

# A Novel Mutation in the Choroideremia Gene in a Turkish Family

Mustafa Iftikhar<sup>1</sup>, Yulia Wolfson<sup>2</sup>, Simrat Sodhi<sup>2</sup>, Bushra Usmani<sup>1</sup>, Hendrik P. N. Scholl<sup>2</sup> and Syed M.A. Shah<sup>3</sup>

## ABSTRACT

Choroideremia is an X-linked recessive genetic disorder caused by mutations in the CHM gene. It is a rare retinal dystrophy that manifests as nyctalopia and vision loss, progressing to blindness in later stages. We report a 21-year Turkish man who presented with nyctalopia for the past 4-5 years. His mother and maternal grandmother had similar, but less pronounced complaints. Fundus examination revealed pigmentary changes and retinal atrophy in both eyes. Optical coherence tomography showed outer retinal loss, with central island of preserved autofluorescence surrounded by absent autofluorescence on fundus autofluorescence examination. Goldmann visual fields were constricted. Microperimetry detected retinal sensitivity losses, and full-field electroretinogram demonstrated extinguished cone responses. Genetic analysis revealed a novel nonsense mutation in the CHM gene, namely p.E480X: c.1438G >T. The mutation causes a premature stop codon in exon 12. This is the first report of a G1438T mutation resulting in an E480X premature stop in the CHM gene.

**Key Words:** Choroideremia, Genetics, Mutation, Retinal degeneration.

## INTRODUCTION

Choroideremia is a rare (1:50,000 males) X-linked recessive retinal dystrophy caused by mutations in the CHM gene (Xq21), which encodes component A of Rab geranyl-geranyl-transferase, also referred to as Rab escort protein-1 (REP-1).<sup>1</sup> It is characterised by progressive chorioretinal degeneration with early retinal thickening, followed by photoreceptor degeneration, retinal pigment epithelium (RPE) depigmentation and retinal remodelling.<sup>1</sup> Clinical manifestations include nyctalopia and peripheral vision loss which gradually progress to central vision loss and eventual blindness in the later stages of the disease.<sup>1</sup> The disease primarily affects males, although female carriers can demonstrate patchy chorioretinal atrophy, with either no symptoms or a mild degree of nyctalopia.

To date, many mutations in the CHM gene have been identified in different ethnic populations, including deletions, insertions, duplications, translocations, splice-site, frameshift, nonsense and missense mutations.<sup>2</sup>

We report a novel mutation identified in the CHM gene of one affected male in a Turkish family, along with related ocular findings.

## CASE REPORT

A 21-year man from a Turkish family presented to the Hospital for difficulty seeing in the dark for the past 4-5 years and reported a recent diagnosis of choroideremia in Turkey. The patient had no other complaints and his past medical history was unremarkable.

The patient had no siblings, and there were no known problems on the paternal side of his family. His mother also reported difficulty seeing in low light situations, although it did not seem to affect her functionality. She was noted to have pigmentary changes on fundus photographs taken in Turkey, along with normal findings on optical coherence tomography (OCT) and microperimetry (MP). Her own mother (the patient's maternal grandmother) also complained of poor night vision. She had 2 sisters, both of whom were healthy and had no visual problems. One of the sisters had an 18-year son who did not have any visual problems. The remainder of the family history was unremarkable, and consanguinity was denied. Figure 1 describes the patient's pedigree.

On examination, the patient's best-corrected visual acuity was 20/20 in both eyes. Intraocular pressure was 10 mmHg in both eyes, extraocular movements were intact, visual fields were full to confrontation and pupils were equal and reactive to light. Slit-lamp examination was normal. Fundus examination revealed waxy pallor of the optic discs along with pigmentary changes and retinal atrophy in both eyes.

OCT demonstrated peripheral loss of outer retinal layers (including the inner segment/outer segment junction and the retinal pigment epithelium) in all quadrants, sparing the fovea (Figure 2A). Fundus autofluorescence revealed a central island of preserved autofluorescence surrounded by absent autofluorescence (Figure 2C). MP demonstrated retinal sensitivity losses within the central 20 degrees (Figure 2D). Goldmann visual fields showed

<sup>1</sup> Medical College, Aga Khan University Hospital, Karachi, Pakistan

<sup>2</sup> Department of Ophthalmology, The Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore MD 21287, USA

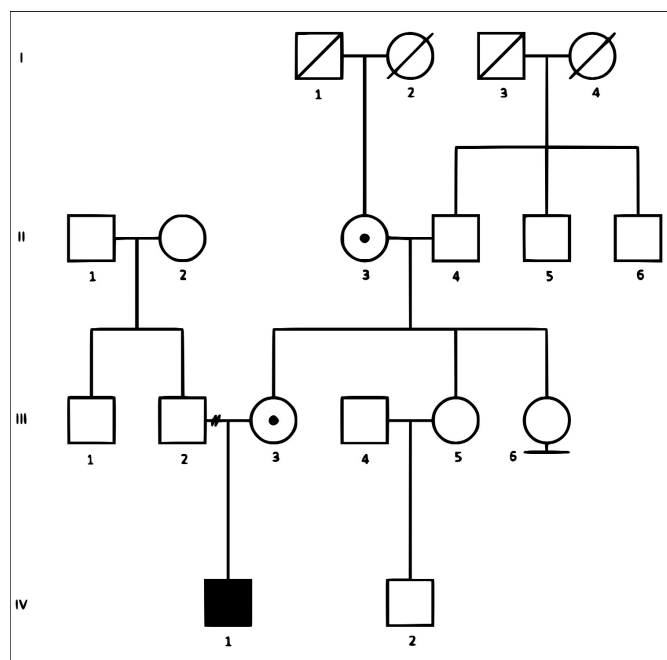
<sup>3</sup> Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Correspondence: Dr. Syed Mahmood Shah, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

E-mail: sms@qvr.net

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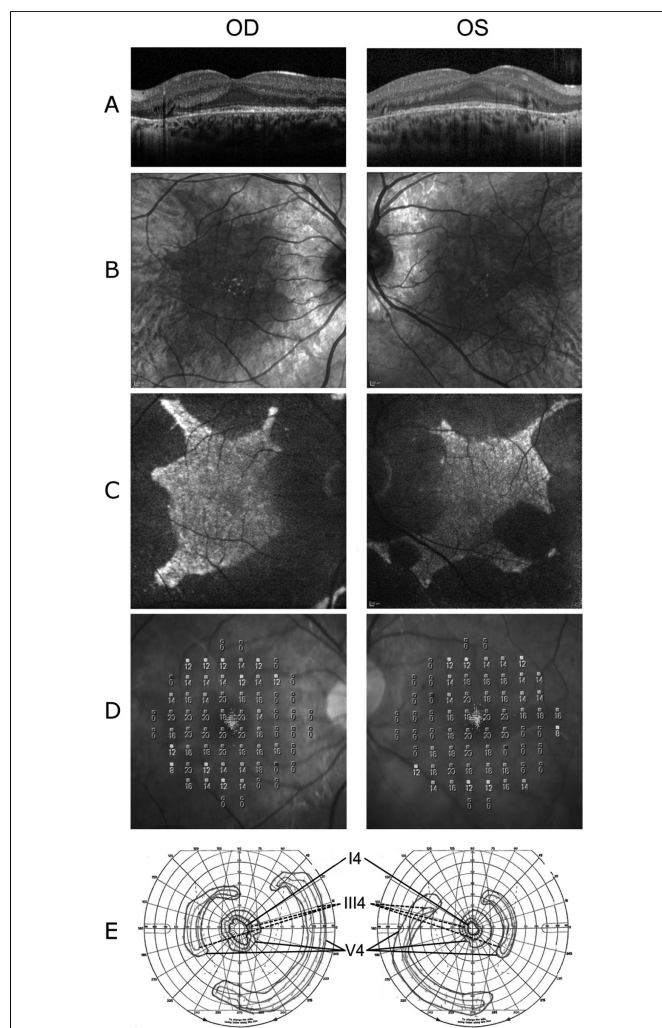
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**Figure 1:** Pedigree of a family with choroideremia. Case IV-1 is the proband and is genetically confirmed. Cases III-3 and II-3 are presumed to be carriers for the mutation.

circumferentially constricted fields (below 20 degrees radius) for all isopters, with temporal peripheral and nasal mid-peripheral crescents of vision (Figure 2E). Full-field electroretinogram demonstrated extinguished rod and cone responses, and dark adaptometry detected an elevated adaptation threshold.

After informed consent was obtained, genomic DNA was extracted from leukocytes of peripheral blood. PCR amplification were carried out at the Casey Institute (CEI) (Portland, OR, USA). Subsequent sequencing was performed at Seqwright Inc. (Houston, TX, USA). Direct testing for mutation in the CHM gene was performed by PCR amplification and DNA sequencing in two directions of all coding exons and exon/intron boundaries. PCR amplification utilised the forward primer 5'-CAAATCT GTTCCAAAATTAATCC-3' and reverse primer 5'-CAACTGTAATAACTGCCCCCTTT-3'. Amplification occurred at 96°C for 3 mins; 94°C for 30s; 10 cycles at 55-65°C for 30s; 72°C for 30s; 94°C for 30s; 30 cycles at 55°C for 30s; 72°C for 30s; 4°C hold in an Applied Biosystems 2720 Thermocycler (Thermo Fisher Scientific, Halthorpe, MD, USA). The PCR product was purified and then subjected to 96-Well High-Throughput DNA sequencing by Seqwright Inc. Sequencing was performed on a bank of ABI Prism 3730xl DNA sequencers (Applied Biosystems) after being subjected to the BigDye Terminator reagent kit. Codon 1 corresponded to the start ATG and nucleotide 1 to the A. The reference sequence for CHM was NM\_000390.2 ' NP\_000381.1. The sensitivity of DNA sequencing was 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analysed.



**Figure 2:** Imaging of a Turkish patient with choroideremia. (A) Spectral-Domain Optical Coherence Tomography demonstrates peripheral loss of outer retinal layers (including the inner segment/outer segment junction and the retinal pigment epithelium). (B) Infrared Reflectance imaging. (C) Fundus Autofluorescence reveals a central island of preserved autofluorescence surrounded by absent autofluorescence. (D) Microperimetry demonstrates retinal sensitivity losses. (E) Goldmann Visual Fields show circumferentially constricted fields (below 20 degrees radius) for all isopters, with temporal peripheral and nasal mid-peripheral crescents of vision.

The patient possessed a novel nonsense mutation in the CHM gene, namely p.E480X: c.1438G >T. The mutation causes a premature stop codon in exon 12.

### DISCUSSION

Choroideremia is a rare genetic disorder caused by mutations in the CHM gene. The CHM gene has been mapped to Xq21.1-q21.3, consists of 15 exons and encodes an intracellular protein known as REP-1. REP-1 is involved in the regulation of intracellular vesicle transport and posttranslational modification in lipid prenylation. It is actively expressed in ocular tissues such as retina, choroid, and RPE. Deficiency of REP-1 results in disruption of intracellular transport and premature cell death.<sup>3</sup> Thus, CHM mutations causing loss of function lead to progressive chorioretinal degeneration.

Clinically, the disease manifests as nyctalopia and reduced peripheral vision followed by loss of visual acuity that may result in legal blindness by the fourth decade of life.<sup>1</sup> Even though most patients with choroideremia have a characteristic phenotype, especially the distinctive autofluorescence appearance as seen in this patient, in some cases it can be difficult to clinically differentiate the disease from other peripheral inherited retinal degenerations such as retinitis pigmentosa.<sup>4</sup> Genetic sequencing can confirm the diagnosis and identify up to 94% of causative mutations, the majority of which are null mutations. This is particularly important because patients with null mutations may benefit from gene replacement therapy, which is being actively tested and has shown promising results so far.<sup>5</sup> Therefore, genetic testing is an important modality in the management of choroideremia patients and will likely play a larger role in the future.

The most common types of mutations identified by genetic testing include insertions or deletions, single point mutations, and mutations that affect splicing. Most small mutations are located earlier in the 700 to 900 region, since it has a higher number of CpG sites where C to T transition can result in a premature stop codon and significantly truncate the protein. We report a novel nonsense mutation in the CHM gene in a Turkish man with choroideremia, p.E480X: c.1438G >T. The mutation causes a premature stop codon in exon 12 and is predicted to lead to a null effect. According to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology 2015 guidelines, this null variant is very likely to be pathogenic

since loss of function is a known mechanism for choroideremia.

We believe that the patient's mother and maternal grandmother were heterozygous for the mutation. Carriers of choroideremia are usually asymptomatic but may display variable severity of the disease depending on the lyonisation in the retinal cells. Given the patient's pedigree, family history, and genetic results, the mother was presumed to be a carrier for the mutation and it was deemed unnecessary to test her.

This is the first report of a G1438T mutation resulting in an E480X premature stop in the CHM gene in a Turkish male with choroideremia.

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