL. Opsin activation of transduction in the rods of dark-reared Rpe65 knockout mice. *J Physiol* 2005;568:83–95. [PubMed: 15994181]
36. Fain GL. Why photoreceptors die (and why they don't). *Bioessays* 2006;28(4):344–354. [PubMed: 16547945]
37. Clarke G, Collins RA, Leavitt BR, et al. A one-hit model of cell death in inherited neuronal degenerations. *Nature* 2000;406:195–199. [PubMed: 10910361]
38. Katz ML, Redmond TM. Effect of Rpe65 knockout on accumulation of lipofuscin fluorophores in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 2001;42(12):3023–3030. [PubMed: 11687551]
39. Moiseyev G, Chen Y, Takahashi Y, Wu BX, Ma JX. RPE65 is the isomerohydrolase in the retinoid visual cycle. *Proc Natl Acad Sci U S A* 2005;102(35):12413–12418. [PubMed: 16116091]
40. Fan J, Rohrer B, Moiseyev G, Ma JX, Crouch RK. Isorhodopsin rather than rhodopsin mediates rod function in RPE65 knock-out mice. *Proc Natl Acad Sci USA* 2003;100(23):13662–13667. [PubMed: 14578454]
41. Fan J, Rohrer B, Frederick JM, Baehr W, Crouch RK. Rpe65^{-/-} and Lrat^{-/-} mice: comparable models of Leber congenital amaurosis. *Invest Ophthalmol Vis Sci* 2008;49(6):2384–2389. [PubMed: 18296659]

FIGURE 1.

Photoreceptor layer thickness topography of two young patients with *RPE65*-LCA compared with patterns in two older patients. (A, B) Cross-sectional scans along the vertical meridian in a 15-year-old normal subject and a 10-year-old patient with *RPE65*-LCA. (C) Normal topography of ONL thickness as an average map based on a group of six normal subjects. (D, E) Patients at 6 and 10 years of age compared with (F, G) two patients in their early 20s. Traces of major blood vessels and location of the optic nerve head are overlaid on each map (depicted as right eyes). T, temporal; N, nasal; S, superior; I, inferior. *Bottom left*: color scale for ONL thickness.

FIGURE 2.

Photoreceptor layer thickness topography of seven other young patients with *RPE65*-LCA. (A–G) Individual ONL topography maps of patients with *RPE65*-LCA ranging in age from 7 to 17 years. (H) Average topography based on the results of all nine patients. *Inset*: SD of thickness, in gray scale. *Bottom left*: ONL thickness color scale.

FIGURE 3.

Summary maps of photoreceptor ONL loss topography in young patients with *RPE65-LCA*. (A) Average photoreceptor losses mapped to a color scale across an expanse of central retina in the nine young patients (ages, 6–17 years). All data are depicted as a right eye. (B) Profiles of individual data sets along four radii extending from the fovea (F) along the superior-temporal (S-T), temporal (T), superior (S), and inferior (I) meridians. *Gray traces*: individual data; *bold black traces*: average profile.

TABLE 1Molecular Characteristics of the *RPE65*-LCA Patients

Patient	Age at Visit (y)	Mutation	Reference *
P1 [†]	6	R44Q/R44Q	11
P2 [†]	7	R44Q/R44Q	11
P3	7	R91W/R91W	11
P4 [‡]	10	L341S/IVS2-2A>C	This study
P5 [‡]	12	L341S/IVS2-2A>C	This study
P6	12	L408P/L408P	11
P7	13	V287F/V287F	11
P8	14	R91Q/R91W	11
P9	17	97del20bp/97del20bp	10, 11, 13
P10	20	G40S/R91Q	11, 13
P11	23	R91W/1059insG	11, 13

* Previous reports of genotype and/or phenotype by the authors.

^{†,‡} Patients are siblings.