TITLE: Clinical and molecular findings in a cohort of 152 Brazilian severe early onset inherited retinal dystrophy patients

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ABSTRACT:

Leber congenital amaurosis (LCA) and early-onset retinal dystrophy (EORD) are severe inherited retinal dystrophy that can cause deep blindness childhood. They represent 5% of all retinal dystrophies in the world population and about 10% in Brazil. Clinical findings and molecular basis of syndromic and non-syndromic LCA/EORD in a Brazilian sample (152 patients/137 families) were studied. In this population 15 genes were found to be related to the phenotype, 38 new variants were detected and four new complex alleles were discovered. Among 123 variants found, the most common were *CEP290*: c.2991+1655A>G, *CRB1*: p.Cys948Tyr, and *RPGRIP1*: exon10-18 deletion.

INTRODUCTION

Leber congenital amaurosis (LCA) is the most severe and earliest onset inherited retinal dystrophy. Affected individuals usually present, in the first year of life with severe visual impairment, nystagmus and occasionally a systemic manifestation (Francis, 2006). Phenotypic variability in fundus abnormality, refractive errors, photophobia or light-seeking behavior, nyctalopia, nystagmus, low visual acuity, and Franceschetti's oculo-digital sign, are also commonly observed in these patients (Chung & Traboulsi, 2009; den Hollander, Roepman, Koenekoop, & Cremers, 2008).

Early-onset retinal dystrophy (EORD) can be considered as belonging to the same LCA spectrum but a milder form, where signs and symptoms appear after the first year of life up to 5-7 years-old (Weleber, Francis, Trzupek, & Beattie, 2004), but still a disease that severely compromises vision. Clinically, LCA and EORD are similar and may represent a continuum; the distinction between them is an extinguished or markedly diminished ERG response before the first year of life for individuals with LCA (Foxman, Heckenlively, Bateman, & Wirtschafter, 1985).

LCA/EORD affect from 1 in 30,000 (Koenekoop, 2004) to 1 in 81,000 (Stone, 2007) individuals, and may be less rare in inbreeding populations (Sherwin, Hewitt, Ruddle, & Mackey, 2008). It represents almost 5% of all hereditary retinal dystrophies in the world (Weleber et al., 2004), while in Brazil our group demonstrated the frequency is double (10.8%) (Motta, Martin, Filippelli-Silva, Salles, & Sallum, 2018) in our cohorts which may be due to referral bias of severely affected children to the three largest specialist centres in São Paulo, Rio de Janeiro and Belo Horizonte.

The inheritance pattern of LCA/EORD is mainly autosomal recessive, but autosomal dominant forms involving the *CRX*, *IMPDH1*, and *OTX2* genes have been reported (Alström & Olson, 1957; Daiger, Rossiter, Greenberg, Christoffels, & Hide, 1998; Kumaran, Moore, Weleber, & Michaelides, 2017; Wright, Chakarova, Abd El-Aziz, & Bhattacharya, 2010). Twenty-five genes have already been associated with this group of diseases, most of them also associated with other retinopathies including some syndromes (Daiger et al., 1998).

The most commonly mutated genes in Brazilian LCA/EORD patients are *CEP290*, *CRB1*, *RPE65*, and *RPGRIP1* (Motta et al., 2018). The purpose of this study was to present the clinical and genetic findings from 152 Brazilian patients with isolated or syndromic LCA/EORD.

MATERIALS AND METHODS

Medical records of five specialized services in hereditary retinopathies in Brazil were reviewed for this retrospective study. One hundred fifty-two patients (from 137 families) with syndromic and non-syndromic Leber congenital amaurosis/early-onset retinal dystrophy and a conclusive genetic test with LCA-related genes were included. Between January 1998 and June 2019, 107 patients (97 families) attended the Universidade Federal de São Paulo or the Instituto de Genética Ocular (São Paulo), 40 patients (35 families) at the INRET Clínica e Centro de Pesquisa, and five patients/families at the Instituto de Olhos Carioca (Rio de

Janeiro). This study was approved by the Research Ethics Committee of the Universidade Federal de São Paulo (0415/2016) and it was also performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained when it was necessary to perform molecular tests.

Medical and family histories and genetic data were collected. All patients were evaluated ophthalmologically by Dr. Sallum, Dr. Porto or Dr. Resende. The clinical diagnosis of Leber congenital amaurosis and early-onset retinal dystrophy was based on detailed clinical examination, visual function, signs/symptoms, ophthalmologic features and age of onset. In addition to these findings, impairment of other systems characterized syndromic forms, such as Joubert and Senior-Løken syndromes.

The genetic data collected were obtained from different types of tests: Next-Generation Sequencing from a 224 gene IRD panel (93 patients), from a 280-300 gene IRD panel (21 patients), from 20 gene LCA panel (20 patients), from whole exome (one patient); SNP array (10 patients); Sanger Sequencing from one gene analysis (two patients), from segregation analysis (five patients). Novel variants were classified as pathogenic or likely pathogenic when representing a loss of function variant (frameshift or nonsense or copy number variation or affecting a canonical splice-site). The pathogenicity of novel missense variants were evaluated by eight predictor softwares: DANN (Quang, Chen, & Xie, 2015), FATHMM, FATHMM-MKL, LRT, MutationAssessor, MutationTaster, PROVEAN and, SIFT. The databases consulted (on March, 2020) were: HGMD, gnomAD, and ClinVar.

RESULTS

In this Brazilian sample of 137 families (152 patients), 123 variants in 15 LCA-associated genes were identified, including 38 novel variants. Table 1 summarises the genotypes identified in the affected individuals and table 2 summarises the variant data with the allele count in this cohort, total allele frequency from all populations of the gnomAD database,

classification according to the ACMG guidelines (Richards et al., 2015) and previous reports of the variants. Additional data, including chromosome coordinate, allele frequency in this study and variant classification according to type, ClinVar, and HGMD are shown in the supplementary table 1. Clinical characteristics and genetic aspects of the Brazilian LCA/EORD cohort according to the causal gene are presented below.

Visual cycle gene defects

RPE65 (OMIM *180069)

Biallelic pathogenic variants in the *RPE65* gene have been associated with LCA2 (OMIM #204100) and retinitis pigmentosa 20 (RP20; OMIM #613794). It is estimated that LCA2 accounting for approximately 5% to 10% of all LCA/EORD cases (Kumaran et al., 2017).

This gene encodes the retinal pigment epithelium-specific 65KDa protein that is critical for regeneration of 11-*cis* retinol in the vitamin A visual cycle and abundant in the retinal pigment epithelium (RPE) (Moiseyev, Chen, Takahashi, Wu, & Ma, 2005). The RPE65 isomerhydrolase activity (conversion of all-*trans* retinyl ester to 11-*cis* retinol) occurs when it complexes with lecithin retinol acyltransferase (LRAT) (Moiseyev et al., 2005).

In keeping with other studies, children evaluated in this study with LCA due to *RPE65* usually manifest light-seeking behavior. In general, neonates had little/no vision at birth. In the following months, vision could slowly arise, but always with a development pattern under that expected for a normal child. Without exceptions, all these patients could attend mainstream education with low vision adaptations like magnification and sitting close to the board at school. Reaching teenage years, best corrected visual acuity (BCVA) was usually 20/200 or 20/400 with declining visual fields and central vision loss through adulthood (Chung et al., 2019). By the third decade, a cane is required for mobility. In the following decade, they can only read electronic screens. By the fifth decade, vision has usually declined to hand movements and light perception.

Patients with biallelic *RPE65* variants had fundus appearance in keeping with previous reports; only mild granular appearance of the RPE and mildly narrowed vessels (Figure 1a). In the granulated regions of the retina, posteriorly, areas of confluent atrophy of the RPE appeared. Fundus autofluorescence was diffuse and severely reduced.

Sixteen variants in the *RPE65* gene were considered as pathogenic in LCA/EORD in 22 families (29 patients) of this study representing the second most common cause of LCA (16.06%) among Brazilian families. Two recurrent variants were the most frequent (p.Arg91Gln and p.Leu341Ser), which were present in five families each. Interestingly, the combination of these two variants in a compound heterozygous was found in LCA (family 76) and EORD (patient 126.1) patients. Other variants common to both phenotypes were p.Gly187Glu (families 75, 127 and 128) and p.Trp402Ter (patients 89.1 and 125.1). In addition, one novel variant (p.Asp62Asn) was identified in one compound heterozygous patient and, was classified as disease-causing by eight pathogenicity predictors (DANN, FATHMM, LRT, MutationAssessor, MutationTaster, PROVEAN, FATHMM-MKL, and SIFT).

LRAT (OMIM *604863)

The lecithin retinol acyltransferase (LRAT) is encoded by the *LRAT* gene and is responsible for the synthesis of all-trans-retinyl esters from all-trans-retinol. Homozygous or compound heterozygous pathogenic variants in the *LRAT* gene have been associated with LCA14 (OMIM #613341), and represent less than 1% of all LCA/EORD causes (Kumaran et al., 2017).

In this cohort, the least severe LCA phenotypes were those caused by abnormalities in LRAT activity, six of 119 patients (LCA, table 1). Their visual acuities were around 20/60 at about 8 years old. As these patients did had less severe vision loss, even with the presence of nystagmus, many parents had difficulty noticing any visual impairment in their children at preschool age.

Since LRAT and RPE65 act as functional partners (LRAT catalyses the preceding reaction in the visual cycle), it is expected that mutations disrupting LRAT function may lead to similar disease to those that disrupt RPE65 activity. The characteristic lack of autofluorescence throughout the retina was remarkably similar in cases with defects in these genes in our cohort. In addition, both presented very mild granulate appearance of the RPE with fine white dots deposits.

Three disease-causing variants were found in seven LCA/EORD families/patients in this cohort. One of three variants was novel and was classified as damaging by seven predictors (DANN, LRT, MutationAssessor, MutationTaster, PROVEAN, FATHMM-MKL, and SIFT). This novel variant (c.298G>A, p.Gly100Ser) was present in four families, of which three affected individuals were homozygous. The previous reported variant c.163C>G:p.Arg55Gly was found in LCA (patient 59.1) and EORD (patient 116.1) cases and, the other c.346T>C:p.Phe116Leu was found in two LCA patients (56.1 and 60.1).

RDH12 (OMIM *608830)

LCA13 (OMIM #612712) is caused by biallelic mutations in *RDH12* gene and accounting for approximately 10% of LCA/EORD cases (Kumaran et al., 2017). Autosomal dominant and recessive retinitis pigmentosa caused by *RDH12* pathogenic variant have also been reported (Benayoun et al., 2009; Fingert et al., 2008).

This gene encodes the retinol dehydrogenase 12 (RDH12), which is expressed predominantly in the inner segment of photoreceptors, where plays catalysing the reduction of all-trans retinal to all-trans retinol. As reviewed by Sarkar and Moosajee (2019), some studies suggest that RDH12 protects the retina from excessive illumination by counteracting accumulation of all-trans-retinal or avoiding a build-up of toxic lipid peroxidation products in the photoreceptor.

Within this cohort, patients with biallelic *RDH12* variants demonstrated extensive bone spicule pigmentary deposits Two fundus features were identified independently or in

conjunction depending on the disease phase: (1) intense bone spicules deposits in a reticular pattern at the vascular arcades; (2) macular yellowish atrophy (Figure 1b). When the macular involvement was absent in the early months/years of life, the child's visual behavior was less affected, leading to a diagnosis of early-onset retinal dystrophy. Some cases of *RDH12*-retinopathy were variable, for example affected siblings of family 71 demonstrated variable disease and even asymmetry (Figures 1c and 1d).

Twelve patients of eleven families had biallelic variants in *RDH12*, the most common variant being c.698T>A:p.Val233Asp that was identified in six patients of five families including one homozygote. This variant and c.806_810delCCCTG were found in two compound heterozygous patients with LCA (72.1) and EORD (121.1). Two sisters of family 71 had three variants in this gene, the parents analysis identified a maternal complex allele p.[Val42Ala; Ala109Pro] in *trans* with p.Val233Asp. Both variants of complex allele have not been previously described. p.Ala109Pro was classified as disease-causing by eight pathogenicity predictors (DANN, FATHMM, LRT, MutationAssessor, MutationTaster, PROVEAN, FATHMM-MKL, and SIFT) and its position is highly conserved evolutionarily. On the other hand, p.Val42 position is less conserved than p.Ala109 and, five pathogenicity predictors classified it as deleterious whereas three classified it as tolerated. Therefore, the potential pathogenic effect of these variants cannot be ruled out alone or as a complex allele.

Phototransduction defects

GUCY2D (OMIM *600179)

GUCY2D was the first gene associated with recessive Leber congenital amaurosis (Perrault et al., 1996) (LCA1; OMIM #204000), and accounts for 10% to 20% of LCA/EORD cases (Kumaran et al., 2017). In addition, it is reported as a disease-causing gene in dominant cone-rod dystrophy (Kelsell et al., 1998) (CORD6; OMIM #601777). This gene encodes retinal

guanylyl cyclase 1 (RetGC) that acts in the recovery process of the phototransduction cascade, controlling the level of cGMP in photoreceptor (Weleber et al., 2004).

Eleven variants in the *GUCY2D* gene were found to be associated with non-syndromic LCA/EORD in 11 families (12 patients) of this study. In two families, we found a pathogenic complex allele comprising two novel variants (p.[Phe415LeufsTer73;Asp558Asn]) in *trans* with a splice altering or a missense variant. Four additional novel variants were identified in this cohort, two affecting canonical splicing site (c.1956+1G>A and c.1957-2A>G) and two missense variants (p.His658Tyr and p.Gly1000Glu). One of these, p.His658Tyr, was found in the homozygous state in one individual, while the variant p.Gly1000Glu was identified in one compound heterozygous patient. Both variants were classified as damaging by seven different predictors. The most frequent variant in *GUCY2D* was the nonsense p.Ser448Ter, which was present in four families, being homozygous in patients in three of the families.

Patients in this cohort with biallelic *GUCY2D* variants had a typical severe phenotype of classical LCA, such as low vision and nystagmus at birth. In addition typical of *GUCY2D* LCA, a relatively normal fundus appearance with normal retinal reflex and a very mild granular aspect of the RPE was observed (Figure 2). Photophobia was seen in some cases.

AIPL1 (OMIM *604392)

AIPL1 encodes aryl hydrocarbon receptor-interacting protein-like 1, which acts indirectly in the phototransduction process (Kirschman et al., 2010) as a photoreceptor-specific co-chaperone for retinal cGMP phosphodiesterase (PDE6). AIPL1 in a complex with HSP90 allows the correct folding and assembly of PDE6 (Sacristan-Reviriego & van der Spuy, 2018). The absence of AIPL1 leads to destabilization of PDE6 and consequently the death of photoreceptor due to increased cGMP levels (Ramamurthy, Niemi, Reh, & Hurley, 2004).

Biallelic pathogenic variants in *AIPL1* are associated with recessive LCA4 (Sohocki, Bowne, et al., 2000) (OMIM #604393), accounting for less than 5% of all LCA/EORD cases (Kumaran et al., 2017). In addition, mutations in the *AIPL1* gene have already been ascribed

as causes of recessive cone-rod dystrophy and retinitis pigmentosa (Sohocki, Perrault, et al., 2000).

Only one patient in this study had biallelic pathogenic variants in *AIPL1*, associated with nystagmus, low vision and his light-seeking behavior noted from birth. He could see shades and shadows but he had difficulty in recognizing people. At age 1, the fundus had a relatively normal appearance with very mild granular RPE pigmentation. Both *AIPL1* variants are already reported in the literature (table 2).

Cilia/ciliary transport defects

CEP290 (OMIM *610142)

Biallelic pathogenic variants in the *CEP290* gene, also known as *NPHP6*, have been associated with a broad spectrum of ciliopathy, characterized by the severity and clinical presentation and ranging from lethal Meckel syndrome type 4 (MKS4; OMIM #611134) to LCA10 (OMIM #611755). Other *CEP290*-related ciliopathies (Coppieters, Lefever, Leroy, & De Baere, 2010) are Bardet-Biedl syndrome-14 (BBS14; OMIM #615991), Joubert syndrome-5 (JBTS5; OMIM #610188) and Senior-Løken syndrome-6 (SLSN6; OMIM #610189). Among all LCA cases, *CEP290* accounts for approximately 15-20% (Kumaran et al., 2017).

CEP290 encodes a centrosomal protein with a molecular weight of 290kDa, which is involved in ciliogenesis and located in centrosomes and the connecting cilia of photoreceptors, interacting microtubule-based transport proteins such as retinitis pigmentosa GTPase regulator (RPGR) (den Hollander et al., 2008).

Since the first year of life, patients in this cohort with biallelic mutations in this gene had nystagmus and low vision; usually visual acuity was light perception at best. Franceschetti's oculo-digital sign was frequent, which could be related to the appearance of enophthalmos

and keratoconus (Figure 3a) in some affected children. Fundus appearances had white dots on the periphery that, over time, became pigmented (Figure 3b and 3c).

As aforementioned, *CEP290* defects can lead to isolated ocular or syndromic disease with renal or central nervous system involvement. In this sample, thirty patients from 29 families had biallelic pathogenic *CEP290* variants. Twenty-six patients from 25 families had non-syndromic LCA/EORD and four had Joubert syndrome (patients 132.1, 133.1, 134.1, and 135.1) according to clinical features. None had associated renal disease identified during clinical follow-up. Magnetic resonance imaging showed all Joubert syndrome patients had the typical central nervous system abnormality, known as the molar tooth sign, leading to psychomotor impairment.

Pathogenic variants in the *CEP290* gene were the main cause of childhood-onset inherited retinal dystrophies (21.17%) among these Brazilian families. Moreover, its deep intronic variant c.2991+1655A>G was the most frequent (15 patients/families) not only among *CEP290* patients but among all LCA/EORD cases as well. Notably, c.2991+1655A>G occurs in seven patients in *cis* with the novel missense variant c.3911T>C:p.Met1304Thr, including one homozygous patient. Currently, with many developing gene therapies, it is relevant to understand if the new allele complex c.[2991+1655A>G;3911T>C] is more pathogenic than the deep intronic variant alone.

In addition to p.Met1304Thr, eight additional novel variants were detected. Two frameshift (c.353_354insGCAATTG and c.2737_2741delGAAAA), two in canonical splice site (c.1522+1G>C and c.1623+2C>A), one nonsense (c.881C>G:p.Ser294Ter) and three missenses (p.Ile5Thr, p.Glu1568Asp and p.Leu1826Pro). The latter were classified as deleterious by at least five predictors (DANN, MutationTaster, PROVEAN, FATHMM-MKL, and SIFT).

The *CEP290* variants c.1666delA, c.2052+1_2052+2delGT, p.Arg908Ter, p.Lys1575Ter and c.6271-8T>G were present in non-syndromic LCA as well as Joubert syndrome patients.

RPGRIP1 (OMIM *605446)

It is estimated that biallelic pathogenic variants in the *RPGRIP1* gene are responsible for about 5% of LCA/EORD cases (Kumaran et al., 2017). Retinitis pigmentosa GTPase interacting protein 1 is encoded by *RPGRIP1* and binds to RPGR protein. RPGRIP1 is a component of the connecting cilium, acting on the anchoring of the RPGR in this structure, which connects the inner to the outer segment of the photoreceptors (Koenekoop, 2005) and appears to be required for disk morphogenesis (Zhao et al., 2003).

Fourteen LCA families (10.22%) presented pathogenic variants in the *RPGRIP1* gene presenting with very early retinitis pigmentosa. The fundus had preserved macula with normal color and reflex and a white granular aspect around it. Night blindness was present in some patients since the early years. However, bone spicule pigmentation appeared later.

Four of eight *RPGRIP1* variants were novel; one of them was a gross deletion of exon 10 to 18 that was the most frequent variant in this gene (nine patients from seven families). The other three novel missense variants were p.Arg267Gln, p.Gly671Glu and p.Tyr823Cys, all of them were classified as disease-causing by at least seven computational predictors.

LCA5 (OMIM *611408)

Lebercilin is a ciliary protein, encoded by the *LCA5* gene. It is widely expressed at the microtubules, centrosome, and primary cilia (den Hollander et al., 2007). And lebercilin interacts with intraflagellar transport complex proteins (Coussa, Lopez Solache, & Koenekoop, 2017; den Hollander et al., 2007).

In spite of the broad expression of lebercilin, biallelic pathogenic variants in *LCA5* cause only Leber congenital amaurosis (Daiger et al., 1998; den Hollander et al., 2007) (LCA5; OMIM #604537) accounting for about 2% of all LCA cases (Kumaran et al., 2017).

In this study, 3 individuals from 2 families had homozygous variants in *LCA5* (1.46%). Of the two *LCA5* variants, one was nonsense (c.838C>T, p.Arg280Ter) and the second was located

in the last nucleotide of exon 5 and already reported as aberrant splicing-causing variant (c.955G>A, p.Ala319Thr/p.?) (Ramprasad et al., 2008).

LCA5 patients presented severe low vision and nystagmus. The fundus had an intense white granular aspect with irregular retinal reflex including the macula. In addition, there was an atrophic ring surrounded a relatively preserved fovea.

Two male twins of family 53 had behavioral problems and very poor interaction with the environment and other people, which were not only related to blindness, but also to the intellectual disability they both had.

SPATA7 (OMIM *609868)

It is estimated that biallelic pathogenic variants in *SPATA7* gene are responsible for about 3% of LCA/EORD cases (Kumaran et al., 2017). Besides LCA3 (OMIM #604232), mutations in this gene have been associated with autosomal recessive juvenile retinitis pigmentosa (Daiger et al., 1998).

Spermatogenesis associated protein 7 (SPATA7) is a ciliary protein found in the primary cilium and in the connecting cilia. It is suggested that SPATA7 is essential for the assembly and localization of the ciliary RPGRIP1 protein complex, and consequently for the protein trafficking via the connecting cilia (Eblimit et al., 2015).

These patients presented with mottled pigmentation in the retinal posterior pole in the first years, bone spicule pigmentary deposits, and a mild atrophic ring surrounded the fovea, as well as, abnormal fundus reflex (Figure 3d). The electrophysiology showed partially preserved macular function in the first years of life.

Five variants in the *SPATA7* gene were identified in three unrelated patients of this study. Three variants are novel, a 26bp deletion encompassing the 3' end of exon 1 and the flanking intronic region: c.8_19+14del, the frameshift deletion: c.699_700delTT and a copy number variation that causes the whole gene deletion.

IQCB1 (OMIM * 609237)

IQ Motif Containing B1, also known as Nephrocystin-5 is involved in ciliogenesis and interacts with retinitis pigmentosa GTPase regulator protein (RPGR) and calmodulin (Otto et al., 2005). The *IQCB1* (or *NPHP5*) gene encodes nephrocystin-5, which is located in the photoreceptor connecting cilia and renal epithelial primary cilia (Otto et al., 2005). Most frequently, *IQCB1* biallelic pathogenic variants lead to Senior-Løken syndrome type 5 (Otto et al., 2005) (OMIM # 609254), in which there is renal impairment associated with LCA. In addition, *IQCB1* variants also cause non-syndromic LCA (Stone et al., 2011).

In this sample, five unrelated patients harboured biallelic pathogenic variants in *IQCB1*, four patients with non-syndromic LCA and one with Senior-Løken syndrome. All had clinical features similar to *CEP290* with a small crowded elevated optic disc with reflex around it. The only patient with Senior-Løken syndrome (patient 136.1) presented with impairment of kidney function starting at 18 years of age. Therefore for the first years her diagnosis was LCA and latter it changed to Senior-Løken. Interestingly, the patient 136.1 with Senior-Løken syndrome had the same genotype as a non-syndromic LCA patient 51.1 (c.1518_1519delCA homozygous). The latter patient is still a child; so future kidney problems cannot be excluded and regular investigation of renal function should be undertaken in all patients with a molecular diagnosis of *IQCB1*-LCA. All *IQCB1* variants found in this study were already reported and classified as pathogenic (table 2).

AHI1 (OMIM * 608894)

Functional alterations in the Jouberin protein are related to Joubert syndrome type 3 (OMIM # 608629), a ciliopathy with many anomalies in the central nervous system such as malformations of the corpus callosum (molar tooth sign) and cerebellar vermis hypoplasia (Valente et al., 2006, 2005), in addition to retinal dystrophy, nystagmus, and nephronophthisis (Brancati, Dallapiccola, & Valente, 2010; Parisi, 2005). However, non-syndromic retinitis pigmentosa cases have also been reported (Nguyen et al., 2017).

The Abelson Helper Integration 1 (*AHI1*) gene encodes Jouberin, which is more sharply expressed in the brain and testis (Dixon-Salazar et al., 2004; Ferland et al., 2004). This

protein is located in primary cilium and is required for ciliogenesis and involved in intracellular trafficking (Lancaster et al., 2011; Lee et al., 2014; Westfall et al., 2010).

In this cohort, three patients with Joubert syndrome had bialleic variants in *AHI1*. They presented central nervous system abnormalities (molar tooth sign) (Figure 3e) and fine motor coordination skill impairment. Night blindness with associated visual field defect was present. At the fundus, there was mottled pigmentation of the RPE, intense early reticular bone spicules in the equatorial region and partial atrophy around the fovea.

Three of five variants found were previously unreported, the c.2623+1G>T and c.2742delT were classified as pathogenic/likely pathogenic, whereas p.Arg610Pro was considered as a variant of uncertain significance, but five pathogenicity predictors (DANN, LRT, MutationTaster, FATHMM-MKL, and SIFT) classified it as deleterious. The latter is located in the first WD40-repeat (WD1), other missense variants in the WD1 have been reported to cause Joubert syndrome (Ben-Salem, Al-Shamsi, Gleeson, Ali, & Al-Gazali, 2014; Knopp et al., 2015; Suzuki et al., 2016). In addition to them, a nonsense variant in the same residue (p.Arg610Ter) has already been associated with Joubert syndrome(Reuter et al., 2017; Romano et al., 2006).

NPHP4 (OMIM * 607215)

The *NPHP4* gene encodes nephrocystin-4, a component of a protein complex involved in several cellular functions including cell division and apical junctions' organization. Nephrocystin-4 is present in primary cilia, centrosomes, basal bodies, and the cortical actin cytoskeleton (Hildebrandt, Attanasio, & Otto, 2009; Mollet et al., 2005). *NPHP4* biallelic pathogenic variants are associated most frequently with isolated cystic kidney disease (nephronophthisis type 4 - OMIM #606966), however, its syndromic form with early-onset retinal degeneration, Senior-Løken Syndrome type 4 (OMIM #606996), has also been reported (Hoefele et al., 2005).

Abnormal *NPHP4* function was responsible for retinopathy in two patients in this series, one without renal impairment (patient 117.1), and one syndromic case (patient 137.1). The fundi, observed in these patients, had intense and linear bone spicules pigmentary deposits.

Three *NPHP4* variants were found in the patient 137.1 with Senior-Løken syndrome. The known variant c.2203C>T:p.Arg735Trp on one allele, in *trans* with a previously unreported complex allele (p.[Thr984Met;Glu989Lys]), the impact of this combination on the protein is not known although the missense variant p.Glu989Lys is observed in 0.2% of African alleles in the gnomAD database and no previously reported nephronophthisis cases suggesting that on its own it is unlikely to represent a pathogenic allele.

Other functional pathway defects

CRB1 **(OMIM *604210)**

Biallelic pathogenic variants in the *CRB1* gene have been associated with a spectrum of inherited retinal dystrophies including LCA (LCA8, OMIM # 613835), rod-cone dystrophy (RP12, OMIM #600105), cone-rod dystrophy, macular dystrophy and early-onset retinal dystrophy (Bujakowska et al., 2012; Khan, Aldahmesh, Abu-Safieh, & Alkuraya, 2014; Motta et al., 2017). Among all LCA cases, LCA8 accounts for approximately 10% (Kumaran et al., 2017). The Crumbs Homolog 1 protein encoded by *CRB1* is involved in photoreceptor morphogenesis and the establishment and maintenance of apico-basal polarization and adherent junctions of epithelial cells (Jacobson et al., 2003; Pocha & Knust, 2013; Richard et al., 2006).

In this cohort, CRB1-retinopathy patients presented with typical findings such as thickened and disorganized retina, nummular pigmentation (Figure 4a) and vessel abnormalities (Figure 4b). Cystic macular edema and Coats disease were seen in the late phases of the disease in some patients.

Mutations in *CRB1* are the third commonest cause of non-syndromic LCA/EORD in Brazilian patients. Twenty patients of 19 families (13.87%) had biallelic variants in *CRB1*. In total, 14 different variants were found, including two previously unreported variants: a frameshift deletion (c.2533_2539delGGTGGAT, p.Gly845SerfsTer9) and a tandem duplication of exons 6 and 7. In this study, the second most frequent variant among all LCA/EORD cases and the most frequent among *CRB1*-related LCA/EORD patients was p.Cys948Tyr (10 patients from nine families). Interestingly, the second most common *CRB1* variant affects the same amino acid residue (p.Cys948Arg), and was detected in five families (homozygous in patients in three of the families). Both p.Cys948Tyr and p.Cys948Arg were found in LCA and EORD patients, however, p.Cys948Tyr was less frequent in EORD (nearly 67% of *CRB1*-LCA patients and 14% of *CRB1*-EORD patients have at least one p.Cys948Tyr variant).

NMNAT1 (OMIM *608700)

Nicotinamide mononucleotide adenylyl-transferase 1 is an enzyme encoded by *NMNAT1* and acts in the nicotinamide adenine dinucleotide (NAD) biosynthesis, catalyzing the formation of NAD+ from nicotinamide mononucleotide (NMN) and ATP. Biallelic pathogenic variants in *NMNAT1* have been associated with LCA9 (OMIM #608553) (Chiang et al., 2012).

Eight variants in the *NMNAT1* gene were associated with LCA in seven unrelated patients in this study. Three variants are previously unreported, two were classified as likely pathogenic (c.759delGinsTA and a duplication of exons 2 to 4) and an intronic variant found downstream of the untranslated first exon (c.-57+21C>T) was classified as a variant of uncertain significance. The most common *NMNAT1* variant was p.Glu257Lys that was identified in five compound heterozygous families. Clinically, patients had thin retina with narrow vessels and pale optic discs. In addition, Coats disease and atrophic macular areas could be seen in some of them (Figure 4c). Special education for blind children was needed for *NMNAT1*-retinopathy patients because of their severe low vision with nystagmus since the first years of life.

CRX (OMIM *602225)

Cone-rod homeobox protein is a transcription factor encoded by the *CRX* gene and is crucial for the differentiation and maintenance of cones and rods from early development (den Hollander et al., 2008). Its role is related to photoreceptor outer segments elongation and phototransduction cascade (Weleber et al., 2004).

Pathogenic variants in *CRX* have been associated with autosomal dominant cone-rod dystrophy-2 (CORD2; OMIM #120970), autosomal dominant retinitis pigmentosa, autosomal dominant and recessive LCA (LCA7; OMIM #613829) (Chacon-Camacho & Zenteno, 2015; Weleber et al., 2004). Approximately 1% of LCA is caused by a mutation in the *CRX* gene (Kumaran et al., 2017).

In this Brazilian series, one patient had a pathogenic variant identified in the *CRX* gene, who had peripheral small areas of hypoautofluorescence with a hyperautofluorescent border representing RPE atrophy outside the arcades. Family history and segregation supported that the novel truncating variant found in the terminal exon, c.500_5001delCA (p.Ser167Ter) was the autosomal dominant LCA-causing variant in keeping with previous reported variants (Hull et al., 2014).

DISCUSSION & CONCLUDING REMARKS

In this study, we report the clinical and molecular findings in syndromic and non-syndromic LCA/EORD in a Brazilian cohort of 137 molecularly diagnosed families (152 affected patients). The most commonly mutated genes were *CEP290* (~21%), *RPE65* (~16%), *CRB1* (~14%), *RPGRIP1* (~10%), *GUCY2D* (~8%) and *RDH12* (~8%), together they accounted approximately 77% of the cases. These findings are in keeping with previous reports (Kumaran et al., 2017), except for the apparent frequency of some genes: *RPE65* (5%- 10%), *CRB1* (10%), and mainly *RPGRIP1* (5%).m

It is clear that LCA/EORD are not independent disease entities, but rather represent a spectrum of severe retinopathy with low vision and nystagmus observed in the first months of

life. The determination of each subtype of the disease is only achieved through molecular genetic testing because in many cases the ophthalmic aspects are similar and in some cases are indistinguishable (e.g. *LCA5*, *SPATA7*, *IQCB1* and *RPGRIP1* patients). Due to the advances in diagnoses and gene therapies, perhaps in the near future, inherited retinopathies will be only classified according to the related gene, such as, for example, *CEP290*-related IRD instead of simply Leber Congenital Amaurosis.

Many of these genes discussed here are also associated with extra-ocular disease, mainly with renal or central nervous system involvement, broadening the spectrum of LCA-EORD to ciliopathies and syndromic disease. Sometimes, the extra-ocular findings may appear later, making it difficult to make an accurate diagnosis early and genotype/phenotype correlations are often complex or unknown. Therefore it is important to follow those patients with adequate clinical and examination and investigations to be able to identify the systemic afflictions for effective care and treatment. For this reason, monitoring with other health professionals is important for better guidance for patients and families.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

WEB RESOURCES

ClinVar, http://www.ncbi.nlm.nih.gov/clinvar/

FATHMM, http://fathmm.biocompute.org.uk/

FATHMM-MKL, http://fathmm.biocompute.org.uk/fathmmMKL.htm

gnomAD, http://gnomad.broadinstitute.org/

HGMD, http://portal.biobase-international.com/hgmd/pro/start.php

LRT, http://www.genetics.wustl.edu/jflab/lrt_query.html

Mutation Assessor, http://mutationassessor.org/

MutationTaster, http://www.mutationtaster.org/

OMIM, http://www.omim.org/

PROVEAN, http://provean.jcvi.org/index.php

RetNet – Retinal Information Network, https://sph.uth.edu/retnet/home.htm

SIFT, http://sift.bii.a-star.edu.sg/

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FIGURE LEGENDS:

Figure 1: Color fundus photographies related to abnormalities in retinoid visual cycling genes. (a) *RPE65* patient 79.1 with only mild granulate aspect of the RPE and mild narrow vessels. (b) *RDH12* patient 70.1 with intense bone spicules deposits and macular yellowish atrophy. (c and d) *RDH12* patient 71.1 with different fundus aspects, only left eye (d) presents macular atrophy.

Figure 2: *GUCY2D* color fundus photographies of patient 48.1 with normal retinal reflex and a very mild granular aspect of the RPE.

Figure 3: Images related to abnormalities in ciliary transport genes. (a) Keratoconus of *CEP290* patient 10.1. (b) Fundus appearance of *CEP290* patient 107.1 with white dots on the periphery. (c) Fundus autofluorescence of *CEP290* patient 107.1. (d) Color fundus photograph of *SPATA7* patient 106.1 with mottled pigmentation in the retinal posterior pole, and a mild atrophic ring surrounded the fovea. (e) Magnetic resonance imaging of *AHI1* patient 131.1 showing the molar tooth sign.

Figure 4: (a and b) Color fundus photographies of *CRB1* patient 31.1 showing (a) nummular pigmentation with perivascular sparing and (b) vascular tortuosity. (c) Fundus appearance of *NMNAT1* patient 64.1 with macular atrophy.

TABLES:

Table1: Data of LCA/EORD patients

Family	Patient ID	Onset	Currant VA OD ; OE	Gene	cDNA and Protein Changes	Zygosity
LEBER CONGENITAL AMAUROSIS PATIENTS						
1	1.1	since birth	LP	*AIPL1*	c.727_729delAAG ; p.Lys243del	Heterozygous
					c.834G>A ; p.Trp278Ter	Heterozygous
2	2.1	since birth	LP	*CEP290*	c.353_354insGCAATTG ; p.Cys118TrpfsTer6	Heterozygous
					c.508A>T ; p.Lys170Ter	Heterozygous
3	3.1	N/A	N/A	*CEP290*	c.6271-8T>G ; p.?	Homozygous
4	4.1	since birth	NLP	*CEP290*	c.384_387delTAGA ; p.Asp128GlufsTer34	Heterozygous
					c.2446C>T ; p.Arg816Cys	Heterozygous
					c.4704G>C ; p.Glu1568Asp	Heterozygous
5	5.1	since birth	NLP	*CEP290*	c.384_387delTAGA ; p.Asp128GlufsTer34	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
6	6.1	since birth	LP	*CEP290*	c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.3911T>C ; p.Met1304Thr	Heterozygous
					c.6271-8T>G ; p.?	Heterozygous
7	7.1	since birth	20/400 ; 20/400	*CEP290*	c.164_167delCTCA ; p.Thr55SerfsTer3	Heterozygous
					c.4723A>T ; p.Lys1575Ter	Heterozygous
	7.2	since birth	CF	*CEP290*	c.164_167delCTCA ; p.Thr55SerfsTer3	Heterozygous
					c.4723A>T ; p.Lys1575Ter	Heterozygous
8	8.1	2 months	NLP	*CEP290*	c.384_387delTAGA ; p.Asp128GlufsTer34	Heterozygous
					c.4723A>T ; p.Lys1575Ter	Heterozygous
9	9.1	since birth	NLP	*CEP290*	c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.6012-12T>A ; p.?	Heterozygous
10	10.1	3 months	NLP	*CEP290*	c.1666delA ; p.Ile556PhefsTer17	Heterozygous
					c.2052+1_2052+2delGT ; p.?	Heterozygous
11	11.1	since birth	20/30 ; 20/30	*CEP290*	c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.6271-8T>G ; p.?	Heterozygous
12	12.1	before 1 year	N/A	*CEP290*	c.1451delA ; p.Lys484ArgfsTer8	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.3911T>C ; p.Met1304Thr	Heterozygous
13	13.1	3 months	NLP	*CEP290*	c.1623+2C>A ; p.?	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.3911T>C ; p.Met1304Thr	Heterozygous
14	14.1	6 months	HM	*CEP290*	c.2695C>T ; p.Gln899Ter	Heterozygous
					c.5777G>C ; p.Arg1926Pro	Heterozygous
15	15.1	1 year	20/400 ; 20/60	*CEP290*	c.881C>G ; p.Ser294Ter	Heterozygous
					c.1522+1G>C ; p.?	Heterozygous
16	16.1	before 1 year	LP	*CEP290*	c.1451delA ; p.Lys484ArgfsTer8	Heterozygous
					c.5477T>C ; p.Leu1826Pro	Heterozygous
17	17.1	since birth	HM	*CEP290*	c.1247T>G ; p.Leu416Ter	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
18	18.1	since birth	LP	*CEP290*	c.2737_2741delGAAAA ; p.Glu913Ter	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.3911T>C ; p.Met1304Thr	Heterozygous
19	19.1	since birth	LP	*CEP290*	c.2991+1655A>G ; p.Cys998Ter	Homozygous
					c.3911T>C ; p.Met1304Thr	Homozygous
20	20.1	2 months	NLP	*CEP290*	c.1219_1220delAT ; p.Met407GlufsTer14	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
21	21.1	2 months	HM	*CEP290*	c.2991+1655A>G ; p.Cys998Ter	Homozygous
22	22.1	since birth	LP	*CEP290*	c.1219_1220delAT ; p.Met407GlufsTer14	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
23	23.1	4 months	LP	*CEP290*	c.2722C>T ; p.Arg908Ter	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous

Table1 cont.

Family	Patient ID	Onset	Currant VA OD ; OE	Gene	cDNA and protein changes	Zygosity
24	24.1	4 months	HM	*CEP290*	c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.3911T>C ; p.Met1304Thr	Heterozygous
					c.6271-8T>G ; p.?	Heterozygous
25	25.1	since birth	NLP	*CEP290*	c.1451delA ; p.Lys484ArgfsTer8	Heterozygous
					c.2991+1655A>G ; p.Cys998Ter	Heterozygous
					c.3911T>C ; p.Met1304Thr	Heterozygous
26	26.1	2 months	20/800 ; 20/400	*CRB1*	c.2842T>C ; p.Cys948Arg	Heterozygous
					c.3462_3463delTG ; p.Cys1154Ter	Heterozygous
27	27.1	1 year	20/60 ; 20/100	*CRB1*	c.2843G>A ; p.Cys948Tyr	Heterozygous
					c.3676G>T ; p.Gly1226Ter	Heterozygous
28	28.1	since birth	20/400 ; 20/400	*CRB1*	c.1633T>C ; p.Ser545Pro	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
	28.2	since birth	20/400 ; 20/400	*CRB1*	c.1633T>C ; p.Ser545Pro	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
29	29.1	before 1 year	20/400 ; 20/400	*CRB1*	c.984G>A ; p.Trp328Ter	Homozygous
30	30.1	since birth	HM	*CRB1*	c.2843G>A ; p.Cys948Tyr	Homozygous
31	31.1	1 year	20/80 ; 20/50	*CRB1*	c.2843G>A ; p.Cys948Tyr	Homozygous
32	32.1	before 1 year	20/3200 ; 20/3200	*CRB1*	c.2533_2539delGGTGGAT ; p.Gly845SerfsTer9	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
33	33.1	since birth	20/1600 ; 20/1600	*CRB1*	c.984G>A ; p.Trp328Ter	Homozygous
34	34.1	1 year	LP	*CRB1*	c.2842T>C ; p.Cys948Arg	Homozygous
35	35.1	3 months	HM	*CRB1*	c.2842T>C ; p.Cys948Arg	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
36	36.1	before 1 year	CF ; HM	*CRB1*	c.2533_2539delGGTGGAT ; p.Gly845SerfsTer9	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
37	37.1	since birth	20/200 ; 20/200	*CRB1*	c.984G>A ; p.Trp328Ter	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
38	38.1	5 months	20/200 ; 20/200	*CRX*	c.500_501delCA ; p.Ser167Ter	Heterozygous
39	39.1	since birth	20/125 ; 20/125	*GUCY2D*	c.1343A>C ; p.Ser448Ter	Homozygous
40	40.1	3 months	NLP	*GUCY2D*	c.1245delT ; p.Phe415LeufsTer73	Heterozygous
					c.1672G>A ; p.Asp558Asn	Heterozygous
					c.1956+1G>A ; p.?	Heterozygous
	40.2	since birth	LP	*GUCY2D*	c.1245delT ; p.Phe415LeufsTer73	Heterozygous
					c.1672G>A ; p.Asp558Asn	Heterozygous
					c.1956+1G>A ; p.?	Heterozygous
41	41.1	since birth	LP	*GUCY2D*	c.2302C>T ; p.Arg768Trp	Homozygous
42	42.1	since birth	LP	*GUCY2D*	c.1245delT ; p.Phe415LeufsTer73	Heterozygous
					c.1672G>A ; p.Asp558Asn	Heterozygous
					c.2598G>C ; p.Lys866Asn	Heterozygous
43	43.1	since birth	LP	*GUCY2D*	c.389delC ; p.Pro130LeufsTer36	Heterozygous
					c.2999G>A ; p.Gly1000Glu	Heterozygous
44	44.1	since birth	NLP	*GUCY2D*	c.1343A>C ; p.Ser448Ter	Homozygous
45	45.1	2 months	LP	*GUCY2D*	c.1343A>C ; p.Ser448Ter	Heterozygous
					c.1957-2A>G ; p.?	Heterozygous
46	46.1	2 months	HM	*GUCY2D*	c.1972C>T ; p.His658Tyr	Homozygous
47	47.1	since birth	HM ; LP	*GUCY2D*	c.1957-2A>G ; p.?	Homozygous
48	48.1	since birth	LP	*GUCY2D*	c.1343A>C ; p.Ser448Ter	Homozygous
49	49.1	since birth	LP	*IQCB1*	c.394-1G>A ; p.?	Homozygous
50	50.1	since birth	LP	*IQCB1*	c.1504C>T ; p.Arg502Ter	Homozygous
51	51.1	since birth	LP	*IQCB1*	c.1518_1519delCA ; p.His506GlnfsTer13	Homozygous
52	52.1	1 year	20/40 ; 20/50	*IQCB1*	c.214C>T ; p.Arg72Ter	Heterozygous
					c.1465C>T ; p.Arg489Ter	Heterozygous
53	53.1	since birth	LP	*LCA5*	c.838C>T ; p.Arg280Ter	Homozygous
	53.2	since birth	LP	*LCA5*	c.838C>T ; p.Arg280Ter	Homozygous
54	54.1	3 months	20/800 ; 20/800	*LCA5*	c.955G>A ; p.Ala319Thr / p.?	Homozygous
55	55.1	1 year	20/200 ; 20/100	*LRAT*	c.298G>A ; p.Gly100Ser	Homozygous

Table1 cont.

Family	Patient ID	Onset	Currant VA OD ; OE	Gene	cDNA and protein changes	Zygosity
56	56.1	6 months	20/80 ; 20/80	*LRAT*	c.298G>A ; p.Gly100Ser	Heterozygous
					c.346T>C ; p.Phe116Leu	Heterozygous
57	57.1	since birth	20/1600 ; 20/1600	*LRAT*	c.298G>A ; p.Gly100Ser	Homozygous
58	58.1	since birth	20/100 ; 20/60	*LRAT*	c.298G>A ; p.Gly100Ser	Homozygous
59	59.1	before 1 year	N/A	*LRAT*	c.163C>G ; p.Arg55Gly	Homozygous
60	60.1	since birth	20/320 ; 20/320	*LRAT*	c.346T>C ; p.Phe116Leu	Homozygous
61	61.1	since birth	LP	*NMNAT1*	c.716T>C ; p.Leu239Ser	Heterozygous
					c.769G>A ; p.Glu257Lys	Heterozygous
62	62.1	3 months	HM	*NMNAT1*	exon 2-4 duplication ; p.?	Heterozygous
					c.769G>A ; p.Glu257Lys	Heterozygous
63	63.1	since birth	LP	*NMNAT1*	c.37G>A ; p.Ala13Thr	Heterozygous
					c.293T>G ; p.Val98Gly	Heterozygous
64	64.1	4 months	NLP	*NMNAT1*	c.-57+21C>T ; p.?	Heterozygous
					c.759delGinsTA ; p.Leu253PhefsTer5	Heterozygous
65	65.1	3 months	NLP	*NMNAT1*	c.507G>A ; p.Trp169Ter	Heterozygous
					c.769G>A ; p.Glu257Lys	Heterozygous
66	66.1	since birth	NLP	*NMNAT1*	c.293T>G ; p.Val98Gly	Heterozygous
					c.769G>A ; p.Glu257Lys	Heterozygous
67	67.1	since birth	LP	*NMNAT1*	c.507G>A ; p.Trp169Ter	Heterozygous
					c.769G>A ; p.Glu257Lys	Heterozygous
68	68.1	since birth	20/800 ; 20/800	*RDH12*	c.806_810delCCCTG ; p.Ala269GlyfsTer2	Homozygous
69	69.1	since birth	20/400 ; 20/100	*RDH12*	c.184C>T ; p.Arg62Ter	Heterozygous
					c.698T>A ; p.Val233Asp	Heterozygous
70	70.1	since birth	20/400 ; 20/400	*RDH12*	c.146C>T ; p.Thr49Met	Heterozygous
					c.598T>C ; p.Tyr200His	Heterozygous
71	71.1	1 year	20/200 ; 20/200	*RDH12*	c.125T>C ; p.Val42Ala	Heterozygous
					c.325G>C ; p.Ala109Pro	Heterozygous
					c.698T>A ; p.Val233Asp	Heterozygous
	71.2	1 year	20/200 ; 20/200	*RDH12*	c.125T>C ; p.Val42Ala	Heterozygous
					c.325G>C ; p.Ala109Pro	Heterozygous
					c.698T>A ; p.Val233Asp	Heterozygous
72	72.1	N/A	20/500 ; 20/125	*RDH12*	c.698T>A ; p.Val233Asp	Heterozygous
					c.806_810delCCCTG ; p.Ala269GlyfsTer2	Heterozygous
73	73.1	since birth	20/400 ; 20/400	*RDH12*	c.698T>A ; p.Val233Asp	Homozygous
74	74.1	before 1 year	N/A	*RPE65*	c.247T>C ; p.Phe83Leu	Homozygous
75	75.1	since birth	N/A	*RPE65*	c.560G>A ; p.Gly187Glu	Homozygous
	75.2	since birth	N/A	*RPE65*	c.560G>A ; p.Gly187Glu	Homozygous
76	76.1	1 year	20/400 ; 20/400	*RPE65*	c.272G>A ; p.Arg91Gln	Heterozygous
					c.1022T>C ; p.Leu341Ser	Heterozygous
	76.2	1 year	20/125 ; 20/125	*RPE65*	c.272G>A ; p.Arg91Gln	Heterozygous
					c.1022T>C ; p.Leu341Ser	Heterozygous
77	77.1	since birth	20/400 ; 20/400	*RPE65*	c.137G>A ; p.Gly46Glu	Heterozygous
					c.272G>A ; p.Arg91Gln	Heterozygous
	77.2	since birth	N/A	*RPE65*	c.137G>A ; p.Gly46Glu	Heterozygous
					c.272G>A ; p.Arg91Gln	Heterozygous
78	78.1	since birth	20/200 ; 20/200	*RPE65*	c.370C>T ; p.Arg124Ter	Heterozygous
					c.1022T>C ; p.Leu341Ser	Heterozygous
	78.2	3 months	20/400 ; CF	*RPE65*	c.370C>T ; p.Arg124Ter	Heterozygous
					c.1022T>C ; p.Leu341Ser	Heterozygous
79	79.1	since birth	20/1600 ; 20/800	*RPE65*	c.247T>C ; p.Phe83Leu	Homozygous
	79.2	since birth	20/200 ; 20/200	*RPE65*	c.247T>C ; p.Phe83Leu	Homozygous
80	80.1	since birth	20/400 ; 20/200	*RPE65*	c.11+5G>A ; p.?	Heterozygous
					c.1583G>T ; p.Gly528Val	Heterozygous
81	81.1	1 year	20/40 ; 20/40	*RPE65*	c.370C>T ; p.Arg124Ter	Homozygous
82	82.1	since birth	HM	*RPE65*	c.61G>T ; p.Glu21Ter	Heterozygous
					c.1022T>C ; p.Leu341Ser	Heterozygous

Table1 cont.

Family	Patient ID	Onset	Currant VA OD ; OE	Gene	cDNA and protein changes	Zygosity
83	83.1	before 1 year	20/1600 ; 20/1600	*RPE65*	c.184G>A p.Asp62Asn	Heterozygous
					c.292_311del p.Ile98HisfsTer26	Heterozygous
84	84.1	since birth	20/800 ; 20/800	*RPE65*	c.272G>A ; p.Arg91Gln	Heterozygous
					c.1101A>G ; p.Arg367=	Heterozygous
85	85.1	since birth	20/100 ; 20/200	*RPE65*	c.1022T>C ; p.Leu341Ser	Homozygous
86	86.1	before 1 year	LP	*RPE65*	c.247T>C ; p.Phe83Leu	Homozygous
87	87.1	before 1 year	20/800 ; 20/1600	*RPE65*	c.247T>C ; p.Phe83Leu	Homozygous
88	88.1	since birth	20/800 ; 20/800	*RPE65*	c.1336dupA ; p.Arg446LysfsTer4	Homozygous
89	89.1	since birth	N/A	*RPE65*	c.1205G>A ; p.Trp402Ter	Homozygous
90	90.1	since birth	20/200 ; 20/200	*RPGRIP1*	c.1611G>A; p.Gln537=	Heterozygous
					c.2759_2760insT ; p.Gln920HisfsTer14	Heterozygous
91	91.1	since birth	N/A	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
	91.2	since birth	N/A	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
92	92.1	since birth	N/A	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
	92.2	since birth	N/A	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
93	93.1	since birth	20/800 ; 20/800	*RPGRIP1*	c.800G>A ; p.Arg267Gln	Heterozygous
					exon 10-18 deletion ; p.?	Heterozygous
94	94.1	since birth	N/A	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
95	95.1	since birth	20/800 ; 20/800	*RPGRIP1*	c.2012G>A p.Gly671Glu	Homozygous
96	96.1	4 months	CF	*RPGRIP1*	c.2759_2760insT ; p.Gln920HisfsTer14	Homozygous
97	97.1	4 months	LP	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
98	98.1	since birth	N/A	*RPGRIP1*	c.2759_2760insT ; p.Gln920HisfsTer14	Homozygous
99	99.1	since birth	HM	*RPGRIP1*	c.2941C>T ; p.Arg981Ter	Homozygous
	99.2	since birth	20/800 ; 20/800	*RPGRIP1*	c.2941C>T ; p.Arg981Ter	Homozygous
100	100.1	3 months	20/800 ; 20/800	*RPGRIP1*	c.2012G>A p.Gly671Glu	Heterozygous
					c.2759_2760insT ; p.Gln920HisfsTer14	Heterozygous
101	101.1	1 year	20/150 ; 20/150	*RPGRIP1*	exon 10-18 deletion ; p.?	Heterozygous
					c.2468A>G ; p.Tyr823Cys	Heterozygous
102	102.1	6 months	LP	*RPGRIP1*	c.800+1G>A ; p.?	Homozygous
103	103.1	N/A	N/A	*RPGRIP1*	exon 10-18 deletion ; p.?	Homozygous
104	104.1	since birth	N/A	*SPATA7*	c.700dupT ; p.Ser234PhefsTer2	Heterozygous
					c.708_711delACAA ; p.Lys236AsnfsTer9	Heterozygous
105	105.1	since birth	20/200 ; 20/400	*SPATA7*	c.699_700delTT ; p.Ser234Ter	Homozygous
106	106.1	5 months	20/200 ; 20/200	*SPATA7*	exon 1-11 deletion ; p.?	Heterozygous
					c.8_19+14del ; p.?	Heterozygous

EARLY-ONSET RETINAL DISTROPHY PATIENTS

Family	Patient ID	Onset	Currant VA OD ; OE	Gene	cDNA and protein changes	Zygosity
107	107.1	4 years	20/30 ; 20/25	*CEP290*	c.14T>C ; p.Ile5Thr	Heterozygous
					c.4962_4963delAA ; p.Glu1656AsnfsTer3	Heterozygous
108	108.1	3 years	20/30 ; 20/200	*CRB1*	c.2842T>C ; p.Cys948Arg	Homozygous
109	109.1	4 years	20/800 ; 20/800	*CRB1*	exon 6-7 duplication ; p.?	Heterozygous
					c.2843G>A ; p.Cys948Tyr	Heterozygous
110	110.1	5 years	20/400 ; 20/400	*CRB1*	c.276_294delinsTGAACACTGTAC;p.Arg92SerfsTer54	Heterozygous
					c.2506C>A ; p.Pro836Thr	Heterozygous
111	111.1	N/A	20/60 ; 20/80	*CRB1*	c.4142C>T ; p.Pro1381Leu	Homozygous
112	112.1	6 years	20/150 ; 20/800	*CRB1*	c.2042G>A ; p.Cys681Tyr	Heterozygous
					c.2506C>A ; p.Pro836Thr	Heterozygous
113	113.1	2 years	20/60 ; 20/60	*CRB1*	c.2291G>A ; p.Arg764His	Heterozygous
					c.4168C>T ; p.Arg1390Ter	Heterozygous
114	114.1	7 years	20/800 ; 20/800	*CRB1*	c.2842T>C ; p.Cys948Arg	Homozygous
115	115.1	N/A	20/80 ; 20/70	*GUCY2D*	c.1052A>G ; p.Tyr351Cys	Homozygous
116	116.1	before 2 years	N/A	*LRAT*	c.163C>G ; p.Arg55Gly	Homozygous
117	117.1	2 years	CF	*NPHP4*	c.3146C>T ; p.Pro1049Leu	Heterozygous
					c.3574C>T ; p.Arg1192Trp	Heterozygous
118	118.1	4 years	20/400 ; 20/400	*RDH12*	c.184C>T ; p.Arg62Ter	Homozygous
119	119.1	2 years	20/60 ; 20/60	*RDH12*	c.698_699delTCinsAA ; p.Val233Glu	Homozygous

Table1 cont.

Family	Patient ID	Onset	Currant VA OD ; OE	Gene	cDNA and protein changes	Zygosity
120	120.1	2 years	20/400 ; 20/400	*RDH12*	c.178G>C ; p.Ala60Pro	Heterozygous
					c.677A>G ; p.Tyr226Cys	Heterozygous
121	121.1	3 years	20/320 ; 20/400	*RDH12*	c.698T>A ; p.Val233Asp	Heterozygous
					c.806_810delCCCTG ; p.Ala269GlyfsTer2	Heterozygous
122	122.1	before 7 years	N/A	*RDH12*	c.278T>C ; p.Leu93Pro	Heterozygous
					c.295C>A ; p.Leu99Ile	Heterozygous
123	123.1	N/A	20/40 ; 20/30	*RPE65*	c.272G>A ; p.Arg91Gln	Homozygous
124	124.1	N/A	20/200 ; 20/400	*RPE65*	c.65T>C ; p.Leu22Pro	Heterozygous
					c.272G>C ; p.Arg91Pro	Heterozygous
125	125.1	N/A	N/A	*RPE65*	c.1205G>A ; p.Trp402Ter	Homozygous
126	126.1	*N/A*	counting fingers	*RPE65*	c.272G>A ; p.Arg91Gln	Heterozygous
					c.1022T>C ; p.Leu341Ser	Heterozygous
127	127.1	*N/A*	20/400 ; 20/400	*RPE65*	c.560G>A ; p.Gly187Glu	Homozygous
128	128.1	N/A	20/800 ; 20/500	*RPE65*	c.560G>A ; p.Gly187Glu	Homozygous
	128.2	7 years	HM	*RPE65*	c.560G>A ; p.Gly187Glu	Homozygous
	128.3	N/A	20/500 ; 20/500	*RPE65*	c.560G>A ; p.Gly187Glu	Homozygous
JOUBERT SYNDROME PATIENTS†						
129	129.1	since birth	N/A	*AHI1*	c.1205delC ; p.Pro402LeufsTer3	Heterozygous
					c.2212C>T ; p.Arg738Ter	Heterozygous
130	130.1	since birth	20/60 ; 20/60	*AHI1*	c.1829G>C ; p.Arg610Pro	Heterozygous
					c.2742delT ; p.Leu915CysfsTer64	Heterozygous
131	131.1	since birth	LP	*AHI1*	c.2623+1G>T	Homozygous
132	132.1	2 months	NLP	*CEP290*	c.2722C>T ; p.Arg908Ter	Heterozygous
					c.6271-8T>G ; p.?	Heterozygous
133	133.1	since birth	LP	*CEP290*	c.2722C>T ; p.Arg908Ter	Heterozygous
					c.6271-8T>G ; p.?	Heterozygous
134	134.1	2 months	NLP	*CEP290*	c.1666delA ; p.Ile556PhefsTer17	Heterozygous
					c.4723A>T ; p.Lys1575Ter	Heterozygous
135	135.1	2 months	NLP	*CEP290*	c.1666delA ; p.Ile556PhefsTer17	Heterozygous
					c.2052+1_2052+2delGT ; p.?	Heterozygous
SENIOR-LØKEN SYNDROME PATIENTS†						
136	136.1	since birth	LP	*IQCB1*	c.1518_1519delCA; p.His506GlnfsTer13	Homozygous
137	137.1	10 months	HM	*NPHP4*	c.2203C>T ; p.Arg735Trp	Heterozygous
					c.2951C>T ; p.Thr984Met	Heterozygous
					c.2965G>A ; p.Glu989Lys	Heterozygous

VA: Visual Acuity; LP: light perception; NLP: no light perception; CF: counting fingers; HM: hand movement at 1 foot; N/A: not available

† Syndromic form of Leber congenital amaurosis

Table2: Likely and causal variants identified in Brazilian patients with non-syndromic and syndromic Leber congenital amaurosis

Causative gene	Transcript	Nucleotide change	Consequence	Patients evaluated		gnomAD† Total AF (%)	ACMG Classification	Some References
				Allele Count	Number of Homozygotes			
AHI1	NM_017651.4	c.1205delC	p.Pro402LeufsTer3	1	0	-	Pathogenic	(Porto et al., 2017)
AHI1	NM_017651.4	c.1829G>C	p.Arg610Pro	1	0	0.0008158	VUS	This study
AHI1	NM_017651.4	c.2212C>T	p.Arg738Ter	1	0	0.001427	Pathogenic	(Chaki et al., 2011; Porto et al., 2017)
AHI1	NM_017651.4	c.2623+1G>T	p.?	2	1	-	Pathogenic	This study
AHI1	NM_017651.4	c.2742delT	p.Leu915CysfsTer64	1	0	-	Likely Pathogenic	This study
AIPL1	NM_014336.4	c.727_729delAAG	p.Lys243del	1	0	0.000398	VUS	(Stone, 2007)
AIPL1	NM_014336.4	c.834G>A	p.Trp278Ter	1	0	0.03352	Pathogenic	(Srikrupa et al., 2018; Weisschuh et al., 2018)
CEP290	NM_025114.3	c.14T>C	p.Ile5Thr	1	0	-	VUS	This study
CEP290	NM_025114.3	c.164_167delCTCA	p.Thr55SerfsTer3	2	0	0.00179	Pathogenic	(Bachmann-Gagescu et al., 2015; Helou et al., 2007)
CEP290	NM_025114.3	c.353_354insGCAATTG	p.Cys118TrpfsTer6	1	0	-	Pathogenic	This study
CEP290	NM_025114.3	c.384_387delTAGA	p.Asp128GlufsTer34	3	0	0.005349	Pathogenic	(Perrault et al., 2007)
CEP290	NM_025114.3	c.508A>T	p.Lys170Ter	1	0	0.001985	Pathogenic	(Stone et al., 2017)
CEP290	NM_025114.3	c.881C>G	p.Ser294Ter	1	0	-	Pathogenic	This study
CEP290	NM_025114.3	c.1219_1220delAT	p.Met407GlufsTer14	2	0	0.007505	Pathogenic	(Bachmann-Gagescu et al., 2015; Perrault et al., 2007)
CEP290	NM_025114.3	c.1247T>G	p.Leu416Ter	1	0	-	Pathogenic	(Porto et al., 2017)
CEP290	NM_025114.3	c.1451delA	p.Lys484ArgfsTer8	3	0	-	Pathogenic	(Otto et al., 2011)
CEP290	NM_025114.3	c.1522+1G>C	p.?	1	0	-	Pathogenic	This study
CEP290	NM_025114.3	c.1623+2C>A	p.?	1	0	-	Likely Pathogenic	This study
CEP290	NM_025114.3	c.1666delA	p.Ile556PhefsTer17	3	0	-	Pathogenic	(Bachmann-Gagescu et al., 2015; Huang et al., 2018)
CEP290	NM_025114.3	c.2052+1_2052+2delGT	p.?	2	0	0.002046	Pathogenic	(X. Wang et al., 2013)
CEP290	NM_025114.3	c.2446C>T	p.Arg816Cys	1	0	0.006271	VUS	(Landrum et al., 2018)
CEP290	NM_025114.3	c.2695C>T	p.Gln899Ter	1	0	-	Pathogenic	(Coppieters, Casteels, et al., 2010; Xiong et al., 2015)
CEP290	NM_025114.3	c.2722C>T	p.Arg908Ter	3	0	0.001236	Pathogenic	(Landrum et al., 2018)
CEP290	NM_025114.3	c.2737_2741delGAAAA	p.Glu913Ter	1	0	-	Likely Pathogenic	This study
CEP290	NM_025114.3	c.2991+1655A>G	p.Cys998Ter	17	2	0.01278	Pathogenic	(den Hollander et al., 2006; Porto et al., 2017)
CEP290	NM_025114.3	c.3911T>C	p.Met1304Thr	8	1	-	VUS	This study
CEP290	NM_025114.3	c.4704G>C	p.Glu1568Asp	1	0	-	VUS	This study

Table 2 cont.

Causative gene	Transcript	Nucleotide change	Consequence	Patients evaluated		gnomAD† Total AF (%)	ACMG Classification	Some References
				Allele Count	Number of Homozygotes			
CEP290	NM_025114.3	c.4723A>T	p.Lys1575Ter	4	0	0.006051	Pathogenic	(Bachmann-Gagescu et al., 2015; Stone et al., 2017)
CEP290	NM_025114.3	c.4962_4963delAA	p.Glu1656AsnfsTer3	1	0	0.004067	Pathogenic	(Coutelier et al., 2018; Perrault et al., 2007)
CEP290	NM_025114.3	c.5477T>C	p.Leu1826Pro	1	0	-	VUS	This study
CEP290	NM_025114.3	c.5777G>C	p.Arg1926Pro	1	0	0.0004187	VUS	(Wiszniewski et al., 2011)
CEP290	NM_025114.3	c.6012-12T>A	p.?	1	0	0.001785	VUS	(Itoh et al., 2018; Suzuki et al., 2016)
CEP290	NM_025114.3	c.6271-8T>G	p.?	7	1	0.001122	VUS	(Porto et al., 2017; Xiong et al., 2015)
CRB1	NM_201253.3	c.276_294delinsTGAACACTGTAC	p.Arg92SerfsTer54	1	0	-	Likely Pathogenic	(Motta et al., 2017)
CRB1	NM_201253.3	c.984G>A	p.Trp328Ter	5	2	-	Pathogenic	(Motta et al., 2017; X. Wang et al., 2013)
CRB1	NM_201253.3	exon 6-7 duplication	p.?	1	0	-	Pathogenic	This study
CRB1	NM_201253.3	c.1633T>C	p.Ser545Pro	2	0	-	VUS	(Porto et al., 2017)
CRB1	NM_201253.3	c.2042G>A	p.Cys681Tyr	1	0	0.0003983	Likely Pathogenic	(Eisenberger et al., 2013; Weisschuh et al., 2018)
CRB1	NM_201253.3	c.2291G>A	p.Arg764His	1	0	0.001771	VUS	(Corton et al., 2013; Motta et al., 2017)
CRB1	NM_201253.3	c.2506C>A	p.Pro836Thr	2	0	0.02796	VUS	(Henderson et al., 2011; Motta et al., 2017)
CRB1	NM_201253.3	c.2533_2539delGGTGGAT	p.Gly845SerfsTer9	2	0	0.0003982	Pathogenic	This study
CRB1	NM_201253.3	c.2842T>C	p.Cys948Arg / p.?	8	3	-	Likely Pathogenic	(Motta et al., 2017; Soens et al., 2017)
CRB1	NM_201253.3	c.2843G>A	p.Cys948Tyr	12	2	0.02027	Likely Pathogenic	(Motta et al., 2017; Porto et al., 2017)
CRB1	NM_201253.3	c.3462_3463delTG	p.Cys1154Ter	1	0	-	Likely Pathogenic	(Motta et al., 2017)
CRB1	NM_201253.3	c.3676G>T	p.Gly1226Ter	1	0	0.001998	Pathogenic	(Carss et al., 2017; Motta et al., 2017)
CRB1	NM_201253.3	c.4142C>T	p.Pro1381Leu	2	1	-	VUS	(Henderson et al., 2011; Tsang et al., 2014)
CRB1	NM_201253.3	c.4168C>T	p.Arg1390Ter	1	0	0.001197	Pathogenic	(Motta et al., 2017; Srikrupa et al., 2018)
CRX	NM_000554.6	c.500_501delCA	p.Ser167Ter	1	0	-	Likely Pathogenic	This study
GUCY2D	NM_000180.3	c.389delC	p.Pro130LeufsTer36	1	0	0.00205	Pathogenic	(Perrault et al., 1996)
GUCY2D	NM_000180.3	c.1052A>G	p.Tyr351Cys	2	1	0.0008305	VUS	(Zulliger et al., 2015)
GUCY2D	NM_000180.3	c.1245delT	p.Phe415LeufsTer73	3	0	-	Likely Pathogenic	This study
GUCY2D	NM_000180.3	c.1343C>A	p.Ser448Ter	7	3	0.003313	Likely Pathogenic	(Perrault et al., 2000)
GUCY2D	NM_000180.3	c.1672G>A	p.Asp558Asn	3	0	0.00813	VUS	This study
GUCY2D	NM_000180.3	c.1956+1G>A	p.?	2	0	0.0003991	Pathogenic	This study
GUCY2D	NM_000180.3	c.1957-2A>G	p.?	3	1	-	Pathogenic	This study

Table 2 cont.

Causative gene	Transcript	Nucleotide change	Consequence	Patients evaluated		gnomAD† Total AF (%)	ACMG Classification	Some References
				Allele Count	Number of Homozygotes			
GUCY2D	NM_000180.3	c.1972C>T	p.His658Tyr	2	1	-	VUS	This study
GUCY2D	NM_000180.3	c.2302C>T	p.Arg768Trp	2	1	0.01415	Likely Pathogenic	(Thompson et al., 2017; Zulliger et al., 2015)
GUCY2D	NM_000180.3	c.2598G>C	p.Lys866Asn	1	0	-	VUS	(Coppieters, Casteels, et al., 2010)
GUCY2D	NM_000180.3	c.2999G>A	p.Gly1000Glu	1	0	-	VUS	This study
IQCB1	NM_001023570.4	c.214C>T	p.Arg72Ter	1	0	0.003891	Pathogenic	(Carss et al., 2017; Stone et al., 2017)
IQCB1	NM_001023570.4	c.394-1G>A	p.?	2	1	0.0004008	Pathogenic	(Porto et al., 2017)
IQCB1	NM_001023570.4	c.1465C>T	p.Arg489Ter	1	0	0.002784	Pathogenic	(Estrada-Cuzcano et al., 2011; X. Wang et al., 2013)
IQCB1	NM_001023570.4	c.1504C>T	p.Arg502Ter	2	1	0.0007955	Pathogenic	(Barbelanne et al., 2013; Porto et al., 2017)
IQCB1	NM_001023570.4	c.1518_1519delCA	p.His506GlnfsTer13	4	2	0.008486	Pathogenic	(Barbelanne et al., 2013; Estrada-Cuzcano et al., 2011)
LCA5	NM_001122769.3	c.838C>T	p.Arg280Ter	4	2	-	Pathogenic	(Carss et al., 2017; Consugar et al., 2015)
LCA5	NM_001122769.3	c.955G>A	p.Ala319Thr / p.?	2	1	-	VUS	(Ramprasad et al., 2008)
LRAT	NM_004744.5	c.163C>G	p.Arg55Gly	4	2	0.003187	VUS	(González-Del Pozo et al., 2018)
LRAT	NM_004744.5	c.298G>A	p.Gly100Ser	7	3	-	VUS	This study
LRAT	NM_004744.5	c.346T>C	p.Phe116Leu	3	1	-	VUS	(Porto et al., 2017)
NMNAT1	NM_022787.4	c.-57+21C>T	p.?	1	0	-	VUS	This study
NMNAT1	NM_022787.4	exon 2-4 duplication	p.?	1	0	-	Likely Pathogenic	This study
NMNAT1	NM_022787.4	c.37G>A	p.Ala13Thr	1	0	0.02124	VUS	(Sasaki et al., 2015)
NMNAT1	NM_022787.4	c.293T>G	p.Val98Gly	2	0	0.001786	VUS	(Chiang et al., 2012; Sasaki et al., 2015)
NMNAT1	NM_022787.4	c.507G>A	p.Trp169Ter	2	0	0.004243	Pathogenic	(Chiang et al., 2012; Thompson et al., 2017)
NMNAT1	NM_022787.4	c.716T>C	p.Leu239Ser	1	0	0.001415	VUS	(Perrault et al., 2012; Sasaki et al., 2015)
NMNAT1	NM_022787.4	c.759delGinsTA	p.Leu253PhefsTer5	1	0	-	Likely Pathogenic	This study
NMNAT1	NM_022787.4	c.769G>A	p.Glu257Lys	5	0	0.06949	Likely Pathogenic	(Ceyhan-Birsoy et al., 2019; Chiang et al., 2012)
NPHP4	NM_015102.5	c.2203C>T	p.Arg735Trp	1	0	0.04348	VUS	(Hoefele et al., 2005)
NPHP4	NM_015102.5	c.2951C>T	p.Thr984Met	1	0	0.004647	VUS	This study
NPHP4	NM_015102.5	c.2965G>A	p.Glu989Lys	1	0	0.04463	VUS	(Landrum et al., 2018)
NPHP4	NM_015102.5	c.3146C>T	p.Pro1049Leu	1	0	-	VUS	This study
NPHP4	NM_015102.5	c.3574C>T	p.Arg1192Trp	1	0	0.1810	VUS	(French et al., 2012; Gast et al., 2016)

Table 2 cont.

Causative gene	Transcript	Nucleotide change	Consequence	Patients evaluated		gnomAD† Total AF (%)	ACMG Classification	Some References
				Allele Count	Number of Homozygotes			
RDH12	NM_152443.2	c.125T>C	p.Val42Ala	2	0	-	VUS	This study
RDH12	NM_152443.2	c.146C>T	p.Thr49Met	1	0	0.001768	VUS	(Janecke et al., 2004; Srikrupa et al., 2018)
RDH12	NM_152443.2	c.178G>C	p.Ala60Pro	1	0	-	VUS	(Abu-Safieh et al., 2013)
RDH12	NM_152443.2	c.184C>T	p.Arg62Ter	3	1	0.005659	Pathogenic	(Porto et al., 2017; Srikrupa et al., 2018)
RDH12	NM_152443.2	c.278T>C	p.Leu93Pro	1	0	0.001767	VUS	(Ávila-Fernández et al., 2010; Bravo-Gil et al., 2017)
RDH12	NM_152443.2	c.295C>A	p.Leu99Ile	1	0	0.006009	VUS	(Bravo-Gil et al., 2016; Zhang et al., 2016)
RDH12	NM_152443.2	c.325G>C	p.Ala109Pro	2	0	-	VUS	This study
RDH12	NM_152443.2	c.598T>C	p.Tyr200His	1	0	-	Likely Pathogenic	(Xu et al., 2014)
RDH12	NM_152443.2	c.677A>G	p.Tyr226Cys	1	0	-	VUS	(Janecke et al., 2004)
RDH12	NM_152443.2	c.698T>A	p.Val233Asp	7	1	0.001205	VUS	(Coppieters, Casteels, et al., 2010)
RDH12	NM_152443.2	c.698_699delTCinsAA	p.Val233Glu	2	1	-	VUS	This study
RDH12	NM_152443.2	c.806_810delCCCTG	p.Ala269GlyfsTer2	4	1	0.01587	Pathogenic	(Aleman et al., 2018; X. Wang et al., 2013)
RPE65	NM_000329.3	c.11+5G>A	p.?	1	0	0.007781	VUS	(Kumaran et al., 2018; Ripamonti et al., 2014)
RPE65	NM_000329.3	c.61G>T	p.Glu21Ter	1	0	-	Pathogenic	(Chung et al., 2019)
RPE65	NM_000329.3	c.65T>C	p.Leu22Pro	1	0	0.002785	VUS	(Li et al., 2014; Xiong et al., 2015)
RPE65	NM_000329.3	c.137G>A	p.Gly46Glu	2	0	-	VUS	(Chung et al., 2019)
RPE65	NM_000329.3	c.184G>A	p.Asp62Asn	1	0	-	VUS	This study
RPE65	NM_000329.3	c.247T>C	p.Phe83Leu	10	5	-	Likely Pathogenic	(Chung et al., 2019; Motta et al., 2019)
RPE65	NM_000329.3	c.272G>C	p.Arg91Pro	1	0	-	VUS	(Simonelli et al., 2007)
RPE65	NM_000329.3	c.272G>A	p.Arg91Gln	8	1	0.0046	Likely Pathogenic	(Chung et al., 2019; Philp et al., 2009)
RPE65	NM_000329.3	c.292_311del	p.Ile98HisfsTer26	1	0	0.004376	Pathogenic	(Lotery et al., 2000; Riera et al., 2017)
RPE65	NM_000329.3	c.370C>T	p.Arg124Ter	4	1	0.005674	Pathogenic	(Chung et al., 2019; Porto et al., 2017)
RPE65	NM_000329.3	c.560G>A	p.Gly187Glu	12	6	0.0007965	Likely Pathogenic	(Motta et al., 2019; Porto et al., 2017)
RPE65	NM_000329.3	c.1022T>C	p.Leu341Ser	8	1	-	Pathogenic	(Chung et al., 2019; Morimura et al., 1998)
RPE65	NM_000329.3	c.1101A>G	p.Arg367= / p.?	1	0	-	VUS	(Soens et al., 2017)
RPE65	NM_000329.3	c.1205G>A	p.Trp402Ter	4	2	0.003189	Pathogenic	(Stone, 2007; Xiong et al., 2015)
RPE65	NM_000329.3	c.1336dupA	p.Arg446LysfsTer4	2	1	-	Pathogenic	(Chung et al., 2019; J. Wang et al., 2014)

Table 2 cont.

Causative gene	Transcript	Nucleotide change	Consequence	Patients evaluated		gnomAD† Total AF (%)	ACMG Classification	Some References
				Allele Count	Number of Homozygotes			
RPE65	NM_000329.3	c.1583G>T	p.Gly528Val	1	0	-	VUS	(Redmond et al., 2005; Thompson et al., 2000)
RPGRIP1	NM_020366.3	c.800G>A	p.Arg267Gln	1	0	0.002013	VUS	This study
RPGRIP1	NM_020366.3	c.800+1G>A	p.?	2	1	0.001208	Pathogenic	(Weisschuh et al., 2018; Xiong et al., 2015)
RPGRIP1	NM_020366.3	exon 10-18 deletion	p.?	16	7	-	Likely Pathogenic	This study
RPGRIP1	NM_020366.3	c.1611G>A	p.Gln537= / p.?	1	0	-	VUS	(Soens et al., 2017)
RPGRIP1	NM_020366.3	c.2012G>A	p.Gly671Glu	3	1	0.0004012	VUS	This study
RPGRIP1	NM_020366.3	c.2468A>G	p.Tyr823Cys	1	0	-	VUS	This study
RPGRIP1	NM_020366.3	c.2759_2760insT	p.Gln920HisfsTer14	6	2	-	Likely Pathogenic	(Dryja et al., 2001)
RPGRIP1	NM_020366.3	c.2941C>T	p.Arg981Ter	4	2	0.0004014	Likely Pathogenic	(Carss et al., 2017; Weisschuh et al., 2018)
SPATA7	NM_018418.5	exon 1-11 deletion	p.?	1	0	-	Likely Pathogenic	This study
SPATA7	NM_018418.5	c.8_19+14del	p.?	1	0	-	Likely Pathogenic	This study
SPATA7	NM_018418.5	c.699_700delTT	p.Ser234Ter	2	1	0.001991	Likely Pathogenic	This study
SPATA7	NM_018418.5	c.700dupT	p.Ser234PhefsTer2	1	0	-	Likely Pathogenic	(Porto et al., 2017)
SPATA7	NM_018418.5	c.708_711delACAA	p.Lys236AsnfsTer9	1	0	0.0003982	Likely Pathogenic	(Porto et al., 2017)

VUS: Variant of Uncertain Significance. More detailed version of this table can be found in the supplementary material.
† Accessed on March, 2020