Clinical Characteristics of a Large Choroideremia Pedigree Carrying a Novel CHM Mutation

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Objective: To describe a large family with a novel mutation in CHM.

Methods: Family members were characterized using clinical examination, wide-field fundus photography, wide-field autofluorescence, and spectral domain optical coherence tomography. The CHM mutation was identified with the National Institutes of Health–sponsored eyeGene program.

Results: A novel nonsense CHM mutation (T1194G), resulting in a premature stop (Y398X) and loss of the final one-third C-terminal portion of the protein, was identified. A large pedigree was generated from information provided by the twice-married proband. Seven men (aged 27-39 years) and 7 women (aged 22-89 years) were evaluated. Affected men showed characteristic peripheral chorioretinal atrophy with islands of macular sparing. Female carriers exhibited a wide range of variability, from mild pigmentary alterations to significant chorioretinal atrophy with severe vision loss. Older women tended to have a more severe phenotype. Autofluorescence demonstrating subfoveal loss or absence of retinal pigment epithelium correlated with vision loss in both sexes. Spectral domain optical coherence tomography demonstrated dynamic changes and remodeling of the outer retina over time, including focal thickening, drusenlike deposits, and disruption to photoreceptor inner segment and outer segment junctions in young female carriers.

Conclusions: CHM (T1194G) is a novel mutation that manifests a wide range of phenotypic variability in a single family with a trend toward more severe phenotypes in older female carriers. Our findings emphasize the importance of considering X-linked diseases by carefully evaluating pedigrees in women with severe manifestations of disease.

Clinical Relevance: These findings demonstrate a novel CHM mutation that emphasizes severe posterior pole carrier phenotypes, age-related changes, and early choroideremia disease.

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CHOROIDEREMIA IS AN X-linked disorder associated with progressive vision and visual field loss in middle-aged men. Funduscopic examination reveals scalloped areas of retinal pigment epithelium (RPE) and choroid loss.1,2 Histologic evaluation reveals pathologic damage to RPE, choroid, and photoreceptors.2

The CHM gene (Xq21.2) (OMIM 300390) is responsible for choroideremia.3 It encodes a 653-amino acid protein, Rab escort protein 1 (REP1), which is localized to rod photoreceptor inner segments4 and functions in vesicular transport through its interaction with RAB GTPases (Rabs).1,5-8 Although numerous mutations have been identified in this 15-exon gene, including deletions, translocations, missense mutations, nonsense mutations, and splice-site mutations,3,8-11 genotype-phenotype relationships have been difficult to establish.12

Whereas young affected males show peripheral scalloped chorioretinal lesions that tend to spare the posterior pole in the later stages of the disease, female carriers show subtle retinal peripheral pigmentary changes.3,13,14 Female carriers are technically heterozygous; however, lyonization of the X chromosome in each somatic cell results in a mosaic pattern of cells across the body in which each cell preferentially expresses either the maternal or paternal X chromosome. Genetic evidence exists that CHM can partially escape X inactivation.15 The presence of varying levels of small REP1 transcripts through this mechanism may allow for potentially greater variability in the manifestation of choroideremia in female carriers.5,13

We describe a family with a novel nonsense CHM mutation (T1194G),
Table. Genotype-Positive Female Carriers Arranged by Descending Age

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>T1194G</th>
<th>Vision (OD; OS)</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:3/F/89</td>
<td>Positive</td>
<td>20/400; 20/200</td>
<td>Diffuse scalloped retina and RPE atrophy with pigment clumping</td>
</tr>
<tr>
<td>III:1/F/81</td>
<td>Positive</td>
<td></td>
<td>Diffuse scalloped retina and RPE atrophy with pigment clumping</td>
</tr>
<tr>
<td>III:5/F/48</td>
<td>Positive</td>
<td>20/20; 20/25</td>
<td>Moderate macular RPE changes and autofluorescence irregularities</td>
</tr>
<tr>
<td>III:3/F/44</td>
<td>Positive</td>
<td>20/20; 20/20</td>
<td>Moderate macular RPE changes and autofluorescence irregularities</td>
</tr>
<tr>
<td>IV:6/F/30</td>
<td>Positive</td>
<td>20/20; 20/20</td>
<td>Mild macular pigmentary mottling</td>
</tr>
<tr>
<td>IV:6/F/22</td>
<td>Positive</td>
<td>20/20; 20/20</td>
<td>Mild macular pigmentary mottling</td>
</tr>
<tr>
<td>IV:1/M/39</td>
<td>Positive</td>
<td>20/40; 20/25</td>
<td>Peripheral pigment clumping with macular RPE atrophy</td>
</tr>
<tr>
<td>IV:2/M/34</td>
<td>Positive</td>
<td>20/20; 20/100</td>
<td>Peripheral pigment clumping with macular RPE atrophy</td>
</tr>
<tr>
<td>IV:3/M/32</td>
<td>Positive</td>
<td>20/20; 20/20</td>
<td>Peripheral pigment clumping with macular RPE atrophy</td>
</tr>
<tr>
<td>IV:4/M/27</td>
<td>Positive</td>
<td>20/20; 20/20</td>
<td>Peripheral pigment clumping with macular RPE atrophy</td>
</tr>
<tr>
<td>IV:5/M/39</td>
<td>Positive</td>
<td>20/20; 20/25</td>
<td>Peripheral pigment clumping with macular RPE atrophy</td>
</tr>
<tr>
<td>IV:9/M/28</td>
<td>Positive</td>
<td>20/30; 20/25</td>
<td>Peripheral pigment clumping with macular RPE atrophy</td>
</tr>
<tr>
<td>IV:10/F/39</td>
<td>NT</td>
<td>20/20; 20/20</td>
<td>Normal examination findings and autofluorescence</td>
</tr>
<tr>
<td>IV:11/M/31</td>
<td>NT</td>
<td>20/40; 20/30</td>
<td>Normal examination findings and autofluorescence</td>
</tr>
</tbody>
</table>

Abbreviations: HM, hand motions; NT, not tested; RPE, retinal pigment epithelium.

METHODS

All patients were examined at the Doheny Eye Institute from February 2010 to March 2011. The research protocol was approved by the institutional review board of the University of Southern California and was in accordance with the tenets set forth in the Declaration of Helsinki. All study participants were informed of the purpose of the examination, volunteered, and signed a written informed consent form before examination and phlebotomy. Ophthalmic examination included best-corrected visual acuity (BCVA), slitlamp biomicroscopy, and dilated fundus examination. A family history was taken, and a pedigree was created. Genotyping was performed on blood samples submitted to the National Institute of Health–sponsored eyeGene program for 12 of the 14 patients.

All patients underwent wide-field fundus photography (pseudocolor red-green) and fundus autofluorescence (green light; wavelength, 532 nm) using a scanning laser ophthalmoscope (Optos 200Tx; Optos PLC). The female proband and selected female carriers underwent OCT in which individual OCT slices were registered to confocal scanning laser ophthalmoscopy infrared reflectance images (793 nm) and fundus autofluorescence images (488 nm) (Spectralis HRA+OCT; Heidelberg Engineering). To compare the same areas of the retina across time, registered infrared images obtained at baseline served as a reference for future OCT images. The fundus autofluorescence images were manually overlaid on infrared images using the retina vessels and optic nerve as invariable landmarks to obtain pseudoregistration of autofluorescence with OCT images.

RESULTS

The proband is an 89-year-old Hispanic woman who was referred for evaluation of a degenerative retinal disorder. Ocular history was significant for poor vision that required spectacles in her 20s, subsequent gradual vision decline, and cataract extraction with intraocular lens placement in the right eye without visual improvement. The patient’s distance BCVA measured 20/400 OD and 20/200 OS. Discussion with the proband (II:3) revealed 2 large families from 2 marriages with several offspring affected by visual problems (Table and Figure 1). The proband had 16 children, many of whom had a diagnosis of retinitis pigmentosa. Seven men (aged 27-39 years) and 7 women (aged 22-89 years) were examined.

Molecular analysis revealed that the proband was heterozygous for a T1194G mutation in the CHM gene, resulting in a nonsense Y398X mutation. This mutation resulted in the truncation of the final one-third of the protein. All family members who were genotyped had the same mutation.

Clinical examination of the affected men revealed scalloped areas of choroid and RPE loss typically seen in choroideremia (Figure 2). We correlated wide-field fundus autofluorescence to vision by outlining islands of preserved RPE (Figure 2). Most men had preservation of subfoveal RPE commensurate with BCVA of between 20/20 and 20/40. In one grandson (IV:2), whose BCVA was 20/100 OS, autofluorescence revealed a crescent-shaped remnant RPE island that excluded the fovea.

Examination of female carriers identified a wide range of phenotypic variability. Older carriers, such as the proband (II:3) and a 61-year-old niece (III:1), demonstrated scalloped areas of RPE atrophy and choroid loss reminiscent of male choroideremia (Figure 3). Unlike findings in males, the atrophic areas in these female carriers were centered in the posterior pole rather than in the periphery. Wide-field autofluorescence demonstrated scalloped areas of RPE loss with variable islands of preserved RPE (Figure 3). Subfoveal atrophy ex-
plained their poor vision (ranging from 20/200 to hand motions). Optical coherence tomography of the proband revealed outer retinal atrophy, choroidal thinning, and increased OCT signal transmission in the choroid, suggestive of RPE loss (Figure 4).

Younger female carriers, ranging in age from 22 to 48 years, demonstrated good central vision with a milder phenotype typical of choroideremia carriers (Figure 3). The older woman (III:3) in the young carriers cohort showed the most abnormal phenotype. Examination of her macula revealed pigmentary changes and autofluorescence irregularities. Comparing fundus photography between younger and older carriers identified progressively increasing chorioretinal involvement with age. Older carriers also showed generalized decreased autofluorescence, with residual areas of autofluorescence disturbance representing RPE disease (Figure 3).

The OCT of the female carriers demonstrated dynamic changes and remodeling over time. Areas of outer retinal thickening (Figure 5) and focal drusenoid RPE deposits (Figure 5) associated with inner segment and outer segment (IS/OS) loss were interspersed with areas of intact IS/OS and relatively normal external limiting membrane and RPE. Registration of OCT images to infrared reflectance images (which were manually registered to autofluorescence) allowed point-to-point comparison of abnormal areas on the OCT to autofluorescence images. The drusenoid deposits correlated with areas of hyperautofluorescence (Figure 5). Furthermore, comparison with images of affected areas examined 1 year later showed remodeling over time. In some cases, the condition increased (eg, a single drusenoid deposit doubled; Figure 5). The local outer segment deposits and elongated outer segments appeared, over time, to evolve into RPE thickening, leading to the appearance of dual RPE deposits, suggestive of underlying RPE dysfunction. These changes were corroborated by the autofluorescence images. Other areas showed improvement with disappearance of subretinal deposits along with retention of relatively normal surrounding IS/OS junction and RPE (Figure 5).

**COMMENT**

To our knowledge, this is the first report of the T1194G mutation resulting in an Y398X premature stop in the CHM gene, a novel mutation that results in a wide range of phenotypic severity in female carriers. The findings in women ranged from typical pigmentary changes to more severe, scalloped atrophic changes that, unlike the atrophic changes in men, showed a predilection toward the posterior pole (Figure 3). Men showed characteristic scalloped atrophic choroideremia changes (Figure 2).

The T1194G mutation resulted in loss of the final one-third of REP1. Most described mutations of CHM are nonsense mutations, splice error mutations, or...
frameshift mutations, leading to premature stop codons that result in an abnormal protein or loss of expression from nonsense-mediated decay. Rare missense mutations have been described, resulting in presumably misfolded and nonfunctional proteins. Although no clear genotype-phenotype correlation exists (eg, patients in whom the entire choroideremia CHM gene is missing do not necessarily have worse phenotypes), a limited understanding of the structure or function of the CHM protein identifies critical residues. The CHM protein shares significant sequence similarities with the guanine nucleotide dissociation inhibitor family identifying 2 important sequence-conserved regions for Rab binding. Crystal structure analysis of a Rab7:Rep1 complex also identifies a critical C-terminus mobile lid that coordinates Rab:Rep binding. This finding agrees with observations that recombinant REP1 lacking the final 70 amino acids cannot mediate geranylgeranylation with geranylgeranyl transferase II, which is critical for Rab membrane attachment. The premature stop seen in the T1194G mutation is downstream of the sequence-conserved regions and thus spares them. However, the T1194G mutation results in the absence of the critical C-terminus domain. Therefore, the significance of the T1194G mutation is manifested through either a truncated protein product without the critical C-terminus or nonsense-mediated decay.

Without clear genotype-phenotype correlation, an argument for a skewed pattern of X-chromosome inactivation in female carriers is typically used to explain phenotypic variation. Although this is a reasonable concept, experimental demonstration of asymmetric X inactivation in CHM has been difficult. Attempts to demonstrate asymmetric X inactivation in 2 Mexican cohorts did not show skewed X inactivation in one family and showed paradoxical skewed X inactivation away from the mutated CHM in another family. The latter study was conducted using isolated peripheral whole-blood leukocytes; hence, peripheral asymmetric X inactivation may not necessarily reflect X inactivation in the eyes. Furthermore, studies using primary female fibroblast cell lines suggest that cells can undergo biallelic expression of CHM, implying the possibility of partial X-inactivation escape for CHM. Ultimately, the best insight into X inactivation in choroideremia may be gained by studying chorioretinal tissue from carriers. The T1194G pedigree showed high intrafamilial phenotypic variability, especially among women. Rare cases of severely affected choroideremia carriers have been described, which is thought to be due to asymmetric X inactivation. Although some reports support an increased prevalence of skewed X inactivation beyond the age of 30 years, the range and rate of variability, with a trend for progressively worse phenotypes with advancing age, argue against a random event in the T1194G cohort. A relationship between age and disease progression in choroideremia carriers is in agreement with some but not all reports in the literature. The use of vision rather than retinal changes as a marker for progression in these studies may explain this. Most choroideremia carriers do not progress to the advanced level of disease seen among carriers in this series. The use of vi-
studies point toward the RPE26 or retina4 as the site of disease onset, with secondary damage to adjacent structures. However, this theory has been discredited by findings in mutant mouse models of choroideremia. In these cases, careful analysis of the pedigree is important to exclude the possibility of X-linked transmission.

The primary cellular site of the pathologic changes in choroideremia continues to be an active area of research. The name of the disease implies that the choroid is the site of disease onset, with secondary damage to adjacent structures. However, this theory has been disputed by findings in mutant mouse models of choroideremia in which histopathologic analysis revealed early damage to retina and RPE.9,24,25 Although mild inflammation has been observed in the choroid at the junction of normal and atrophic retina in affected males,2 most studies point toward the RPE26 or retina1 as the site of disease onset. Examination of the edge of atrophic areas in affected men, using topographic analysis and laminar reflectivity patterns of time-domain OCT, suggested that retinal thickening precedes or coincides with photoreceptor abnormalities as the earliest finding, followed by outer retinal atrophy and loss of lamination.27 Our examination of young, mildly affected carriers with the T1194G mutation allowed us to explore early changes in choroideremia and to show similar findings. Areas of outer retinal thickening and drusenlike deposits occur near areas of IS/OS segment disruption. Point-to-point correlation of autofluorescence to OCT in female carriers revealed that hyperautofluorescent areas colocalized with the drusenlike deposits and RPE thickening on OCT. This finding may reflect the accumulation of indigestible autofluorescent material, such as photoreceptor outer segments, leading to enlargement of RPE cells. Regardless of the exact sequence of disease onset, whether in the RPE or photoreceptors, early changes in choroideremia are clearly dynamic, as evidenced by the OCT findings. Registered areas of the retina showed either improvement or worsening of abnormalities over time (Figure 5).

In conclusion, T1194G is a novel mutation in CHM, leading to the loss of the final one-third of REP1. The mutation identified this family as important because it may be associated with a more severe, age-dependent phenotype in female carriers and may implicate more complicated mechanisms for choroideremia disease expression or age-related skewed X inactivation. Our analysis of the mild female carriers also implies that the photoreceptors or RPE may be the primary site of disease onset. More important, findings in this large family suggest that physicians need to consider X-linked diseases even when severe manifestation of disease is seen in females, such as in cases of choroidal sclerosis.28 In these cases, careful analysis of the pedigree is important to examine the possibility of X-linked transmission.

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Figure 5. Optical coherence tomography (OCT) of the female carriers demonstrating dynamic changes and remodeling. A and B, OCT image of a young female carrier (III:3) shows early outer retinal thickening (arrows) with focal retinal pigment epithelium hyperreflective drusenlike deposits (single and double asterisks) associated with the loss of the inner segment and outer segment junction. C, Autofluorescence images demonstrated macular irregularity and areas of drusenlike deposits (single and double asterisks) that appeared hyperautofluorescent. D and E, OCT 1 year later of III:3 shows persistent outer retina thickening (D, arrow) and dynamic changes with both worsening (comparing A and D asterisks) and improvement (comparing B and E double asterisks) of drusenlike material. Choroid and choriocapillaris appeared relatively normal. F, Autofluorescence obtained 1 year later showed an increase in the size of the drusenlike material correlating to an enlarged area of hyperautofluorescence (comparing top line C and F) and resolution of the drusenlike material corresponding to a lessening of hyperautofluorescence (comparing bottom line C and F).


15. Carrel L, Willard HF. Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. Proc Natl Acad Sci U S A. 1999;96(13):7364-7369.


