Retinal sensitivity and ganglion cell-related retinal layer thickness in the normal aging process

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Abstract

Aging-associated changes in visual field (VF) sensitivity were compared prospectively and longitudinally with the circumpapillary retinal nerve fiber layer thickness (cpRNFLT) and macular ganglion cell-inner plexiform layer thickness (GCIPLT) changes in the corresponding retinal areas of the same eyes (72 eyes of 37 normal Japanese subjects; mean age, 51.3 years). The Humphrey Field Analyzer 24-2 test (HFA 24-2) and spectral-domain optical coherence tomography (SD-OCT) measurements of the cpRNFLT and GCIPLT in a 0.6-mm-diameter circle corresponding to the four central points of HFA 24-2 adjusted for retinal ganglion cell displacement ($GCIPLT_{4TestPoints}$) were performed every 3 months for 3 years. The tiem changes of the mean sensitivity over the entire field (VF_{mean}) and the four central points ($VF_{4TestPoints}$), cpRNFLT, and $GCIPLT_{4TestPoints}$ were analyzed using a linear mixed model. The aging-associated decline rates of VF_{mean} and $VF_{4TestPoins}$ were 0.12 and 0.19 decibels/year (p<0.001), which significantly accelerated with increased subjects' age (0.009 and 0.010 decibels/year, p<0.001, respectively) without changes in the ocular media. Those of the CpRNFLT and $GCIPLT_{4TestPoints}$ were not significant in both (p>0.114), but significantly accelerated with increased subjects' age (0.021 and 0.010 μm/year, p=0.001 and 0.004, respectively). These results have implications in studying physiological aging- or desease-related changes in these parameters.

Histologic studies in human eyes have estimated that about 7,000 retinal ganglion cells (RGCs) are lost during normal aging.[1-5] In accordance with these histologic findings, previous cross-sectional or longitudinal studies in normal human eyes have shown that visual field (VF) sensitivity measured using standard automated perimetry (SAP) [6-9] and the thickness of the RGC-related retinal layers determined by spectral-domain optical coherence tomography (SD-OCT) showed aging-associated declines.[10-19] Previous studies of glaucomatous eyes with early or pre-perimetric damage have reported that changes in the circumpapillary retinal nerve fiber layer thickness (cpRNFLT) measured by SD-OCT generally preceded glaucomatous changes in the VF sensitivity detected using SAP standard test programs such as the Humphrey Field Analyze 24-2 Swedish Interactive Thresholding Algorithm (HFA-24, Carl Zeiss Meditec, Dublin, CA).[20-23] Both glaucoma and the normal aging process reduce the thickness of the RGC-related retinal layers measured using SD-OCT and VF sensitivity measured by SAP.[6-19] However, the comparisons of the aging-associated declines measured by the two tests have not been reported in the same normal subjects' eyes, while comparisons of declines in the two tests in the same glaucomatous eyes have been studied.[20-23] In the current study, we performed prospective measurements of the longitudinal time changes in the cpRNFLT and macular ganglion cell-inner plexiform layer thickness (GCIPLT) and the SAP sensitivity in the corresponding retinal areas in the same normal eyes.

Results

A total of 76 eyes of 38 normal subjects were enrolled; two eyes of one subject and one eye of one subject were excluded because of development of small retinal hemorrhages of unknown origin and epiretinal membrane or vitreoretinal traction, respectively, during follow-up, and one eye of one subject was excluded because reliable SD-OCT measurements could not always be obtained mainly due to saccades and blinking. The demographics of the remaining 72 eyes of 37 subjects are shown in Table 1. Inter-visit test-retest variability calculated as the minimal detectable change (MDC) (MDC = 1.96 $\times\sqrt{2}\times$standard error of measurements) [24] was 3.3 μm for the cpRNFLT and 1.6 μm for the mean of the GCIPLT in a circular retinal area with a diameter of 0.6 mm (approximately 2 degrees of visual angle) corresponding to the four central test points of the HFA 24-2, adjusted for RGC displacement [25] ($GCIPLT_{4TestPoints}$), respectively.

During the 3-year prospective follow-up, ocular transparent media including lens showed no changes on biomicroscopic examination. The mean sensitivity over the entire field (VF_{mean}) and that of the four central test points of the HFA 24-2 ($VF_{4TestPoints}$) of an eye with average parametric values of this cohort (Table 1) showed significantly negative longitudinal time changes (agin-associated declines) of -0.12 decibels [dB]/year (p<0.001) and -0.19 dB/year (p<0.001), respectively, which were significantly more negative by -0.009 and -0.010 dB/year with older baseline ages (P<0.001), and by -0.11 and -0.14 dB/dB with higher baseline sensitivities (P<0.001), respectively. Further,

the VF_{mean} and $VF_{4TestPoints}$ measurement results themselves were also significantly lower by 0.021 and 0.028 dB/year with older baseline age (p<0.001), and higher by 0.60 and 0.43 dB/dB with higher baseline sensitivity, respectively (Tables 2 and 3).

On the other hand cpRNFLT and the mean of the GCIPLT in a circular retinal area with a diameter of 0.6 mm (approximately 2 degrees of visual angle) corresponding to the four central test points of the HFA 24-2, adjusted for RGC displacement [25], $GCIPLT_{4TestPoints}$, of an eye with average parametric values of this cohort (Table 1) showed no significant longitudinal time changes (aging-rassociated declines) (p=0.352 and 0.114, respectively); however, the aging-related declines of cpRNFLT and $GCIPLT_{4TestPoints}$ were significantly more greater by 0.021 and 0.010 μm/year with older baseline age (p=0.001 and 0.004, respectively). The cpRNFLT and $GCIPLT_{4TestPoints}$ measurement results themselves were also significantly thinner by 0.025 μm with older baseline age (p<0.001) in both, by 0.15 and 0.09 μm with longer axial length (AL) (p=0.030 and 0.019, respectivly), and by 0.62 and 0.19 $\mu m/mm^2$ with greater disc area (p<0.001 and P=0.048, respectivly) (Tables 4 and 5).

In the current study, the longitudinal aging-associated declines in the VF sensitivity and SD-OCT-measured thicknesses of the RGC-related retinal layers in the corresponding area were measured in the same eyes of normal Japanese subjects (mean age, 51 years). The longitudinal aging-related decline rates of the VF_{mean}, the mean sensitivity of the all test points of HFA 24-2, and $VF_{4TestPoints}$, the mean sensitivity of the four

central test points of HFA 24-2, were -0.12 and -0.19 dB/year, respectively, which were significantly more negative along with older baseline age and higher VF sensitivity. This aging-associated declines in the VF sensitivity currently estimated from longitudinal VF measurement results from normal subjects were comparable to those estimated by previous cross-sectional studies (-0.1 ~-0.2 dB/year). [6-9] The current study further confirmed significant acceleration of the aging-associated decline rates in the VF sensitivity previously observed only in cross-sectional datasets [7-9] by longitudinal observation. Since no significant changes were seen in the ocular media including lens in the current subjects during the prospective follow-up period, acceleration of aging-associated declines in the VF sensitivity currently observed would be mainly attributed to post-retinal neuronal factors rather than pre-retinal ones.

The aging-associated changes in the thickness of the RGC-related retinal layers in the retinal area corresponding to the VF_{mean} and $VF_{4TestPoints}$ measurement sites adjusted for RGC displacement [25], i.e., the cpRNFLT and $GCIPLT_{4TestPoints}$, respectively, showed the same trends as each other; the longitudinal aging-associated decline rates during follow-up (coefficients for duration) were not significant, but they were significantly more negative by -0.01 ~ 0.02 μm/year along with older subjects' age (coefficients for interaction between the baseline age and duration were significantly negative), being compatible with increases aging-associated decline in the VF sensitivity along with older subjects' age observed in the same eyes. The baseline age, AL, and disc area had significantly negative effects on the cpRNFLT and $GCIPLT_{4TestPoints}$

126 measurement results themselves, which agreed with previous cross-sectional SD-OCT
127 study results for the cpRNFLT and age [10,13,14], AL [14], and disc area [10] and for
128 GCIPLT and age [11,12,15] and AL,[11,15] suggestinng little differences in the SD-OCT
129 characteristics between the current normal subjects and those in previous studies.
130 Leung et al. followed 70 eyes of 35 normal subjects every 4 months for 30 months
131 using Cirrus HD-OCT (Carl Zeiss Meditec), and reported a significant longitudinal
132 aging-associated decline in the cpRNFLT of −0.52 μm/year,[16]; they also followed 72
133 eyes of 40 normal subjects every 4 months for a mean of 40 months and reported a
134 significant aging-associated decline of the macular GCIPLT of −0.32 μm/year.[17]
135 Vianna et al. followed 37 eyes of 37 normal subject every 6 months for a median of 4.5
136 years using Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) and
137 reported significant longitudinal aging-associated decline in the cpRNFLT
138 −0.44μm/year.[18] Hammel et al. measured the aging-related decreases in the
139 cpRNFLT and macular GCIPLT in 28 normal subjects using Cirrus HD-OCT for a median
140 of 1.7 years with 6 visits and also reported a significant longitudinal aging-associated
141 decline in the cpRNFLT of −0.48 μm/year but not in the macular GCIPLT.[19] In the
142 current study, we used the 3D-OCT 2000 (Topcon) and measured the cpRNFLT and
143 GCIPLT corresponding to the central four test points of the HFA 24-2 ($GCIPLT_{4TestPoints}$)
144 every 3 months for 3 years in 72 eyes of 37 normal Japanese subjects and could not
145 detect significant longitudinal aging-associated changes in these parameters. This
146 finding was rather unexpected, since both the cpRNFLT and $GCIPLT_{4TestPoints}$

147 measurement results themselves were significantly and negatively correlated with the
148 subjects' ages in the current cohort (coefficients for age at baseline were significantly
149 negative). To re-confirm that time changes in the cpRNFLT and $GCIPLT_{4TestPoints}$ during
150 the follow-up period were not significant, we conducted simple linear regression
151 analyses using only duration (time lapse after entry) as an explanatory variable, i.e.,
152 time changes in the cpRNFLT or $GCIPLT_{4TestPoints}$ = A× duration + B. The coefficient for
153 duration, A, was calculated to be 0.0042 μm/year (p=0.470) and p=0.0024 μm/year
154 (p=0.538) for the cpRNFLT and $GCIPLT_{4TestPoints}$, respectively.
155 Larger test-retest variability, fewer measurements, and a shorter follow-up period
156 should reduce the power to detect a significant trend. The inter-session test-retest
157 variability values of the measurement results with the current SD-OCT instrument were
158 3.3 and 1.6 μm, respectively, for the minimum detectable change in the cpRNFLT and
159 $GCIPLT_{4TestPoints}$, which were favorably compared with those reported in the literature
160 for measurement results with other SD-OCT instruments.[24,30] The follow-up period
161 and interval in the current study were similar to (3 years vs. 1.7 ~4.5 years,
162 respectively) and shorter than (3 months vs. 3.5 ~ 6 months, respectively) those
163 adopted by previous studies.[16-19] Thus, it seems rather unlikely that a shorter
164 follow-up period and fewer measurements mainly accounted for an undetected
165 significant trend in longitudinal decline in the cpRNFLT and $GCIPLT_{4TestPoints}$ in the
166 current subjects.
167 The current study indicated that in older subjects, the longitudinal aging-associated

declines in the cpRNFLT and $GCIPLT_{4TestPoints}$ were significantly more negative (coefficients for interaction between the baseline age and duration were significantly negative) by -0.02 and -0.01 μm/year, respectively, suggesting that in subjects somewhat older than the current ones with mean age of 51 years, the longitudinal aging-associated change rates of the cpRNFLT and $GCIPLT_{4TestPoints}$ would be sufficiently negative to be detected. For example, at age 65 years (14 years older than the mean age of the current subjects), a longitudinal aging-associated decline rate of the cpRNFLT ($GCIPLT_{4TestPoints}$) would be 0.067 - 0.02×14=-0.21 μm/year (-0.064 – 0.01×14=-0.20 μm/year). Assuming similar variations in the cpRNFLT and $GCIPLT_{4TestPoints}$ measurement results, i.e., similar standard error values for estimates, and the same values for other explanatory variables, a coefficient for duration of -0.15 μm/year would be significantly different from zero, i.e., a significant decline rate of -0.15 μm/year would be obtained for the cpRNFLT or $GCIPLT_{4TestPoints}$. Thus, it is likely that subjects older than the current ones could show significant longitudinal aging-related declines in the cpRNFLT and $GCIPLT_{4TestPoints}$. The somewhat younger ages of subjects in the current study than in the previous studies by Leung et al.[16,17] and Vianna et al.[18] (51 vs. 56, 58 and 65 years, respectively) might be partly responsible for the discrepancy between the current and their results. Relative rates of aging-associated declines in the cpRNFLT were reportedly less than predicted by aging-associated decline in the number of RGCs, which was attributed to the presence of differential aging-associated declines of the non-neuronal components in the

RNFL.[31,32] A racial difference in the cpRNFLT has been reported [13,14] and a comparison of the BMO-MRW and cpRNFLT between normal Japanese and Caucasians subjects suggested a difference in the ratio of the amount of RGC axons to that of the non-neuronal components in the RNFL between them.[33] If there are racial differences in the amount of aging-associated changes in the non-neuronal component between Japanese subjects and those of other races, this might partly explain the discrepancy between the current result and those of Leung et al, Vianna et al., and Hammel et al.[16-19]. Not only aging-associated changes in the retinal neurons but also those in the neurons in the central visual pathway should be related to a physiologic aging-associated decline in the perceived VF sensitivity in a specific retinal area. Since the GCIPLT or cpRNFLT in a specific retinal area is related to the number of RGCs or RGC axons but not neurons in the central visual pathway, the physiologic aging processes at age 50 years might be reflected with more sensitivity in the perceived VF sensitivity decline than in the thickness of the RGC-related retinal layers in the corresponding retinal area. A significant aging-associaed decline in the perceived VF sensitivity not associated with a significant aging-associated decline in the cpRNFLT or macular GCIPLT in the corresponding area currently observed in norma Japanese eyes might be at least partly explained as above. On the other hand, glaucomatous damage primarily affects the RGCs and their axons and secondarily the neurons in the central visual pathway. In this case, the perceived SAP sensitivity decline may be dampened by the plasticity of the visual cortex and normal cerebral adaptation to chronic

deterioration in the visual input caused by slowly progressing glaucomatous RGC damage.[34]

Whatever the causes of the discrepancy in the aging-associated decline rates in the cpRNFLT or macular GCIPLT between the current and previous longitudinal studies, the current finding that a significant acceleration of aging-associated decline rate was detected not only in the VF sensitivity, but also in the cpRNFLT and macular GCIPLT is interesting. Acceleratoin of aging-associated decline rates of cpRNFLT and macular GCIPLT along with increase in subjects' age is compatible with, and may be one of the post-retinal neural factors for acceleration of ageing-associated decline rates of VF sensitivity currently found and previously reported in cross-sectional datasets [7-9] Further, it underlines again that an age-matched normal control group must be always provided in studying diseases-caused thinning of cpRNFLT or macular GCIPLT, since physiological aging-associated thinning rates of these RGC-related retinal layers thicknesses were dependent on subjects' age.

The current study had several limitations. First, the number of normal subjects was small and the follow-up period short, which would result in a lower statistical power to detect significant time changes in the RGC-related retinal layer thickness measured using a current SD-OCT instrument. As discussed previously, however, the cpRNFLT or GCIPLT time change rates of about 0.3~0.5 μm/year have been reported in previous studies with a similar number of normal subjects and follow-up periods,[16-19] and the test-retest variation of the current SD-OCT measurement results was thought to be

231 reasonably satisfactory based on modern technology. These results suggested that the
232 statistical power to detect the time change rates of the cpRNFLT or GCIPLT in the
233 current normal subjects should be comparable to those of previous studies that
234 detected significant aging-related declines of the cpRNFLT or macular GCIPLT.[16-19]
235 The mean age of the current normal Japanese subjects was about 50 years and as
236 discussed previously, the physiologic aging-related decline of the cpRNFLT and
237 $GCIPLT_{4Testpoints}$ were calculated to be about 0.2 μm/year at age 65 years, which could
238 be detected under the same study conditions. Thus, it should be noted that the current
239 results were applicable in Japanese subjects aged around 50 years.
240 In summary, acceleration of aging-associated decline rates of VF sensitivity
241 associated with acceleration of aging-associated decline rate or cpRNFLT or macular
242 GCIPLT in the corresponding retinal area along with increased subjects' age may have
243 implications for studying not only the physiologic aging-related changes in visual
244 function, but also the diseases-caused rates of thinning in the cpRNFLT or macular
245 GCIPLT. Further, the physiologic aging processes was more evidently found in the
246 declines in the HFA-measured VF sensitivity than in the cpRNFLT or macular GCIPLT in
247 corresponding retinal areas measured using a current SD-OCT instrument in norma
248 Japanese aged around 50 years.
249
250 **Methods**
251 **Subjects.** Self-reported healthy Japanese individuals were recruited at the Tajimi Eye

252 Clinic (Tajimi, Gifu, Japan). After subjects were screened verbally and the medical
253 histories recorded, an ocular examination was performed that included measurements
254 of the uncorrected and autorefraction-corrected visual acuity (VA) with the Landolt
255 chart at 5 meters and the corneal curvature using an autorefractometer (KR-800A,
256 Topcon, Tokyo, Japan). In addition, the central corneal thickness and AL were measured,
257 respectively, using a specular microscope (SP-3000P, Topcon) and the IOLMaster (Carl
258 Zeiss Meditec). The VFs were examined using SAP (HFA 24-2 SITA program, Carl Zeiss
259 Meditec). The VF examination was repeated if it was considered unreliable or outside
260 the normal limits. The SD-OCT examination was followed by dilated optic disc stereo
261 photography and fundus photography, dilated funduscopy, slit-lamp biomicroscopy,
262 and intraocular pressure (IOP) measurements by Goldmann applanation tonometry. A
263 pair of sequential stereoscopic optic nerve head photographs at a parallax of about 8
264 degrees (30-degree angle of view) and non-stereoscopic fundus photographs
265 (45-degree angle of view) was obtained using a digital fundus camera (TRC-NW7,
266 Topcon) after pupillary dilation with 1.0% tropicamide. All ocular examinations were
267 performed bilaterally.
268 The inclusion criteria were age between 20 and 80 years; normal eye examinations
269 without any clinically significant cataract, ocular media, vitreoretinal, or choroidal
270 abnormalities; IOP of 21 mmHg or lower; best-corrected decimal VA of 1.0 or higher;
271 spherical refraction of $\pm$ 6 diopters (D) or less; astigmatism of 2 D or less; AL of 26 mm
272 or less; no previous ocular surgeries; normal VF test results with the glaucoma

hemifield test, and mean deviation and pattern standard deviation within normal limits. Subjects were excluded if the VF results were unreliable based on the perimetrist's notes and reliability indices with fixation loss and false positive rates of over 20% and over 15%, respectively; the optic disc stereo photographs were of insufficient quality; or the OCT images were of insufficient quality (typically truncated B-scans and scans with a manufacturer-authorized image quality score of 30 or lower). After enrollment, routine ophthalmic examinations, SD-OCT and VF measurements were performed every 3 months for 3 years.

The Review Board and Ethics Committee of Gifu Prefecture Medical Association approved the study (reference number, 25-1-001), which adhered to the tenets of the Declaration of Helsinki. The study was registered in the University Hospital Medical Information Network Clinical Trial Registry (UMIN-000012412). Each subjects provided written informed consent after they received a full explanation of the study protocol.

SD-OCT. SD-OCT data sets were obtained using a 3D-OCT 2000 (Topcon) with the horizontal 3-dimensional (3D) scan protocol in which data were obtained from 6.0 × 6.0-mm-square areas (512 A-scans × 128 frames) centered on the disc and with the vertical 3D scan protocol in which data were obtained from a 7.0 x 7.0-mm-square area (512 A-scans × 128 frames) centered on the fovea over a period of about 1.5 seconds for each scan. The magnification was corrected according to the manufacturer-provided formula that was based on refractive error, corneal radius, and

AL.[26] The data obtained in the presence of eye movements were discarded and the examination was repeated. Images also were excluded if they 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 had an image quality score of 30 or less. OCT measurements were repeated 3 times with several-second intervals, and the image with the best quality was used. The disc barycenter was determined using the raster-scan data, and the fovea identified in the OCT image as the pixel that was thinnest between the inner limiting membrane and the photoreceptor inner/outer segment junction adjacent to the fixation point. The RNFL and GCIPL were segmented automatically in all B-scan images.[27] To minimize variability due to misplacement of the measurement location and/or segmentation error, an experienced researcher (T.K.) checked the locations of the disc barycenter and fovea and all layer segmentation in all images. The cpRNFLT was measured along the 3.4-mm-diameter circle centered on the disc center. The macular GCIPLT in a 0.6-mm-diameter circular retinal area (corresponding to about 2 degrees of visual angle) corresponding to each of the four central test points of the HFA 24-2 and adjusted for the RGC displacement [25] was sequentially obtained and the mean of these 4 measurement results were obtained ($GCIPLT_{4TestPoints}$). The diameter of the retinal area (2 degrees) was similar to the grid size of the HFA 10-2 test program and roughly twice as large as the size-III stimulus point movements during fixation, such as drift.

Inter-visit test-retest variabilities of the cpRNFLT and $GCIPLT_{4TestPoints}$ measurements

315 with the current instrument were calculated using the two measurements obtained at
316 enrollment during two separate sessions.
317
318 **Data analysis.** The results are expressed as the mean (standard deviation). The effects
319 of aging (duration or time lapse from the baseline measurement) on the cpRNFLT,
320 mean VF sensitivity (dB) on the HFA 24-2 VF (VF_{mean}), $GCIPLT_{4TestPoints}$, and the mean VF
321 sensitivity of the central 4 test points of the HFA 24-2 ($VF_{4TestPoints}$) were analyzed, using
322 the time changes of the cpRNFLT, $VF_{mean,}$ $GCIPLT_{4TestPoints}$, or $VF_{4TestPoints}$ values during
323 follow-up as dependent variables and using the linear mixed model, which considers
324 correlations between the paired eyes and measurement values from the same eyes.
325 The explanatory variables were duration (time lapse from the baseline measurement),
326 baseline age, baseline VF_{mean} or $VF_{4TestPoints}$, and AL [28,29] for the time changes of the
327 VF_{mean} and $VF_{4TestPoints}$, respectively. For the time changes of the cpRNFLT or
328 $GCIPLT_{4TestPoints}$, the variables were duration (time lapse from the baseline
329 measurement), baseline age, baseline cpRNFLT or $GCIPLT_{4TestPoints}$, AL [11,15], and disc
330 area [10,16,17], respectively. The JMP® Pro 13 software (SAS Institute Inc., Cary, NC,
331 USA) was used for analysis and $p < 0.05$ was considered statistically significant.
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Data Availability Statement

Datasets generated and/or analyzed during the current study are available from the the orresponding author on reasonable request.

Author Contributions

M.A., A.I. and Y.T. designed the current study, A.I. collected the data, M.A., A.I. and Y.T. analyzed the data, M.F. and Y.O. carried out statistical calculation, and M.A. and A. I. drafted the manuscript. All authors reviewed the manuscript.

Additional Information

Competing financial interests: Makoto Araie holds a patent licensed to Topcon without any royalties, is a consultant of Santen and Topcon. Makoto Fujii and Yuko Ohno declare no competing financial interests. Yuki Tanala is an employee of Santen, and Tsutomu Kikawa is an employee of Topcon. Aiko Iwase holds a patent licensed to Topcon without any royalties, is a consultant of Santen and has received speaker honoraria from Carl Zeiss Meditec and Santen. All authors attest that they meet the current ICMJE requirements to qualify as authors.