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Genetics and phenotypes of *RPE65* mutations in inherited retinal degeneration: A study from a tertiary eye care center in Brazil

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Purpose: Biallelic variants in the retinal pigment epithelium–specific 65-kDa protein (*RPE65*) gene are linked to several inherited retinal diseases (IRDs), including Leber congenital amaurosis (LCA), early-onset severe retinal dystrophy (EOSRD), and retinitis pigmentosa (RP). This study screened patients from a tertiary center in Brazil with IRDs for *RPE65* variants to characterize the associated phenotypes.

Methods: LCA, EOSRD, and RP diagnoses were based on predefined clinical criteria. Patients underwent comprehensive clinical evaluations and retinal imaging. Genomic DNA was analyzed using a next-generation sequencing panel for IRDs, covering 238 genes.

Results: *RPE65* variants were identified in seven of the 68 patients screened. Of these, three were homozygous, and four were compound heterozygous for the identified mutant alleles. A total of six variants were detected, of which one was novel. The p.Leu341Ser (c.1022T>C) mutation was the most prevalent, being found in four of seven patients. Visual loss onset ranged from birth to the third decade of life. A consistent clinical feature observed in all patients was some degree of pigmentary change upon peripheral retinal examination.

Conclusions: *RPE65* variants were found in 10.3% of cases in this series, associated with LCA, EOSRD, and RP. These variants were consistently linked with pigmentary changes in the peripheral retina and exhibited variable manifestations regarding arteriolar attenuation, disc pallor, and macular appearance. In this series, the prevalence of the p.Leu341Ser (c.1022T>C) mutation was 57%.

Inherited retinal dystrophies (IRDs) encompass a diverse group of progressive diseases that severely impact vision. Among the various phenotypes within IRDs, Leber congenital amaurosis (LCA) and retinitis pigmentosa (RP) represent a spectrum associated with significant visual impairment. LCA, first described by Theodore Leber in 1869, refers to severe recessively inherited early-onset rod-cone dystrophy. A milder form, early-onset severe retinal dystrophy (EOSRD), was identified by Leber in 1916 [1]. LCA typically presents at birth or within the first year, characterized by nystagmus, photophobia, and severely impaired rod-cone responses on electroretinogram (ERG) [2,3]. EOSRD manifests within the first five years of life, characterized by some preservation of vision and minimal ERG responses [1]. RP generally appears in the first or second decade of life, with progressive visual loss affecting both peripheral and central vision [4]. These disorders exhibit significant genetic heterogeneity across populations, with a mutation in the *RPE65* gene being one of the possible causes [2,5,6]. Over time, *RPE65* mutations associated with LCA, EOSRD, and RP lead to loss of light perception at any intensity along with a profound reduction in navigational vision [7].

The *RPE65* gene, located on chromosome 1p31, encodes a 65-kDa retinoid isomerase enzyme essential for vitamin A metabolism in the retinoid cycle [8]. The prevalence of *RPE65*-associated LCA varies globally, ranging from 1% to 20%, while *RPE65*-associated RP is found in less than 1% to 4% of cases [9]. In a Brazilian study, the *RPE65* gene accounted for 16 of 78 LCA cases (20.5%), with no *RPE65* mutations identified in patients with RP [10]. Understanding the genetic burden of isolated mutations is crucial for identifying candidates for emerging therapies such as gene replacement using AAV-*RPE65* constructs [11]. This study investigates *RPE65* mutations in patients with IRD phenotypes, including LCA, EOSRD, and RP, at our institution and describes the associated clinical features.

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METHODS

Patient recruitment and screening: This study adhered to the Declaration of Helsinki and received approval from the local ethics committee of Ezequiel Dias Foundation (No. 53981621.1.3001.9507). Informed consent was obtained from all patients or their legal representatives. Patients with LCA, EOSRD, and RP were recruited from the Retina Department of São Geraldo Hospital, Minas Gerais, Brazil, which is a tertiary reference center for the whole state. They underwent a comprehensive ophthalmic evaluation and retinal imaging, including fundus photography (Optos Daytona, Optos, Dunfermline, Scotland or Topcon, Tokyo, Japan), fundus autofluorescence (Optos), optical coherence tomography (OCT; Heidelberg, Germany), and full-field electroretinography (Roland Consult, Brandenburg, Germany) consistent with the International Society for Clinical Electrophysiology of Vision standards.

Diagnoses were based on clinical criteria of LCA, EOSRD, and RP. LCA was diagnosed with visual loss from birth or within the first year, presenting nystagmus or absence of fixation and poor pupillary light reflex. Fundus appearance can range from normal, pseudopapillary edema, maculopathy, depigmented fundus, peripheral pigmentary retinopathy to typical RP-like abnormalities [12]. EOSRD is considered a milder form of LCA yet severe when diagnosed between one and five years of age. RP is diagnosed when onset occurs after age five years. Its initial symptom is reduced night vision, which is followed by a progressive loss of the visual field in a concentric pattern. Function at the macula is usually relatively well preserved until later stages of the disease. Fundus abnormalities typically include bone spicule pigmentation predominantly in the periphery, attenuation of retinal vessels, and a pallor of the optic nerve head [1,2,4,13]. The study sample, totaling 68 individuals, was screened based on patients followed by the Retina Department who met the diagnostic criteria mentioned above (Appendix 1). Syndromic patients were not included. Saliva samples were collected for genomic DNA extraction. Mutations were identified using a next-generation sequencing panel targeting 238 genes associated with IRDs.

RESULTS

Case reports: A total of 68 patients were screened from June 2022 to May 2023. *RPE65* variants were identified in seven patients. Another 29 variants were also identified, with greater prevalence in *RHO* (eight patients), *RPGR* (six patients), *CRB1* (four patients), *USH2A* (four patients), and *EYS* (three patients).

Of the *RPE65* mutations, three were homozygous, and four were compound heterozygous for the identified mutant alleles. We detected six variants; one variant was novel. The p.Leu341Ser (c.1022T>C) mutation was the most prevalent in four of seven patients. The patients' clinical features and genetic mutations are summarized in Table 1, and their pedigrees are shown in Figure 1. The following is an in-depth account of the cases that had the *RPE65* mutation in this study.

Cases 1, 2, and 3: c.1022T>C; p.(Leu341Ser): Three male siblings, aged 25, eight, and three years, were referred to the Retina Department presenting vision loss. They were children of a nonconsanguineous Caucasian couple, with consanguinity reported only in the maternal family. Sanger sequencing confirmed the homozygous mutation in the patients and heterozygous status in both parents.

The 25-year-old sibling had a history of nystagmus and difficulty in fixing and following light since age three months (Table 1). Fundus examination showed diffuse mottling of the retinal pigment epithelium (RPE), vascular attenuation, optic disc pallor, and macular atrophy in both eyes (OU). OCT showed severe outer retinal thinning and loss of the ellipsoid zone in OU (Figure 2).

The eight-year-old sibling had diminished vision, as noted by his parents since birth. He also presented squint from two years old and difficulty with night vision since four years old (Table 1). The retinal signs included subtle granular retinal pigment epithelial degeneration in the retina. The OCT of OU demonstrated sparse disruptions in the ellipsoidal zone (Figure 2).

The younger brother, at age three years, had a history of diminished vision, as noted by the parents since the age of one month and nystagmus that began at age one year (Table 1). At the current fundus examination, he had pigmentary mottling in the background retina. Despite the limitations imposed by age and low vision, it was possible to obtain OCT images that showed apparently preserved retinal thickness in the macula (Figure 2).

Case 4: c.1022 T>C;p.(Leu341Ser) and c.560G>A;p.(Gly187Glu): A 20-year-old woman presented vision loss and nystagmus at the clinical examination, noted by the parents since one year old, as well as diminished vision and light sensitivity since the age of two years (Table 1). Fundus examination showed severe central macular atrophy, optic disc pallor, vascular attenuation, and diffuse RPE mottling. OCT revealed extensive retinal thinning and photoreceptor loss. Full-field ERG showed minimal rod and cone responses

Table 1. Mutations and clinical features of patients with RPE 65-associated retinal dystrophy.

Patient	Gender	Genetic variant details	Consanguinity in parents	Age and mode at onset of signals and symptoms	Current age and signals and symptoms	Current Best corrected visual acuity (OD/OS)	Refractive examination (OD/OS)
1	Male	c.1022T>C; p.(Leu341Ser)	No	3 months of life; Nystagmus and difficulty of fixing and following the light	25 y.o.; Nystagmus, nyctalopia and photophobia	Hand movements; Counting fingers close to the face	+1,50 - 1,00 x 135; +1,50 - 0,50 x 45
2	Male	c.1022T>C; p.(Leu341Ser)	No	Since birth; Decreased vision	8 y.o.; Nyctalopia and Strabismus	20/63; 20/200	+1,50 -1,00 x 170; +1,50 -1,50 x 10
3	Male	c.1022T>C; p.(Leu341Ser)	No	1 month of life; Decreased vision	3 y.o.; Nystagmus and nyctalopia	20/400; 20/400	+6,00 -1,00 x 180; +6,00 - 0,50 x180
4	Female	c.1022 T>C;p.(Leu341Ser) and c.560G>A;p.(Gly187Glu)	No	Close to 2 Years of life; Decreased vision, light sensibility	20 y.o.; Light sensibility	20/250; 20/320	+2,50 -1,00 x 14; +3,00 -1,75 x 175
5	Male	c.370CT>C;p.(Arg124*) and c.1022 T>C;p.(Leu341Ser)	No	3 Years of life.	23 y.o.	20/100; 20/200	-8,50 -2,00 x 15; -7,50 -1,50 x 165
6	Female	c.11+5 G>A and c.1520C>T; p.(Ala507Val)	No	Before 9 year-old; Diminished vision and difficulty with night vision	12 y.o.; Nyctalopia	20/60; 20/60	+0,75 -1,00 x 20; +0,75 -1,00 x 160
7	Female	c.271C>T; p. (Arg91Trp) and c.560G>A; p.(Gly187Glu)	No	The third decade; Nyctalopia	59 y.o.; Nyctalopia	20/60; 20/60	-6,00 -1,00 x160; -5,50 -1,00 x165

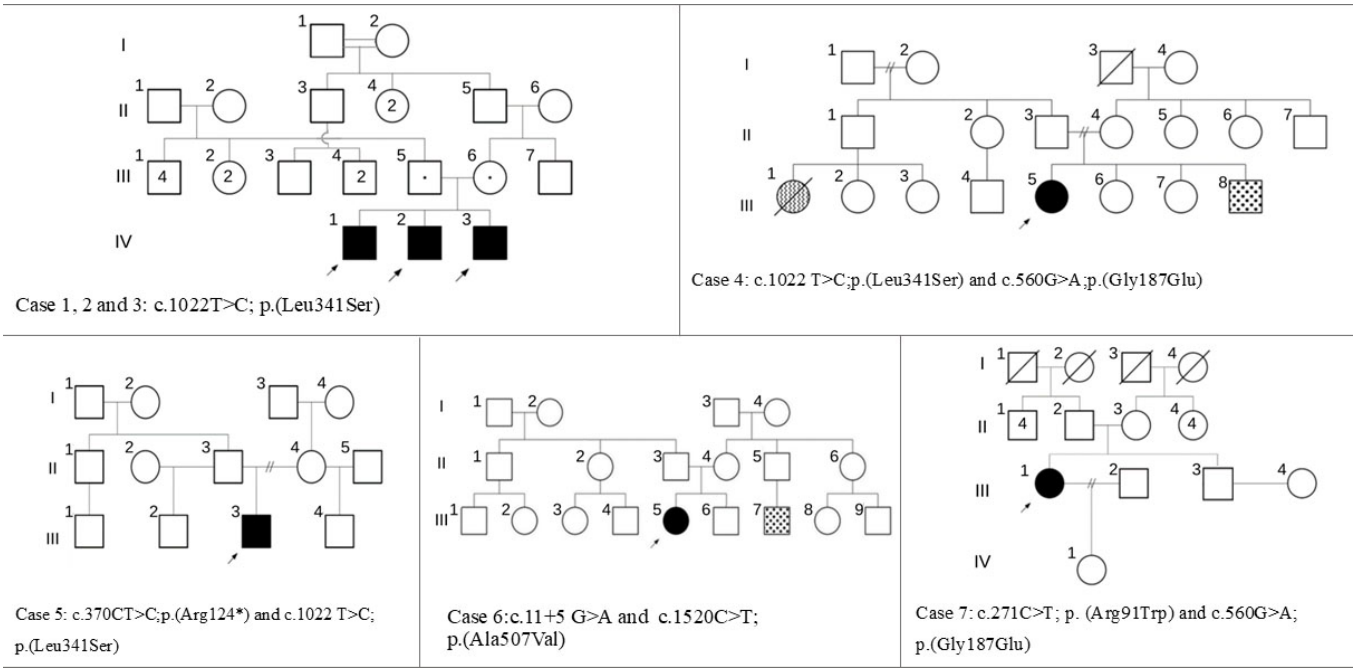

Figure 1. Pedigree information of seven cases with the genetic variant found for *RPE65* gene. The arrow denotes the proband. In case 4, the highlighted pedigree III-1 refers to an individual with intellectual disability, and III-8 with speech delay. In case 6, the highlighted pedigree III-7 refers to an individual with autism.

(Figure 3). The patient denied consanguinity, and no family members were available for testing.

Case 5: c.370CT>C;p.(Arg124) and c.1022 T>C;p.(Leu341Ser):* A 23-year-old male patient reported visual difficulties starting at age three years (Table 1). He also had high myopia and classic RP-like retinal features. OCT showed photoreceptor irregularities and disruptions in the ellipsoidal zone (Figure 4). The parents were nonconsanguineous.

Case 6: c.11+5 G>A (intronic) and c.1520C>T; p.(Ala507Val): A 12-year-old girl presented with reduced vision and nyctalopia since the age of nine years. Fundus examination showed mild retinal changes with preserved subfoveal and perifoveal ellipsoid zones on OCT. The full-field ERG showed reduced rod and cone responses OU (Figure 5). She reported nonconsanguineous parents.

Case 7: c.271C>T; p. (Arg91Trp) and c.560G>A; p.(Gly187Glu): A 59-year-old woman experienced nyctalopia starting in her third decade of life (Table 1). Despite visual acuity preservation (a best-corrected visual acuity of 20/60 in both eyes), she had a severely constricted visual field. Fundus examination revealed diffuse RPE degeneration, vascular attenuation, and optic disc pallor.

Genetic analysis: In cases 1, 2, and 3, the variant p.Leu341Ser (c.1022T>C) was identified, in homozygosity, in the *RPE65* gene. This variant results in a dysfunctional protein product, classified as pathogenic by the in silico functional prediction software dbNSFP [14]. Therefore, it is pathogenic and has also been previously reported in patients with IRDs [15-17]. Molecular analysis confirmed the mutation in homozygosity in the probands and was present in heterozygosity in both parents.

In case 4, the p.Leu341Ser variant described above and the p.Gly187Glu (c.560G>A) mutation were identified in the *RPE65* gene. Based on dbNSFP prediction, the variant p.Gly187Glu (c.560G>A) is pathogenic and has been previously reported [18,19]. Parental genotyping was not performed to define the phase of the variants, and the compound heterozygosity was assumed based on the phenotype [20].

In case 5, the p.Leu341Ser and p.Arg124* variants were found in the *trans* phase, confirmed through parental genotyping. The p.Arg124* variant (c.370C>T) promotes the replacement of arginine in position 124 by a premature stop codon, which leads to premature translation arrest and loss of function. In silico prediction indicates that the premature stop codon most likely induces nonsense-mediated decay of the mRNA product, resulting in severely impaired protein translation or nonfunctional truncated protein. It is pathogenic and has already been published in the medical literature and deposited in the ClinVar variant repository [21].

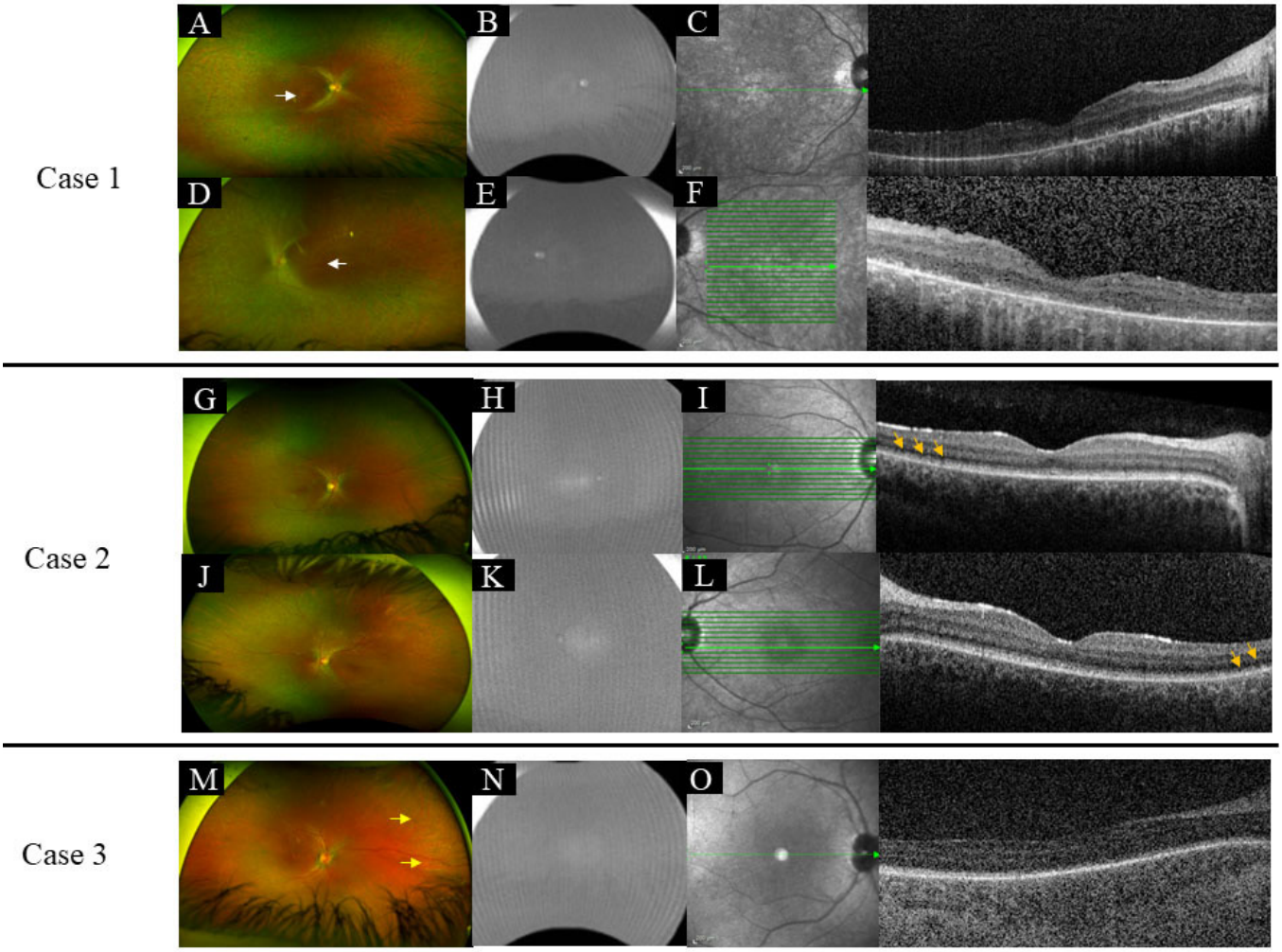

Figure 2. Images of Cases 1, 2 and 3 with *RPE65* gene mutation. Cases 1, 2, and 3 the variant c.1022T>C; p.(Leu341Ser) was identified in the *RPE65* gene. Case 1. Fundus of the 25-year-old sibling shows (**A** and **D**) attenuation of blood vessels, diffuse mottling of the retinal pigment epithelial with subtle pigments, slight pallor of the optic disc, and macular atrophy (highlighted by the white arrows). The fundus autofluorescence of both eyes (OU) shows diffuse background hypo-autofluorescence (**B** and **E**). Optical coherence tomography (OCT) of the OU (**C** and **F**) shows thinning of the outer retina and total loss of the ellipsoid zone of photoreceptors. Case 2. The proband at the age of eight years, with the fundus (**G** and **J**) showing subtle granular retinal pigment epithelium degeneration. The optic discs, the blood vessels, and the macula appear to have no clinically significant change. Fundus autofluorescence of OU presents an insufficient image for analysis of possible retinal changes (**H** and **K**). The OCT (**I** and **L**) shows sparse disruptions in the ellipsoidal zone and outer nuclear membrane (highlighted by the orange arrows). Case 3. The three-year-old sibling with the right eye (OD) fundus presenting pigmentary mottling in the mid-peripheral retina (**M**, highlighted by the yellow arrow) and optic disc, blood vessels, and macula with no notable change. The fundus autofluorescence shows an insufficient image for analysis (**N**). The OCT (**O**) shows no changes in retinal thickness.

Two heterozygous variants were identified in the *RPE65* gene in case 6: the c.11+5G>A and the p.Ala507Val (c.1520C>T). The c.11+5G>A variant presents an alteration in the splice donor site in intron 1, predicted to be a noncoding protein variant due to its splicing effect by dbNSFP. It is pathogenic and has already been described in the medical literature [22,23]. The p.Ala507Val variant has not been published in the medical literature or deposited in the ClinVar repository. In silico predictors suggest that this replacement is deleterious. Genotyping of the parents was performed, and it was identified that the variants are in compound heterozygosity.

Case 7 presented the p.Gly187Glu variant, already related in case 4, and p.Arg91Trp. This variant is pathogenic and has already been described in the literature, including functional studies demonstrating impaired RPE65 protein activity and reduced protein expression despite normal mRNA expression,

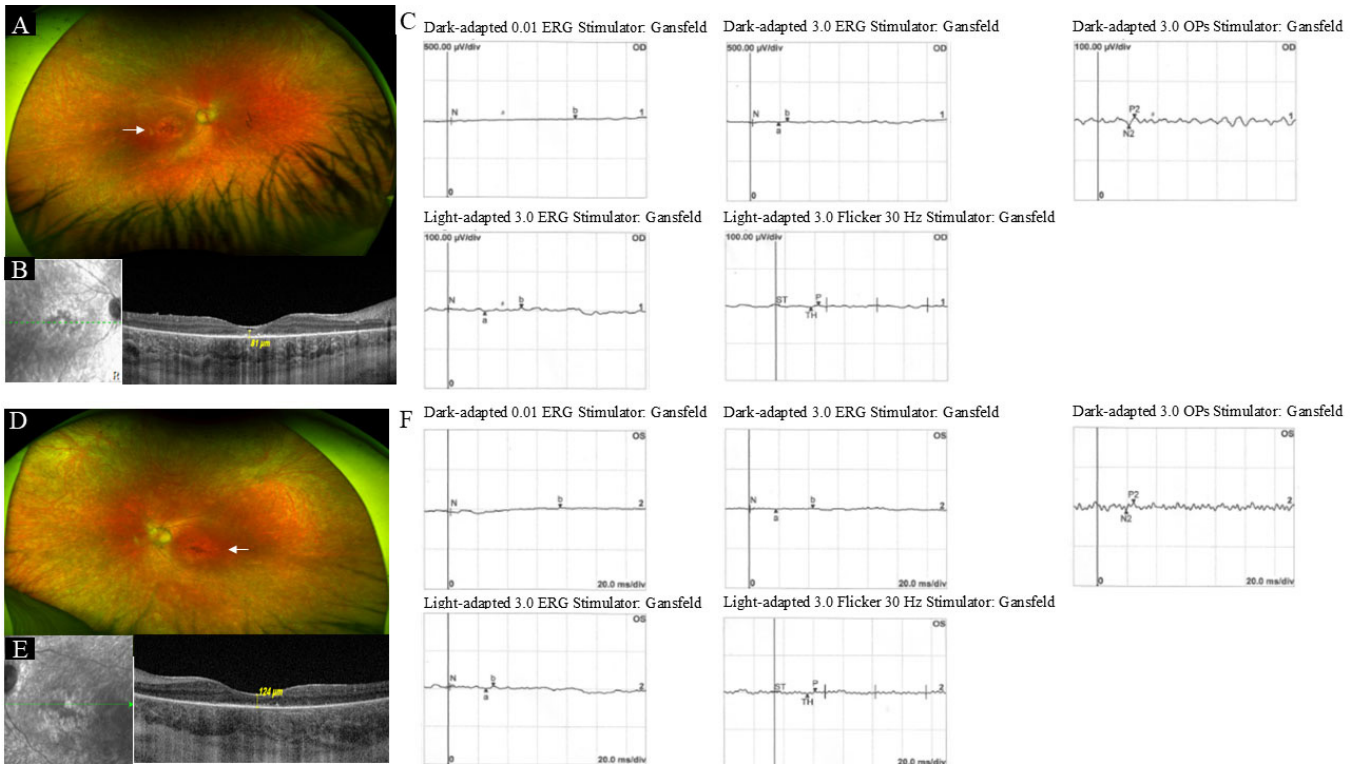

Figure 3. Images and full-field electroretinogram of Case 4 with *RPE65* gene mutation. Case 4 shows a proband with mutation c.1022 T>C;p.(Leu341Ser) and c.560G>A;p.(Gly187Glu) in the *RPE65* gene at the age of 20 years with fundus (**A** and **D**) showing severe central macular atrophy (highlighted by the white arrows), slight pallor of the optic disc, attenuation of blood vessels, and diffuse retinal pigment epithelium mottling degeneration. Optical coherence tomography shows severe overall retinal thinning and photoreceptor loss in the macula (**B** and **E**). The full-field electroretinogram shows minimal rod and cone responses in right and left eyes (**C** and **F**).

suggesting that this mutation reduces translation efficiency or decreases protein stability, causing protein degradation [16,24-28]. Parental genotyping was performed, confirming the *trans* phase of the variants.

DISCUSSION

In this study, we present the genetic and clinical analysis of seven patients with IRDs in whom mutations in the *RPE65* gene were identified, including three patients with LCA (cases 1, 2, and 3), two patients with EOSRD (cases 4 and 5), and two patients with RP (cases 6 and 7). The phenotype-genotype correlation of *RPE65* mutations has been extensively explored in previous studies, involving patients from different countries [2,18,29-31]. However, not many studies involving the Brazilian population are reported. This study includes a cohort from a tertiary center for the state of Minas Gerais, a reference hospital for IRDs that supports the 853 cities from the state [17,32]. These mutations not only result in substantial visual impairment but are also the target of gene therapy. A deeper understanding of their impact on the eye can help optimize the timing of treatment.

The genetic analysis of these patients showed that three had a homozygous biallelic mutation, while four had variants for which the *cis* or *trans* configurations were unclear. For three of these four patients, further studies confirmed compound heterozygosity. In one case, parental testing was not possible, but based on previous disease characterization, the authors assumed it is also compound heterozygosity. Among the six mutations identified in the reported cases, one involved premature protein termination, one was intronic, and five were missense mutations. One missense variant was novel (Table 1). Regardless of the different mutations in the *RPE65* gene described above, all seven patients had significant visual impairment.

Regarding the prevalence of mutations, four of the seven patients carried the p.Leu341Ser (c.1022T>C) variant. Although it is not often identified in other studies, this variant was a significant finding in our study, being associated with LCA and EOSRD [15,18,29,30,33].

None of the patients were born from consanguineous marriages (Figure 1). The most consistent clinical feature observed in all patients was some degree of pigmentary

change upon peripheral retinal examination. While vascular attenuation and optic disc pallor were prevalent, they were not universal, possibly due to differences in genetic mutations and age at presentation. Notable variants include c.271C>T; p.(Arg91Trp) and c.560G>A; p.(Gly187Glu) in patient 7, who, at 59 years old, had 20/60 vision in both eyes. Considering her age, this patient has relatively preserved visual acuity compared to others, but her severely compromised visual field underscores the aggressive nature of the *RPE65* mutation.

Our study and other research highlight the association between *RPE65* mutation and disease severity or progression [18,26,34,35]. However, other variables interfere in a genetic manifestation, as seen in the three siblings with the p.Leu341Ser (c.1022T>C) mutation. Despite having the same variant and living in the same environment, their disease manifestations varied. Analyzing visual acuity and OCT data, the eight-year-old sibling appears less affected than the three- and 25-year-old siblings.

Recent advances in gene therapy, including the viral vector–based gene delivery system for *RPE65* (Voretigene Neparvovec; Spark Therapeutics, Philadelphia, PA) approved by the US Food and Drug Administration, offer new treatment possibilities for patients with biallelic *RPE65* variants. Our series of seven cases highlights the prevalence of *RPE65*-related IRDs in our population and documents the associated clinical characteristics. These observations provide valuable insights into the natural history of the disease that helps to identify the possible benefits of this treatment.

Challenges in our setting include limited access to widespread genotyping and genetic counseling. Further functional studies characterizing *RPE65* variants would also be beneficial in understanding the physiologic consequences of each mutation, to clarify genotype-phenotype correlations. A limitation of our study is the variability in patient ages at presentation, ranging from childhood to adulthood, with diagnoses based on vision loss history and various clinical signs. A strength of this study is the inclusion of a series of LCA, EOSRD, and RP cases diagnosed at a single center.

In summary, the *RPE65*-related retinal diseases in our series were characterized by pigmentary retinal changes as a predominant feature, as demonstrated by other studies developed in different ethnic and geographic environments [2,18,30,36]. The frequency of *RPE65* variants in our sample (10.3%) was consistent with those reported in other studies. We found a higher prevalence of p.Leu341Ser (c.1022T>C) mutation in four of the seven patients (57%) when compared with the literature. Considering recent advances, including a better understanding of the importance of genetic alterations in the pathophysiology of IRDs, we believe that genotyping should be extended to as many patients as possible to identify those who could benefit from future treatments.

APPENDIX 1. SUPPLEMENTAL TABLE 1.

To access the data, click or select the words "Appendix 1." Total mutations distribution among the 68 patients group.

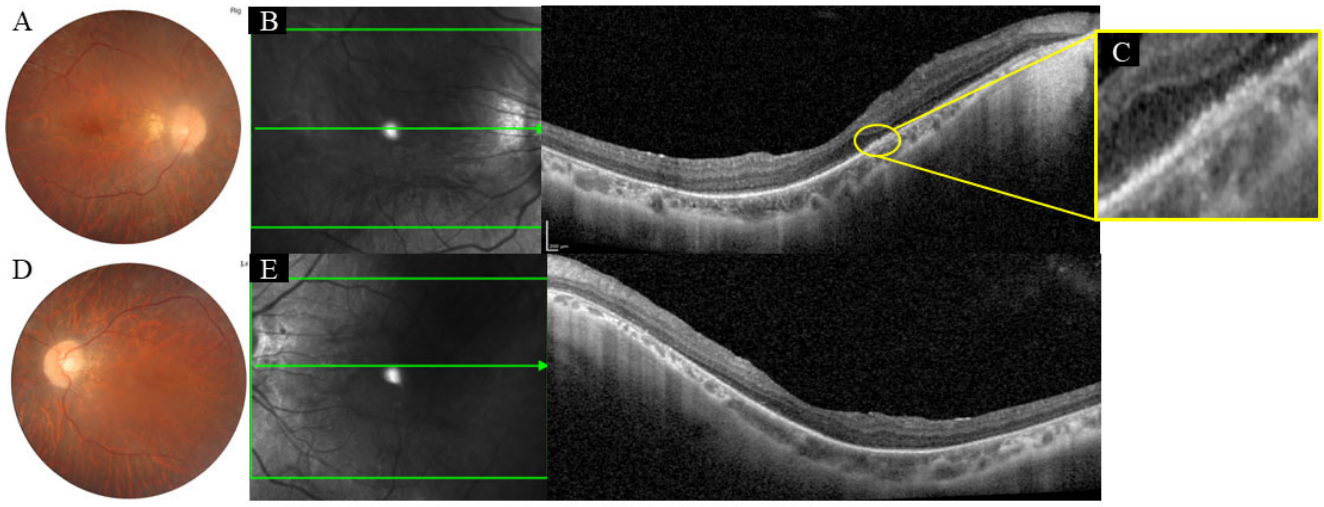

Figure 4. Images of Case 5 with *RPE65* gene mutations. Case 5 with mutation c.370CT>C;p.(Arg124*) and c.1022 T>C;p.(Leu341Ser) at age 23 years with fundus (**A**, **D**) showing classic retinitis pigmentosa–like features, in addition to myopia features such as tilted disc and peripapillary atrophy. Optical coherence tomography of both eyes (**B**, **C**, right eye and **E**, left eye) demonstrates disruptions in the ellipsoidal zone, in addition to abnormal curvature of the eye and thin choroid.

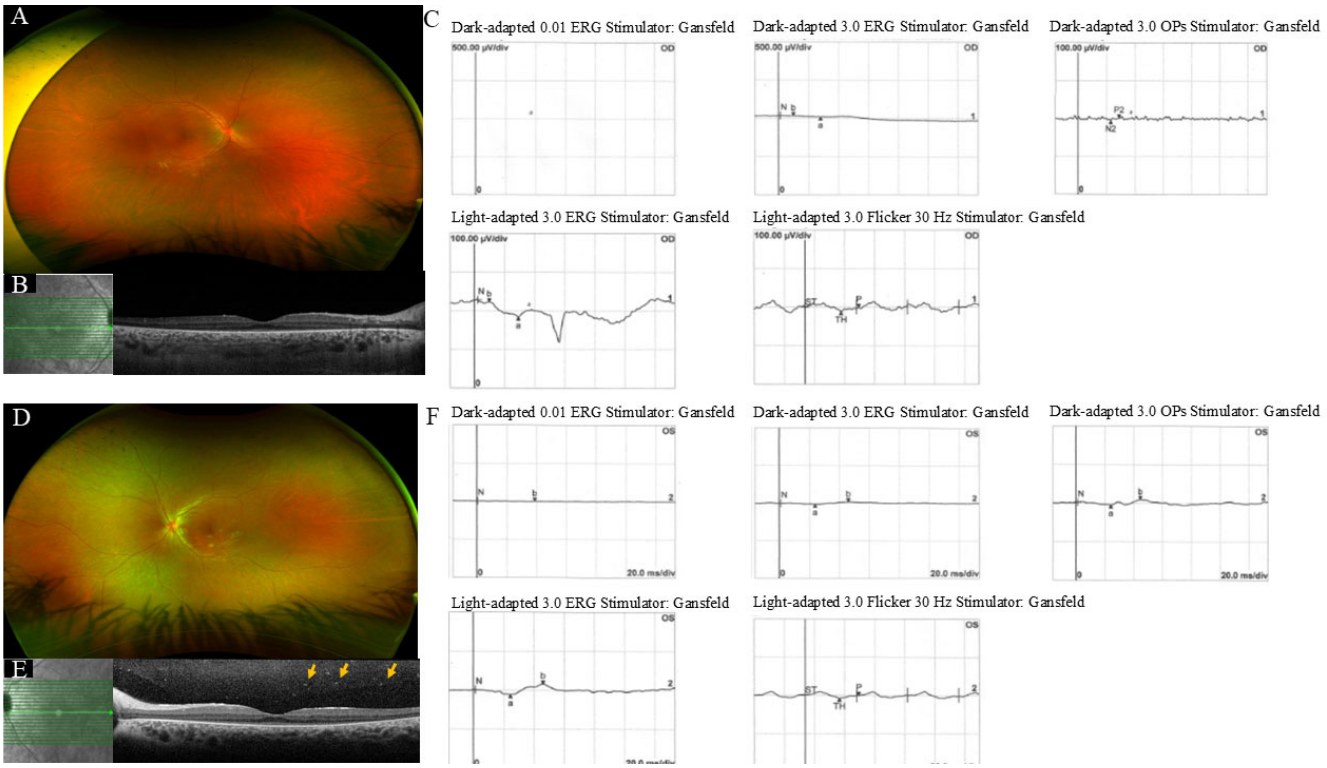

Figure 5. Images and full-field electroretinogram of Case 6 with *RPE65* gene mutation. Case 6 shows a proband with mutation c.11+5 G>A and c.1520C>T; p.(Ala507Val) at the age of 12 years. The fundus (**A** and **D**) shows diffuse pigmentary changes in the retina, and blood vessels, optic disc, and macula apparently preserved in both eyes (OU). OU optical coherence tomography (**B** and **E**) shows subfoveal outer retina preserved, and perifoveal atrophy with loss of the ellipsoid zones. In the left eye (OS) vitreous cavity, there are signs of vitreous degeneration (highlighted by the orange arrow). The full-field electroretinogram shows minimal rod and cone responses in OU (**C** and **F**).

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