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Potential involvement of more than one locus in trait manifestation for individuals with Leber congenital amaurosis

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Abstract

Leber congenital amaurosis (LCA) is a clinically and genetically heterogeneous retinal dystrophy. The causes of LCA have been unraveled partially at the molecular level. At least 14 genes have been reported that, when mutated, result in LCA. To understand the roles of the known genes in LCA, a group of outbred subjects from 60 apparently either recessive families, with one or more affected individuals, or isolated patients were evaluated. One affected individual from each family underwent comprehensive mutational analysis by direct DNA sequencing of all coding regions and splice junctions of 13 LCA genes. Mutations were identified in 70% of individuals. *CEP290* made the largest contribution to the identified mutations, providing 43% of those mutant alleles. We identified seven families in which affected individuals with two mutant alleles, sufficient to cause disease, had an additional mutation at a second LCA locus. Our findings suggest that mutational load can be important to penetrance of the LCA phenotype.

Introduction

Leber congenital amaurosis (LCA, MIM#204000) is a clinically heterogeneous disorder of the retina that affects approximately 2–3 per 100,000 individuals in the general population and accounts for 5% of all inherited retinal dystrophies (Koenekoop 2004). The inclusion criteria for establishing a diagnosis of LCA are profound visual impairment present at birth or at an early age, infantile nystagmus, oculodigital signs, and a diminished or non-recordable electroretinographic response (ERG). Additional clinical features or later findings may include keratoconus, neural hearing deficits, developmental delay, seizures, and a variety of fundus appearances (Lewis 1988). This clinical diversity likely reflects both its genetic and allelic heterogeneity. To date, 14 genes have been identified in which mutant alleles can cause LCA (Table 1). The sequencing of known LCA genes allows identification of mutations in 65–70% of LCA patients, leaving a substantial fraction of LCA clinical cases for which the genetic etiology is not yet determined (Dharmaraj et al. 2000; Hanein et al. 2004; Lotery et al. 2000; Yzer et al. 2006, den Hollander et al. 2006, 2008).

The LCA genes encode proteins involved in various biological pathways that include retina development (CRB1, CRX), phototransduction (GUCY2D, AIPL1), vitamin A metabolism (RPE65, LRAT, RDH12), cellular transport (TULP1), and ciliary processes (RPGRIP1, CEP290, LCA5) (Table 1) (den Hollander et al. 2001, 2006, 2008; Sohocki et al. 2000a; Swaroop et al. 1999; Cremers et al. 2002; Perrault et al. 2004; Thompson et al. 2005). The discovery of mutations in the ciliary proteins, RPGRIP1, CEP290, and LCA5, emphasizes the emerging role of disrupted ciliary functions in the pathogenesis of selected LCA cases and places LCA in a group of disorders now classified as ciliopathies (Badano and Katsanis 2002; Badano et al. 2006a, b). Mutations of these genes are responsible for nearly a third of causally definable LCA cases (den Hollander et al. 2006). The ciliary proteins participate in transport and communication between inner and outer segments of rods and cones and thereby their dysfunction can trigger photoreceptor death (Abd-El-Barr et al. 2007).

The discovery of *CEP290/NPHP6*, encoding a 290 kDa centrosomal protein, highlighted a new pathogenetic mechanism underlying some LCA. The *CEP290* mutations are frequent and detected in 21% of patients, thus making *CEP290* a major contributor to LCA. The protein localizes to centrosomes of dividing cells and to the connecting cilium of photoreceptors. It appears that CEP290 cooperates with Rab8 to promote ciliogenesis (Tsang et al. 2008). Mutations in *CEP290* are found in patients with LCA among other ciliopathies including Joubert syndrome (JBTS, MIM#610188), Meckel Syndrome (MS, MIM#610142), McKusick-Kaufman syndrome (MKKS, MIM#236700), Senior–Loken syndrome (SLSN, MIM#610189) and Bardet Biedl Syndrome (BBS, MIM#2099000), each of which has retinal dystrophy accompanied by systemic features.

Most patients with LCA resulting from mutations in *CEP290* have an intronic mutation c. 2991+1655A>G that affects splicing and results in a putatively hypomorphic allele (den Hollander et al. 2006). The severity of the CEP290-dependent phenotypes might correlate inversely with CEP290 activity. Less severe phenotypes, including LCA or nephronophthisis, involving just one system would result from hypomorphic mutations, while multisystem disorders result from complexity similar to what has been proposed for alterations in *ABCA4* residual activity for Stargardt disease, cone rod dystrophy, retinitis pigmentosa, and age-related macular degeneration (Shroyer et al. 1999, 2001a, b; Lewis et al. 1999; Wiszniewski et al. 2005). Another hypothesis suggests the existence of second-site modifiers that could directly or indirectly interact with CEP290 and thus moderate or modulate the phenotype (Khanna et al. 2009; Coppieters et al. 2010). *RPGRIP1L* is the first identified second-site modifier that does not cause LCA alone but is proposed to modulate the retinal phenotype in LCA patients with known pathogenic mutations at another locus (Khanna et al. 2009). Interestingly, the presence of a third mutant allele in 7% of subjects from an LCA cohort study has been reported, and interpreted to reflect a modifier effect on the LCA phenotype (Zernant et al. 2005).

The presence of mutations in more than one disease locus is observed frequently in patients with ciliopathies. In fact, the genetic concept of triallelic inheritance whereby two mutant alleles at one locus and one mutant allele at another locus are required for trait manifestation was first described in BBS (Katsanis et al. 2001; Eichers et al. 2004). In patients with BBS, mutations in more than one locus are found in approximately 10% of patients, and it has been shown that the third mutant allele may modify the severity of the disease (Hichri et al. 2005; Khanna et al. 2009). Thus, the third mutant allele may be associated with the either penetrance of the clinical phenotype or variability of expression. The ability to discern the latter depends on both the type of trait and the methods applied to assay phenotypic manifestations. Digenic triallelic inheritance has now been reported in Waardenburg syndrome, Usher syndrome, and some forms of right ventricular hypertrophy (Ebermann et al. 2010; Xu et al. 2010; Chiang et al. 2009).

Here, we report mutational analysis of 13 LCA genes in 60 unrelated North American families with a clinical diagnosis of Leber congenital amaurosis. We found mutations in 84/120 (70%) alleles in 42/60 (70%) of patients. In addition, we identified a third disease-associated mutant allele in a second locus in 7/60 (12%) patients. Our findings further support the concept that mutational load may be important to disease manifestations.

Materials and methods

Patient enrollment

After informed consent, individuals and families were enrolled for this study in a protocol approved by the Institutional Review Board for Human Subject Research at Baylor College of Medicine, Houston, TX. The inclusion requirement was a clinical diagnosis of LCA based on (1) evidence of substantial reduction of vision in the first year of life, (2) infantile nystagmus with or without oculodigital blindisms, and (3) markedly diminished ERG responses. An additional inclusion criterion of multiple affected family members, incorporated early in the recruitment process, was later relaxed to allow increased enrollment. Anticoagulated blood samples were obtained and DNA extraction done by standard methods. DNA samples on control individuals were obtained from the Baylor Human Polymorphism Resource through the Kleberg Genotyping Center at Baylor College of Medicine. Ninety-six control samples from healthy unrelated non-Hispanic white individuals were also studied.

Mutation detection

Samples of genomic DNA from an affected individual in each family were amplified by PCR with primers in the introns flanking each exon or cluster of exons for each of the genes. To detect the common *CEP290* mutation: c.2991+1655A>G localized to IVS 26, the intronic region was amplified and sequenced using published primers (Li et al. 2009).

Data were analyzed with ABI Sequence Analysis version 3.2 (Applied Biosystems, Foster City, CA), Sequencher version 4.1 (GeneCodes, Ann Arbor, MI), and Mutation Surveyor v3.24 (SoftGenetics).

Each sequence variation identified in an affected family member was tested for co-segregation with the phenotype in samples from all available family members and, if the variation was not previously reported, it was tested in the control DNA sample sets and assessed with three predictive software tools: MutationTaster (http://neurocore.charite.de/MutationTaster/), PolyPhen (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://blocks.fhcrc.org/sift/SIFT.html).

Results

Subjects with LCA

To investigate LCA genes mutations, we enrolled 60 unrelated patients and analyzed coding sequences of 13 LCA genes. The LCA families were recruited in North America and patients had various ethnic backgrounds. In all subjects, ophthalmic manifestations consistent with LCA were present in the newborn period. All subjects presented with non-syndromic retinal disease, except for Ar 187-04 who was also diagnosed with non-disjunction trisomy 21 Down syndrome. In each family, the parents of the affected individuals had no known retinal disease or visual impairment (other than refractive error). In 31/60 families, two or more siblings were diagnosed with LCA. No significant difference in severity of retinal disease was apparent between affected siblings.

Mutation analysis

Of the 60 families, 42 (70%) had one or more mutations in the 13 LCA genes tested, 34 (57%) had two or three mutations, and 7 (12%) had three mutations in two different LCA genes (Table 2). The distribution of disease alleles in the tested genes among these 60 families was: 36 (43%) with *CEP290* mutations, 13 (15%) with *GUCY2D* mutations, 13 (15%) with *AIPL1* mutations, 8 (9%) with *CRB1* mutations, 6 (7%) with *RPE65* mutations, 4 (5%) with *RPGRIP1* mutations, 3 (4%) with *LCA5* mutations and 1 (1%) with *CRX* mutations. No coding sequence mutations were detected in five known LCA genes: *RD3*, *RDH12*, *TULP1*, *SPATA7*, and *LRAT*. We detected 52 different mutant alleles including 33 reported and 19 novel (12 in *CEP290*, 3 in *AIPL1*, 3 in *CRB1*, and 1 in *RGRIP1*). None of the novel alterations was found in the 96 control samples nor was any documented in human mutation and SNP databases.

Potential triallelic inheritance

Mutations in more than one gene were detected in seven subjects who were compound heterozygotes for mutations in one LCA gene and also carried a heterozygous mutation in another LCA gene, giving a total of three mutated LCA alleles. These patients tested positive for two mutant alleles at *CEP290* with an additional mutations at either *AIPL1*, *RPGRIP1* or *LCA5*; two mutant alleles at *GUCY2D* with an additional mutation at either *AIPL1* or *RPGRIP1*; two *AIPL1* mutations with one at *GUCY2D*; and two RPE65 mutations with one in *AIPL1* (Table 3). No particular pattern or combination of mutated genes was observed in subjects with three independent mutated alleles. The severe retinal

phenotype in these patients could not be further distinguished from other LCA patients with compound heterozygous mutations at a single locus (Table 3).

Discussion

We report an analysis of 13 of 14 known LCA genes in a cohort of North American patients with a clinical diagnosis of Leber congenital amaurosis. We recruited 60 families with one or more family members affected with LCA. Patients fulfilled clinical criteria for LCA, and anamnestic data were evaluated by a single physician (R.A.L.). Our results show that mutations in known LCA genes are present in 42/60 (70%) subjects diagnosed with this condition. A similar rate of mutation detection in known LCA genes, with an average frequency of 65–70%, has been reported in other studies conducted in Europe and North America (den Hollander et al. 2008; Perrault et al. 2010).

We found a substantial predominance of *CEP290* mutations in our study, affecting 19/60 (32%) patients and comprising 43% of all identified mutant alleles. We did not detect mutations in *RD3*, *RDH12*, *TULP1*, *SPATA7*, and *LRAT*, likely reflecting a low frequency of mutations in these genes in subjects with LCA from our unrelated North American cohort. Mutations in these genes have been reported in 1–4% of patients with LCA. We did not perform mutational analysis of the recently identified LCA gene—IMPDH1, in which mutations have been identified in only a few families. Overall, we were able to identify 52 different mutations including 33 previously reported and 19 unpublished. We studied the prevalence of new sequence variants in the general population and were unable to identify these alterations in a control population of 96 individuals, or in LCA mutation databases or SNP databases suggesting that carrier states are quite rare and these LCA-associated mutations are unlikely to represent benign variants. We were not able to perform segregation studies for a few sequence variants in affected families because DNA samples from other family members were not available.

Our studies show that 7/60 (12%) patients with Leber congenital amaurosis have three mutated alleles in two LCA loci. In 4/7 subjects with three mutated alleles all three mutations were reported previously as pathogenic (Gerber et al. 2001; Ito et al. 2004; Dharmaraj et al. 2000; Akey et al. 2002; Booij et al. 2005; Sohocki et al. 2000b; Morimura et al. 1998). In the remaining 3/7 individuals, one or two variants have been identified for the first time (Table 4). In the group of patients with three mutated alleles, a total number of 17 different alleles were detected including: two nonsense mutations and four single nucleotide deletions, that represent likely disease causing variants; seven missense mutations predicted in silico to be disease causing changes by at least one predictive tool; and four missense alleles: p.D1114G (*RPGRIP1*), p.P701S (*GUCY2D*), p.R302L (*AIPL1*) and p.G439E (*LCA5*), not classified by applied predictive tools as likely to be pathogenic. Sequence variants p.D1114G and p.P701S have been reported previously as possible modifiers of the LCA phenotype (Zernant et al. 2005). The latter variant was observed in 2% of general population and in 5% of patients with LCA. There are no functional studies assessing the physiological effects of this allele but available segregation studies for p.P701S in multiple families strongly suggest a likely pathogenic effect. Another allele, p.R302L was also not classified as pathogenic by predictive programs. This variant was originally identified as a homozygous mutation in a large consanguineous family from India with at least three individuals affected with LCA. Epidemiologic studies did not reveal the presence of the allele in control DNA samples (Sohocki et al. 2000b). The newly reported *LCA5* variant, p.G439E is predicted to be benign by available bioinformatic tools but at this point we are lacking additional data from segregation studies and functional assays to asses reliably its pathogenicity. In silico analysis is a powerful tool to study the effects of sequence substitutions on protein expression and some biophysical properties of the encoded

proteins; however, further studies with biological systems are required to elucidate any potential pathological consequences of sequence variants. The reliable classification of sequence variants is frequently challenging, especially for genes exclusively expressed in the retina wherein functional studies of mutant alleles in vitro are difficult.

The significance of the third mutated allele is unknown but one potential interpretation is that the penetrance or trait manifestation can be associated with triallelic inheritance as has been observed for other ciliopathies. An alternative explanation is that this combination of three mutant alleles occurred randomly and is not associated with trait manifestations. Inconsistent with this latter hypothesis is our observation that the putative third mutant allele is often one that is in a gene infrequently found causative in this North American population studied (e.g. *RPGRIP1*, *LCA5*). Furthermore, the occurrence of a mutation in another gene appears more frequently than would be expected by chance ($p = 1.9 \times 10^{-6}$ by binomial test, assuming the background LCA mutant allele frequency at 0.01). If the mutation of one of the genes is responsible for causing LCA in affected family members, then the chances of having a mutation in another LCA gene should be no different than the population risk. Mutations in more than one LCA locus were reported in 22/300 (7%) patients with Leber congenital amaurosis by Zernant et al. (2005). Those authors proposed that the additional mutation might exert an additive effect on the LCA phenotype as some patients with three hits presented with an apparently more severe disease as determined by clinical examination and onset of clinical symtoms (Zernant et al. 2005).

Non-Mendelian inheritance has been described previously for a number of hereditary eye disorders. Mutations in *peripherin* and RDS can result in a digenic retinitis pigmentosa (RP7), wherein affected individuals are double heterozygotes (Kajiwara et al. 1994). The pleiotropic disorder Bardet–Biedl syndrome is characterized by early-onset retinal dystrophy and multisystem involvement. There are at least 14 BBS loci and mutations in two BBS loci have been described in less than 10% of families with BBS. The third mutant allele may be required for penetrance (Katsanis et al. 2001). However, it has been proposed that the third allele may modify the severity of clinical phenotype (Badano et al. 2006a).

Since the discovery of triallelic inheritance, much progress has been made to identify genes that modify the clinical outcome in patients with retinal diseases. One of these genes, *RPGRIP1L*, modifies the severity of retinal dystrophy in patients with LCA and other ciliopathies including SLS, BBS, and MKS, although mutations in this gene alone are not sufficient to cause LCA. The *RPGRIP1L* variants were found in LCA patients among whom mutation studies revealed homozygous, compound heterozygous, and heterozygous mutations in LCA-causing genes (Khanna et al. 2009). We hypothesize that the third mutated allele may contribute to the penetrance of retinal deterioration in patients with LCA. However, it is challenging to assess its clinical effect as LCA is a condition of very early onset, rapid progression, and relatively homogenous clinical presentation.

Our study provides evidence that mutational load may be important to trait manifestation in LCA. Further studies are required to better comprehend the molecular underpinnings of these genetic observations.

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Table 1

LCA genes, their encoded protein product functions and reported mutational frequencies among patients with LCA

Gene			Function	Mutation frequency (%)
MIM#610142	*CEP290*	Centrosome protein 290 kDa	Ciliary protein	21
MIM#600179	*GUCY2D*	Photoreceptor-specific guanylate cyclase	Signal transduction	12
MIM#604210	*CRB1*	Crumbs homolog 1	Photoreceptor polarity determination	10
MIM#180069	*RPE65*	Retinal pigment epithelium 65 kDa protein	Vitamin A metabolism	6
MIM#604392	*AIPL1*	Aryl hydrocarbon receptor-interacting protein like 1	Chaperone protein	5
MIM#605446	*RPGRIP1*	Retinitis pigmentosa GTPase regulator interacting protein 1	Ciliary protein	4
MIM#608830	*RDH 12*	Retinal dehydrogenase 12	Vitamin A metabolism	3
MIM#611408	*LCA5*	Lebercilin	Microtubule transport	2
MIM#602225	*CRX*	Photoreceptor-specific homeobox gene	Photoreceptor differentiation control	1
MIM#602280	*TULP1*	Tubby-like gene 1	Vesicular trafficking	<1
MIM#604863	*LRAT*	Lecithin retinol acyltransferase	Vitamin A metabolism	<1
MIM#609868	*SPATA7*	Spermatogenesis-associated protein 7	Unclear	<1
MIM#146690	*IMPDH1*	Inosine monophosphate dehydrogenase 1	Purine synthesis	<1
MIM#610612	*RD3*	Retinal degeneration 3	Unclear	<1

Literature reports based on the following studies: den Hollander et al. (2006, 2008), Dharmaraj et al. (2000), Hanein et al. (2004), Lotery et al. (2000) and Yzer et al. (2006)

Table 2

Mutations in families affected with LCA

Pedigree	Allele I		Allele II	
	Nucleotide	Translation	Nucleotide	Translation
GUCY2D				
Ar-039	c.2743A>G	P.I915V	c.2516delC	p.T838RfsX27
Ar-085	c.1343C>A	p.S448X	c.1343C>A	p.S448X
Ar-123	c.2303G>A	p.R768Q	c.2303G>A	p.R768Q
Ar-695	C.2302C>T	p.R768W	c.692_693insG	p.K232EfsX86
Ar-712	c.1343C>A	p.S448X	c.1343C>A	p.S448X
Ar-098	c.2101C>T	p.P701S	?	?
Ar-666	c.2248G>T	p.E750X	?	?
Ar-785	c.2302C>T	p.R768W	?	?
RPE65				
Ar-073	c.284A>G	p.E95G	c.284A>G	p.E95G
Ar-089	C.271C>T	p.R91W	c.1102T>C	p.Y368H
Ar-782	c.1022T>C	p.L341S	c.355delT	p.S121LfsX6
CRX				
Ar-589	c.705delC	p.L236SfsX135	?	?
AIPL1				
Ar-072	c.834G>A	p.W278X	c.834G>A	p.W278X
Ar-098	c.834G>A	p.W278X	c.809G>A	p.R270H
Ar-553	c.834G>A	p.W278X	c.834G>A	p.W278X
Ar-770	c.266G>A	p.C89Y	c.266G>A	p.C89Y
Ar-123	c.834G>A	p.W278X	?	?
Ar-164	c.971G>T	p.R324L	?	?
Ar-177	c.905G>T	p.R302L	?	?
Ar-782	c.971G>T	p.R324L	?	?
CRB1				
Ar-170	c.3038A>C	p.Q1013P	c.2548G>A	p.G850S
Ar-597	c.2843G>A	p.C948Y	c.2843G>A	p.C948Y
Ar-611	c.2401A>T	p.K801X	c.2688T>A	p.C896X
Ar-115	c.2401A>T	p.K801X	?	?
Ar-785	c.853A>C	p.S285R	?	?
RPGRIP1				
Ar-848	c.2367+1G>A	Splice	c.2710+1G>A	splice
Ar-021	c.3341A>G	P.D1114G	?	?
Ar-039	c.1753C>T	p.P585S	?	?
CEP290				
Ar-21	c.2991+1655A>G	p.C998X;WT	c.5777G>C	p.R1926P
Ar-51	c.6277delG	p.V2093SfsX4	?	?
Ar-81	c.2991+1655A>G	p.C998X;WT	c.829G>C	p.E277Q

Pedigree	Allele I		Allele II	
	Nucleotide	Translation	Nucleotide	Translation
Ar-86	c.2991+1655A>G	p.C998X;WT	c.1429C>T	p.R447X
Ar-107	c.2991+1655A>G	p.C998X;WT	c.3178delA	p.T1060LfsX5
Ar-110	c.2991+1655A>G	p.C998X;WT	c.4452_4455delAGAA	p.E1484LfsX3
Ar-113	c.2991+1655A>G	p.C998X;WT	c.1236delG	p.L412X
Ar-137	c.2991+1655A>G	p.C998X;WT	c.1066-1G>A	splice
Ar-163	c.2991+1655A>G	p.C998X;WT	c.1830delA	p.L611FfsX5
Ar-177	c.2991+1655A>G	p.C998X;WT	c.5493delA	p.A1831PfsX19
Ar-187	c.2991+1655A>G	p.C998X;WT	c.5813_5817delCTTTA	p.T1938DfsX16
Ar-612	c.2213delT	p.L738X	c.4438-3delC	splice
Ar-681	c.2991+1655A>G	p.C998X;WT	c.4962_4963delAA	p.E1655NfsX3
Ar-746	c.2991+1655A>G	p.C998X;WT	C.4882C>T	p.Q1628X
Ar-757	c.2991+1655A>G	p.C998X;WT	?	?
Ar-759	c.2991+1655A>G	p.C998X;WT	c.2505_2506delAG	p.G836IfsX2
Ar-863	c.2991+1655A>G	p.C998X;WT	c.5850delT	p.F1950LfsX15
Ar-864	c.2991+1655A>G	p.C998X;WT	c.3185delT	p.L1062RfsX3
Ar-889	c.2991+1655A>G	p.C998X;WT	c.5218C>T	p.Q1740X
LCA5				
Ar-588	c.835C>T	p.Q279X	c.835C>T	p.Q279X
Ar-863	c.1316G>A	p.G439E	?	?

? unknown

Table 3

Clinical presentation of LCA patients with mutations identified in two loci

Pedigree	Gene	Mutation	Age of onset	Visual Acuity	ERG	Clinical findings
Ar-21-04	RPGRIP1	p.D1114G	Birth	LPO	NR	Nystagmus, vitreous cells, narrow versus, severe "taporetinal dystrophy"
	CEP290	c.2991+1655A>G				
	CEP290	p.R1926P				
Ar-39-04	GUCY2D	p.I915V	<3 months	N/A	NR	Nystagmus, keratoconus in early teens, hyperopia (+7.50 spherical equivalent), "retinitis pigmentosa" —like retinal dystrophy
	GUCY2D	p.T838RfsX27				
	RPGRIP1	p.P585S				
Ar-98-11	GUCY2D	p.P701S	Birth	5/160	NR	Optic atrophy, diffuse vascular attenuation and retinal atrophy with midperiheral bone spicules
	AIPL1	p.W278X				
	AIPL1	p.R270H				
Ar-123-04	GUCY2D	p.R768Q	Birth	LPO	NR	KCNS at age of 12 years, diffuse retinal atrophy and optic nerve atrophy, hyperopia (+8.00 spherical equivalent), autism, consanguinity (1st cousin parents)
	GUCY2D	p.R768Q				
	AIPL1	p.W278X				
Ar-177-04	AIPL1	p.R302L	Birth	No fixation	NR	Slow pupils, high hyperopia (+7.75 spherical equivalent), nystagmus, mild optic nerve atrophy, midretinal pigment degeneration
	CEP290	p.A1832PfsX19				
	CEP290	c.2991+1655A>G				
Ar-782-04	RPE65	p.L341S	Birth	LP	NA	Sandy diffuse peripheral pigmentary granularity and clumping, optic nerve atrophy
	RPE65	p.S121LfsX6				
	AIPL1	p.R324L				
Ar-863-03	CEP290	c.2991+1655A>G	Birth	NLP	NR	Sluggish pupils, high hyperopia, nystagmus
	CEP290	p.F1950LfsX15				
	LCA5	p.G439E				

NR non-recordable, *NA* not available, *LP* light perception, *LPO* light perception only, *NLP* no light perception

Table 4

Characteristics of sequence variants identified in LCA patients with triallelic inheritance

Pedigree	Gene	Mutation	Reference	Grantham score[a]	MutationTaster prediction	PolyPhen prediction	SIFT prediction
Ar-21-04	RPGRIP1	p.D1114G	1	94	B	B	B
	CEP290	c.2991+1655A>G	2	–	–	–	–
	CEP290	p.R1926P	3	103	D	D	D
Ar-39-04	GUCY2D	p.I915V	This report	29	D	B	B
	GUCY2D	p.T838RfsX27	3	–	–	–	–
	RPGRIP1	p.P585S	This report	74	B	D	B
Ar-98-11	GUCY2D	p.P701S	4	74	B	B	B
	AIPL1	p.W278X	5	–	–	–	–
	AIPL1	p.R270H	6	29	D	D	B
Ar-123-04	GUCY2D	p.R768Q	7	43	D	D	D
	GUCY2D	p.R768Q	7	43	D	D	D
	AIPL1	p.W278X	5	–	–	–	–
Ar-177-04	AIPL1	p.R302L	5	102	B	B	B
	CEP290	p.A1832PfsX19	8	–	–	–	–
	CEP290	c.2991+1655A>G	2	–	–	–	–
Ar-782-04	RPE65	p.L341S	9	145	D	D	D
	RPE65	p.S121LfsX6	This report	–	–	–	–
	AIPL1	p.R324L	This report	102	D	B/D	B
Ar-863-03	CEP290	c.2991+1655A>G	2	–	–	–	–
	CEP290	p.F1950LfsX15	10	–	–	–	–
	LCA5	p.G439E	This report	85	B	B	B

B benign variant, *D* disease causing variant, *B/D* variant of unknown significance

(1) Gerber et al. (2001), (2) den Hollander et al. (2006), (3) Stone (2007), (4) Dharmaraj et al. (2000), (5) Sohocki et al. (2000b), (6) Simonelli et al. (2007), (7) Lotery et al. (2000), (8) Brancati et al. (2007), (9) Morimura et al. (1998), (10) Perrault et al. (2007)

[a]Substitutions determined as conservative (0–50), moderately conservative (51–100), moderately radical (101–150), radical (>150)