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Screening of a large cohort of Leber congenital amaurosis and retinitis pigmentosa patients identifies novel *LCA5* mutations and new genotype-phenotype correlations

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Abstract

To investigate the prevalence of sequence variants in *LCA5* in patients with Leber congenital amaurosis (LCA), early onset rod-cone dystrophy (EORD) and autosomal recessive retinitis pigmentosa (RP), to delineate the ocular phenotypes, and to provide an overview of all published

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LCA5 variants in an online database. Patients underwent standard ophthalmic evaluations after providing informed consent. In selected patients, optical coherence tomography (OCT) and fundus autofluorescence imaging was possible. DNA samples from 797 unrelated patients with LCA and 211 with the various types of RP were screened by Sanger sequence analysis of all *LCA5* exons and intron/exon junctions. Some LCA patients were pre-screened by APEX technology or selected based on homozygosity mapping. *In silico* analyses were performed to assess the pathogenicity of the variants. Segregation analysis was performed where possible. Published and novel *LCA5* variants were collected, amended for their correct nomenclature, and listed in a Leiden Open Variation Database (LOVD). Sequence analysis identified 18 new probands with 19 different *LCA5* variants. Seventeen of the 19 *LCA5* variants were novel. Except for two missense variants and one splice site variant, all variants were protein-truncating mutations. Most patients expressed a severe phenotype, typical of LCA. However, some LCA subjects had better vision and intact inner segment/outer segment (IS/OS) junctions on OCT imaging. In two families with *LCA5* variants, the phenotype was more compatible with EORD with affected individuals displaying preserved islands of RPE. One of these milder families harbored a homozygous splice site mutation, a second family was found to have a combination of a stop mutation and a missense mutation. This is the largest *LCA5* study to date. We sequenced 1008 patients (797 with LCA, 211 with arRP) and identified 18 probands with *LCA5* mutations. Mutations in *LCA5* are a rare cause of childhood retinal dystrophy accounting for ~2% of disease in this cohort and the majority of *LCA5* mutations are likely null. The *LCA5* protein truncating mutations are predominantly associated with LCA. However, in two families with the milder EORD, the *LCA5* gene analysis revealed a homozygous splice site mutation in one and a stop mutation in combination with a missense mutation in a second family, suggesting that this milder phenotype is due to residual function of lebercilin and expanding the currently known phenotypic spectrum to include the milder early onset RP. Some patients have remaining foveal cone structures (intact IS/OS junctions on OCT imaging) and remaining visual acuities, which may bode well for upcoming treatment trials.

Keywords

LCA; RP; retinal dystrophy; blindness; *LCA5*; lebercilin

Introduction

Leber congenital amaurosis (LCA) is an infantile onset, severe retinal dystrophy that presents with profound visual impairment, inability to fixate and nystagmus from birth or within the first few weeks of life (von Leber, 1869). It is the most severe form of a spectrum of disorders arising within the first few years of life, which affect both rod and cone photoreceptors, termed childhood or early onset retinal dystrophies (EORD) (Gu, et al., 1997). The term juvenile retinitis pigmentosa (RP) has also been used to describe EORD. Autosomal recessive retinitis pigmentosa (arRP) falls into this spectrum of disorders with onset typically later in childhood or beyond. LCA accounts for 3-5% of childhood blindness in the developed world and has an incidence of 2-3 per 100,000 live births (Heckenlively, et al., 1988). Depending on the age at diagnosis, the retinal appearance may be normal or there may be a variety of abnormalities including vascular narrowing, macular atrophy, peripheral

white dots at the level of the retinal pigment epithelium (RPE), and retinal pigmentation. The full-field electroretinogram (ERG) is usually nondetectable (<10uV) with conventional ERG testing or severely decreased before the age of one year (Foxman, et al., 1985; Franceschetti and Dieterle, 1954).

LCA is predominantly inherited as an autosomal recessive trait, although rare dominant forms have been reported (Swaroop, et al., 1999). Nineteen causative genes have thus far been reported; *AIPL1* [MIM# 604392] (Sohocki, et al., 2000), *CABP4* [MIM# 608965] (Aldahmesh, et al., 2010), *CEP290* [MIM# 610142] (den Hollander, et al., 2006), *CRB1* [MIM# 604210] (den Hollander, et al., 2001), *CRX* [MIM# 602225] (Freund, et al., 1998), *GUCY2D* [MIM# 600179] (Perrault, et al., 1996), *IMPDH1* [MIM# 146690] (Bowne, et al., 2006), *IQCB1* [MIM# 609237] (Estrada-Cuzcano, et al., 2011), *KCNJ13* [MIM# 603208] (Sergouniotis, et al., 2011), *LCA5* [MIM# 611408] (den Hollander, et al., 2007), *LRAT* [MIM# 604863] (Thompson, et al., 2001), *MERTK* [MIM# 604705] (Gal, et al., 2000), *NMNAT1* [MIM# 608700] (Chiang, et al., 2012; Falk, et al., 2012; Koenekoop, et al., 2012; Perrault, et al., 2012), *RD3* [MIM# 180040] (Friedman, et al., 2006), *RDH12* [MIM# 608830] (Janecke, et al., 2004), *RPGRIP1* [MIM# 605446] (Dryja, et al., 2001), *RPE65* [MIM# 180069] (Gu, et al., 1997; Marlhens, et al., 1997), *SPATA7* [MIM# 609868] (Wang, et al., 2009) and *TULP1* [MIM# 602280] (Hanein, et al., 2004). Interestingly, mutations in some of these genes, such as *CEP290* (Littink, et al., 2010), *CRB1* (den Hollander, et al., 1999), *LRAT* (Thompson, et al., 2001), *MERTK* (Gal, et al., 2000), *RPE65* (Morimura, et al., 1998) and *SPATA7* (Wang, et al., 2009), may also be associated with milder forms of retinal dystrophy.

The LCA5 locus (MIM# 604537) was mapped to chromosome 6q11-q16 by traditional linkage studies in a multi-generational kindred of the Old Order River Brethren (Dharmaraj, et al., 2000). Further homozygosity mapping in consanguineous and non-consanguineous families led to a refinement of the critical region (den Hollander, et al., 2007; Mohamed, et al., 2003) and the causative gene, *LCA5*, was identified (den Hollander, et al., 2007). *LCA5* comprises nine exons, seven of which encode Lebercilin, a 697-amino acid protein that contains four coiled-coil domains (den Hollander, et al., 2007). Lebercilin is widely expressed in human tissues including cilia. *In situ* hybridization of mouse embryos at day 12.5 post-coitum detected almost ubiquitous, low level staining. At embryonic day 14.5, staining of the eye, inner ear, kidney, and regions of the central and peripheral neural system, gut and the ciliated epithelium of the nasopharynx, trachea, and lungs was more pronounced. In the adult eye, expression was limited to the photoreceptor cell layer. In ciliated cell lines, lebercilin localizes to the ciliary axoneme. In mouse and rat retina, it is found between the outer and inner segments of the photoreceptor layer. In a human embryonic kidney cell line, HEK293, recombinant lebercilin was found to interact with 24 proteins, many of which are associated with centrosomal or ciliary functions, and the intraflagellar transport machinery (Boldt, et al., 2011). *Lca5* inactivation in mice led to very severe and rapid retinal degeneration. At 1 month post-natally, there was a significant reduction in photoreceptor cells, and after 4 months, the photoreceptor layer was completely absent (Boldt, et al., 2011).

To date, 12 different mutations in *LCA5* have been reported. In patients of all families except one, the phenotype has been typical of LCA (Abu-Safieh, et al., 2013; Ahmad, et al., 2011; den Hollander, et al., 2007; Gerber, et al., 2007; Jacobson, et al., 2009; Li, et al., 2011; Ramprasad, et al., 2008; Vallespin, et al., 2010a; Vallespin, et al., 2010b). Despite its ubiquitous expression and its crucial functions in the cilia (Boldt, et al., 2011), the phenotype associated with *LCA5* mutations has thus far been confined to the retina. In this paper we present the results of screening for mutations in *LCA5* in a large number of subjects with LCA and EORD (n = 797), and arRP (n = 211), and describe the associated phenotypes.

Materials and Methods

Patients

In total, we sequenced 1008 patients. We ascertained 797 probands with LCA and EORD, living in Belgium (n = 47), Canada and the USA (n = 300), Germany (n = 65), the Netherlands (n = 54), South Africa (n = 18), Sweden (n = 20), Switzerland (n = 42), China (n = 100), Pakistan (n = 1) and the United Kingdom (n = 150). In addition, we included 211 persons with isolated or arRP from the Netherlands. Many LCA and arRP patients were not originally from their current place of residence and were actually from India, Pakistan, Russia and other countries. Approximately 50% of the persons with LCA were previously analyzed using various Asper Ophthalmics LCA allele-specific primer extension (APEX) mutation chips. None of the DNAs of persons with arRP were previously analyzed for *LCA5* mutations using APEX. LCA, early-onset retinal degeneration (EORD), and RP may be considered a continuum of retinal dystrophies, in which LCA represents the extreme end of severity, as far as age of onset and functional outcome are concerned. In our current study, LCA was diagnosed in children who were born with severe visual loss, inability to fixate at around 6 weeks of age, nystagmus and absent or non-detectable ERGs. EORD was diagnosed in children born with reduced, but clinically identifiable, visual function and fixation, with or without nystagmus, and with progressive night-blindness and subsequent vision loss. arRP was diagnosed in patients without nystagmus, a variable onset of progressive night-blindness and visual loss after age 2 years.

Clinical Investigations

All patients and family members provided informed consent as part of research projects approved by the local research ethics committees, and the study was conducted in accordance with the principles of the Declaration of Helsinki. Clinical evaluation, including monocular best-corrected visual acuity (BCVA) by projected Snellen charts, slit-lamp biomicroscopy and fundus examination was performed on all patients who could cooperate with testing. Where possible, patients underwent retinal imaging using a Topcon TRC 501A retinal camera (Topcon Corporation, Tokyo, Japan), optical coherence tomography using a SPECTRALIS® Spectral domain OCT scanner (Heidelberg Engineering, Germany), and retinal autofluorescence (AF) imaging using a confocal scanning laser ophthalmoscope (Zeiss Prototype; Carl Zeiss Inc., Oberkochen, Germany). Pattern and full-field electroretinography (PERG and ERG) were performed, where possible, using the recommendations of the International Society for Clinical Electrophysiology of Vision

(ISCEV) or a modified Paediatric ERG protocol with skin electrodes as previously described (Flitcroft, et al., 2005; Kriss, 1994; Marmor, et al., 2009). Patient #14 (Berman-Gund Laboratory ID: 048-038 was evaluated by full-field ERG testing using Burian Allen contact lens electrodes, narrow bandpassed filtering and computer averaging that can extend the range of detectability of 30Hz cone ERGs to 0.05 uV (Berson, et al., 1993). Goldmann perimetry was performed in patient #16.

Blood samples were collected in EDTA tubes and DNA was extracted using the Puregene blood extraction kit (Invitrogen, Paisley, UK) following manufacturer's instructions, or by a standard salting out procedure (Miller, et al., 1988).

Mutation screening

Nucleotide numbering reflects cDNA with +1 corresponding to the A of the ATG translation initiation codon (RefSeq NM_181714.3), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. Primers used to amplify the coding exons and the intron-exon boundaries of *LCA5* were designed using those published previously (den Hollander, et al., 2007). In addition, 194 persons with LCA and 94 persons with arRP were analysed for the presence of an exon 1 deletion identified in the original *LCA5* family (den Hollander, et al., 2007). To this end, primers 5'- TTCCGTGCAATTTAGGGATT-3' and 5'-CGGGGTTTTTGTTTTGTTTG-3' were designed to amplify a deletion breakpoint fragment of 117 bp. All standard polymerase chain reactions (PCR) were performed in a total volume of 30 μl containing 200 μM dNTPs (Bioline, London, UK), 20 μM of each primer, 1× reaction buffer with 1.5 mM $MgCl_2$ (Bioline) with 1 unit of Biotaq (Bioline) and 100 ng of DNA. PCR was carried out on a PTC200 DNA engine thermal cycler (Bio-Rad, Hemel Hempstead, UK). Cycling conditions were as follows: 5 minutes denaturation at 94°C followed by 35 cycles of 94°C for 30 seconds, annealing temperature for 30 seconds and extension at 72°C for 30 seconds. A final extension of 72°C for 5 minutes completed the cycling conditions.

PCR products were visualised on a 2% agarose gel containing 0.05% ethidium bromide. The products were cleaned using multiscreen PCR filter plates (Millipore, Watford, UK) prior to sequencing. PCR products were sequenced directly using the ABI Prism Big Dye terminator Kit (V3.1) in a 10μl reaction. Samples were purified using the Montage cleanup kit (Millipore, Watford, UK) prior to being run on an ABI applied biosystems 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Electropherograms were analysed for sequence changes using DNAStar computational software (DNAStar, Inc., USA). Any sequence changes identified were checked visually.

Mutations were tested in normal controls when possible, and co-segregation was performed in the families, when possible. Mutation frequencies were analyzed in the exome variant server database, 1000 genomes database and dbSNP database. *In silico* analyses were then performed to assess pathogenicity, including Blosum62, Polyphen2, and PhyloP.

Patient #1 was originally identified to harbor a heterozygous mutation in *LCA5* by microarray analysis using the Asper LCA Apex chip. His second mutation was identified following *LCA5* sequence analysis. Patient #18 was selected for *LCA5* sequence analysis

because homozygosity mapping of two affected persons (IV:2 and IV:3) of family W12-2240 (Fig. 1) showed a large, significant homozygous region surrounding *LCA5*. To identify this region, we had employed the CytoScan® High-Density array containing probes to detect 750,000 single nucleotide polymorphisms (Affymetrix, Santa Clara, CA).

Results

Mutational Analysis

A total of 797 patients with LCA, EORD and 211 with arRP were screened for sequence variants in protein-coding exons (i.e. exons 3 through 9) of *LCA5*. Eighteen probands were identified with two disease-causing variants. In all, 19 different *LCA5* variants were identified (Table 1), 17 of these variants are novel. Homozygous variants were found in 10 probands, compound heterozygous variants in six probands, and heterozygous protein truncating variants in two probands (Table 1).

All but two mutations were predicted to lead to premature termination of the lebercilin protein. One of the missense mutations that we identified was in patient #4 involving the first amino acid of the lebercilin protein (p. Met1Ile). The other missense change is a novel change in patient #16 involving a histidine to arginine change at position 164 (p. His164Arg). The underlying c.491A>G variant was not found in 8600 alleles of 'control' individuals in the Exome Variant Server database and once in the 1000 genomes database (rs183669161). Unfortunately we do not have parental DNA to test for segregation. The PhyloP score of c.491A is 3.92, Polyphen2 predicts damage, and the histidine at position 164 is fully conserved between human, rhesus, mouse, dog, opossum, chicken, Western clawed frog and zebrafish. In an affected half-brother (IV:1) and an affected cousin (V:1) of patient #7 (Fig. 1A), the same homozygous splice site variant (c.1231+2insT) was found, and two unaffected persons carried this variant in the heterozygous state. This variant is predicted to completely inactivate this splice site based on SpliceSiteFinder, MaxEntSplice and NNSplice analysis. Homozygosity mapping using DNA from two persons with LCA from family W12-2240 (Fig. 1B: patients IV:2 and IV:3) yielded 10 shared homozygous regions larger than 5 Mb. The third largest homozygous region on chromosome 6 (18.9 Mb) encompassed *LCA5*. In all three affected individuals (Fig. 1B), the c.1151delC (p. Pro384Glnfs*18) variant was identified in the homozygous state in *LCA5*. This variant was present in the heterozygous state in five unaffected members (Fig. 1B).

The ethnic origins of the 18 probands with *LCA5* mutations were as follows: European Caucasian: 7 (British, Slovakian-Romani, Spanish); Asian: 7 (Pakistan, Afghanistan, Indian-Mauritian, Iraqi, Taiwanese and Chinese); North American: 3; Mexican: 1.

Clinical phenotype

Of the 18 probands with *LCA5* variants identified in this study, all but two patients had a clinical diagnosis of LCA, with onset of severe visual loss at birth or within the first few months of life, and nystagmus (Table 2). However, patients #7 and #16 received a diagnosis of EORD. Patient #7 was born with congenital nystagmus, and developed nightblindness at age 3, visual field loss at age 6 and visual loss at age 8. In patient #16 the visual loss started

after the age of 40 years. In addition, patient #16 did not have nystagmus. All of our LCA and EORD patients had nyctalopia and severe visual field constriction. General health was good in the majority of patients, except in patient #2 who had global developmental delay and patient #3 who had behavioral problems. Fourteen of the 16 LCA patients were legally blind with visual acuity ranging from 1.0 logMAR to light perception. One patient, #11, with LCA and nystagmus, had visual acuities of 0.48 right (20/60) and 0.54 left (20/70) at age 6 years (Table 2). Patient #16, with EORD, had a visual acuity of 0.30 (20/40) at age 55 years. Most patients had a hypermetropic refraction (Table 2). The high myopic correction in patient 6 may be attributed to keratoconus. A full-field electroretinogram was performed in eight patients and was nondetectable (<10uV) with conventional ERG testing in five patients. A small cone ERG response could be quantified in patient #14 and trace cone ERG responses (unquantified) could be detected in patient #16 (Table 2). Anterior segments were normal in all but one of the oldest patients (patient #6), who had bilateral keratoconus at age 37 years. This patient also developed bilateral nuclear sclerotic and posterior subcapsular lenticular opacities in adulthood.

Fundus examination most commonly revealed widespread retinal pigment epithelial atrophy and granularity, with peculiar, small, white dots at the level of the RPE in the retinal periphery, which were seen in 10 of 14 patients (71%) in whom funduscopic data were available (Fig. 2). Figs. 2b and 2c show the white dots clearly, and document the round, evenly spaced, similar sized lesions. Intraretinal pigment migration, when present, was minimal, situated in the far retinal periphery, and seen in the oldest patients, patients #1, #6, #7, #14 and #16 (Fig. 2A, 2D and 2E). Macular atrophy was noted in only three patients, #3, #16 and #17 (Fig. 2B, 2E and 2F); the macula was otherwise normal on funduscopy in the remaining patients. In one patient, the macular lesion was particularly severe, with features of a "macular coloboma" (Fig. 2F). Optic discs were normal in appearance (pink disc color) in all but one patient (patient #14), who had optic disc drusen (Fig. 2D). In one family with arRP (patient #7), we identified a pumpkin colored maculopathy and intra-retinal pigmentation in the periphery with faintly preserved islands of RPE (Fig. 3).

OCT data, available for patients #1, #11, and #16 showed preservation of the central foveal inner segment/outer segment (IS/OS) junctions and of the outer segments in the two younger patients (#1 and #11) (Fig. 4A and 4B). In the older patient (#16) OCT imaging demonstrated macular atrophy, disruption of retinal lamination and the presence of a hyporeflective well-circumscribed area in the outer nuclear layer, with a hyperreflective border. This may represent an area of outer retinal tubulation (also known as rosettes) (Fig. 4C). OCT image acquisition was otherwise not possible due to nystagmus. Fundus autofluorescence (FAF) imaging data was available in two patients (Fig. 5). FAF in patient #1 revealed an overall hypofluorescence in the macula, with a central hyperfluorescent signal in the fovea (Fig. 5A). FAF images of patient #16 showed severe hypofluorescence in the macula corresponding to significant RPE atrophy (Fig. 5B). Goldmann perimetry in patient #16 revealed a small central island, with a relatively large preserved nasal field and a small temporal field.

Discussion

LCA5 mutations are a rare cause of LCA. Previously, only 12 different mutations had been identified and published in 16 families with LCA. In the current study, which is the largest study to date, we have screened the coding region of *LCA5* in 1008 patients, 797 with LCA and EORD and 211 with arRP, ascertained from eight different countries. We identified mutations in 18 new LCA patients. We discovered 19 different *LCA5* mutations, 17 of which are novel. Mutations in *LCA5* thus account for ~2% of patients with LCA, EORD and arRP in our cohort. All 29 different *LCA5* variants have now been included in the new Leiden Open Variation Database (LOVD; http://www.LOVD.nl/LCA5). In two patients (#14 and #15), we identified heterozygous variants. The second allele may have been missed because the variant may be located outside the protein-coding exons, or because these variants are causative in a more complex inheritance model (such as digenic), or that these are coincidental findings of heterozygous variants that play no role in the LCA phenotype.

The majority of *LCA5* mutations found both in this study and in those published previously are likely null mutations due to premature termination of the protein. Only one missense mutation in this gene (p. Ser202Pro) has been previously reported in LCA, but without accompanying clinical information (Vallespin, et al., 2010b). We also identified a missense variant, p. Met1Ile (the start codon), in patient #4, who carried a diagnosis of LCA, in combination with the most common *LCA5* stop mutation p. Gln279*. If the next methionine in the open reading frame were to be used instead (at position 336), as the alternative start codon, then two coiled-coiled domains in the lebercilin protein would be lacking, very likely rendering the truncated lebercilin inactive. Therefore, this missense mutation may also be a null allele. We also identified another missense mutation, p. His164Arg. This mutation was found in combination with a novel nonsense mutation, p. Tyr23*, in LCA patient #16, but segregation data are lacking. Thus, we cannot exclude that these variants are in *cis* configuration and that they are not causative for the phenotype in patient #16.

Previously, and in the majority of patients in this study, the phenotype reported in association with *LCA5* mutations is consistent with LCA with severely reduced vision at, or near birth, nystagmus, and a non-detectable (<10 µV) ERG (Ahmad, et al., 2011; den Hollander, et al., 2007; Dharmaraj, et al., 2000; Gerber, et al., 2007; Jacobson, et al., 2009; Mohamed, et al., 2003; Ramprasad, et al., 2008). High hypermetropia is common. The visual acuity is reported to range between 0.20 to light perception and there is extensive peripheral field loss. In our study, the majority of patients had a clinical diagnosis of LCA, with fundus examination revealing widespread atrophy of the retina and RPE but with little intra-retinal pigment migration. On the clinical examination we commonly identified scattered white dots at the level of the RPE (Fig. 2A, 2B, 2C, 2D – white arrows). White dots can be found in other genetic types of LCA, such as *RPE65*, *LRAT* and *CEP290*. In *RPE65* type LCA, Weleber suggested that the white dots might represent accumulations of trans-retinyl esters (Weleber, et al., 2011). The biochemical make-up of the white dots associated with *LCA5* mutations is as yet unknown. Our *LCA5* associated retinal phenotypes are consistent with the retinal changes observed in other patients with *LCA5* mutations (Figs. 2 and 3) (Ahmad, et al., 2011; Dharmaraj, et al., 2000; Gerber, et al., 2007; Mohamed, et al., 2003; Ramprasad, et al., 2008).

However, in this study, for the first time, we identified an Iraqi family with two affected sibs who carried a diagnosis of EORD, because they maintained some useful vision until age 10. In addition, these siblings had preservation of the peripheral islands of RPE (Fig. 3), a phenotype similar to PPRPE documented by Heckenlively and later found to be associated with LCA and arRP patients with *CRB1* mutations (den Hollander, et al., 1999; Heckenlively, 1982). We have thus expanded the phenotypic spectrum associated with *LCA5* mutations, which now includes both EORD and a new retinal phenotype consisting of preserved islands of peripheral RPE (Fig. 3).

There is little published data on retinal imaging such as OCT and fundus autofluorescence imaging in patients with *LCA5* mutations. In this regard, we here contribute three new patients with LCA5 mutations and new OCT and FAF data. However, it is a limitation of the present study that we were unable to obtain good quality autofluorescence imaging and OCT on the entire cohort. This was, in part, due to the inherent difficulties of such imaging in patients with nystagmus, their ages and their remote living conditions. Jacobson *et al*. reported a low autofluorescent signal corresponding to macular atrophy in two siblings with a homozygous *LCA5* mutation, in whom mutations in *CRB1* had previously been excluded (Jacobson, et al., 2009). We demonstrated similar macular hypoautofluorescence in one subject who in addition had a small area of hyperautofluorescence at the fovea, suggesting continued metabolic activity of the foveal cones and RPE. In our study, OCT imaging was possible in three subjects and the findings revealed surprising preservation of the central foveal IS/OS junctions and of the outer segment structures (in the younger patients, age 17 years and 6 years respectively). In the older patient, age 55 years, OCT imaging demonstrated macular atrophy, loss of the retinal lamination and probable outer retinal tubulation (also known as rosettes), a late stage process in the degeneration of photoreceptors that has been described recently in a number of retinal conditions (Fig. 4C) (Sergouniotis, et al., 2012; Zweifel, et al., 2009). Previously published OCT findings have demonstrated loss of the photoreceptor and outer nuclear layers, and abnormal retinal lamination eccentric to the fovea, but with evidence of a retained photoreceptor layer at the fovea (Jacobson, et al., 2009). This suggests that in the early stages of the disease there may be viable photoreceptors (cones) in the foveal region, which may be amenable to therapeutic intervention. It is of interest that patient 14 at age 19, with an advanced stage of disease, still had a detectable 30 Hz cone ERG in each eye (Table 2) with narrow bandpassed filtering and computer averaging. In future clinical trials, this technique of ERG recording which is not performed in the present ISCEV protocol could be considered as a method of objectively monitoring efficacy or lack of efficacy as undoubtedly most patients with remaining vision will have a detectable cone ERG with this technique.

Lebercilin is expressed in a number of tissues; however there have been no reports of extraocular abnormalities in *LCA5* mutations. One patient with *LCA5* retinopathy died from asphyxia and the authors propose a possible link between the cause of death and the presence of lebercilin in the bronchial ciliated epithelial cells (Ramprasad, et al., 2008). In the present study, one patient had global developmental delay while another experienced behavioral problems. Whether this is related to abnormal extraocular expression of lebercilin remains to be determined.

LCA5-associated LCA is a rare form of severe childhood onset retinal dystrophy. Most *LCA5* mutations are null and are associated with a severe phenotype. Missense mutations are rare and may be associated with a milder rod-cone dystrophy (EORD) suggesting that such mutations may result in some residual protein function. *LCA5* type LCA may be amenable to treatment, but this is more likely to be successful in young patients.

Acknowledgments

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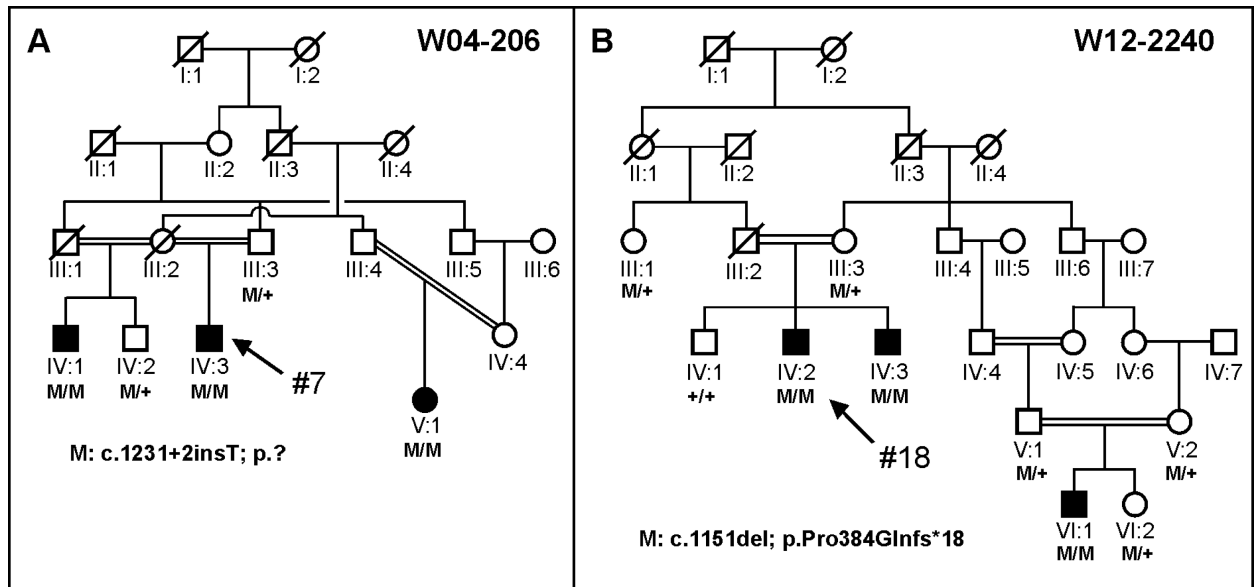
Figure 1. Pedigrees of patients #7 and #18

A. An EORD family with proband #7 is indicated with an arrow. A half-brother and cousin also show the clinical characteristics of arRP and carry the splice variant (M: c.1231+2insT) in the homozygous state. One unaffected brother and the father of the proband carry this variant in the heterozygous state. B. An LCA family with proband #18. A frequent Pakistani *LCA5* variant (c.1151del) is found in the homozygous state in three persons with LCA and in the heterozygous state in five unaffected persons.
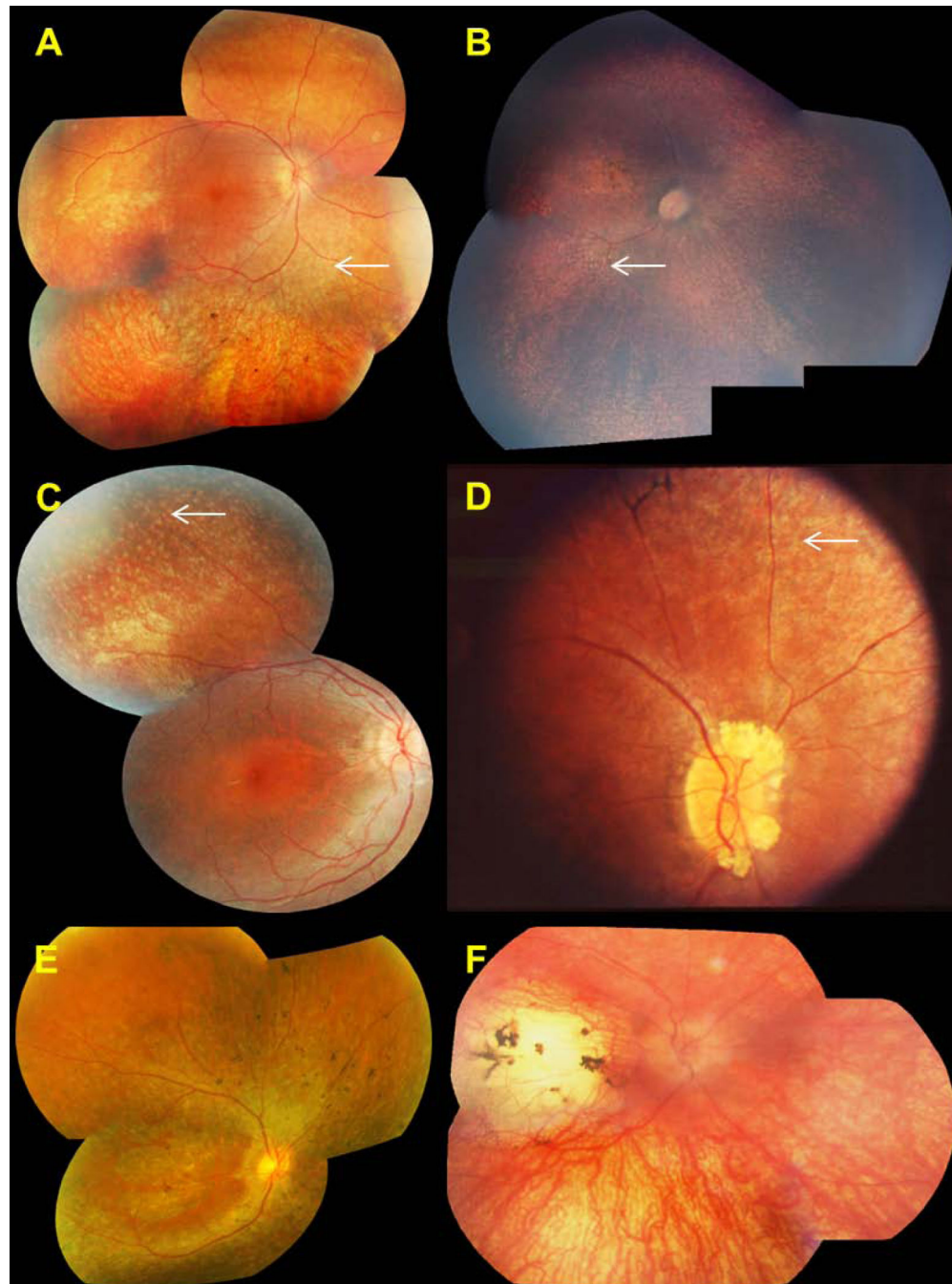
Figure 2. Retinal phenotype of patients with *LCA5* mutations
Right eye shown for each patient.

A. Patient #1, age 17 years, with widespread RPE atrophy, mid-peripheral retinal white dots (white arrow) and peripheral intra-retinal pigmentation

B. Patient #3, age 5 years, with mid-peripheral retinal white dots (white arrow) and macular atrophy

C. Patient #4, age 8.5 years, with widespread RPE atrophy and mid-peripheral retinal white dots (white arrow)

D. Patient #14, age 19 years, with retinal white dots (white arrow), peripheral intra-retinal pigmentation and optic disc drusen

E. Patient #16, age 55 years, with widespread RPE atrophy, foveal preservation and peripheral intra-retinal pigmentation

F. Patient #17, age 18.5 years with marked macular atrophy and hypopigmented but relatively normal peripheral retina. Intra-retinal pigment was seen in the oldest patients (panels A, D and E)
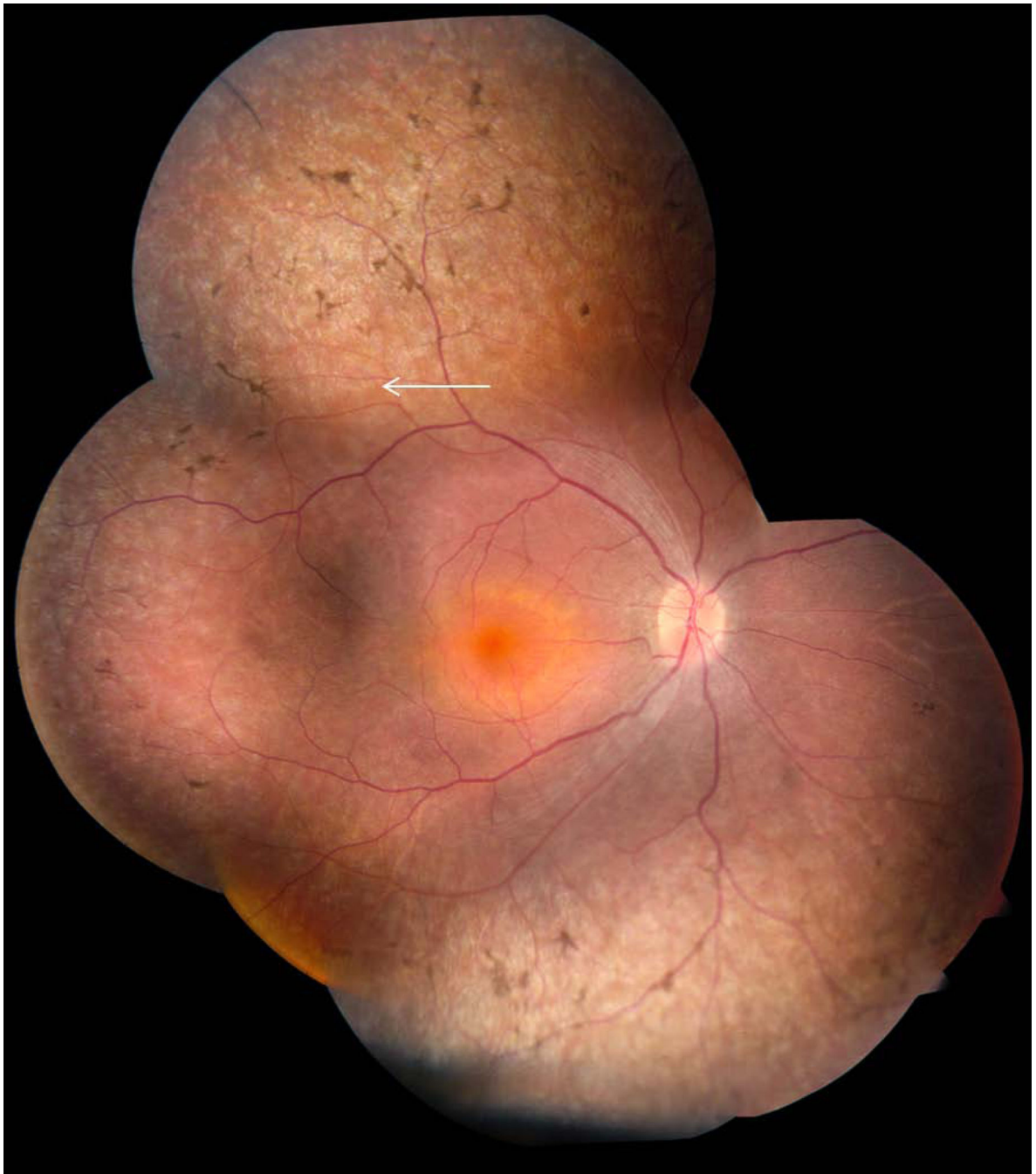
Figure 3. Retinal phenotype of arRP patient #7, right eye, age 21 years
There is widespread RPE atrophy, peripheral preserved islands of RPE (white arrow), peripheral intra-retinal pigmentation and a 'pumpkin' colored macula
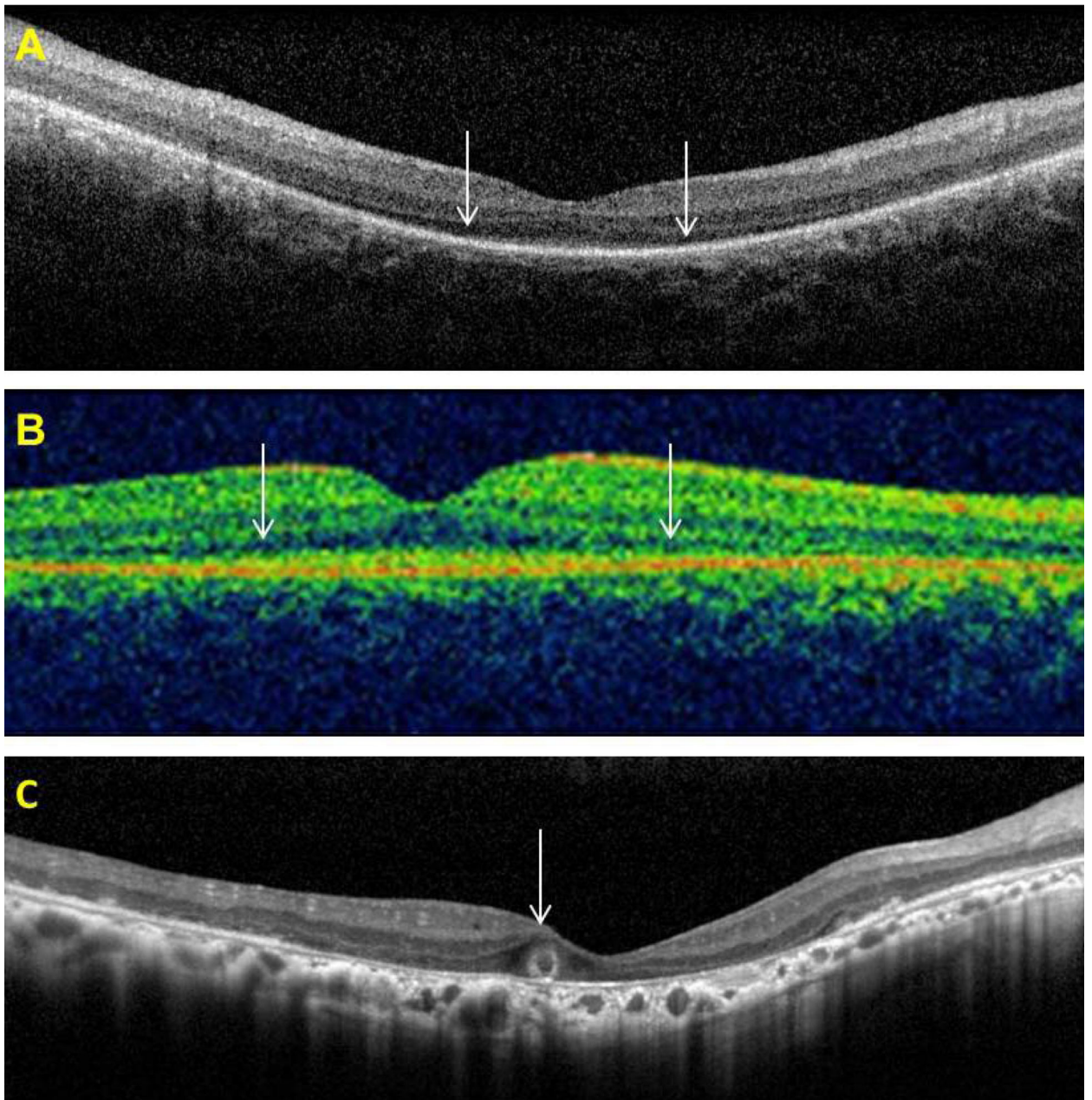
Figure 4. Optical coherence tomography imaging of patients with *LCA5* variants
A. Patient #1 (age 17 years) and B. Patient #11 (age 6 years) - white arrows demarcate the extent of preservation of the central foveal inner segment/outer segment (IS/OS) junctions and of the outer segments
C. Patient #16 (age 55 years) demonstrating macular atrophy, disruption of retinal lamination and a presumed area of outer retinal tubulation (white arrow)

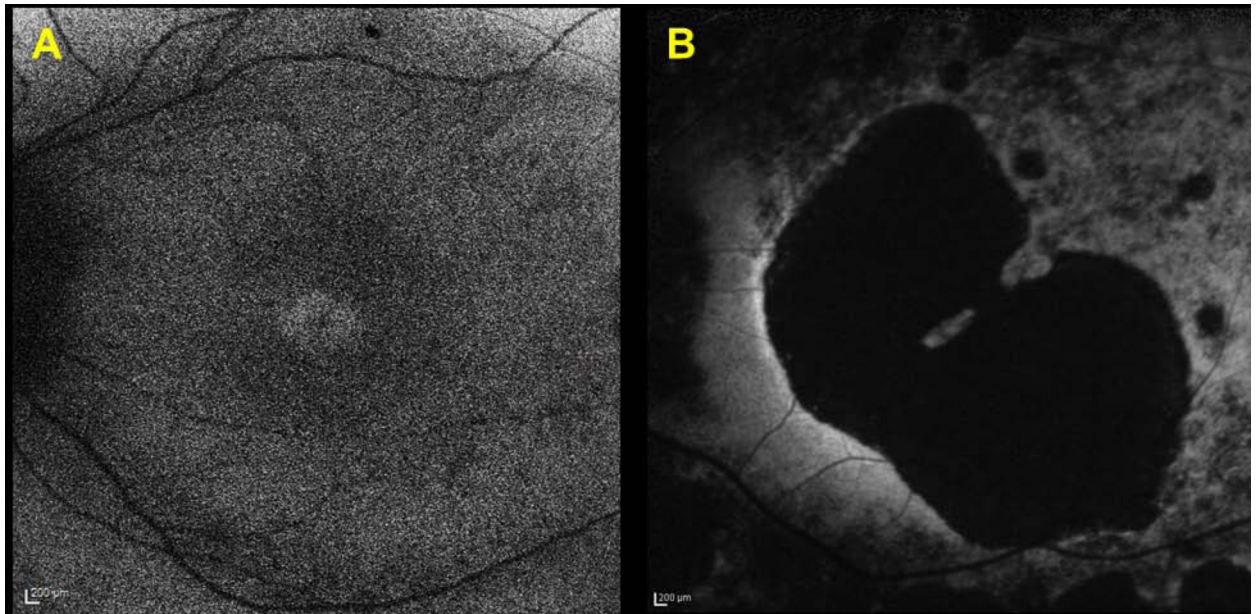**Figure 5. Fundus autofluorescence imaging of patients with *LCA5* variants**
A. Patient #1 (age 17 years), demonstrating overall macular hypofluorescence, with a hyperfluorescent foveal signal, suggesting metabolic activity of the foveal cones and RPE complex
B. Patient #16 (age 55 years), showing a large area of macular hypofluorescence corresponding to RPE atrophy

Table 1

Mutations identified in *LCA5*

Patient	Allele 1		Allele 2		Segregation	Reference (Allele 1/Allele 2)
	DNA variant	Protein variant	DNA variant	Protein variant		
1	c.835C>T	p.(Gln279*)	c.1756A>T	p.(Lys586*)	Yes	den Hollander *et al* 2007/Novel
2	c.1151delC	p.(Pro384Glnfs*18)	c.1151delC	p.(Pro384Glnfs*18)	Yes	den Hollander *et al* 2007
3	c.633_639del	p.(Glu211Aspfs*13)	c.633_639del	p.(Glu211Aspfs*13)	M-yes; F-NT	Novel
4	c.3G>A	p.(Met1Ile)	c.835C>T	p.(Gln279*)	NT	Novel/den Hollander *et al* 2007
5	c.1676C>A	p.(Ser559*)	c.1676C>A	p.(Ser559*)	Yes	Novel
6	c.367C>T	p.(Gln123*)	c.1756T>A	p.(Lys586*)	NT	Novel/Novel
7	c.1231+2insT	p.?	c.1231+2insT	p.?	Yes	Novel
8	c.142A>T	p.(Arg48*)	c.142A>T	p.(Arg48*)	NT	Novel
9	c.142A>T	p.(Arg48*)	c.142A>T	p.(Arg48*)	NT	Novel
10	c.1543_1544delinsA	p.(Ser515Lysfs*78)	c.1543_1544delinsA	p.(Ser515Lysfs*78)	NT	Novel
11	c.795T>G	p.(Tyr265*)	c.1759_1760insAG	p.(Leu587Glnfs*7)	NT	Novel/Novel
12	c.835C>T	p.(Gln279*)	c.835C>T	p.(Gln279*)	NT	den Hollander *et al* 2007
13	c.42_45del	p.(Lys15Glnfs*95)	c.1207C>T	p.(Gln403*)	NT	Novel/Novel
14	c.1730dup	p.(Leu577Phefs*12)	+	+	NT	Novel
15	c.2011C>T	p.(Arg671*)	+	+	NT	Novel
16	c.69C>G	p.(Tyr23*)	c.491A>G	p.(His164Arg)	NT	Novel/Novel
17	c.439_449dup	p.(Glu151*)	c.439_449dup	p.(Glu151*)	Yes	Novel
18	c.1151delC	p.(Pro384Glnfs*18)	c.1151delC	p.(Pro384Glnfs*18)	Yes	den Hollander *et al* 2007

Mutation nomenclature is based on NM_181714.3

F, father; M, mother; NT, not tested; +, not identified

Table 2

Clinical Characteristics of persons with *LCA5* variants

Patient[#]	Ethnicity	Diagnosis	Age of onset	Age at exam (years)	General Health	Latest VAR (LogMAR)	Latest VAL (LogMAR)	RE Spherical Equivalent (Dioptres)	LE Spherical Equivalent (Dioptres)	Anterior segment	Fundus	ERG
1	British Caucasian	LCA	3 months	17	Good	1	1	+ 4.00	+ 4.38	Normal	Widespread RPE atrophy, white dots, vascular attenuation, minimal BS pigmentation inferiorly	Undetectable
2	Afghani	LCA	Birth	4	Global developmental delay	LP	LP	+ 2.50	+2.00	Normal	White dots in retinal periphery, normal macula, normal discs	Rudimentary full field rod function; undetectable cone function
3	Slovakian Romani	LCA	3 months	8	Behavioral issues	LP	LP	+ 6.50	+ 6.50	Normal	Widespread RPE atrophy, white dots, macular atrophy	Undetectable
4	British Caucasian	LCA	Infancy	8.5	Good	1.1	1.1	N/A	N/A	Normal	Widespread RPE atrophy, white dots, normal discs and macula	N/A
5	Indian Mauritian	LCA	6 weeks	1	N/A	LP	LP	+ 6.00	+ 6.00	Normal	N/A	N/A
6	British Caucasian	LCA	Birth	37	Good	CF	LP	− 13.26	− 1.94	Bilateral KC; bilateral NS; PSCLO	Asteroid hyalosis LE, widespread RPE atrophy, white dots, minimal BS pigmentation, vascular thinning, normal macula	N/A
7	Iraqi	EORD	Birth	21	Good	1	1.2	N/A	N/A	Normal	Salt and pepper' fundus, peripheral preserved RPE islands pattern, minimal BS pigmentation, pumpkin coloured macula	N/A
8	Spanish	LCA	Birth	3	Good	CF	CF	N/A	N/A	Normal	White dots in retinal periphery	N/A
9	Spanish	LCA	Birth	3	Good	CF	CF	N/A	N/A	Normal	White dots in retinal periphery	N/A
10	Mexican	LCA	Birth	3	Good	HM	HM	N/A	N/A	Normal	N/A	N/A
11	Chinese	LCA	Birth	6	Good	0.48	0.54	+ 3.25	+ 3.88	Normal	White dots in posterior pole	Undetectable
12	US	LCA	Birth	20	Good	HM	HM	+ 5.00	+ 5.00	Normal	Granularity throughout fundus	Undetectable
13	Chinese	LCA	Birth	16	Good	HM	HM	N/A	N/A	Normal	White dots in retinal periphery	Not able to perform
14[#]	US	LCA	Birth	19	Good	1	1	+ 2.25	+ 2.25	Normal	White dots in retinal periphery and posterior pole; minimal BS pigmentation; right optic disc drusen	Cone b-wave: 0.26 µV OD, 0.56 µV OS##
15	US	LCA	Birth	1	Good	N/A	N/A	N/A	N/A	Normal	N/A	N/A
16	Taiwan	EORD	43 years	55	Good	0.3	1.66	− 0.75	− 0.13	Normal	Relatively pink optic discs, attenuated vessels with vascular sheathing, macular RPE atrophy with preserved foveae, widespread RPE atrophy, with superficial and deep intra-retinal pigmentation	Trace of cone function in OS
17	British Caucasian	LCA	Birth	18.5	Good	LP	LP	+ 9.00	+ 9.00	Normal	Bilateral well-circumscribed atrophic macular lesions. Relatively normal hypo-pigmented mid-peripheral and peripheral retinae. No white dots, normal optic nerves	N/A
18	Pakistani	LCA	Birth	20	Good	LP	LP	N/A	N/A	Normal	N/A	Undetectable

ArRP, autosomal recessive retinitis pigmentosa; BS, bone spicule; CF, counting fingers; ERG, electroretinogram; HM, hand movements; KC, keratoconus; LCA, Leber congenital amaurosis; LP, light perception; N/A, not available; NS, nuclear sclerosis; OD, right eye; OS, left eye; PPRPE, preserved para-arteriolar retinal pigment epithelium; PSCLO, posterior capsular lens opacity; RPE, retinal pigment epithelium; VAL, visual acuity left eye; VAR, visual acuity right eye.

[#] represents patient ID Berman-Gund Laboratory ID: 048-038.

Patient 14 was evaluated by full-field ERG testing using narrow bandpassed filtering and computer averaging that can extend the range of detectability of 30Hz cone ERGs to 0.05 uV (Berson, et al. 1993)