Clinical and Molecular Genetics of Leber’s Congenital Amaurosis: A Multicenter Study of Italian Patients

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PURPOSE. To identify the molecular basis of Leber’s congenital amaurosis (LCA) in a cohort of Italian patients and to perform genotype-phenotype analysis.

METHODS. DNA samples from 95 patients with LCA were analyzed by using a microarray chip containing disease-associated sequence variants in eight LCA genes. In addition, all patients in whom no mutations were identified by microarray were subjected to sequence analysis of the CEPI290 gene. Patients with mutations identified underwent a detailed ophthalmic evaluation.

RESULTS. Disease-causing mutations were identified in 28% of patients, and twelve novel variants were identified. Mutations occurred more frequently in the RPE65 (8.4%), CRB1 (7.4%), and GUCY2D (5.2%) genes. Mutations in CEPI290 were found in only 4.2% of the patients analyzed. Clinical assessment of patients carrying RPE65 or CRB1 mutations revealed the presence of retinal dysmorphologies in the first decade of life. RPE65 mutations were almost always associated with normal macular thickness, as assessed by optical coherence tomography (OCT), whereas CRB1 mutations were associated with reduced retinal thickness and a coarsely laminated retina. Fundus autofluorescence was mostly observed in patients with RPE65 and GUCY2D mutations and was not elicitable in patients carrying CRB1.

CONCLUSIONS. RPE65 gene mutations represented a significant cause of LCA in the Italian population, whereas GUCY2D and CEPI290 mutations had a lower frequency than that found in other reports. This finding suggests that the genetic epidemiology of LCA in Italy is different from that reported in the United States and in Northern European countries. Autofluorescence in patients with RPE65 mutations was more frequently associated with preserved retinal thickness, which suggests that these mutations are not associated with progression of retinal degeneration. Therefore, normal retinal thickness (identified by OCT) and fundus autofluorescence may be the means with which to identify patients with LCA who carry RPE65 mutations, which are expected to be a potential gene therapy target in the near future. (Invest Ophthalmol Vis Sci. 2007;48:4284–4290) DOI:10.1167/iovs.07-0068

Leber’s congenital amaurosis (LCA) is a group of hereditary retinal dystrophies characterized by severe loss of visual function early in life.1,2 The clinical features usually include severely reduced or absent scotopic and photopic electroretinogram (ERG), roving eye movements/nystagmus, digito-ocular signs (eye poking or rubbing), and an apparently normal or salt-and-pepper pigmented fundus.3 Although an early-onset and severe disease, LCA has a variable expression,4,5 which may reflect, at least in part, its high genetic heterogeneity.

LCA is usually inherited as an autosomal recessive trait, although dominant inheritance has also been reported.5–9 Thus far, mutations in 10 retinal genes have been shown to cause LCA; namely AIPL1,10 CRB1,11 CRX,12 GUCY2D,13 RDH12,14 RPE65,15 RPGRIP1,16 TULP1,17 IMPDH118 and, more recently, CEP290.19 Because of the increasing number of LCA-causing genes, it has been difficult to classify patients with LCA on a molecular basis and consequently to evaluate phenotype-genotype correlations. Hanein et al.20 proposed a genotype-phenotype correlation scheme in which patients are divided into two groups. One group consists of patients whose symptoms fit the traditional definition of LCA (i.e., congenital or very early cone–rod dystrophy with mutations in the GUCY2D, AIPL1, and RPGRIP1 genes). The other group consists of patients affected by severe progressive rod–cone dystrophy with mutations in the RPE65, TULP1, CRB1, and CRX genes. However, other reports suggest that this classification is oversimplified.5,21

A correct molecular classification of patients with LCA is important because a treatment strategy based on gene therapy22,23 may be available for this condition in the near future. The advent of a genotyping LCA microchip based on the allele-specific primer extension (APEX) technique perhaps will lead to a more precise molecular classification of this condition.24 Using this chip, it is possible to screen simultaneously for more than 300 known LCA-causing mutations.

The purpose of this study was to perform a comprehensive mutation analysis of Italian patients by using the LCA gene
microarray chip combined with the analysis of a sequence variant in the recently identified CEP290 gene that is reported to be responsible for approximately 20% of LCA cases.\textsuperscript{19} We also performed detailed ophthalmic evaluations in patients carrying mutations in the attempt to identify genotype–phenotype correlations that may improve the diagnostic and prognostic evaluation of patients with LCA.

Materials and Methods

Patient Selection
The diagnostic criteria for LCA are severe, or occasionally moderately severe, visual impairment during the first year of life and, with a few exceptions, nystagmus and nondetectable or severely reduced rod and cone electroretinogram amplitudes.\textsuperscript{25} Ophthalmic examination included best corrected visual acuity by projected Snellen charts or Teller Acuity Cards, measurement of objective refractive error after cycloplegia, biometry, slit lamp biomicroscopy, dilated fundus examination, and electrophysiology recordings obtained according to ISCEV (International Society for Clinical Electrophysiology of Vision) standards.\textsuperscript{26} Ninety-five unrelated patients of Italian origin with a clinical diagnosis of LCA were selected for the study in three centers (i.e., the Department of Ophthalmology, Second University of Naples; Center of Child Neuro-ophthalmology, Department of Child Neurology and Psychiatry of the IRCCS C. Mondino Foundation; and the Department of Ophthalmology at the University of Cagliari). Patients with neurologic or other systemic abnormalities, identified using previously described diagnostic procedures\textsuperscript{3} were not included in this study. Genetic counseling assessed that in most cases the family structure of patients was consistent with autosomal recessive inheritance. In particular, 20 patients had at least one affected sibling, 22 patients had consanguineous parents, whereas the remaining 53 represented simplex cases.

Mutation Analyses

Blood samples were collected from the 95 selected patients. All procedures were approved by the Ethics Boards of the participating institutes and adhered to the tenets of the Declaration of Helsinki. All samples were acquired after written informed consent was obtained from the patient or, in the case of children, their legal guardians. Genomic DNA was extracted from blood samples using standard techniques.\textsuperscript{27} We used a genotyping microarray based on the APEX (arrayed primer extension technology)\textsuperscript{28} available at Asperbio (http://www.asperbio.com/Lecer BergenCongenitalAmaurosisDNAtest.htm; Tarfu Estonia) for the analysis of 344 mutations in the AIPL1, CRB1, RPE65, CRX, RPGRIP1, GUCY2D, MERTK, and LRAT genes that can be responsible for LCA or severe forms of retinal degeneration. Please note that the version of the genotyping chip analyzed was recently replaced by a new version containing 423 mutations in 10 genes, including CEP290. All the sequence variations identified with the microchip were validated by direct sequencing of polymerase chain reaction (PCR) products spanning the predicted mutations.

We analyzed the AIPL1, CRB1, RPE65, and GUCY2D genes using PCR and oligonucleotide primer pairs that amplify the coding exons and intron–exon junctions of these genes. The sequences of the primers and the PCR conditions were mostly retrieved from previous reports,\textsuperscript{10,11,15,27} with some slight modification, and are listed in Supplementary Table S1, online at http://www iovs.org/cgi/content/full/48/9/4284/DC1. Concerning the CEP290 gene, we used the oligonucleotide primers and PCR conditions described by Den Hollander et al.\textsuperscript{19} for the amplification of the genomic fragment spanning the c.2991+1655A mutation and the primers and the oligonucleotide primers and PCR conditions described by Valente et al.\textsuperscript{30} for the amplification of the genomic fragments spanning all coding exons. All amplified products were subject to denaturing high performance liquid chromatography (dHPLC) and all products with a dHPLC pattern different from control samples were sequenced by dye termination chemistry (Prism Big Dye Terminator Cycle Sequencing V2.0 kit; Applied Biosystems, Inc. [ABI], Foster City, CA). In all cases where two putative mutations were identified in the same patient, we tested other members of the family, both affected and not affected, to verify whether the sequence variations segregated with the LCA phenotype. All the novel sequence variants identified corresponding to missense variations were also tested in at least 100 control chromosomes by dHPLC analysis.

Phenotype Analysis

A more detailed ophthalmic evaluation was performed in patients harboring LCA gene mutations. Autofluorescence was recorded with a standard confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph; Heidelberg Engineering, Heidelberg, Germany). To amplify the autofluorescence signal, we aligned the best five images obtained using the software integrated in the instrument and calculated a mean image.

Cross-sectional retinal reflectivity profiles were obtained with optical coherence tomography (OCT); Carl Zeiss Meditec, Inc., Dublin, CA). Subjects underwent OCT imaging using 512 A-scans over a 3-mm transverse scanning length, for an optimal sampling rate of 400 A-scans per second, centered on the fovea. According to the manufacturer, the longitudinal resolution of the present model is 8 to 10 μm, whereas the transverse resolution is ∼20 μm. The precise location and orientation of each scan were determined using the OCT simultaneous view video images. Because nearly all patients were affected by nystagmus, which complicates the recording, OCT scans and autofluorescence were obtained in 12 patients.

Results

Molecular Studies

We used a combined approach to perform an extensive mutation analysis in genes involved in the pathogenesis of LCA in 95 unrelated Italian patients with a diagnosis of LCA or early-onset retinal degeneration. We first used a microarray chip that allows the simultaneous analysis of 344 known mutations in six LCA genes (see the Materials and Methods section). All patients with heterozygous sequence variations in any of the genes analyzed were subject to complete screening of the gene in which the putative first mutation was identified. In addition, to independently assess the efficacy of the LCA gene microarray chip as well as to get further insight into the frequency of novel mutations in the Italian LCA population, all the patients who were negative after the microchip screening underwent a detailed screening for mutations in the RPE65, CRB1, and AIPL1 genes. The latter two analyses were performed by dHPLC and direct sequencing of the complete coding sequences and all intron–exon boundaries of the genes under study to identify novel mutations that could not be detected with the LCA microchip. Finally, we also screened this set of patients for mutations in the CEP290 gene that was recently suggested to be responsible for LCA in over 20% of cases.\textsuperscript{19} For this analysis, we first analyzed all patients for the c.2991+1655A→G (p.C998X) sequence variation that so far has constantly been detected in LCA cases due to CEP290 mutations. The patients in which the latter mutation was identified were then analyzed for sequence variations throughout the entire gene.

The integrated approach used in this study led to the identification of 48 sequence variations potentially responsible for the LCA phenotype in 27 of the 95 patients analyzed (28%; Table 1). Twenty-one of these patients were either homozygotes or compound heterozygotes for the mutations, whereas in six patients, the second mutation could not be identified after complete screening of the gene. Overall, we detected 31 different mutations, including 19 previously described and 12 newly identified: three in CRB1, two in GUCY2D, two in RPE65, one in AIPL1, and four in CEP290. Causative mutations were identified in the RPE65, CRB1, AIPL1, GUCY2D, and CEP290 genes, whereas no mutations were detected in the
Table 1. Mutations Identified in the LCA Patients Analyzed

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<th>Mutation 2</th>
<th>Clinical Analysis</th>
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Novel mutations are shaded. NF, not found.

*Mutations found by dHPLC and/or sequencing.

**RPGRIP1, CRX, MERTK, and LRAT genes using the LCA microchip. Overall, by using the LCA microchip, we were able**
**to identify most patients with mutations in the genes analyzed, excluding the CEP290 gene that, at the time of the analysis,**
**was not represented yet on the array. In particular, the microchip analysis alone was sufficient to identify at least one mutated allele in 87% (20/23) of the patients and both mutated alleles in 70% of the patients (12/17).**

**Besides known mutations, the microchip also allowed us to identify two novel mutations in the GUCY2D gene, which represented allelic variants of previously described mutations (described later). The complete analysis of the CRB1, RPE65, and A1PL1 genes by dHPLC and sequencing yielded only three additional patients with mutations in the genes analyzed as they (patients A25, A137, and A9; Table 1) were carrying novel mutations not represented in the LCA microchip. In addition, the latter analysis allowed us to identify the second (novel) mutation in three patients in whom the first mutation was identified by microchip analysis. In the following sections, we detail, on a gene-by-gene basis, the molecular findings obtained.**

**RPE65. Eight (8.4%) of the patients displayed mutations in RPE65, three of which were compound heterozygotes, and the remaining five were homozygous (Table 1). We found two novel mutations in this gene: a 2-bp deletion leading to a frameshift of the protein at amino acid position 146 (p.C146fs) followed by a premature termination at position 156 and a missense mutation at amino acid position 313 (p.H313R) affecting a histidine residue highly conserved in vertebrates.**

**CRB1. We found mutations in CRB1 in seven (7.4%) of the patients. However, only four of them displayed two mutations (two homozygous and two compound heterozygotes), whereas in the remaining three cases, we could not find a second mutation after complete analysis of the gene. Two new missense mutations were identified in this gene: p.C438Y and p.R1361H. The latter variation occurred in the last exon of an alternative transcript of the CRB1 gene (GenBank accession AF154671; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD).**

**A1PL1. Mutations in this gene were found in three (3.2%) of the patients: one of them was homozygous and the second was a compound heterozygote, and in the remaining one, we found only one mutation in the heterozygous state. In the compound heterozygous patient, we identified a novel missense mutation, p.R270H, affecting an amino acid residue extremely conserved across evolution.**

**GUCY2D. We found sequence variations with a potential pathogenetic role in this gene in five (5.2%) of the patients. Three of these patients had homozygous mutations, whereas in the remaining ones we found only one heterozygous mutation. A sequence variant found in one of the homozygous patients (i.e., the p.P701S, previously reported to be responsible for LCA4,31) was detected in three additional patients (of the 95 analyzed) in the heterozygous state. However, we did not detect a second mutation in the GUCY2D in these three patients. Furthermore, we found the same variation to be present at a very similar frequency in heterozygosity in a population of healthy individuals (2 heterozygous of 100 analyzed). For all these reasons, we do not have sufficient evidence to conclude that the p.P701S represents a pathogenetic mutation in these patients.**
three patients. However, based on our results as well as on previous reports\(^2\) that indicate that the p.P701S variation in homozygosity consistently segregated with the disease, we consider a pathogenetic role of this mutation in patient A95 to be highly likely (Table 1). Finally, we identified, by microchip analysis, two novel missense variations in RPE65 (4.2%), and CRB1 (7.4%), followed by GUCY2D (5.2%), CEP290 (4.2%), and AIPL1 (3.2%). We could not find any mutation in 71.9% of the patients analyzed.

Finally, in none of the patients analyzed, we found putative mutations in more than one gene thus making unlikely the possibility of digenic or triallelic inheritance. However, we identified several polymorphisms in the genes analyzed (see Supplementary Table S2, \(\text{http://www.iovs.org/cgi/content/full/48/9/4284/DC1}\)) and we cannot exclude a possible role of any of these polymorphisms as a modifier allele on the LCA phenotype.

Genotype–Phenotype Correlations
The patients with LCA in which mutations were identified underwent a detailed ophthalmic evaluation that was extended to other affected members of the same family. Twenty-four patients (mean age, 19.1 ± 13.7 [SD] years) from 20 families were available for this study. Anamnestic records revealed that six of the 24 patients (four with RPE65 mutations and two with CRB1 mutations) had minimal visual acuity during the first 8 to 12 years of life that permitted reading and writing without Braille. The capability of reading and writing is currently retained in one RPE65 and one CRB1 patient, whereas it was lost in the other four patients at approximately 13 to 14 years of age.

The clinical features of the patients with LCA analyzed are reported in Table 2. Five patients (21%; mean age, 27 ± 14.5 years), one with a mutation in AIPL1, two in GUCY2D, and two in CRB1, reported photophobia and three patients (12%);
mean age, 35 ± 10 years) reported night blindness, including two with the \textit{RPE65} mutation and one with the \textit{CRB1} mutation. Six patients (25%; mean age, 18 ± 11 years) reported light-gazing; five of these had an \textit{RPE65} mutation and one a \textit{GUCY2D} mutation. The remaining 10 patients (42%; mean age 10 ± 9 years) did not report any symptoms. Visual acuity was severely decreased in all patients. Two patients had total blindness, 11 had light perception, 4 had hand motion, and 7 had visual acuity between 20/200 and 20/1000. Refraction was available in 19 patients, 15 (79%) had hyperopia ranging from +1 to +9.25 D, and 4 (21%) had myopia between −1 and −6.5 D. Biometric analysis confirmed the refractive data: axial length was between 21.92 and 18.3 mm in myopic patients and between 22.26 and 25.83 mm in myopic subjects. Nystagmus was observed in 75% of patients.

Keratoconus was identified only in one patient with an \textit{AIPL1} mutation and in one with a \textit{GUCY2D} mutation. Light posterior subcapsular lens opacities were found in three patients with a mutation in \textit{AIPL1}, \textit{CRB1}, and \textit{RPE65}, respectively. Fundus examination revealed salt-and-pepper retinal dystrophy in all patients with \textit{GUCY2D} mutations (Fig. 2C). Patients with \textit{RPE65} mutations had salt-and-pepper retinal dystrophy (Fig. 2A), which in two cases was associated with macular atrophy. The appearance of the fundus oculi was more heterogeneous among patients with \textit{CRB1} mutations. In fact, three subjects had salt-and-pepper retinal dystrophy, preserved para-arteriole retinal pigment epithelium (PPRPE) was present in one subject, and pigmentary retinopathy (Fig. 2B) with different degrees of macular lesion was found in four patients. \textit{AIPL1} mutations were associated with salt-and-pepper retinal dystrophy in one patient and with retinitis pigmentosa and macular atrophy in another (data not shown). Electroretinograms were extinguished in 22 of 25 patients, the only exceptions being represented by two patients with \textit{CRB1} mutations and one with an \textit{RPE65} mutation (Table 2).

Twelve patients underwent OCT analysis (Table 3). Five of them (four with \textit{RPE65} mutations and one with a \textit{GUCY2D} mutation) had a normal retinal thickness (Figs. 2G, 2I), whereas the other six (three with mutations in \textit{CRB1}, one with a mutation in \textit{AIPL1} and two with mutations in \textit{RPE65}) had reduced retinal thickness associated with indistinct retinal layers in patients with \textit{CRB1} mutations (Fig. 2H).

The autofluorescence analysis showed fundus autofluorescence in 4 (all with normal macular thickness) of the 12 patients examined, 3 with \textit{RPE65} mutations (Fig. 2D) and 1 with a mutation in \textit{GUCY2D} (Fig. 2F). No fundus autofluorescence was elicitable in the remaining eight patients (only one with normal macular thickness): four with \textit{CRB1} (Fig. 2E), three with \textit{RPE65}, and one with \textit{AIPL1} mutations.

**DISCUSSION**

We performed a molecular analysis of a group of Italian patients with LCA using an approach mainly based on the use of

**FIGURE 2.** Examples of ophthalmic findings in patients with mutations in LCA genes. The patients analyzed were: A136b (A, D, G), \textit{RPE65} mutation; A78 (B, E, H), \textit{CRB1} mutation; and A57 (C, F, I), \textit{GUCY2D} mutation (see also Table 2). (A–C) Fundus photographs: salt-and-pepper retinal dystrophy in patients A136b (A) and A57 (B) and bone spicular pigmentation with retinal atrophy in patient A78 (B). (D–F) Fundus autofluorescence (AF): please note the presence of AF in patients A136b (D) and A57 (E) and the absence of AF in patient A78 (E). (G–I) OCT of the posterior pole crossing the fovea, showing a normal retinal profile in patients A136b (G) and A57 (I) and an abnormal lamination pattern in patient A78 (H). NFL, nerve fiber layer; IPL, inner plexiform layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PR, photoreceptors; RPE, retinal pigment epithelium.
an LCA genotyping chip. Using this strategy, we identified 48 putative causative mutations in 28% of the patients analyzed. We confirmed that the LCA microchip was very effective in identifying the mutated LCA gene. The complete mutation analysis, by dHPLC and direct sequencing, of the \textit{RPE65}, \textit{AIPL1}, and \textit{CRB1} genes in all the patients in which the microarray did not reveal any mutated allele failed to detect the presence of any false negatives for the mutated alleles present in the array. In addition, the complete analysis by dHPLC and sequencing allowed us to identify only three additional patients with mutations in any of the three analyzed genes, which could not be detected by the microarray analysis because they were novel (patients A25, A137, and A9; Table 1). These results confirm the reliability of this microchip as a first-level tool for mutation analysis in patients with LCA.

The frequency of the involvement of the genes analyzed in the LCA Italian families analyzed is reported in Figure 1. The values observed point out the significant differences with respect to previous mutation analyses performed on patients from the United States and other European countries. On one hand, we found that the prevalence of \textit{RPE65} mutations is higher in the Italian population than in Northwest Europe and in the United States, as recently described,\textsuperscript{21,24} and more similar to the values observed in a study in which most of the analyzed families originated from the Mediterranean area.\textsuperscript{20} On the other hand, we found that the frequency of \textit{GUCY2D} mutations (5.2%) in the Italian LCA population is considerably lower with respect to previous reports\textsuperscript{20,21,24} and that mutations in the \textit{CEP290} gene, which was recently reported to be involved in 20% of cases in patients with LCA,\textsuperscript{19} were found in only 4.2% of the Italian patients analyzed. These data confirm that, similar to what observed in other highly heterogeneous retinal inherited disorders,\textsuperscript{25} the genetic epidemiology of LCA in Italy, and possibly in southern Europe and in the Mediterranean area is different from that reported in northern Europe and the United States.

In our study of phenotype-genotype correlations, we found that retained visual function in the first decade of life occurred only in carriers of \textit{RPE65} and \textit{CRB1} mutations. This finding coincides with reports that a large number of subjects with a minimal visual acuity (ranging from 2/400 to 20/50) in their first decade of life were carriers of \textit{RPE65}\textsuperscript{53–55} and \textit{CRB1} \textsuperscript{21,36}

In terms of symptoms, photophobia was the most frequent complaint in \textit{GUCY2D} and \textit{AIPL1} patients, whereas light gazing and night blindness were more frequent in \textit{RPE65} patients. These findings are in agreement with previous observations that reported a frequent association of night blindness with \textit{RPE65} mutations\textsuperscript{53–57} and not with \textit{GUCY2D} mutations.\textsuperscript{38,39} and, in contrast, a frequent association of photophobia with \textit{GUCY2D} mutations and a lack of photosensitivity in \textit{RPE65} patients.\textsuperscript{35,34,38–40} Based on the latter reports, Hanein et al.\textsuperscript{20} proposed a possible correlation between the visual symptoms observed and the LCA genotype whereas another recent report\textsuperscript{41} suggested that both photophobia and night blindness do not seem to be reliable clinical features that can be used to direct gene analysis. Our results seem to support the hypotheses of Hanein et al.\textsuperscript{20} but, for a more definite assessment of this question, it is necessary to analyze additional collections of patients.

The fundus examination confirms the frequent retinal phenotype associated with \textit{RPE65} and \textit{GUCY2D} mutations characterized by salt-and-pepper retinal dystrophy that does not appear to be related to disease duration. Fundus abnormalities were more heterogeneous in carriers of \textit{CRB1} mutations. In fact, we observed salt-and-pepper retinal dystrophy in younger patients and subsequently massive spicular and not nummular pigmentation at the posterior pole, which was reported to be a phenotypic feature of carriers of \textit{CRB1} mutations.\textsuperscript{21,25,36} Moreover, most of our \textit{RPE65} subjects (67%) had a normal retinal thickness and the autofluorescence signal was elicitable in 75% of them (Table 3). In contrast, all four carriers of \textit{CRB1} mutations had a coarse OCT lamination pattern, as previously reported.\textsuperscript{41} A thinner retina and no autofluorescence at the posterior pole (Table 3). These data suggest that a normal OCT profile is more frequently associated with fundus autofluorescence.

Despite the severity of this disease, patients with LCA who have \textit{RPE65} mutations retain minimal visual capabilities up to 8 to 12 years and a greater integrity of retinal tissue, as shown by normal retinal thickness associated with partially preserved fundus autofluorescence. This observation suggests that \textit{RPE65} mutations prevent the progression of retinal degeneration in patients with LCA and starkly contrasts with the disease progression in carriers of \textit{CRB1} mutations. Therefore, the results of our analysis suggest that the occurrence in the same patient of minimal visual acuity during the first decade of life, salt-and-pepper retinal dystrophy, normal retinal thickness (identified with OCT), and presence of fundus autofluorescence may be indicative of the presence of mutations in the \textit{RPE65} gene.

This study represents the first example of an integrated molecular and clinical characterization of patients with LCA that include OCT and autofluorescence analyses performed

### Table 3. Summary of OCT and Autofluorescence Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>Mutation</th>
<th>Age (y)</th>
<th>Autofluorescence</th>
<th>OCT (Macular Thickness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A61</td>
<td>\textit{AIPL1}</td>
<td>p.W278X; p.R270H</td>
<td>31</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A78</td>
<td>\textit{CRB1}</td>
<td>p.I852T; p.T745M</td>
<td>45</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A78b</td>
<td>\textit{CRB1}</td>
<td>p.I852T; p.T745M</td>
<td>40</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A73</td>
<td>\textit{CRB1}</td>
<td>p.T745M; p.C438Y</td>
<td>24</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A77</td>
<td>\textit{CRB1}</td>
<td>p.K801X</td>
<td>25</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A57</td>
<td>\textit{GUCY2D}</td>
<td>p.L325R het</td>
<td>31</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>A136b</td>
<td>\textit{RPE65}</td>
<td>p.E102K</td>
<td>25</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>A136c</td>
<td>\textit{RPE65}</td>
<td>p.E102K</td>
<td>25</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>A137</td>
<td>\textit{RPE65}</td>
<td>p.Cys146fs</td>
<td>17</td>
<td>Yes</td>
<td>Normal</td>
</tr>
<tr>
<td>A120</td>
<td>\textit{RPE65}</td>
<td>p.R91P</td>
<td>35</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A135</td>
<td>\textit{RPE65}</td>
<td>p.Y144D</td>
<td>35</td>
<td>No</td>
<td>Reduced</td>
</tr>
<tr>
<td>A136</td>
<td>\textit{RPE65}</td>
<td>p.E102K</td>
<td>27</td>
<td>No</td>
<td>Normal</td>
</tr>
</tbody>
</table>
simultaneously on a significant number of patients analyzed (Table 2) and the small number of gene-specific genotypes identified can somehow limit the conclusions that can be drawn, nevertheless we believe that the present study provides valuable information for a better prognostic evaluation of patients with LCA and for a more efficient identification of patients who could potentially benefit from future experimental therapies.

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References