

Relationships Between Serum Antioxidant and Oxidant Statuses and Visual Function in Retinitis Pigmentosa

Masataka Ishizu,¹ Yusuke Murakami,¹ Kohta Fujiwara,¹ Jun Funatsu,¹ Shotaro Shimokawa,¹ Shunji Nakatake,¹ Takashi Tachibana,¹ Toshio Hisatomi,^{1,2} Yoshito Koyanagi,¹ Masato Akiyama,¹ Yukihide Momozawa,³ Tatsuro Ishibashi,¹ Koh-Hei Sonoda,¹ and Yasuhiro Ikeda^{1,4}

¹Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

²Department of Ophthalmology, Fukuoka University Chikushi Hospital, Fukuoka, Japan

³RIKEN Center for Integrative Medical Sciences, Kanagawa, Japan

⁴Department of Ophthalmology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan

Correspondence: Yusuke Murakami, Department of Ophthalmology, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan; ymuraka3@med.kyushu-u.ac.jp

Submitted: February 20, 2019

Accepted: September 17, 2019

Citation: Ishizu M, Murakami Y, Fujiwara K, et al. Relationships between serum antioxidant and oxidant statuses and visual function in retinitis pigmentosa. *Invest Ophthalmol Vis Sci.* 2019;60:4462-4468. <https://doi.org/10.1167/iovs.19-26927>

PURPOSE. To investigate the serum changes of antioxidant/oxidant markers and the relationship between these factors and visual function in patients with retinitis pigmentosa (RP).

METHODS. Fifty-two RP patients <40 years old and 25 controls were included. Serum samples were analyzed for superoxide dismutase 3 (SOD3) activity, glutathione peroxidase (GPx), potential antioxidant (PAO), and hexanoyl-lysine (HEL). The relationships between these markers and visual parameters, including best-corrected visual acuity (BCVA), mean deviation (MD), and average retinal sensitivity of 4 or 12 central points on static perimetry tests (Humphrey Field Analyzer, the central 10-2 program) were examined in the RP patients.

RESULTS. Although there was no significant difference in the serum SOD3 activity between RP patients and controls, serum SOD3 activity in the severe degeneration group with macular involvement (16.3 ± 11.3 U/mL) was significantly lower compared with those in the mild degeneration group (those with midperipheral scotomas; 28.5 ± 16.6 U/mL, $P = 0.0459$). SOD3 was significantly related to visual acuity ($r = -0.3701$, $P = 0.0069$) and the average retinal sensitivity of four central points ($r = 0.3463$, $P = 0.0137$) in RP patients. The linear trends of these two parameters across SOD3 levels were also significant ($P = 0.0264$ and 0.0172 , respectively). There was no consistent correlation between other serum antioxidant/oxidant markers and visual parameters.

CONCLUSIONS. Lower serum SOD3 activity was associated with the severe retinal degeneration in RP patients. Our results suggest that serum SOD3 activity may be related to disease severity in RP.

Keywords: retinitis pigmentosa, oxidative stress, superoxide dismutase

The term “retinitis pigmentosa” (RP) refers to a heterogeneous group of inherited retinal degeneration disorders with mutations in more than 60 genes.^{1,2} Although there are shared clinical characteristics in RP, both the onset and the progression of the disease vary significantly among individuals.³ In typical RP, rod cells are primarily impaired due to genetic mutations, and this impairment is followed by gradual and progressive cone cell loss that results in severe vision loss. The cone cell death in RP has been demonstrated to be attributed to microenvironmental changes during retinal remodeling, such as oxidation,⁴ inflammation,⁵ and metabolic alterations.^{6,7} Although genotype-phenotype relationships have been reported in a subset of RP patients,⁸⁻¹¹ the associations between environmental factors and retinal degeneration are less well characterized.

Oxidative stress is the result of an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system. Although ROS are generated as products of normal metabolic reactions in aerobic organisms, the production of ROS is substantially elevated under damaged and inflammatory conditions.¹² To counter ROS, the cells

constitutively express antioxidant enzymes (such as superoxide dismutase [SOD]) that catalyze the dismutation of superoxide anion into hydrogen peroxide and oxygen. In mammals, there are three SOD isozymes: cytoplasmic Cu/Zn-SOD (SOD1), mitochondrial Mn-SOD (SOD2), and extracellular Cu/Zn-SOD (SOD3), which localize in the cytoplasm, mitochondria, and extracellular space, respectively.^{13,14}

The oxidation of proteins and lipids was increased in the aqueous humor and vitreous samples of RP patients, along with decreased antioxidant capacity, including reduced SOD3 activity.^{15,16} However, two research groups investigating the serum redox alterations in RP obtained controversial results. In 2013, Martínez-Fernández de la Cámara et al.¹⁵ reported the increased formation of thiobarbituric acid reactive substances (TBARS) and increased nitrotyrosine (which are the markers of oxidative/nitrosative stress) and decreased antioxidant SOD3 activity in the serum of 56 RP patients, whereas Campochiaro et al.¹⁶ later demonstrated no significant differences in the serum protein carbonyl content, oxidized glutathione ratio, or SOD3 level among seven to nine RP and seven control subjects. To address this discrepancy in results, we here performed an



independent analysis of the antioxidant/oxidant markers in 52 patients with RP and 25 control subjects. We also evaluated the associations between the RP patients' serum antioxidant/oxidant status and central visual function to characterize the relationship between antioxidant status and disease severity.

PATIENTS AND METHODS

Ethics Statement

This study was carried out under approval from the Institutional Review Board of Kyushu University Hospital (Fukuoka, Japan) and was conducted in accordance with the tenets of the Declaration of Helsinki on Biomedical Research Involving Human Subjects. All participating subjects provided written informed consent and were examined at Kyushu University Hospital.

Patients and Control Subjects

The study included 52 patients with RP and 25 age- and gender-matched healthy control subjects <40 years old. Because an individual's systemic redox status is influenced by systemic disorders, such as hypertension, diabetes, cardiovascular disease, and inflammatory disorders,^{17–21} we excluded subjects who had any systemic disease and those who had a history of other ocular diseases or intraocular surgery.

The diagnosis of typical RP was based on a history of night blindness, peripheral vision constriction, and/or ring scotoma, and markedly reduced or nonrecordable a- and b-wave amplitudes on electroretinography, in addition to ophthalmoscopic findings (i.e., characteristic fundus changes such as attenuated retinal vessels, and bone spicule-like pigment clumping in the midperipheral and peripheral retina). Patients with cone-rod or cone dystrophy, Bietti crystalline retinopathy, or macular dystrophy were excluded from the study. Genetic analysis of 83 RP causative genes was previously performed for 40 of 52 RP patients.²² The genetic inheritance patterns were determined based on the detected variants.

All of the control subjects underwent a health screening, including examinations of their vision, hearing, height, weight, and blood pressure, a urinary strip test, a chest X-ray, and a physician examination within the past 1 year, and they had results without specific abnormal findings.

Ophthalmic Examination

Best-corrected visual acuity (BCVA) was measured for all of the RP patients with full subjective refraction using a Landolt ring chart (CV-6000; Tomey, Nagoya, Japan; or AVC-36; Kowa, Nagoya, Japan) at 5 m or single Landolt test cards (HP-1258; Handaya, Tokyo, Japan). The BCVA was based on the minimum Landolt C letter that the subject was able to correctly answer >60% (3/5) of the time. For the statistical analyses, the decimal acuity values were converted into the logMAR.

Automated static perimetry testing was performed with a Humphrey Field Analyzer (HFA) (Humphrey Instruments, San Leandro, CA, USA) using the central 10–2 SITA Standard Program. The lens was corrected as appropriate for the test distance. When the test reliability was not sufficient (fixation loss >20%, false positive >15% or false negative >33%), the test was repeated. If the test reliability did not meet the criteria in the repeated examination, the results of static perimetry tests were not used for the analysis. The mean deviation (MD) and the average retinal sensitivity of 4 or 12 central points were obtained as described.²³ Because of the insufficient test reliability, the perimetry testing data from two of the 52 RP patients were excluded. Basically, these ophthalmic data

described in this article were derived from the patients' right eyes. In the generalized mixed model analysis, we used the data derived from two eyes of each single person. One of the 52 RP patients was excluded from this analysis because of his amblyopic left eye.

For a subgroup analysis, the RP patients were divided into three subsets based on the status of Goldmann perimetry and visual acuity (VA): mild, those with midperipheral scotomas and good central VA ($\geq 20/20$); moderate, those with preserved central islands and good central VA ($\geq 20/20$); and severe, those with reduced central VA ($< 20/20$).

Serum and Whole Blood Preparation

Whole blood (8 mL) without EDTA was obtained from an antecubital vein of each subject by using the collection tube SIM-L1008SQ3 (Kyokuto Pharmaceutical Industrial Co., Tokyo, Japan) at the same visit as the visual testing. After centrifugation for 5 minutes at 1200g, the serum was collected and dispensed into 1.5-mL tubes and frozen at -80°C within 1 hour from blood drawing. The samples were kept in a freezer until analyzed, and thawed 30 minutes before examination. The freeze-thaw process was conducted once for each sample. For the glutathione peroxidase (GPx) measurement, whole blood with EDTA was obtained and kept in a freezer until analyzed.

Evaluation of the Serum Antioxidant/Oxidant Status

The serum antioxidant/oxidant status was analyzed in serum from the RP patients and the healthy controls. The serum samples were analyzed for SOD3 activity, GPx, potential antioxidant (PAO), and hexanoyl-lysine (HEL). All of the measurements were performed in duplicate and the average values were used for the analysis.

SOD3 activity was measured by using a SOD3 Activity Assay kit (Northwest Life Science Specialties, Vancouver, WA, USA), according to the manufacturer's instructions. The serum SOD activity levels are expressed as U/mL.

GPx was measured by a glutathione peroxidase assay kit (Northwest Life Science Specialties), according to the manufacturer's instructions. The serum GPx levels are expressed as mU/mL.

PAO was measured by using a test kit for potential antioxidant (Japan Institute for the Control of Aging, Fukuroi, Shizuoka, Japan), according to the manufacturer's instructions. The serum PAO levels are expressed as micromolars.

HEL was measured by using a HEL ELISA kit (Japan Institute for the Control of Aging) according to the manufacturer's instructions. The serum HEL levels are expressed as nanomolars.

Statistical Analyses

The data are presented as the arithmetic mean \pm SD. Sex ratio differences were examined by χ^2 tests. Mean values were compared between the control subjects and the RP patients by the Wilcoxon rank sum test. Comparisons among the multiple groups were first conducted by the Kruskal-Wallis test. When the *P* value was less than 0.05 in the Kruskal-Wallis test, the Steel-Dwass test was performed as a post hoc test. The correlation between the serum antioxidant/oxidant status and the various visual parameters was analyzed using Spearman's rank correlation coefficient. The linear trends in visual parameters across the levels of serum antioxidant/oxidant markers were also tested using linear regression model with the generalized estimating equation to take into account correlation between two eyes of each single person.^{24,25}

TABLE 1. Baseline Characteristics of the Control Subjects and RP Patients With Different Disease Severity

	Control Subjects, <i>n</i> = 25	RP Patients			<i>P</i> Value
		Mild, <i>n</i> = 17	Moderate, <i>n</i> = 10	Severe, <i>n</i> = 25	
Age, y	30.2 ± 3.8 (23–38)	30.0 ± 6.0 (20–38)	30.3 ± 5.7 (20–36)	30.0 ± 7.2 (20–39)	0.9826*
Males:females	10:15	6:11	4:6	16:9	0.2101†
SOD3 activity, U/mL	23.3 ± 12.1	28.4 ± 16.6	25.0 ± 10.0	16.3 ± 11.3	0.0201*
GPx, mU/mL	3.4 ± 0.7	3.3 ± 0.6	3.3 ± 0.6	3.6 ± 0.7	0.3797*
PAO, μM	1127.1 ± 139.1	992.2 ± 178.2	990.6 ± 161.4	1051.3 ± 195.6	0.0607*
HEL, nM	10.4 ± 9.3	7.6 ± 5.7	9.5 ± 6.7	10.0 ± 6.5	0.7099*
VA, logMAR		−0.06 ± 0.06	−0.03 ± 0.04	0.50 ± 0.40	< 0.0001*
MD value, dB		−9.3 ± 7.8	−15.1 ± 10.4	−22.8 ± 9.2‡	0.0005*
Average retinal sensitivity of 4 central points, dB		32.4 ± 3.5	31.8 ± 6.4	17.8 ± 10.6‡	< 0.0001*
Average retinal sensitivity of 12 central points, dB		31.3 ± 4.2	28.7 ± 9.0	15.8 ± 10.7‡	< 0.0001*
Inheritance mode, <i>n</i>					
AD		2	1	2	
RP causative gene,§ <i>n</i>		<i>RP1</i> , 1; <i>IMPDH1</i> , 1	<i>RP1</i> , 1	<i>RP1</i> , 1; <i>PRPF31</i> , 1	
AR		5	1	4	
RP causative gene,§ <i>n</i>		<i>EYS</i> , 3; <i>PDE6A</i> , 1; <i>USH2A</i> , 1	<i>EYS</i> , 1	<i>EYS</i> , 1; <i>USH2A</i> , 1; <i>TULP1</i> , 1; <i>PRCD</i> , 1	
X-linked		0	0	1	
RP causative gene,§ <i>n</i>				<i>RP2</i> , 1	
ND		6	8	10	
NT		4	0	8	

Data are the mean ± SD. Inheritance pattern was presumed based on the result of sequencing. The bolded values indicate statistical significance, *P* < 0.05. AD, autosomal dominant; AR, autosomal recessive; ND, not determined; NT, not tested; XL, X-linked.

* Kruskal-Wallis test.

† χ^2 test.

‡ *n* = 23.

§ These genes were determined in the previous genomic sequence analysis of 83 RP causative genes.²²

which was performed with the SAS software package (version 9.4; SAS, Cary, NC, USA). The values of serum antioxidant/oxidant markers were treated as continuous variables and were transformed into logarithms to improve the skewed distribution. All other statistical analyses were performed with JMP Pro13 software (SAS). Two-sided *P* < 0.05 values were considered significant.

RESULTS

The Serum Antioxidant Status and Oxidant Status in the RP Patients and Controls

The characteristics and genetic information of the participants are summarized in Supplementary Tables S1 and S2. There were no significant differences in age or sex distribution between the two groups. We assessed the participants' serum SOD3 activity, GPx, and PAO as antioxidant markers. There was no significant difference in the SOD3 activity or GPx levels between the RP patients and controls. The serum PAO levels were slightly lower in the RP patients (1020.3 ± 182.9 μM) compared with the control subjects (1127.1 ± 139.1 μM, *P* = 0.0182; Supplementary Table S1). We also analyzed serum HEL as an oxidant marker. There was no significant difference between the RP patients and controls.

To investigate the antioxidant/oxidant levels according to the degree of retinal degeneration, we divided the patients into three subsets based on the status of visual field and VA (mild: those with midperipheral scotomas; moderate: those with preserved central islands; and severe: those with macular involvement). The characteristics of each group are shown in Table 1. The serum SOD3 activity in the severe degeneration group (16.3 ± 11.3 U/mL) were significantly lower compared

with those in the mild degeneration group (28.5 ± 16.6 U/mL, *P* = 0.0459), and showed a trend for decrease compared with the moderate degeneration group (25.0 ± 10.0 U/mL, *P* = 0.0751), as shown in Figure 1 and Supplementary Table S3. There was no significant difference in the serum SOD3 activity between controls (23.3 ± 12.1 U/mL) and each RP patient group. Among four markers investigated, only SOD3 activity was significantly changed along with the disease severity.

To examine the possibility of oxidative modification of the samples during storage and preparation, we compared the levels of antioxidants/oxidants in blood samples of healthy controls that were frozen immediately, 1 hour, or 3 hours after blood collection. The levels of GPx and PAO did not change largely by the time from the sample collection to storage. SOD3 activity and HEL showed low variability between samples frozen immediately and 1 hour after collection, but were slightly decreased (SOD3) or increased (HEL) in samples frozen 3 hours after collection (Supplementary Table S4). Because all the samples used for the initial analyses were frozen within 1 hour from blood drawing, the data of GPx, PAO, SOD3, and HEL were considered to be reliable in terms of oxidative modification during sample preparation.

The Associations Between Serum Antioxidant/Oxidant Status and Visual Function in the RP Patients

We next investigated the relationships between serum antioxidant/oxidant markers and visual parameters in the RP patients. Spearman's rank testing showed that the patients' serum SOD3 activity levels were significantly correlated with the VA (*r* = −0.3701, *P* = 0.0069) and with the average retinal sensitivity of four central points on HFA 10–2 program (*r* =

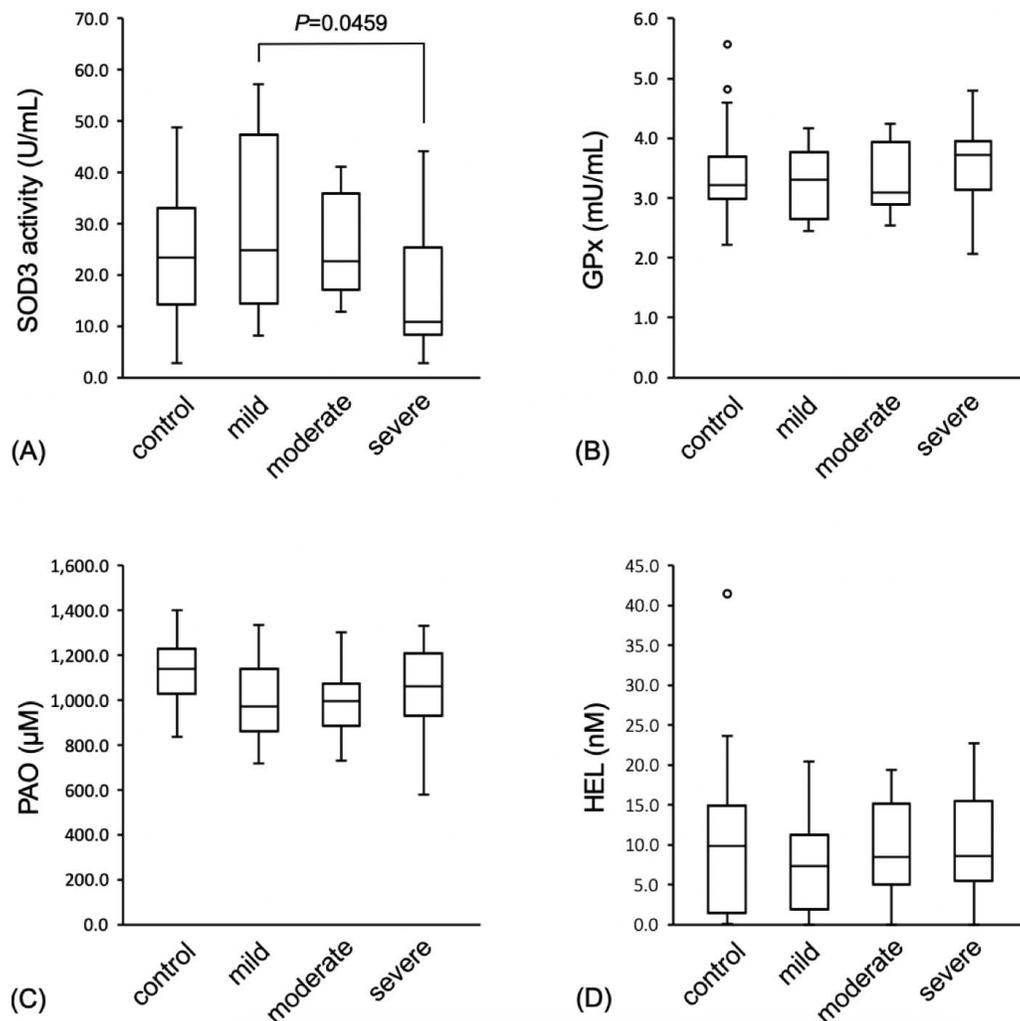


FIGURE 1. Box plots of the serum antioxidant/oxidant values ([A] SOD3; [B] GPx; [C] PAO; [D] HEL) in the control subjects and RP patients with different disease severity. The patients were divided into three subsets based on visual status of Goldmann perimetry and VA (mild: those with midperipheral scotomas; moderate: those with preserved central islands; and severe: those with macular involvement). Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line of box, 25th percentile; horizontal bar within box, median. Whiskers extend from the box to 1.5 times the interquartile range. Circles represent outliers. *P* value was analyzed by the Steel-Dwass test.

0.3463, $P = 0.0137$), but not with the MD value ($r = 0.1249$, $P = 0.3876$), as shown in Figure 2 and Table 2. The correlation between SOD3 and the average sensitivity of 12 central points showed a trend but not statistical significance ($r = 0.2382$, $P = 0.0957$). On the other hand, GPx was negatively correlated with the average sensitivity of 12 central points ($r = -0.2997$, $P = 0.0345$).

We also analyzed the linear trends in visual parameters across the levels of serum antioxidant/oxidant markers using the linear regression model with the generalized estimating equation to take into account correlation between two eyes of each single person. The analysis showed that the increased serum SOD3 activity was significantly associated with the elevation of VA ($\beta = -0.1607$, $P = 0.0264$) and the average retinal sensitivity of four central points ($\beta = 5.3841$, $P = 0.0172$; Table 3). The significant correlations between GPx and visual parameters were not found in this analysis.

DISCUSSION

Oxidative stress has been implicated in the pathogenesis of various retinal and neurodegenerative diseases, including RP.

Rod cells, which account for 95% of photoreceptor cells in the human retina, are packed with mitochondria and are highly metabolically active. After mutation-induced rod cell death in RP, oxygen consumption would therefore be reduced and the remaining cone cells might be exposed to a higher level of oxygen.²⁶ In addition, activated microglial cells may contribute to the local accumulation of oxidative stress via the production of ROS during retinal degeneration.

Indeed, in an experimental model of RP, significant oxidation of nucleic acids, proteins, and lipids was observed in the retinas from an early stage to a late stage of retinal degeneration,⁴ and exogenous antioxidant treatment or the overexpression of antioxidant genes in the retina suppressed cone photoreceptor cell death and preserved their function in RP models.²⁷⁻²⁹ SOD family molecules are endogenous antioxidant enzymes that catalyze the dismutation of superoxide anion, and experimental studies have shown that a combined retinal overexpression of cytoplasmic SOD1 or mitochondrial SOD2 with glutathione peroxidase or catalase was able to slow the photoreceptor cell death in RP models,^{30,31} although the function of extracellular SOD3 in RP has not been fully elucidated.

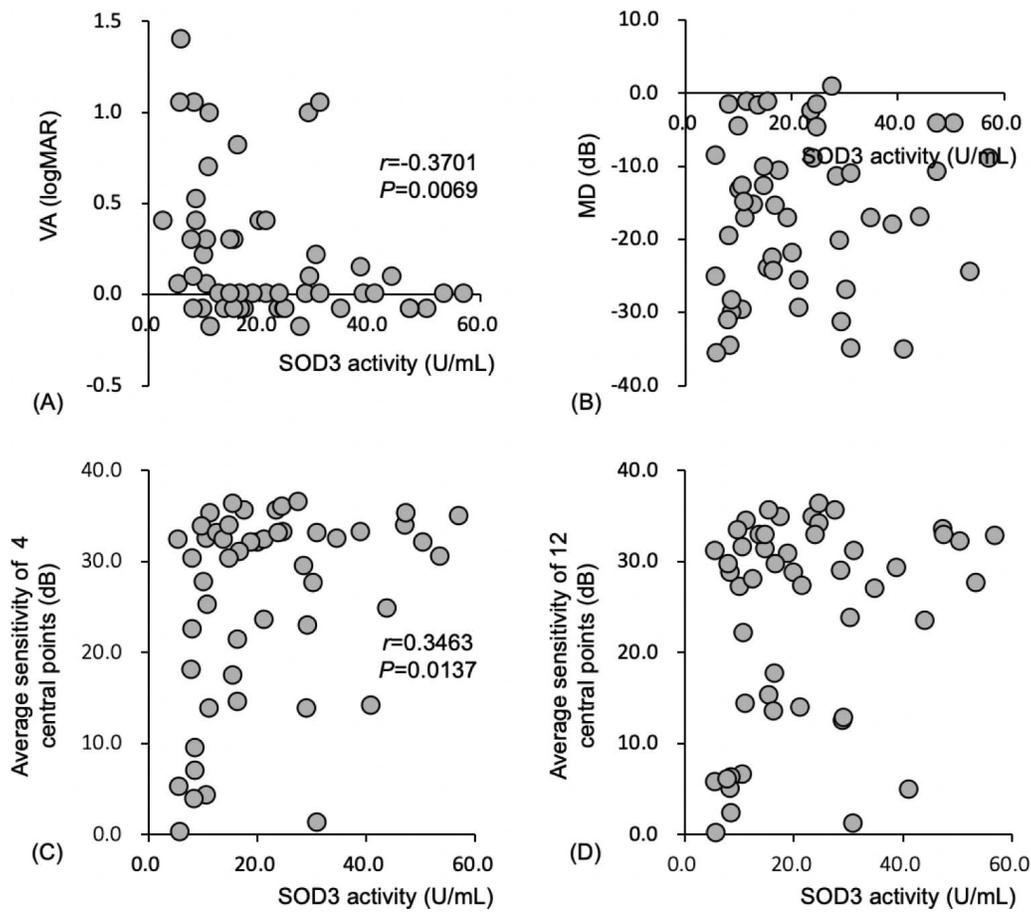


FIGURE 2. Relationships between the serum SOD3 activity value and visual parameters in patients with RP. Scatterplots showing the relationship between SOD3 activity and VA (A), the MD value in HFA 10–2 tests (B), the average sensitivity of four central points (C), and the average sensitivity of 12 central points (D).

Regarding human samples, two groups have already demonstrated the changes of antioxidant/oxidant markers in serum (e.g., SOD3, nitrotyrosine, and TBARS) and aqueous humor (e.g., SOD3, total antioxidant capacity, carbonyl content of protein, and glutathione/glutathione disulfide) of RP patients.^{15,16} Martínez-Fernández de la Cámara et al.¹⁵ also reported the association between better visual field and higher levels of ocular antioxidants, which is classified by the fuzzy clustering according the patients' nicotinamide adenine dinucleotide phosphate oxidase, SOD3, protein and total antioxidant capacity values. However, the relations between serum antioxidant/oxidant molecules and visual function have not been fully addressed in prior studies.

In the present study, we measured the levels of the participants' serum SOD3, GPx, and PAO as antioxidant markers and HEL as an oxidant marker. In the analysis based on the disease severity, the serum SOD3 activity in the severe degeneration group was lower compared with those of the mild degeneration group. In addition, our data demonstrated that higher serum SOD3 activity is related to better VA and the central retinal sensitivity in RP patients. These findings suggest that RP patients with severe vision loss also have lower SOD3 activity.

There are several limitations in our study. First, the sample size was small due to the single-center nature of the study. To minimize the influence of systemic problems leading to

TABLE 2. Correlation Between the Levels of Serum Antioxidant/Oxidant Markers and Visual Function in RP Patients

	SOD3		GPx		PAO		HEL	
	r	P Value	r	P Value	r	P Value	r	P Value
Age	-0.1837	0.1924	0.2436	0.0818	-0.1817	0.1973	0.2328	0.0968
VA	-0.3701	0.0069	0.1999	0.1554	-0.0388	0.7846	0.1237	0.3821
MD value*	0.1249	0.3876	-0.2292	0.1094	-0.1476	0.3065	-0.0678	0.6398
Average retinal sensitivity of 4 central points*	0.3463	0.0137	-0.2399	0.0933	-0.0313	0.8290	-0.0589	0.6846
Average retinal sensitivity of 12 central points*	0.2382	0.0957	-0.2997	0.0345	-0.0502	0.7290	-0.0692	0.6332

The bolded values indicate statistical significance, $P < 0.05$.

* $n = 50$.

TABLE 3. Increase in Visual Parameters Per Every 1-Log-Transformed Serum Antioxidant/Oxidant Marker in RP Patients

	SOD3		GPx		PAO		HEL	
	Increase in Visual Parameter, SE	P Value	Increase in Visual Parameter, SE	P Value	Increase in Visual Parameter, SE	P Value	Increase in Visual Parameter, SE	P Value
VA,* logMAR	-0.1607 (0.0703)	0.0264	0.4554 (0.2586)	0.0843	-0.0494 (0.2691)	0.8550	-0.0025 (0.0667)	0.9706
MD value,† dB	2.4093 (2.3801)	0.3164	-11.5622 (7.7612)	0.1427	-8.4174 (8.0242)	0.2994	-0.4289 (2.0100)	0.8320
Average retinal sensitivity of 4 central points,† dB	5.3841 (2.1827)	0.0172	-11.4301 (7.4561)	0.1317	0.0162 (7.8049)	0.9984	0.5554 (1.8524)	0.7657
Average retinal sensitivity of 12 central points,† dB	4.6491 (2.3688)	0.0552	-13.6375 (7.8701)	0.0894	-2.8322 (8.2823)	0.7339	0.1813 (2.0068)	0.9284

Values are shown as the β estimate and SE. Serum antioxidant/oxidant markers were transformed to the logarithmic scale. The bolded values indicate statistical significance, P < 0.05.

* n = 51.

† n = 49.

oxidative stress throughout the body, we examined relatively young patients (<40 years old). This also contributed to the small sample size. A larger number of patients should be explored in further studies.

Second, we did not directly test foveal center sensitivity, and used the average sensitivity of four central points on the HFA 10-2 program as a parameter of central visual function. A number of studies including ours have demonstrated the usefulness of HFA 10-2 programs to evaluate the macular function and progression in RP patients.³²⁻³⁴ We previously reported that the MD and the average retinal sensitivity of four central points were strongly correlated with ellipsoid zone (EZ) width in RP patients ($r = 0.8348$, $P < 0.0001$; and $r = 0.8127$, $P < 0.0001$, respectively).³⁵ Certainly the HFAs are subjective examinations and the results can be influenced by fixation loss or other factors; however, these findings indicate that HFA parameters are reliable for assessing the severity of degeneration in RP patients. Microperimetry may be a better option for evaluating macular function because it can accurately project a light onto the specific point of the retina using an auto-tracking system. Indeed, Asahina et al.³⁶ reported that the retinal sensitivity measured by microperimetry rather than those by HFA were correlated with EZ area in RP patients. Igarashi et al.³⁷ also demonstrated superior test-retest repeatability with microperimetry (intraclass correlation coefficient [ICC]: 0.81); however, HFA still showed good repeatability (ICC: 0.77) in RP patients. The length of EZ was also frequently analyzed as a structural parameter to reflect the degeneration status of RP^{38,39}; however, our study did not include EZ width data because the resolution of optical coherence tomography images was not sufficiently high to precisely determine the EZ width.

Finally, we could not compare the ocular and serum antioxidant levels in the participants. In this study, we included relatively young patients, younger than 40 years, to avoid the influence of systemic diseases, and the institutional review board in our university does not allow us to obtain aqueous humor samples unless patients undergo ocular surgeries. It is important to clarify whether serum SOD3 levels fall in response to disease progression in RP. In addition, because of the cross-sectional aspect of the present study, cause-effect relationships between SOD3 activity and disease progression of RP remain unknown. An independent study to investigate the antioxidant/oxidant levels in both aqueous and serum samples as well as a follow-up study assessing the longitudinal changes of serum SOD3 activity and their relationships to the functional and structural progression of disease will be necessary.

In conclusion, our results demonstrate the serum antioxidant/oxidant profiles in RP patients. Of note, lower serum

SOD3 activity was correlated with the severe vision loss in RP patients. Our results suggest that serum SOD3 activity may be related to disease severity in RP.

Acknowledgments

Supported by Japanese Ministry of Education, Culture, Sports, Science, and Technology Grants 16H06268 and 19K09952 (YM), The Uehara Memorial Foundation Grant (YM), The Kaibara Morikazu Medical Science Promotion Foundation Grant (YM), the Charitable Trust Fund for Ophthalmic Research in Commemoration of Santen Pharmaceutical's Founder (YM), and the Bayer Retina Award (YD).

Disclosure: M. Ishizu, None; Y. Murakami, None; K. Fujiwara, None; J. Funatsu, None; S. Shimokawa, None; S. Nakatake, None; T. Tachibana, None; T. Hisatomi, None; Y. Koyanagi, None; M. Akiyama, None; Y. Momozawa, None; T. Ishibashi, None; K.-H. Sonoda, None; Y. Ikeda, None

References

- Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. *Lancet*. 2006;368:1795-1809.
- Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. *Clin Genet*. 2013;84:132-141.
- Hamel C. Retinitis pigmentosa. *Orphanet J Rare Dis*. 2006;1:40.
- Shen J, Yang X, Dong A, et al. Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J Cell Physiol*. 2005;203:457-464.
- Yoshida N, Ikeda Y, Notomi S, et al. Clinical evidence of sustained chronic inflammatory reaction in retinitis pigmentosa. *Ophthalmology*. 2013;120:100-105.
- Punzo C, Kornacker K, Cepko CL. Stimulation of the insulin/mTOR pathway delays cone death in a mouse model of retinitis pigmentosa. *Nat Neurosci*. 2009;12:44-52.
- Camacho ET, Punzo C, Wirkus SA. Quantifying the metabolic contribution to photoreceptor death in retinitis pigmentosa via a mathematical model. *J Theor Biol*. 2016;408:75-87.
- Berson EL, Rosner B, Weigel-DiFranco C, Dryja TP, Sandberg MA. Disease progression in patients with dominant retinitis pigmentosa and rhodopsin mutations. *Invest Ophthalmol Vis Sci*. 2002;43:3027-3036.
- Sandberg MA, Rosner B, Weigel-DiFranco C, McGee TL, Dryja TP, Berson EL. Disease course in patients with autosomal recessive retinitis pigmentosa due to the USH2A gene. *Invest Ophthalmol Vis Sci*. 2008;49:5532-5539.
- Talib M, van Schooneveld MJ, van Genderen MM, et al. Genotypic and phenotypic characteristics of CRB1-associated

- retinal dystrophies: a long-term follow-up study. *Ophthalmology*. 2017;124:884-895.
11. Comander J, Weigel-DiFranco C, Maher M, et al. The genetic basis of pericentral retinitis pigmentosa - a form of mild retinitis pigmentosa. *Genes*. 2017;8:E256.
 12. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal*. 2014;20:1126-1167.
 13. Behndig A, Svensson B, Marklund SL, Karlsson K. Superoxide dismutase isoenzymes in the human eye. *Invest Ophthalmol Vis Sci*. 1998;39:471-475.
 14. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic Biol Med*. 2002;33:337-349.
 15. Martínez-Fernández de la Cámara C, Salom D, Sequedo MD, et al. Altered antioxidant-oxidant status in the aqueous humor and peripheral blood of patients with retinitis pigmentosa. *PLoS One*. 2013;8:e74223.
 16. Campochiaro PA, Strauss RW, Lu L, et al. Is there excess oxidative stress and damage in eyes of patients with retinitis pigmentosa? *Antioxid Redox Signal*. 2015;23:643-648.
 17. Pedro-Botet J, Covas MI, Martin S, Rubies-Prat J. Decreased endogenous antioxidant enzymatic status in essential hypertension. *J Hum Hypertens*. 2000;14:343-345.
 18. Gawlik K, Naskalski JW, Fedak D, et al. Markers of antioxidant defense in patients with type 2 diabetes. *Oxid Med Cell Longev*. 2016;2016:2352361.
 19. Ho E, Karimi Galougahi K, Liu CC, Bhindi R, Figtree GA. Biological markers of oxidative stress: applications to cardiovascular research and practice. *Redox Biol*. 2013;1:483-491.
 20. Poulianiti KP, Kaltsatou A, Mitrou GI, et al. Systemic redox imbalance in chronic kidney disease: a systematic review. *Oxid Med Cell Longev*. 2016;2016:8598253.
 21. Griffith B, Pendyala S, Hecker L, Lee PJ, Natarajan V, Thannickal VJ. NOX enzymes and pulmonary disease. *Antioxid Redox Signal*. 2009;11:2505-2516.
 22. Koyanagi Y, Akiyama M, Nishiguchi KM, et al. Genetic characteristics of retinitis pigmentosa in 1204 Japanese patients. *J Med Genet*. 2019;56:662-670.
 23. Fujiwara K, Ikeda Y, Murakami Y, et al. Assessment of central visual function in patients with retinitis pigmentosa. *Sci Rep*. 2018;8:8070.
 24. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. *Biometrics* 1988; 44:1049-1060.
 25. Katz J, Zeger S, Liang KY. Appropriate statistical methods to account for similarities in binary outcomes between fellow eyes. *Invest Ophthalmol Vis Sci* 1994;35:2461-2465.
 26. Yu DY, Cringle SJ, Su EN, Yu PK. Intraretinal oxygen levels before and after photoreceptor loss in the RCS rat. *Invest Ophthalmol Vis Sci*. 2000;41:3999-4006.
 27. Komeima K, Rogers BS, Lu L, Campochiaro PA. Antioxidants reduce cone cell death in a model of retinitis pigmentosa. *Proc Natl Acad Sci U S A*. 2006;103:11300-11305.
 28. Komeima K, Rogers BS, Campochiaro PA. Antioxidants slow photoreceptor cell death in mouse models of retinitis pigmentosa. *J Cell Physiol*. 2007;213:809-815.
 29. Yoshida N, Ikeda Y, Notomi S, et al. Laboratory evidence of sustained chronic inflammatory reaction in retinitis pigmentosa. *Ophthalmology*. 2013;120:e5-e12.
 30. Usui S, Komeima K, Lee SY, et al. Increased expression of catalase and superoxide dismutase 2 reduces cone cell death in retinitis pigmentosa. *Mol Ther*. 2009;17:778-786.
 31. Usui S, Oveson BC, Iwase T, et al. Overexpression of SOD in retina: need for increase in H2O2-detoxifying enzyme in same cellular compartment. *Free Radic Biol Med*. 2011;51:1347-1354.
 32. Iijima H. Correlation between visual sensitivity loss and years affected for eyes with retinitis pigmentosa. *Jpn J Ophthalmol*. 2012;56:224-229.
 33. Ikeda Y, Hisatomi T, Yoshida N, et al. The clinical efficacy of a topical dorzolamide in the management of cystoid macular edema in patients with retinitis pigmentosa. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:809-814.
 34. Ikeda Y, Yoshida N, Notomi S, et al. Therapeutic effect of prolonged treatment with topical dorzolamide for cystoid macular oedema in patients with retinitis pigmentosa. *Br J Ophthalmol*. 2013;97:1187-1191.
 35. Murakami Y, Funatsu J, Nakatake S, et al. Relations among foveal blood flow, retinal-choroidal structure, and visual function in retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2018;59:1134-1143.
 36. Asahina Y, Kitano M, Hashimoto Y, et al. The structure-function relationship measured with optical coherence tomography and a microperimeter with auto-tracking: the MP-3, in patients with retinitis pigmentosa. *Sci Rep*. 2017;7:15766.
 37. Igarashi N, Matsuura M, Hashimoto Y, et al. Assessing visual fields in patients with retinitis pigmentosa using a novel microperimeter with eye tracking: the MP-3. *PLoS One*. 2016; 11:e0166666.
 38. Birch DG, Locke KG, Feliuss J, et al. Rates of decline in regions of the visual field defined by frequency-domain optical coherence tomography in patients with RPGR-mediated X-linked retinitis pigmentosa. *Ophthalmology*. 2015;122:833-839.
 39. Strampe MR, Huclenpähler AL, Higgins BP, et al. Intraobserver repeatability and interobserver reproducibility of ellipsoid zone measurements in retinitis pigmentosa. *Trans Vis Sci Tech*. 2018;7(3):13.