A Novel Potentially Causative Variant of NDUFAF7 Revealed by Mutation Screening in a Chinese Family With Pathologic Myopia

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PURPOSE. Pathologic myopia described as myopia accompanied by severe deformation of the eye besides excessive elongation of eye, is usually a genetic heterogeneous disorder characterized by extreme, familial, early-onset vision loss. However, the exact pathogenesis of pathologic myopia remains unclear. In this study, we screened a Han Chinese family with pathologic myopia to identify the causative mutation and explore the possible pathogenic mechanism based on evaluation of the biological functions of the mutation.

METHODS. We identified the mutations in a family with pathologic myopia by single nucleotide polymorphism array combined with short tandem repeat microsatellite marker analysis and exome sequencing. Mutations were validated among family members by direct Sanger sequencing. The subcellular localization of the protein variant was investigated by immunofluorescence, and the stability of the mutant protein was determined by immunoblotting. Intracellular levels of adenosine triphosphate and reactive oxygen species and complex I activity were measured by traditional biochemical methods to determine the functional role of the disease-associated mutation.

RESULTS. The novel missense mutation: c.798C>G (p.Asp266Glu) in NDUFAF7, cosegregated with the disease and the resulting amino acid substitution affected a highly conserved residue in its protein. The mutation D266E in NDUFAF7 impaired complex I activity, which resulted in decreased ATP levels in cultured cells.

CONCLUSIONS. We propose that the heterozygous mutation (c.798C>G) in NDUFAF7 may contribute to the pathogenesis of pathologic myopia, possibly by interfering with the phototransduction cascade. Mitochondrial dysfunction during eye development may lead to pathologic myopia.

Keywords: pathologic myopia, NDUFAF7, causative mutation

High myopia is an extreme form of myopia, usually defined by an ocular axial length >26 mm or a refractive error <−6.00 diopters (D). High myopia is a leading cause of blindness worldwide, with a relatively high prevalence of 1% to 5% in Asian countries, and as high as 4.1% in China.1–4

High myopia can be complicated by pathology in 8% of high myopia in that there might be other genetic variants that play a role in this process compared to high myopia without pathology.3–8 Pathologic myopia (PM) generally causes irreversible visual impairment, which involves not only elongation of the eye, but also characteristic pathologic changes in the retina, choroid, and sclera, such as posterior sclera staphyloma, macular degeneration, lacquer cracks, and choriotireal atrophy.3 Pathologic myopia is highly heritable, and genetic linkage studies have identified over 20 loci for PM, with autosomal dominant, autosomal recessive, or X-linked recessive modes of inheritance.10–13 More than 70 genes related to refractive variation have been screened out by association studies.14 Furthermore, recent studies have indicated that environment is another factor influencing the growth of the eye.15 Environmental factors, such as higher level of education, spending more time on near-work, less time on outdoor activities, and increasing urbanization have been known to be associated with PM.16–19 The complex nature of PM means that its etiology remains unclear.

In this study, we aimed to screen the causative mutation from a Han Chinese family with pathologic myopia and evaluated its biological functions. We demonstrated that one mutation in NDUFAF7 might be responsible for the development of myopia. Our study establishes a connection between mitochondrial defect and pathologic myopia. The results of this study may provide valuable insights for the further treatment of PM.

MATERIALS AND METHODS

Patients

A Han Chinese family with PM was recruited from the department of ophthalmology at the People’s Hospital of
Wenshan prefecture, Yunnan Province. All patients provided informed consent. The study was approved by the Shenzhen Research Institute of Population and Family Planning Review Board and adhered to the tenets of the Declaration of Helsinki. The diagnosis of PM was based on progressive loss of peripheral vision, age-related decrease in visual acuity, and waxy pale discs. Medical and ophthalmic histories were obtained, and ophthalmologic examinations were carried out. None of the family members had any history of other ocular or systemic abnormalities. A total of 50 healthy Han Chinese individuals who were unrelated to each other or to the patients were recruited as controls. All the patients in the family were female, and we therefore enrolled more female control individuals (2:1, female: male). The age of the controls ranged from 50 to 65.4 years (mean 52.9 ± 2.7 years), and the average axial length for the right eye (OD) was 23.11 ± 0.72 mm. All controls originated from the same geographical area as the patients.

**Genome-wide Genetic Analysis Based on Single-Nucleotide Polymorphism (SNP) and Short Tandem Repeat (STR) Genotyping**

Blood samples were collected and genomic DNA was extracted by using a blood kit (NucleoSpin; Macherey-Nagel, Düren, Germany). Six DNA samples from family members (proband, her two parents, her daughter, and her two siblings) were genotyped using a commercial SNP array (Human Mapping 250K SNP GeneChip Sty Array; Affymetrix, Santa Clara, CA, USA). SNP genotypes were obtained following the Affymetrix GeneChip Mapping protocol. The 262,270 SNPs were searched for regions containing ≥10 consecutive SNPs and segregating in all patients. To clarify the boundaries of regions, we performed STR microsatellite marker analysis in family members based on SNP haplotype identified core regions, using a commercial mapping set (ABI PRISM Linkage Mapping Sets v2.5 kit; Applied Biosystems, Inc., Foster City, CA, USA). The STR markers were amplified by PCR using standard protocols. The PCR products were electrophoresed using a 16-capillary genetic analyzer (ABI 3130XL; Applied Biosystems, Inc.). Genotyping data were analyzed using commercial software (GeneMapper Software v3.2; Applied Biosystems, Inc.).

**Mutation Screening**

Exome capture and sequencing of the proband (II-3) were performed using life whole exome capture and sequencing technology (Ion PGM 200; Thermo Fisher Scientific, Waltham, MA, USA). Data analysis was performed as described previously, with slight modifications. Considering the low explained heritability of PM, we thus expect the variants to be rare in population samples. Minor allele frequency (MAF) of each variant was obtained from a known database, including the Single Nucleotide Polymorphism Database (dbsNP build 148); 1000 Genomes (phase 3); and the Exome Aggregation Consortium (ExAC). Briefly, a variant located in the above-identified cosegregating regions and meeting one of the following requirements would be considered as a candidate mutation: newly discovered, an MAF of <0.01, or although reported, its MAF is not available in these databases. The subsequent Sanger sequencing for those candidate variants was performed in family members to determine if they cosegregated in patients. Potential causative variants were filtered further by heterozygous inheritance according to the dominant trait of the pedigree.

**Phylogenetic Sequence Analysis**

NDUFAF7 amino acid sequences across species were extracted from the National Center for Biotechnology Information Homologene database and aligned using the multiple alignment application in the DNAMAN tool (version 7). Phylogenetic analysis was performed using the neighbor-joining procedure.

**DNA Constructs**

As previously reported, NDUFAF7 has two transcripts of 1.3 and 1.0 kb, respectively. Here, we focused our studies on the long isoform. The cDNA for full-length NDUFAF7 (NM_144736) was amplified by reverse transcription-PCR from a sample of human brain total RNA (6565530; Clontech, Mountain View, CA, USA) using the forward and reverse primers 5’-ATGAGTTTACTGCTGAGGTACG-3’ and 5’-CTGCAAGCAAGTTCAC-3’, respectively. The PCR product was purified and cloned into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). Disease-associated mutation was introduced using a site-directed mutagenesis kit (Q5, E0552S; New England Biolabs, Ipswich, MA, USA). The accuracy of the clones was verified by Sanger sequencing. We created C-terminal tagged constructs using PCR primers incorporating a FLAG tag downstream of NDUFAF7 (primers available upon request).

**Cell Culture and Immunoblotting**

Human retinal pigment epithelium (ARPE)-19 (eye); 293T (kidney); and E6E7 (cervical) cells were grown at 37°C in an atmosphere of 5% CO2 in high-glucose Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (10099-141; Gibco, Grant Island, NY, USA). DNA construct was transiently transfected into cells using transfection reagent (Lipofectamine 2000; Life Technologies, Carlsbad, CA, USA) in growth medium in a six-well plate according to the manufacturer’s instructions. After incubation for a further 48 hours, the cells were harvested and lysed with radio immunoprecipitation assay buffer for 30 minutes on ice. Whole-cell proteins (20 μg) were separated by SDS-PAGE and immunoblotted with 1 μg/ml of rabbit polyclonal FLAG (F7425; Sigma-Aldrich Corp., St. Louis, MO, USA) and rabbit monoclonal glyceraldehyde 3-phosphate dehydrogenase (GAPDH, 1:10,000, ab128915; Abcam, Cambridge, UK) antibodies.

**Immunofluorescence and Confocal Microscopy**

Cells were seeded on coverslips. After transfection with the desired construct, the cells were stained with red fluorescent dye (100 ng/mL MitoTracker Red; Life Technologies, Washington, DC, USA) for 30 minutes to label mitochondria, washed with PBS, fixed with 4% formaldehyde for 15 minutes at room temperature, and washed three times with PBS for 5 minutes each. After blocking for 1 hour, cells were incubated with anti-FLAG antibody (1:2000) overnight at 4°C; washed with PBS; and then incubated with secondary antibody (1:2000) for 1 hour. The coverslips were mounted on glass slides and examined with a confocal laser-scanning microscope (SP5; Leica, Heidelberg, Germany). AlexaFluor 488 goat anti-rabbit antibody (ab150077; Abcam) was used as the secondary antibody.

**Evaluation of Reactive Oxygen Species (ROS) Levels**

Intracellular ROS were measured using a ROS detection kit (ENZ-51011; Enzo, Farmingdale, NY, USA) based on 2′,7′-dichlorodihydrofluorescein (DCF) oxidation.
Measurement of ATP Content and Mitochondrial Complex I Enzyme Activity

ATP content was measured using an ATP bioluminescence assay kit (Beyotime Institute of Biotechnology, Jiangsu, China). Briefly, cells were harvested and lysed with lysis buffer, centrifuged at 12,000g for 5 minutes at 4°C, and 20 μL of supernatant was mixed with 100 μL of luciferase reagent. ATP levels were then measured in a commercial detection system (Promega Corp.). The protein concentration was determined using a protein assay kit (Coomassie; Thermo Fisher Scientific). Total ATP levels were expressed as nmol/mg protein. Complex I enzyme activity was determined using a complex I enzyme activity microplate assay kit (Abcam) according to the manufacturer’s instructions. Briefly, cells were lysed and centrifuged at 12,000g for 20 minutes at 4°C, 150 µg of lysate was then loaded on a test plate and incubated for 3 hours at room temperature. Assay solution (200 μL) was finally added to each reaction well and the optical density at 450 nm was measured at approximately 1-minute intervals for 30 minutes. The activity was expressed in a kinetic mode as millioptical-density/minute based on the enzyme activity proportional to the increase in absorbance at 450 nm. To account for possible effects of the transfection mixture and transfection procedure, cells transfected with pcDNA3.1-EGFP expression vector were used as a control.

Statistical Analysis

Data are expressed as mean ± SD. Statistical significance was examined using 2-tailed Student’s t-tests and a significant difference was determined at \( P < 0.05 \).

RESULTS

Clinical Features of PM Family

The selected family with PM originated from Yunnan Province in Southwestern China. The family included fraternal twins, which included the proband (II-3). The proband was a 55-year-old female who complained of painless, progressive age-related bilateral vision loss. Ophthalmologic evaluation revealed a spherical refractive error of −21.5 D in the OD and −20.25 D in the OS. The OD and OS axial length were 31.32 and 31.56 mm, respectively, and myopic conus appeared in both eyes. Fundus examination showed prominent posterior staphyloma, tessellation, attenuated retinal vessels, patchy atrophy, and severe peripapillary atrophy (Fig. 1). The retinal nerve fiber thickness was significantly reduced in the myopic eyes compared with the normative reference provided by Fourier domain optical coherence tomography. Her daughter (III-2) was also diagnosed with PM and had refractive errors of OD −7.5 D and OS −7.25 D. All patients in the family had normal intraocular pressures <21 mm Hg, with no retinitis pigmentosa (RP) or night blindness. The detailed characteristics of the available family members are summarized in Supplementary Table S1.

Mutations Associated With PM

We performed whole-genome SNP genotyping in six immediate family members to identify regions cosegregating with PM (Fig. 2). However, because of the small pedigree structure, none of the SNPs had a two-point LOD score >1.0 calculated using MERLIN 1.1.2 software. Cosegregated regions were therefore detected by SNP haplotype analysis based on the presence of at least 10 consecutive SNPs, which identified 10 core segregating regions in this family (Supplementary Table S2). The boundaries of the cosegregating regions were defined by performing microsatellite-marker analysis for these core regions. Seven regions were considered most likely to cosegregate with the disease (Supplementary Table S3), including the largest region spanning approximately 96 Mb between markers D2S165 and D2S347 on chromosome 2 (Fig. 2), which harbored 1025 genes, including known proteins, pseudogenes, and hypothetical proteins. Based on whole-exome sequencing data from the proband (II-3), we identified 62 variants in the cosegregated regions (Supplementary Tables S4, S5) and validated these variants by Sanger sequencing in all available family members. The following seven variants were finally found to cosegregate with the disease: c.465C>A (p.Arg155Ser) of glycolipid transfer protein domain-containing protein 1 (GLTPD1) on chromosome 1; c.3527T>C (p.Gln1176Arg) of chromosome 2 open reading frame 71.
(C2ORF71) and c.798C>G (p.Asp266Glu) of NADH:ubiquinone oxidoreductase complex assembly factor 7 (NDUFAF7) on chromosome 2; c.310G>A (p.Val104Ile) of rhodopsin (RHO) and c.3199G>A (p.Val1067Ile) of zinc finger and BTB domain containing 38 (ZBTB38) on chromosome 3; and c.1117G>T (p.Asp373Tyr) of heparan sulfate-glucosamine 3-sulfotransferase 4 (HS3ST4) and c.4807C>T (p.Arg1603Cys) of KIAA0556 on chromosome 16 (Supplementary Table S5). All variants had been reported as SNPs in dbSNP148 except the missense variant c.798C>G in NDUFAF7. We determined if these mutations were common variants by screening control samples from 50 healthy individuals. All the controls were negative for these seven mutations, except for the mutation in C2ORF71 (Supplementary Table S6). C2ORF71, an RP-associated gene, knockdown of which resulted in visual defects in zebrafish, suggesting that it may play a functional role in the development of normal vision. However, C2ORF71 serves as a causative gene in an autosomal recessive inheritance pattern, and importantly, the c.3527T>C mutation in C2ORF71 showed a relatively high mutation frequency of 2%, being present in a heterozygous state in two of the 50 matched controls (Supplementary Table S6). We therefore excluded the possibility that C2ORF71 c.3527T>C was responsible for the development of PM. GLTPD1 is a ubiquitously expressed lipid transfer protein that is specifically responsible for the trafficking of ceramide-1-phosphate from the Golgi to the plasma membrane and its loss of function would result in disease states associated with inflammation. HS3ST4 encodes the enzyme heparin sulfate 3-O-sulfotransferase 4, which is strongly expressed in the hippocampus and is
thought to play a role in herpes simplex virus type 1 pathogenesis and in memory loss through involvement in the immune response.\textsuperscript{25,26} KIAA0556 encodes a ciliary protein located at the base of cilia, which was reported to be associated with Joubert syndrome and recognized as an autosomal recessive causative gene.\textsuperscript{27,28} ZBTB38 is strongly associated with human height, and encodes a protein that regulates height by specifically binding methylated DNA to repress the transcription of apoptosis genes.\textsuperscript{29,30} Although these four mutations have been found to be related to other diseases and no evidences so far indicates that they are involved in myopia according to their function, we still could not exclude their contribution to myopia development in such a small pedigree. Whereas in the case of NDUFAF7, as a mitochondrial assembly factor, it is involved in mitochondrial respiratory chain system,\textsuperscript{31} and previous research showed that the decline in ATP synthesis via mitochondrial energy metabolism leads to severe visual impairment.\textsuperscript{32} Thus, we decided to focus on the function investigation of the mutant (MT) NDUFAF7 detected as a de novo missense mutation (p.D266E) in this family. As for RHO c.510G>A, it was found in RP patients, and no clear evidence of its pathogenicity has been reported.\textsuperscript{33,34}

**Structural Implications of RHO Amino Acid Substitution**

The V104I mutation in RHO was first discovered in a family with Leber congenital amaurosis by Macke et al.\textsuperscript{33} but did not segregate with the disease and was therefore reported as a polymorphism. Li et al.\textsuperscript{34} subsequently detected it in the heterozygous state in two sporadic Chinese patients with RP and considered it as a possibly benign variation or recessive allele. RHO is a member of the G-protein–coupled receptor protein family and is composed of seven transmembrane helices and one C-terminal domain that can bind to G protein (Supplementary Fig. S1A). It plays a vital role in visualizing objects and is a protein of interest in searching for inherited diseases.\textsuperscript{31} It is thought to play a role in herpes simplex virus type 1 oxidative phosphorylation system. As a methyltransferase, NDUFAF7 methylates Arg-85 in the NDUFS2 subunit of complex I as an early step during the assembly of complex I. Methylation of arginine is required for the formation of the 400 kDa subcomplex, an early assembly intermediate of complex I.\textsuperscript{31} Complex I is not only responsible for generating the transmembrane proton motive force,\textsuperscript{39} but also for mediating electron transfer.\textsuperscript{39,40} It is therefore conceivable that the D266E mutation in NDUFAF7 may influence electron transfer via affecting the assembly of complex I, resulting in alteration to intracellular ROS levels. Overexpression of wild-type (WT) NDUFAF7 resulted in an increase of ROS compared with control cells grown without plasmid transfection, as a result of excessive assembled complex I. However, the D266E mutation profoundly attenuated the increase in ROS levels in the tested cell lines, suggesting that this mutation suppressed intracellular ROS production. These results demonstrated a negative effect of the D266E mutation on electron transfer. Low efficiency in electron transfer usually suppresses ROS formation.\textsuperscript{41} Importantly, the attenuation of ROS production by the mutation was more significant in ARPE-19 cells (>40%) than in the other two cell lines (Fig. 4). ARPE-19 cells are retinal pigment epithelia cells that localize exclusively to the retina. Overall, these results suggest that D266E mutation is likely to be associated with eye disease, based on its more deleterious effect on eye tissue cells.

**NDUFAF7 Molecular Evolution**

A phylogenetic tree of NDUFAF7 can be used for species classification and reveal the evolutionary relationship between species. The D266E mutation occurs within an evolutionarily relatively conserved region based on sequence alignment analysis. The aspartate at position 266 is highly conserved in mammalian species and is completely conserved in the Eutheria clade. Interestingly, an amino acid substitution (glutamate) at 266 occurs in the platypus, a kind of primitive mammal animal laying eggs like a bird, and glutamate at 266 is present as a normal residue in the Aves clade (Fig. 3). The conversion from aspartate to glutamate at residue 266 is thus likely to be harmful in humans, which may be a violation of laws of evolution in the Eutheria clade. In brief, NDUFAF7 p.D266E should not be a common variant in mammalian species.

**D266E Mutation in NDUFAF7 Associated With Reduced Intracellular ROS Levels**

NDUFAF7 is an assembly factor of complex I (NADH ubiquinone oxidoreductase) that is the first complex of the oxidative phosphorylation system. As a methyltransferase, NDUFAF7 methylates Arg-85 in the NDUFS2 subunit of complex I as an early step during the assembly of complex I. Methylation of arginine is required for the formation of the 400 kDa subcomplex, an early assembly intermediate of complex I. Complex I is not only responsible for generating the transmembrane proton motive force,\textsuperscript{39} but also for mediating electron transfer.\textsuperscript{39,40} It is therefore conceivable that the D266E mutation in NDUFAF7 may influence electron transfer via affecting the assembly of complex I, resulting in alteration to intracellular ROS levels. Overexpression of wild-type (WT) NDUFAF7 resulted in an increase of ROS compared with control cells grown without plasmid transfection, as a result of excessive assembled complex I. However, the D266E mutation profoundly attenuated the increase in ROS levels in the tested cell lines, suggesting that this mutation suppressed intracellular ROS production. These results demonstrated a negative effect of the D266E mutation on electron transfer. Low efficiency in electron transfer usually suppresses ROS formation.\textsuperscript{41} Importantly, the attenuation of ROS production by the mutation was more significant in ARPE-19 cells (>40%) than in the other two cell lines (Fig. 4). ARPE-19 cells are retinal pigment epithelia cells that localize exclusively to the retina. Overall, these results suggest that D266E mutation is likely to be associated with eye disease, based on its more deleterious effect on eye tissue cells.

**D266E Mutation in NDUFAF7 Causes Reduced Intracellular ATP Levels**

ROS are important indicators reflecting the mitochondrial respiratory chain activity, which is proportional to ATP production.\textsuperscript{42} We investigated if the D266E mutation altered ATP production. Wild-type and mutant proteins, respectively, were expressed in ARPE-19 cells, with cells transfected with pcDNA3.1-green fluorescent protein (GFP) as a control to account for the effects of transfection and protein overexpression. The D266E mutation resulted in an approximately 19% decrease in ATP levels compared with wild-type NDUFAF7, indicating that the mutation had an inhibitory effect (Fig. 5). Overexpression of wild-type NDUFAF7 was associated with a significant increase in ATP levels compared with GFP-transfected cells (P < 0.05), whereas a negligible (P > 0.1) change was observed in cells expressing mutant NDUFAF7. Collectively, the D266E mutation in NDUFAF7 suppressed the production of ATP.

**D266E Mutation in NDUFAF7 Causes Reduced Mitochondrial Complex I Activity**

NDUFAF7 contains the DUF185 methyltransferase motif; however the D266E mutation does not affect this domain, suggesting that this mutation might not affect its catalytic function in methylation. According to HOPE analysis (http://
www.cmbi.ru.nl/hope/home), D266 is located on the surface of the NDUFAF7 protein, and its replacement by glutamate would thus extend the branch on the surface, potentially disturbing the interactions between NDUFAF7 and its substrate NDUFS2, thereby influencing the efficiency of methylation and subsequent assembly of complex I. Consistent with the result for ATP, complex I activity was reduced by approximately 9% in cells transfected with mutant compared with wild-type NDUFAF7, while complex I activities were similar in mutant- and GFP-transfected cells ($P > 0.1$; Fig. 6). We considered that
the D266E mutation in NDUFAF7 may affect the contact of NDUFAF7 with NDUFS2, thus lowering its binding to NDUFS2, impairing the methylation efficiency, and affecting complex I assembly. In contrast, excess mutant protein in mutant-transfected cells may rescue the defect of efficiency in complex I assembly. There was accordingly no significant change in complex I activity in mutant NDUFAF7-expressing cells. An inhibitory effect on complex I activity was also previously observed in the cells in the absence of NDUFAF7 using siRNA.

These experiments, together with the predicted structural variation, suggest that the mutation at position 266 in NDUFAF7 might suppress the assembly of complex I but have no effect on protein methylation function.

Cellular Localization and Stability of Mutant NDUFAF7 Protein

We determined if the D266E mutation may decrease complex I activity by affecting the subcellular localization or stability of NDUFAF7. NDUFAF7 has been confirmed to locate in the mitochondrial matrix and immunofluorescence showed an identical localization of the mutant protein with the mitochondrial compartment in ARPE-19 cells (Fig. 7A). We also detected the location of the mutant protein in two other, noncancerous somatic cell lines, E6E7 and 293T, and in HeLa cancer cells. Without exception, mutant NDUFAF7 was localized in the mitochondria (data not shown) in all tested cells, suggesting that the D266E mutation has no effect on the subcellular localization of NDUFAF7. We also determined if the D266E mutation affected protein stability by subjecting whole-cell proteins to immunoblot analysis (Fig. 7B). Protein expression levels of mutant NDUFAF7 were identical to those of wild-type NDUFAF7, indicating no effect of the D266E mutation on NDUFAF7 protein stability. The lack of effect on the subcellular location and stability of NDUFAF7, together with no effect on methylase function, suggest that the mutation might inhibit methylation efficiency of NDUFAF7 for substrate, resulting in impaired complex I assembly in eye tissue of affected individual.

DISCUSSION

The genetic basis of PM has attracted increasing interest in recent years. Twenty-two myopia loci have been documented...
in the Online Mendelian Inheritance in Man to date, of which 16 are high myopia loci. Although many candidate genes have been reported, the precise pathogenic mechanism responsible for PM remains unknown.

In the current study, the usefulness of two-point linkage analysis is limited due to the small size of the pedigree, even when considering the superiority of SNP haplotype markers as an increase of heterozygosity in linkage statistics (data not shown). Thus, cosegregation analysis has been performed for this family. However, the size of cosegregated regions identified in this small pedigree are so large that we have to choose whole-exome sequencing to screen the candidate mutations in order to avoid missing the real causative mutation in the pedigree (Supplementary Table S3). Finally, we identified a variant in NDUFAF7 (p.Asp266Glu) that might contribute to the development of PM. The decrease in complex I activity in mutant NDUFAF7-expressing cells, resulted in the decrease of ATP production. ATP is able to be utilized for guanosine diphosphate (GDP) regeneration by the nucleoside-diphosphate kinase reaction: ATP + guanosine diphosphate (GDP) → ADP + GTP. The resulting decreased GTP level then weakens the photopic and scotopic responses of photoreceptor cells. Photoreceptors have previously been shown to have high energy demands, consuming approximately 2.5 × 10⁷ ATP molecules per second in the dark and 10⁸ ATP per second in the light. ⁴⁸ The retina usually consumes energy more rapidly than the brain, which is known to require high levels of energy supply. ⁴⁹ A decrease in ATP supply will affect postsynaptic responses in both the light and the dark (Fig. 8). When exposed to light, 11-cis-retinal is converted to all-trans-retinal, causing a conformational change in rhodopsin, which then activates the α-subunit of G-protein by GDP/GTP exchange. The activated G-protein further activates its effector, cyclic guanosine monophosphate (cGMP) phosphodiesterase, which hydrolyzes cGMP to 5′GMP. The resulting decrease in cGMP causes closure of the cyclic nucleotide gated (CNG) channels in the plasma membrane, blocking the inward flow of sodium and calcium and hyperpolarizing the membrane. This change in membrane potential is transmitted as a neural signal through the rod bipolar cells of the retina to the ganglion cells, and ultimately to the brain. Intracellular ATP levels were generally reduced in cells with D266E-mutated NDUFAF7, resulting in reduced local GTP regeneration, and negatively affecting the light-activated phototransduction cascade in photoreceptor cells. The reduced production of activated G-protein causes only partial CNG channels to close, leading to incomplete hyperpolarization (under-hyperpolarization; Fig. 8), thus decreasing the light sensitivity of photoreceptor cells.

In contrast, high concentrations of cGMP are produced in the dark by transmembrane guanylyl cyclases (GC-E/F) located at the disc membrane, to maintain CNG channels in the open state. Open CNG channels allow the influx of sodium and calcium to depolarize the photoreceptor synaptic terminal. The D266E mutation is associated with reduced GTP concentrations, contributing to reduced levels of the second messenger cGMP, incomplete opening of CNG channels, and insufficient depolarization (under-depolarization; Fig. 8).

In summary, the D266E mutation in NDUFAF7 affects the state of CNG channels by altering cellular cGMP concentrations, leading to insufficient hyperpolarization or depolarization of photoreceptor cells and consequently low light sensitivity. However, the decrease in ATP levels is relatively small (~19%), indicating that the damage to photoreceptors is probably cumulative. This may also explain why the severity of eye damage gradually increases with age. The short of energy supply might lead to optical nerve, a high energy-consuming tissue, remaining at the insufficient levels of ATP during its development. However, the degree of myopia of patient II-3 was more serious than patient III-2. Patient II-3 was born in the 1960s, which was a resource-scarce period in China, suggesting that poor living conditions might have amplified the damaging effects of the D266E mutation on eye development. Recent studies have also indicated that the differences in the environments for child growth can cause a strong myopic shift from one generation to the next. ⁵⁰ Moreover, the congenital vision loss in patient II-3 was also likely to be associated with defective mitochondria inherited from her mother (I-2) at the oocyte stage. Overall, favorable living conditions could slow
the progression of myopia in carries of the D266E mutation, but age-related visual deterioration will still occur, as seen in patient III-2 who may have experienced better living conditions than the proband.

Early genetic screens have highlighted the importance of mitochondria in visual function. Gronlund et al. 58 recently performed a variety of ophthalmologic assays in 59 patients with mitochondrial disease associated with known mutations. Of these patients, 81% had ophthalmologic defects such as reduced eye movement, external ophthalmoplegia, strabismus, nystagmus, low visual acuity, photophobia, optic atrophy, and refractive errors.58 However, little is known about the precise role of mitochondria in eye disease. The mutation identified in the current study has established a mechanistic connection between PM and mitochondria defects. Overall, the results suggest that the D266E mutation in NDUFAF7 has a profound impact on vision development. Future studies are needed to elucidate the mechanisms whereby the mutation influences optical nerve development and to define its synergistic effect with the environment.

We do not know whether four other variants in GLTPD1, ZBTB38, HS3ST4, and KIAA0556 should be considered benign or possible myopia-causing mutations. Although these mutation-containing genes have been found in other diseases irrelevant to myopia,24–30 they may still play some minor roles in PM as genetic modifiers or the helper of mutated NDUFAF7 due to their cosegregation with PM in the family. Further analysis of additional patients and performing function investigation for them may provide some suggestive evidence to clarify their roles in PM disease.

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