Novel RP1L1 Variants and Genotype–Photoreceptor Microstructural Phenotype Associations in Cohort of Japanese Patients With Occult Macular Dystrophy

Kaoru Fujinami,1–3 Shuhei Kameya,4 Sachiko Kikuchi,4 Shinji Ueno,5 Mineo Kondo,6 Takaaki Hayashi,7 Kei Shinoda,8 Shigeki Machida,9,10 Kazuki Kuniyoshi,11 Yuichi Kawamura,12 Masakazu Akahori,12 Kazutoshi Yoshitake,12 Satoshi Katagiri,7 Ayami Nakanishi,5 Hiroyuki Sakuramoto,11 Yoko Ozawa,2 Kazuo Tsubota,2 Kunihiko Yamaki,4 Atsushi Mizota,8 Hiroko Terasaki,5 Yozo Miyake,11 Takeshi Iwata,12 and Kazushige Tsunoda 1

1Laboratory of Visual Physiology, Division for Vision Research, National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center, Meguro-ku, Tokyo, Japan
2Department of Ophthalmology, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan
3UCL Institute of Ophthalmology, London, United Kingdom
4Department of Ophthalmology, Nippon Medical School Chiba Hokusoh Hospital, Inzai, Chiba, Japan
5Department of Ophthalmology, Nagoya University Graduate School of Medicine, Showa-ku, Nagoya, Aichi, Japan
6Department of Ophthalmology, Meie University Graduate School of Medicine, Tsu, Mie, Japan
7Department of Ophthalmology, The Jikei University School of Medicine, Nishishimbashi, Minato-ku, Tokyo, Japan
8Department of Ophthalmology, Teikyo University School of Medicine, Itabashi-ku, Tokyo, Japan
9Department of Ophthalmology, Iwate Medical University School of Medicine, Morioka, Iwate, Japan
10Department of Ophthalmology, Dokkyo Medical University Koshigaya Hospital, Koshigaya, Saitama, Japan
11Department of Ophthalmology, Kinki University Faculty of Medicine, Osaka-Sayama City, Osaka, Japan
12Division of Molecular and Cellular Biology, National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center, Meguro-ku, Tokyo, Japan
13Aichi Medical University, Nagakute, Aichi, Japan

Correspondence: Kazushige Tsunoda, Division for Vision Research, National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center, 2-5-1 Higashigakouka, Meguro-ku, Tokyo 152-8902, Japan; tsunodakazushige@kankakuki.go.jp.
KF and SKam contributed equally to the work presented here and should therefore be regarded as equivalent authors.

Submitted: March 31, 2016
Accepted: July 24, 2016

PURPOSE. To determine the clinical and genetic characteristics of Japanese patients with occult macular dystrophy (OMD) in a nationwide multicenter study.

METHODS. Twenty-three patients from 21 families with clinically diagnosed OMD were studied at 10 institutions throughout Japan. Ophthalmologic examinations including spectral-domain optical coherence tomography were performed. Patients were classified into two phenotype groups: a classical group having both blurred ellipsoid zone and absence of interdigitation zone of the photoreceptors, and a nonclassical group lacking at least one of these two features. Whole-exome sequencing, direct sequencing, and in silico molecular analysis were performed to detect the pathogenic RP1L1 variants. Statistical associations between the phenotype and genotypes based on the presence of pathogenic RP1L1 variants were investigated.

RESULTS. There were 12 families with the classical findings and 9 families with the nonclassical findings. Nine pathogenic RP1L1 missense variants were identified in 12 families (57%) including three reported variants, namely, p.R45W, p.S1199C, and p.G1200A, and six novel variants, p.G221R, p.T1194M, p.G1200D, p.G1200V, and p.V1201G. The pathogenic missense variants in seven families (33%) were located between amino acid numbers 1196 and 1201. A significant association was found between the photoreceptor microstructural phenotypes and molecular genotypes.

CONCLUSIONS. The spectrum of the morphologic phenotypes and pathogenic RP1L1 variants was documented in a well-characterized Japanese cohort with OMD. A unique motif including six amino acids (1196–1201) downstream of the doublecortin domain could be a hot spot for RP1L1 pathogenic variants. The significant association of the morphologic phenotypes and genotypes indicates that there are two types of pathophysiology underlying the occult macular dysfunction syndrome: a hereditary OMD with the classical phenotype (Miyake’s disease), and a nonhereditary OMD-like syndrome with progressive occult maculopathy.

Keywords: ocular macular dystrophy, RP1L1, macular dystrophy, electroretinogram, Miyake’s disease
Ocull macular dystrophy (OMD; Online Mendelian Inheritance in Man [OMIM] 613587), first described by Miyake et al.1–3 in 1989, is an inherited macular dystrophy characterized by a progressive decrease in the visual acuity in eyes with an essentially normal-appearing fundus and normal fluorescein angiograms. The full-field electroretinograms (ERGs) are usually normal; however, the focal macular ERGs, multifocal ERGs (mfERGs), and pattern ERGs are abnormal.1-7 These findings suggested that the retinal dysfunction is confined to the macula.4-7

Characteristic changes in the microstructure of the photoreceptors have been detected by spectral-domain optical coherence tomography (SD-OCT) in eyes with OMD, and these changes have been subsequently used in the diagnosis of OMD.3,8,9 The most prominent alterations in the SD-OCT images are the disruptions or absence of the two highly reflective lines in the macular area: the ellipsoid zone (EZ) and the interdigitation zone (IZ).3,8 The absence of the IZ at the fovea is the initial sign of this disorder.3,9 A thickened and blurred EZ in the early stage and disrupted or absent EZ in the late stage are common features of eyes with OMD.3,8 A thinning of the photoreceptor and outer nuclear layers becomes more apparent with increasing time, but the retinal pigment epithelium (RPE) remains unchanged.3,8

In 2010, linkage analyses of two families with autosomal dominant inheritance OMD detected causative mutations in the retinitis pigmentosa 1-like 1 (RP1L1) gene (OMIM 608581).10 The RP1L1 gene was originally identified by human and mouse genomes sequencing, and it contained four exons that span 50 kb on chromosome 8p.11,12 The RP1L1 protein has a maximal length of 2,480 amino acids with a predicted molecular weight of 252 kDa. Immunohistochemistry showed that it is expressed in the rod and cone photoreceptors of cynomolgus monkeys.10 The RP1L1 protein was suggested to be involved in the morphologic and functional maintenance of photoreceptors.3,11,13

Since the discovery of the causative RP1L1 mutations in patients with OMD, a number of OMD cases with RP1L1 mutations have been reported.7,8,14-19 The most common mutation is c.133C>T; p.Arg45Trp in exon 2.7,20,21 In addition, extensive retinal dysfunction such as generalized cone dysfunction and generalized rod-cone dysfunction has been documented in patients with biallelic RP1L1 gene aberrations.7,20,21 However, the spectrum of RP1L1 variants and clinical characteristics of patients with OMD have not been completely determined because there have been no studies on a large cohort of patients with a definitive diagnosis of OMD in a specific population.

Thus, the purpose of this study was to determine the clinical and molecular genetic characteristics of a well-described cohort of patients with OMD in the Japanese population. This study also provided an opportunity to determine whether there was a significant association between the photoreceptor microstructures and the molecular genotypes in eyes with OMD.

METHODS

Patients

The protocol of this study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the participating institutions: National Institute of Sensory Organs (NISO); National Hospital Organization, Tokyo Medical Center; Nippon Medical School Chiba Hokusoh Hospital; Nagoya University Graduate School of Medicine; Mic University Graduate School of Medicine; The Jikei University School of Medicine; Teikyo University School of Medicine; Iwate Medical University School of Medicine; Kinki University Faculty of Medicine; and Aichi Medical University. A signed informed consent was obtained from all patients.

A cohort of 23 Japanese patients with clinically diagnosed OMD were studied between 2008 and 2012. All of the patients met the established criteria for the clinical diagnosis of OMD including a progressive decrease of the visual acuity in both eyes or a decrease of central vision, essentially normal fundus appearance, normal full-field ERGs, and localized macular dysfunction detected by focal macular or multifocal ERGs.1-3 A full medical history with detailed family history was obtained from all.

Clinical Investigation, Data Uploading, and Morphologic Classification

Comprehensive ophthalmologic examinations were performed on all patients, and also on two unaffected family members who were used for cosegregation analyses (Fig. 1; subjects 2H-2 and 7H-1). The clinical evaluations included measurements of the best-corrected decimal visual acuity (BCVA), visual field testing, electrophysiological assessments, ophthalmoscopy, fundus autofluorescence (AF) imaging, and SD-OCT.1-3,5,8,9,22-24 All of the clinical data and images were uploaded into the NISO database, and the diagnosis and data quality were confirmed by two of the authors (KF and KTsun).

The classical characteristic SD-OCT findings were defined as those in patients with the p.Arg45Trp mutation.3,8,10 All patients were classified into one of the two groups based on the microstructural changes of the photoreceptors: one group with the classical SD-OCT findings in which there was blurring of the EZ and absence of the IZ, and a second nonclassical group in which at least one of the two classical features was lacking.

Exome Sequencing, Targeted Analysis for Retinal Disease–Causing Genes on RetNET, and Variant Classification

After informed consents were obtained, blood samples were collected from the 23 patients and from two unaffected family members for cosegregation analyses.

Genomic DNA was extracted from the peripheral blood with the Gentra Puregene Blood Kit (Qiagen, Tokyo, Japan) and sheared with the Covaris Ultrasonicator (Covaris, Woburn, MA, USA). Exome sequencing and targeted sequence analysis were done according to the published protocol of NISO, a customized analysis protocol for the Japanese population.25-26 Paired-end sequence library construction and exome capturing were performed by the Agilent Bravo automated liquid-handling platform with SureSelect XT Human All Exon kit V3-5 + UTRs kit (Agilent Technologies, Santa Clara, CA, USA). Enriched libraries were sequenced with the Illumina HiSeq2000 sequencer (San Diego, CA, USA; read length 2 × 101 bp).

Reads were aligned to the University of California, Santa Cruz, California, United States (UCSC) human genome 19 reference sequence with Burrows-Wheeler Aligner software.27 Duplicated reads were removed by Picard MarkDuplicates module, and mapped reads around insertion–deletion polymorphisms (INDELs) were realigned by the Genome Analysis Toolkit (GATK).28 Base-quality scoring was recalibrated by GATK. Mutation calling was performed by the GATK Unified Genotyper module.

Called single-nucleotide variants (SNVs) and INDELs were annotated by the snpEff software (snpEff score; “high,”...
moderate,” or “low”). All called SNVs and INDELs of the 238 genes registered as retinal disease–causing genes on the RetNet database were selected for further analysis (https://sph.uth.edu/retnet/home.htm; in the public domain). The identified variants were filtered with allele frequency (less than 1%) of the Human Genetic Variation Database (HGVD; http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.htm; in the public domain), which is specific for the Japanese population. Depth and coverage for the targeted areas were interrogated using the integrative Genomics Viewer (http://www.broadinstitute.org/igv/; in the public domain).

Filtered variants were classified into two groups: variants with “high” damage predicted on SnpEff (major variants) and variants with “moderate” damage predicted on SnpEff (sub-major variants).

**Direct Sequencing of RP1L1 Gene**

The RP1L1 variants identified by exome sequencing and targeted analysis were further confirmed by direct sequencing in all patients and the two unaffected family members. The targeted exons, 2, 3, and 4, of the RP1L1 gene were amplified by PCR using the established primer pairs (Trascript ID: NM_178857.5). Both DNA strands were sequenced by a sequencer, 3730xl DNA Analyzer using the BigDye Terminator kit V3.1 (Life Technologies Corporation, Carlsbad, CA, USA).

**In Silico Molecular Genetic Analysis; Prediction, Frequency, and Conservation Scores**

All identified variants were analyzed using three software prediction programs: SnpEff, Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org/; in the public domain), and PolyPhen2 (http://genetics.bwh.harvard.edu/pph/index.html; in the public domain).

The allelic frequency of all of the variants was estimated with reference to two databases, the HGVD and the ExAC Browser (Beta; http://exac.broadinstitute.org; in the public domain).

Conservation in the positions of the identified variants was evaluated with primate PhyloP and phastCons scores provided by UCSC based on the human genome 19 coordinates. Higher PhyloP and phastCons scores were assigned to higher conservation.

Overall, the pathogenicity prediction of all variants, confirmed by direct sequencing, was classified into one of the two categories, pathogenic or less likely pathogenic, based on the results of software prediction, allelic frequency, and conservation score. Variants classified as pathogenic met three criteria: high pathogenicity on the prediction program (damaging on SIFT and probably damaging on PolyPhen2 HDIV), low frequency (less than 1% on the ExAC Browser database for East Asian), and higher preservation score (more than 0.1 on PhyloP 46-way primate and 0.005 on Phast Cons 46-way primate).

---

**Figure 1.** Pedigrees of 23 Japanese families with occult macular dystrophy (OMD). The solid squares (men) and circles (women) represent the affected patients. Unaffected family members are represented by white icons. The slash symbol indicates deceased individuals. The generation number is shown on the left. The proband of each pedigree is marked by an arrow and the clinically examined individuals are indicated by a cross.
Table 1. Key Phenotypic Features of 23 Affected Individuals With Occult Macular Dystrophy (OMD)

<table>
<thead>
<tr>
<th>Fm ID</th>
<th>Pt ID</th>
<th>Inheritance</th>
<th>Sex</th>
<th>Age</th>
<th>Onset</th>
<th>RE</th>
<th>LE</th>
<th>BCVA</th>
<th>ERG</th>
<th>Central Dysfunction</th>
<th>Funduscopy</th>
<th>Blurring of EZ</th>
<th>Absence of IZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-II-2</td>
<td>AD</td>
<td>Male</td>
<td>14</td>
<td>7</td>
<td>0.8</td>
<td>0.7</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>1</td>
<td>1-II-3</td>
<td>AD</td>
<td>Female</td>
<td>45</td>
<td>40</td>
<td>0.15</td>
<td>0.5</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>2</td>
<td>2-II-1</td>
<td>Isolated</td>
<td>Female</td>
<td>31</td>
<td>25</td>
<td>0.2</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>3</td>
<td>3-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>36</td>
<td>20</td>
<td>0.3</td>
<td>0.6</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>4</td>
<td>4-II-1</td>
<td>AD</td>
<td>Male</td>
<td>46</td>
<td>33</td>
<td>0.15</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Pale disc</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>5</td>
<td>5-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>42</td>
<td>34</td>
<td>0.2</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>6</td>
<td>6-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>42</td>
<td>38</td>
<td>0.2</td>
<td>0.1</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>7</td>
<td>7-II-1</td>
<td>AD</td>
<td>Male</td>
<td>49</td>
<td>48</td>
<td>0.3</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Pale disc</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>8</td>
<td>7-II-2</td>
<td>AD</td>
<td>Female</td>
<td>79</td>
<td>79</td>
<td>0.3</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>9</td>
<td>7-II-3</td>
<td>AD</td>
<td>Male</td>
<td>48</td>
<td>30</td>
<td>0.3</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>10</td>
<td>8-II-1</td>
<td>AD</td>
<td>Male</td>
<td>51</td>
<td>25</td>
<td>0.1</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>11</td>
<td>10-II-1</td>
<td>Isolated</td>
<td>Female</td>
<td>52</td>
<td>48</td>
<td>0.2</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>12</td>
<td>11-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>52</td>
<td>45</td>
<td>0.7</td>
<td>0.1</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>13</td>
<td>12-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>52</td>
<td>44</td>
<td>0.5</td>
<td>0.4</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>14</td>
<td>13-II-1</td>
<td>Isolated</td>
<td>Female</td>
<td>58</td>
<td>53</td>
<td>1.2</td>
<td>1.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>15</td>
<td>14-II-1</td>
<td>AD</td>
<td>Female</td>
<td>54</td>
<td>40</td>
<td>0.08</td>
<td>0.1</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>16</td>
<td>15-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>64</td>
<td>62</td>
<td>0.1</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>17</td>
<td>16-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>57</td>
<td>47</td>
<td>0.2</td>
<td>0.2</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>18</td>
<td>17-II-1</td>
<td>AD</td>
<td>Female</td>
<td>58</td>
<td>48</td>
<td>0.4</td>
<td>0.3</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>19</td>
<td>18-II-1</td>
<td>AD</td>
<td>Female</td>
<td>65</td>
<td>61</td>
<td>0.5</td>
<td>0.5</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>20</td>
<td>19-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>69</td>
<td>64</td>
<td>0.6</td>
<td>0.6</td>
<td>Normal</td>
<td>(+)</td>
<td>Hard druzen</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>21</td>
<td>20-II-1</td>
<td>Isolated</td>
<td>Male</td>
<td>72</td>
<td>40</td>
<td>0.1</td>
<td>0.1</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>22</td>
<td>21-II-1</td>
<td>Isolated</td>
<td>Female</td>
<td>66</td>
<td>60</td>
<td>1</td>
<td>1</td>
<td>Normal</td>
<td>(+)</td>
<td>Normal</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Autosomal dominant inheritance was confirmed by full clinical examinations in three families (families 1, 7, and 18). An autosomal dominant family history was found reported in five families (families 4, 8, 9, 14, and 17, by direct questioning). AD, autosomal dominant; Fm, family; LE, left eye; Pt, patient; RE, right eye.

Association Between Microstructural Phenotype Classification and Genotype Classification

All families were classified into one of two genotype groups based on the presence of pathogenic RP1L1 variants: the RP1L1-positive group and the RP1L1-negative group.

Fisher’s exact test was used to determine the significance of the association between the microstructural phenotype classification and genotype classification with commercially available software, Excel Toki 2012 (Social Survey Research Information Co., Ltd., Tokyo, Japan). P values < 0.05 were considered statistically significant.

RESULTS

Demographics, Clinical Findings, and Photoreceptor Microstructural Classification

Twenty-three patients from 21 Japanese families with a clinical diagnosis of OMD were studied. The key phenotypic findings are shown in Table 1, and detailed clinical data are presented in Supplementary Table S1. The pedigree charts of the 21 families are shown in Figure 1. Autosomal dominant inheritance was confirmed by full clinical examinations in three families (families 1, 7, and 18), and an autosomal dominant family history was found in five families (families 4, 8, 9, 14, and 17) by direct questioning.

There were 10 women (43%) and 13 men (57%). The median age at the initial examination was 52.0 years with a range of 7 to 79 years. The decimal BCVA median age at the initial examination was 52.0 years with a range of 7 to 79 years. The decimal BCVA was 0.8 and 1.2 for the right and left eyes, respectively. All of the patients complained of decreased central vision, and 15 had photophobia (15/23, 65%). The visual fields were determined in 17 patients; a central scotoma was detected in 16 patients (16/17, 94%), and no visual field defect was detected in 1 patient (1/17, 6%). There were two patients with normal-tension glaucoma and glaucoma-associated visual field defects (2/17, 12%).

Electrophysiological recordings showed localized macular dysfunction in all patients. A pale optic disc was detected in three patients (3/23, 13%), and hard druzen was found in one patient (1/23, 4%). One patient had an epiretinal membrane in the left eye, and the affected eye was excluded from all the imaging analyses. The AF images were normal in 14 patients (14/23, 61%); there were hyperautofluorescent changes in the parafveal area in 5 patients (5/23, 22%), and a ring AF enhancement at the foveola in 4 patients (4/23, 17%).

Spectral-domain OCT showed that the IZ was not present in 18 patients (18/23, 78%) and blurred EZ was detected in 14 patients (14/23, 61%). There were five patients (5/23, 22%) who had neither absence of IZ nor blurred EZ. None of the patients had RPE atrophy. Representative SD-OCT images from 10 subjects are shown in Figure 2.

Exome Sequencing Analysis and Candidate Variant Detection

Exome sequencing and targeted analysis were performed on all 23 affected individuals and two unaffected family members. Adequate data quality was verified and proper analysis was performed on all of the subjects (Supplementary Table S2). After filtration, 13 variants were identified in the RP1L1 gene by targeted exome analysis in the 23 patients (Table 2). RP1L1 variants were detected in 16 patients from 14 families. No variant was found in seven patients from seven families.
Direct Sequencing of 13 Candidate RP1L1 Variants Identified by Exome Analysis

Direct sequencing of the other 13 variants detected by targeted exome analysis was performed, and all of these variants were verified (Table 2). Cosegregation analysis with unaffected family members in terms of the candidate variants was performed in two families (family 2, c.133C>T, p.Arg45Trp; family 7, c.3596C>G, p.Ser1199Cys). These variants were not detected in the unaffected family members.

Detection of Disease-Causing Variants With In Silico Molecular Genetic Analysis

A summary of the results of the in silico molecular genetic evaluations of the variants identified by exome analysis is presented in Table 3. Nine missense variants met the criteria and were classified as pathogenic. The pathogenic missense variants included three already reported variants, namely, c.133C>T, p.Arg45Trp; c.3596C>G, p.Ser1199Cys; and c.3599G>C, p.Gly1200Ala. There were also six pathogenic variants that have not been reported, namely, c.661G>A, p.Gly221Arg; c.3581C>T, p.Thr1194Met; c.3581C>T, p.Thr1196Ile; c.3599G>T, p.Gly1200Val; c.3599G>A, p.Gly1200Asp; and c.3602T>G, p.Val1201Gly. Four variants were determined to less likely be pathogenic, namely, c.2026A>T, p.Ser676Cys; c.3514C>A, p.Leu1172Ile; c.4650T>G, p.Asn1550Lys; and c.6063delC, p.Asp2021GlufsTer3.

Two missense variants, c.3581C>T, p.Thr1194Met and c.3587C>T, p.Thr1196Ile, were identified in a patient (11-II-1), and analysis of each exome sequence read with the integrative Genomics Viewer revealed that these two variants are located on the same chromosome (in cis). It is not possible to determine which of the variants are pathogenic or indeed if it is a combination of the two variants that leads to disease. However, there are 14 alleles comprising four different amino acid changes at the former position (p.Thr1194) present in the ExAC dataset. Furthermore, this residue exhibits incomplete conservation throughout mammalian RP1L1 orthologues. Therefore the latter residue (c.3587C>T, p.Thr1196Ile), which is completely con...
served throughout mammalian orthologues and unaltered in the ExAC dataset, may be more likely to be pathogenic if altered. This patient also had a homozgyous frameshift variant with premature termination (c.6063delC, p.Asp2021GlufsTer3), which had relatively higher allele frequency and significantly lower conservation scores and was classified as less likely pathogenic: 0.5% in the normal Japanese population on HGVD and 0.174% of the East Asian population on ExAC; PhyloP and Phastcons scores of 1.11 and 0.02. It was uncertain whether this homozgyous frameshift variant has a considerable clinical impact, since the missense variant, c.3587C>T, p.Thr1196Ile, is most likely disease causing in this patient.

Overall, 9 disease-causing variants were identified in 14 patients (14/23, 61%) from the 12 families (12/21, 57%). One common variant, c.133C>T, p.Arg45Trp, was detected in five patients from four families, and another common variant, c.3596G>G, p.Ser1199Cys, was found in three patients from two families. Six novel pathogenic variants were identified in five patients from five families (5/21, 26%). Of the nine pathogenic variants, six were located between amino acid numbers 1196 and 1201, which is downstream of the doublecortin domain.1

Major Variants of Other Retinal Disease–Causing Genes on RetNet

Among the nine patients without pathogenic RP1L1 variants, two major variants of other retinal disease–causing genes were detected in the RetNet in three patients; c.5797C>T, p.Arg1933Ter of the RP1 gene in two subjects and c.1023C>A, p.Tyr341Ter of the RP2 gene in one subject (Table 4).

Association Between Photoreceptor Microstructural Phenotype Classification and Genotype Classification

All 23 patients were classified into one of the two groups based on the changes in the microstructures of the photoreceptors detected in the SD-OCT images. There were 14 patients in the classical group with both EZ blurring and IZ absence, and 9 patients in the nonclassical group with only one of the two microstructure abnormalities (Table 1). The classical SD-OCT findings were identified in 12 families and nonclassical findings in 9 (Tables 1, 5; Fig. 1). No discordance of the microstructural classification was found in the pedigrees of three families including multiple affected members (families 1, 7, and 18). Pathogenic RP1L1 variants were detected in 12 families, and no pathogenic RP1L1 variants were detected in 9 families. There was a significant association between the microstructural classification and genotype classification (P = 0.000371, Table 5).

Six of the eight families with autosomal dominant OMD inheritance or history were identified as having pathogenic RP1L1 variants (6/8, 75%). All of these RP1L1-positive families had the classical SD-OCT findings, while two autosomal dominant families with nonclassical SD-OCT findings did not have RP1L1 pathogenic variants (families 8 and 17, Fig. 1).

**DISCUSSION**

Our results indicated that there was a significant association between the genotype and the photoreceptor microstructural phenotype; that is, the presence of pathogenic RP1L1 variants...
<table>
<thead>
<tr>
<th>Fm ID</th>
<th>Ex</th>
<th>Nucleotide Change, Amino Acid Change</th>
<th>Allele Frequency, %</th>
<th>Prediction</th>
<th>Conservation Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ExAC</td>
<td>SIFT/Polyphen2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HGVD  EA  SA  Eu  La  Af</td>
<td>HDIV  PhyloP  Cons</td>
<td>dbSNP ID  Report  Pathogenicity</td>
</tr>
<tr>
<td>1, 2, 3, 10</td>
<td>2</td>
<td>c.135C&gt;T, p.Arg45Trp</td>
<td>0.000 0.012 0.000 0.003 0.000 0.000 0.003</td>
<td>D/PROD</td>
<td>0.46 0.09 rs267607017</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>c.661G&gt;A, p.Gly221Arg</td>
<td>0.000 0.000 0.000 0.000 0.009 0.000 0.001</td>
<td>D/PROD</td>
<td>0.46 0.16 rs76709509</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>c.2026A&gt;T, p.Ser670Cys</td>
<td>0.100 0.012 0.000 0.000 0.000 0.000 0.001</td>
<td>T/B</td>
<td>−1.60 0.02 rs752248086</td>
</tr>
<tr>
<td>12</td>
<td>4</td>
<td>c.351C&gt;A, p.Leu1172Ile</td>
<td>0.300 0.081 1.757 0.202 0.069 0.000 0.371</td>
<td>D/PROD</td>
<td>−0.30 0.01 rs143870426</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>c.581C&gt;T, p.Thr194Met</td>
<td>0.000 0.000 0.055 0.000 0.000 0.000 0.007</td>
<td>D/PROD</td>
<td>0.56 0.01 rs552391475</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>c.587C&gt;T, p.Thr196Ile</td>
<td>0.000 0.000 0.000 0.000 0.000 0.000 0.000</td>
<td>D/PROD</td>
<td>0.56 0.01 ND</td>
</tr>
<tr>
<td>5, 7</td>
<td>4</td>
<td>c.5596C&gt;G, p.Ser1199Cys</td>
<td>0.000 0.000 0.000 0.000 0.000 0.021 0.002</td>
<td>D/PROD</td>
<td>0.56 0.03 ND</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>c.5599G&gt;T, p.Gly1200Val</td>
<td>0.000 0.000 0.000 0.000 0.000 0.000 0.000</td>
<td>D/PROD</td>
<td>0.56 0.01 ND</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>c.5599G&gt;C, p.Gly1200Ala</td>
<td>0.000 0.000 0.000 0.000 0.000 0.000 0.000</td>
<td>D/PROD</td>
<td>0.56 0.01 ND</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>c.5599G&gt;A, p.Gly1200Asp</td>
<td>0.000 0.000 0.000 0.000 0.000 0.000 0.000</td>
<td>D/PROD</td>
<td>0.56 0.01 ND</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>c.3602T&gt;G, p.Val1201Gly</td>
<td>0.000 0.000 0.000 0.000 0.000 0.000 0.000</td>
<td>D/PROD</td>
<td>0.46 0.01 ND</td>
</tr>
<tr>
<td>14</td>
<td>4</td>
<td>c.4650T&gt;G, p.Asp1550Tyr</td>
<td>0.000 0.000 0.000 0.000 0.000 0.000 0.000</td>
<td>D/PROD</td>
<td>−1.73 0.00 rs760013790</td>
</tr>
<tr>
<td>11</td>
<td>4</td>
<td>c.3605delC, p.Asp2021GluTer3</td>
<td>0.500 0.174 0.000 0.000 0.000 0.001 0.012</td>
<td>D/PROD</td>
<td>−1.11 0.02 rs576305644</td>
</tr>
</tbody>
</table>

All identified variants were analyzed using three software prediction programs: Snpeff, Sorting Intolerant from Tolerant (SIFT; http://sift.jcvi.org; in the public domain), and PolyPhen2 (http://genetics.bwh.harvard.edu/pph/index.html; accessed on September 1, 2015). The allelic frequency of all of the variants was estimated in reference to two databases; the HGVD (http://www.genome.med.kyoto-u.ac.jp/SnpDB/about.htm, in the public domain) and the ExAC Browser (Beta; http://exac.broadinstitute.org; in the public domain). Conservation in the positions of the identified variants was evaluated with primate PhyloP 46-way primate and phastCons 46-way primate scores provided by UCSC based on the human genome 19 coordinates (http://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&g=cons46way; in the public domain). Variants classified as pathogenic met three criteria: high pathogenicity on the prediction program (damage on SIFT and probably damaging on Polyphen2 HDIV), low frequency (less than 1% on the ExAC Browser database for East Asian), and higher preservation scores (more than 0.1 on PhyloP 46way primate and 0.005 on Phast Cons 46way primate). Af, African; B, benign; D, damaging; EA, East Asian; Eu, European (non-Finish); Ex, exon; Hetero, heterozygous; Homo, homozygous; HGVD, human genetic variation database; La, Latino; LLP, less likely pathogenic; ND, not detected; P, pathogenic; PROD, probably damaging; SA, South Asian; T, tolerated.
was significantly associated with an abnormality of both EZ blurring and IZ absence of the photoreceptor microstructures. Thus, patients presenting clinically with occult macular dysfunction syndrome can be separated into those with hereditary OMD caused by genetic mutations (including Miyake's disease; \textit{RP1L1}-associated retinal disorder) and those with nonhereditary OMD-like syndrome with progressive occult maculopathy showing clinical signs resembling OMD.

Consistent changes of the photoreceptor microstructures were detected in the SD-OCT images in 11 of the 12 families in the pathogenic \textit{RP1L1}-positive group (Fig. 1; Table 5). A concordance of the microstructural phenotype and the presence of pathogenic \textit{RP1L1} variants was found in two families with pathogenic \textit{RP1L1} mutations (families 1, 7). These findings are keeping with the fact that the mutations of the \textit{RP1L1} gene result in specific damage in the photoreceptor microstructures in a unique manner.\textsuperscript{3,11,13,18} Our results therefore support the assumption that the \textit{RP1L1} protein is involved in the morphologic and functional maintenance of photoreceptors.

The most common pathogenic variant, \textit{c.133C>T},\textit{p.R45W}, was detected in five patients from four families in our study, which is a hot spot as previously reported.\textsuperscript{3} The missense pathogenic variants in seven patients were located between amino acid numbers 1196 and 1201, which is downstream of the doublecortin domain.\textsuperscript{7} In earlier reports, three pathogenic variants, \textit{p.S1199C}, \textit{p.S1199P}, and \textit{p.G1200A}, were also located in this region.\textsuperscript{7,16} This six amino acid residue sequence (TSSSGV), which is highly conserved throughout mammalian orthologues\textsuperscript{20} (NCBI HomoloGene; http://www.ncbi.nlm.nih.gov/homologene; in the public domain; accessed on June 1, 2016), may therefore be considered a second mutation hot spot for OMD. Furthermore, these results indicate that this significantly unique motif could have an important function in the \textit{RP1L1} protein.

There was still another pathogenic missense mutation, \textit{p.G221R}, not found around the aforementioned hot spot regions. A patient (19-II-4) with this mutation had neither the EZ blurring nor IZ absence in the SD-OCT images. Two possibilities explain this atypical photoreceptor microstructural phenotype. One is that the patient is currently at a very early stage of OMD although he is 69 years old with an onset at 64 years of age. Another possibility is a limitation in the \textit{in silico} prediction, which did not exclude this variant because the analysis was technically imperfect in evaluating the actual pathogenesis.

### Table 4. Two Major Variants of Retinal Disease–Causing Genes on RetNet Identified in RP1L1-Negative OMD

<table>
<thead>
<tr>
<th>ExAC Allele Frequency, %</th>
<th>ExAC AF</th>
<th>EA</th>
<th>SA</th>
<th>Eu</th>
<th>La</th>
<th>Af</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>dbSNP ID</td>
<td>HGVD</td>
<td>Phast Conservation Score</td>
<td>PhyloP State</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele Frequency, %</td>
<td>Pos</td>
<td>ExAC</td>
<td>Ex</td>
<td>Chr</td>
<td>Exon Acid Change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dbSNP ID</td>
<td>HGVD</td>
<td>Phast Conservation Score</td>
<td>PhyloP State</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| All patients were classified into one of the two groups based on the microstructural changes of the photoreceptors: a group with the classical SD-OCT findings in which there was blurring of the EZ and absence of the IZ. The second group was the nonclassical group with at least one of the two classical features lacking. All families were classified into one of two genotype groups based on the presence of pathogenic \textit{RP1L1} variants: the \textit{RP1L1}-positive group and the \textit{RP1L1}-negative group.

---

**Japanese Cohort With Occult Macular Dystrophy**

\textit{IOVS} September 2016 | Vol. 57 | No. 11 | 4844
Sporadic cases that had bilateral central cone dysfunction p.Y341X of the pathogenicity remains uncertain. Further genetic analyses mutations in RP1L1 blurring and IZ absence. This indicates that the classical SD-variants in this cohort of OMD patients. Eight of these patients > p.K203RfsX28; c.1637G high prevalence in the control group. 33,34 The other variant, interpreted as negative due to nonsegregation with disease and RP1 variant of the variants. There have been two reports describing the RP2 other type is a retinopathy with clinical signs of occult macular dysfunction syndrome, that is, clinical and ERG findings similar to hereditary OMD but not related to the Mendelian genetic abnormality. We refer to the non-Mendelian form as OMD-like syndrome (progressive occult maculopathy). Our results suggest that the classical SD-OCT findings and autosomal dominant family history, as well as the presence of known RP1L1 mutations, can help differentiate the two types.

There are limitations in our study. The applied sequencing method, the targeted genes for analysis, and the protocol for pathogenicity prediction may not be completely accurate. Large deletions in the targeted region could have been missed; proper analysis for the repetitive region (presumably around c.3900-4128) was not available with exome sequencing, and genes not listed on RetNet were not analyzed. More comprehensive gene screening including whole-exome analysis for genes with no prior association with inherited retinal disease in those unsolved families, as well as analysis methods such as whole genome sequencing, could help to further determine the genetic aberrations of our cohort—although short-read sequencing technology may still not allow clear delineation of the RP1L1 repeat region particularly in individuals who may harbor different numbers of repeats on each allele. Although the in silico analysis was useful in predicting the pathogenicity, multilateral approaches including functional analyses should be incorporated in future studies, for example, creating knock-in mice with the identical mutations, protein interaction assays with mutant proteins, and in vitro investigations of induced pluripotent stem cell-derived photoreceptors.

In conclusion, this study investigated the photoreceptor microstructural and molecular genetic characteristics of a Japanese cohort with OMD in a multicenter study. The findings delineated the spectrum of the disorder in both the photoreceptor microstructural phenotype and genotype. The results have highlighted the importance of knowledge of the photoreceptor microstructures for a more accurate diagnosis of the RP1L1-associated OMD (Miyake’s disease). In addition, the presence of other hereditary disorders, for example, non-RP1L1 hereditary OMD or non-Mendelian hereditary disorders with mostly nonclassical photoreceptor microstructural findings, an OMD-like syndrome, is implied in the RP1L1-negative patients with central cone dysfunction and normal fundus.

**Acknowledgments**

The authors thank the patients and their families for participation in this study. We thank Duo Hamasaki, PhD, of the Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, Florida, for discussions and editing of our manuscript. Supported by research grants from the Japan Agency for Medical Research and Development, the Ministry of Health, Labor and Welfare, and Japan, Grants-in-Aid for Scientific Research, Japan Society for the Promotion of Science. KF is supported by Foundation Fighting Blindness, United States. The authors have no proprietary or commercial interest in any materials discussed in this article.

Disclosure: K. Fujinami, None; S. Kameya, None; S. Kikuchi, None; S. Ueno, None; M. Kondo, None; T. Hayashi, None; K.
References


