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Choroideremia Associated with a Novel Synonymous Mutation in Gene Encoding REP-1

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Abstract

Purpose—To report a novel synonymous mutation in *CHM* and the associated phenotype in an affected man and carrier mother.

Methods—Case report.

Results—A 34-year-old man with a long history of progressive night blindness and visual field constriction was diagnosed with choroideremia based on ocular examination and multimodal retinal imaging. Extensive chorioretinal degeneration was noted on SD-OCT and FAF imaging. Candidate *CHM* gene sequencing revealed a hemizygous c.1359C>T, p.(S453S) variant. This variant was heterozygous in the mother of the proband who exhibited the classic carrier phenotype of choroideremia on FAF imaging.

Conclusions—A novel c.1359C>T, p.(S453S) variant in *CHM* is the first identified synonymous mutation associated with disease manifestation in an affected man and carrier phenotype in a heterozygous mother.

Keywords

Choroideremia; Synonymous mutation

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

PATIENT ANONYMITY

Clinical images and genetic information are not identifiable to a specific individual in this study. Informed consent for participation was obtained as outlined in the Columbia University Medical Center IRB-approved protocol, AAAR0284.

INTRODUCTION

Choroideremia (CHM, OMIM 303100) is a rare X-linked retinal degenerative disease causing progressive blindness in approximately 1 in 50,000 individuals, with a particularly high prevalence in Finland.¹ Affected males typically present with night blindness followed by decreased peripheral vision and eventually severe blindness around the sixth decade of life. Visual acuity can be stable, and even remain 20/20, if degeneration does not involve the fovea.² The molecular etiology involves dysfunction of the *CHM* gene product, which encodes the Rab escort protein-1 (REP-1). REP-1 is thought to have an important role in protein transport in photoreceptors and the retinal pigment epithelium (RPE) via prenylation, a type of lipid modification, of Rab proteins in the retina. A build-up of unprenylated Rabs is hypothesized to cause the extensive chorioretinal degeneration that is observed clinically.³ Fundoscopy typically shows pale fundi due to illumination of the sclera behind degenerated retinal layers. Peripheral thinning of the photoreceptor outer segments and loss of RPE on spectral domain optical coherence tomography (SD-OCT) with large corresponding areas of hypoautofluorescence on short-wavelength fundus autofluorescence imaging (SW-FAF) is the most ubiquitous clinical presentation.⁴

The phenotype of CHM is unique, allowing for expedient identification at specialized centers.⁵ Almost all known pathogenic variants identified are null mutations and direct sequencing of *CHM* can confirm the diagnosis. In fact, one retrospective analysis found that 94% of clinically diagnosed cases could be confirmed by directly sequencing the 15 exons and flanking intronic regions of *CHM*.⁵ Studies seeking to analyze the mutation spectrum of CHM typically find the majority of disease-causing variants to be nonsense mutations, followed by deletions.^{5,6} Large genomic rearrangements are frequent in CHM which often necessitate additional assays for insertions and deletions. To date, analyses have revealed deletions, insertions, duplications, translocations, nonsense, splice-site, frameshift and missense mutations in *CHM*.⁶ Here we present the first 'silent', or synonymous, mutation associated with CHM and a pronounced carrier phenotype. This case report serves to identify a rare variant that is believed to be disease causing in a patient who could thus be considered for future gene therapy trials.

CASE REPORT

An otherwise healthy 34-year-old man of Cuban descent (Fig. 1, III:1) presented to the Harkness Eye Institute Electrophysiology Clinic of Columbia University for an evaluation of progressive vision loss due to a suspected retinal degeneration. The patient reported an onset of progressive night blindness since the age of 23. A positive family history includes an affected maternal grandfather who suffered from a similar condition and notably, severe visual impairment later in life of unknown origin (Fig. 1, I:3). There was no history of vision loss in his mother. Visual acuity was best-corrected to 20/20 in both eyes and a clear cornea and unremarkable anterior segment were observed on slit-lamp examination. Dilated fundus examination showed a clear vitreous. There was extensive chorioretinal degeneration of the peripheral retina with scalloped preservation of the retinal pigment epithelium (RPE) in the central macula bilaterally. No intraretinal pigment migration was observed; however sub-retinal hyperpigmentation was present. In addition, peripheral regions of the fundus were

pale (Table 1). FAF imaging revealed extensive RPE loss outside the central macula and peripapillary area. Spatially corresponding SD-OCT scans confirmed extensive outer retinal degeneration in these areas with evident RPE loss yielding increased reflective transmittance to the choroidal layers in areas of hypoautofluorescence on FAF (Fig. 2). The patient was referred for genetic screening of *CHM* at the Casey Eye Institute (Portland, Oregon) in accordance with the clinical findings. The genetic results were inconclusive and identified a novel hemizygous, synonymous mutation in exon 11, c.1359C>T, p.(S453S). Larger genomic aberrations in *CHM* were not detected in the proband utilizing array comparative genome hybridization (aCGH) performed at Molecular Visions Laboratory (Hillsboro, Oregon). It was recommended for the mother to be evaluated.

The mother (Fig. 1, II:2) of the proband presented at 63-years-old with no visual complaints. Past medical history included arthritis and diabetes mellitus. Past ocular history was unremarkable except a history of refractive surgery in both eyes. Vision was 20/30 bilaterally without correction. Slit lamp examination of the anterior segment and funduscopy were unremarkable (Table 1). SD-OCT revealed intact retinal layers with intermittent areas of decreased reflectance in the RPE layer (Fig. 3.). The choroid was found to be thin, measuring 167µm and 183 µm thick subfoveally in the right and left eyes, respectively. FAF imaging revealed a mosaic pattern of patchy hypoautofluorescence in a fishnet pattern up to the parafoveal area (Fig. 3), consistent with a *CHM* carrier phenotype. The patient was heterozygous for the c.1359C>T, p.(S453S) variant identified in the proband, confirming parental segregation.

DISCUSSION

Choroideremia is one of several inherited retinal dystrophies that have a specific phenotype warranting candidate single-gene testing. With imminent phase III clinical trials and ongoing natural history studies, identification of male patients harboring hemizygous *CHM* mutations has become increasingly important. The present case identifies the first synonymous mutation associated with choroideremia and illustrates how the genotypic spectrum of an inherited retinal dystrophy can be expanded by clinical correlation and assessment of family members. Although the identified c.1359C>T, p.(S453S) variant does not alter the predicted protein sequence, it alters the nucleotide sequence of the third codon of exon 11 (TCC to TCT), close to the flanking intron region. This nucleotide is weakly conserved (phyloP: 2.09 [-20.0;10.0]), however the effect is predicted to eliminate the binding site for the Serine/arginine-rich splicing factor 1 (SF2/ASF). Interestingly, non-coding variation within this region, namely c.1380-6T>G, c.1380-1G>A, and c.1350-1G>A are reported to cause skipping or truncation of exon 11 and are associated with disease phenotype.^{7,8} The identified c.1359C>T variant is exceedingly rare in the general population, as it has not been identified in 123,136 exome sequences and 15,496 whole-genome sequences (<http://gnomad.broadinstitute.org/>; accessed April 2017).

Presence of classic carrier signs in a mother who is heterozygous for this novel variant, further supports pathogenicity of this variant. The lyonization phenotype of heterozygous carriers in several X-linked retinal dystrophies are previously well-characterized, including *RPGR*-associated retinitis pigmentosa, ocular albinism, and choroideremia.⁹ Various

mosaic-like patterns are observable in carrier females of these conditions due to neighboring cells expressing either functional or dysfunctional protein product, as a result of random X-chromosome inactivation, or lyonization. FAF imaging of the mother in our study showed an extensive patchy appearance of the fundus with areas of reticular hypopigmentation and hyperpigmentation of the RPE in the macula. Subtle aberrations of the RPE were observed on SD-OCT along with thinning of the observable choroid. These features were without significant visual consequence and are consistent with previous reports and histopathologic studies.^{4,10–13} However, axial and refractive status was not documented, which could explain the visual acuity and thin choroid on SD-OCT. Identification of a carrier phenotype on FAF imaging in this case allowed for expedient confirmation of the diagnosis.

Although a novel variant was found to segregate with specific features of choroideremia in this family, contribution of an additional or stronger variant within the same allele remains possible as candidate gene sequencing cannot routinely detect large insertions or deletions in the gene. Negative findings from the aCGH test decrease the probability of this occurrence, although further assessment of this synonymous variant with a REP1 prenylation assay and mRNA sequencing (RNA-seq and splicing assays) may further elucidate its pathogenicity. Patients with this variant may benefit from traditional gene-based therapies as the approach involves supplementing target cells with a wild-type copy of the gene. Choroideremia is already being approached in clinical trials with conventional gene therapy.¹⁴ Each trial utilizes an adeno-associated viral vector delivering functional *CHM*. Initial results from one of the phase I/IIa clinical trials suggest gene therapy for choroideremia is safe. Furthermore, all patients had increased retinal sensitivity in the treated area of the retina in a vector dosage-dependent manner.² Patients with the c.1359C>T, p.(S453S) variant and a classic choroideremia phenotype should be considered for enrollment into treatment trials.

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References

1. Sankila EM, Tolvanen R, van den Hurk JA, et al. Aberrant splicing of the CHM gene is a significant cause of choroideremia. *Nat Genet.* 1992 May; 1(2):109–13. [PubMed: 1302003]
2. MacLaren RE, Groppe M, Barnard AR, et al. Retinal gene therapy in patients with choroideremia: initial findings from a phase 1/2 clinical trial. *Lancet.* 2014 Mar 29; 383(9923):1129–37. [PubMed: 24439297]
3. Pereira-Leal JB, Hume AN, Seabra MC. Prenylation of Rab GTPases: molecular mechanisms and involvement in genetic disease. *FEBS Lett.* 2001 Jun 8; 498(2–3):197–200. [PubMed: 11412856]
4. Ma KK, Lin J, Boudreault K, et al. Phenotyping Choroideremia and Its Carrier State with Multimodal Imaging Techniques. *Retin Cases Brief Rep.* 2017 Winter;11(Suppl 1):S178–S81. [PubMed: 27599108]

5. Simunovic MP, Jolly JK, Xue K, et al. The Spectrum of CHM Gene Mutations in Choroideremia and Their Relationship to Clinical Phenotype. *Invest Ophthalmol Vis Sci*. 2016 Nov 01; 57(14): 6033–9. [PubMed: 27820636]
6. Sanchez-Alcudia R, Garcia-Hoyos M, Lopez-Martinez MA, et al. A Comprehensive Analysis of Choroideremia: From Genetic Characterization to Clinical Practice. *PLoS One*. 2016; 11(4):e0151943. [PubMed: 27070432]
7. McTaggart KE, Tran M, Mah DY, et al. Mutational analysis of patients with the diagnosis of choroideremia. *Hum Mutat*. 2002 Sep; 20(3):189–96. [PubMed: 12203991]
8. Esposito G, De Falco F, Tinto N, et al. Comprehensive mutation analysis (20 families) of the choroideremia gene reveals a missense variant that prevents the binding of REP1 with Rab geranylgeranyl transferase. *Hum Mutat*. 2011 Dec; 32(12):1460–9. [PubMed: 21905166]
9. Wuthisiri W, Lingao MD, Capasso JE, Levin AV. Lyonization in ophthalmology. *Curr Opin Ophthalmol*. 2013 Sep; 24(5):389–97. [PubMed: 23892913]
10. Bonilha VL, Trzupek KM, Li Y, et al. Choroideremia: analysis of the retina from a female symptomatic carrier. *Ophthalmic Genet*. 2008 Sep; 29(3):99–110. [PubMed: 18766988]
11. MacDonald IM, Chen MH, Addison DJ, et al. Histopathology of the retinal pigment epithelium of a female carrier of choroideremia. *Can J Ophthalmol*. 1997 Aug; 32(5):329–33. [PubMed: 9276121]
12. Syed N, Smith JE, John SK, et al. Evaluation of retinal photoreceptors and pigment epithelium in a female carrier of choroideremia. *Ophthalmology*. 2001 Apr; 108(4):711–20. [PubMed: 11297488]
13. Ghosh M, McCulloch C, Parker JA. Pathological study in a female carrier of choroideremia. *Can J Ophthalmol*. 1988 Jun; 23(4):181–6. [PubMed: 3395922]
14. Sengillo JD, Justus S, Cabral T, Tsang SH. Correction of Monogenic and Common Retinal Disorders with Gene Therapy. *Genes (Basel)*. 2017 Jan 27.8(2)

SUMMARY STATEMENT

A novel synonymous mutation in *CHM* was identified in a patient with choroideremia and his carrier mother. To our knowledge, this is the first silent mutation identified in an exon of *CHM* associated with classic disease phenotype.

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Fig. 1. Pedigree segregating a novel synonymous choroideremia variant

Proband (III: 1) exhibits symptoms and characteristic imaging findings for choroideremia and harbors a hemizygous c.1359C>T, (p.S453S) variant in *CHM*. Mother of the proband (II: 2) exhibits characteristic choroideremia carrier phenotype on retinal imaging.

*; denotes obligate carriers who were not examined and did not undergo *CHM* sequencing.

Fig. 2. Retinal imaging of the proband

Digital color photo of the right (A) and left (B) eyes depicting classic pale-appearing peripheral fundus due to illumination of the sclera posterior to the degenerating choroid. SW-FAF imaging of the right (C) and left (D) eyes revealing areas of extensive RPE loss and areas of foveal sparing. SD-OCT imaging of the right (E) and left (F) shows chorioretinal thinning and diminished visualization.

Fig. 3. Retinal imaging of the unaffected carrier mother

SW-FAF imaging of the right (A) and left (B) eyes reveals mosaic-like RPE loss which spares the central macula in a fishnet pattern. SD-OCT imaging of the right (C) and left (D) eyes reveals a relatively thin choroid and sparsely scattered hyporeflectance of the RPE.

Table 1

Clinical Features of the Proband and Carrier Mother.

Individual	Age/Sex	Visual Acuity	Fundoscopy
Proband (III:1)	34/M	20/20cc OU	Pale peripheral fundus; Subretinal pigment; extensive RPE atrophy with macular sparing
Mother (II:2)	62/F	20/30sc OU	Posterior pattern of pigment mottling resembling a “fishnet with knots”

cc, with correction; sc, without correction; OU, both eyes