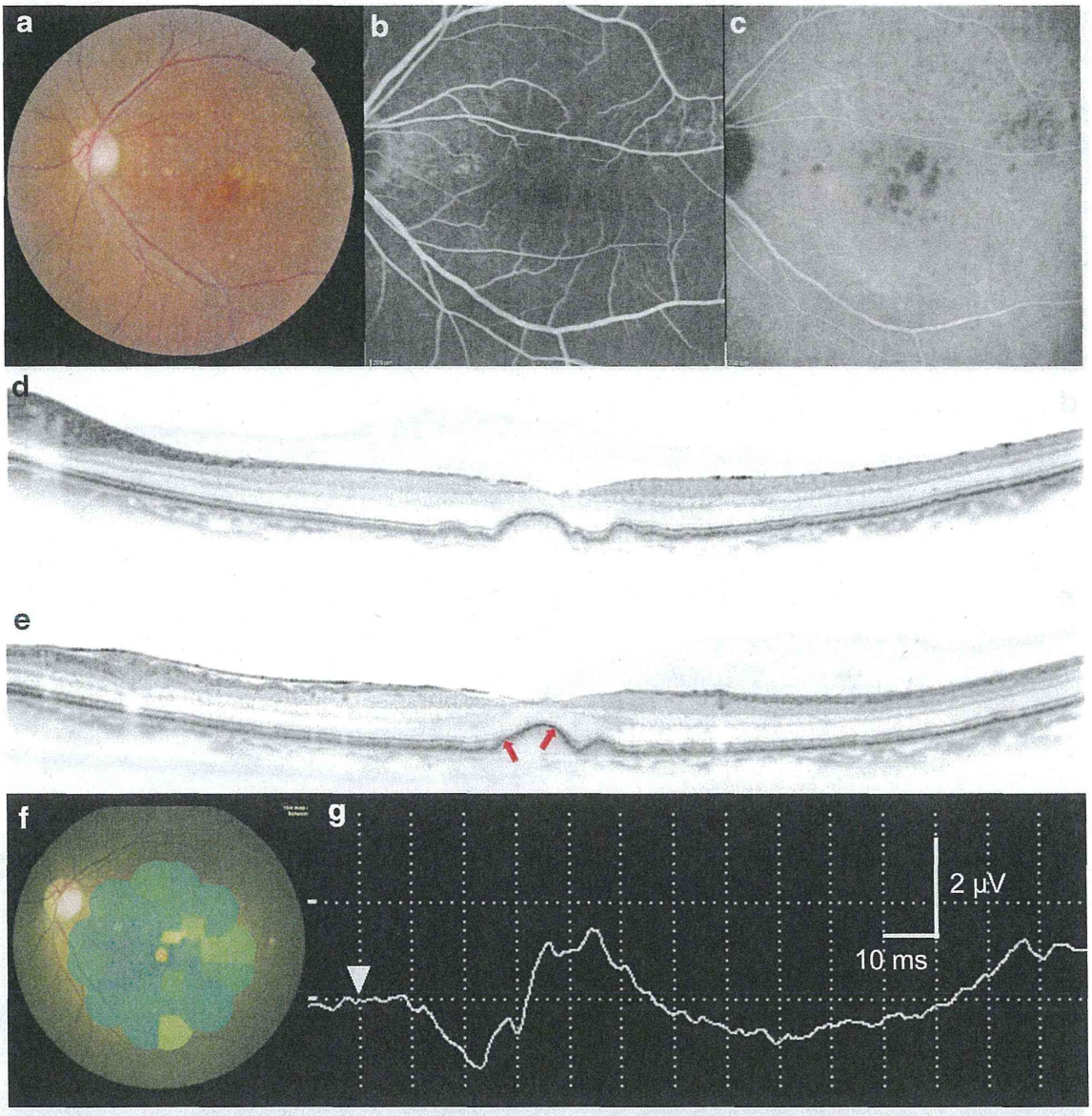

Fig. 5 Macular function in an eye with large drusen. **a** Fundus photograph shows multiple large drusen in the macular area (1.0 on a Landolt chart, OD). **b**, **c** *Fluorescein* and *indocyanine green* angiograms reveal no choroidal neovascularization. *Horizontal* **d** and *vertical* **e** sections obtained with OCT show multiple large drusen beneath and affecting the fovea. The junction of the inner and outer segments of photoreceptors (between the *arrows*) was discontinued. **f** Microperimetry shows preserved retinal sensitivity within the macular area except for the fovea. **g** Focal macular electroretinogram shows that the amplitude of all of the waves was relatively preserved. *Arrowhead* beginning of stimulus

control group, 13 eyes had small drusen in the macular area and 7 had no drusen. All eyes showed good macular function (Fig. 1).

All functional parameters were measured, with VA, fmERG, and microperimetry showing significant variation between the groups (Table 1). All eyes with neovascular AMD showed marked morphologic changes in the neurosensory retina. In this group, cystoid macular edema was seen in 4 eyes (21 %), serous retinal detachment in 14 eyes (74 %), and PED in 17 eyes (89 %); foveal thickness of the neurosensory retina (384 ± 256 μm) was significantly increased compared with the control eyes (224 ± 27 μm) (Fig. 2). Consistent with these morphologic changes, macular function (VA, fmERG, and microperimetry) was significantly deteriorated in the neovascular AMD group (Figs. 3, 4).

In the large drusen group, all eyes showed multiple large drusen in the macular area; the mean number of drusen measuring 125–250 μm was 10.3 ± 4.2 and that of drusen measuring at least 250 μm was 3.1 ± 2.1. These eyes showed minimal morphologic changes in the neurosensory retina. No eyes in this group showed cystoid macular

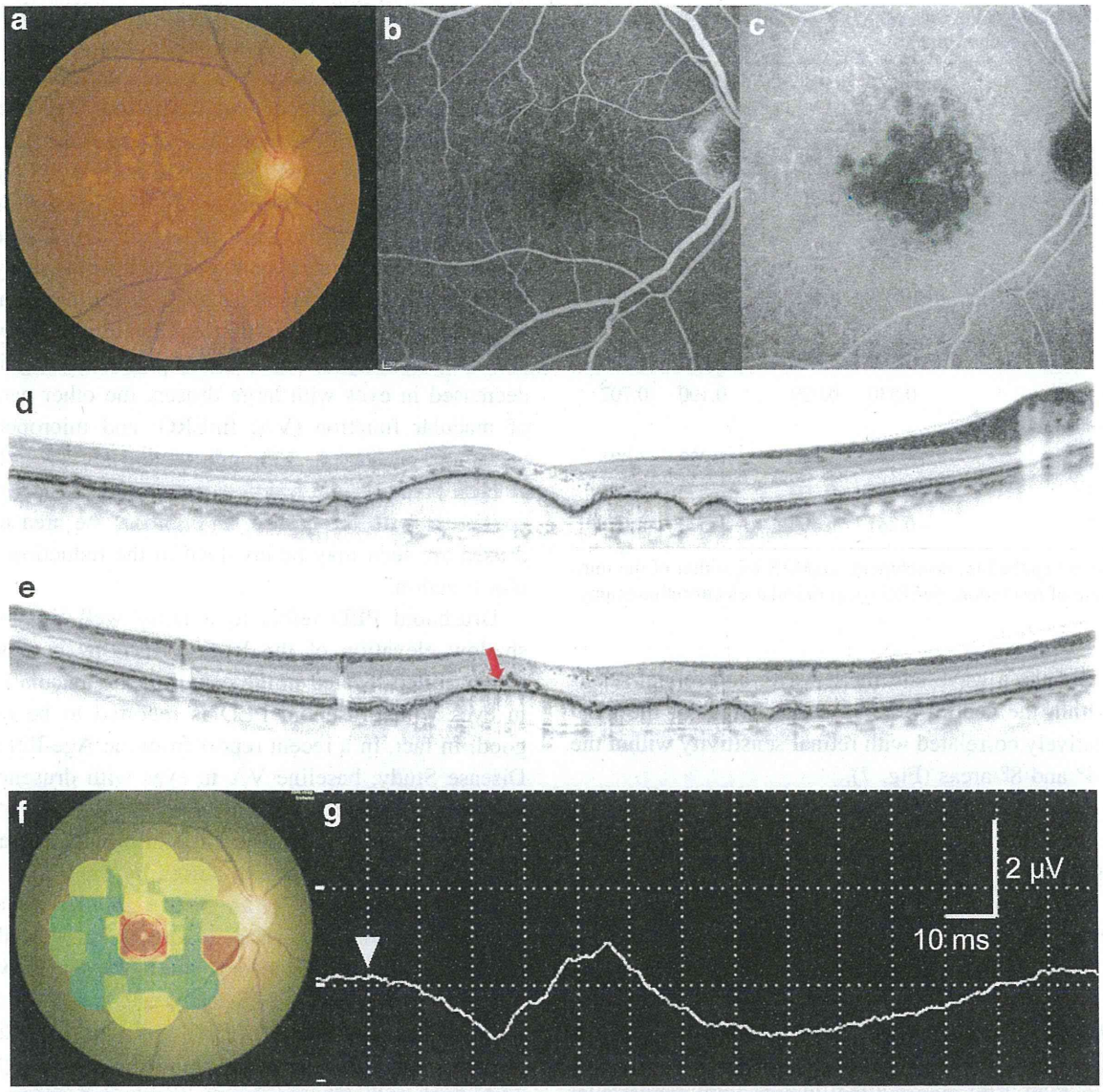

Fig. 6 Macular function in an eye with drusenoid pigment epithelial detachment (PED). **a** Fundus photograph of drusenoid PED under the fovea (0.7 on a Landolt chart, OD). **b** *Fluorescein* angiogram reveals no choroidal neovascularization. **c** From the late-phase *indocyanine green* angiogram, the area of drusenoid PED was calculated as 6.26 mm^2. *Horizontal* **d** and *vertical* **e** sections obtained with OCT show drusenoid PED. The height of the PED was 258 µm. The *red arrow* indicates hyperreflective foci. **f** Retinal sensitivity map obtained with microperimetry shows a marked reduction in retinal sensitivity consistent with drusenoid PED. **g** In the focal macular electroretinogram, the amplitude of each wave was reduced to 60–75 % of normal amplitudes. *Arrowhead* beginning of stimulus

edema, serous retinal detachment, or a vitelliform lesion. Foveal thickness of the neurosensory retina (196 ± 40 µm) was no different from that in the control group (Fig. 2). In this large drusen group, while retinal sensitivity at the central point was significantly decreased, the other parameters of macular function (VA, fmERG, and microperimetry) were preserved (Figs. 3, 5).

In the drusenoid PED group, all eyes had drusenoid PED of at least 1/2 disc diameter within the macular area. The mean area of the PED was 4.78 ± 3.74 mm^2, and the mean height was 266 ± 178 µm. In eyes with drusenoid PED, the foveal thickness between the ILM and the Bruch membrane (377 ± 164 µm) was significantly greater than that in the control eyes (224 ± 27 µm, $P = 0.013$). However, the structure of the neurosensory retina was well preserved, and the foveal thickness between the ILM and RPE (200 ± 49 µm) did not differ from that in the control group (Fig. 2). On the other hand, the macular function of these eyes was significantly deteriorated. VA, amplitude of the a-wave and of the b-wave, and retinal sensitivity measured with the MP1 were significantly decreased when compared with the control eyes (Figs. 3, 6). Table 2 shows the correlation between the size of the drusenoid PED and macular function and between the area of the PED and the

Table 2 Correlation between size of drusenoid pigment epithelium detachment and macular function

	Area of drusenoid PED		Height of drusenoid PED	
	r	*P* value	*r*	*P* value
Visual acuity in logMAR	0.058	0.820	0.432	0.074
Amplitude of fmERG				
a-wave	−0.427	0.077	−0.118	0.642
b-wave	−0.445	0.067	−0.312	0.207
Latency of fmERG				
a-wave	0.635	0.006	−0.090	0.732
b-wave	0.530	0.029	0.100	0.702
Retinal sensitivity				
Center point	−0.472	0.056	−0.423	0.091
Within 4°	−0.682	0.003	−0.625	0.007
Within 8°	−0.761	0.0004	−0.533	0.028

PED pigment epithelium detachment, *logMAR* logarithm of the minimum angle of resolution, *fmERG* focal macular electroretinography

latencies of the a-wave and the b-wave, and retinal sensitivity within the central 4° or 8°. The height of the PED was negatively correlated with retinal sensitivity within the central 4° and 8° areas (Fig. 7).

Discussion

Eyes with neovascular AMD often have a severe decrease in VA. In addition, because such eyes often show serous retinal detachment, subretinal hemorrhage, retinal edema, or PED in the macular area, they may well have a reduction in function in the macular area. With the use of fmERG, Nishihara et al. [26] reported that in eyes with neovascular AMD, the amplitude of each wave was reduced to 29 to 35 % of that of the control eyes. With the use of microperimetry, Sulzbacher et al. [24] and Hautamäki et al. [27] more recently reported that retinal sensitivity was markedly decreased within the area of CNV, macular edema, hemorrhage, subretinal fluid, and PED in eyes with neovascular AMD. In our patients with neovascular AMD, cystoid macular edema was seen in 21 %, serous retinal detachment in 74 %, and PED in 89 % of the patients, and the thickness of the fovea in the neurosensory retina was significantly increased. In eyes with neovascular AMD, severe macular dysfunction is based on the morphologic changes caused by the exudative change resulting from the CNV.

Eyes with drusen often maintain good VA. However, as the number or size of the drusen increases, they may cause a functional disturbance in the macular area. So far, several electrophysiologic assessments have been performed to study the macular function in eyes with drusen [23, 28–33]. Falsini et al. [33] documented an abnormality of the focal ERG threshold in eyes with more than 20 soft drusen, although they did not investigate the correlation between each drusen and the local sensitivity loss. With the use of microperimetry, Midena et al. [16] reported that retinal sensitivity in eyes with large drusen (>125 μm) was severely deteriorated. Iwama et al. [34] reported that eyes with confluent soft drusen often show focal areas with reduced retinal function consistent with irregularity of the RPE line or of the junction between the inner and outer segments of the photoreceptors. In the current study, while retinal sensitivity at the central point was significantly decreased in eyes with large drusen, the other parameters of macular function (VA, fmERG, and microperimetry) were well preserved. Although we did not assess function at each point, retinal function may be focally deteriorated, consistent with the drusen. In addition, the area in which drusen are seen may be involved in the reduction of macular function.

Drusenoid PED refers to a fairly well-circumscribed, shallow elevation of the RPE formed by confluent soft drusen, often located in the center of the macula [35]. VA in eyes with drusenoid PED is reported to be relatively good. In fact, in a recent report from the Age-Related Eye Disease Study, baseline VA in eyes with drusenoid PED was * 20/32, with * 90 % of eyes having VA better than 20/40 [35]. So far, however, little information is available on the macular dysfunction caused by drusenoid PED. In the current study, VA, amplitude of the a-wave and b-wave, and retinal sensitivity measured with the MP1 were significantly decreased when compared with the control eyes. In addition, the area and height of the PED were correlated with the fmERG and with the retinal sensitivity within the macular area—correlations that are consistent with the previously mentioned report of confluent drusen [34]. Photoreceptor damages, which could be observed as discontinuity of the junction of the inner and outer segments and as presence of hyper reflective foci in the OCT image (Fig. 6) [36, 37], might result in decreased macular function in eyes with drusenoid PED. Falsini et al. [33] also discussed that focal ERG sensitivity loss in eyes with drusen might result from photoreceptor drop out, as could be slightly seen in the OCT images of eyes with large drusen in our study (Fig. 5).

The prognosis of drusenoid PED was initially thought to be relatively good [38, 39]; however, a recent cohort study reported a high rate of progression to more advanced AMD [35]. Roquet et al. [25] documented that presence of metamorphopsia and drusenoid PED of greater than two disc diameters were risk factors of CNV occurrence within 2 years. Recently, other research groups have reported results of pilot studies on the early treatment of drusenoid PED without CNV by photodynamic therapy or by

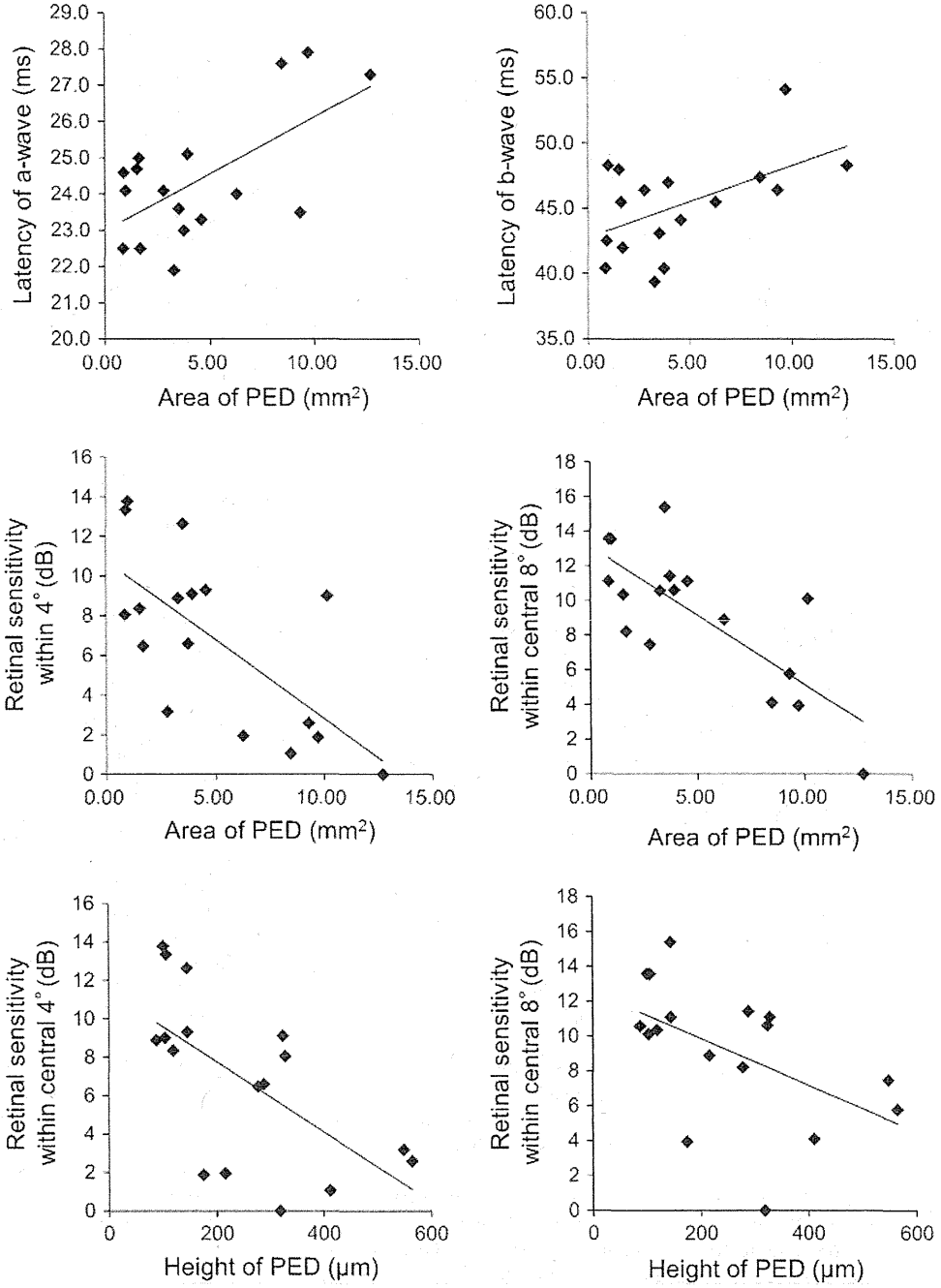

Fig. 7 Scattergram of the size of the drusenoid pigment epithelial detachment and macular functions measured with focal macular electroretinography or microperimetry. *PED* pigment epithelium detachment

antivascular endothelial growth factor therapy [40–43]. Gallego-Pinazo et al. [41] successfully treated six patients with drusenoid PED using intravitreal ranibizumab. However, Krishnan and Lochhead [42] reported rapid development of geographic atrophy after intravitreal injection of pegaptanib in an eye with drusenoid PED. In a recent report from the Age-Related Eye Disease Study, 19 % of eyes with drusenoid PED developed central geographic atrophy, and 23 % of these developed neovascular AMD [35]. When geographic atrophy develops in the extrafoveal region, VA measurement does not reflect a visual disturbance. The effect of treatment for drusenoid PED remains controversial. Multimodal measurements of macular function would be most helpful to evaluate the treatment efficacy of drusenoid PED.

There are various limitations to the current study. First, the eligible patients and controls in this study were all Japanese, and the genetic background may well have influenced the characteristics of AMD, so our results should be confirmed in another population. Second, the sample size of each group was small, so it is possible that we did not detect small differences between groups. Third,

the current study excluded central geographic atrophy, primarily because this is a relatively rare feature of AMD in Japanese patients. Finally, this was a cross-sectional study, so we could not offer any information regarding changes in macular function over time. Further longitudinal studies are necessary to fully elucidate the macular function in eyes with AMD of various stages and to study the treatment effects and the natural course of eyes with AMD, especially those with AMD in the early stage. Multimodal evaluations of the entire macular function should be of great help in these endeavors.

Conflicts of interest K. Ogino, None; A. Tsujikawa, None; K. Yamashiro, None; S. Ooto, None; A. Oishi, None; I. Nakata, None; M. Miyake, None; A. Takahashi, None; A. A. Ellabban, None; N. Yoshimura, None.

References

1. Friedman DS, O'Colmain BJ, Munoz B, Tomany SC, McCarty C, de Jong PT, et al. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol. 2004;122:564–72.
2. Bressler NM. Age-related macular degeneration is the leading cause of blindness. JAMA. 2004;291:1900–1.
3. Resnikoff S, Pascolini D, Etya'ale D, Kocur I, Pararajasegaram R, Pokharel GP, et al. Global data on visual impairment in the year 2002. Bull World Health Organ. 2004;82:844–51.
4. Ishibashi T, L-JSG. Maintenance therapy with pegaptanib sodium for neovascular age-related macular degeneration: an exploratory study in Japanese patients (LEVEL-J study). Jpn J Ophthalmol. 2013;57:417–23.
5. Yoshida Y, Kohno T, Yamamoto M, Yoneda T, Iwami H, Shiraki K. Two-year results of reduced-fluence photodynamic therapy combined with intravitreal ranibizumab for typical age-related macular degeneration and polypoidal choroidal vasculopathy. Jpn J Ophthalmol. 2013;57:283–93.
6. Yoshizawa C, Saito W, Hirose S, Kitamei H, Noda K, Ishida S. Photodynamic therapy combined with intravitreal bevacizumab and sub-tenon triamcinolone acetonide injections for age-related macular degeneration. Jpn J Ophthalmol. 2013;57:68–73.
7. Tezel TH, Del Priore LV, Flowers BE, Grosof DH, Benenson IL, Zamora RL, et al. Correlation between scanning laser ophthalmoscope microperimetry and anatomic abnormalities in patients with subfoveal neovascularization. Ophthalmology. 1996;103:1829–36.
8. Ogino K, Tsujikawa A, Murakami T, Muraoka Y, Akagi-Kurashige Y, Ishihara K, et al. Evaluation of macular function using focal macular electroretinography in eyes with macular edema associated with branch retinal vein occlusion. Invest Ophthalmol Vis Sci. 2011;52:8047–55.
9. Ogino K, Tsujikawa A, Nakamura H, Miyamoto K, Murakami T, Muraoka Y, et al. Focal macular electroretinogram in macular edema secondary to central retinal vein occlusion. Invest Ophthalmol Vis Sci. 2011;52:3514–20.
10. Miyake Y. Focal macular electroretinography. Nagoya J Med Sci. 1998;61:79–84.
11. Springer C, Bültmann S, Völcker HE, Rohrschneider K. Fundus perimetry with the Micro Perimeter 1 in normal individuals: comparison with conventional threshold perimetry. Ophthalmology. 2005;112:848–54.
12. Rohrschneider K, Springer C, Bültmann S, Völcker HE. Microperimetry—comparison between the micro perimeter 1 and scanning laser ophthalmoscope—fundus perimetry. Am J Ophthalmol. 2005;139:125–34.
13. Ritter M, Bolz M, Sacu S, Deak GG, Kiss C, Pruente C, et al. Effect of intravitreal ranibizumab in avascular pigment epithelial detachment. Eye (Lond). 2010;24:962–8.
14. Parisi V, Perillo L, Tedeschi M, Scassa C, Gallinaro G, Capaldo N, et al. Macular function in eyes with early age-related macular degeneration with or without contralateral late age-related macular degeneration. Retina. 2007;27:879–90.
15. Ozdemir H, Karacorlu M, Senturk F, Karacorlu SA, Uysal O. Microperimetric changes after intravitreal bevacizumab injection for exudative age-related macular degeneration. Acta Ophthalmol. 2012;90:71–5.
16. Midena E, Vujosevic S, Convento E, Manfre A, Cavarzeran F, Pilotto E. Microperimetry and fundus autofluorescence in patients with early age-related macular degeneration. Br J Ophthalmol. 2007;91:1499–503.
17. Midena E, Radin PP, Pilotto E, Ghirlando A, Convento E, Varano M. Fixation pattern and macular sensitivity in eyes with subfoveal choroidal neovascularization secondary to age-related macular degeneration: a microperimetry study. Semin Ophthalmol. 2004;19:55–61.
18. Landa G, Su E, Garcia PM, Seiple WH, Rosen RB. Inner segment–outer segment junctional layer integrity and corresponding retinal sensitivity in dry and wet forms of age-related macular degeneration. Retina. 2011;31:364–70.
19. Dinc UA, Yenerel M, Gorgun E, Oncel M. Assessment of macular function by microperimetry in intermediate age-related macular degeneration. Eur J Ophthalmol. 2008;18:595–600.
20. Calabrèse A, Bernard JB, Hoffart L, Faure G, Barouch F, Conrath J, et al. Wet versus dry age-related macular degeneration in patients with central field loss: different effects on maximum reading speed. Invest Ophthalmol Vis Sci. 2011;52:2417–24.
21. Bolz M, Simader C, Ritter M, Ahlers C, Benesch T, Prunte C, et al. Morphological and functional analysis of the loading regimen with intravitreal ranibizumab in neovascular age-related macular degeneration. Br J Ophthalmol. 2010;94:185–9.
22. Yodoi Y, Tsujikawa A, Kameda T, Otani A, Tamura H, Mandai M, et al. Central retinal sensitivity measured with the micro perimeter 1 after photodynamic therapy for polypoidal choroidal vasculopathy. Am J Ophthalmol. 2007;143:984–94.
23. Falsini B, Serrao S, Fadda A, Iarossi G, Porrello G, Cocco F, et al. Focal electroretinograms and fundus appearance in non-exudative age-related macular degeneration: quantitative relationship between retinal morphology and function. Graefes Arch Clin Exp Ophthalmol. 1999;237:193–200.
24. Sulzbacher F, Kiss C, Kaider A, Eisenkoelbl S, Munk M, Roberts P, et al. Correlation of SD-OCT features and retinal sensitivity in neovascular age-related macular degeneration. Invest Ophthalmol Vis Sci. 2012;53:6448–55.
25. Roquet W, Roudot-Thoraval F, Coscas G, Soubrane G. Clinical features of drusenoid pigment epithelial detachment in age related macular degeneration. Br J Ophthalmol. 2004;88:638–42.
26. Nishihara H, Kondo M, Ishikawa K, Sugita T, Piao CH, Nakamura Y, et al. Focal macular electroretinograms in eyes with wet-type age-related macular degeneration. Invest Ophthalmol Vis Sci. 2008;49:3121–5.
27. Hautamäki A, Oikkonen J, Onkamo P, Immonen I. Correlation between components of newly diagnosed exudative age-related macular degeneration lesion and focal retinal sensitivity. Acta Ophthalmol. doi:10.1111/j.1755-3768.2012.02556.x.
28. Piccardi M, Ziccardi L, Stifano G, Montrone L, Iarossi G, Minnella A, et al. Regional cone-mediated dysfunction in age-related maculopathy evaluated by focal electroretinograms: relationship

with retinal morphology and perimetric sensitivity. Ophthalmic Res. 2009;41:194–202.
29. Li J, Tso MO, Lam TT. Reduced amplitude and delayed latency in foveal response of multifocal electroretinogram in early age related macular degeneration. Br J Ophthalmol. 2001;85:287–90.
30. Gerth C, Delahunt PB, Alam S, Morse LS, Werner JS. Cone-mediated multifocal electroretinogram in age-related macular degeneration: progression over a long-term follow-up. Arch Ophthalmol. 2006;124:345–52.
31. Feigl B, Brown B, Lovie-Kitchin J, Swann P. Functional loss in early age-related maculopathy: the ischaemia postreceptoral hypothesis. Eye (Lond). 2007;21:689–96.
32. Feigl B, Brown B, Lovie-Kitchin J, Swann P. Cone- and rod-mediated multifocal electroretinogram in early age-related maculopathy. Eye (Lond). 2005;19:431–41.
33. Falsini B, Fadda A, Iarossi G, Piccardi M, Canu D, Minnella A, et al. Retinal sensitivity to flicker modulation: reduced by early age-related maculopathy. Invest Ophthalmol Vis Sci. 2000;41:1498–506.
34. Iwama D, Tsujikawa A, Ojima Y, Nakanishi H, Yamashiro K, Tamura H, et al. Relationship between retinal sensitivity and morphologic changes in eyes with confluent soft drusen. Clin Exp Ophthalmol. 2010;38:483–8.
35. Cukras C, Agron E, Klein ML, Ferris FL 3rd, Chew EY, Gensler G, et al. Natural history of drusenoid pigment epithelial detachment in age-related macular degeneration: Age-Related Eye Disease Study Report No. 28. Ophthalmology. 2010;117:489–99.
36. Ogino K, Murakami T, Tsujikawa A, Miyamoto K, Sakamoto A, Ota M, et al. Characteristics of optical coherence tomographic hyperreflective foci in retinal vein occlusion. Retina. 2012;32:77–85.
37. Bolz M, Schmidt-Erfurth U, Deak G, Mylonas G, Kriechbaum K, Scholda C. Optical coherence tomographic hyperreflective foci: a morphologic sign of lipid extravasation in diabetic macular edema. Ophthalmology. 2009;116:914–20.
38. Hartnett ME, Weiter JJ, Garsd A, Jalkh AE. Classification of retinal pigment epithelial detachments associated with drusen. Graefes Arch Clin Exp Ophthalmol. 1992;230:11–9.
39. Casswell AG, Kohen D, Bird AC. Retinal pigment epithelial detachments in the elderly: classification and outcome. Br J Ophthalmol. 1985;69:397–403.
40. Querques G, Bux AV, Delle Noci N. Foveal geographic atrophy following intravitreal pegaptanib sodium (Macugen) for drusenoid pigment epithelium detachment. Eur J Ophthalmol. 2009;19:890–3.
41. Gallego-Pinazo R, Marina A, Suelves C, Frances-Munoz E, Millan JM, Arevalo JF, et al. Intravitreal ranibizumab for symptomatic drusenoid pigment epithelial detachment without choroidal neovascularization in age-related macular degeneration. Clin Ophthalmol. 2011;5:161–5.
42. Lee NY, Kim KS. Photodynamic therapy treatment for eyes with drusenoid pigment epithelium detachment. Korean J Ophthalmol. 2008;22:194–6.
43. Krishnan R, Lochhead J. Regression of soft drusen and drusenoid pigment epithelial detachment following intravitreal anti-vascular endothelial growth factor therapy. Can J Ophthalmol. 2010;45:83–4.

Association Between *ZIC2*, *RASGRF1*, and *SHISA6* Genes and High Myopia in Japanese Subjects

Maho Oishi,[1,2] Kenji Yamashiro,[1] Masahiro Miyake,[1,2] Yumiko Akagi-Kurashige,[1,2] Kyoko Kumagai,[1] Isao Nakata,[1,2] Hideo Nakanishi,[1,2] Munemitsu Yoshikawa,[1,2] Akio Oishi,[1] Norimoto Gotoh,[1] Akitaka Tsujikawa,[1] the Nagahama Study Group, Ryo Yamada,[2] Fumihiko Matsuda,[2] and Nagahisa Yoshimura[1]

[1]Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan
[2]Center for Genomic Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan

Correspondence: Kenji Yamashiro, Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, 54 Kawahara, Shogoin, Sakyo, Kyoto 606-8507, Japan; yamashro@kuhp.kyoto-u.ac.jp.

See the appendix for the members of the Nagahama Study Group.

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Purpose. We investigated the association of genetic variations, which were identified recently in a large-scale genome-wide association study (GWAS) to confer risk of refractive error and common myopia in Caucasians, with high myopia in Japanese subjects.

Methods. The 5 single-nucleotide polymorphisms (SNPs) from the 5 genes *TOX*, *RDH5*, *ZIC2*, *RASGRF1*, and *SHISA6*, were genotyped in 1339 unrelated highly myopic Japanese patients and 3248 healthy Japanese participants in the Nagahama Study. In addition, genotypes were compared between high myopia patients without choroidal neovascularization (CNV) and patients with myopic CNV.

Results. Significant associations between rs8000973 near *ZIC2* ($P = 7.16 \times 10^{-7}$), rs4778879 in *RASGRF1* ($P = 3.40 \times 10^{-7}$), and rs2969180 in *SHISA6* ($P = 0.033$) and high myopia were observed. Odds ratios (95% confidence intervals) were 1.33 (1.19–1.49), 0.78 (0.71–0.86), and 1.11 (1.01–1.22) for the rs8000973 C allele, rs4778879 A allele, and rs2969180 G allele, respectively. The effect of the rs2969180 allele G contrasted with that observed in the original report, whereas the effect of the other 2 SNPs agreed. Further analysis using controls with −1.0 diopter (D) ≤ spherical equivalent ≤ +1.0 D showed a significant association between *ZIC2* and *RASGRF1*, but not *SHISA6*. Among the patients with high myopia, 516 had myopic CNV in either eye, while 823 patients did not have myopic CNV in eyes. No evaluated genes showed a significant association with the development of myopic CNV.

Conclusions. *ZIC2* and *RASGRF1* are susceptibility genes, not only for common myopia, but also for high myopia.

Keywords: high myopia, ZIC2, RASGRF1, SHISA6, CNV

Myopia, or nearsightedness, is the most common ocular disorder worldwide. Recent studies reported that the prevalence of myopia is approximately 20% to 42% in the Caucasian population, and much higher (40%–70%) in East Asian populations.[1–4] High myopia is distinguished from common myopia by an excessive increase in the axial length of the eye[5,6] and is considered important because of its association with various ocular complications that lead to blindness.[7–10] For example, choroidal neovascularization (CNV) beneath the fovea is one of the most vision-threatening complications of high myopia.[11,12]

Previous studies have indicated the involvement of genetic and environmental factors in the progression of myopia.[13–16] Family-based linkage analyses and twin studies have identified MYP1-19 loci and several candidate genes,[17,18] but genetic screening studies have achieved limited success. Since 2009, several genome-wide association studies (GWAS) have reported candidate genes for myopia,[19–26] but none of the reported genes or loci, except for the 15q14 locus, showed a consistent association with either common or high myopia in later studies.[27–30] Moreover, although some loci were reported to be associated with common and high myopia,[25,27,31] it still is not clear whether common myopia and high myopia share the same genetic background.

Recently, Verhoeven et al.[32] and Kiefer et al.[33] conducted a large-scale GWAS independently, and reported multiple new susceptibility loci for refractive error and common myopia. To investigate whether these loci cause high myopia in Japanese subjects, we performed a large-scale, case-control study on high myopia. In addition, we investigated the contribution of these genetic variations to the occurrence of CNV in high myopic eyes.

Methods

All procedures used in this study adhered to the tenets of the Declaration of Helsinki. The institutional review boards and the ethics committees of each institution involved approved the protocols of this study. All patients were fully informed of the purpose and procedures of this study, and written consent was obtained from each patient.

Table 1. Characteristics of the Study Population

	Patients, High Myopia*	Controls†
Patients, *n*	1339	3248
Age in y, mean ± SD	57.13 ± 14.90	52.20 ± 14.12
Sex, *n* (%)		
Male	442 (33.0%)	1092 (33.6%)
Female	897 (67.0%)	2154 (66.4%)
Axial length, mm ± SD		
Right eyes	29.25 ± 1.87	24.11 ± 1.39
Left eyes	29.12 ± 1.83	24.07 ± 1.39
Refraction of the phakic eyes, D‡		
Right eyes	−12.39 ± 4.66	−1.73 ± 2.85
Left eyes	−12.54 ± 4.59	−1.64 ± 2.80

* Axial length of ≥26.0 mm in eyes.

† Healthy individuals recruited from Nagaha cohort study.

‡ For calculations of refraction, eyes that had undergone cataract surgery or corneal refractive surgery were excluded.

Patients and Controls

A total of 1339 unrelated highly myopic Japanese patients was recruited from Kyoto University Hospital, Tokyo Medical and Dental University Hospital, Fukushima Medical University Hospital, Kobe City Medical Center General Hospital, Ozaki Eye Hospital, and Otsu Red-Cross Hospital. All patients underwent comprehensive ophthalmic examinations, including dilated indirect and contact lens slit-lamp biomicroscopy, automatic objective refraction, and measurements of the axial length by applanation A-scan ultrasonography (UD-6000; Tomey, Nagoya, Japan) or partial coherence interferometry (IOLMaster; Carl Zeiss Meditec, Dublin, CA). Patients with an axial length of ≥26.0 mm in both eyes were placed into the high myopia group. For control subjects, we included 3248 unrelated healthy Japanese subjects (control 1) from the Nagahama Prospective Genome Cohort for the Comprehensive Human Bioscience dataset (The Nagahama Study). Automatic objective refraction and measurements of the axial length by partial coherence interferometry (IOLMaster; Carl Zeiss Meditec) were performed on all participants. For subanalysis, subjects with a spherical equivalent between −1.0 and +1.0 diopters (D) in both eyes also were included as a control group (control 2). All participants were Japanese, and subjects with any history of ocular disease were eliminated from the control group.

To evaluate the contribution of single-nucleotide polymorphisms (SNPs) to the occurrence of CNV in myopic eyes, the high myopia group was divided into 2 groups: CNV and no CNV. The inclusion criteria for the CNV group were clinical presentation and angiographic manifestations of macular CNV or Fuchs' spot in at least 1 eye.

SNP Selection

Verhoeven et al.[32] reported 26 loci (29 potential candidate genes) associated with refractive error and common myopia in a large-scale multi-ethnic GWAS. Of these loci, 8 also were reported to be associated with myopia and replicated in another recent large-scale GWAS including Caucasian participants.[33] For our analysis, we selected 8 SNPs in these 8 loci that were evaluated in the original report. Among these 8 SNPs, 3 showed extremely low minor allele frequency (MAF) in the Japanese population according to the HapMap data (rs12205363 in *LAMA2*, rs1656404 near *PRSS56*, and rs1960445 near *BMP3*; MAF 0.00, 0.01, and 0.02, respectively). In addition to these 3 SNPs, rs524952 in *GJD2* also was excluded as we had confirmed its association previously with high myopia.[27] Selected SNPs included rs7837791 near *TOX*, rs3138144 in *RDH5*, rs8000973 near *ZIC2*, and rs2969180 in *SHISA6*. Although negated by Kiefer et al.[33] at the replication stage ($P = 0.08$), rs4778879 in *RASGRF1* was included because its association with myopia still is disputed despite numerous replication studies.

Genotyping

Genomic DNAs were prepared from peripheral blood by using a DNA extraction kit (QuickGene-610L; Fujifilm, Minato, Tokyo, Japan). Genotyping of samples from 1339 high myopic patients was performed using a commercially available assay (TaqMan SNP assay with the ABI PRISM 7700 system; Applied Biosystems, Foster City, CA). For the control group, 3712 individuals from the Nagahama study were genotyped using HumanHap610K Quad Arrays, HumanOmni2.5M Arrays, and/or HumanExome Arrays (Illumina, Inc., San Diego, CA). To ensure high-quality genotype data, a series of quality control (QC) filters were applied to the data from each platform, including MAF cutoffs (MAF > 0.01), Hardy-Weinberg equilibrium (HWE; $P > 1 \times 10^{-7}$), genotypic success rate (>95%), individual call rate (>99%), and estimated relatedness (PI-HAT < 0.35). The QCs were performed using PLINK (ver.1.07 available in the public domain at http://pngu.mgh.harvard.edu/purcell/plink/). The fixed dataset consisted of 3248 individuals. Genotype data directly assessed by arrays was used for analyses. Because directly genotyped data of SNP rs4778879 in *RASGRF1* in controls was not available, we analyzed genotype counts of SNP rs6495367 whose linkage disequilibrium value (r^2) is 1.0 compared to rs4778879 (HapMap phase II + III rel 28 JPT).

Statistical Analyses

Data are presented as the mean ± SD. Deviations in the genotype distribution from the HWE were assessed for each group by using the HWE exact test. The χ^2 test for the trend or its exact counterpart was used to compare the genotype distribution of 2 groups. To adjust for age and sex, we performed multiple regression and logistic regression analyses. Two subjects in the control group were excluded from multiple regression and logistic regression analyses because of lack of information regarding age or sex. Statistical analyses were performed using SPSS software (version 21.0; SPSS Science, Chicago, IL). A P value of <0.05 was considered statistically significant. To analyze CNV, a P value of <0.01 (= 0.05/5) was considered statistically significant after Bonferroni correction. Power calculations were performed using R software, package "pwr" (v 3.0.0; R Foundation for Statistical Computing, Vienna, Austria; available in the public domain at http://www.r-project.org/).

Results

Basic information of the study population is shown in Table 1. The mean age of the 1339 high myopia cases was 57.13 ± 14.90 years and the male-to-female ratio was 33.0%:67.0%. The average axial length of cases was 29.19 ± 1.85 mm. Among the 2678 eyes included in the study, 1920 (71.7%) were phakic, and the mean refraction of the phakic eyes was −12.68 ± 4.54 D. The mean age of the 3248 control subjects was 52.20 ± 14.12 years, and the male-to-female ratio was 33.6%:66.4%. The average axial length of controls was 24.09 ± 1.39 mm, and the mean refraction of the 5572 (85.8%) phakic eyes was −1.68 ±

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Table 2. Genotype Frequency, Associations, and Odds Ratios (ORs) in the High Myopia Patients and Controls (Control 1)

				Genotype Frequency								
SNP	Chr	Position	Genes	Genotype	High Myopia	Control 1	Nominal P*	Adjusted P†	Adjusted OR†	95% CI†	N‡	HWE P§
rs7837791	8	60179086	*TOX*	GG	22.1%	21.7%	0.47	0.62	1.02	0.93–1.12	3239	0.76
				TG	50.9%	50.0%						
				TT	27.0%	28.3%						
rs3138144	12	56114769	*RDH5*	CC	19.2%	20.9%	0.41	0.28	0.95	0.85–1.05	1848	0.49
				CG	50.1%	48.7%						
				GG	30.7%	30.4%						
rs8000973	13	100691367	*ZIC2*	CC	10.2%	6.5%	8.64E-07	7.16E-07	1.33	1.19–1.49	1849	0.76
				TC	42.8%	38.6%						
				TT	47.0%	54.8%						
rs4778879	15	79372875	*RASGRF1*	AA	17.9%	(GG) 23.5%‖	1.46E-07	3.40E-07	0.78	0.71–0.86	3244‖	0.88‖
				GA	49.0%	(GA) 50.1%‖						
				GG	33.1%	(AA) 26.4%‖						
rs2969180	17	11407901	*SHISA6*	GG	24.5%	20.7%	0.023	0.033	1.11	1.01–1.22	3240	0.10
				AG	49.0%	51.2%						
				AA	26.5%	28.1%						

Chr, chromosome; CI, confidence interval.
* Differences in the observed genotypic distribution were examined by χ^2 test for trend.
† Age and sex adjustment was performed based on a logistic regression model.
‡ Number of control subjects who were genotyped directly.
§ The HWE test results for control subjects who were genotyped directly.
‖ Data of SNP rs6495367, whose linkage disequilibrium value (r^2) is 1.0 compared to SNP rs4778879.

2.82 D. Among the control group, 999 subjects had a spherical equivalent between −1.0 and +1.0 D in both eyes, and these subjects were used as control 2. Their average axial length was 23.38 ± 0.79 mm, and the mean refraction of the 1998 (100%) phakic eyes was −0.11 ± 0.53 D.

Genotype counts, associations examined using the χ^2 test for trend analysis, odds ratios for the 5 SNPs between high myopia cases and controls, number of control subjects who were genotyped directly, and the results of the HWE exact test in controls are shown in Table 2. The SNPs rs8000973 near *ZIC2*, rs4778879 in *RASGRF1*, and rs2969180 in *SHISA6* showed significant association with high myopia ($P = 7.16 \times 10^{-7}$, 3.40×10^{-7}, and 0.033, respectively). The odds ratios (95% confidence intervals) were 1.33 (1.19–1.49) for the rs8000973 C allele, 0.78 (0.71–0.86) for the rs4778879 A allele, and 1.11 (1.01–1.22) for the rs2969180 G allele. The effect of the rs2969180 allele G was contrasting to that obtained in the previous study, whereas the other 2 SNPs showed the same trend as that observed in the original report. The distributions of the genotypes for all the five SNPs were in HWE. When control group 2 was evaluated, the SNPs rs8000973 and rs4778879 showed significant association with high myopia

Table 3. Genotype Frequency, Associations, and ORs in the High Myopia Patients and Control 2

				Genotype frequency							
SNP	Chr	Position	Genes	Genotype	High Myopia	Control 2*	Nominal P†	Adjusted P‡	Adjusted OR‡	95% CI‡	N§
rs7837791	8	60179086	*TOX*	GG	22.1%	20.7%	0.20	0.24	1.07	0.95–1.21	997
				TG	50.9%	49.9%					
				TT	27.0%	29.4%					
rs3138144	12	56114769	*RDH5*	CC	19.2%	20.5%	0.26	0.21	0.91	0.79–1.05	567
				CG	50.1%	51.5%					
				GG	30.7%	28.0%					
rs8000973	13	100691367	*ZIC2*	CC	10.2%	7.0%	1.33E-05	1.29E-05	1.43	1.22–1.67	568
				TC	42.8%	34.7%					
				TT	47.0%	58.3%					
rs4778879	15	79372875	*RASGRF1*	AA	17.9%	(GG) 26.4%‖	1.28E-07	1.01E-07	0.72	0.64–0.82	998‖
				GA	49.0%	(GA) 47.8%‖					
				GG	33.1%	(AA) 25.8%‖					
rs2969180	17	11407901	*SHISA6*	GG	24.5%	21.2%	0.043	0.076	1.11	0.99–1.25	996
				AG	49.0%	49.5%					
				AA	26.5%	29.3%					

* Healthy individuals with spherical equivalent between −1.00 and +1.00 in eyes.
† Differences in the observed genotypic distribution were examined by χ^2 test for trend.
‡ Age and sex adjustment was performed based on a logistic regression model.
§ Number of control subjects who were genotyped directly.
‖ Data of SNP rs6495367 whose linkage disequilibrium value (r^2) is 1.0 compared to SNP rs4778879.

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TABLE 4. Characteristics of the High Myopic Patients With CNV and With No CNV

	CNV	No CNV
Patients, *n*	516	823
Age in y, mean ± SD	60.99 ± 13.28	54.56 ± 15.56
Sex, *n* (%)		
Male	112 (21.7%)	330 (40.1%)
Female	404 (78.3%)	493 (59.9%)
Axial length, mm ± SD		
Right eyes	29.29 ± 1.71	29.22 ± 1.96
Left eyes	29.10 ± 1.69	29.13 ± 1.91

(Table 3, $P = 1.29 \times 10^{-5}$ and 1.01×10^{-7}, respectively). In contrast, rs2969180 in *SHISA6* showed a marginal association with high myopia (nominal $P = 0.043$ and adjusted $P = 0.076$). The SNPs in *RDH5* (rs3138144) and near *TOX* (rs7837791) showed no association with high myopia for all settings examined in this study.

Among the 1339 high myopic patients, 516 had CNV, while 823 did not. The demographics of the CNV group and the no CNV group are shown in Table 4. There was no difference in the axial lengths in each group ($P > 0.05$), whereas the age and female ratios were significantly higher in the CNV group ($P < 0.05$), as was reported previously.[12,34] The results of the association between the genetic variants and myopic CNV in this study are shown in Table 5. None of these 5 SNPs showed significant associations with CNV occurrence in the high myopia patients after Bonferroni correction.

DISCUSSION

In the present study, we showed that SNPs rs8000973 near *ZIC2* and rs4778879 in *RASGRF1*, which were reported recently as susceptibility loci for common myopia, were significantly associated with high myopia in Japanese subjects. Our study also suggested that rs2969180 in *SHISA6* is associated with high myopia. Although it is unclear whether common and high myopia share the same genetic background, our results indicated the existence of some overlap.

The association between the 15q25 locus/*RASGRF1* region and myopia still is controversial; however, our findings strongly suggested the contribution of the 15q25 locus/*RASGRF1* region to high myopia. The 15q25 locus/*RASGRF1* region was reported initially by Hysi et al.[21] to be associated with refractive error and common myopia in a large-scale GWAS by using Caucasian cohorts. However, later studies could not replicate its association with common myopia,[28–30] and its association with high myopia remains controversial. We showed that this locus had a weak association ($P = 0.031$ for rs8027411 and $P = 0.047$ for rs17175798) with high myopia in Japanese subjects,[27] but a Chinese study showed no association of 15q25 with moderate or high myopia. In contrast with these 2 reports on high myopia, our study used a larger number of cases and a larger control group, which differed from that used in our previous study, and the examined SNP also was different from those in previous reports. Because rs4778879 showed weak linkage disequilibrium with previously investigated SNPs, the number of samples would lead to the contradictory results obtained for high myopia between the present and previous studies. Further study on common myopia by using a relatively larger number of samples may confirm the association between the 15q25 locus/*RASGRF1* region and common myopia.

The risk allele in rs8000973 near *ZIC2* and rs4778879 in *RASGRF1* was the same as that observed in the previous study, but the effect of rs2969180 in *SHISA6* differed from that observed in the previous study. Of the SNPs examined in this study, the MAFs in the control group and those obtained from the HapMap data were fairly consistent. The significance of the association of *SHISA6* was weaker than that of *ZIC2* and *RASGRF1* when compared with the population controls (control 1), and it was marginal when compared with the subjects with emmetropic refractive error (−1.0 to +1.0 D) in eyes (control 2). In control 1, the average axial length and mean refraction of the phakic eyes were slightly shifted to a myopic range (24.09 ± 1.39 mm, and −1.68 ± 2.82 D, respectively) as a logical outcome of the high prevalence of myopia (40%–70%) in the Japanese population. Because control 1 included high myopia participants, as the Japanese general population includes 1% to 5% high myopia, analysis of control 1 may have less power to detect the genetic association with high myopia. Although using emmetropic subjects as

TABLE 5. Genotype Frequency, Associations, and ORs in the High Myopia Patients With CNV and With No CNV

					Genotype Frequency					
SNP	Chr	Position	Genes	Genotype	CNV, %	No CNV, %	Nominal *P**	Adjusted *P*†	Adjusted OR†	95% CI†
rs7837791	8	60179086	*TOX*	GG	22.9	21.6	0.50	0.33	0.92	0.78–1.09
				TG	47.5	52.9				
				TT	29.5	25.5				
rs3138144	12	56114769	*RDH5*	CC	19.7	18.9	0.94	0.80	0.98	0.83–1.16
				CG	49.3	50.6				
				GG	31.1	30.6				
rs8000973	13	100691367	*ZIC2*	CC	11.2	9.7	0.11	0.14	1.14	0.96–1.37
				TC	44.7	41.6				
				TT	44.1	48.7				
rs4778879	15	79372875	*RASGRF1*	AA	17.	18.1	0.60	0.65	0.96	0.81–1.14
				GA	48.5	49.2				
				GG	34.0	32.6				
rs2969180	17	11407901	*SHISA6*	GG	23.2	25.3	0.12	0.04	0.84	0.71–0.99
				AG	47.6	49.8				
				AA	29.2	24.9				

* Differences in the observed genotypic distribution were examined by χ^2 test for trend.
† Age and sex adjustment was performed based on a logistic regression model.