Novel Variants in Phosphodiesterase 6A and Phosphodiesterase 6B Genes and Its Phenotypes in Patients With Retinitis Pigmentosa in Chinese Families

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Research Article

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Abstract

Background: Retinitis pigmentosa (RP) is a genetically heterogeneous disease with 65 causative genes identified to date. However, only approximately 60% of RP cases genetically solved to date, predicating that many novel disease-causing variants are yet to be identified. The purpose of this study is to identify novel variants in phosphodiesterase 6A and phosphodiesterase 6B genes and present its phenotypes in patients with retinitis pigmentosa in Chinese families.

Methods: Five retinitis pigmentosa patients with PDE6A variants and three with PDE6B variants were identified through a hereditary eye disease enrichment panel (HEDEP), all patients' medical and ophthalmic histories were collected, and ophthalmological examinations were performed, then we analysed the possible causative variants. Sanger sequencing was used to verify the variants.

Results: We identified 20 mutations sites in eight patients, two heterozygous variants were identified per patient of either PDE6A or PDE6B variants, others are from CA4, OPTN, RHO, ADGRA3 variants. We identified two novel variants in PDE6A: c.1246G > A;p.(Asp416Asn) and c.1747T > A;p.(Tyr583Asn). Three novel mutations in PDE6B: c.401T > C;p.(Leu134Pro), c.2293G > C;p.(Ala765Pro) and c.1610-1612del;p.(537-538del).CA4: c.243G > A;p.(Trp81*) and RHO: c.688G>A;p.(Val230Ile) are novel variants and maybe affecting the phenotype. Among them, c.401T > C;p.(Leu134Pro) variant in PDE6B is non- pathogenic; RHO: c.688G>A;p.(Val230Ile) is conflicting interpretations of pathogenicity;Other novel variants are all pathogenic.

Conclusions: This study reveals novel and known variants in Chinese families with PDE6A and PDE6B mutations in autosomal recessive RP, expanding the clinical and genetic findings of photoreceptor-specific enzyme deficiencies.

Background

Retinitis pigmentosa (RP,OMIM 268000) is a heterogeneous group of inherited retinal dystrophy (IRD) characterized by night blindness, retinal degeneration with bone spicule pigmentation, constricted visual fields, and progressive disease course. The prevalence of RP is approximately 1 per 4000 persons[1].

Retinitis pigmentosa (RP) is a genetically heterogeneous disease with 65 causative genes identified to date. However, only approximately 60% of RP cases genetically solved to date, predicating that many novel disease-causing variants are yet to be identified(https://sph.uth.edu/retnet/sum-dis.htm 2021.04.28). The gene therapy and stem cell therapy for retinitis pigmentosa has a promising future, so the identification of novel causative variants is becoming increasingly important.

Phosphodiesterase 6(PDE6)enzyme is a heterotetrameric protein consisting of alpha(PDE6A;180071), beta (PDE6B; 180072), and 2 gamma subunits (PDE6G; 180073) [2] . Both alpha and beta subunits are required for full phosphodiesterase activity, the mechanisms by which PDE6A and PDE6B mutations lead to RP are probably similar, studies found PDE6A and PDE6B subunits are enzymatically equivalent[3], either of which is associated with recessive RP, may lead to rod death and secondarily affecting the cone photoreceptor cells[4] .

Mutations in PDE6A are found in a very low percentage of patients with RP as showed first in a study by Huang and coworkers, suggesting a frequency of <1%[5]. Screening of about 160 patients with recessive RP in North America in a subsequent study found a frequency of mutations of approximately 3–4%[6].Mutations in PDE6B are found in a frequency of about 4% in patients from North America [1, 7–9].There is no statistics date about incidence rate in Chinese family. Because of the low incidence, many novel disease-causing variants are yet to be identified. The purpose of this study is to report the causative variants of Chinese RP families with PDE6A and PDE6B variants, expanding the clinical and genetic findings of photoreceptor-specific enzyme deficiencies.

Materials And Methods

Patients

Eight patients from eight unrelated families were enrolled in this retrospective study. We identified five RP patients with PDE6A mutations and three with PDE6B mutations. All patients were recruited from the Department of Ophthalmology, Beijing Tongren Eye Center. Clinical diagnosis of RP was made based on clinical evaluation and electroretinograms. All medical and surgical records for the patient were reviewed. The ophthalmic examinations performed in the study patient included decimal best-corrected visual acuity (BCVA), slit lamp, funduscopy, fundus photography, visual field testing, electroretinography (ERG), optical coherence tomography (OCT) and fluorescein angiography(FFA). One hundred Chinese Han healthy individuals were selected as the control group.

Mutation screening by HEDEP

Blood samples were obtained from the patients, and genomic DNA was extracted by using standard protocols. A specific hereditary eye disease enrichment panel (HEDEP) based on targeted exome capture technology was used to collect the protein coding regions of 441 hereditary eye disease genes. Exon-enriched DNA libraries were then subjected to high-throughput sequencing using the Illumina HiSeq platform. Targeted gene enrichment, high-throughput sequencing, and data analysis were performed as described previously[10] . Briefly, exons of the target genes and adjacent portions of introns were captured by probe hybridization; enriched target genes were then sequenced with the Illumina HiSeq platform. Specific pathogenic mutations were verified by Sanger sequencing.

Mutation validation by Sanger sequencing

Specific pathogenic mutations were verified by Sanger sequencing using four programs to evaluate the identified missense variants included mutation taster (MutationTaster),the PolyPhen2 http://genetics.bwh.harvard.edu/pph2/ SIFT(http://sift.bii.a-star.edu.sg/index.html), and PROVEAN (http:// provean.jcvi.org/index.php) programs.
BDGP(https://www.fruitfly.org/seq_tools/splice.html),Netgene(http://www.cbs.dtu.dk/services/NetGene2/) were used to evaluate the identified splicing variants. Meanwhile, the frequency of the identified variants in health population was assess use gnomAD. All mutations were evaluated regarding pathogenicity following American College of Medical Genetics and Genomics (ACMG) criteria.

Results

Genetic Screening

In this study, probands P01 to P05 were PDE6A variants while P06 to P08 were PDE6B variants, all of them were heterozygous variants ,20 mutations sites were identified in them, including 11 missense mutations, one nonsense mutation, three splicing mutations and one delete mutation. We identified two novel variants in PDE6A,three novel mutations in PDE6B,CA4: c.243G > A;p.(Trp81*) in P01 and RHO: c.688G>A;p.(Val230Ile) in P08 are novel variants and maybe affecting the phenotype. Among them, c.401T >C;p.(Leu134Pro) variant in P06 is non-pathogenic; RHO: c.688G>A;p.(Val230Ile) is conflicting interpretations of pathogenicity;other novel variants are all pathogenic(Table 1).

Table 1 variants identified in this study

Family	Gene	Nucleotide variant	Protein variant	Polyphen	Mutation Taster	SIFT	PROVEN	VF in gnomAD	Previously reported
P01	PDE6A	c.1349T > C	p.(Phe450Ser)	Benign	Disease causing	Tolerated	Neutral	0.016%	Yes[11]
	PDE6A	c.1246G > A	p.(Asp416Asn)	Probably damaging	Disease causing	Deleterious	Deleterious	NA	No
	CA4	c.243G > A	p.(Trp81*)	NA	NA	NA	NA	NA	No
P02	PDE6A	c.1685G>A	p.(Arg562Gln)	Possibly damaging	Disease causing	Deleterious	Deleterious	0.0028%	Yes[12]
	PDE6A	c.1407 + I G > C	p.?	NA	NA	NA	NA	0.0080%	Yes[13–15]
P03	PDE6A	c.2275 -2A > G	p.?	NA	NA	NA	NA	NA	Yes[16]
	PDE6A	c.1957C > T	p.(Arg653*)	NA	NA	NA	NA	0.0028%	Yes[17]
P04	PDE6A	c.1747T > A	p.(Tyr583Asn	Possibly damaging	Disease causing	Tolerated	Deleterious	NA	No
	PDE6A	c.1651A > G	p.(Lys551Glu)	Benign	Disease causing	Deleterious	Deleterious	NA	Yes[12]
	OPTN	c.1634G > A	p . (Arg545Gln)	Benign	Disease causing	Tolerated	Neutral	0.3103%	Yes[18, 19]
P05	PDE6A	c.1651A > G	p.(Lys551Glu)	Benign	Disease causing	Deleterious	Deleterious	NA	Yes[12]
	PDE6A	c.285C > A	p.(Ser95Arg)	Possibly damaging	Disease causing	Deleterious	Deleterious	NA	Yes[12]
P06	PDE6B	c.401T > C	p.(Leu134Pro)	Probably damaging	Disease causing	Deleterious	Deleterious	0.0037%	No
	PDE6B	c.2293G > C	p.(Ala765Pro)	Benign	Polymorphism	Deleterious	Neutra	0.04182%	No
P07	PDE6B	c.385G > A	p.(Glu129Lys)	Probably damaging	Disease causing	Deleterious	Deleterious	0.0014%	Yes[20]
	PDE6B	c.1610-1612del	p. 537-538del	NA	NA	NA	NA	NA	No
P08	PDE6B	c.1467+IG > C	p.?	NA	NA	NA	NA	0.0008%	Yes[21]
	PDE6B	c.2204T > C	p.(Leu735Pro)	Probably damaging	Disease causing	Deleterious	Deleterious	0.0004%	Yes[12]
	RHO	c.688G>A	p.(Val230Ile)	Probably damaging	Disease causing	Tolerated	Neutral	0.0039%	No
	ADGRA3	c.921-IG > A	p.?	NA	NA	NA	NA	NA	No

VF in gnomAD: the variants frequency in health population in gnomAD; *NA*: data not available

Novel variants and clinical findings

In P01 (Fig. 1a-d), the proband was a 12-year old man who presented with 0.4 vision in her right eye and 0.5 vision in his left eye. He was found night blindness by his parents when he was 6 years old, then ophthalmic examination revealed poor vision correction. Fundus photographs show relatively mild retinal degeneration, swelling of the nerve fiber layer causes unclear optic disc boundaries and tortuous venous of both eyes (Fig. 1a), macular foveal becomes shallower and central macular thicknesses were 296 microns in the right(Fig. 1b)(OCT date was not available in the left).

Variants of c.1246G > A;p.(Asp416Asn M2) in PDE6A gene and c.243G > A;p.(Trp81*M3) in CA4 have not been reported in RP cases previously, predict the effect of missense changes on protein structure and function (Polyphen, Mutation Taster, SIFT, PROVEN) predicted

PDE6A c.1246G>A; p.(Asp416Asn) to be probably damaging, disease causing, deleterious and deleterious. Nonsense mutation in CA4: c.243G > A; p.(Trp81*) leads to a premature termination of protein translation and can causes autosomal dominant hereditary retinitis pigmentosa, it maybe pathogenic and affecting the phenotype of P01. We don't find the variants frequency in health population of the two variants in Genome Aggregation Database (gnomAD), predicted PDE6A c.1246G>A;p.(Asp416Asn) and CA4 c.243G > A;p.(Trp81*) were the causative variants for this RP family.

In P04 (Fig. 2a-f), the proband was a 36-year old man who presented with 0.6 vision in his right eye and 0.5 vision in his left eye. Ophthalmoscopy showed extensive intraretinal pigment migrations extending from the mid-periphery equatorial region to the arcades in both eyes with extensive arterial attenuation, macular and peripapillary atrophy, only a small central foveal island was sparing(Fig. 2a). OCT images show high-density deposits on the surface of RPE layer in macula, residual intraretinal vacuoles and an entirely disrupted and atrophy of the retina and macular, the outer retinal structures are lost(Fig. 2b). Fluorescein angiographic show "bull's eye" macular atrophy and bonespicule hyperpigmentation blocks fluorescence in large-scale of the posterior pole and spot strong fluorescence(Fig. 2c). The full-field ERG shows a decrease in rod and cone amplitude in rod response and combined rod-cone response, as well as a delayed implicit time. The 30Hz Flicker cone response also shows a decreased amplitude(Fig. 2e).

The c.1634G > A;p.(Arg545Gln) variant of OPTN has been described before with respect to open angle glaucoma and it was a benign variant[22]. So,it does not associated with the phenotype.Variants of c.1747T > A;p.(Tyr583Asn,M1)in PDE6A gene has not been reported in RP cases previously, predict the effect of missense changes on protein structure and function (Polyphen, Mutation Taster, SIFT, PROVEN) predicted it to be probably damaging, disease causing, tolerated and deleterious. We don't find the variants frequency in health population of the two variants in Genome Aggregation Database (gnomAD), predicted DE6A c.1747T > A;p.(Tyr583Asn) was the causative variants for P04 RP family.

In P06 (Fig. 3a-e), the proband was a 42-year old woman who presented with 0.02 vision in her right eye and 0.4 vision in her left eye. Anterior segment examination show posterior subcapsular cataracts serious in left than right, so the fundus images are not clear in left eye, ophthalmoscopy showed attenuated vessels, and mid-peripheral bone-spicule pigmentation(Fig. 3a). Significant macular atrophy and exudes in outer plexus layer can been seen in right eye and serious than left, there were macular epiretinal membrane in right eye and extensive epiretinal membranes with thickened hyaloid in left eyes. The outer nuclear layer (ONL) and disruption of the ellipsoid zone (EZ) and external limiting membrane (ELM) can been seen in bilateral eye(Fig. 3b). Fluorescein angiographic show bonespicule hyperpigmentation blocks fluorescence, and the hyperfluorescent spots clearly demarcate the atrophic areas(Fig. 3c). Phenotypic differences between the two eyes illustrate that macular atrophy may significant affect vision than extensive epiretinal membranes.

Variants of c.401T > C;p.(Leu134Pro,M1) and c.2293G > C;p.(Ala765Pro,M2) in PDE6B have not been reported in RP cases previously, predict the effect of missense changes on protein structure and function (Polyphen, Mutation Taster, SIFT, PROVEN) predicted PDE6B c.401T > C;p.(Leu134Pro) to be probably damaging, disease causing, deleterious and deleterious; Missense variants of PDE6B c.2293G > C;p.(Ala765Pro) predicted to be benign, polymorphism, deleterious and neutral, the frequency of the two mutations are 0.0037% and 0.04182%, respectively in health population of the two variants in Genome Aggregation Database (gnomAD)， predicted PDE6B c.401T > C;p.(Leu134Pro)were the causative variant for this RP family, but variant of c.2293G > C;p.(Ala765Pro,M2) may has no relationship with phenotype of this family.

In P07(Fig. 4a-d), the proband was a 42-year old man, anterior segment examination show posterior subcapsular cataracts in both eyes,so the fundus images are not clear, attenuated vessels, and mid-peripheral bone-spicule pigmentation(Fig. 4a). OCT images show thinning of the retinal and the ellipsoid zone (EZ) is retained only in macular area(Fig. 4b).

Variants of c.1610-1612del;p.(537-538del,M2) has not been reported in RP cases previously, the deletion causes frameshift mutation, the protein structure and function of PDE6B were changed, we don't find the variant frequency in health population in Genome Aggregation Database (gnomAD), predicted PDE6B c.1610-1612del;p.(537-538del) was the causative variants for this RP family.

In P08(Fig. 5a-c),the proband was a 47-year old woman, fundus photographs show macular atrophy and peripapillary atrophy, attenuated vessels, and mid-peripheral bone-spicule pigmentation(Fig. 5a).

Variants of c.688G>A;p.(Val230Ile) in RHO gene and c.921-IG > A in ADGRA3

have not been reported in RP cases previously, variant of RHO is associated with autosomal dominant retinitis pigmentosa (adRP), predict the effect of missense changes on protein structure and function (Polyphen, Mutation Taster, SIFT, PROVEN) predicted RHO: c.688G>A;p.(Val230 Ile) to be probably damaging, disease causing, tolerated and neutra. The RHO variant has not been described in the literature, but affects a conserved amino acid residue and might also be relevant for the phenotype.The variants frequency in health population of the variant in Genome Aggregation Database (gnomAD) is 0.0039%, so, the variant is conflicting interpretations of pathogenicity, it maybe affecting the

phenotype. The splicing mutation of ADGRA3 just has pathogenicity while exist another mutation at the same time can lead to arRP, so it was not the causative variant for this RP family.

Clinal findings of known variants

In P02 (Fig.6a-d), the proband was a 28-year old man who presented with 0.8 vision in both eyes. He had night blindness since infancy, fundus photographs show moderate retinal degeneration, retinal arteriolar attenuation (Fig.6a). OCT images of P02 show nearly normal thickness of macular and mild macular epiretinal membrane, conserved *IS/OS* line shorter than normal fundus(Fig.6b). .

In P03 (Fig.7a-f2),the proband was a 34-year old woman who presented with 0.3 best corrected visual acuity in both eyes (OD:-10.50DS/+2.00DC×90°, OS:-9.50DS/+1.25DC×75°). She had night blindness since infancy, the cataract surgery had done for both eyes, because of posterior capsular opacity, the fundus images can't presented clearly. Fundus photographs show macular atrophy and an entirely disrupted ellipsoid zone in the right eye(Fig.7a), epiretinal membrane, cystoid macular edema, outer retinoschisis,lamellar macular hole in the left eye(Fig.7b), pathological myopia maybe the reason of those phenotype. Visual fields were reduced to a small central(Fig.7c). ERGs to all stimuli were not detectable(Fig.7e).

In P05 (Fig. 8a-e), the proband was a 47-year old man who presented with 0.01 vision in his right eye and HM vision in his left eye. He had night blindness since infancy, fundus photographs show gray retinal with severe chorioretinal atrophy with bone spicule pigmentation in the area from macular to the peripheral retina, compatible with macular atrophy and structure change(Fig. 8a). OCT images show macular epiretinal membrane and vitreomacular traction and an entirely disrupted ellipsoid zone in both eyes, disappearance of the foveal depression of the right eye(Fig. 8b).Fluorescein angiographic bonespicule hyperpigmentation blocks fluorescence, and the hyperfluorescent spots clearly demarcate the atrophic areas(Fig. 8c).

Discussion

The phosphodiesterase 6 enzyme is involved in hydrolysis of cGMP in the photoreceptors during the transduction of light signals. PDE6A gene locates at chromosome 5, the human PDEA gene comprises 22 exons spanning approximately 45 to 50 kb and encodes for a protein containing 860 amino acids [23, 24]. PDE6B gene locates at chromosome 4 and encodes for a protein containing 854 amino acids[2]. The mechanisms by which PDE6A and PDE6B mutations lead to RP are probably similar, it has been hypothesized to be due to an increased Ca^{2+} influx[25], and/or increased accumulation of cGMP[26] . The mutation of PDE6A causes retinitis pigmentosa 43, which affects the function of PDE6B[27]. Phenotypic analysis revealed no substantial differences between the two groups except for night blindness as a presenting symptom that was noted to be more prevalent in the PDE6A than PDE6B group[28].We identified five RP patients with PDE6A variants and three with PDE6B variants, all of our patients complaint night blindness since the memory which consistent with previous studies that reveal that nyctalopia occurs in early childhood[9, 29–33].ERG presenting extinguish in most cases or only 30Hz mild reserved in previous studies[9, 29, 31, 32, 34] ,and we come to the same conclusion. The EZ width was reduced in all patients and was highly symmetric between the eyes[35–37], and we come to the same conclusion. When it comes to the complications of PDE6A and PDE6B variants, the most worth to pay attention to being macular abnormalities, such as vitreomacular traction, epiretinal membrane, cystoid macular edema, retinoschisis、lamellar macular hole. More than half of our patients (P03,P04,P05,P06,P08)have those changes. One mechanism to explain this may be that loss of the photoreceptors elicits new glial barriers, causing Muller cells to migrate[8].

In P01, swelling of the nerve fiber layer causes unclear optic disc boundaries and tortuous venous of both eyes and macular foveal becomes shallower which different from other patients, variant of c.1349T>C;p.(Phe450Ser,M1) predicted may has no pathogenicity by online tools. Variant of c.243G>A;p.(W81*) is a nonsense mutation and leads to a premature termination of CA4 protein translation,CA4 variant phenotype is Retinitis Pigmentosa 17-autosomal dominant inheritance, we presume that c.243G>A;p.(W81*) of CA4 variant maybe affect the phenotype, therefor, CA4 variant may worsen or maybe the reason of the occur of RP of this family.

Retinoschisis and lamellar macular hole in P03 have not been reported in PDE6A and PDE6B variants so far as we know. But this patient accompany with refractive error (OD:-10.50DS/+2.00DC×90°,OS:-9.50DS/+1.25DC×75°).So, one mechanism to explain this may be that it related to pathological myopia but are rarely associated with RP in the literature[38, 39].

In P04,OPTN variant generally causes open angle glaucoma, in an autosomal dominant manner of genetic[18, 19], the proband carries c.1634G>A;p.(R545Q) heterozygous mutation, which has been included by HGMD database. Our patient doesn't identify of glaucoma, and we predicted it to be benign by online tools. Therefor it could be non-pathogenic mutation.

In P06, we identified two heterozygous mutations of c.401T > C;p.(Leu134Pro,M1) and c.2293G > C;p.(Ala765Pro,M2) in PDE6B. Predict the effect of missense changes on protein structure and function (Polyphen, Mutation Taster, SIFT, PROVEN) predicted missense variants of PDE6B c.2293G > C;p.(Ala765Pro,M2) predicted to be benign, polymorphism, deleterious and neutral, the frequency of the mutations is

0.04182% in health population of the variant in Genome Aggregation Database (gnomAD),the heterozygous mutations of c.401T > C;p.(Leu134Pro,M1) in PDE6B just has pathogenicity while exist another mutation at the same time can lead to arRP, but exist another mutation of PDE6B c.2293G > C;p.(Ala765Pro,M2) predicted to be non-pathogenic by online tools. Analyzing the cause of this patient still along with phenotype of RP due to following reasons[40]: (1) there were larger deletions or rearrangements not detectable by Sanger sequencing; (2) there were deeper intronic mutations, which caused aberrant splicing, but were not examined in our study; (3) there were mutations in regulatory regions, which were not examined in our study; (4) there may be additional genes are responsible forRP.

In P08,variants of c.688G>A;p.(Val230Ile) in RHO gene and c.921-IG > A in ADGRA3 were noval variants, chromosome 3 that comprised the rhodopsin gene (RHO/NM_000539.3) and chromosome 4 that comprised adhesion G protein-coupled receptor A3 (ADGRA3/NM_145290.4),also named G protein-coupled receptor 125(GPR125) [41]were detected. RHO variant can causes RP, generally be autosomal dominant inheritance, but can rarely be recessive inheritance[42], pedigree P08 carries RHO c.688G>A;p.(Val230Ile) is a single site heterozygote mutation. ADGRA3 protein is a G protein-coupled receptor of unknown function, Leen Abu-Safieh[43]found a novel splice-site mutation in a second isolated Saudi RP patient, it is associated with recessive retinitis pigmentosa; The heterozygous mutation of ADGRA3 should exist with another mutation at the same time can lead to autosomal recessive RP (arRP). So, in this arRP family, the RHO variant is conflicting interpretations of pathogenicity, it maybe affecting the phenotype.The variant of ADGRA3 may not worsen the phenotype. But due to the complexity and limitations of the detection technology of gene mutation, at present, we can't completely exclude this sequencing may be pathogenic although another pathogenic site does not detected .

One limit of our study is present fundus images only in posterior pole, the periphery fundus can't present well because of equipment incomplete. Besides, eight cases are a small sample to present the clinical and genetic features of PDE6A and PDE6B variants, we need to collect more sample to analyse in future. Overall, this study reveals novel and known mutations in Chinese families with PDE6A and PDE6B mutations in autosomal recessive RP. These findings expand the clinical and genetic findings of photoreceptor-specific enzyme deficiencies.

Conclusion

In conclusion, We identified two novel variants in PDE6A, three novel mutations in PDE6B ,one novel variant in CA4 and one novel variant in RHO. Among them, c.401T > C;p.(Leu134Pro) variant in PDE6B is non- pathogenic; RHO: c.688G>A;p.(Val230Ile) is conflicting interpretations of pathogenicity;Other novel variants are all pathogenic. This study expanding the clinical and genetic findings of photoreceptor-specific enzyme deficiencies.

Abbreviations

RP Retinitis Pigmentosa

PDE6A Phosphodiesterase 6A

PDE6B Phosphodiesterase 6B

HEDEP Hereditary eye disease enrichment panel

IRD Inherited retinal dystrophy

BCVA Best-corrected visual acuity

Declarations

Ethics approval and consent to participate

The study was performed in accordance with the ethical standards of the Declaration of Helsinki (1964) and its subsequent amendments. All experiments involving patient DNA, as well as DNA from related individuals, were approved by the Clinical Research Ethics Committee in Beijing Tongren Hospital, Capital Medical University. Written informed consent was obtained from all participants or guardians on behalf of minors/child participants; the ethics committees approved this consent procedure (TREC2015-XJS07).

Consent for publication

Informed consent for publication is obtained from all participants or guardians on behalf of minors/child participants.

Availability of data and materials

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests

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Authors contributions

Yuyu Li conceived and designed the study and wrote the first draft, Ruyi Li and Hehua Dai were responsible for patient data, Genlin Li conducted data analyses and designed the study.All authors read and approved the final manuscript.

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Not applicable

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Figures

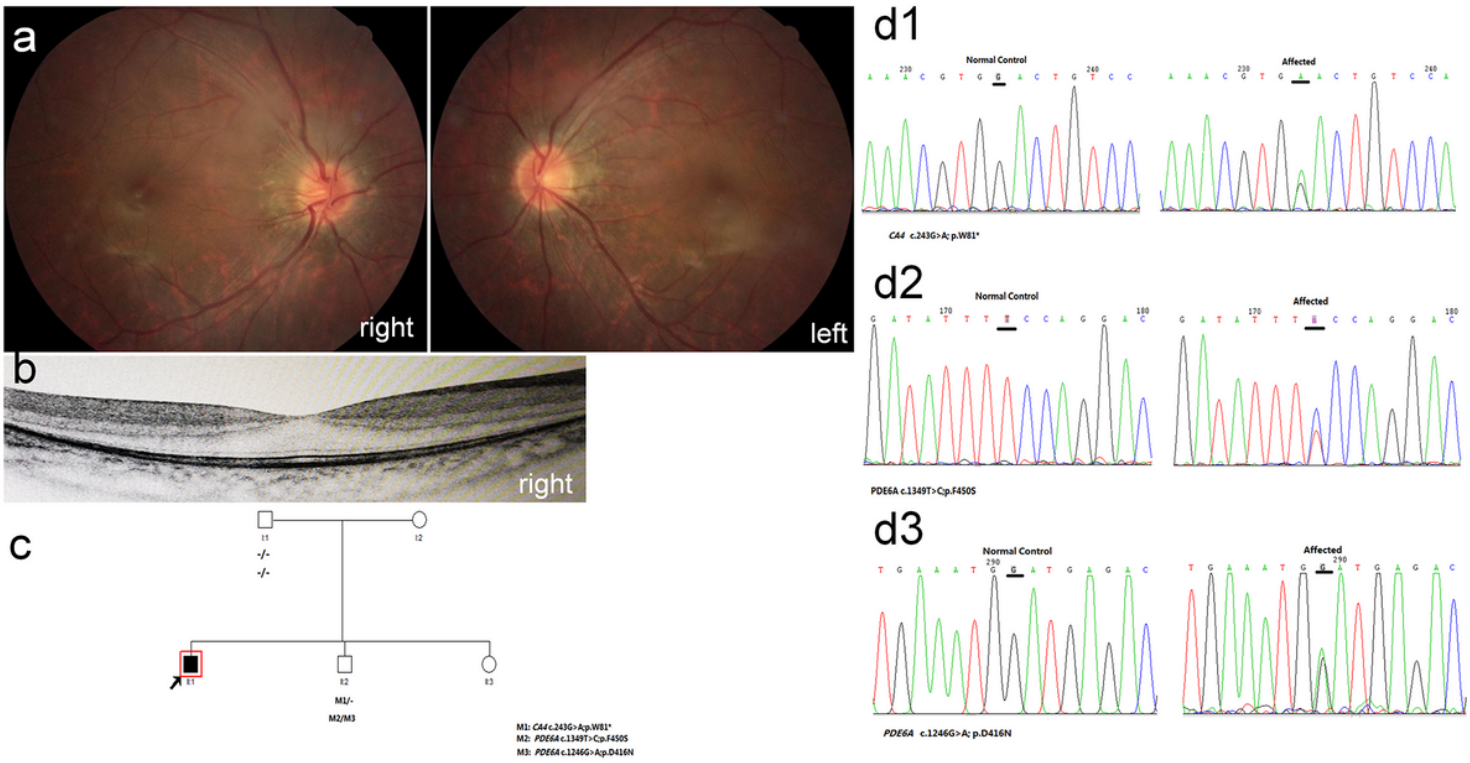

Figure 1

Clinical observations and genetic testing in the PDE6A variant of P01. a Fundus photographs show welling of the nerve fiber layer, macular foveal becomes shallower. b OCT images show central macular thicknesses were 296 microns in the right and the inner segment/outer segment (IS/OS)junction layer disappears at the periphery of the macular. c The pedigree of P01. d1-d3 Sequence chromatogram of P01.

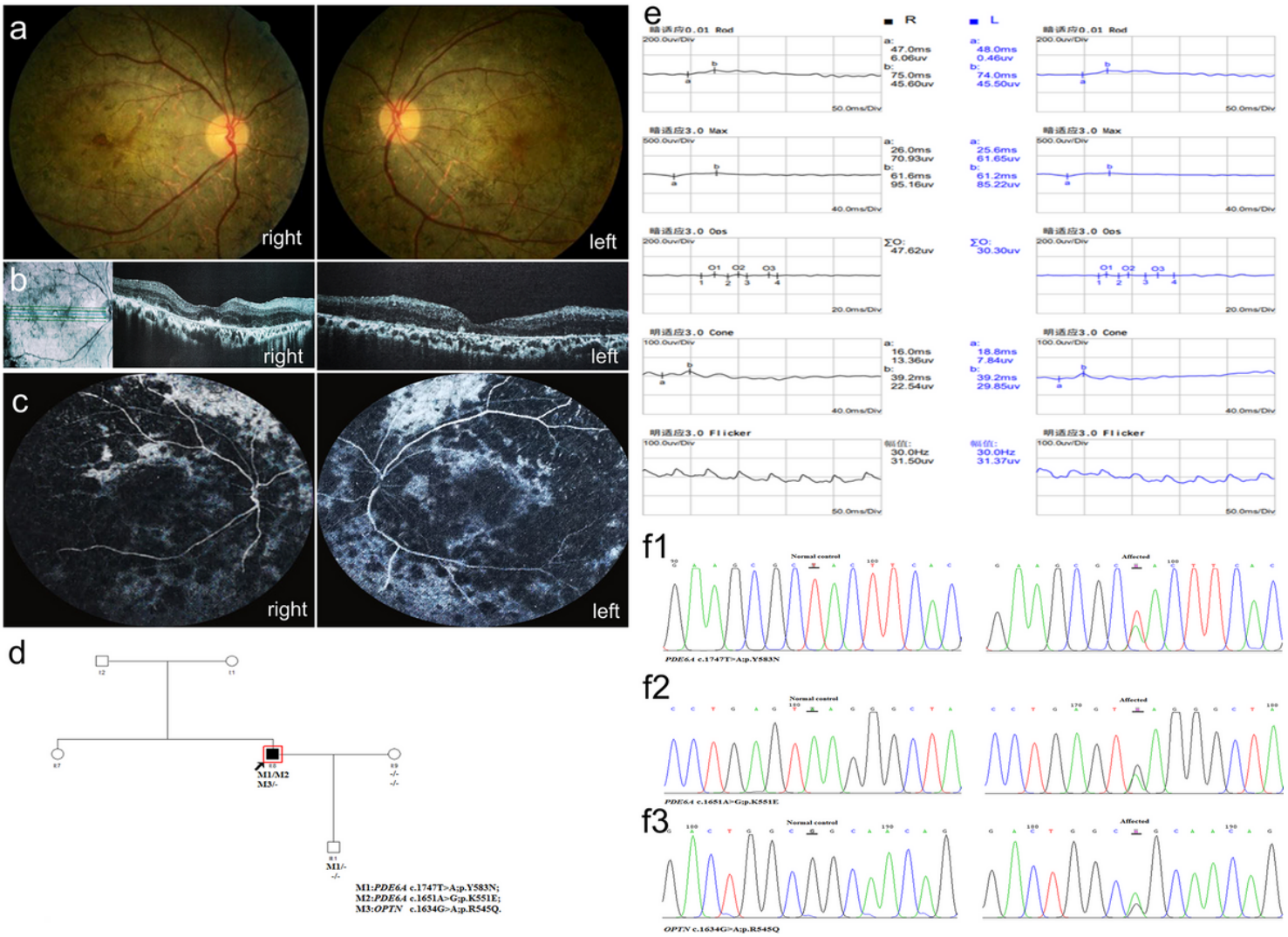

Figure 2

Clinical observations and genetic testing in the PDE6A variant of P04. a Fundus photographs show extensive intraretinal pigment migrations and arterial attenuation in both eyes, macular and peripapillary atrophy. b OCT images show high-density deposits on the surface of RPE layer in macula, residual intraretinal vacuoles and an entirely disrupted and atrophy of the retina and macular, the outer retinal structures are lost. c Fluorescein angiographic show “bull’s eye” macular atrophy (Fig. 4c). (Fig. 4e). d The pedigree of P04. e The full-field ERG shows a decrease in rod and cone amplitude in rod response and combined rod-cone response, as well as a delayed implicit time. The 30Hz Flicker cone response also shows a decreased amplitude. f1-f3Sequence chromatogram of P04.

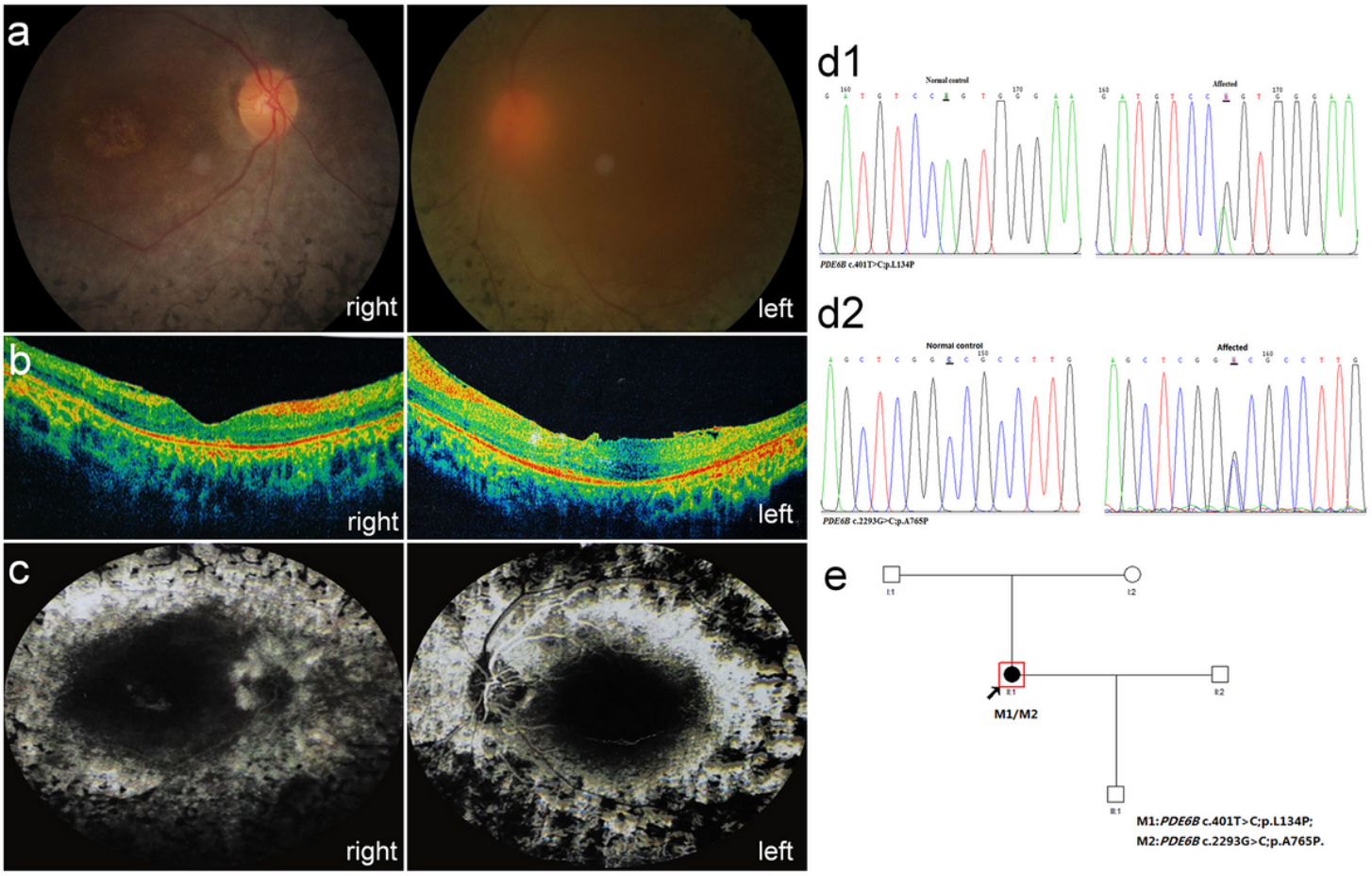

Figure 3

Clinical observations and genetic testing in the PDE6B variant of P06. a Fundus photographs of P06, because of posterior subcapsular cataracts, the fundus images are not clear, attenuated vessels, and mid-peripheral bone-spicule pigmentation. b OCT images show macular epiretinal membrane and residual intraretinal vacuoles. c Fluorescein angiographic show bonespicule hyperpigmentation blocks fluorescence, and the hyperfluorescent spots clearly demarcate the atrophic areas. d1-d2 The pedigree of P06. e Sequence chromatogram of P06.

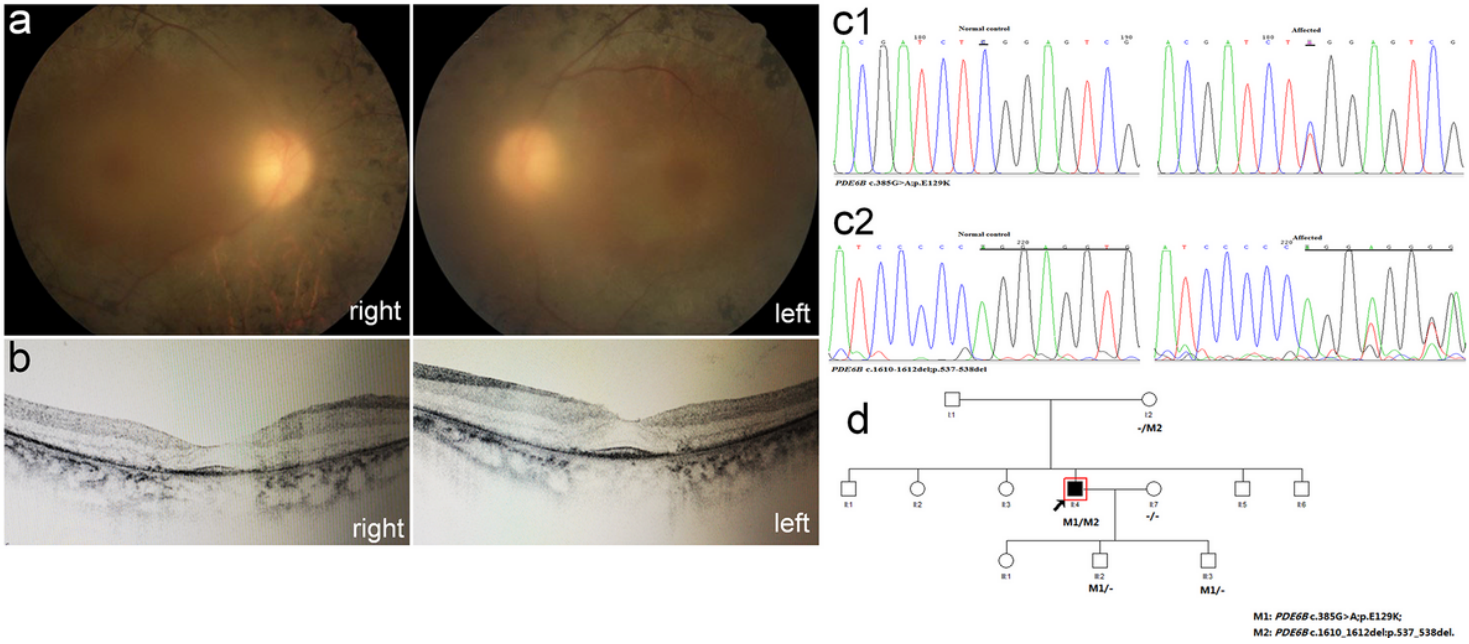

Figure 4

Clinical observations and genetic testing in the PDE6B variant of P07. a Fundus photographs of P07, because of posterior subcapsular cataracts, the fundus images are not clear, attenuated vessels, and mid-peripheral bone-spicule pigmentation. b OCT images show thinning of the retinal and the ellipsoid zone (EZ) is retained only in macular area. c The pedigree of P07. d Sequence chromatogram of P07.

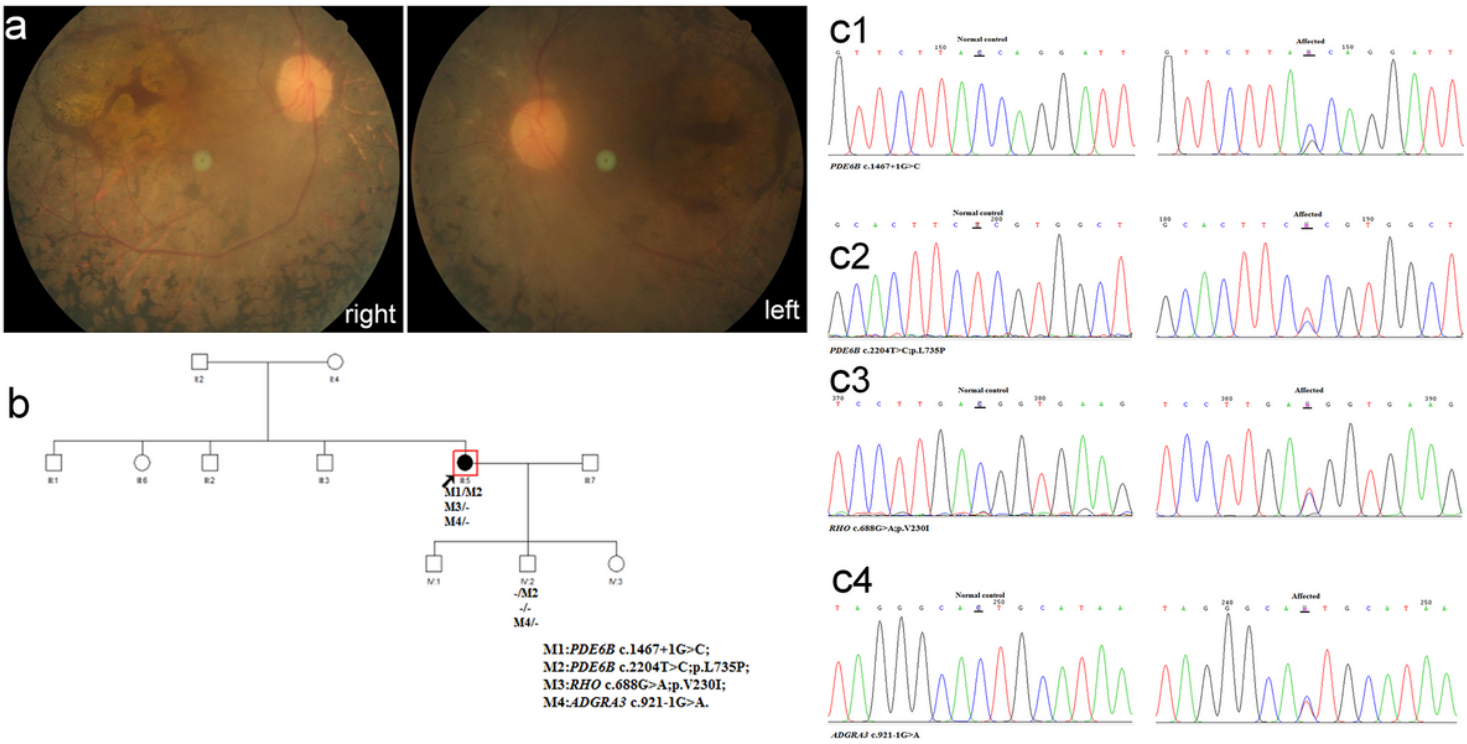

Figure 5

Clinical observations and genetic testing in the PDE6B variant of P08. a Fundus photographs of P08, 47 years old, show macular atrophy and peripapillary atrophy, attenuated vessels, and mid-peripheral bone-spicule pigmentation. b The pedigree of P08. c Sequence chromatogram of P08.

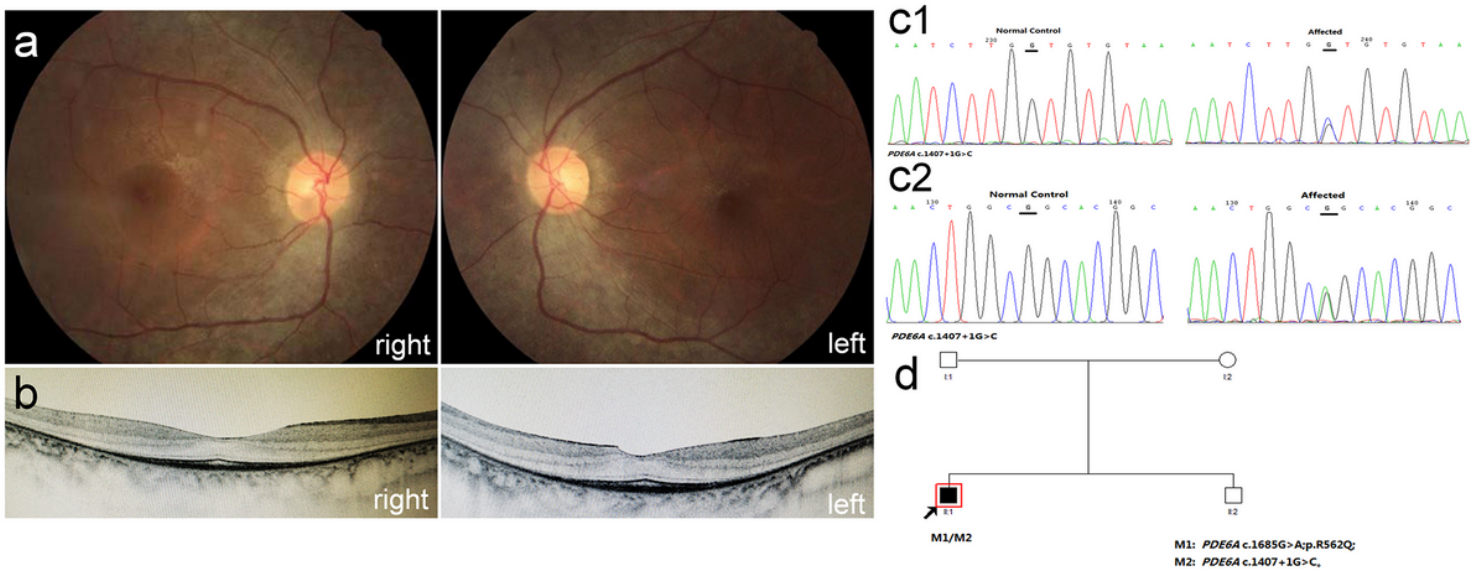

Figure 6

Clinical observations and genetic testing in the PDE6A variant of P02. a Fundus photographs show moderate retinal degeneration, retinal arteriolar attenuation. b OCT images show nearly normal thickness of macular and mild macular epiretinal membrane, conserved IS/OS line shorter than normal fundus. c The pedigree of P02. d1-d3 Sequence chromatogram of P02.

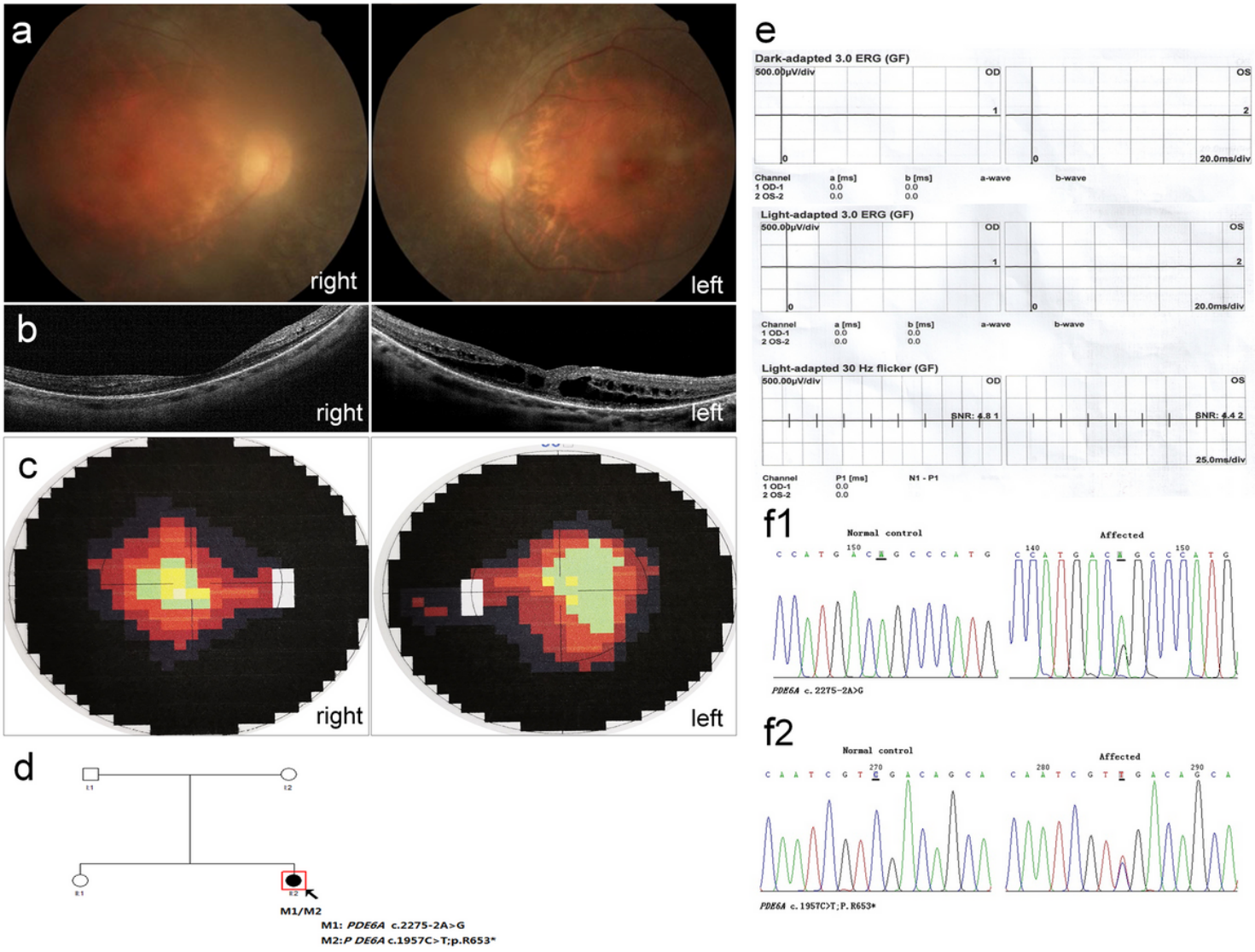

Figure 7

Clinical observations and genetic testing in the PDE6A variant of P03. a Fundus photographs of P03, the cataract surgery had done for both eyes, but because of posterior capsular opacity, the fundus images can't presented clearly, markedly severe retinal degeneration with visible atrophic choroidal vessels in the posterior retina and waxy temporal pallor of the optic disc. b OCT images show macular atrophy and an entirely disrupted ellipsoid zone in the right eye, epiretinal membrane, cystoid macular edema, outer retinoschisis□lamellar macular hole in the left eye. c visual fields were reduced to a small central. d The pedigree of P03. e ERGs to all stimuli were not detectable. f1-f2 Sequence chromatogram of P03.

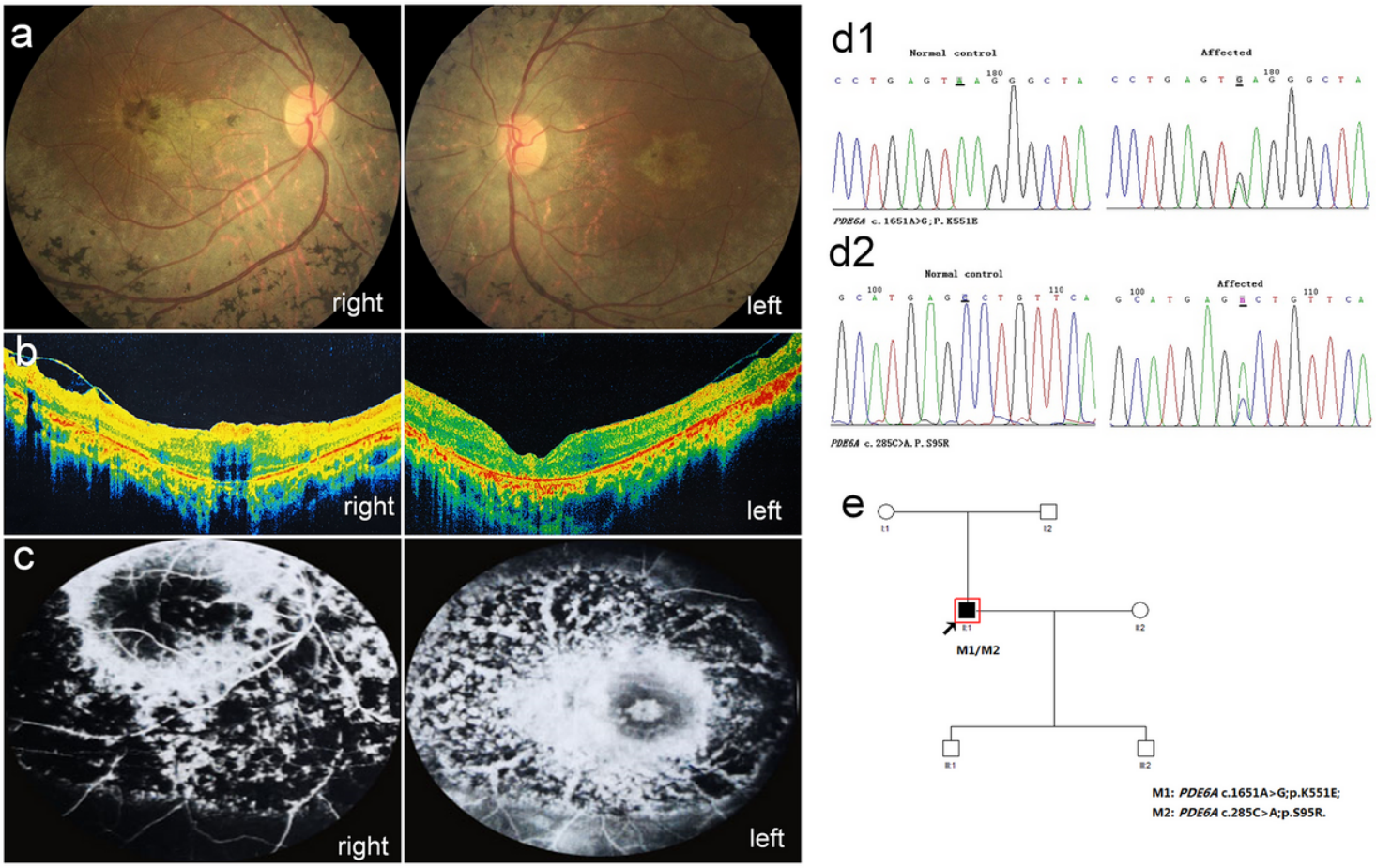

Figure 8

Clinical observations and genetic testing in the PDE6A variant of P05. a Fundus photographs show severe chorioretinal atrophy with bone spicule pigmentation in the area from macular to the peripheral retina, compatible with macular atrophy and structure change. b OCT images show macular epiretinal membrane and vitreomacular traction and an entirely disrupted ellipsoid zone in both eyes, disappearance of the foveal depression of the right eye. c Fluorescein angiographic bonespicule hyperpigmentation blocks fluorescence, an d the hyperfluorescent spots clearly demarcate the atrophic areas. d The pedigree of P05. e Sequence chromatogram of P05.