Original Article

Leber congenital amaurosis: A clinical and genetic study from a tertiary eye care center

Abhishek Upadhyaya[1,2], Srikanta Kumar Padhy[3], Nithin Teja[1,2], Goura Chattannavar[4], Shreya Dutta[1], Venkatesh Pochaboina[5], Jeyapoorani Balasubramanian[5], Chitra Kannabiran[6], Brijesh Takkar[1,7], Manjushree Bhate[4], Subhadra Jalali[1], Deepika C Parameswarappa[1]

Purpose: To assess the clinical phenotypes and genetic mutations in patients with Leber congenital amaurosis (LCA) from a tertiary eye care center in India. **Design:** Retrospective observational study. **Methods:** The study includes patients with a clinical diagnosis of LCA who underwent genetic testing from January 2016 to December 2021. The clinical exome of the patients was analyzed by targeted next-generation sequencing. The genetic variants found were classified as per standard American College of Medical Genetics and Genomics (ACMG) criteria and ClinVar database. **Results:** There were 35 patients (19 females, 16 males) of LCA. Family history was positive in 29% (10/35) and a history of consanguinity was noted in 54% (19/35) of the patients. The mean presenting best-corrected visual acuity was 2.48 ± 0.59 logMAR. Retinal pigment epithelial abnormalities and macular involvement were seen in 83% (58/70) and 23% (16/70) of the eyes, respectively, at presentation. The most common causative genes for LCA in our cohort were: *GUCY2D* (20%, 7/35), *CRB1 (*14%, 5/35), *RPE65 (*11%, 4/35), *RPGRIP1 (*11%, 4/35), and *LCA5 (*9%, 3/35). Autosomal recessive inheritance was seen in 94% (33/35). Macular involvement at presentation was seen in *CRB1* (3/5), *NMNAT1* (2/2), and one each of *RPE65*, *LCA5*, and *RDH12* patients. The genetic testing cost was reduced from 23,800 INR to 15,000 INR per test in the study duration. **Conclusions:** Genetic screening of LCA cases identified various genotypes, with *GUCY2D* being the most common. Increased awareness and reduced costs of genetic testing would benefit both patients and caregivers. With promising clinical trial outcomes, genotyping is crucial for better patient selection and treatment.

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Leber congenital amaurosis (LCA) belongs to a group of inherited retinal diseases (IRDs). It is one of the severe forms of IRD with the earliest presentation within 6 months to 1 year of life leading to legal blindness.[1–3] The prevalence of LCA varies from 1:30000 to 1:80000. It contributes to 5% of all IRDs and 20% of childhood blindness; however, the occurrence varies depending on geographic location.[4–6] The higher occurrence of LCA and IRDs in the South Indian population can be attributed to a constricted gene pool with an increased frequency of consanguineous marriages.[7]

Typical clinical features of LCA include nyctalopia, photoaversion, eye-poking behavior called the oculodigital sign, orbital fat atrophy, enophthalmos, nystagmoid eye movements as early as 6 weeks from birth, profound vision loss from light perception to 20/200, fixation loss, amaurotic pupils, fundus appearance that may vary from normal to presence of pigmentary changes, macular atrophy, or a presentation similar to typical retinitis pigmentosa (RP).[1–3,5,8] The retinal features can vary from a normal-looking fundus to the presence of one or more features, including bone spicule pigmentation, nummular pigmentation, salt and pepper pigmentation, peripheral yellow to white spots, attenuated vessels, macular atrophy, macular pigmentation, macular pseudocoloboma, and pseudo optic disc edema.[4,5,9] Other common associations of LCA include high refractive errors (hypermetropia to myopia), keratoconus, and cataract.[5,10] Rare systemic associations include the presence of coexisting mental retardation, hearing loss, obesity, renal issues, autism, and olfactory dysfunction. Syndromes which include ciliopathies such as Joubert

[1]Srimati Kanuri Santhamma Center for Vitreoretinal Diseases, Anant Bajaj Retina Institute, Kallam Anji Reddy Campus, L V Prasad Eye Institute, Hyderabad, Telangana, [2]Standard Chartered – LVPEI Academy for Eye Care Education, L V Prasad Eye Institute, Hyderabad, Telangana, [3]Vitreoretina and Uveitis Services, Anant Bajaj Retina Institute, Mithu Tulasi Chanrai Campus, L V Prasad Eye Institute, Bhubaneswar, [4]Jasti V. Ramanamma Children's Eye Care Centre, Child Sight Institute, L V Prasad Eye Institute, Hyderabad, Telangana, [5]Department of Genetic Counselling, L V Prasad Eye Institute, Hyderabad, Telangana, [6]Kallam Anji Reddy Molecular Genetics Laboratory, L V Prasad Eye Institute, Hyderabad, Telangana, [7]Indian Health Outcomes, Public Health and Economics Research Center, Kallam Anji Reddy Campus, L V Prasad Eye Institute, Hyderabad, Telangana, India

Correspondence to: Dr. Deepika C Parameswarappa, Kallam Anji Reddy Campus, L V Prasad Eye Institute, Banjara Hills, Hyderabad – 500 034, Telangana, India. E-mail: deepikacpd@gmail.com

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syndrome, Alstrom syndrome, Batten disease, Senior Loken syndrome, and other peroxisomal diseases present with similar ocular manifestations as observed in LCA. It mostly overlaps with a milder form of the same disease called severe early childhood-onset retinal dystrophy or early-onset RP presenting between 1 and 5 years of life.[11,12]

Identification of genes associated with LCA is important to understand the pathogenesis, progression, and future risk to family members and for developing and/or implementing targeted therapy. In 2017, US-FDA approved the Voretigene neparvovec-rzyl gene replacement therapy for biallelic *RPE65*-associated retinal dystrophy due to its safety and efficacy.[13] As more therapies become available, it is important to know the disease-associated genotypes of LCA patients in a given ethnicity.[14] The load of LCA and early-onset RP is huge in developing countries of Asia like India.[15,16] Despite the burden of LCA and RP, there is sparse literature on the genetics of LCA in developing countries and in the Indian population.[7,17–20] Moreover, the prevalence of mutations in the associated genes for LCA in Western and Asian countries can differ from each other due to ethnicity and patterns of inbreeding.[7,16,18,21] With this background, we aim to report mutations in known LCA genes and clinical presentation of the disease in a cohort of patients presenting to a tertiary eye care center in southern India.

Methods

This is a retrospective review of clinically diagnosed LCA cases who underwent genetic testing from a tertiary eye care institute between January 2016 and December 2021. The study was approved by the Institutional Review Board (approval no.: LEC-BHR-R-06-23-1056) and adhered to the tenets of the declaration of Helsinki.

Clinical diagnosis of LCA was based on the following criteria: (i) Children with onset of one or more symptoms during the first year of life (nyctalopia, photoaversion, eye poking, orbital fat atrophy, enophthalmos, nystagmoid eye movements, amaurotic pupils), or (ii) Severe visual impairment (<20/200 or logMAR 1 as per WHO—International Classification of Diseases (ICD-11) effect on January 1, 2022), and (iii) Nonrecordable electroretinogram (ERG) in both scotopic and photopic phases whenever available.[22] Multigenerational pedigree details were taken whenever available. A comprehensive ophthalmic evaluation was carried out. Best-corrected visual acuity (BCVA) was checked in smaller children with the help of age-appropriate Teller acuity and LEA symbol matching charts depending on the child's cooperation when feasible and converted to logMAR. A vision of logMAR 3 was considered for children who were unable to fixate on the light.[23,24] Fundus imaging and full-field ERG were performed in cooperative children. Two of the patients were examined under anesthesia. Fundus photography and ERG were not possible in patients with very severe visual impairment and poor cooperation due to nystagmus. Among patients who underwent ERG testing, both light-adapted and dark-adapted responses were undetectable. The study comprises patients who could financially afford the genetic testing from a commercially available certified genetic laboratory.

The whole blood (ethylenediaminetetraacetic acid) sample was collected from the probands after obtaining informed consent from the guardians. DNA was isolated from blood leukocytes and quantitated using a NanoDrop spectrophotometer. The DNA samples were sequenced using targeted next-generation sequencing (NGS) for clinical exome which covered ~96% of all positions at 20× coverage or higher on the Illumina MiSeq Sequencer to identify the mutations. Sequencing of the protein-coding regions of approximately 41 Mb of the human exome (targeting approximately 99% of regions in consensus coding sequences and RefSeq) was performed using Illumina NovaSeq platform at a mean depth of 100–150× and >90% of bases covered at 30× depth. The following databases and in silico algorithms are used to annotate and evaluate the impact of the variant in the context of human disease: 1000 genomes, gnomAD, (https://gnomad.broadinstitute.org/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), human gene mutation database (https://www.hgmd.cf.ac.uk/ac/index.php) Online Mendelian Inheritance in Man (OMIM), for presence in normal populations and any previous associations with the disease, to evaluate their pathogenicity. The conservation score for the variants (GERP score), Grantham score, and indirect evidence for protein structure change in in silico models were also looked into. Standard criteria as given by the American College of Medical Genetics and Genomics and Association for Molecular Pathology (ACMG-AMP) guidelines were used to reclassify the evidence for the pathogenicity of variants.[25] Descriptive statistics using mean ± standard deviation and median with interquartile range (IQR) were used to elucidate the demographic data.

Results

The study included 35 unrelated LCA patients who met the clinical criteria and had genetic reports available. There were 19 females (54%) and 16 males (46%). The presenting age range was from 3 months to 13 years. The median age at presentation to the tertiary center was 24 months (IQR: 7.60). 28.5% (10/35) of the patients had a positive family history of similar clinical features. 54.3% (19/35) of the patients were born to parents with a history of consanguineous marriage. However, the precise degree of consanguinity remained indistinct due to retrospective data constraints. The majority of patients exhibited symptoms such as nystagmoid eye movements (62.9%, 22/35), avoidance of eye contact with parents (40%, 14/35), and oculodigital sign (34.3%, 12/35).

The mean BCVA (n = 35/35, 100%) noted at the time of presentation in this cohort was 2.48 ± 0.59 SD logMAR. Out of this cohort, eight patients (n = 8/35, 23%) at the time of presentation were unable to even fixate on the light. The pupillary reactions were sluggish in 11 patients and normal in rest of the patients with no paradox. The majority of the patients had hyperopia (n = 32/35, 91%). The other three patients had a myopic refractive error. The median spherical equivalent (n = 35/35, 100%) was +5.00 diopters (IQR: 2.60–6.50).

At presentation, 77% (54/70) of the eyes exhibited no abnormalities in the optic disc. Optic disc pallor was documented in 20% (14/70) of eyes, and one patient displayed pseudodisc edema with optic disc drusen in both eyes. Retinal pigment epithelium (RPE) changes in the background retina were seen in 82.8% (58/70) of the eyes with the clinical presentation varying from fine pigmentary changes to mottling to diffuse granularity. Fundus imaging (by Clarus 500, Zeiss Inc., Germany or Optos, Silverstone P200T, Optos

PLC, Dunfermline, United Kingdom) and a full-field ERG (by RETeval, LKC Technologies or MonPack, Metrovision Technologies) were performed in 40% (14/35) and 46% (16/35) of the patients, respectively.

On exome sequencing, mutations were found in the following genes: *GUCY2D* (20%, 7/35), *CRB1* (14.3%, 5/35), *RPE65* (11.4%, 4/35), *RPGRIP1* (11.4%, 4/35), *LCA5* (8.6%, 3/35), *AIPL1* (8.6%, 3/35), *NMNAT1* (5.7%, 2/35), *SPATA7* (5.7%, 2/35), *CEP290* (5.7%, 2/35), *PRPH 2* (2.9%, 1/35), *RDH12* (2.9%, 1/35), and *IMPDH1* (2.9%, 1/35). The most common genes detected to cause LCA were *GUCY2D* followed by *CRB1*, *RPE65*, and *RPGRIP1*. Table 1 highlights the clinical features of the most common genes identified in this study. The clinical details, genetic variants found, classification, and inheritance pattern of all the patients are provided in Supplementary Table 1. The most common inheritance pattern was autosomal recessive 94% (33/35). Five patients with normal-looking fundus had variants in *GUCY2D* (3 months and 5 years), *CRB1* (7 months and 1 year), and *LCA5* (8 months). The fundus features of *CRB1* were round-shaped pigmentation with macular involvement and paravascular sparing of RPE (cases 8, 10, and 11) [Fig. 1]. Case 11 also had a thickened retina with remodeling in OCT. Macular involvement was seen in *CRB1* (3/5), *NMNAT1* (2/2) and one each of *RPE65*, *LCA5*, and *RDH12* patients. One patient with *RPGRIP1* mutation in our cohort presented with coats-like exudation [Fig. 2—case 16] in the right eye and underwent intravitreal bevacizumab injection along with focal laser at the age of 10 years. Focal laser was performed on the telangiectatic vessels under general anesthesia. The exudation was due to the coat vasculopathy and there was no vasoproliferative tumor noted.

There were no syndromic LCA in the current cohort of 35 patients or they lacked detailed evaluation. But there were nine patients (*4 GUCY2D, 2 NMNAT1*, 1 each of *CRB1*, *RPGRIP1*, and *LCA5*) where the patient records mentioned speech therapy. One patient had high myopia and astigmatism with keratoconus (*RPE65*) and six patients had strabismus. Another patient had bilateral cataract (*NMNAT1*—case 25) at the age of 9 years and subsequently underwent bilateral cataract surgery with intraocular lens implantation. However, the vision remained unchanged both before and after the cataract surgery as following light and fixating on objects.

The follow-up data was available in 80% (28/35) of patients. The median duration of follow-up was 51 months (IQR: 21.25,117). The mean BCVA at the last follow-up was 2.36 ± 0.58 SD logMAR. The duration of follow-up in our institute ranged from one single visit to 28 years of follow-up. The median amount spent per genetic testing was 18,000 INR (range 15,000–23,800 INR). 89% (31/35) of patients availed the services of center for sight enhancement (CSE) and rehabilitation offered by our institute.

Discussion

Our study reports the diverse genotypic variants and clinical characteristics observed in a cohort of 35 Indian patients with LCA. The most common causative genes for LCA in our cohort were *GUCY2D*, *CRB1*, *RPE65*, *RPGRIP1*, *LCA5*, and *AIPL1*. 94% of the genetic mutations were homozygously inherited

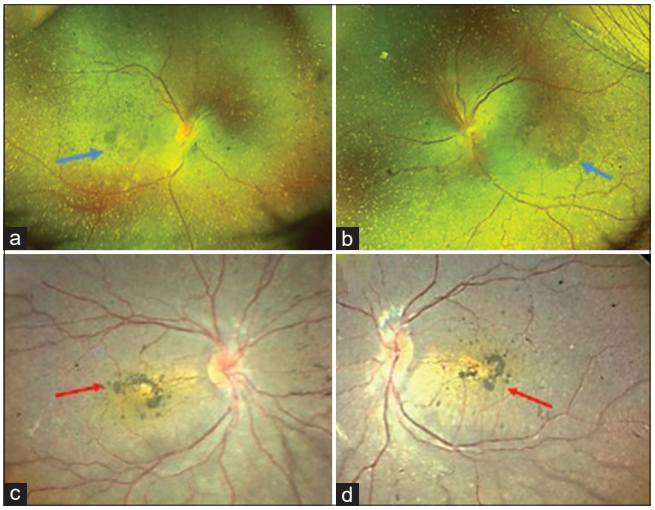

Figure 1: Case 8 with nummular macular pigmentation (blue arrow) (a: right eye and b: left eye) and atrophy in the macular area along with pigmentary changes throughout the fundus in both eyes. The patient on genetic testing was found to be positive for *CRB1* (homozygous: c.2230C>T/p.Arg744*). Case 10 with *CRB1* mutation (c18_21delTAAC/p.Asn7Thrfs*15 and c.1651C>C/T/p.Gln551*) showing nummular macular pigmentation (c: right eye and d: left eye) (red arrow), peripheral pigmentary changes, and paravascular sparing of retinal pigment epithelium

Table 1: Highlighting the clinical features of commoner genes

Gene (number of patients)	Median age at presentation to hospital (in months)	Mean (±SD) BCVA at presentation in logMAR	Common phenotypic features
GUCY2D (7)	12	2.73±0.20	• 5 patients (cases 1–5): RPE changes±mild optic disc pallor • 2 patients (cases 6 and 7): Normal-looking fundus Fundus imaging was not available (NA) for any of the patients
CRB1 (5)	12	2.44±0.67	• 3 patients (cases 8, 10, and 11): Round-shaped pigmentation with macular involvement and paravascular sparing of RPE • Case 11 had a thickened retina with remodeling in optical coherence tomography • Cases 9 and 12: Normal-looking fundus (fundus imaging NA)
RPGRIP1 (4)	66	2.77±0.13	• 3 patients (cases 13, 14, and 16): RPE changes, bony spicules, and attenuated vessels, no specific phenotype seen • 1 patient (case 15) did not have fundus imaging—also the variant needs validation

in an autosomal recessive pattern. 5/35 patients (Cases 3, 10, 12, 18, and 28) in our cohort were heterozygous with an autosomal recessive inheritance and there was a lack of segregation analysis from parents. In the current cohort, 57% (20/35) had pathogenic variants and 9% (3/35) were likely pathogenic. There were 17 nonsense (stop), 13 missense and 4 other variant types (deletion, stop loss, spice site intronic), detected [Supplementary Table 1]. On clinical examination, 12/35 (34%) patients with LCA had variants of uncertain significance (VUSs) and one among them had a likely benign variant (Case 15; *RPGRIP1*, c. 930 + 3A>G splice site variant). This variant of Case 15 can be deleterious by predictive tools but it is also present in the control population, hence this is unclear at this stage. The identified VUSs were homozygous in 9/12 patients and one patient with *IMPDH1* variant was heterozygous showing an autosomal dominant pattern. In the current cohort from a developing country with a less studied population for genetic variants, it is expected to find more VUSs in a genetic report. Ideally, all the VUS should be validated with functional analysis to identify pathogenicity. However, it is difficult because of the lack of facilities for the same. Hence with the available knowledge, we have reclassified VUSs as per ACMG criteria for better understanding.[25]

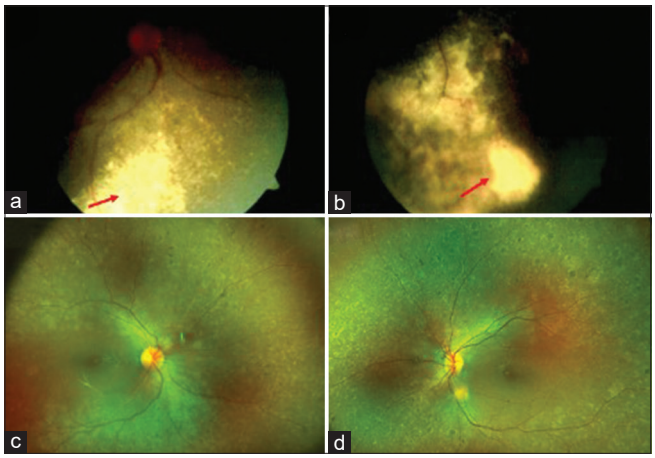

Figure 2: Case 16 with LCA at the age of 10 years had subretinal exudation (red arrow) (a and b) in the right eye with Coats-like picture. The patient underwent intravitreal bevacizumab injection and focal laser therapy. The fundus pictures (c: right eye and d: left eye) show mid-peripheral and peripheral pigmentary changes at the last follow-up visit (age: 15). The patient on genetic testing was found to be positive for *RPGRIP1* mutations (homozygous: c.2171T>A/p.Val724Glu)

Each patient with *GUCY2D* (c. 3085C>A/pArg1029Ser) and *CRB1* (c. 2434C>G/p. Gln812Glu) mutation [Supplementary Table 1, Cases 3 and 12] had only one variant found and another variant was not identified by the current NGS technique used for analysis. The probable explanation for this could be a missing variant in the deep intronic sequence which needs further testing with whole genome analysis and validation. It may also be possible that the identified variant was an incidental finding and not associated with the disease. All the patients with VUS and compound heterozygous mutations in our study had clinical phenotypes matching to LCA criteria mentioned in the methodology.

To date, at least 26 genes have been identified in LCA (https://sph.uth.edu/Retnet/20th February 2024). The genes affected alter the function of retinal photoreceptors and retinal pigment epithelial cells. *GUCY2D*, *AIPL1*, *RD3*, and *KCNJ13* affect the phototransduction pathway. *RPE65*, *LRAT*, and *RDH12* affect the function of retinoid cycle. *LCA5*, *CEP290*, *RPGRIP1*, *SPATA7*, *TULP1*, and *QCB1* affect ciliary transportation and *CRX*, *CRB1*, *GDF6*, *PRPH 2* affect photoreceptor morphogenesis.[1,2,26] LCA is mostly inherited in an autosomal recessive pattern except for *CRX*, *IMPDH1*, and *OTX2* which are inherited in an autosomal dominant pattern.

Comprehensive studies in Western and Chinese populations have explored genotypic variants and their phenotypic relationships in LCA with the most common genes noted as *CEP290*, *GUCY2D*, *CRB1*, *RPE65*, and *RDH12*. In contrast, Indian research on the LCA's genetic profile remains limited.[17–21,27] The most prevalent mutation noted in our patient cohort was in *GUCY2D* (20%, 7/35). *CEP290* mutations reported commonly in the LCA cohort of the Western part of

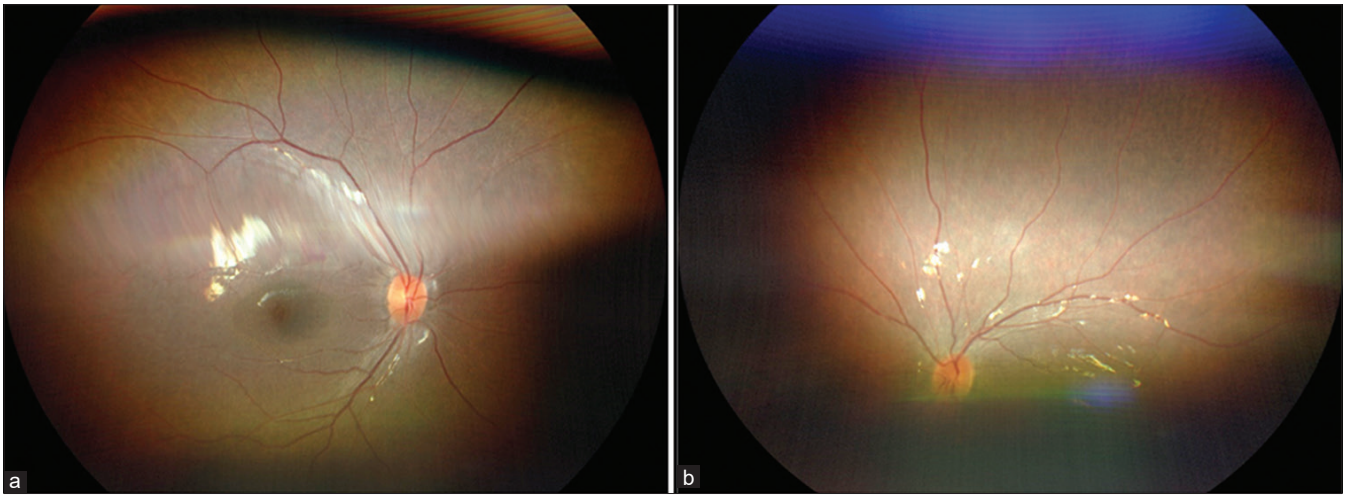

Figure 3: Case 22 at 3 years of age had a near normal-looking fundus and subtle retinal pigment epithelial changes (a: right eye and b: left eye). The child on genetic testing was found to be positive for *AIPL1* mutations (homozygous: c.809G>T/p.Arg270Leu)

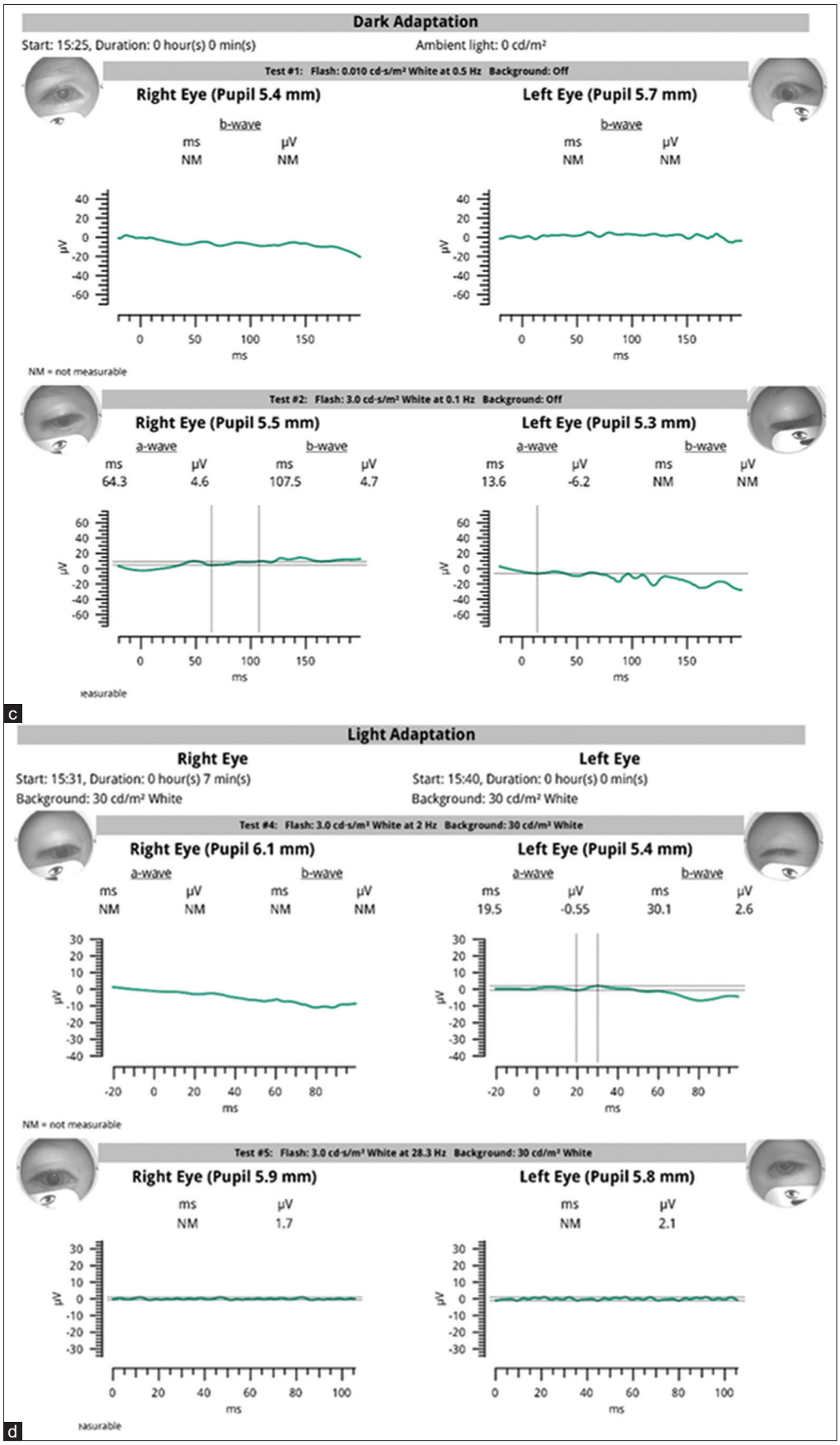

Figure 3: Contd... Case 22 at 3 years of age had a near normal-looking fundus and subtle retinal pigment epithelial changes with nondetectable scotopic (c) and photopic (d) responses on electroretinogram. The child on genetic testing was found to be positive for *AIPL1* mutations (homozygous: c.809G>T/p.Arg270Leu)

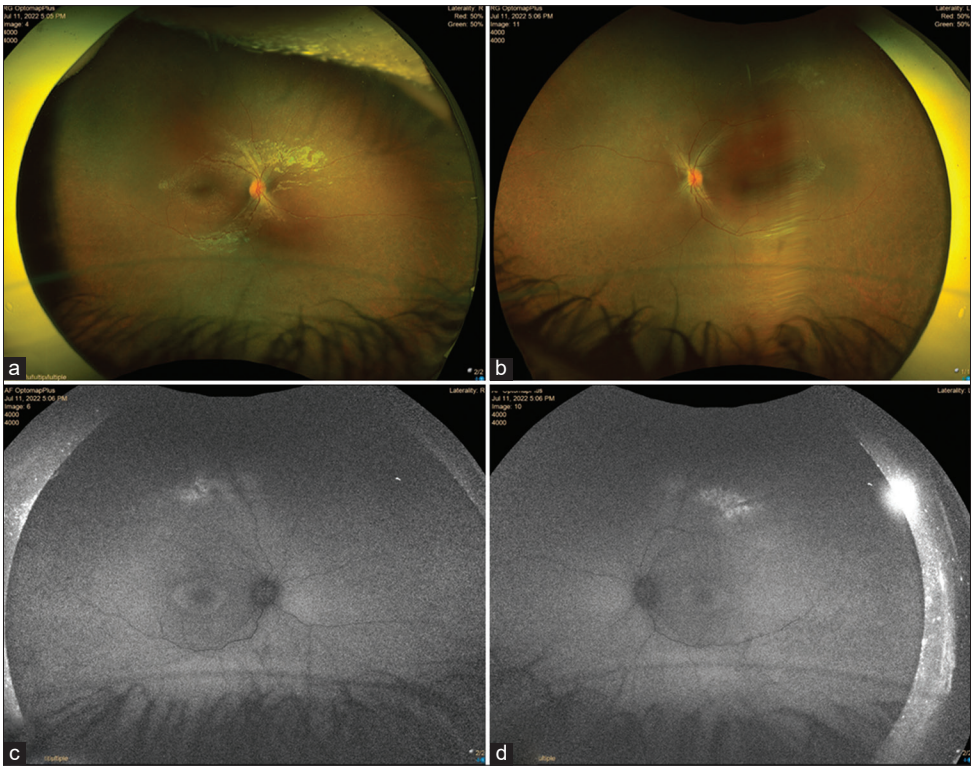

Figure 4: Case 24 with attenuated vessels and RPE changes (a: right eye and b: left eye). Autofluorescence showing hyperautofluorescent ring (c: right eye and d: left eye). The child on genetic testing was found to be positive for *SPATA7* mutations (homozygous: c.288T>A/p.Cys96*)

the world were seen less often in our cohort with only two patients having mutations in *CEP290*.[10–12] Another Indian study on a large cohort of patients noted that *GUCY2D* LCA is the most common, similar to our study.[17] Correlating with the clinical picture present in this subset, all the patients had severely diminished vision at presentation. LCA cases associated with *GUCY2D* mutations typically display early-life relatively stationary but extremely poor vision, characterized by nystagmoid eye movements, oculodigital signs, and pronounced photophobia.[9,28] Out of the seven patients with *GUCY2D* mutation in our study, five patients had RPE changes ranging from RPE granularity to mottling, and two of the patients had a normal-looking fundus [Table 1]. In the study by Srikrupa *et al.*,[17] patients with *GUCY2D* mutations showed minimal peripheral granularity. Among our patients with *GUCY2D* mutations, five underwent vision therapy/stimulation exercises, speech therapy, and skill training at the hospital-approved rehabilitation center.

The second most prevalent mutation in our cohort was in *CRB1* (14%, 5/35). Three patients with *CRB1* LCA had the characteristic phenotype of round-shaped pigmentation with macular involvement [Fig. 1] and paravascular sparing of RPE. These features are typical of *CRB1* which is well known in literature.[17,29,30] Apart from this, we observed macular involvement in *NMNAT1*, *LCA5*, and *RDH12*. The cohort of individual genes is very low to define a common feature in our series.

We did not find any specific phenotype in *RPGRIP1* LCA. Four patients with *RPE 65* mutations are part of another study from our group "*RPE65* mutations in LCA, early-onset severe retinal dystrophy, and retinitis pigmentosa from a tertiary eye care centre in India."[31] Hence the information regarding *RPE65* mutations is limited in the current manuscript to avoid repetition. Biallelic *RPE65* mutations were the third most common causative for LCA in our cohort. Identifying *RPE65* mutations in the current era of gene therapy is very crucial for early patient identification and appropriate therapy. One of the study patients with *AIPL1* mutation had mild RPE changes but a completely extinguished ERG [Fig. 3]. This underscores the pivotal role of ERG as an essential tool, even in cases where the fundus appears nearly normal, but with markedly compromised vision indicating a structure-function dissociation [Figs. 3 and 4].

There were 829 patients tagged with a diagnostic code of LCA during the study period of 6 years. In our study, 35/829 (4.22%) patients or guardians bore the cost of genetic testing. The low percentage highlights the challenges in affordability of the genetic tests in our country. The diagnosis of 829 cases is by multiple physicians without a uniform criterion. Also, the number 829 could include a few cases that might not be LCA genetically or of a different diagnosis or patients who underwent genetic testing on a research basis or outside our care. The number 829 is also high for a study duration of 6 years as ours is a tertiary referral institute. As we have not reviewed all 829 cases completely, there could be a marginal underestimate or overestimate of the total number of patients undergoing genetic analysis. Genetic testing expenses have come down significantly due to increased knowledge, demand, and awareness among patients and clinicians. Despite this reduction, affordability remains an issue for many patients in developing countries. This emphasizes the need for clinicians to collaborate with certified genetic labs, focusing on narrower gene panels and cost-reduction

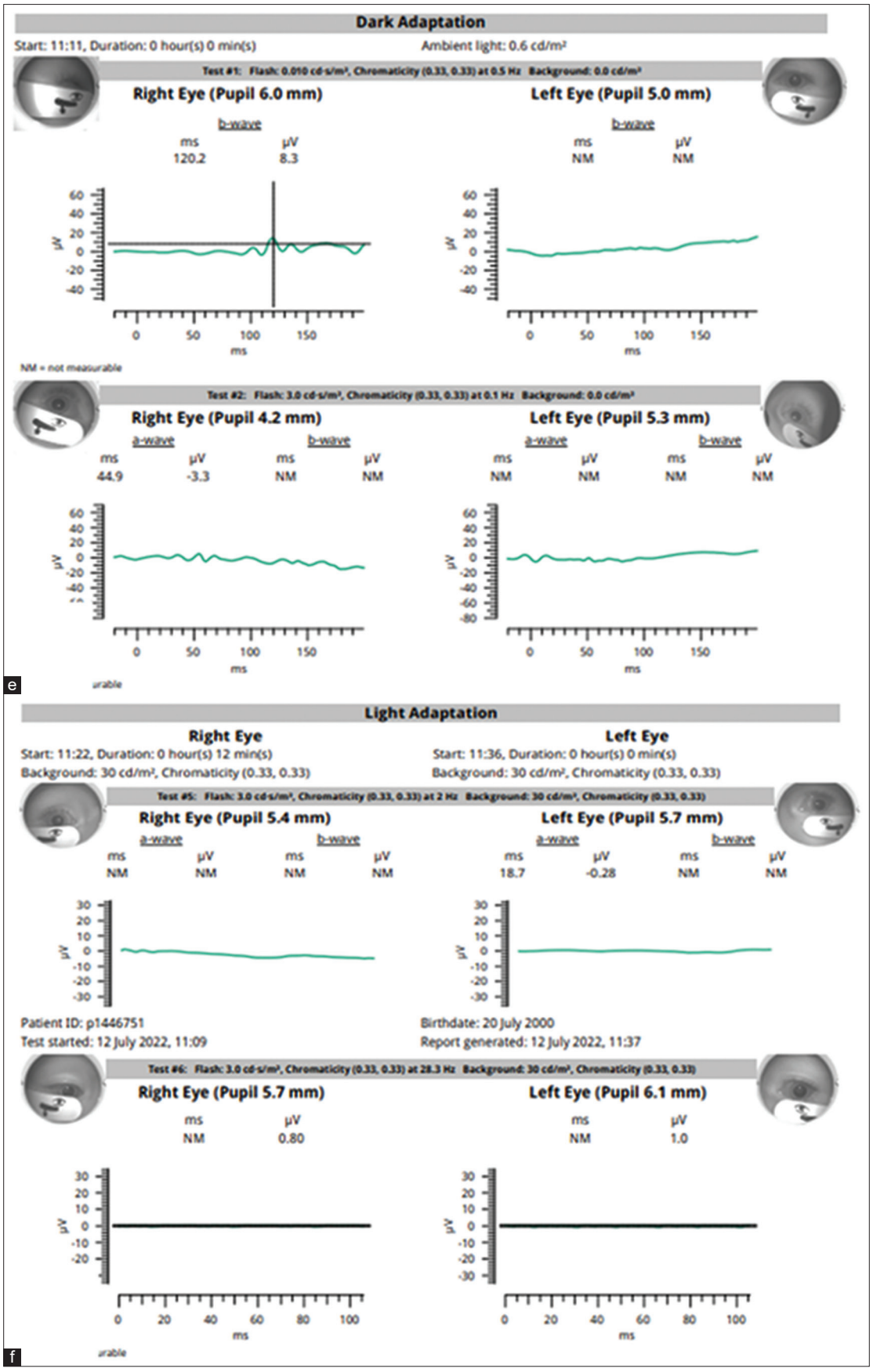

Figure 4: Contd... Case 24 with attenuated vessels and RPE changes Electroretinogram showing non-detectable scotopic (e) and photopic (f) responses. The child on genetic testing was found to be positive for *SPATA7* mutations (homozygous: c.288T>A/p.Cys96*)

strategies to enhance accessibility. Genotyping retinal/ocular disorders in developing countries faces numerous challenges, including inadequate infrastructure, limited access to genetic testing platforms, high testing costs, and a shortage of proficient genetic counselors/geneticists to validate test results.[27] The challenges faced at the regional group level include individualizing patterns of genetic defects in different ethnicities, and training ophthalmologists and geneticists.[14]

Our study has several limitations, primarily stemming from its retrospective design from tertiary care, lack of fundus photos in all, genetic testing from multiple laboratories, absence of family member evaluations along with segregation analysis of parents, especially in compound heterozygous variants, and lack of complete validation of VUSs. The study is limited to patients who could afford the genetic testing which might affect the overall proportion of mutations found. However, its strength lies in the extensive cohort of LCA patients from India. We attempted to correlate fundus features with genetic variants, while also addressing the issue of cost affordability.

Conclusion

Our study identified prevalent genes associated with the LCA cohort that afforded genetic testing in southern India: *GUCY2D*, *CRB1*, *RPE65*, and *RPGRIP1*. Autosomal recessive inheritance was the most common pattern observed. While fundus features were diverse (except *CRB1*), all patients exhibited severe vision impairment, emphasizing the narrow window for gene or cell replacement therapies. Notably, five patients displayed nearly normal fundus appearance yet had profound vision loss and undetectable ERG, underscoring ERG's role in LCA assessment. These five patients with structure–function dissociation can be good candidates for exploring therapies. Over 6 years, the cost of genetic testing exhibited a decreasing trend, prompting the need for proactive collaboration with genetic laboratories and expanded testing efforts.

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Conflicts of interest: There are no conflicts of interest.

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*Refer to Supplementary Table 1 for clinical details and genetic variants of all the patients.

Supplementary Table 1: Highlighting the clinical details and genetic variants found in all patients

Case-Age at presentation/ Gender	Presenting visual acuity and fundus features	Zygosity; Inheritance pattern	Genomic position variant change and Exon position	Type of variant change	Variant Classification (ACMG* nomenclature)
		GUCY2D			
1–9 m/M	†BE – Fixing and following light Fundus – Mild pallor of optic disc with §RPE changes	Homozygous Autosomal Recessive	c.2213_2215delAAG (p.Glu738del) Exon 11	Deletion	Pathogenic (PS4, PM2, PM4, PP4, PP5)
2–7 m/M	†BE – No dazzle reflex for light Fundus – §RPE changes	Homozygous Autosomal Recessive	c.1654dup (p.Ile552Asnfs*5) Exon 7	Duplication (Frameshift and nonsense)	Pathogenic (PVS1, PM2, PP4)
3–2 y/F	†BE – Not fixating on light Fundus – §RPE changes	Heterozygous Autosomal Recessive	c.3085C>A (p.Arg1029Ser) Exon 17	Missense	Variant of unknown significance (PM2)
4–1 y/M	†BE – Fixing and following light Fundus – Mild pallor of optic disc with attenuated vessels and §RPE changes	Homozygous Autosomal Recessive	c.582G>T (p.Trp194Cys) Exon 2	Missense	Variant of unknown significance (PM2)
5–12 y/F	†BE – ‡HM Fundus – Attenuated vessels and §RPE changes	Homozygous Autosomal Recessive	c.2197G>A (p.Ala733Thr) Exon 11	Missense	Variant of unknown significance (PM2)
6–3 m/F	†BE – No dazzle reflex for light Fundus – Normal looking clinically	Homozygous Autosomal Recessive	c.1918T>C (p.Trp640Arg) Exon 9	Missense	Variant of unknown significance (PM2, PM5)
7–5 y/F	Visual acuity – †BE – Not fixating on light Fundus – Normal looking clinically	Homozygous Autosomal Recessive	c.2132C>A (p.Pro711Gln) Exon 11	Missense	Variant of unknown significance (PM2, PP3)
		CRB 1			
8–1 y/F	†BE – Fixing and following light Fundus imaging – §Yellow RPE changes with round-shaped pigmentation in the macula	Homozygous Autosomal Recessive	c.2230C>T (p.Arg744*) Exon 7	Nonsense	Pathogenic (PVS1, PS4, PM2, PP5)
9–7 m/M	Visual acuity - †BE - Fixing and following light Fundus – Normal looking clinically	Homozygous Autosomal Recessive	c.3152G>A (p.Trp1051*) Exon 9	Nonsense	Pathogenic (PVS1, PS4, PM2, P5)
10–3 y/F	†BE – 20/260 TAC at 55cm Fundus imaging – §RPE changes with round-shaped pigment clumps in the macula and paravascular sparing of RPE. FAF – Paravascular hypoautofluorescence and macular hypoautofluorescence corresponding to pigments	Compound Heterozygous Autosomal Recessive	1. c18_21delTAAC (p.Asn7Thrfs*15) Exon 1 2. c.1651C>T (p.Gln551*) Exon 6	1. Deletion (Frameshift & nonsense) 2. Nonsense	Likely pathogenic Pathogenic (PVS1, PM2, PP5)
11–6 m/F	†BE – Fixing and following light Fundus imaging – Round-shaped pigment clumps, macular scar with atrophy OCT thickened retina with vitreoretinal interface abnormalities	Homozygous Autosomal Recessive	c.2194_2195insG CTATGGAGACACC ATCAGCCTCTCCA TGTTTGTCCGAAC (p.Gln747Trpfs*56) Exon 7	Insertion (Frameshift and nonsense)	Likely pathogenic
12–1 y/F	†BE – Not Fixing and following light Fundus – Normal looking clinically	Heterozygous Autosomal Recessive, Autosomal dominant	c.2434C>G (p.Gln812Glu) Exon 7	Missense	Variant of unknown significance/ (PM2, PP2)
		RPGRIP1			
13–3 y/F	†BE – ¶PL, #PR accurate Fundus imaging – §RPE changes, bony spicules and attenuated vessels	Homozygous Autosomal Recessive	c.2668C>T (P.Arg890*) Exon 16	Nonsense	Pathogenic (PVS1, PM2, PM3, PP4, PP5)

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Supplementary Table 1: Contd...

Case-Age at presentation/ Gender	Presenting visual acuity and fundus features	Zygosity; Inheritance pattern	Genomic position variant change and Exon position	Type of variant change	Variant Classification (ACMG* nomenclature)
	OCT parafoveal outer retinal loss with central sparing				
14–5 m/M	Visual Acuity – †BE – Not fixing light Fundus photos Minimal §RPE changes	Homozygous Autosomal Recessive	c.1250_1251insG (p.Asp418Glyfs*3) Exon 11	Insertion (Frameshift and nonsense)	Pathogenic (PVS1, PM2, PP4)
15–8 y/F	†BE – ¶PL, #PR accurate Fundus – Diffuse §RPE changes	Homozygous Autosomal Recessive	c.930+3A>G (5' splice site proximal) Intron 7	Intronic	Variant of unknown significance/Benign (PP3, PP4/BS1, BS2, BP6, BA1) (This variant can be deleterious by predictive tools but it is also present in common population, hence this is unclear at this stage – needs validation)
16–8 y/M	Visual Acuity – †BE – ¶PL Presenting complaints – Nystagmoid eye movements Fundus imaging – Diffuse §RPE degeneration changes from the vascular arcades and beyond with chorioretinal atrophic patches	Homozygous Autosomal Recessive	c.2171T>A (p.Val724Glu) Exon 14	Missense	Variant of unknown significance (PM2, PP3)
		LCA5			
17–2 y/F	†BE – Fixing and following light Fundus – Mild pallor of optic disc with attenuated vessels and §RPE changes	Homozygous Autosomal Recessive	c.1151delC (p.Pro384Glnfs*18) Exon 8	Deletion (Frameshift and nonsense)	Pathogenic, (PVS1, PM2, PP5, PP4)
18–3 y/F	†BE – Fixing and following light Presenting complaints – Nyctalopia, Nystagmoid eye movements, strabismus Fundus – Optic disc drusen, §RPE changes and macular pigmentation	Compound Heterozygous Autosomal Recessive	c.1A>G (P.MET1?) Exon 8 c.1105C>T (p.Gln369*) Exon 7	Missense Nonsense	Pathogenic (PVS1, PS1, PM2, PM3, PP5) Pathogenic/PVS1, PM2, PP4
19–8 m/F	†BE – Fixing and following light Presenting complaints – Nystagmoid eye movements, poor eye contact Fundus – Normal looking clinically	Homozygous Autosomal Recessive	c.536_537delAA (p.Gln179Argfs*7) Exon 8	Deletion (Frameshift and nonsense)	Pathogenic (PVS1, PM2, PP4, PP5)
		AIPL1			
20–3 y/F	†BE – 20/380 TAC at 55 cm Fundus – Mild optic disc pallor with attenuated vessels	Homozygous Autosomal Recessive	c.834G>A (p.Trp278*) Exon 6	Nonsense	Pathogenic (PVS1, PS3, PM2, PM3, PS4, PP1, PP4, PP5)
21–2 y/F	†BE - Fixing and following light Fundus imaging - Minimal §RPE changes and attenuated vessels	Homozygous Autosomal Recessive	c.733_735delGAG (p.Glu245del) Exon 5	Deletion (Inframe)	Variant of unknown significance (PM2, PM4)
22–4 m/F	†BE – Fixing and following light Presenting complaints – Difficulty in making eye contact Fundus imaging – Minimal §RPE changes FAF – parafoveal and mid-peripheral hyperautofluorescent ring	Homozygous Autosomal Recessive	c.809G>T (p.Arg270Leu) Exon 6	Missense	Variant of unknown significance (PM2, PP3)
		SPATA 7			
23–3 m/F	†BE – Not fixating on light Fundus imaging –§RPE changes and attenuated vessels	Homozygous Autosomal Recessive	c.960dupA (p.Pro321Thrfs*6) Exon8	Duplication (frameshift and nonsense)	Pathogenic (PVS1, PM2, PM3, PP4, PP5)

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Supplementary Table 1: Contd...

Case-Age at presentation/ Gender	Presenting visual acuity and fundus features	Zygosity; Inheritance pattern	Genomic position variant change and Exon position	Type of variant change	Variant Classification (ACMG* nomenclature)
24–2 y/M	†BE – Fixing and following light Fundus imaging –§RPE changes and attenuated vessels FAF – Parafoveal hyperautofluorescent ring	Homozygous Autosomal Recessive	c.288T>A (p.Cys96*) Exon 5	Nonsense	Pathogenic (PVS1, PM2, PM3, PP4, PP5)
		NMNAT1			
25–7 m/M	†BE - Fixing and following light Fundus – Pale disc with §RPE changes in background retina and macula	Homozygous Autosomal Recessive	c.838T>C (p.Ter280Gln) Exon 5	Stop loss	Likely pathogenic (PM2, PM4, PP4, PP5)
26–5 m/M	†BE - No dazzle reflex for light Fundus – §RPE changes with macular atrophy	Homozygous Autosomal Recessive	c.25G>A (p.Val9Met) Exon 2	Missense	Pathogenic (PS3, PM2, PM3, PP1, PP2, PP3, PP5)
		CEP290			
27–6 y/F	†RE – 20/500, LE -20/600 Fundus – Pale disc with §RPE changes	Homozygous Autosomal Recessive	c.2483G>T (p.Ser828Ile) Exon 23	Missense	Variant of unknown significance (PM2, PP3)
28–1 y/M	†BE – Fixing and following light Fundus – Minimal §RPE changes	Compound Heterozygous Autosomal Recessive	c.5445_5448delAACT (p.Thr1816Ilefs*3) Exon 40 c.451C>T (p.Arg151*) Exon 7	Deletion (frameshift and nonsense) Nonsense	Pathogenic (PVS1, PM2, PM3, PP5) Pathogenic (PVS1, PM2, PM3, PP5)
		PRPH 2			
29–7 m/F	†BE – Fixing and following light Fundus – §RPE changes	Homozygous Autosomal Recessive	c.818G>A (p.Trp273*) Exon 2	Nonsense	Pathogenic (PVS1, PM2, PP4)
		RDH12			
30–5 y/M	†BE – 20/100 Fundus imaging – Pallor of optic disc, bony spicules with macular pseudocoloboma, and pigmentation OCT outer retinal and choroidal thinning with posterior bowing corresponding to pseudocoloboma picture	Homozygous Autosomal Recessive	c.505C>T (p.Arg169Trp) Exon 7	Missense	Pathogenic (PS4, PM1, PM2, PM3, PP2, PP3, PP4, PP5)
		IMPDH1			
31-5y/F	†BE – Fixing and following light Fundus imaging – §RPE changes as fine yellow dots with attenuated vessels	Heterozygous Autosomal Dominant	c.1363G>A (p.Val455Met) Exon 13	Missense	Variant of unknown significance (PM2, PP3, PP4)

M: male, Female, OCT: Optical coherence tomography, FAF: Fundus autofluorescence. *ACMG-AMP: American College of Medical Genetics and Genomics and Association for Molecular Pathology (PM – Pathogenic moderate, PP – Pathogenic Supporting, PVS – Pathogenic Very Strong, PS – Pathogenic Strong, BP – Benign Supporting, BS – Benign Strong, BA- Benign alone), †BE: Both eyes, ‡HM: Hand motion appreciation,§RPE: Retinal Pigment Epithelium, ||CF CF: Counting fingers close to face, ¶PL: Perception of light, #PR: Projection of rays, m: Months, y: years, M: Male, F: Female, TAC: Teller Acuity Charts. *The ACMG (American College of Medical Genetics and Genomics) and Association for Molecular Pathology (ACMG-AMP) is the most updated standard criteria used to classify the pathogenicity evidence for any genetic variants.(Reference 24) The mutational change is weighted as very strong (PVS1), strong (PS1–4), moderate (PM1–6), supporting (PP1–5), benign stand-alone (BA1), benign strong (BS1–4) or benign supporting (BP1–6). The numbering refers to different criteria (tables 3 & 4 of reference). The variant is labelled with one or more of the above notation resulting in a combined score. The combined score is classified by using table 5 in reference to understand the pathogenicity. *Four patients previously published had biallelic *RPE65* pathogenic mutations. (Reference 30)