Differences in Relationship Between Macular Inner Retinal Layer Thickness and Retinal Sensitivity in Eyes With Early and Progressed Glaucoma

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Submitted: September 6, 2015
Accepted: February 5, 2016

PURPOSE. To determine if the relationship between retinal sensitivity and macular inner retinal layer thickness differs between primary open-angle glaucoma (POAG) with mild and advanced central visual field (VF) damage.

METHODS. One eye of 153 POAG patients was included. Using spectral-domain optical coherence tomography, we measured the average thickness of the macular ganglion cell-inner plexiform layers (GCIPLT) and the macular nerve fiber layer/GCIPL (ganglion cell complex [GCC]) in a 0.9-mm-diameter ganglion cell displacement–adjusted circular area corresponding to the four central test points of the Humphrey Perimeter 24-2 program and correlated the results with the average retinal sensitivity (1/Lambert) at the corresponding test points, with adjustment for other confounding factors.

RESULTS. Ninety-three eyes had mild central and 60 eyes advanced central VF damage with an average total deviation (TD) of the four test points of greater than or equal to −4 decibels (dB) (mild group) and less than −4 dB (more severe group), respectively; the average mean deviations were −5.0 and −9.8 dB, respectively. In the mild group, the GCC and GCIPLT were correlated significantly and positively with the average retinal sensitivity with partial regression coefficient of 0.007 and 0.005, respectively, and in the more severe group with partial regression coefficient of 0.019 = 0.007 + 0.012 (P = 0.007) and 0.010 = 0.005 + 0.005 (P = 0.078), respectively. The axial length and disc size were correlated with GCIPLT with marginal significance (P = 0.052 and P = 0.042).

CONCLUSIONS. The relationship between the macular GCC and GCIPL thickness and retinal sensitivity at the corresponding retinal areas differed between POAG with mild and advanced central VF damage.

Keywords: ganglion cell complex, macula, retinal sensitivity

Glaucoma damages the retinal ganglion cell (RGC) bodies and axons and is associated with characteristic patterns of visual field (VF) defects and changes in the appearance of the optic nerve head (ONH). Thinning of the RGC-related retinal layers caused by RGC loss is associated with decreased VF sensitivity. Structural and functional tests are indispensable for assessing the extent of glaucomatous damage, especially in early glaucoma.1

A number of studies have used optical coherence tomography (OCT) to study correlations between standard automated perimetry (SAP)-determined sensitivity and OCT-determined thicknesses of the RGC-related retinal layers such as the circumpapillary retinal nerve fiber layer (cpRNFL); combined macular RNFL (mRNFL); ganglion cell layer (GCL) and inner plexiform layer (IPL); macular ganglion cell complex (GCC); and the combined macular GCL and IPL (GCIPL).2–14 Previous studies have agreed that the correlation between the OCT-measured thicknesses of the RGC-related retinal layers and corresponding SAP sensitivities is weak and insignificant when the SAP sensitivities are normal.2–15 This finding has been explained by the fact that substantial RGC loss (i.e., at least 10% to approximately 20%) resulting from glaucomatous damage is needed to lower the measured SAP sensitivity to outside the normal range.2,5,15 and it is reasonable to assume that the SAP sensitivities on the linear scale (1/Lambert = 10dB value/10) or the decibel (dB) scale decrease linearly with decreases in the thicknesses of the RGC-related retinal layers or the density of the RGCs on the dB scale, respectively, once the RGC loss exceeds the critical point. In studying the relationship between the SAP sensitivities and thicknesses of the RGC-related retinal layers in the corresponding retinal areas, the GCC or GCIPL thickness in the macular region where most RGCs are located is advantageous over adopting the cpRNFL thickness, as the axons comprising the cpRNFL originate from different retinal...
regions, and interindividual variation is believed to exist between the VF regions and the disc sectors.\textsuperscript{16–19}

We recently reported that the GCC or GCIPL thickness corresponding to the central four test points of the Humphrey Field Analyzer (HFA) 24-2 program (Carl Zeiss Meditec, Dublin, CA, USA) was correlated significantly and positively with the corresponding linear scale SAP sensitivity at the four test points in normal eyes.\textsuperscript{14} although the slope of the regression line was much flatter than those reported in glaucomatous eyes.\textsuperscript{2–4,13} This finding suggested that the stage of glaucoma may also affect the relationship between the SAP sensitivities and thicknesses of the RGC-related layers in the corresponding retinal areas. In fact, Ajtony et al.\textsuperscript{5} reported that correlation with those advanced VF damage, after adjusting for the linear scale SAP sensitivity relationship in the macular region in the slope characterizing the GCC or GCIPL thickness and the corresponding retinal area depends on the disease stage or not.

In the current study, we investigated if there is a difference in the slope characterizing the GCC or GCIPL thickness and the linear scale SAP sensitivity relationship in the macular region between POAG eyes with mild central VF damage and those with those advanced VF damage, after adjusting for the possible effect of age, disc size, and axial length.\textsuperscript{20–25}

Materials and Methods

Subjects

Patients with POAG were recruited using identical inclusion criteria at the four participating institutes (University of Tokyo, Kanazawa University, Kyoto University, and Tajimi Municipal Hospital). The institutional review board of each institution approved the study protocol and adhered to the tenets of the Declaration of Helsinki. Each subject provided written informed consent after obtaining an explanation of the study protocol. Patients fulfilling the inclusion criteria who were seen at one of the four institutions and agreed to participate in the study were consecutively enrolled and underwent the following ocular measurements: refraction and corneal curvature radius with an automatic refractometer (ARK-900; Nidek, Tokyo, Japan), best-corrected visual acuity, axial length with the IOLMaster (Carl Zeiss Meditec), and IOP by Goldmann applanation tonometry, and the following examinations: slit-lamp, dilated funduscopy, and testing with the HFA 24-2 Swedish Interactive Threshold Algorithm Standard (SITA-S) program (Carl Zeiss Meditec). Patients were included if they were accustomed to VF testing using an HFA that provided reliable and reproducible VF test results (fixation loss, false-positives, or false-negatives <20%) and had apparent glaucomatous changes in the ONH and/or apparent RNFL defects confirmed by the panel of glaucoma specialists (MA, MH, and AI) on stereo-fundus photographs and digitally constructed red-free photographs. The ONH changes included an apparent rim notch in the superior or inferior portion with a width of 0.1 or less disc diameter and a vertical cup-to-disc ratio over 0.7 in one eye that was greater than that of the fellow eye by a factor of 0.2 or more. The presence of glaucomatous VF defects was not a concern when apparent ONH findings and/or RNFL defects with a width greater than the major retinal vessel diameter at the disc margin were observed.

Other inclusion criteria included eyes with a refractive error of $-6.0$ diopeters (D) or higher and less than 3.0 D; and the absence of a clinically relevant cataract or a history of other ocular pathologic changes that could affect the results of HFA or OCT examinations, including incisional intraocular or refractive surgeries. The VF was checked using the HFA 24-2 SITA-8 program within 3 months of the OCT examination and confirmed to reproduce the VFDs found in the previous tests. Glaucomatous VFDs were defined according to the criteria of Andrews and Patella.\textsuperscript{26} Eyes with POAG were grouped based on the mild (mild group) or advanced central VF damage (more severe group) according to the extent of damage at the four central test points of the HFA 24-2 program. If both eyes of a participant fulfilled the inclusion criteria, the eye with better reliability and reproducibility of visual field test results (fixation loss, false-positives, or false-negatives) was included.

Optical Coherence Tomography Measurement

Optical coherence tomography scanning was performed using an SD-OCT1000 instrument (Topcon, Tokyo, Japan) equipped with a nonmydriatic fundus camera function. Data sets were obtained using the raster-scan protocol, where data were obtained from a 6.0 $\times$ 6.0-mm$^2$ area (512 $\times$ 128 pixels) centered on the fixation point over a period of approximately 2.5 seconds. To obtain accurately sized fundus images, the magnification was corrected according to the formula provided by the manufacturer based on the refractive error, corneal radius, and axial length. The correspondence of the fundus photographs and OCT images was confirmed automatically using an OCT projection image and localization of the major retinal vessels.

The data obtained during eye movements were discarded and the examination repeated. Images that were affected by involuntary blinking or saccades, indicated by breaks, shifting of the vessels, or the presence of a straight line across the fundus OCT image, or those with a quality factor less than 60% were also excluded. In the macular area, the fovea was identified automatically in the OCT image as the pixel with the thinnest total retinal thickness adjacent to the fixation point. The mRNFL and GCIPL were segmented automatically in all B-scan images.\textsuperscript{27} An experienced examiner (AT) confirmed the image segmentation. Data for the GCC were obtained with the following formula: (mRNFL + GCIPL).

Immediately after the SD-OCT measurement, a color fundus photograph centered on the disc was taken with the nonmydriatic fundus camera function of the SD-OCT device. The disc area in the fundus photographs was identified by determining the area inside a closed spline curve fitted to seven manually determined points on the disc margin. The magnification was corrected as described previously.\textsuperscript{25}

Relationship Between GCC and GCIPL Thickness and the Linear Scale VF Sensitivity in Corresponding Retinal Areas

The GCC and GCIPL thicknesses (GCCT and GCIPLT) in a circular retinal area with a 0.9-mm diameter ($\sim$3° of visual angle) corresponding to the four central test points of the HFA 24-2 program, adjusted for RGC displacement, were assessed in the SD-OCT raster-scan data. Ganglion cell displacement was estimated with the following formula: $y = 1.29 \times (x + 0.46)^{0.67}$, where $y$ indicates ganglion cell eccentricity and $x$ indicates cone eccentricity.\textsuperscript{28} The dB values for the SAP sensitivity in the four central test points of the HFA 24-2 test program were anti-logged to obtain the sensitivity in the linear scale [$(1/\text{Lambert})^{2.46}$] of which the mean of the four central test points was calculated using the above four anti-logged dB values (LSAP$_4$ test points). Age,\textsuperscript{20,23,24,25} axial length or refraction,\textsuperscript{21,23,25} and disc size\textsuperscript{20} are external factors that reportedly can affect the OCT-measured thicknesses of the cRNFL, GCC, or GCIPL. The laterality and gender were excluded from the external factors according to the result of

\[ 
\text{GCC/GCIPL Thickness and Sensitivity in Glaucoma} \]

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mean sensitivity of the 4 central test points,

\[ \text{Mean sensitivity} = \text{TD4 test points, dB} \]

\[ \text{Mean sensitivity} = \text{GCIPL4 test points, dB} \]

\[ \text{Mean sensitivity} = \text{GCC4 test points, dB} \]

The effects of age, axial length, and disc size on the relationship between the GCC (GCIPL) thickness in the four retinal areas were considered by multiple linear regression analysis:

\[ \text{GCC4 test points} = \text{A1} \times \text{age} + \text{A2} \times \text{Axial length (mm)} + \text{A3} \times \text{disc size} + \text{A4} \times \text{LSAP4 test points} + \text{A5} \times \text{GLA} \times \text{LSAP4 test points} + \text{A6} \times \text{A7} \times \text{GLA} + \text{error} \]

where GCC4 test points (GCIPL4 test points) indicates the mean of the measured GCC (GCIPL) thickness in the four retinal areas in the mild and more severe groups, and GLA indicates a category variable that adopts 0 in the mild group and 1 in the more severe group. The effects of age, axial length, and disc size on the relationship between the GCC4 test points (GCIPL4 test points) and LSAP4 test points were assumed to be independent of disease stage. When the partial regression coefficients A1 and A2 both differed significantly from 0, it was thought that the GCC4 test points (GCIPL4 test points) changed along with the LSAP4 test points decrease, but the slope characterizing the relationship between them differed significantly between the mild and more severe groups after correction of other confounding factors. The data were analyzed using SPSS software (21.0J for Windows; SPSS Japan Inc., Tokyo, Japan).

### RESULTS

A total of 153 eyes of 153 patients with POAG who met the criteria were included and divided into those with mild central VF damage (mild group) and those with advanced central VF damage (more severe group). The mild group had the mean of the TDs at the four central test points of the HFA 24-2 program (TD4 test points) of −4 dB or higher, and the more severe group had a TD4 test points less than −4 dB. The mild group included 93 eyes of 93 patients whose age, MD, and TD4 test points averaged 58.3 years, −3.0 dB, and −0.8 dB, respectively (Table 1). The more severe group included 60 eyes of 60 patients whose age, MD, and TD4 test points averaged 58.3 years, −3.0 dB, and −0.8 dB, respectively (Table 2). A simple regression analysis between GCC4 test points (GCIPL4 test points) (μm) and LSAP4 test points (1/Lambert) without adjustment for other confounding factors yielded regression coefficient (slope) of 0.007 ± 0.002 (P = 0.001) in the former group and the difference in the regression coefficient between the former and latter groups was 0.011 ± 0.004 (P = 0.011) with the R² value of 0.399.

The results of the main multiple regression analysis are summarized in Tables 3 and 4. Multicolinearity among the explanatory variables was judged to be negligible. For the GCC4 test points (μm), the LSAP4 test points (1/Lambert) were correlated significantly with partial regression coefficient of 0.007 ± 0.002 (P = 0.002). The difference in the partial regression coefficient for the LSAP4 test points between the mild and more severe groups (A3) was 0.012 ± 0.004 (P = 0.008).
Confounding factors, the slope ($C_0 \cdot A_x$) was plotted against the mean thickness of the ganglion cell–inner plexiform layer (GCIPL) in a 0.9-mm-diameter circular retinal area corresponding to the four central test points using the HFA 24-2 program after correcting for RGC displacement.

For the GCIPL$_T_4$ test points (µm), the LSAP$_4$ test points (1/Lambert) was correlated significantly with partial regression coefficients of 0.005 ± 0.002 (SE) ($P = 0.005$) and the difference in the partial regression coefficient for the LSAP$_4$ test points between the early and progressed groups ($A_2$) was 0.005 ± 0.003 ($P = 0.078$). That is, when GCIPL$_T_4$ test points (µm) was plotted against the LSAP$_4$ test points (1/Lambert) after adjustment for other confounding factors, the slope ($\Delta$ GCIPL$_T_4$ test points/$\Delta$ LSAP$_4$ test points) tended to be approximately two times greater in the more advanced than in the mild group (0.010 ± 0.005 ± 0.005 vs. 0.005). Further, the axial length and disc size was negatively ($P = 0.052$ and $P = 0.043$) correlated with the GCIPL$_T_4$ test points, respectively. The $R^2$ value for regression was 0.347.

Supplementary analyses examined the relationship between the GCCT (GCIPL) and LSAP at two test points in the upper (lower) hemifield or at each of the four central test points using the same equation. In these analyses, retinal areas with mild VF damage were defined as those with the mean or individual total deviation value (TD) of −4.0 dB or higher, and retinal areas with more severe VF damage as those with the mean or individual TD value less than −4.0 dB.

Hemifield-based analyses revealed the following results. At the retinal area corresponding to the two central test points in the upper hemifield, 90 eyes (90 cases) had mild central VF damage (mean TD ≥ −4 dB) and 65 eyes (65 cases) advanced central VF damage (mean TD < −4 dB). The LSAP was correlated significantly with the GCCT (GCIPL) with a partial regression coefficient of 0.008 ± 0.003 (0.005 ± 0.002) ($P < 0.008$) and the difference in the partial regression coefficient between the both groups was 0.015 ± 0.006 (0.006 ± 0.0061), respectively ($P = 0.002$ and 0.133). At the retinal areas corresponding to the two central test points in the lower hemifield, 136 eyes (136 cases) had mild central VF damage (mean TD ≥ −4 dB) and 17 eyes (17 cases) advanced central VF damage (mean TD < −4 dB). The partial regression coefficient of the LSAP for the GCCT (GCIPL) was 0.009 ± 0.002 (0.006 ± 0.002) ($P < 0.001$), whereas the differences in the partial regression coefficient between the both groups were 0.008 ± 0.011 (0.004 ± 0.009) ($P > 0.500$).

Pointwise analyses revealed the following results. At the retinal area corresponding to the superotemporal test point, the LSAP was correlated significantly with the GCCT (GCIPL) with a partial regression coefficient of 0.012 ± 0.003 (0.008 ± 0.002) ($P < 0.001$) and the difference in the partial regression coefficient between the group with TD ≥ −4.0 dB ($n = 88$) and...
that TD < −4.0 dB (n = 65) group was 0.050 ± 0.016 (0.032 ± 0.011) (P < 0.006). At the retinal areas corresponding to the inferior and superior nasal and inferior temporal test points, the numbers of eyes with TD ≥ −4.0 dB and those with TD < −4.0 dB were 121 to 143 and 10 to 32 of 153, respectively. The partial regression coefficient of the LSAP for the GCC (GCCPLT) was 0.007 to approximately 0.009 (0.005 ~ 0.007) (P < 0.002), respectively, whereas the differences in the partial regression coefficient between the both groups were 0.009 to approximately 0.037 (0.001 ~ 0.009) (P = 0.147 ~ 0.792), respectively.

**DISCUSSION**

Greenfield et al. first reported that the MD values were correlated significantly with the mean macular thickness measurements obtained using time-domain OCT in eyes with moderately advanced glaucoma. The GCCP and GCC thicknesses also were reported to be correlated with the global or corresponding regional SAP sensitivity (in dB or the linear scale) in groups with only glaucomatous eyes or groups with glaucomatous and normal eyes. Although correlation between cpRNFL, GCC, or GCIPL thickness and retinal sensitivity was the subject of many previous studies, to the best of our knowledge, the current study is the first to report a significant difference in the relationship between the GCC (GCCPLT) thickness and SAP linear scale sensitivity in corresponding macular regions between eyes with mild and advanced central VF. That is, (ΔGCC (GCCPLT) test points/Δ LSAP (test points)) was approximately 2.7 (2.2) times greater, or given a same reduction in the retinal sensitivity, reduction in RGC-related inner retinal layer thickness will be approximately 2.5 times greater, in the macular area with advanced glaucomatous damage than in that with mild glaucomatous damage. Because many clinicians are focusing their attention to functional and structural test results obtained from the macular area of glaucoma eyes, we suppose that the current result would be clinically interesting. The higher significance (P = 0.008 vs. P = 0.078) for the intergroup difference in the GCC/LASP relationship than that in the GCIPLSAP relationship is probably attributable to the fact that the GCC included the macular RNFL thickness, in which a change parallels that in the GCIPL. Using another SD-OCT instrument, Cho et al. plotted the average GCC thickness against the mean sensitivity over the entire HFA 24-2 test field in the linear scale in glaucomatous eyes with a mean MD of −7.0 dB. If the average GCC thickness/mean sensitivity in the linear scale is approximated from the figure they presented, the value of approximately 0.025 will be obtained, which is not far from that currently obtained for (ΔGCC (GCCPLT) test points/ΔLSAP (test points)) after adjustment for other confounding factors, 0.019, in the current POAG eyes with advanced central VF damage with a mean MD of −9.8 dB.

That finding also could be reproduced by the upper hemifield-based or pointwise analysis at the retinal area corresponding to the superotemporal test point from the central four test points. However, in the lower hemifield or at retinal areas corresponding to the other central four test points, the difference in ΔGCC (GCCPLT)/ΔLASP between the eyes with mild and advanced central glaucomatous damage did not reach significance. In these cases, only 10 to 32 of the 153 eyes were classified into the group with advanced central glaucomatous damage, probably because the central four test points, especially those in the lower hemifield, are more likely to be spared until the very late stage of glaucoma. This small sample size in one arm may have substantially lowered the statistical power to detect an intergroup difference.

The difference between the current eyes with mild and advanced central glaucomatous damage may indicate that the relationship between GCC/GCIPLT or the number of RGCs and the perceived sensitivity at the corresponding retinal area is affected by the extent of the glaucomatous damage. Ajtony et al. reported that correlation between cpRNFL thickness and MD value in POAG eyes fit a curvilinear regression model rather than a linear regression model, which agrees with the current results obtained in the macular area. One possible explanation may be plasticity in the visual cortex or normal cerebral adaptation to chronic modifications in the visual input caused by slowly progressing glaucomatous damage.

In the current mild group, the mean sensitivity of the central four test points in the dB scale averaged 32.1 dB, whereas it was 32.9 dB in normal eyes in our previous report. The corresponding GCCPLT test points (GCCPLT test points) in the mild group was 106.8 (79.0) μm, and that in the normal eyes calculated under the same conditions was 121.5 (90.3) μm. The intergroup difference in GCCPLT test points (GCCPLT test points), 14.7 (11.3) μm, was highly significant (P < 0.001) after adjustment for the intergroup differences in age, axial length, and SAP sensitivity, corresponding to approximately 35% of the dynamic range of the GCCPLT test points (GCCPLT test points) we approximated previously. Further, it is obvious that the (ΔGCCPLT test points (GCCPLT test points)/Δ LSAP (test points)) value of the current mild group, 7 (5) μm/1000 (1/Lambert), cannot explain the above difference in the GCCPLT test points (GCCPLT test points), 14.7 (11.3) μm, and the difference in the LSAP (test points), (2064 ~ 1625 ≈ 440 [1/Lambert]), seems to be compatible with the (ΔGCCPLT test points (GCCPLT test points)/Δ LSAP (test points)) value of the more severe group, 19 (11) μm/1000 (1/Lambert).}

In normal eyes, the GCCPLT test points (GCCPLT test points) was correlated strongly with age, whereas age was not correlated significantly in the current eyes. The effect of glaucoma on the GCC/LASP test points (GCCPLT test points) may be much stronger than that of physiologic aging in eyes with POAG. In POAG eyes, the GCCPLT test points was negatively correlated with a partial regression coefficient of approximately 1.2 μm/mm to axial length, which agreed with the value reported by Mwanza et al. in normal eyes, approximately 1.0 μm/mm. These findings suggested that the effect of refraction must be corrected when studying the relationship between the RGC-related retinal layer thickness and SAP sensitivity in both normal and POAG eyes. Disc size also was negatively correlated only with the GCCPLT test points in the current POAG eyes. Mwanza et al. reported that the GCIPLT was not correlated with disc size in normal eyes. Further, the relationship between the disc size and glaucomatous damage remains controversial. Thus, it seems difficult to discuss the pathophysiologic relationship between the GCIPLT and disc size in POAG eyes based on the current results, and this issue deserves future study. The possibility of a cohort effect in this study may not be excluded.
The current study had several limitations, the first being its cross-sectional design. Disease stage-dependent differences, if they exist, should be investigated in a longitudinal study. Second, we could not include a large number of POAG eyes with very advanced regional damage, because it was difficult to recruit enough patients with a dense central scotoma who met the inclusion criteria. The structure/function relationship in very advanced POAG awaits future studies. Third, we assumed that there was a linear relationship between the GCC test points (GCCPLT test points) and SAP sensitivity in the linear scale. However, the current results suggested that the value of AGCCT (AGCIPLT/ALSAP) may be even greater in eyes with very advanced POAG, and fitting to an exponential curve may have to be considered. As discussed above, however, the value of (AGCCT test points [AGCIPLT test points]/ALSAP test points) derived from current multiple linear regression analysis could reasonably explain the difference in GCC test points (GCCPLT test points) and LSAP test points between the mild and more severe POAG group. This result may indicate that the linear regression model was an acceptable approximation as far as the current subjects were concerned. Forth, structure-function relationship in the macular area should be better studied by adopting HFA 10-2 test results. What was reported in the current communication is the relationship at the most central four test points of HFA 24-2 test program. Future studies using HAF 10-2 test results should yield more information on the disease stage-dependent difference in the structure-function relationship in the macular area.

In summary, we found that the relationship between the GCC (GCPIPL) thickness in retinal areas corresponding to the central four test points of the HFA 24-2 program and SAP sensitivity in the linear scale at these points differed between the eyes with mild central VF damage and those with advanced central VF damage from POAG. This finding suggested that not only the number or density of RGCS but also other local or central nervous system factors relating to perceived visual sensitivity may be modified along with progression of POAG from its early stage to the moderately advanced stage.

Acknowledgments

Supported by Grants-in-Aid for Scientific Research by the Ministry of Health, Labor and Welfare of Japan (H18-Sensory-General-001).

Disclosure: M. Araie, Alcon Japan (C, R), Allergan (C), Bosch-Lomb (C), Carl Zeiss Meditec (R), Kowa (C, R), Otsuka (R), Pfizer Japan (C), Santen (C, R), Senju (C, R), Topcon (C, R) P; H. Murata, None; A. Iwase, Alcon Japan (R), Carl Zeiss Meditec (R), Kowa (R), Otsuka (R), Pfizer Japan (R), Santen (R), Senju (R), Topcon (R), P; M. Hangai, Nidek (C, F, R); K. Sugiyama, None; N. Yoshimura, Nidek (C, F, R).

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