Relationship Between Macular Curvature and Common Causative Genes of Retinitis Pigmentosa in Japanese Patients

Yoshito Koyanagi,1–4 Shinji Ueno,1 Yasuki Ito,1 Taro Kominami,1 Shiori Komori,1 Masato Akiyama,2–5 Yusuke Murakami,2 Yasuhiro Ikeda,2,6 Koh-Hei Sonoda,2 and Hiroko Terasaki1

1Department of Ophthalmology, Nagoya University Graduate School of Medicine, Nagoya, Japan
2Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
3Laboratory for Statistical Analysis, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan
4Laboratory for Statistical and Translational Genetics, RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan
5Department of Ocular Pathology and Imaging Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
6Department of Ophthalmology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Correspondence: Shinji Ueno, Department of Ophthalmology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan; ueno@med.nagoya-u.ac.jp.

Received: February 28, 2020
Accepted: June 18, 2020
Published: August 4, 2020

Citation: Koyanagi Y, Ueno S, Ito Y, et al. Relationship between macular curvature and common causative genes of retinitis pigmentosa in Japanese patients. Invest Ophthalmol Vis Sci. 2020;61(10):6. https://doi.org/10.1167/iovs.61.10.6

PURPOSE. To determine the relationship between the macular curvature and the causative genes of retinitis pigmentosa (RP).

METHODS. We examined the medical records of the right eyes of 65 cases with RP (31 men and 34 women; average age, 47.6 years). There were 31 cases with the EYS variants, 11 cases with the USH2A variants, six cases with the RPGR variants, 13 cases with the RP1 variants, and four cases with the RP1L1 variants. The mean curvature of Bruch’s membrane was calculated within 6 mm of the fovea as the mean macular curvature index (MMCI, 1/μm). We used multiple linear regression analysis to determine the independence of the causative genes contributing to the MMCI after adjustments for age, sex, axial length, and width of the ellipsoid zone.

RESULTS. The median MMCI was $-31.2 \times 10^{-7}/\mu m$ for the RPGR eyes, $-16.5 \times 10^{-7}/\mu m$ for the RP1L1 eyes, $-13.0 \times 10^{-7}/\mu m$ for the RP1 eyes, $-9.8 \times 10^{-7}/\mu m$ for the EYS eyes, and $-9.0 \times 10^{-7}/\mu m$ for the USH2A eyes. Compared with the EYS gene as the reference gene, the RPGR gene was significantly related to the MMCI values after adjusting for the other parameters ($P = 5.30 \times 10^{-5}$). In contrast, the effects of the other genes, USH2A, RP1, and RP1L1, were not significantly different from that of the EYS gene ($P = 0.26$, $P = 0.49$, and $P = 0.92$, respectively).

CONCLUSIONS. The RPGR gene had a stronger effect on the steep macular curvature than the other ciliopathy-related genes.

Keywords: macular curvature, causative genes, retinitis pigmentosa, spectral-domain optical coherence tomography, mean macular curvature index

Retinitis pigmentosa (RP) is the most common hereditary retinal degenerative disease worldwide.1 It is characterized by a reduction in vision, visual field constriction, and night blindness, which develop due to the dysfunction and death of the rod photoreceptors. These changes lead to the death of the cone cells and a reduction of the visual acuity.2,3

To date, 89 genes causing nonsyndromic RP have been registered in the Retinal Information Network (https://sph.uth.edu/retnet/). Accessed on November 28, 2019). The recent adaptation of high-throughput DNA sequencing technologies has accelerated the identification of the causative genes of Japanese patients with RP, and these studies have revealed the major causative genes in this population.1–4 Some of these major genes were categorized into the ciliopathy-related genes (e.g., the USH2A, RPGR, RP1, and RP1L1 genes),7–12 and it was also suggested that the function of the EYS protein is associated with the cilium.1,13,14

Recently, several studies have reported that steep macular curvatures were observed in the eyes of patients with various inherited retinal disorders, including retinal ciliopathy, Joubert syndrome, Leber’s congenital amaurosis, and RP without high myopia.15–20 In our earlier study, we established that the mean macular curvature index (MMCI) can be an additional parameter of the ocular anatomy that can be used to quantify the degree of macular curvature. The MMCI was determined from the spectral-domain optical coherence tomography (SD-OCT) images. We showed that the macular curvature of eyes with RP was steeper than that of normal eyes.21 We also found a significant relationship between the age, axial length (AL), and the width of the ellipsoid zone...
FIGURE 1. Fundus photographs and SD-OCT images of representative cases with RP for each causative gene. A: Case 1 is a 50-year-old female that was a case with EYS-related retinitis pigmentosa (RP) with an ellipsoid zone (EZ) of 635 μm. The mean macular curvature index (MMCI) was $-0.3 \times 10^{-5} \text{μm}^{-1}$. The axial length (AL) was 24.81 mm. B: Case 2 is a 48-year-old female with EYS-related RP with an EZ of 2762 μm. Bruch’s membrane has a steeper curvature than that of Case 1. The MMCI was $-36.0 \times 10^{-5} \text{μm}^{-1}$. The AL was 24.52 mm. C: Case 3 is a 53-year-old male with USH2A-related RP and an EZ of 3266 μm. The MMCI was $-8.0 \times 10^{-5} \text{μm}^{-1}$. The AL was 23.81 mm. D: Case 4 is a 34-year-old male who is a case of RPGR-related RP with an EZ of 2333 μm. The OCT image shows a steep macular curvature. The MMCI was $-47.8 \times 10^{-5} \text{μm}^{-1}$. The AL was 25.83 mm. E: Case 5 is a 23-year-old female who is a case of RP1-related RP without an intact EZ (0 μm). This case had a relatively flat macular line. The MMCI was $-5.62 \times 10^{-5} \text{μm}^{-1}$. The AL was 21.81 mm. F: Case 6 is a 67-year-old male who is a case of RP1L1-related RP without an intact EZ (0 μm). The MMCI was $-18.01 \times 10^{-5} \text{μm}^{-1}$. The AL was 24.09 mm.

Considering the genetic and clinical heterogeneity of RP, comparisons of the MMCIs associated with the causative genes is important for further understanding of the pathology of RP. Therefore, the aim of this study was to assess the relationship between the macular curvature and the causative genes for RP.

METHODS

Subjects

We reviewed the medical records of the right eye of 72 cases with typical RP from two facilities, Nagoya University Hospital (n = 37) and Kyushu University Hospital (n = 35), which were examined between 2002 and 2019. These cases had been genetically diagnosed with the five most frequent causative genes of RP (EYS, USH2A, RPGR, RP1, and RP1L1) in the Japanese population. The clinical diagnosis was based on the history of night blindness, ring scotoma and/or constriction of the visual fields, and severe rod-cone dysfunction or nonrecordable electroretinograms. In addition, an attenuation of the retinal vessels and bone spicule-like pigment clumping in the mid-peripheral and peripheral retina were detected by experienced ophthalmologists. We excluded seven cases (four EYS eyes, one USH2A eye, one RPGR eye, and one PR1 eye) due to incomplete clinical data, such as the visual acuity (n = 2), AL (n = 1), and SD-OCT images (n = 4). In the end, we studied 65 cases.

This retrospective study was approved by the ethics committee of each hospital (Nagoya University 16-0538-3, Kyushu University 2019-136) and was conducted in accordance with the tenets of the Declaration of Helsinki on biomedical research involving human subjects. The institutional review boards also waived the need for a written informed consent from each patient because the study design was a retrospective chart examination.

Measurements of Ocular Parameters

The best-corrected visual acuity (BCVA) was measured on the same day as the SD-OCT images were taken. For the statistical analyses, we converted the decimal value to logMAR units. We used an IOLMaster (Carl Zeiss Meditec, Dublin, CA, USA) to measure the AL of the eyes. We selected the horizontal scanned OCT images consisting of 100 averaged images with the eye-tracking system functioning for the analyses. To adjust the size of each image, we corrected for the differences in the pixel resolution between the longitudinal and transverse directions. We measured the width of the EZ between the borders where the EZ band touched the upper surface of the retinal pigment epithelium with the built-in calipers using the Heidelberg Eye Explorer software (Heidelberg Engineering, Heidelberg, Germany). If the entire length of the EZ line exceeded the size of the OCT image, the borders of the EZ line were set to be that of the OCT image.

Measurement of Macular Curvature

We calculated the MMCI as an objective index of the macular curvature as described in detail. Briefly, we quantitatively analyzed the reflective line corresponding to Bruch’s membrane across the fovea (yellow line in Fig. 1A in Komori...
et al.21) using MATLAB software (The MathWorks, Inc., Natick, MA, USA). We marked 12 points on the Bruch's membrane line beginning from the fovea (yellow triangles in Fig. 1A in Komori et al.21). The marks were separated by 760 μm in the OCT images. Using cubic spline interpolation, the approximate curvature of the marked points was calculated by the software (yellow and red lines in Fig. 1B in Komori et al.21). The curvatures in the 6-mm range including the fovea were selected from the calculated curve. To reduce the effects of the optic nerve head, curvature values outside this range were not used. Using all measured values for the local curvature in 1-μm steps, the mean curvature between ±3 mm from the central fovea (red line in Fig. 1B in Komori et al.21) was calculated. Plus values of MMCI indicated a convex shape and minus values indicated a concave shape.

### Genetic Diagnosis

Blood samples were collected for the genetic analyses. Genetic diagnosis was performed as described in detail.6,24,25 A summary of the genetic diagnosis of the RP cases is presented in Table 1.

| ID | Age, y | Gender | Causative Genes | Genetic Inheritance Form | Variant 1 | Zygosity 1 | Variant 2 | Zygosity 2 |
|----|--------|--------|-----------------|--------------------------|-----------|------------|-----------|------------|
| N-27 | 27     | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| N-38 | 54     | M      | EYS             | AR                       | p.(Ser1653fs) | Hetero     | p.(Tyr2935*) | Hetero     |
| N-122| 49     | M      | RPGR            | XL                       | p.(Glu746fs)  | Hemi       | —         | —         |
| N-71 | 44     | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| N-109| 59     | M      | USH2A           | AR                       | p.(Ser4748Phe) | Hetero     | p.(Cys934Trp) | Hetero     |
| N-167| 34     | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| N-168| 29     | M      | USH2A           | AR                       | p.(Thr3667Pro) | Hetero     | p.(Val164Phe) | Hetero     |
| N-169| 51     | F      | EYS             | AR                       | p.(Ala2736Pro) | Hetero     | p.(Gln203*) | Hetero     |
| N-180| 61     | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| N-183| 70     | F      | EYS             | AR                       | p.(Tyr2935*)  | Hetero     | p.(Asn404fs) | Hetero     |
| N-208| 48     | F      | USH2A           | AR                       | p.(Ser5060Pro) | Hetero     | p.(Ser4748Phe) | Hetero     |
| N-224| 39     | F      | EYS             | AR                       | p.(Ala2948Pro) | Homo       | —         | —         |
| N-225| 37     | M      | RPGR            | XL                       | p.(Glu1746fs) | Hemi       | —         | —         |
| N-250| 71     | M      | RP1             | AD                       | p.(Glu661*)   | Hetero     | —         | —         |
| N-257| 48     | F      | EYS             | AR                       | p.(Ser1653fs) | Hetero     | p.(Ala2498Pro) | Hetero     |
| N-259| 46     | F      | EYS             | AR                       | p.(Gly2186Glu) | Homo       | —         | —         |
| N-303| 60     | F      | EYS             | AR                       | p.(Asp498fs)  | Hetero     | p.(Cys211Tyr) | Hetero     |
| N-37 | 57     | F      | EYS             | AR                       | p.(Gly2799fs) | Hetero     | p.(Arg1870Trp) | Hetero     |
| N-39 | 31     | F      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| NA0048 | 19 | F      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| NA0070 | 38 | F      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| N-75 | 64     | F      | USH2A           | AR                       | p.(Cys934Trp)  | Hetero     | p.(Glu2186Glu) | Hetero     |
| NA0209 | 19 | M      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| N-228| 77     | F      | EYS             | AR                       | p.(Glu2186Glu) | Hetero     | p.(Tyr2935*) | Hetero     |
| N-235| 47     | M      | RPGR            | XL                       | p.(Glu1802fs) | Hemi       | —         | —         |
| N-294| 43     | M      | USH2A           | AR                       | p.(Cys934Trp)  | Homo       | —         | —         |
| N-298| 22     | M      | RPGR            | XL                       | p.(Thr575fs)  | Hemi       | —         | —         |
| NA1048 | 23 | M      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| N-1201| 36    | M      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| NAI209| 29    | F      | RP1             | AR                       | p.(Tyr1352Alafs*9) | Homo | —         | —         |
| OPH-783| 35   | M      | RPGR            | XL                       | p.?         | Hemi       | —         | —         |
| OPH-209| 49   | M      | EYS             | AR                       | p.(Ser1653fs) | Hetero     | p.(Trp2640*) | Hetero     |
| OPH-499| 64   | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| OPH-39 | 69    | M      | RPGR            | XL                       | p.(Gly718fs)  | Hemi       | —         | —         |
| OPH-753| 48   | F      | USH2A           | AR                       | p.(Cys934Trp)  | Homo       | —         | —         |
| OPH-423| 48   | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| OPH-985| 42   | M      | EYS             | AR                       | p.(Tyr2935*)  | Hetero     | p.(Glu2186Glu) | Hetero     |
| OPH-616| 47   | M      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| OPH-16 | 44    | M      | RP1             | AR                       | p.(Arg58*)    | Hetero     | p.(Glu501*)  | Hetero     |
| OPH-302| 54   | M      | USH2A           | AR                       | p.(Arg1870Trp) | Hetero | p.(Glu2752Arg) | Hetero     |
| OPH-570| 54   | M      | USH2A           | AR                       | p.(Arg58*)    | Hetero     | p.(Glu2752Arg) | Hetero     |
| OPH-908| 40   | M      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| OPH-458| 29   | F      | RP1             | AD                       | p.(Arg727fs)  | Homo       | —         | —         |
| OPH-698| 53   | F      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| OPH-16 | 44    | M      | USH2A           | AR                       | p.(Glu208Arg) | Hetero     | —         | —         |
| OPH-182| 23   | F      | RP1             | AD                       | p.(Cys1399fs) | Hetero     | —         | —         |
| OPH-980| 53   | M      | EYS             | AR                       | p.(Ser1653fs) | Homo       | —         | —         |
| OPH-613| 47   | M      | EYS             | AR                       | p.(Tyr2935*)  | Hetero     | —         | —         |
| OPH-159| 58   | M      | RP1             | AD                       | p.(Arg872fs)  | Hetero     | —         | —         |
TABLE 1. Continued

| ID       | Age, y | Gender | Causative Genes | Genetic Inheritance Form | Variant 1              | Zygosity 1 | Variant 2              | Zygosity 2 |
|----------|--------|--------|-----------------|--------------------------|------------------------|------------|------------------------|------------|
| OPH-767  | 51     | M      | EYS             | AR                       | p.(Cys211Tyr)           | Homo       | p.(Leu2938Met)         | Hetero     |
| OPH-43   | 52     | F      | USH2A           | AR                       | p.(Pro560Ala)           | Homo       | p.(Gly2752Arg)         | Homo       |
| OPH-552  | 46     | F      | EYS             | AR                       | p.(Gln3101fs)           | Homo       | —                      | —          |
| OPH-129  | 68     | M      | RP1L1           | AR                       | p.(Ala1009fs)           | Homo       | —                      | —          |
| OPH-824  | 53     | F      | EYS             | AR                       | p.(Tyr2935*)            | Homo       | —                      | —          |
| OPH-864  | 50     | F      | EYS             | AR                       | p.(Tyr2935*)            | Homo       | —                      | —          |
| OPH-459  | 36     | F      | USH2A           | AR                       | p.(Tyr3701*)            | Hetero     | p.(Ile3620Thr)         | Hetero     |
| OPH-465  | 66     | M      | RP1L1           | AR                       | p.(Arg568*)             | Homo       | —                      | —          |
| OPH-293  | 59     | F      | EYS             | AR                       | p.(Ser1653fs)           | Hetero     | p.(Met12Thr)           | Hetero     |
| OPH-279  | 50     | F      | EYS             | AR                       | p.(Ser1653fs)           | Homo       | —                      | —          |
| OPH-327  | 52     | M      | RP1L1           | AR                       | p.(Arg568*)             | Homo       | —                      | —          |
| OPH-791  | 42     | M      | RP1           | AD                       | p.(Gln689p)             | Hetero     | —                      | —          |
| OPH-222  | 78     | M      | RP1           | AD                       | p.(Arg872fs)            | Hetero     | —                      | —          |
| OPH-617  | 50     | F      | EYS             | AR                       | p.(Ser1653fs)           | Homo       | —                      | —          |
| OPH-51   | 43     | F      | EYS             | AR                       | p.(Tyr2935*)            | Hetero     | p.(Ser696fs)           | Hetero     |

The noncanonical splice site variant (c.28+5G→A) in RPGR was detected in OPH-783. NA0209 & NA1209 and NA0048 & NA1048 were siblings. AD, autosomal dominant; AR, autosomal recessive; Hetero, heterozygous; Hemi, hemizygous; Homo, homozygous; XL, X-linked.

Statistical Analyses

We determined the significance of the differences in the age, BCVA, AL, EZ width, and MMCI for the five causative genes by Kruskal-Wallis tests. We also compared the MMCI of each variant for each gene by Kruskal-Wallis tests. We used multiple linear regression analyses to determine the independence of the causative genes contributing to the MMCI. To adjust for the clinical factors, we included the sex (male or female), age (years, continuous), EZ width (μm, continuous), and AL (mm, continuous) as covariates in the analysis. The MMCI of the EYS eyes were defined as the reference value because the EYS is the most common causative gene of RP in the Japanese population, and the distribution of MMCI was similar to that of all the RP cases in our previous study (Table 2).1 We quantified the effects of the other genes relative to that of the EYS value. The MMCI values were converted to absolute square numbers to treat them as normal distributions. We considered a P value of <0.05 to be statistically significant. The R software version 3.4.4 was used for all statistical analyses (available in the public domain at http://www.R-project.org/).

RESULTS

Clinical and Genetic Characteristics of Patients With RP

The median BCVA of all the patients with RP was 0.30 logMAR units, and the median AL of all the patients with RP was 24.0 mm. In all of the eyes, the EZ was fully or partially disrupted. The median MMCI for all RP eyes was $-12.6 \times 10^{-5}$ μm⁻¹.

The clinical and genetic characteristics of the patients with RP for each causative gene are presented in Table 1 and Table 2. There were 31 cases with the EYS variants (10 men and 21 women), 11 cases with USH2A variants (6 men and 5 women), 6 cases with RPGR variants (6 men), 13 cases with RP1 variants (6 men and 7 women), and 4 cases with RP1L1 variants (3 men and 1 woman). Of the 13 RP1-related patients with RP, there were eight ARRP cases, and seven of them had an Alu insertion in the RP1 gene, which has been reported as a frequent causative variant of patients with RP1-related RP in Japan.25 The common variant in EYS [p.(Ser1653fs)] was detected in 16 cases, and none of these patents were relatives.26 No significant differences were observed in the AL, BCVA, or the EZ width among the different causative genes (Table 2). However, we found significant differences in the age and MMCI among the different causative genes (P = 0.01 and P = 0.02, respectively).

Representative Fundus Photographs and SD-OCT Images of Patients With RP for Each Causative Gene

The fundus photographs and horizontally scanned SD-OCT images of six right eyes of representative patients with RP with different causative genes (cases 1–6) are shown in Table 2. The fundus photographs and horizontally scanned SD-OCT images with different causative genes (cases 1–6) are shown.
in Figure 1. In the EYS eyes, one case had a flat macula (Fig. 1A) and another case had a steep macula (Fig. 1B). The USH2A (Fig. 1C), RP1 (Fig. 1E), and RP1L1 eyes (Fig. 1F) had relatively flat maculae. In contrast, the RPGR eye had a steep macula (Fig. 1D).

Distribution of MMCIs of RP Eyes Among Causative Genes

The distribution of the MMCIs of the RP eyes among the causative genes is shown in Figure 2. The MMCIs ranged from $-52.62 \times 10^{-5} \mu m^{-1}$ to $-0.26 \times 10^{-5} \mu m^{-1}$. Interestingly, the MMCI of all RPGR-related RP cases was less than $-24 \times 10^{-5} \mu m^{-1}$, indicating that the RPGR eyes had the steepest curvature. In contrast, all of the MMCIs of the USH2A and RP1L1 eyes were greater than $-20 \times 10^{-5} \mu m^{-1}$, indicating that these eyes had a flatter macular curvature. In addition, we observed a wide range of MMCIs in the EYS and RP1 eyes (Table 2 and Fig. 2). The median MMCI was $-31.2 \times 10^{-5}/\mu m$ for the RPGR eyes, $-16.5 \times 10^{-5}/\mu m$ for the RP1L1 eyes, $-13.0 \times 10^{-5}/\mu m$ for the RP1 eyes, $-9.8 \times 10^{-5}/\mu m$ for the EYS eyes, and $-9.0 \times 10^{-5}/\mu m$ for the USH2A eyes. The RPGR eyes had the steepest curvatures and were significantly steeper than that of the RP1L1 ($P = 0.01$), RP1 ($P = 0.003$), USH2A ($P = 0.0002$), and EYS ($P = 0.003$) eyes.

Correlations Between MMCI and Clinical Parameters

The significance of the correlations between age, BCVA, AL, and EZ width and the MMCI was determined for all RP eyes. The MMCI was significantly correlated with the AL and the EZ width ($P = 0.03$ and $P = 0.02$, respectively), although the correlation coefficients were relatively low. On the other hand, the MMCI was not correlated with age or the BCVA ($P = 0.87$ and $P = 0.63$, respectively). The significant correlation observed between MMCI and AL was consistent with the results of our previous study.21

Table 3. Results of Multiple Linear Regression Analysis for Independence of Causative Genes Contributing to MMCIs

| Gene    | Estimate | Standard Error | P Value |
|---------|----------|----------------|---------|
| USH2A   | $3.92 \times 10^{-5}$ | $3.47 \times 10^{-5}$ | 0.26 |
| RPGR    | $-2.42 \times 10^{-4}$ | $4.80 \times 10^{-5}$ | $5.30 \times 10^{-6}$ |
| RP1     | $-2.50 \times 10^{-5}$ | $3.60 \times 10^{-5}$ | 0.49 |
| RP1L1   | $5.57 \times 10^{-6}$ | $5.50 \times 10^{-3}$ | 0.92 |

Results of multiple linear regression analysis for independence of causative genes contributing to the MMCIs. To adjust for the clinical factors, we included the sex (male or female), age (years, continuous), EZ width (μm, continuous), and AL (mm, continuous) as covariates in the analysis. The effect of the EYS gene on MMCI was defined as a reference. We quantified the effects of other genes. The MMCI values were converted to absolute square numbers to treat them as normal distributions.

Multiple Linear Regression Analysis for Independence of Causative Genes Contributing to MMCIs

Multiple linear regression analyses were performed to assess the independence of the causative genes contributing to the MMCIs after adjustment for age, sex, AL, and EZ width (Table 3). We used the effect of the EYS on MMCI as a reference and quantified the effects of the other genes relative to that of EYS. The MMCI values were converted to absolute square numbers to treat them as being normally distributed. Compared with the effect of EYS as the reference gene, RPGR significantly affected the MMCI values ($P = 5.30 \times 10^{-6}$). On the other hand, the effects of the other genes, USH2A, RP1, and RP1L1, were not significantly affected compared with the EYS gene ($P = 0.26$, $P = 0.49$, and $P = 0.92$, respectively).

Variant-Based Analyses

We compared the MMCIs of each homozygous variant of each AR gene, including p.(Ser1653fs), p.(Tyr2935*), p.(Glu1886Glu), p.(Cys111Tyr), and p.(Gln691fs) in EYS; p.(Cys934Trp), p.(Glu768Arg), and p.(Pro560Ala) in USH2A; and p.(Arg658*) and p.(Ala1009fs) in RP1L1 (Table 1). For RPGR, we compared the MMCIs of each hemizygous variant [p.(Glu746fs), p.(Glu802fs), p.(Thr575fs), p.(Arg718fs)] and one splice-site variant (c.28+5G>A) (Table 1). We also compared the MMCIs of homozygous variants of RP1L1-related ADRP [p.(Tyr1352Alafs*9) and p.(Cys1399fs)] or heterozygous variants of RP1L1-related ADRP [p.(Glu661*), p.(Arg872fs), and p.(Gln689*)], because the inheritance pattern of this gene can be autosomal recessive or autosomal dominant (Table 1). Significant differences in the MMCIs of each variant were not observed for each gene (Kruskal-Wallis tests). We further examined the MMCI of EYS-related RP cases with the most frequent homozygous variants [p.(Ser1653fs) in EYS] and observed that MMCI varied even within the same variants (Supplementary Fig. S1).

Discusion

Our findings showed that the steepness of macular curvature in RP eyes differs among the different causative genes for RP. More specifically, the eyes with the RPGR gene had the steepest curvature compared to the other ciliopathy-related
genes. Earlier studies have reported the findings in RPGR eyes complicated by high myopia\textsuperscript{29–31} and on the relationship between RPGR-related RP and pathologic myopia.\textsuperscript{31,34} However, the macular curvature in these eyes was not mentioned. Our study showed that the macular curvature in eyes with RPGR-related RP was steeper than that in eyes with RP caused by other genes, and the effects of RPGR on MMCIs were significant even after adjusting for AL. These findings indicate that the RPGR gene affected the macular curvature independently. Considering that RPGR-related X-linked RP is the target of current clinical trials of adeno-associated viral-mediated gene replacement therapy,\textsuperscript{11,33,34} the MMCIs may be a useful parameter to evaluate the characteristics of this macula structure in the preoperative evaluations for the prevention of complications of subretinal injection, such as a macular hole. An observation of the MMCIs before injection into the subretinal space could provide information on the appropriate angle, site, and injection dose in clinical trials.

We also found that the distribution of the MMCIs differed among the causative genes, although the MMCIs of RP caused by each gene was lower than that of controls ($-6.63 \pm 5.63 \times 10^{-5} \mu m$).\textsuperscript{21} The EYS eyes had a wide range of MMCIs, and in contrast, the USH2A and RP1L1 eyes had a flat macula and a narrow range of distribution of the MMCIs. We could differentiate the effects on the specific phenotype of RP among causative genes using the MMCIs.

Our results also indicated that there are morphologic differences among the ciliopathy-related genes. Although most of the genes examined, USH2A, RPGR, RP1, and RP1L1, encode proteins located in the photoreceptor cilia,\textsuperscript{7–12} earlier studies have reported differences in the location of the encoded protein in the photoreceptors.\textsuperscript{10} Interestingly, the protein of RPGR is located only in the basal body of the cilium.\textsuperscript{10} Thus, we hypothesize that the abnormalities of the basal body of the cilia might lead to structural changes and steep macular curvature. However, the mechanisms that lead to this difference in MMCIs among genes need additional experimental data.

In a previous study, Khan et al.\textsuperscript{18} reported their findings in cases of recessive early-onset retinal dystrophy with macular staphyloma caused by the C21orf2 gene, which encodes a protein that is localized to the photoreceptor primary cilium. However, reports on macular curvature in inherited retinal dystrophy, including RP, and detailed evaluations of the relationship between macular curvature and visual function are limited. Therefore, an investigation of the relationship between increased macular curvature and visual function is necessary. The results of this study showed the steepest curvature in RPGR-related RP, which was previously described to have a severe course.\textsuperscript{1,28} This suggests that MMCIs are somehow related to disease severity. If the steep curvature led to RP progression, the reduction of macular curvature might be a potential target for treatment. Increasing the number of cases, including cases with other causative genes, will also be important in future studies.

This study has several strengths. We collected a relatively large number of cases with genetically identified causative genes and quantified the macular curvature for each causative gene. In addition, we verified the effects of the genes on the MMCIs after adjusting for other parameters.

There are also limitations in this study. This was a cross-sectional and retrospective analysis with potential selection biases. RP is a rare disease, and the number of genetically solved cases is limited. The RPGR-related RP is known to have a severe course, and it is assumed that the EZ line was shorter at a younger age. However, the number of subjects with RP caused by each gene in this study was small, and it is difficult to evaluate the clear trends by age and EZ line for each gene. In variant-based analysis, we could not detect significant differences in the phenotype-genotype correlation because most of the cases consisted of only one patient if we divided groups by each variant. There were several cases with the most frequent homozygous variant [p.(Ser1653fs) in EYS], and our results suggest that the MMCIs also varied among cases caused by the same causative variant. However, further comparisons with other variants were difficult; therefore, the small size of our sample was a limitation for variant-based statistical evaluations.

In addition, most of the RPGR-related RP cases were caused by frameshift variants except for one case (OPH-783). Previous reports have suggested that frameshift variants of the RPGR cause more severe clinical alterations; therefore, this may have limited our results (Table 1). Further multicenter studies are needed to collect cases to reduce the selection bias.

In conclusion, our results suggest that the steepness of the macular curvature is significantly associated with the causative genes for RP. The RPGR gene had a stronger effect on the steep macular curvature than the other ciliopathy-related genes.

Acknowledgments

The authors thank Duco Hamasaki (Bascom Palmer Eye Institute) for the discussions and editing the final version of the manuscript, as well as Yukihide Momozawa and the members of the Laboratory for Genotyping Development, RIKEN Center for Integrative Medical Sciences.

Supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI (grant 19K00928 to SU) and Takayama Retina Research Award (to SU).

Disclosure: Y. Koyanagi, None; S. Ueno, Novartis Pharma K.K. (F), Tomey Corporation (F), Nidek Company (F), Canon Life Care Solutions (F), HOYA Company (F); Y. Ito, Alcon Japan (F), Bayer Health Care (F), Canon Life Care Solutions (F), Carl Zeiss Meditec (F), Kowa Pharmaceutical (F), Novartis Pharma K.K. (F), Pfizer Japan (F), Santen Pharmaceutical (F); T. Kominnami, None; S. Komori, None; M. Akiyama, Nidek Company (F); Y. Murakami, None; Y. Ikeda, Nideck Company (F), HOYA Corporation (F, C), Otsuka Pharmaceutical (F), Santen Pharmaceutical (F), Novartis Pharma K.K. (F), Senju Pharmaceutical (F), Kowa Pharmaceutical (F), Bayer Health Care (F); K.-H. Sonoda, None; H. Terasaki, Nideck Company (F), Rohto Pharmaceutical (F), Otsuka Pharmaceutical (F), Pfizer Pharmaceutical (F), Bayer Japan (F), Santen Pharmaceutical (F), Alcon Japan (F), Novartis Pharma K.K. (F), Carl Zeiss Meditec (F, C), Senju Pharmaceutical (F), Kowa Pharmaceutical (F), Bayer Health Care (F, C), Nakamoto (F), HOYA Corporation (F, Astellas Pharma (F), Ono Pharmaceutical (C), Sanwa Kagaku Kenkyusho (F), Aichi Ophthalmologists Association (F), Nitten Pharmaceutical (F), Takeda Pharmaceutical Company (F), Chiba Ophthalmologist Association (F), Japan Medical Association (F), Fukushima Ophthalmologist Association (F)

References

1. Verbakel SK, van Huet RAC, Boon CJF, et al. Non-syndromic retinitis pigmentosa. Prog Retin Eye Res. 2018;66:157–186.
2. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet. 2006;368:1795–1809.
3. Campochiaro PA, Mir TA. The mechanism of cone cell death in retinitis pigmentosa. Prog Retin Eye Res. 2018;62:24–37.
4. Oishi M, Oishi A, Gotoh N, et al. Comprehensive molecular diagnosis of a large cohort of Japanese retinitis pigmentosa and Usher syndrome patients by next-generation sequencing. *Invest Ophthalmol Vis Sci.* 2014;55:7369–7375.

5. Maeda A, Yoshida A, Kawai K, et al. Development of a molecular diagnostic test for retinitis pigmentosa in the Japanese population. *Ipn J Ophthalmol.* 2018;62:451–457.

6. Koyanagi Y, Akiyama M, Nishiguchi KM, et al. Genetic characteristics of retinitis pigmentosa in 1204 Japanese patients. *J Med Genet.* 2019;56:662–670.

7. Adams NA, Awadein A, Toma HS. The retinal ciliopathies. *Ophthalmic Genet.* 2007;28:113–125.

8. Liu Q, Zhang Q, Pierce EA. Photoreceptor sensory cilia and inherited retinal degeneration. *Adv Exp Med Biol.* 2010;664:223–232.

9. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. *N Engl J Med.* 2011;364:1533–1543.

10. Estrada-Cuzcano A, Roepman R, Cremers FP, den Hollander AI, Mans DA. Non-syndromic retinal dystrophy: translating gene discovery into therapy. *Hum Mol Genet.* 2012;21:R111–R124.

11. Dias MF, Joo K, Kemp JA, et al. Molecular genetics and emerging therapies for retinitis pigmentosa: basic research and clinical perspectives. *Prog Retin Eye Res.* 2018;63:107–131.

12. Takahashi VKL, Xu CI, Takiuti JT, et al. Comparison of structural progression between ciliopathy and non-ciliopathy associated with autosomal recessive retinitis pigmentosa. *Orphanet J Rare Dis.* 2019;14:187.

13. Alfano G, Krucezke PM, Shah AZ, et al. EYS is a protein associated with the ciliary axoneme in rods and cones. *PloS One.* 2016;11:e0166397.

14. Lu Z, Hu X, Liu F, et al. Ablation of EYS in zebrafish causes mislocalisation of outer segment proteins, F-actin disruption and cone-rod dystrophy. *Sci Rep.* 2017;7:46098.

15. Xu X, Fang Y, Yokoi T, et al. Posterior staphylomas in eyes with retinitis pigmentosa without high myopia. *Retina.* 2019;39:1299–1304.

16. Kuniyoshi K, Sakuramoto H, Yoshitake K, et al. Longitudinal clinical course of three Japanese patients with Leber congenital amaurosis/early-onset retinal dystrophy with RDH12 mutation. *Doc Ophthalmol.* 2019;128:219–228.

17. Toma C, Rubio G, Marzi F, et al. Macular staphyloma in patients affected by Joubert syndrome with retinal dystrophy: a new finding detected by SD-OCT. *Doc Ophthalmol.* 2018;137:25–36.

18. Khan AO, Eisenberger T, Nagel-Wolfrum K, Wolfrum U, Bolz HJ. C21orf2 is mutated in recessive early-onset retinal dystrophy with macular staphyloma and encodes a protein that localises to the photoreceptor primary cilium. *Br J Ophthalmol.* 2015;99:1725–1731.

19. Parmeggiani F, De Nadai K, Piovan A, Binotto A, Zamengo S, Chizzolini M. Optical coherence tomography imaging in the management of the Argus II retinal prostheses system. *Eur J Ophthalmol.* 2017;27:e16–e21.

20. Smirnov VM, Marks C, Drumare I, Defoort-Dhellemmes S, Dhaenens CM. Severe retinitis pigmentosa with posterior staphyloma in a family with c.886C>A p.(Lys296Glu) RHO mutation. *Ophthalmic Genet.* 2019;40:365–368.

21. Komori S, Ueno S, Ito Y, et al. Steeper macular curvature in eyes with non-highly myopic retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 2019;60:3135–3141.

22. Kaplan J, Bonneau D, Frezal J, Munnich A, Dufer JI. Clinical and genetic heterogeneity in retinitis pigmentosa. *Hum Genet.* 1990;85:635–642.

23. Kominami T, Ueno S, Kominami A, et al. Associations between outer retinal structures and focal macular electroretinograms in patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 2017;58:5122–5128.

24. Kominami A, Ueno S, Kominami T, et al. Case of cone dystrophy with normal fundus appearance associated with biallelic POC1B variants. *Ophthalmic Genet.* 2018;39:255–262.

25. Nikopoulos K, Cisarova K, Quinodoz M, et al. A frequent variant in the Japanese population determines quasi-Mendelian inheritance of rare retinal ciliopathy. *Nat Commun.* 2019;10:2884.

26. Hosono K, Ishigami C, Takahashi M, et al. Two novel mutations in the EYS gene are possible major causes of autosomal recessive retinitis pigmentosa in the Japanese population. *PloS One.* 2012;7:e31036.

27. Iwanami M, Oshikawa M, Nishida T, Nakadomari S, Kato S. High prevalence of mutations in the EYS gene in Japanese patients with autosomal recessive retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 2012;53:1033–1040.

28. Tee JI, Smith AJ, Hardcastle AJ, Michaelides M. RPGR-associated retinopathy: clinical features, molecular genetics, animal models and therapeutic options. *Br J Ophthalmol.* 2013;97:1022–1027.

29. Tee JI, Yang Y, Kalitezos A, Webster A, Bainbridge J, Michaelides M. Natural history study of retinal structure, progression, and symmetry using ellipsoid zone metrics in RPGR-associated retinopathy. *Am J Ophthalmol.* 2019;198:111–123.

30. Mawatari G, Fujinami K, Liu X, et al. Clinical and genetic characteristics of 14 patients from 13 Japanese families with RPGR-associated retinal disorder: report of eight novel variants. *Hum Genome Var.* 2019;6:34.

31. Parmeggiani F, Barbaro V, De Nadai K, et al. Identification of novel X-linked gain-of-function RPGR-ORF15 mutation in Italian family with retinitis pigmentosa and pathologic myopia. *Sci Rep.* 2016;6:39179.

32. Sanchez Tocino H, Diez Montero C, Villanueva Gomez A, Lobo Valentin R, Montero-Moreno JA. Phenotypic high myopia in X-linked retinitis pigmentosa secondary to a novel mutation in the RPGR gene. *Ophthalmic Genet.* 2019;40:170–176.

33. Cehajic Kapetanovic J, McClements ME, Martinez-Fernandez de la Camara C, MacLaren RE. Molecular strategies for RPGR gene therapy. *Genes (Basel).* 2019;10:674.