Characterizing the Phenotype and Genotype of a Family With Occult Macular Dystrophy

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Objective: To characterize the phenotype of a white patient with occult macular dystrophy (OMD) and her clinically unaffected family members and to determine whether similar mutations were present in the RP1L1 gene in this family. Occult macular dystrophy is a rare macular dystrophy with central cone dysfunction hidden behind a normal fundus appearance that has been attributed to a mutation in the retinitis pigmentosa 1–like 1 (RP1L1) gene in 4 Japanese families.

Methods: In this observational cross-sectional study of 1 white family with OMD, patients meeting the clinical criteria for OMD and their family members were evaluated by use of multifocal electroretinography, the Farnsworth D-15 color vision test, automated perimetry, spectral-domain optical coherence tomography (SD-OCT), fundus autofluorescence, and fundus photography. Fluorescein angiography was performed only on the proband. Members of this family were screened for genetic mutations in the RP1L1 gene.

Results: In the family studied, the clinically affected proband was noted to have loss of the foveal outer segments and absence of bowing of the inner segment/outer segment junction on SD-OCT scans. In addition, 1 clinically unaffected family member also demonstrated loss of the foveal photoreceptor outer segments and, therefore, decreased bowing of the inner segment/outer segment junction on SD-OCT scans. The fundus autofluorescence images of the eyes of the proband and her family members were normal. Although mutations in the RP1L1 gene have been identified in sporadic and autosomal dominant OMD pedigrees, no mutations in the RP1L1 gene were found in any of the participants.

Conclusions: Loss of the outer segments of foveal photoreceptors can be detected and quantified by use of SD-OCT in patients with OMD. Similar findings are present in some clinically unaffected family members and may represent subclinical manifestations of the disease. Although mutations in the RP1L1 gene have been described in several Japanese families with OMD, there were no such mutations in this white family of European descent, which suggests that inherited OMD is a genetically heterogeneous disorder.

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unaffected family members in 1 white pedigree with OMD clinically, electrophysiologically, and with SD-OCT, and we also screened these individuals for genetic mutations in the $RP1L1$ gene.

**METHODS**

The protocol and consent forms were approved by the Johns Hopkins Hospital institutional review board. Families with OMD were recruited from the Wilmer Eye Institute in Baltimore, Maryland. For all recruited participants, written informed consent was obtained.

**INCLUSION AND EXCLUSION CRITERIA**

Patients who met the following clinical criteria for OMD were included in our study: normal fundus appearance, normal fluorescein angiogram, and abnormal macular function suggested by an abnormal multifocal electroretinogram (mfERG). Patients were judged to have an abnormal mfERG of clinical significance if the amplitude of the central element (ring 1, subtending $<2.4^\circ$) was below the fifth percentile of normative values or if the implicit times exceeded the 95th percentile of normative values. All available immediate family members of affected patients were recruited to participate in our study (participants indicated by cross bars and asterisks in Figure 1). Families were excluded if the proband had an abnormal fundus appearance (eg, retinal flecks, bull’s-eye maculopathy, or macular scarring) or an abnormal full-field ERG.

**CLINICAL ASSESSMENT**

During their initial study visit, participants underwent Snellen best-corrected visual acuity testing and Farnsworth D-15 color vision testing, automated perimetry (Humphrey 10-2 threshold test, SITA Standard) was performed, and color fundus images and fundus autofluorescence images were obtained. Fluorescein angiography was performed for probands only.

**ELECTROPHYSIOLOGIC AND OCT ASSESSMENT**

The mfERGs were recorded one eye at a time with the Visual Evoked Response Imaging System Version 5.2 (EDI) using a Burian-Allen contact lens ERG electrode in accordance with In-
ternational Society for Clinical Electrophysiology of Vision standards. Both eyes were dilated with cyclopentolate hydrochloride, 1%, and phenylephrine hydrochloride, 2.5%. The visual stimuli consisted of a 103 hexagonal array. The luminance of each hexagon was independently modulated according to a binary m-sequence at 75 Hz. The data were compared against a database of 48 normal eyes for 6 distinct rings of hexagons.

Spectralis OCT (Heidelberg Engineering) imaging was also performed for all participants. Horizontal line scans through the fovea, captured by use of SD-OCT, were segmented using computer-assisted manual segmentation in a masked fashion based on a previously published protocol. Participants with other unrelated foveal pathology (eg, foveal drusen or disciform scar) identified during imaging were excluded from segmentation analysis. Participants with other unrelated foveal pathology (eg, foveal drusen or disciform scar) identified during imaging were excluded from segmentation analysis. The horizontal line scan with the most appreciable bowing of the IS/OS junction was selected after manually reviewing all horizontal scans to ensure that the correct line scan through the fovea was chosen, even with eccentric fixation. To quantify the “bowing effect” seen in normal healthy foveal architecture, we subtracted the length of the outer segments at a point 3° nasal to the fovea (see the white double arrow in Figure 2) from the fovea (see the black double arrow in Figure 2) for each eye. We defined the measurement for each individual eye, which we termed the effective foveal outer segment length (eFOSL). The eFOSL is the outer segment length 3° nasal to the fovea (white double arrow) subtracted from the foveal outer segment length (black double arrow).

The same computer-assisted manual segmentation analysis was performed on a set of 62 normal eyes for which SD-OCT images were available to create a normative database for comparison (obtained at the Retina Foundation of the Southwest, Dallas, Texas). The eFOSL was obtained for all normal eyes in the database using horizontal line scans.

### GENETIC TESTING

Genetic testing for the RP1L1 gene was performed on all available participants (indicated by asterisks in Figure 1) at the National Hospital Organization Tokyo Medical Center at the National Institute of Sensory Organs in Japan, using previously described methods. The DNA from peripheral blood was extracted, and direct sequencing was performed on all the exons and flanking regions of the RP1L1 gene. We define mutation as amino acid change that is expected to influence protein function. This mutation should segregate within the family and never be detected in the normal population. The study participants were carefully compared against age-matched normal individuals with no visual abnormalities recorded in the database of single-nucleotide polymorphisms maintained by the National Center for Biotechnology Information.

### RESULTS

In this family (Figure 1), there was 1 clinically affected proband (II-4) with OMD. The patient described central visual loss beginning at 42 years of age, at which point she was found to have a best-corrected visual acuity of 20/400 in the right eye and 20/30 in the left eye, bilateral central...
scotomas, normal results from an ophthalmoscopical examination, and a normal fluorescein angiogram. At that time, she received a diagnosis of normal-tension glaucoma, although no glaucomatous optic nerve cupping was noted. Five years later, her visual acuity in the left eye had decreased further to 20/125. At this time, a mERG revealed a clinically significant decrease of foveal response amplitudes in both eyes, compatible with OMD (Figure 3). Spectral-domain OCT revealed marked loss of the foveal outer segments with a flattening of the normally bowed IS/OS junction (Figure 4A and B). Her eFOSL was markedly abnormal, measuring 0 µm in both eyes (Figure 5).

The proband's father (I-1) did not have any visual complaints. On clinical examination, his best-corrected visual acuity was 20/25 bilaterally. He had mild cataracts and bilateral extrafoveal nonneovascular age-related macular degeneration. His foveas appeared to be normal. A mERG revealed a clinically significant reduction in foveal response amplitude in the right eye and a normal foveal response amplitude in the left eye (Table). He had delayed ring 1 implicit times in both eyes. Spectral-domain OCT revealed marked loss of the foveal outer segments and decreased bowing of the IS/OS junction bilaterally (Figure 4C and D). The eFOSL was decreased in both eyes, measuring 6 µm in the right eye and 2 µm in the left eye (Figure 5).

One brother (II-2) had been evaluated previously by an ophthalmologist and was presumed to have OMD based on clinical history and clinical examination, but neither multifocal electroretinography nor OCT was ever performed. The patient died of an unrelated health condition during the study period and, therefore, was unavailable for further testing.

Another of the proband’s brothers (II-3) denied any visual complaints and had normal results from an ophthalmoscopic examination and normal foveal response amplitudes and normal ring 1 implicit times on a mERG. In this individual, SD-OCT revealed a normal outer segment length with preserved bowing of the IS/OS junction (Figure 4E and F) and a normal eFOSL of 23 µm in the right eye and 17 µm in the left eye (Figure 5).

The proband’s mother (I-2) was unavailable for clinical assessment, multifocal electroretinography, or imaging, but she underwent genetic testing. Fundus autofluorescence images for all 3 tested family members were normal (images not shown except for proband II-4; Figure 3G and H). Sequencing of the exons of the RP1L1 gene of the proband, mother, father, and one brother (I-1, I-2, II-3, and II-4) revealed no mutations.

For patients with OMD, there is a growing amount of data collected with SD-OCT imaging that can be compared against a normative database. Rangaswamy et al12 have developed a segmentation technique to measure the outer segment length on SD-OCT; for normal individuals, the outer segment length is increased centrally and decreased in the periphery, creating a central bowing effect of the IS/OS junction, a normal phenomenon in foveal cone specialization.13,14 Four previous studies4-6,8 have examined structural abnormalities on SD-OCT images of patients with OMD. These studies4-6,8 have described abnormalities in the IS/OS junction and decreased bow-

![Figure 5. The effective foveal outer segment lengths for the proband (II-4) and 2 family members (I-1 and II-3) shown alongside those of individuals with normal eyes. The dotted line represents the 10th percentile effective foveal outer segment length in 62 normal eyes is 12 µm.](https://archopht.jamanetwork.com/)
ing of the IS/OS junction in OMD patients. Park et al\(^6\) examined unilateral or asymmetric cases, and in 3 of 5 cases, SD-OCT revealed morphologic changes in the photoreceptor layers, even in the eyes with normal vision. Park et al\(^6\) suggested that there is a subclinical stage in the better eye that eventually progresses to clinically manifest OMD. In our study, affected OMD eyes did have abnormalities in the photoreceptor outer segments and demonstrated decreased bowing of the IS/OS junction, which, for the first time, we quantified with segmentation analysis and compared against normal eyes. Interestingly, 1 clinically unaffected family member with no visual symptoms (I-1) demonstrated decreased bowing at the IS/OS junction on SD-OCT images. In this case, it is unclear whether the lack of bowing in clinically unaffected patients is simply a variant of normal or is truly a subclinical stage of OMD. Serial examinations over time will reveal whether or not these subclinical phenotypes eventually will progress to clinically manifest OMD in one or both eyes.

Previous studies have not examined clinically unaffected family members with OCT imaging. Abnormalities in the foveal outer segment that were recorded on a mERG were present in successive generations. These findings suggest that OMD could be an autosomal dominant condition with variable penetrance and/or expressivity. Alternatively, some of the OCT findings and mERG findings may be spurious (ie, related to foveal drusen or cataract), and, if so, this could suggest that our proband is an isolated case or has a different inheritance pattern (eg, autosomal recessive).

The \textit{RP1L1} gene, which functions in microtubule assembly and stabilization in photoreceptor axonemes,\(^1\) is responsible for at least 7 Japanese families with autosomal dominantly inherited OMD (T. Iwata, PhD, unpublished data, October 31, 2011). The mutations that have previously been reported include the following mutations in \textit{RP1L1}: 2 substitution mutations (c.3107T>C (p.Arg45Trp) and c.362C>T (p.Arg45Trp)), 1 missense mutation (c.3596 C>G in exon 4), and 1 insertion mutation (c.325insT in exon 2).\(^1,11,13,16\) To date, no mutations in the \textit{RP1L1} gene have been identified in autosomal recessive pedigrees. In this family, no mutations were identified in the \textit{RP1L1} gene. It appears, therefore, that multiple genes may be responsible for inherited OMD, similar to the genetic heterogeneity seen in retinitis pigmentosa. Alternatively, this family could represent an autosomal recessive form of OMD in which the \textit{RP1L1} gene is not implicated.

Our study also highlights that SD-OCT may be a sensitive tool that can detect structural abnormalities in the photoreceptor layer before functional impairment. Spectral-domain OCT has several advantages over multifocal electroretinography. Images are easier to interpret, are faster to obtain, have high spatial resolution, and have good repeatability. Furthermore, in OMD, in which patients may have dense central scotomas, SD-OCT images of the fovea can still be obtained even in patients with eccentric fixation. In contrast, multifocal electroretinography requires a steady central fixation for accurate interpretation of central amplitudes. Fundus autofluorescence reveals nonspecific weak hyperautofluorescent changes in the fovea for about 50% of patients with OMD; however, the autofluorescence images obtained from all tested individuals from this family were normal. This suggests that the abnormality does not lie in the retinal pigment epithelium and that fundus autofluorescence may be a useful tool in distinguishing OMD from other retinal dystrophies in which retinal pigment epithelial disease is characteristic.

In conclusion, a thorough examination of symptomatic and asymptomatic patients in this OMD family revealed a previously uncharacterized possible subclinical phenotype. To our knowledge, this is the first time that loss of foveal outer segments is quantitatively characterized in OMD. This loss may be suggestive of, but not pathognomonic for, OMD. Further genetic testing may allow us to understand the causative mutations and inheritance patterns, thus paving the way for development of potential treatments for OMD.

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Correction

Error in PubMed. In the Global Health Editorial titled “Global Burden of Visual Impairment and Blindness” by Bourne et al, published in the May issue of the Archives (2012;130[5]:645-647), the link to the collaborators’ list was incomplete at the time of publication and posting and is now incorrect in PubMed. The name of one of the members of the GBD Vision Loss Expert Group, Dr Tasanee Braithwaite, should have been cited but was not.