Mutation report

A novel mutation in the *NYX* gene associated with high myopia, but not congenital stationary night blindness

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ABSTRACT

Background: Myopia is a complex eye disorder. The complex form of X-linked congenital stationary night blindness (CSNB1) is usually associated with moderate to high myopia, and is caused by mutations in the *NYX* gene. We explored if NYX mutations could be associated with high myopia, but not CSNB1.

Methods: The coding regions of the NYX gene was sequenced for 204 Chinese males with high myopia (-8.00 dioptres or worse for both eyes). The frequencies of any sequence variations identified were determined in 200 Chinese males without myopia.

Results: A non-synonymous mutation (c.529_530GC>AT or p.Ala177Met) was identified in one male subject with high myopia, but not in 200 male emmetropes. The mutation was predicted to affect the protein function. From ocular electrophysiological tests, the proband was found to have normal rod function, but mildly abnormal in cone function and inner retina function. He did not suffer from CSNB1.

Conclusion: One novel non-synonymous mutation was identified in an adult male presented with high myopia, but not CSNB1.

INTRODUCTION

Myopia or short-sightedness is the most common disease of the eye worldwide and is one of the leading causes accounting for blindness.[1] It is getting more frequent in the younger generations, and is more prevalent in Asian populations than in Caucasian populations. It is a complex disease influenced by genetic and environmental factors, and their interactions.[2,3]

The *NYX* gene (GeneID: 60506 and MIM: 300278) is located at chromosome Xp11.4, and has two exons.[4] It produces a transcript of 2.7 kb, and encodes the cell surface protein nyctalopin (NYX) with 481 amino acids. The exact function of NYX is not yet known. The protein is found in the post-synaptic side of the photoreceptor-to-ON bipolar cell synapse in the retina,[5] and is widely believed to play an important role in synapse transmission.

There are several forms of night blindness in humans. The complete form of X-linked recessive congenital stationary night blindness (CSNB1) is caused by mutations in the *NYX* gene.[6,7] CSNB1 is characterized by a selective defect in the retinal ON pathway and moderate to high myopia.[8] The defect in the retinal ON pathway results in a non-recordable dark-adapted rod-mediated b wave of the electroretinogram (ERG), which is ultimately derived from the depolarizing bipolar cells. The *nob* (meaning *no b wave*) mouse is a naturally occurring mouse model of CSNB and carries a null mutation in the *Nyx* gene.[9,10] Intriguingly, the nob mice were found to show a significant myopic shift (~ 4 dioptres [D]) in refractive error after 2 weeks of form deprivation, relative to the opposite and control eyes.[11] In contrast, wildtype mice produced a similar response only after 6 weeks of form deprivation. This clearly demonstrates the high susceptibility of the nob mice to experimental myopia. More intriguing is the recent identification of missense mutations in two male probands with high myopia, but without night blindness.[12] As such, this study aims to investigate whether *NYX* mutations are associated with high myopia but not congenital stationary night blindness in a cohort of 204 male high myopes.

PATIENTS AND METHODS

Approval was obtained for the study from the Human Subjects Ethics Subcommittee of The Hong Kong Polytechnic University, and the tenets of the Declaration of Helsinki were observed. Unrelated Han Chinese subjects were recruited for our on-going Myopia Genetics Study with the following entry criteria of spherical equivalent (SE) for both eyes: -8.0 D or worse for probands with high myopia, and within ±1.0 D for controls (emmetropes). Subjects were excluded if they showed obvious signs of ocular diseases or other inherited diseases associated with myopia. After giving written informed consent, all subjects received a complete ocular examination (visual acuity, refraction, slit lamp and dilated fundus examination) in the Optometry Clinic of The Hong Kong Polytechnic University as described previously.[13] DNA was extracted from venous blood samples with a modified salting-out method [13] or the FlexiGene DNA Kit (Qiagen).

We screened male high myopes (n=204) in our collection for *NYX* mutations because recessive *NYX* mutations, if any, will show phenotypic effects in hemizygous males. Exon 1 of the *NYX* gene was amplified in one fragment while exon 2 was amplified as two separate fragments with standard protocols. The amplified fragments were sequenced with BigDye Terminator Cycle Sequencing Kit (ver. 1.1; Applied Biosystems) according to the manufacturer's instructions. Primer sequences and reaction conditions are available on request. The allele frequency of any sequence variation identified in the probands was determined in a group of 200 males without myopia by means of restriction fragment length polymorphism technique according to the protocols from the manufacturer (Fermentas). Functional consequences of non-synonymous mutations were predicted using Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/www/SIFT_seq_submit2.html).[14,15] Multiple sequence alignment was extracted from PolyPhen-2 output with the sequence from Chimpanzee added. The NYX protein model and the mutations of interest were generated using the Coot programme (http://www.biop.ox.ac.uk/coot/),[16] and the energy state of each mutant was decided after coordination of systematic prediction and environment-dependent optimization. The protein images were produced using the CCP4MG program (http://www.ysbl.york.ac.uk/~ccp4mg/).[17]

A proband was found to harbour a non-synonymous mutation, and was followed up with ocular electrophysiological tests. Electro-oculogram (EOG) measurements were obtained with a Ganzfeld stimulator (GS-2000; Nicolet Biomedical Inc.) according to the Standard of the International Society for Clinical Electrophysiology of Vision: the amplitudes of EOG in both light and dark phases were recorded and the Arden ratios were calculated.[18] All five basic dark-adapted and light-adapted full-field flash electroretinograms (ERGs) were sequentially recorded with the use of a Ganzfeld stimulator according to the Standard of the International Society for Clinical Electrophysiology of Vision.[19] The amplitude and implicit time of ERG responses were measured.

RESULTS

Four sequence variations were identified by direct sequencing of the *NYX* coding regions in 204 male high myopes: c.529_530GC>AT (p.Ala177Met; 1/204), c.843G>A (rs3810733 or p.Glu281Glu; 2/204), c.1198G>A (p.Gly400Ser; 7/204) and c.1227C>T (p.Thr409Thr; 4/204). Their respective allele frequencies in a group of 200 male emmetropes as determined by restriction digestion were 0/200, 3/200, 7/200 and 14/200. Sequence variations were named using standard nomenclature,[20] and based on NM_022567 for coding sequences and NP_072089 for amino acid sequences. Two changes were synonymous while one non-synonymous change (c.1198G>A; p.Gly400Ser) is a documented polymorphisms.[12] The two-base substitution c.529_530GC>AT altered the amino acid (alanine) at position 177 of the protein (fig 1A), which is conserved in 16 species from human to frog (fig 2). This non-synonymous alteration (p.Ala177Met) was predicted to be *probably damaging* by Polyphen-2, and to *affect protein function* by SIFT. This amino acid is located within the 5th leucine-rich repeat (LRR) of the protein. Protein modeling predicted that the more bulky side chain of methionine would push apart adjacent loops formed from leucine-rich repeats (fig 3) and hence affect the protein function.

The p.Ala177Met mutation was found in a 46 year-old severely myopic man (II-2, fig 1B) with

spherical equivalent of -10.00 D (OD) and -9.00 D (OS) and elongated eyeballs of >28 mm. He first wore spectacles at the age of 8 years. His corrected visual acuity was normal for right eye, but slightly reduced for left eye (0.2, LogMAR). Fundus examination showed that the <<margin?? peripheral retina >> of the left eye was not distinct and the cup/disc ratio of the right eye was at 0.7, and that the fundus of both eyes were tigroid in appearance (fig 1C). He had no problem in walking in dim environment. EOG measurements revealed an abnormal Arden ratio of 1.54 for the left eye, indicating that the function of retinal pigment epithelial cells of the left eye was abnormal. In the flash ERG measurement, the normal scotopic (dark-adapted) rod responses from both eyes (fig 4) implied a normal rod function. The maximal flash mixed rod-cone response, except the a-wave implicit time, and photopic (light-adapted) cone response from both eyes were within normal ranges, though close to the lower limits. In particular, maximal flash response exhibited distinct b-wave. However, the photopic oscillating potentials and 30Hz flicker cone response were abnormal. Taken together, this subject had normal rod function, but was abnormal in cone function and the inner retinal function of both eyes. His parents were not available for examination, but were reported to have neither myopia nor night blindness. The eye examination results for his wife and his son were unremarkable (fig 1B).

DISCUSSION

A novel non-synonymous mutation (p.Ala177Met) was identified in a male proband with high myopia, but not CSNB1. This mutation was not found in 200 male emmetropes. The 177th amino acid is located within the 5th LRR of the human nyctalopin and is conserved in 16 species from human to frog (fig 2). The mutation was predicted to be deleterious and affect the protein function. Another reported *NYX* mutation causing high myopia but not CSNB1 was found 14 residues downstream within the 6th LRR: p.Arg191Gln (or c.572_573GC>AA).[12] Amino acid Arg191 is conserved in 11 of the 16 species aligned (fig 2). Both mutations are predicted by the Coot program to *reduce* the stability of the protein structure (fig 3). Intriguingly, within the 5th and 6th LRRs are three other amino acid residues for which mutations have been reported to cause CSNB1 (associated with high myopia): p.Pro175Arg, p.Leu184Pro and p.Ala187Lys.[6,7] Of these, two mutations (p.Pro175Arg and

p.Ala187Lys) are predicted to *increase* the structural stability of the protein (fig 3). Amino acids Pro175 and Leu184 are conserved in all 16 species compared while Ala187 is only conserved in 7 species from human to rabbit. Even so, it is still difficult to explain why different mutations in the *NYX* gene produce different phenotypes. With 11 LRRs, nyctalopin is predicted to be involved in protein-protein interactions.[21] As such, identification and structural characterization of its protein-protein interactions with other proteins involved in the synapse transmission should be useful in elucidating the different phenotypic effects conferred by different mutations. In fact, it is not uncommon that different mutations in a gene expressed in the eye cause different ocular phenotypes.

This is the third *NYX* mutation reported to date to be associated with high myopia, but not CSNB1. The other two mutations were also reported in Chinese: p.Cys48Trp and p.Arg191Gln.[12] The p.Cys48Trp was a *de novo* mutation identified in 8-year-old boy without ERG results. The p.Arg191Gln mutation was found in an adult. This latter patient and our proband both had normal functions for rods and the corresponding bipolar cells as indicated by normal b-waves in scotopic rod-mediated response and in scotopic maximal flash response, which are characteristically much reduced or absent in CSNB1. This indicates that these mutations did not result in complete loss of function. On the other hand, the patients' cone function was slightly reduced with abnormal 30Hz flicker cone response, and their inner retina function was also abnormal with reduced photopic oscillatory potentials (mediated by amacrine cells in the inner plexiform layer). The patient with the p.Arg191Gln mutation, but not our proband, also had abnormal photopic single-flash cone response. Therefore, there is emerging evidence that the cone-mediated ON pathway may be involved in myopia development.[12]

Myopia is a complex disease.[2,3] On school of thought favours the model of common disease common variants in which several common variants, with small effect size individually, contribute to the disease phenotype.[22] Such common variants are best identified by genetic association studies and a few common myopia susceptibility genes have been quite reproducibly identified by this approach, e.g. *TGFB1* and *HGF*.[13,23-26] Another school of thought favours the model of common

disease rare variants in which many rare variants, with much bigger effect size individually, contribute to the common disease phenotype.[22] Genetic association studies have very limited power in detecting such rare variants, which are in fact best identified by re-sequencing of carefully selected biological plausible candidate genes as has been done in this study and a few other studies.[12,27] Obviously, both models are not mutually exclusive and can co-exist for any given disease as exemplified by myopia and other examples.[28,29] The search for rare variants contributing to common diseases is riskier and much more costly when compared to the search of common variants from a practical viewpoint, but should still be pursued in order to have better understanding of the genetic basis underlying complex diseases such as myopia.

In conclusion, a novel non-synonymous *NYX* mutation (p.Ala177Met) was identified in an adult male presented with high myopia, but not CSNB1.

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Patient consent: Obtained

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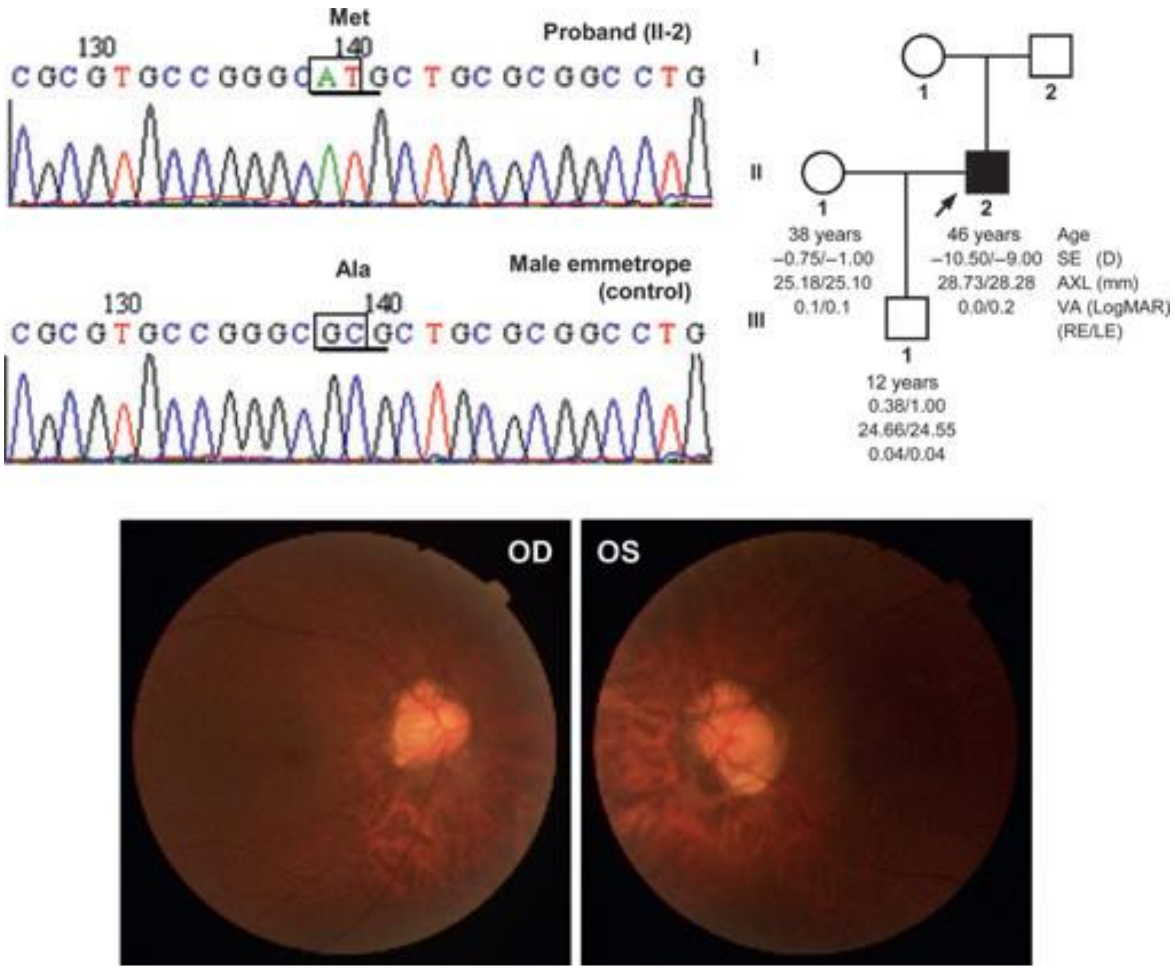

Figure 1. The results of the male proband presented with high myopia, but not congenital stationary night blindness. (A) Sequencing shows the c.529_530GC>AT (p.Ala177Met) mutation in the proband (II-2) in comparison to the sequence from a male control subject. (B) The family tree of the proband (II-2). The ocular data are shown for the right eye (OD) and the left eye (OS), including spherical equivalent (SE) in dioptres (D), axial length (AXL) in mm and visual acuity (VA) in LogMar scale. (C) The proband's tigroid fundus picture is typical of the changes seen in high myopia.

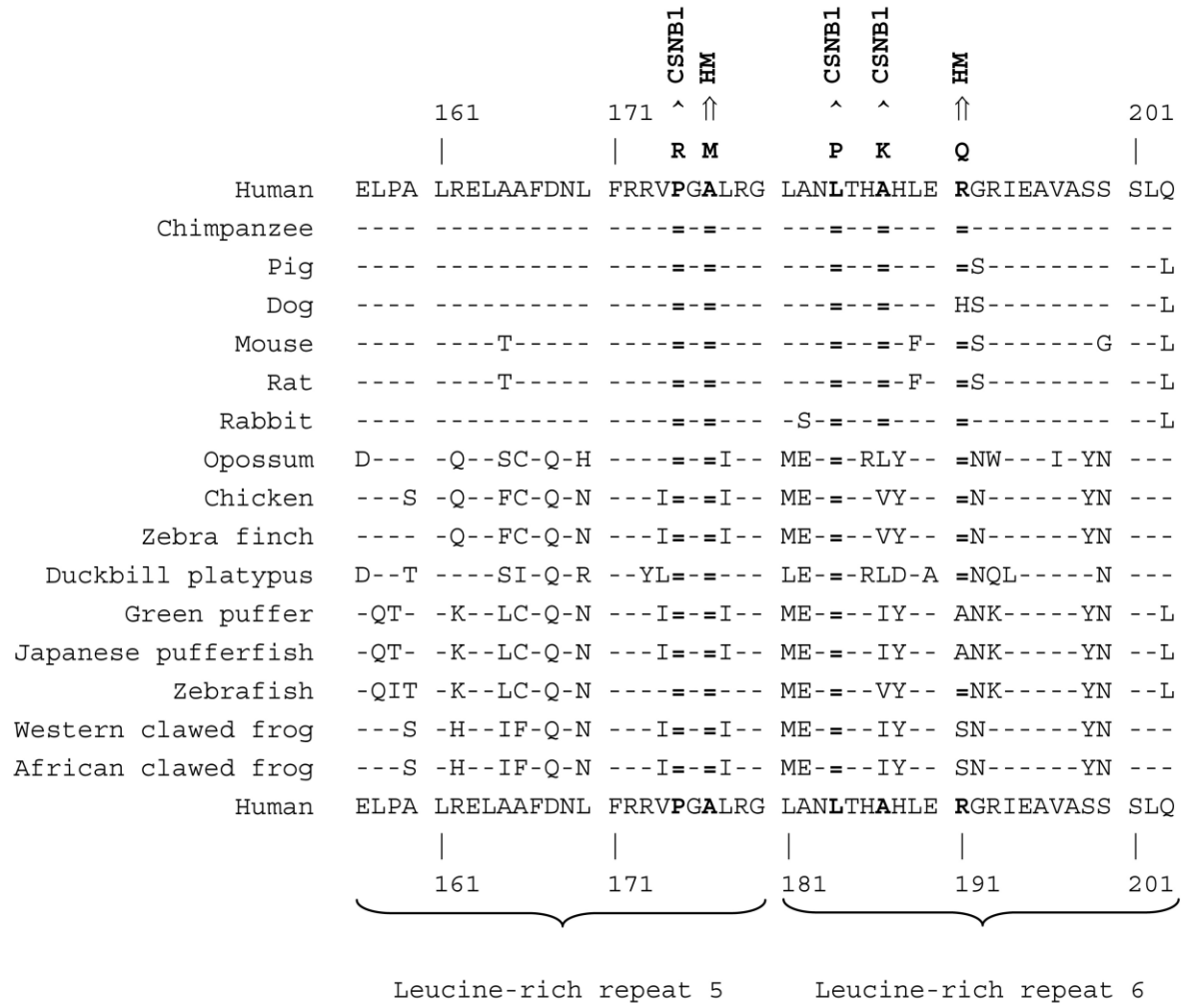

Figure 2. Multiple sequence alignment for the NYX amino acid sequences. The amino acid sequences of the 5th and 6th leucine-rich repeats of the human nyctalopin are aligned with those from 15 other species. The numbering is based on the human protein. Sequence identity with the human protein is indicated as " – " or "=" if mutations have been reported for the amino acid in the human protein (shown in boldface). The mutations cause either congenital stationary night blindness type 1 (CSNB1, associated with myopia) or high myopia (HM, without CSNB1).

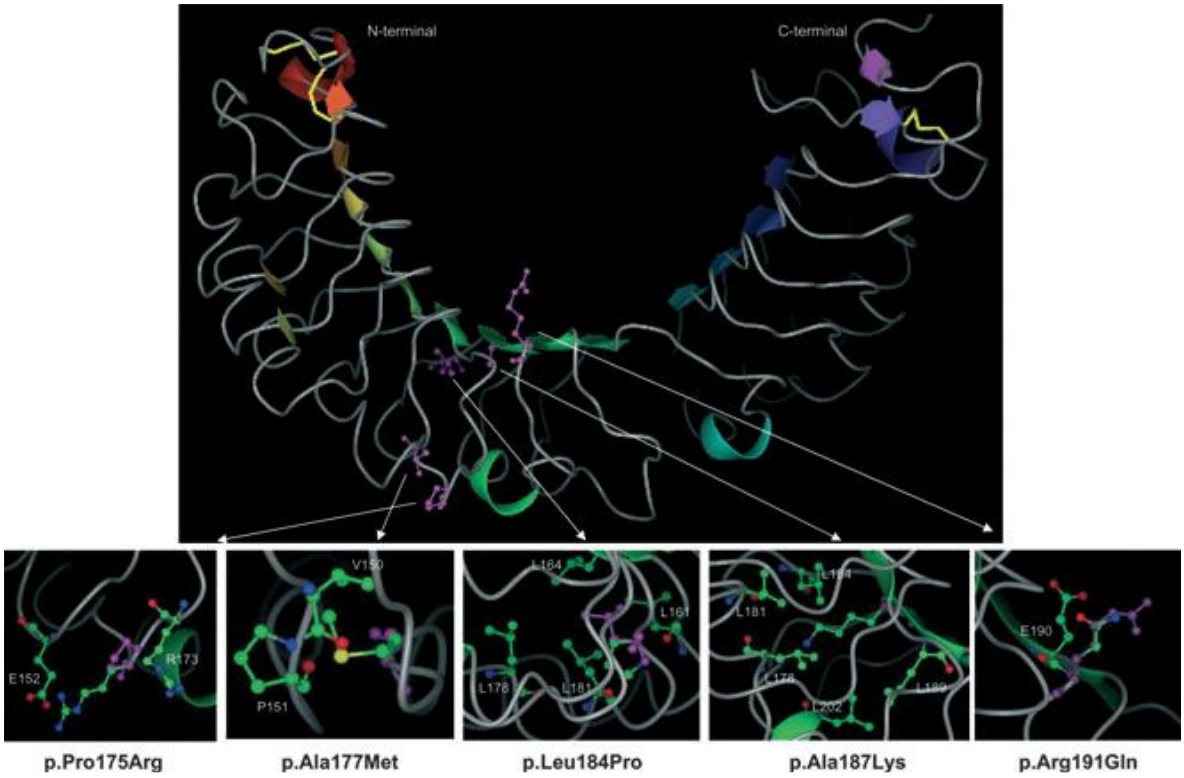

Figure 3. The NYX protein structure and the five mutations within the 5th and the 6th leucine-rich repeats. The protein structure (the top panel) and the mutations (bottom panel) are modeled using the Coot program, and the images are produced using the CCP4MG program. …..

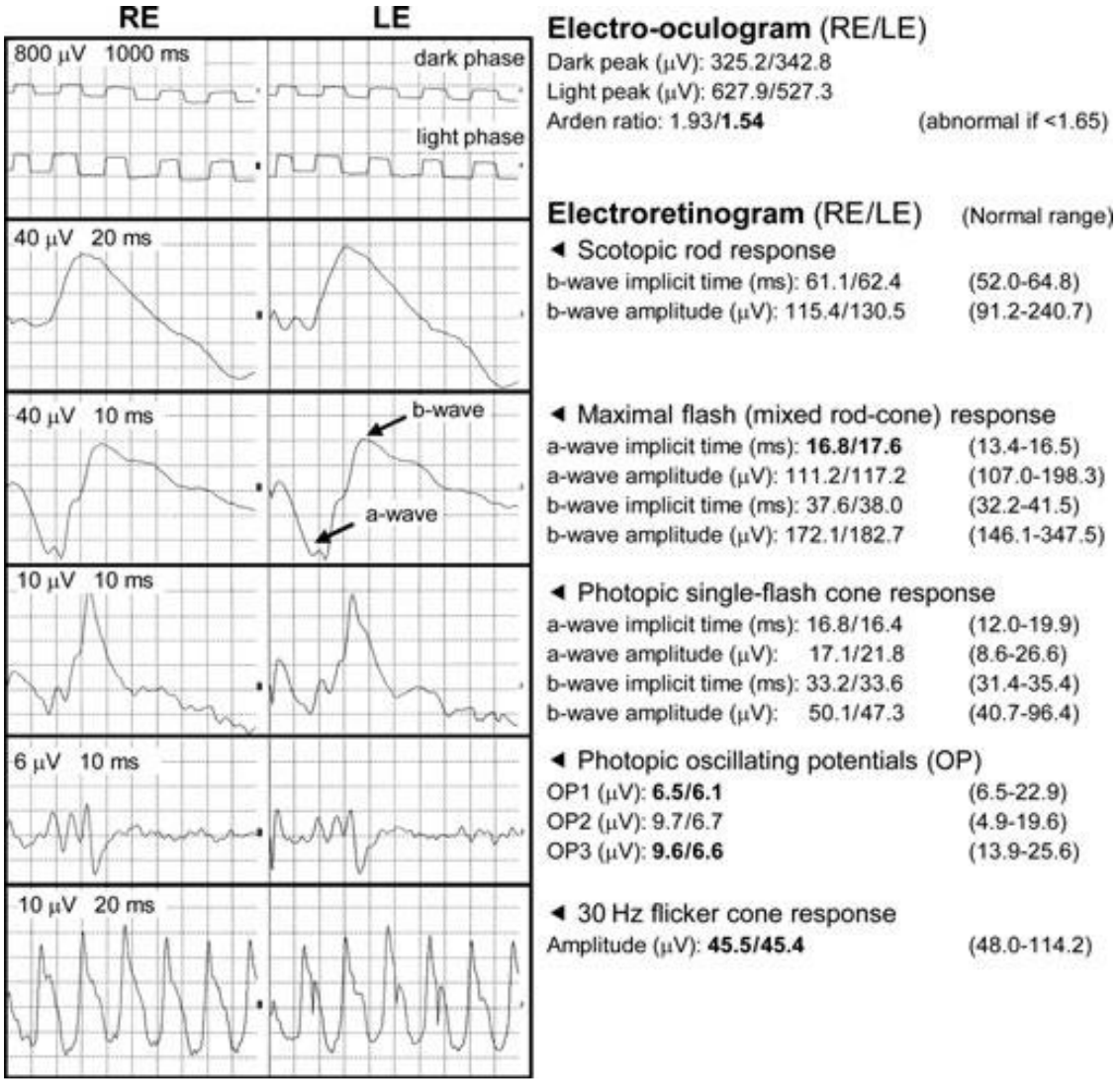

Figure 4. Electrophysiological test results of the male proband presented with high myopia, but not congenital stationary night blindness. The left panel shows the recordings of the electro-oculogram and electroretinogram for the right eye (OD) and the left eye (OS) with the vertical voltage scale (in μV) and the horizontal time scale (in milliseconds) of the grids shown on the top left side for each recording. The right panel shows the corresponding numerical readings together with the normal ranges used in our Optometry Clinic.