High-Resolution Retinal Imaging Reveals Preserved Cone Photoreceptor Density and Choroidal Thickness in Female Carriers of Choroideremia

Kai Suzuki, MD; Kiyoko Gocho, MD, PhD; Keiichiro Akeo, MD; Sachiko Kikuchi, MD, PhD; Daiki Kubota, MD; Satoshi Katagiri, MD; Kaoru Fujinami, MD, PhD; Kazushige Tsunoda, MD, PhD; Takeshi Iwata, PhD; Kunihiro Yamaki, MD, PhD; Tsutomu Igarashi, MD, PhD; Tadashi Nakano, MD, PhD; Hiroshi Takahashi, MD, PhD; Takaaki Hayashi, MD, PhD; Shuhei Kameya, MD, PhD

BACKGROUND AND OBJECTIVE: To characterize the photoreceptors and choroidal morphology of heterozygous female carriers of choroideremia who typically do not have any visual defects but can have severe funduscopic changes.

PATIENTS AND METHODS: This was a clinical case series study. Detailed ophthalmic examinations were performed on six female carriers from four families with choroideremia. The subfoveal choroidal thickness (SFCT) was determined by spectral-domain optical coherence tomography (SD-OCT) and the cone photoreceptor density by adaptive optics (AO) retinal imaging. SFCT and cone densities of the carriers were compared to that of normal eyes of healthy subjects.

RESULTS: The mean age of the carriers was 42.5 years. Fundus photographs showed diffuse, patchy depigmentation; however, the SFCT was within the normal limits. AO retinal imaging revealed preserved cone densities at temporal eccentricities from 2 to 8 angular degrees.

CONCLUSIONS: The findings indicate that despite the presence of distinctive depigmentation of the retinal pigment epithelium in female carriers of choroideremia, their cone photoreceptor densities and SFCT are well-preserved. These observations may account for the good visual acuity and lack of an awareness of visual disturbances.


INTRODUCTION

Choroideremia (OMIM: 303100) is an X-linked disease that leads to a degeneration of the choriocapillaris, retinal pigment epithelium (RPE), and photoreceptors of the retina.1,2 Choroideremia is caused by loss-of-function mutations in the CHM gene that encodes the Rab escort protein-1 (REP-1).3 REP-1 is necessary for the prenylation of ras-related GTPases, also called the Rab proteins.4-6 The Rab proteins are involved in transporting vesicles during endocytosis and exocytosis.7 The characteristic lesion of cho-
Chorioretinal scalloped atrophy in the midperipheral fundus with preservation of the macula. Affected male subjects suffer from a progressive reduction of the central vision, constriction of visual fields, and night blindness beginning at an early age. More extensive chorioretinal atrophy develops at later stages.

Heterozygous female carriers typically have no visual defects but can have severe funduscopic changes such as patchy areas of chorioretinal degenerations. The retinal findings in only a small number of fully affected women have been reported. The phenotype of female carriers of choroideremia is attributable to the inactivation of the X chromosome. In female individuals, one of the two X chromosomes is silenced during early development, and once it is shut down, this state is stably propagated throughout life to reduce the double dose expression of the X chromosome genes. In cases of X-linked genetic diseases, the tissues of female carriers would represent a mixture of some cells expressing a normal gene and others expressing an abnormal gene. Cheung et al. noted that skewed X chromosome inactivation of the normal allele might cause an expression of the mutation with severe visual loss in some choroideremia carriers.

There have been several studies analyzing the photoreceptors by adaptive optics scanning light ophthalmoscopy (AOSLO) in male patients with choroideremia and female carriers. These reports described severe cone loss in choroideremia and occasional patchy cone loss in female carriers. There are also several studies analyzing the subfoveal choroidal thickness (SFCT) by spectral-domain optical coherence tomography (SD-OCT) in patients with choroideremia. These reports reported a severe}

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**Figure 1.** Pedigree charts of choroideremia carriers. The solid squares (men) represent the affected patients with choroideremia. The gray circles (women) represent choroideremia carriers. The generation number is shown on the left.
thinning of the SFCT and loss of the choriocapillaris in patients with choroideremia.

However, statistical analysis of the cone photoreceptor densities beyond the foveal region using a high-resolution retinal camera and SFCT analysis by SD-OCT in female carriers of choroideremia has not been reported. We present our analyses of genetically identified female carriers of choroideremia using an adaptive optics (AO) retinal camera and SD-OCT images.

PATIENTS AND METHODS

The protocol of this study conformed to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the Nippon Medical School and The Jikei University School of Medicine. A signed written informed consent was obtained from all participants after the nature and possible consequences of the study were explained.

Blood samples were collected from the patients and parents, and genomic DNA was isolated from the peripheral white blood cells using a blood DNA isolation kit (NucleoSpin Blood XL; Macherey Nagel, Langenwehe, Germany). Variants of the CHM gene were confirmed by direct sequencing. The coding regions and flanking introns of the CHM gene were amplified by polymerase chain reaction (PCR) using primers produced by Greiner Bio-One (Tokyo, Japan).

Figure 2. Fundus photographs and autofluorescence images of choroideremia carriers. Fundus photographs (A, C, E, G, I, K, and M) and fundus autofluorescence (FAF) images (B, D, F, H, J, L, and N) from Carriers 1 (A, B), 2 (C, D), 3 (E, F), 4 (G, H), 5 (I, J), and 6 (K, L) are shown. Results from a male patient with choroideremia (M, N) are also shown for comparison. Fundus photograph of Case 2 shows almost no abnormality, whereas Cases 1, 4, 5, and 6 show many drusenoid deposits. FAF images of Case 4 show mixed hyper- and hypoautofluorescence.
The PCR products were purified (ExoSAP-IT; ThermoFisher Scientific; Waltham, MA) and were used as the template for sequencing. Both strands were sequenced on an automated sequencer (Bio Matrix Research, Chiba, Japan).

The ophthalmological examinations included measurements of the best-corrected visual acuity (BCVA) using a conventional Landolt C chart and the refractive error (spherical equivalent). Slit-lamp biomicroscopy, ophthalmoscopy, fundus photography, fundus autofluorescence (FAF) imaging, SD-OCT, and full-field electroretinography (ERG) were also performed. The ERGs were recorded using an extended testing protocol conforming to the International Society for Clinical Electrophysiology of Vision standards. The FAF images were acquired with the TRC-NW8Fplus retinal camera (Topcon, Tokyo, Japan), and the SD-OCT images were acquired with a Cirrus HD-OCT (Carl Zeiss Meditec, Oberkochen, Germany). The SFCT was defined as the distance between the hyperscattering line of the RPE and the chorioscleral interface as described by Usui et al. Three masked clinicians measured the SFCT with the built-in caliper function of the OCT software. The SFCT of the participants were compared to that of 37 normal eyes.

**Adaptive Optics (AO) Retinal Image Analyses**

The cone densities of the participants were compared to that of 34 normal eyes. High-resolution retinal images were acquired with an AO retinal camera (rtx1; Imagine Eyes, Orsay, France) at temporal eccentricities of 2° to 8°. The AO retinal images from each retina were stitched together using an automated image editing software (ImageJ; National Institutes of Health, Bethesda, MD). A previously reported protocol named the peak density method described by Feng et al. was used. In this method, the windows selected were those where the cone density was maximal in the near vicinity of each fixed-interval window. The maximum displacement of these sampling windows was 150 μm. These sample windows were manually selected to avoid dark areas caused by the shadows of retinal vessels or changes in background intensity due to choroidal vessels. Then, an automated cone counting soft-
Figure 4. Spectral-domain optical coherence tomography (SD-OCT) images of choroideremia carriers. SD-OCT images from Carriers 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), and 6 (F) are shown. An image from a male patient with choroideremia (G) is also shown for comparison. The SD-OCT findings of Carriers 4 and 5 show drusenoid deposits on the retinal pigment epithelium (D, E). The thinning of the choriocapillaris layer, which was observed in choroideremia patients, is not seen in any of the carriers. Both the choriocapillaris and choroidal vessels are well-preserved in all carriers.
ware (AO detect; Imagine Eyes, Orsay, France) was used to determine the number and position of each cone in the sample window. The results were manually corrected by three investigators. The differences in the cone counts between the three investigators was less than ±2 cones/50 μm x 50 μm sampling window for all windows counted. The mean values of the three investigators were used for the statistical analyses.

Statistical Analyses

The mean ± standard deviation (SD) of the cone density and SFCTs was calculated for the normal control eyes and for the CHM carriers, and the significance of the differences between the two groups was determined by t-tests (Excel; Microsoft, Redmond, WA). A P value of less than .05 was considered statistically significant. Because of the small sample size, each of the cone density was also examined to determine if the values fell within ±2 SD of the control group.

RESULTS

Molecular Genetic Analyses

The results of the Sanger sequencing of the carriers and familial male choroideremia patients and their pedigree charts are shown in Table 1 and Figure 1. All of the variants were splicing or insertion/deletion mutations, which will result in a premature termination codon of the mRNA and processed to nonsense mediated decay. These variants were not found in gnomAD and Japanese specific Human Genetic Variation Database. According to the American College of Medical Genetics and Genomics standards and guidelines, these variants are classified as PVS1, PS1, and PM2. The resulting criteria for these variants will be pathogenic (Table 1).29

Figure 5. Subfoveal choroidal thickness (SFCT) of choroideremia carriers. SFCT was obtained from the optical coherence tomography images shown in Figure 4. The SFCT is defined as the distance from the retinal pigment epithelium to choriocapillaris interface. The SFCT of the carriers are compared to healthy control of our hospital. No significant differences were found between CHM carriers and our healthy controls.
Clinical Findings

The decimal BCVA of both eyes of all carriers was better than 1.0. Fundus photographs of Carrier 2 showed almost no abnormality, whereas Carrier 4 showed severe depigmentation (Figure 2). Carriers 1, 5, and 6 had scattered depigmentation. Fundus autofluorescence (FAF) images of Carrier 4 showed mixed hyper- and hypoauflorescence (i.e., speckled FAF pattern), whereas Carriers 1, 5, and 6 showed only scattered hypoauflorescence. The FAF of Carrier 2 was total hypoauflorescence since she was 10-years-old. Full-field ERGs recorded from Carrier 5 showed no abnormalities of both the rod and cone responses (Figure 3).

SFCT and Retinal Structure Analysis by SD-OCT

The SD-OCT findings of the eyes of Carriers 4 and 5 showed drusenoid deposits on the RPE (Figure 4). The SFCT of the carriers were compared to 37 healthy controls (21 men and 16 women; mean age: 42.9 years ± 22.8 years; range: 10 years to 66 years) of our hospital. No significant differences were found ($P = .406$) between the CHM carriers (mean ± SD: 376 μm ± 26.9 μm) and our healthy controls (mean ± SD: 341 μm ± 65.8 μm) (Figure 5).

Analyses of High-Resolution Images Obtained by AO Retinal Camera

The cone densities of the participants were compared to the normal eyes of 34 healthy subjects (27 men and seven women; mean age: 37.4 years ± 9.1 years; range: 24 years to 55 years) by AO retinal imaging (Table 2, Figure 6). Comparisons of the cone densities of CHM carrier and healthy control group at each angular eccentricity showed that a significant

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**Figure 6.** Adaptive optics retinal images of choroideremia Carriers 1 to 6 are shown. Results from a male patient with choroideremia and normal control are also shown for comparison. Areas from angular eccentricities of 2° and 8° from the foveal center are shown. The size is 200 μm x 200 μm.
difference was observed only at 4° temporal from the fovea with \( P \) value of .0404 (Figure 7). However, each value of the cone densities in all the CHM carriers tested were within two standard deviations through angular eccentricities of 2° to 8° horizontally from the fovea (Figure 7). Therefore, we consider the cone densities of the CHM carriers were not significantly decreased, although group comparisons showed a significant difference at 4° with relatively high \( P \) value. Small, patchy cone losses were observed in all carriers at 2°, as has also been observed in normal controls. The packing of the cone mosaics in the carriers were not disturbed (Figure 6).

**DISCUSSION**

Our data confirmed that the cone photoreceptor densities and SFCT were within the normal limits in female carriers of choroideremia. This normality was present despite the distinct depigmentation of the RPE. However, we could not clearly distinguish individual cones at less than 2° from the fovea, as has been reported for similar imaging devices. Nevertheless, we conclude that the cone photoreceptor densities were well-preserved at 2° to 8° horizontally from the foveal center in the female carriers of choroideremia. Changes in RPE pigmentation (without RPE loss) but not photoreceptors might suggest that photoreceptor changes were secondary to the RPE degeneration. Recent OCT angiography (OCTA) images have shown an atrophy and loss of choriocapillaris in male patients and female carriers with choroideremia. In a case series of seven male patients affected by choroideremia and two female carriers, the authors demonstrated a significant reduction of the choriocapillaris flow in both affected subjects and carriers. Since SD-OCT is limited in its evaluation

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**Figure 7.** Cone densities of choroideremia carriers are compared with normal control at temporal eccentricities from 2 to 8 angular degrees. Results from a male patient with choroideremia are also shown for comparison. The bars represent 2 standard deviations (SDs) of the normal control. Cone densities of all carriers are within two SDs of the normal control.
of the choriocapillaris because of limited lateral resolution and lack of contrast, we could not evaluate the choriocapillaris in the female carriers of choroideremia. However, our findings showed that the SFCT of female carriers were not altered.

Fundus photographs, FAF, and SD-OCT of our carriers showed variations in the degree of depigmentation and drusenoid deposits in the RPE as has been reported. Skewed X chromosome inactivation was suggested to contribute to these phenotypic variations. However, Perez-Cano et al. determined the X-inactivation pattern by androgen receptor polymorphisms in the peripheral leukocytes of patients with choroideremia. They reported that the X-inactivation pattern was random (50:50-65:35) in 10 of 11 asymptomatic female carriers of choroideremia. A skewed pattern (68:32) was observed in only one of 11, and the X chromosome which was preferentially inactivated in the asymptomatic carrier with the skewed pattern was one carrying the mutant allele. On the other hand, only a symptomatic 70-year-old female carrier showed a random 52:48 X-inactivation ratio. Thus, they concluded that the X-inactivation status and abnormal retinal phenotype are not correlated. Combining our data with those of Perez-Cano et al., we can conclude that the majority of female carriers of choroideremia have random X-inactivation pattern with normal cone photoreceptor density and SFCT. This is very important from a point of view of gene therapy and counseling. It is possible to assume that at least one-half of the CHM gene expression is enough to maintain the photoreceptor and choroidal morphology within the foveal area.

Our study has limitations. Because of the limitations of the flood-illuminated AO retinal imaging system, we could not clearly distinguish individual cones at less than 2° from the foveola. Therefore, we could not obtain an estimate of the cone density in the foveal center. We need to examine the foveal cone density in these carriers after there are advancements in the AO technology. Although this study involved only six carriers with choroideremia, a cohort with a large number of carriers allowed us to detect more detailed information of the chorioretinal pathology. SD-OCT is limited in its evaluation of the choriocapillaris. Indeed, the choriocapillaris is poorly resolved with even the highest resolution structural OCTs because of their limited lateral resolution and lack of contrast. OCTA evaluations of CHM carriers are needed for a more detailed information of the choriocapillaris.

In conclusion, our finding that the cone photoreceptor densities and SFCT are well-preserved in female carriers of choroideremia may account for

### Table 1: Summary of Clinical and Genetic Features of Choroideremia Carrier

<table>
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<tr>
<th>Carrier No.</th>
<th>Case ID</th>
<th>Sex</th>
<th>Age</th>
<th>Decent BCVA</th>
<th>logMAR BCVA (OD/OS)</th>
<th>Position</th>
<th>HGVD</th>
<th>c.646delA</th>
<th>p.T216LfsX16</th>
<th>Pathogenicity</th>
<th>Classification</th>
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<td>1</td>
<td>1-I-2</td>
<td>F</td>
<td>66</td>
<td>1.2 / 1.2</td>
<td>–0.08/–0.08</td>
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<td>c.646delA</td>
<td>p.T216LfsX16</td>
<td>Pathogenic</td>
<td>PVS1, PS1, PM2</td>
<td>Pathogenic</td>
<td>This study</td>
</tr>
<tr>
<td>2</td>
<td>1-II-1</td>
<td>F</td>
<td>38</td>
<td>1.0 / 1.0</td>
<td>0/0</td>
<td>X:85217626</td>
<td>c.646delA</td>
<td>p.T216LfsX16</td>
<td>Pathogenic</td>
<td>PVS1, PS1, PM2</td>
<td>Pathogenic</td>
<td>This study</td>
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<tr>
<td>3</td>
<td>1-III-1</td>
<td>F</td>
<td>10</td>
<td>1.0 / 1.0</td>
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<td>c.646delA</td>
<td>p.T216LfsX16</td>
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<td>PVS1, PS1, PM2</td>
<td>Pathogenic</td>
<td>This study</td>
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</tr>
<tr>
<td>5</td>
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<td>F</td>
<td>50</td>
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<td>c.646delA</td>
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<td>Pathogenic</td>
<td>PVS1, PS1, PM2</td>
<td>Pathogenic</td>
<td>This study</td>
</tr>
<tr>
<td>6</td>
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<td>F</td>
<td>47</td>
<td>1.2 / 1.2</td>
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<td>c.646delA</td>
<td>p.T216LfsX16</td>
<td>Pathogenic</td>
<td>PVS1, PS1, PM2</td>
<td>Pathogenic</td>
<td>This study</td>
</tr>
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</table>

BCVA = best-corrected visual acuity; logMAR = logarithm of the minimum angle of resolution; OD = right eye; OS = left eye; HGVD = Human Genetic Variation Database; HGVD = Human Genetic Variation Database; ACMG = American College of Medical Genetics; PVS1 = very strong evidence of pathogenicity 1; PS1 = strong evidence of pathogenicity 1; PM2 = moderate evidence of pathogenicity 2.
the subjective characteristics of carrier females of good visual acuity and absence of visual symptoms.

REFERENCES


TABLE 2

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<th>TABLE 2</th>
<th>Statistical Analysis of Cone Counting (Cones/mm²)</th>
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<tr>
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<tr>
<td>CHM Carrier</td>
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<tr>
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</table>

T = temporal eccentricities in angular degrees; SD = standard deviation