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Correlation between macular blood flow and central visual sensitivity in retinitis pigmentosa

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ABSTRACT.

Purpose: **To investigate the changes in macular blood flow and the correlation between those changes and central visual function in patients with retinitis pigmentosa (RP).**

Methods: **The mean blur rate (MBR), a quantitative blurring index of the laser speckle pattern that represents retinal and choroidal blood flow, was measured by laser speckle flowgraphy. Mean blur rate values in the macular area were compared between 70 patients with RP and 28 control subjects. The relationships between MBR on the one hand and, on the other, visual acuity (VA), mean deviation (MD) and averaged macular sensitivity of static perimetry tests (Humphrey Filed Analyzer, the central 10-2 program) were analysed in patients with RP.**

Results: **Macular MBR was decreased to 75% in patients with RP compared with control subjects ($p < 0.0001$, Student's *t*-test). Spearman's rank testing showed that macular MBR was significantly correlated with VA ($r = -0.261$, $p = 0.0299$), MD values ($r = 0.438$, $p = 0.0002$) and averaged macular sensitivity at the central 4 and 12 points of static perimetry tests ($r = 0.426$ and 0.442, $p = 0.0003$ and 0.0002, respectively). Multivariable-adjusted analysis confirmed that MBR was independently associated with MD ($p = 0.0002$) and macular sensitivity at the central 4 and 12 points ($p < 0.0001$ and 0.0002, respectively).**

Conclusions: **Decreased macular blood flow was associated with reduced macular visual sensitivity in patients with RP. Although the cause–effect relationships remain to be elucidated, these findings suggest that vascular defects may be involved in the pathogenesis of RP such as central vision loss.**

Key words: blood flow – central vision – laser speckle flowgraphy – retinitis pigmentosa

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Introduction

Retinitis pigmentosa (RP) refers to a group of inherited retinal degenerations that result from photoreceptor cell death and that affect over 1 million individuals globally (Hartong et al. 2006). Vision loss in RP typically begins with night blindness and visual field constriction due to rod dysfunction and death, followed by loss of daylight and central vision due to cone cell loss. Previous studies have identified mutations in more than 50 genes that cause RP, but the mechanisms by which these mutations induce rod and cone cell death have not been fully elucidated. It should be noted that because many of the genes associated with RP are expressed exclusively in rods, it is still a puzzle why and how cones – which do not utilize mutant proteins – die subsequent to rod degeneration.

A number of studies have suggested the involvement of impaired ocular blood flow in the pathogenesis of RP. Attenuation of retinal vessels is a classical feature of RP, and previous studies have shown that retinal blood flow, as measured by laser Doppler velocimetry and retinal function imaging, is decreased in patients with RP compared with that in age-matched controls (Grunwald et al. 1996; Beutelspacher et al. 2011). In addition, the intraocular pressure (IOP) pulse amplitude, an indirect measure of pulsatile choroidal blood flow, is reduced in patients with RP who still retain good visual acuity (VA; Langham & Kramer 1990). Falsini et al. (2011) further demonstrated that subfoveal blood flow measured by laser Doppler flowmetry is reduced in patients with RP and correlates with central cone function as assessed by focal electroretinograms (FERGs). These findings suggest that microcirculatory changes may be implicated in the progression of RP, especially in the loss of cone-mediated central visual function.

In this study, we investigated macular blood flow using laser speckle

flowgraphy (LSFG). The laser speckle phenomenon is an interference event when coherent light sources such as lasers are scattered by a diffusing surface. The speckle pattern in the ocular fundus, which appears under the illumination of laser light, varies by the movement of red blood cells, and the LSFG ANALYZER software calculates the mean blur rate (MBR) of speckle pattern in a defined fundus area (Sugiyama et al. 2010). The MBR shows a high level of intrasession reproducibility (Aizawa et al. 2011) and linearly correlates with blood flow as measured by conventional techniques such as the microsphere and hydrogas clearance methods (Tamaki et al. 1994; Sugiyama et al. 1996; Takahashi et al. 2013). By this method, we here evaluated the changes in macular blood flow in patients with RP and its association with cone-mediated visual parameters.

Methods

Ethics statement

This study was approved by the Institutional Review Board of the Kyushu University Hospital (Fukuoka, Japan) and was conducted in accordance with the tenets of the Declaration of Helsinki on biomedical research involving human subjects. The Institutional Review Board waived the need for written informed consent, because the study design comprised a retrospective chart review.

Patients

Patients were recruited from the Kyushu University Hospital in 2011–2014. Seventy patients with a diagnosis of typical RP and 28 control subjects without ocular diseases were included for the comparison of macular blood flow between the two groups. Patients with RP were subsequently analysed for the association between macular blood flow and visual parameters. The baseline characteristics of these patients are shown in Table 1. The diagnosis of typical RP was based on a patient's history of night blindness, visual field constriction and/or ring scotoma, and markedly reduced or non-recordable a-wave and b-wave amplitudes on ERG testing, in addition to ophthalmoscopic findings (e.g. bone spicule-like pigment clumping in the mid-peripheral and peripheral retina and attenuation of retinal vessels). Excluded from the study were patients who had a history of other ocular diseases or intraocular surgery received treatment with a systemic calcium blocker or topical antiglaucoma treatment, or had refractive errors (spherical equivalent) less than −6 dioptres. The best-corrected VA was measured with a standard Japanese decimal VA chart and was converted to the logarithm of the minimum angle of resolution (logMAR) units.

Visual field testing

All patients underwent automated static perimetry testing [Humphrey Field Analyzer (HFA); Humphrey Instruments, San Leandro, CA, USA] using the central 10-2 Swedish Interactive Thresholding Algorithm Standard Program. The lens was corrected as appropriate for the test distance. Visual field testing was repeated if the test reliability was not satisfactory (fixation loss > 20%, false positive > 15% or false negative > 33%). The test was examined twice, and the better result was used for analysis to reduce the learning effect. Visual sensitivity parameters including the mean deviation (MD) and the averaged macular sensitivity at the central 4 and 12 points were analysed as previously described (Iijima 2012; Ikeda et al. 2012, 2013).

Laser speckle flowgraphy

The principles and methods for LSFG measurement have been previously described in detail (Sugiyama et al. 2010; Akiyama et al. 2014). In brief, LSFG-NAVI (Softcare, Fukuoka, Japan) was used to evaluate macular microcirculation. The subject's pupil was dilated with 0.5% tropicamide and 0.5% phenylephrine (Santen, Osaka, Japan) before examination. The subjects were guided to see the internal fixation target, and a 5.9 × 2.9 mm (width × height) area at the posterior pole was defined by the fundus camera equipped with LSFG-NAVI. After switching to a diode laser (830 nm), a speckle pattern from the illuminated fundus was recorded by a CCD camera (750 × 360 pixels) at a frequency of 1/30 seconds for 4 seconds. Thereafter, the MBR of the speckle images was calculated in a 2.4 × 2.4 mm area centred on the fovea by LSFG ANALYZER software (v 3.0.43.0, Softcare, Fukuoka, Japan). The representative speckle images from patients with RP and control subjects are shown in Fig. 1. Three measurements were taken consecutively for each subject, and the averaged value for each subject was used for the statistical analyses. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded before the measurement of LSFG. The ocular perfusion pressure (OPP) was calculated as follows: $2/3[1/3SBP + 2/3DBP] - IOP$.

Table 1. Baseline characteristics of control subjects and patients with RP.

	Control subjects (n = 28)	Patients with RP (n = 70)	p-value
Age	44.5 ± 14.5 (22–74)	47.8 ± 15.8 (15–79)	0.3396
Gender (males:females)	13:15	27:43	0.4798
IOP	12.7 ± 2.2	12.3 ± 2.4	0.4758
SBP	125.1 ± 15.8	123.5 ± 13.8	0.6343
DBP	75.3 ± 10.7	76.7 ± 9.5	0.5376
OPP	47.5 ± 8.0	48.0 ± 6.2	0.7171
MBR	10.6 ± 3.2	7.9 ± 2.6	<0.0001
Visual acuity (logMAR)	−0.08 ± 0.07	0.08 ± 0.21	<0.0001
MD value		−13.2 ± 9.9	
Macular sensitivity (4 points)		29.3 ± 6.2	
Macular sensitivity (12 points)		27.7 ± 7.3	
Inheritance mode, n			
AD		8	
AR		13	
X-linked		0	
Spontaneous		49	

IOP, intraocular pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; OPP, ocular perfusion pressure; MBR, mean blur rate; MD, mean deviation; AD, autosomal dominant; AR, autosomal recessive; RP, retinitis pigmentosa.
Data are expressed as mean ± standard deviation.
Ocular characteristics are derived from the right eyes.

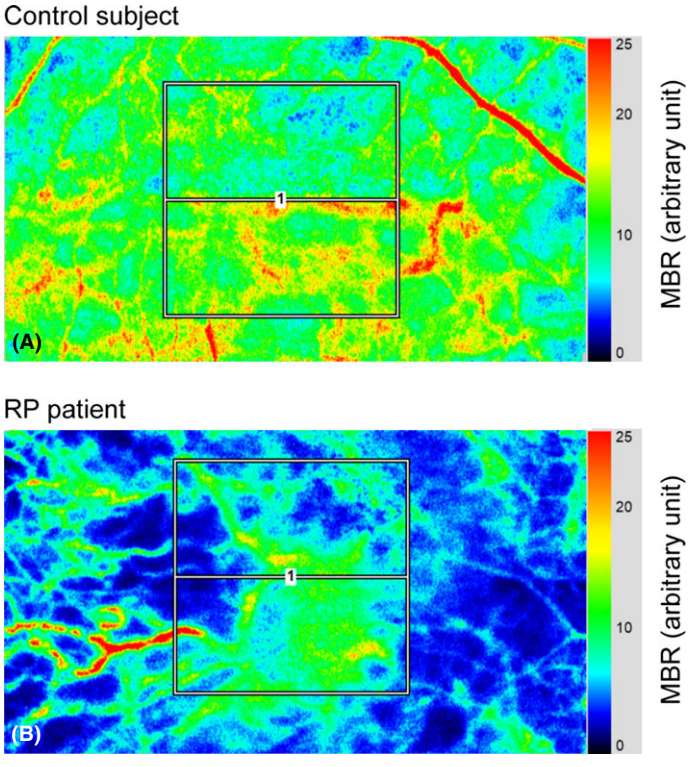

Fig. 1. Representative laser speckle flowgraphy (LSFG) colour map of the posterior pole in control subject (A) and patient with retinitis pigmentosa (RP) (B). Data were obtained from 37-year-old healthy woman with visual acuity (VA) of −0.18 (logMAR) and mean blur rate (MBR) of 11.5 (A) and 27-year-old man associated with RP, with VA of 0.10 (logMAR) and MBR of 7.3. The MBR was measured in the central 2.4 × 2.4 mm square area centred on the fovea.

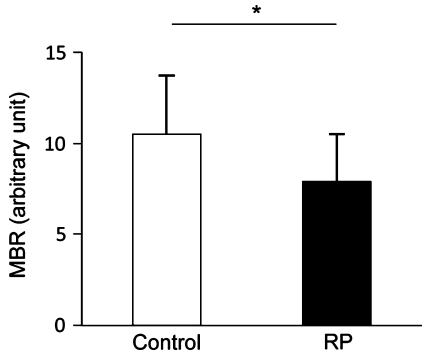

Fig. 2. Macular blood flow in control subjects and patients with retinitis pigmentosa (RP). Mean blur rate (MBR) in the macular area was decreased in patients with RP (7.9 ± 2.6) compared with control subjects (10.6 ± 3.2; *p < 0.0001).

Statistical analysis

The results obtained from the right eye of each subject in both groups were analysed. Data were presented as the arithmetic mean ± standard deviation. Statistical differences between the groups were analysed by unpaired Student's *t*-tests. The relationship between blood flow values and visual parameters was examined by Spearman's rank correlation coefficient and by multivariable-adjusted linear regression analysis. The blood flow values were converted to logarithmic scale to better approximate a normal distribution. All of the statistical analyses were performed with SAS software (version 9.2; SAS Institute Inc., Cary, NC, USA). A p-value of <0.05 was considered statistically significant.

Results

Macular blood flow in patients with RP and control subjects

A total of 70 consecutive eyes of the 70 patients with RP were age-matched with 28 normal eyes of the control subjects. Demographic data of the study population are listed in Table 1. There were no significant differences in age, gender distribution, IOP, SBP, DBP or OPP between the groups. The MBR in the macular area was significantly decreased in patients with RP (7.9 ± 2.6) compared with that in control subjects (10.6 ± 3.2, p < 0.0001; Fig. 2).

Correlation between macular blood flow and visual parameters in patients with RP

We next investigated the correlations between macular blood flow and visual parameters in the 70 patients with RP. Visual acuity showed marginal but statistically significant correlation with MBR ($r = -0.261$, p = 0.0299; Fig. 3A and Table 2). In contrast, MD values of HFA tests were strongly correlated with the corresponding MBR ($r = 0.438$, p = 0.0002; Fig. 3B and Table 2). The averaged macular sensitivity at the central 4 and 12 points of HFA tests was also positively correlated with MBR ($r = 0.426$ and 0.442, p = 0.0003 and 0.0002, respectively; Fig. 3C,D and Table 2). No correlation was found between MBR on the one hand and, on the other, age, gender, IOP, SBP, DBP and OPP. Multivariable-adjusted linear regression analysis showed that macular MBR was independently associated with MD ($\beta = 0.015 \pm 0.003$, p = 0.0002) as well as macular sensitivity at the central 4 and 12 points ($\beta = 0.027 \pm 0.006$ and 0.021 ± 0.005, p < 0.0001 and 0.0002, respectively), even after adjustment for age, gender, IOP, SBP and OPP.

Discussion

In the present study, we evaluated the changes in macular blood flow in patients with RP using LSFG. Our data showed that macular MBR in the patients with RP was decreased to approximately 75% that in the control subjects. These results are consistent with a previous report in which patients with RP had 74% of the subfoveal blood flow in healthy controls, as assessed by laser Doppler flowmetry (Falsini et al. 2011). Zhang et al. (2013) also showed that retinal and choroidal blood flow in patients with RP was half that in healthy controls, as measured by magnetic response imaging. Notably, most of the patients with RP included in these studies retained good VA, suggesting that the reduction in macular blood blow occurs in an early phase of cone degeneration in RP. Moreover, we demonstrated that macular blood flow was significantly correlated with central visual sensitivity on static perimetry tests in patients with RP. These results, taken together with a previous report

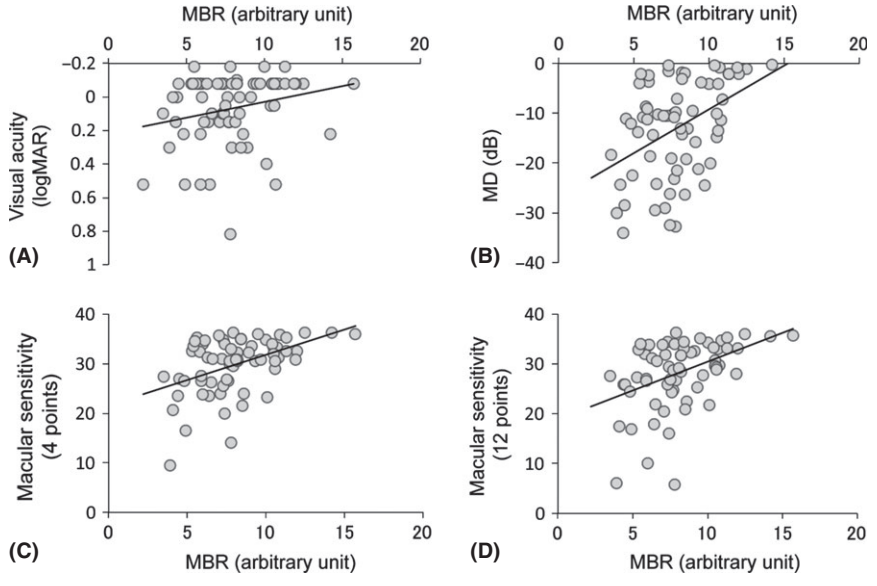

Fig. 3. Scatter plot of the mean blur rate (MBR) and visual acuity (VA) (A), MBR and MD (B), MBR and averaged macular sensitivity in the central 4 points (C), and MBR and averaged macular sensitivity in the central 12 points (D).

Table 2. Correlation coefficient analyses between MBR, visual parameters, and other ocular and systemic parameters.

	MBR	
	r	p
Age	−0.007	0.9512
Gender (males:females)	−0.064	0.5990
IOP	0.155	0.2022
SBP	−0.087	0.4756
DBP	0.104	0.3936
OPP	−0.043	0.7230
Visual acuity (logMAR)	**−0.261**	**0.0299**
MD value	**0.438**	**0.0002**
Macular sensitivity (4 points)	**0.426**	**0.0003**
Macular sensitivity (12 points)	**0.442**	**0.0002**

MBR, mean blur rate; MD, mean deviation; IOP, intraocular pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; OPP, ocular perfusion pressure.
Bold signifies statistically significant values.

demonstrating the association between subfoveal blood flow and foveal FERG response in patients with RP (Falsini et al. 2011), suggest that impaired macular blood flow may be involved in the pathogenesis of RP such as cone cell loss.

Vascular defects have been implicated in various retinal and neurodegenerative diseases. These vascular changes were traditionally thought to occur secondary to neuronal cell loss; however, recent studies have shown that vascular abnormalities may also contribute to the pathogenesis of neurological diseases (Zacchigna et al. 2008). For instance, insufficient expression of vascular endothelial growth factor (VEGF), a potent angiogenic and vascular survival factor, exacerbates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis (ALS; Oosthuyse et al. 2001). Moreover, a genetic variation in the VEGF promoter that lowers VEGF expression increases the risk for ALS (Lambrechts et al. 2003), suggesting a link between a vascular factor and motor neuron degeneration. In RP, it is generally thought that attenuation of retinal and choroidal blood vessels results from primarily rod cell defects. Recent studies using retinal oximetry showed that oxygen saturation in retinal venules is increased in patients with RP compared with control subjects, suggesting the decreased oxygen demand in patients with RP (Eysteinsson et al. 2014; Turksever et al. 2015). These finding may support the idea that decreased blood flow is a consequence of rod degeneration. However, there is a possibility that decreased blood flow conversely affects the late phase of retinal degeneration such as cone cell loss. In line with this hypothesis, previous experimental studies showed that dying cones in mouse models of RP exhibit molecular and morphological characteristics of nutrient starvation, suggesting the insufficient availability of trophic support (Punzo et al. 2009; Murakami et al. 2012). Interestingly, recent clinical studies have shown that oral treatment with calcium blocker nilvadipine or topical administration of BK channel activator unoprostone, each of which increases choroidal blood flow, delays the loss of central visual sensitivity in patients with RP (Nakazawa et al. 2011; Tawada et al. 2013). Moreover, we recently observed that topical unoprostone in one eye increases macular blood flow and preserves central visual function in both treated and contralateral eyes of patients with RP (Akiyama et al. 2014). Because unoprostone topically administered is rapidly distributed to systemic blood vessels (Yamamoto et al. 2012), its protective effect on contralateral eyes could be mediated by affecting blood flow. Although these results should be confirmed in larger and longitudinal studies, ocular microcirculation may be a potential target for protecting central vision in patients with RP.

Laser speckle flowgraphy-NAVI is the updated version of LSFG, which provides better spatial resolution of the blood flow map (Sugiyama 2014). Sufficient data can be obtained in <5 seconds with high reproducibility (Aizawa et al. 2011), and the MBR linearly correlates with ocular blood flow obtained by invasive techniques such as hydrogas clearance methods (Wang et al. 2012; Takahashi et al. 2013), indicating the reliable quantifiability of this method (Sugiyama 2014). In the present study, we evaluated the MBR in a central 2.4 × 2.4 mm area in the macula, which is perfused with both choroidal and retinal blood flow. Alm & Bill (1973) previously investigated the blood flow rates in monkey eyes using a microsphere method and showed that

the ratio of choroid to retinal blood flow exceeds 95% in both foveal and mid-peripheral region. In consistent with these findings, Isono et al. (2003) demonstrated that the speckle pattern observed in LSFG is largely originated from the choroid (over 90% of the total circulation) in a monkey model of branch retinal artery occlusion. These findings suggest that LSFG is useful for evaluating the changes in choroidal circulation in retinal disorders associated with photoreceptor, RPE and choroidal lesions.

In conclusion, the present study demonstrated that decreased macular blood flow is associated with the reduction in macular visual sensitivity in patients with RP. However, because of its cross-sectional design, it was not able to assess the precise cause–effect relationship between blood flow and central vision. Further longitudinal studies investigating whether reduced blood flow is associated with a faster deterioration of cone function will provide a better understanding of the pathophysiology of cone cell loss in RP.

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