Parafoveal Photoreceptor Abnormalities in Asymptomatic Patients With RP1L1 Mutations in Families With Occult Macular Dystrophy

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Oculta
cular macular dystrophy (OMD; OMIM 613587) 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.1–3 Heterozygous mutations in the retinitis pigmentosa 1-like 1 (RP1L1) gene (OMIM 608581) cause this ocular condition,3,5 and OMD with the RP1L1 mutations has been specifically designated as Miyake’s disease.4 The RP1L1 protein was suggested to be involved in the maintenance of the morphologic and functional characteristics of the photoreceptors,7,8 and a number of mutations in the RP1L1 gene have been reported.5,8,9,13–15 The most common mutation is the c.135C>T, p.Arg45Trp mutation in exon 2,7,8,9,11,13–15 and there is another hot spot between amino acid numbers 1194 and 1201 in exon 4, which is downstream of the doublecortin domain.6,9,10 In addition to the typical phenotype of OMD, extensive retinal dysfunction such as generalized cone dysfunction and generalized rod–cone dysfunction has been documented in patients with the biallelic RP1L1 gene.9,16

Similar to other autosomal-dominant ocular disorders, such as autosomal-dominant retinitis pigmentosa,17,18 Best vitelliform macular dystrophy,19,20 and autosomal-dominant optic atrophy,21–23 the phenotype of the carriers of the same RP1L1 mutation varies considerably.5 Some of these RP1L1 carriers were diagnosed to be unaffected based on subjective examinations such as the visual acuity and visual field tests. This has led to the suggestion that the mutations of RP1L1 have relatively low penetrance in some of the autosomal-dominant families.5 However, high penetrance autosomal dominant inheritance has been confirmed in a recent large cohort of OMD.6

Due to the large variations in the ocular symptoms and slow progressive development of the phenotype, the diagnosis of OMD needs to be confirmed multimodally by focal macular or multifocal ERGs,1–3,24 fundus autofluorescence (FAP),25,26 and
spectral-domain optical coherence tomography (SD-OCT). SD-OCT plays an especially important role in the diagnosis of OMD; the presence of pathogenic RP1L1 variants is significantly associated with characteristic abnormalities of the photoreceptor layer in the macular region (e.g., blurring of the ellipsoid zone [EZ] and absence of the interdigitation zone [IZ]). However, the characteristics of the asymptomatic cases with the RP1L1 variants have not been determined.

The purpose of this study was to investigate the retinal properties of seven asymptomatic eyes in four cases with the RP1L1 mutations. The phenotypes of these cases were determined by multimodal imaging and electrophysiologic studies. Three of these cases were either the parent or the child of symptomatic patients carrying the same mutation, and one was affected unilaterally.

**Patients and Methods**

An informed consent form was received from all the subjects for the tests after an explanation of the procedures to be used, and permission was obtained to use their medical data for research. The procedures used adhered to the tenets of the Declaration of Helsinki, and approval to perform this study was obtained from the Review Board/Ethics Committee of the National Institute of Sensory Organs, National Hospital Organization, Tokyo Medical Center.

We selected four families in which asymptomatic family members were present from the 16 families carrying RP1L1 mutations (Fig. 1). In the four families, the clinical data of the seven members (one man and six women, aged 19–82 years) are presented in Table 1. Three of them did not have any visual symptoms in either eye and were diagnosed as asymptomatic. One was unilaterally affected. For families 1, 2, and 3, the clinical phenotypes of the asymptomatic family members were compared to a family member with clinically diagnosed OMD.

**TABLE 1. Clinical Characteristics of Symptomatic and Asymptomatic Cases With RP1L1 Mutations**

<table>
<thead>
<tr>
<th>Family Case ID</th>
<th>Sex</th>
<th>Age at Onset, y</th>
<th>Age at Exam, y</th>
<th>R/L Visual Symptoms</th>
<th>BCVA</th>
<th>Visual Field Test</th>
<th>Amino Acid Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 KA290</td>
<td>F</td>
<td>11</td>
<td>19</td>
<td>Decreased VA</td>
<td>0.1</td>
<td>Relative central scotoma in HFA (10–2)</td>
<td>p.R45W, heterozygous</td>
</tr>
<tr>
<td>#2 KA208</td>
<td>M</td>
<td>26</td>
<td>32</td>
<td>Decreased VA</td>
<td>0.6</td>
<td>Relative central scotoma in HFA (10–2)</td>
<td>p.S1199C, heterozygous</td>
</tr>
<tr>
<td>#3 KA207</td>
<td>F</td>
<td>Unknown</td>
<td>50</td>
<td>Decreased VA</td>
<td>0.2</td>
<td>Relative central scotoma in HFA (10–2)</td>
<td>p.G1200D, heterozygous</td>
</tr>
<tr>
<td>#4 SMOP08</td>
<td>F</td>
<td>Unknown</td>
<td>55</td>
<td>Decreased VA</td>
<td>0.5</td>
<td>Relative central scotoma in HFA (10–2)</td>
<td>p.R45W, heterozygous</td>
</tr>
</tbody>
</table>

**Figure 1.** Pedigrees of four families with OMD. The black squares (men) and circles (women) represent patients with typical phenotype of OMD. Gray circles represent asymptomatic cases with the RP1L1 mutation. The proband of each pedigree is marked by an arrow. Asterisks indicate family members carrying the RP1L1 mutation. Patients without asterisks did not undergo genetic examinations.
In family 4, the findings in the asymptomatic right eye were compared to that of the symptomatic left eye. The diagnosis of OMD was made by the presence of a progressive decrease of the visual acuity, normal ophthalmoscopic appearance of the fundus, normal full-field ERGs, and localized macular dysfunction detected by focal macular or multifocal ERGs.\(^1\)\(^{-3}\)\(^\text{,}\)\(^5\)

Clinical and genetic tests were conducted for the segregation analyses of \(RP1L1\) mutation in two of the asymptomatic cases, KA291 and KA209, whereas, KA243, the 50-year-old daughter of KA007, consulted us to be examined.

For the genetic analyses, DNA was extracted from peripheral blood lymphocytes and screened for mutations in \(RP1L1\) as described in detail.\(^6\) Three heterozygous missense mutations, all of which were previously reported, were detected: c.133C > T, p.Arg45Trp\(^3\) in families 1 and 4; c.3599G > A, p.Ser1199Cys\(^6\) in family 2; and c.3596C > G, p.Ser1199Cys\(^7\) in family 3.\(^10\) To search for suspected genes, whole exome sequencing with targeted analysis for retinal disease-related genes on RetNet (RetNet, http://www.sph.uth.tmc.edu/RetNet; provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX, USA) and inheritance filtration were performed in the four families. Considering the inheritance and associated phenotypic expression of each rare candidate variant, only the \(RP1L1\) variant was determined to be causative. Some of the genetic data and clinical records of two of the affected patients, KA007 and SMOP08, have been published.\(^3\)\(^,\)\(^6\) SMOP08 is the same patient as case 1 in Ref. 5.

**Clinical Examinations**

Comprehensive ophthalmologic examinations were performed on all cases including measurements of the best-corrected visual acuity (BCVA) in decimal units, visual field testing, ophthalmoscopy, electrophysiologic assessments, FAF imaging, and SD-OCT imaging. The visual field assessments were made with the Humphrey visual field analyzer (HFA, model 750i; Carl Zeiss Meditec, Dublin, CA, USA) or with the Goldmann perimeter (GP). The SITA Standard strategy was used with the 30–2 program or the 10–2 program for the HFA analysis. The foveal threshold was also determined before the visual field testing by the HFA. The foveal threshold was also determined before the visual field testing by the HFA.

**Reflectivity Profiles of SD-OCT Images**

Optical coherence tomography images were obtained by SD-OCT (Cirrus HD OCT, version 6.5; Carl Zeiss Meditec) after pupil dilation. Four horizontal (9.0 mm) and vertical (6.0 mm) scans through the foveal center were averaged. To evaluate the microstructures of the photoreceptors, we used the longitudinal reflectivity profiles to assess the signal intensities especially of the EZ and IZ (Figs. 3–5). The longitudinal reflectivity was measured perpendicular to the RPE layer with considerations for the natural curvature of the photoreceptor and RPE layers (Fig. 3). The reflectivity was averaged over 5 pixels in width. The profile was determined either at the foveal center or at parafoveal regions which were 450 to 850 \(\mu m\) from the fovea.

In addition, we measured the OCT signal intensities of the IZ in symptomatic and asymptomatic cases and control eyes quantitatively. For this, we examined 31 eyes from 10 men and 21 women whose mean age was 43.2 ± 12.3 years with a range of 18 to 75 years. The peak intensities of the IZ relative to those of the RPE along the same profiles were calculated at the foveal center and parafoveal regions, which were 600 \(\mu m\) from the center. The distribution of signal intensities was analyzed and plotted by a commercially available software (BellCurve for Excel, version 2.13; Social Survey Research Information Co., Ltd., Tokyo, Japan).

**RESULTS**

In the pedigree charts, the symptomatic cases are indicated by black and the asymptomatic cases by gray (Fig. 1). The family members with \(RP1L1\) mutations are marked with asterisks, and all of them had a heterozygous pathogenic missense mutation in the \(RP1L1\) gene. The clinical characteristics and the results of the ocular examinations of both the symptomatic and asymptomatic cases are listed in Tables 1 and 2, and the fundus photographs and FAF images are shown in Figure 2. The profiles of the OCT reflectivity in each family are shown in Figures 4 and 5.

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**Table 2. Results of Ocular Examinations of Symptomatic and Asymptomatic Cases With \(RP1L1\) Mutations**

<table>
<thead>
<tr>
<th>Family</th>
<th>Case ID</th>
<th>R/L</th>
<th>Fovea</th>
<th>Parafovea</th>
<th>Fovea</th>
<th>Parafovea</th>
<th>FAF Abnormality in Fovea</th>
<th>Reduced Responses in Multifocal ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>KA290</td>
<td>R</td>
<td>Blurred</td>
<td>Blurred</td>
<td>Disappeared</td>
<td>Disappeared</td>
<td>Slight ring-shaped hyperfluorescence</td>
<td>Rings 1–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Blurred</td>
<td>Blurred</td>
<td>Disappeared</td>
<td>Disappeared</td>
<td>Slight ring-shaped hyperfluorescence</td>
<td>Rings 1–2</td>
</tr>
<tr>
<td>#2</td>
<td>KA007</td>
<td>R</td>
<td>Blurred</td>
<td>Blurred</td>
<td>Disappeared</td>
<td>Disappeared</td>
<td>Slight circular hyperfluorescence</td>
<td>Rings 1–3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Blurred</td>
<td>Blurred</td>
<td>Disappeared</td>
<td>Disappeared</td>
<td>Slight circular hyperfluorescence</td>
<td>Rings 1–3</td>
</tr>
<tr>
<td>#3</td>
<td>KA208</td>
<td>R</td>
<td>Blurred</td>
<td>(–)</td>
<td>Disappeared</td>
<td>(–)</td>
<td>Slight circular hyperfluorescence</td>
<td>Ring 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Blurred</td>
<td>Blurred</td>
<td>Disappeared</td>
<td>(–)</td>
<td>Slight circular hyperfluorescence</td>
<td>Ring 2</td>
</tr>
<tr>
<td>#4</td>
<td>SMOP08</td>
<td>R</td>
<td>Blurred</td>
<td>(–)</td>
<td>Disappeared</td>
<td>NE</td>
<td>NE</td>
<td>Ring 1–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L</td>
<td>Blurred</td>
<td>Blurred</td>
<td>Disappeared</td>
<td>NE</td>
<td>NE</td>
<td>Ring 1–2</td>
</tr>
</tbody>
</table>
FIGURE 2. Fundus photographs and FAF images of the both eyes of seven carriers from four families with OMD. Fundus photographs show no abnormalities except for KA007, who had microaneurysms due to diabetic retinopathy. In the FAF images, slight hyperfluorescence of the fovea which is characteristic in OMD can be seen in three cases: ring-shaped hyperfluorescence around the fovea in KA290, and circular hyperfluorescence at the fovea in KA007 and KA243. The family and patient identifications are indicated on the left.
Family 1

There were two symptomatic patients and one asymptomatic family member in Family 1, and all of them had a heterozygous mutation, c.133C > T, p.Arg45Trp, in the RP1L1 gene. KA291, the mother of KA290, did not have any visual symptoms, but her sister and daughter were diagnosed with typical OMD. Her BCVA was 1.0 (OD) and 1.2 (OS). Her visual field test showed a central relative scotoma by HFA (10–2) with normal foveal threshold in both eyes. The ocular fundus and FAF were normal in both eyes. The OCT images of KA291 showed normal appearing EZ and IZ (red asterisk in Fig. 4, left) at the fovea. KA290, the daughter of KA291, was symptomatic with OMD and showed typical OCT findings caused by RP1L1 mutations (Fig. 4, left). The EZ and IZ (red asterisk in Fig. 4, left) at the fovea were absent in both eyes (Fig. 2). The OCT images of KA292 showed normal appearing EZ and IZ (red asterisk in Fig. 4, right) at the fovea. However, the EZ was slightly disrupted and IZ was absent in the parafoveal regions.

Family 2

There was one symptomatic patient and one asymptomatic family member in Family 2, and both of them were heterozygous for the RP1L1 mutation, c.3599G > A, p.Gly1200Asp.6 KA243, the daughter of KA007, did not have any visual symptoms, and her BCVA was 1.0 (OD) and 1.2 (OS). Her visual field test showed a paracentral relative scotoma by HFA (10–2) with normal foveal threshold in both eyes. The OCT images of KA243 showed normal appearing EZ and IZ (red asterisk in Fig. 4, right) at the fovea (Fig. 2). The OCT images of KA007 with OMD showed the typical finding caused by the RP1L1 mutation,3 but the abnormal photoreceptor region was limited more in the fovea than other cases with RP1L1 mutations (Fig. 5, left). The OCT images of KA209 showed normal appearing EZ and IZ (red asterisk in Fig. 5, right) at the fovea; however, the EZ was slightly disrupted and IZ was absent in the parafoveal regions.

Family 3

There were four symptomatic patients and one asymptomatic family member in Family 3. The genetic and clinical examinations were performed on one affected and one unaffected member, and both of them had a heterozygous RP1L1 mutation, c.3596C > G, p.Ser1199Cys.6 KA209, the mother of KA208, did not have any visual symptoms. Her BCVA was 1.2 in both eyes, and her visual field tests did not show any abnormalities. The OCT images of KA209 showed normal appearing EZ and IZ (red asterisk in Fig. 5, right) at the fovea. However, the EZ was slightly disrupted and IZ was absent in the parafoveal regions.

Family 4

Family 4 was a large family with 13 affected patients, and part of the pedigree is shown in Figure 1. All of the affected members that were examined had a heterozygous mutation, c.133C > T, p.Arg45Trp.3,4 SMOP08, the sister of the proband, is a unilaterally symptomatic patient who complained of reduced visual acuity in the left eye since the age of 55 years, but did not have any visual symptoms in the right eye. Her BCVA was 1.2 (OD) and 0.1 (OS), and visual field tests revealed a relative central scotoma in the left eye but was normal in the right eye. The OCT images showed that the left eye of SMOP08 had normal appearing EZ and IZ (red asterisk in Fig. 5, right) at the fovea. However, the EZ was slightly disrupted and IZ was absent in the parafoveal regions.

We have measured the OCT signal intensities of the IZ in symptomatic and asymptomatic cases quantitatively and compared them to those in control eyes. The mean relative signal intensity of the IZ in symptomatic eyes was significantly lower than that in control eyes (p < 0.05). The OCT signal intensity of the IZ in asymptomatic eyes was also lower than that in control eyes, but the difference was not significant (p > 0.05). These findings suggest that the OCT signal intensity of the IZ is a sensitive indicator of disease severity and may be useful for prognosis and monitoring of disease progression.

Additional Figures:

Figure 3. OCT images and longitudinal reflectivity profiles of a normal eye (55-year-old woman). Top: horizontal OCT image with temporal, central, and nasal reflectivity profiles shown below; Bottom: vertical OCT image with inferior, central, and superior reflectivity profiles shown below. Red asterisks indicate sharp reflectivity peaks of IZ, which can be observed in normal eyes. ELM, external limiting membrane; ILM, internal limiting membrane; a.u., arbitrary units.
IZ intensities of the 31 control eyes are shown with the standard deviations in Figure 6. In control eyes, the mean peak intensity of the IZ relative to the RPE was $0.99 \pm 0.07$ (horizontal section) and $0.98 \pm 0.06$ (vertical section) at the foveal center. Those in the parafovea were $1.12 \pm 0.05$ at the nasal region, $1.11 \pm 0.07$ at the temporal region, $1.10 \pm 0.05$ at the superior region and $1.09 \pm 0.06$ at the inferior region.

The relative IZ intensities of the four symptomatic and four asymptomatic eyes are plotted for each eye. The OCT images of these eyes are presented in Figures 4 and 5. The IZ intensities of the symptomatic eyes (red marks in Fig. 6) were lower than that of normals at both the fovea and parafovea, except for that of KA208 which had normal intensities at the parafovea. On the other hand, the IZ intensities of the asymptomatic eyes (black marks in Fig. 6) were almost normal at the fovea and lower than normals at the parafovea.

**DISCUSSION**

Our results showed that asymptomatic cases with the *RP1L1* mutation had photoreceptor abnormalities in the microstructures of the parafoveal region (e.g., absence of the IZ and
The foveal center was preserved normally. The photoreceptor layer spared in the central fovea accounted for the well-preserved visual acuity in these asymptomatic cases.

Among the 40 members of the 16 families with the \textit{RP1L1} mutations in the National Institute of Sensory Organs database, we found 36 affected patients including one unilateral patient (SMOP08), three asymptomatic patients with parafoveal microstructural abnormalities (KA291, KA243 and KA209), and one unaffected case. The only unaffected case was a 60-year-old woman with the \textit{p.R45W} mutation who was previously reported.\textsuperscript{3,4} She did not have any visual symptoms and the photoreceptor layer in the SD-OCT images was normal both in the fovea and parafoveal regions. If we classify the three asymptomatic cases with parafoveal lesions to be affected, the penetrance of \textit{RP1L1} mutation in our cohort would be 97.5\% \textsuperscript{5} (39/40), which is much higher than that reported by another group.\textsuperscript{9} The relatively low penetrance observed in other studies could be partly due to the existence of asymptomatic patients with central foveal sparing. In this study, KA291 and KA209 did not show any detectable abnormalities in visual field tests and multifocal ERGs, and

**Figure 5.** OCT images and longitudinal reflectivity profiles of families \#3 and \#4. In symptomatic eyes (KA208 and left eye of the SMOP08), there is no reflectivity peak of IZ at the fovea. The EZ appears thickened and blurred at the fovea. In KA208, the reflectivity peaks of both EZ and IZ (double asterisks) are clearly observed at the parafovea. In the asymptomatic eyes (KA209 and right eye of the SMOP08), the reflectivity peak of EZ and IZ (red asterisk) are clearly observed at the fovea. In the parafovea, reflectivity peak of IZ is not observed.
only the detailed examinations of the SD-OCT images revealed photoreceptor abnormalities in the parafoveal region. Thus, some of the asymptomatic family members without detailed examinations could have been misclassified to be normal although other factors such as ethnic and environmental differences in the presence of photoreceptor abnormality should be considered to explain the low penetrance in the other studies.9

A sparing of the fovea is commonly observed in different types of macular diseases, such as in ABCA4- and PRPH2-related retinopathies,27–33 mitochondrial retinal dystrophy,34 macular dystrophy with CRB1 mutation,35 and age-related macular degeneration.36–39 The explanations for the physiologic and anatomic sparing of the fovea have been presented in many publications.40–49 However, it should be noted that in most cases with the RP1L1 mutation, the photoreceptor damage progresses simultaneously both in the fovea and parafoveal regions and the mechanisms underlying the central foveal sparing in OMD may be different from that of other disorders which typically have photoreceptor and RPE atrophy concurrently.27–35 Further investigations are needed to understand the underlying mechanism of the heterogeneity in primarily affected regions of the OMD.

The question arises on whether OMD with foveal sparing represents an early stage that will progress to a more advanced stage with foveal atrophy. The right fovea of SMOP08 has still been spared at the age of 82 years (Fig. 5, right), which is not commonly observed in other macular diseases. On the other hand, the photoreceptor structure of KA208 is selectively damaged at the fovea (Fig. 5, left), and the parafoveal region is preserved normal with clearly identified EZ and IZ (double asterisks in Fig. 5, left). These cases indicate that foveal sparing is not the natural course of the OMD and either central foveal or parafoveal can be independently affected in specific cases of OMD. Because none of the asymptomatic patients were followed for a long period of time, the natural course of the central foveal sparing has not been determined. Thus, we do not know whether the central foveal sparing observed in the four asymptomatic cases could be an initial phase or a subtype of macular lesion of OMD. Long-term observations will be able to confirm the natural course of these cases.

In this case series, two cases had the most common mutation, p.Arg45Trp; The other two cases had mutations p.S1199C and p.G1200D, which are located in another hot spot between amino acid numbers 1194 and 1201. Although the number of cases is not sufficient to conclude, particular relationships between the mutations and phenotypes were not suggested in the foveal-sparing phenotype.

In conclusion, we have shown that the asymptomatic patients with the RP1L1 mutation had photoreceptor abnormalities only in the parafoveal regions while the center of the fovea was spared. We conclude that detailed morphologic examination by SD-OCT is a valuable method to determine the genotype-phenotype relationship even in family members without any ocular symptoms. In addition, it is necessary to examine asymptomatic members carefully because some of them may progress to the typical phenotype of OMD.

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