Disc hemorrhage (DH) is considered to be an important sign of structural glaucomatous damage and has been identified as an important risk factor for progression of glaucoma in numerous studies.1–5 Our group has previously reported that DH could be a strong risk factor for progression, particularly in the early stage of open-angle glaucoma (OAG) and normal tension glaucoma, along with inadequate reduction in intraocular pressure.6,7 However, to date, progression of glaucoma has been evaluated by conventional visual field (VF) testing or by deepening or widening of a circumpapillary retinal nerve fiber layer (cpRNFL) defect using conventional red-free photography or optical coherence tomography (OCT).1,2,5,8–13 Therefore, in the past, it was only possible to evaluate the relationship between DH and progression of glaucoma using VF or cpRNFL parameters. Currently, the macular inner retinal structures, including parameters related to the ganglion cell-inner plexiform layer (GCIPL), can be used to evaluate the status of glaucoma in the clinical setting. Furthermore, many studies have shown the performance of GCIPL parameters to be comparable with or better than that of cpRNFL parameters in the diagnosis of glaucoma.14–17 One group reported that eyes with DH showed more rapid retinal ganglion cell (RGC) loss than eyes without DH on the basis of global RGC counts estimated using a model developed by Medeiros and colleagues18,19 from standard automated perimetry (SAP) and OCT. However, there are still no reports of GCIPL parameters being used to monitor progression of glaucoma or to evaluate the relationship between DH and progression of GCIPL.

The purpose of this study was to evaluate the longitudinal rate of change in GCIPL thickness, as measured by spectral-domain (SD) OCT, in early-stage glaucomatous eyes with DH. Using trend-based analysis, we evaluated the GCIPL thinning rate according to the presence of DH and/or its topographic characteristics.

METHODS
The study protocol was approved by the Institutional Review Board of Seoul National University Hospital and followed the tenets of the Declaration of Helsinki for biomedical research. All patients provided informed consent.
Subjects

This study included 46 patients with early-stage OAG and DH who underwent serial SD-OCT measurements for more than 30 months. All the subjects were already enrolled in the ongoing prospective Macular Ganglion Cell Imaging Study that was started in 2011. Patients who had undergone at least four serial GCIPL OCT measurements were considered suitable for inclusion in linear regression analysis and were consecutively enrolled.

All subjects underwent a complete ophthalmologic examination, including visual acuity tests, assessment of manifest refraction, slit-lamp examination, intraocular pressure measurements using Goldmann application tonometry, gonioscopy, dilated fundus examination, color disc photography and red-free RNFL photography. The topographic patterns and recurrence of DH using digital color stereo disc photographs and red-free RNFL photographs. The topographic characteristics of DH were defined as DH detected only once during follow-up. Recurrent DH was defined when any new DH, regardless of the location, was detected during follow-up. The number of DH recurrences detected during follow-up was recorded, and the characteristics of these recurrences, including the location of recurrent DH in relation to the initial DH, were evaluated.

OCT Imaging

A Cirrus HD-OCT 4000 machine was used to obtain the OCT images. The optic disc cube scan and ganglion cell analysis (GCA) protocol for macular cube scanning (macular cube, 6 × 6 mm², 200 × 200 pixels) were used for diagnosis and follow-up of glaucoma in the clinic.

The optic disc cube scan imaged the optic disc region in an area of 6 × 6 mm² (200 × 200 pixels). The RNFL thickness was measured in each pixel, and an RNFL thickness map was generated. A cpRNFL circle measuring 3.46 mm in diameter and consisting of 256 A-scans was then automatically positioned around the optic disc.

The macular cube scan generated four sets of 128 horizontal B-scans, each comprising 512 A-scans, centered on the 6 × 6 mm² macular region. The built-in GCA algorithm (Cirrus HD-OCT software, version 10.0) detected and measured macular GCIPL thickness within a 6 × 6 × 2-mm cube in an elliptical annulus around the fovea. The GCA algorithm identified the outer boundary of the RNFL as well as the outer boundary of the inner plexiform layer. The difference between the RNFL and inner plexiform layer outer boundary segmentation yielded the combined thickness of the GCIPL. All OCT images located a centered optic disc or fovea were well focused and had a signal strength ≥6.

Calculation of Cirrus HD-OCT GCIPL and cpRNFL Thinning Rates and VF Indices Changing Rates

Linear regression analysis versus time was performed for GCIPL thickness in the global, superior, inferior hemiretina, and six sectors (superior, superotemporal, superonasal, inferior, inferotemporal, and inferonasal) for each eye of each subject in order to determine the rate of change in GCIPL thickness (expressed in micrometers per year), as described previously. The rate of change in RNFL thickness was calculated in the same manner as that used in the global, superior, and inferior regions. Images with a signal strength of <6, images that were out of focus in reference to the fovea or optic disc, and cases of algorithm segmentation failure were excluded from the linear regression analysis.

The rate of change in VF parameters was calculated in the same manner as that used for the MD, PSD, and VF index (VF1). Unreliable VF results were excluded from linear regression analysis.

Determination of Glaucoma Progression

Color disc photographs, red-free RNFL photographs, and VF tests were performed and assessed according to the routine follow-up schedule. The patient’s structural progression status was determined by structural changes on disc and RNFL photography, as described previously. Progressive optic disc changes (i.e., focal or diffuse narrowing, neuroretinal rim notching, increased cup-to-disc ratio, adjacent vasculature...
position shift) were identified by comparing serial photograph-
ic disc images and regarded as progression of glaucoma.
Changes in an RNFL defect were determined using serial RNFL
photographs and defined as the appearance of a new defect or
an increase in width or depth of an existing defect. These
changes were considered to indicate structural progression. 25
Two observers (WJL, JWJ) masked to all other patient
information independently evaluated all photographs. In cases
of disagreement in structural progression, a third glaucoma
specialist (KHP) served as an adjudicator.

Functional progression was determined by event analysis
using the commercial guided progression analysis (GPA)
software provided by the VF device. Functional progression
was confirmed when at least three test points were flagged as
having deteriorated significantly at the same test point
locations in three consecutive fields (the software classifies
VF progression as “likely progression”). 1, 21 These changes also
had to have been observed at the final visit.

Statistical Analyses
All statistical tests were performed using statistics software
(PASW Statistics version 18; SPSS, Inc., Chicago, IL, USA) and
MedCalc (MedCalc Software, Ostend, Belgium). The paired t-
test was used to compare the GCIPL thinning rate between
glaucomatous eyes with DH and fellow eyes without DH. The
independent t-test was used to compare the GCIPL thinning
rate between glaucomatous eyes with DH and glaucomatous
controls without DH. Pearson correlation for continuous
variables, the independent t-test for binary categorical vari-
able, and 1-way analysis of variance for multiple categorical
variables was used to evaluate the association between GCIPL
thinning rate obtained from linear regression and the
characteristics of DH. P values < 0.05 were considered to be
statistically significant. The study data are shown as the mean ± standard deviation.

RESULTS

The study included 46 eyes of 46 patients with early-stage OAG
and DH who fulfilled the inclusion criteria. Of these, 32
patients had DH in one eye and 14 had DH in both eyes. Forty-
ine non-DH eyes of 49 patients with early-stage OAG were
enrolled as the control group without DH.

Clinical Demographics

The clinical demographic characteristics of the patients at
baseline are shown in Table 1. Most of the DHs were located in
the inferior portion (41 eyes, 89.1%). The mean number of DH
recurrences was 1.76 ± 0.90, and recurrent DHs were
observed in 23 eyes (50%). Other topographic features of the
DHs are described in Table 1.

Comparison of Ocular Characteristics Between
Eyes With DH and Contralateral Eyes Without DH

A comparison of the ocular characteristics of the eyes with DH
and fellow eyes without DH is provided in Table S2. There were no significant differences between the
two groups of eyes, except for sex and the GCIPL thickness of
the inferotemporal sector at baseline.

Comparison of GCIPL and RNFL Thinning Rate and
VF Parameters Changing Rate Between Groups

A comparison of the GCIPL and RNFL thinning rate in eyes
with DH and fellow eyes without DH is provided in Table 2. In
the eyes with DH, the GCIPL thinning rate in the inferior
hemiretina, inferotemporal sector, inferior sector, and infer-
onal sector was significantly more rapid than that in the
fellow eyes without DH (−1.07 ± 0.75 vs. −0.78 ± 0.85 μm/yr,
P = 0.001; −1.72 ± 0.90 vs. −1.07 ± 0.85 μm/yr, P = 0.020;
−1.30 ± 0.79 vs. −0.42 ± 0.78 μm/yr, P < 0.001; and −1.16 ±
0.29 ± 0.69 μm/yr, P = 0.039, respectively). A representative
case of a patient with glaucoma and DH and trend-based analysis of the GCIPL thinning rate is shown in
Figure 1. The RNFL thinning rate in the inferior portion was
significantly more rapid in the eyes with DH than in the fellow
eyes without DH (−1.87 ± 1.91 vs. −0.85 ± 1.95 μm/yr, P = 0.033).
The rate of change in VF parameters was not significantly different between the groups.

A comparison of the GCIPL thinning rate in eyes with DH
and control eyes without DH is provided in Table 3. The GCIPL
thinning rate in the global, inferior hemiretina, inferotemporal,
and inferior sectors in the eyes with DH was significantly more
rapid than that in the control eyes without DH (−0.78 ± 0.85
vs. −0.32 ± 0.48 μm/yr, P = 0.002; −1.00 ± 0.94 vs. −0.37 ±
0.67 μm/yr, P < 0.001; −1.31 ± 1.07 vs. −0.34 ± 0.75 μm/yr,
P < 0.001; and −1.29 ± 0.95 vs. −0.43 ± 0.99 μm/yr, P < 0.001,
respectively). The RNFL thinning rate in the inferior portion
was significantly more rapid in the eyes with DH than that in
the control eyes without DH (−2.18 ± 1.99 vs. −1.28 ± 1.38
μm/yr, P = 0.012). The rate of change in VF parameters was not
significantly different between the groups.

A comparison of the GCIPL thinning rate between the
groups is shown in Figure 2. The GCIPL thinning rate in the
global average area was significantly more rapid in the eyes
with DH than in the control eyes without DH (Fig. 2A). The
GCIPL thinning rate was significantly more rapid in the inferior
hemiretina in eyes with DH than in the rates in fellow eyes and
control eyes without DH (Fig. 2B). In the inferotemporal area,
eyes with DH showed significantly more rapid GCIPL thinning
than the fellow eyes and control eyes without DH.

Association of Rate of GCIPL Thinning With
Recurrent and Topographic Characteristics of DH

There was no significant association between GCIPL thinning
rate and recurrence of DH, recurrent DH at 1 clock hour (30
degree) of initial DH location, or recurrent DH at the same
location (Table 4). The association between the topographic
characteristics of the DH and the GCIPL thinning rate was
analyzed and is summarized in Supplementary Tables S3 and
S4. There were no significant association between GCIPL
thinning rate and the topographic characteristics of the DH,
including angular extent, corrected length of maximum radial
extent, number, and proximal location. Only the corrected
DH areas were significantly correlated with the rate of GCIPL
thinning of the global average and inferotemporal sectors (r =
0.366, P = 0.015 and r = 0.298, P = 0.049, respectively). The
rates of GCIPL and RNFL thinning of the affected hemiretina
(corresponding to the DH location) were significantly more
rapid than those of the unaffected hemiretina (−1.06 ± 0.94
μm/yr, P < 0.001, and −2.21 ± 1.93 μm/yr, P = 0.004, respectively; Supplementary
Table S5).
TABLE 1. Clinical Characteristics and Demographics of Eyes With DH

<table>
<thead>
<tr>
<th>Patients With DH, n = 46</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral DH</td>
</tr>
<tr>
<td>Location of DH</td>
</tr>
<tr>
<td>Superior portion</td>
</tr>
<tr>
<td>Inferior portion</td>
</tr>
<tr>
<td>Proximal location of DH</td>
</tr>
<tr>
<td>Cup wall</td>
</tr>
<tr>
<td>Cup margin</td>
</tr>
<tr>
<td>Disc rim</td>
</tr>
<tr>
<td>Disc margin</td>
</tr>
<tr>
<td>Angular extent of DH, deg</td>
</tr>
<tr>
<td>Corrected DH area, mm²</td>
</tr>
<tr>
<td>Corrected LMRE of DH, mm</td>
</tr>
<tr>
<td>Number of DH recurrences</td>
</tr>
<tr>
<td>1 time (no recurrence)</td>
</tr>
<tr>
<td>2 times</td>
</tr>
<tr>
<td>3 times</td>
</tr>
<tr>
<td>Over 4 times</td>
</tr>
<tr>
<td>Recurrent DH</td>
</tr>
<tr>
<td>Recurrent at 1 clock hour</td>
</tr>
<tr>
<td>Recurrent at more than 1 clock hour</td>
</tr>
<tr>
<td>Recurrent at same location</td>
</tr>
<tr>
<td>Recurrent at different locations</td>
</tr>
</tbody>
</table>

The data are shown as the mean and standard deviation unless otherwise indicated. LMRE, length of maximum radial extent.

DISCUSSION

OCT parameters are widely used in trials currently to analyze progression of glaucoma. Event-based analysis of OCT GPA focuses on newly developed damage, which evaluates the difference in RNFL thickness between baseline and follow-up examination from the OCT RNFL thickness map. When the differences exceed the test-retest variability, the pixels or parameters are regarded as showing significant changes.

Our group recently performed a study that evaluated the GCIPL thinning rate using a trend-based approach in glaucomatous eyes with a localized RNFL defect and assessed the ability of the GCIPL thinning rate to predict progression of glaucoma. We found that the GCIPL thinning rate was more rapid in eyes that progressed than in eyes that did not progress and that analysis of the GCIPL thinning rate is useful for discriminating the progression of glaucoma. Recently, GPA has been developed for the macular GCIPL protocol and introduced with commercially available software. On this background, we hypothesized that DH, a well-known factor in progression of glaucoma, could affect the GCIPL thinning rate, and performed the present study to test this hypothesis.

Recently, Gracitelli et al. reported that eyes with DH showed faster rates of RGC loss than eyes without DH. Because functional status ultimately reflects dysfunction or loss of RGC, evaluating RGC could be essential when determining the status and progression of glaucoma. Medeiros et al. developed an empirical formula for estimation of RGC counts using a combination of OCT images of the RNFL and SAP sensitivity values and estimated longitudinal rates of RGC loss. The results of our study are consistent with those of the previous studies. However, we believe that our present study has some additional strengths. First, we used a more convenient commercially available method, that is, the GCA software in the Cirrus OCT. Second, we examined rates of GCIPL loss in specific areas (i.e., global average, both hemiretinans, and six sectors in the macular area) that were not evaluated in the previous reports.

In our analysis, the GCIPL thinning rate in the inferior area, including the inferior hemiretina and inferotemporal sectors, was more rapid than in the other sectors, and there was a

<table>
<thead>
<tr>
<th>Rate of GCIPL thickness change, μm/y</th>
<th>Eyes With DH, n = 32</th>
<th>Contralateral Eye, n = 32</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>−0.79 ± 0.80</td>
<td>−0.57 ± 0.90</td>
<td>0.150*</td>
</tr>
<tr>
<td>Superior hemiretina</td>
<td>−0.49 ± 1.10</td>
<td>−0.69 ± 1.57</td>
<td>0.268*</td>
</tr>
<tr>
<td>Inferior hemiretina</td>
<td>−1.07 ± 0.75</td>
<td>−0.44 ± 0.54</td>
<td>0.001*</td>
</tr>
<tr>
<td>Superaonasal sector</td>
<td>−0.32 ± 0.98</td>
<td>−0.51 ± 1.25</td>
<td>0.345*</td>
</tr>
<tr>
<td>Superior sector</td>
<td>−0.60 ± 2.01</td>
<td>−0.83 ± 2.71</td>
<td>0.230*</td>
</tr>
<tr>
<td>Superotemporal sector</td>
<td>−0.53 ± 0.90</td>
<td>−0.72 ± 0.95</td>
<td>0.365*</td>
</tr>
<tr>
<td>Inferotemporal sector</td>
<td>−1.13 ± 1.00</td>
<td>−0.61 ± 0.66</td>
<td>0.020*</td>
</tr>
<tr>
<td>Inferior sector</td>
<td>−1.30 ± 0.79</td>
<td>−0.42 ± 0.78</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Inferonasal sector</td>
<td>−0.78 ± 1.16</td>
<td>−0.29 ± 0.69</td>
<td>0.039*</td>
</tr>
</tbody>
</table>

The data are shown as the mean and standard deviation unless otherwise indicated. Bold values indicates statistically significant with P Value ≤ 0.05.
significant difference between eyes with and without DH, whether fellow eyes or control eyes were used for comparison. The high GCIPL thinning rate in the inferior area would be attributable to the high prevalence of inferiorly located (especially inferotemporal) DH in our study population. This finding is in agreement with a previous report that localized thinning of the RNFL and progression of glaucoma were spatially compatible with the location of DH and that the RNFL thinning rate was most rapid in the inferotemporal area (especially at the 7 o’clock position).12 It is also in accordance with many other studies showing that DHs occur most frequently in the inferotemporal area.1,20,26–28 Recently, Hood29 proposed that early glaucomatous damage involves the macula and is often associated with localized RNFL thinning in a narrow region of the disc known as the macular vulnerability zone. In our study, we showed that location of progression in the macula corresponds to the macular vulnerability zone in which DH frequently occurred.

The clinical significance of recurrent DH remains controversial. Some studies have reported more pronounced progressive changes at the optic disc in eyes with recurrent DH than in eyes without recurrent DH.1,13 However, the present study demonstrated that recurrence of DH was not associated with the GCIPL thinning rate as part of structural progression. Our results are partially consistent with other studies showing that recurrent DH is not in itself associated with greater...
The progression of glaucoma. Eyes with recurrent DH may have received intensified treatment and may have been followed up more closely. Intensifying treatment can slow the rate of RNFL thinning in eyes with DH and should be considered when interpreting the clinical significance of recurrent DH.

The present study has some limitations. First, the number of GCIPL image acquisitions was limited. The mean number of GCIPL acquisitions in the eyes with DH was only 5.1 ± 0.8 (range, 4–7) and the mean duration of follow-up of the GCIPL using OCT was only 51.1 ± 10.8 (range, 31–80) months. Future studies with large sample sizes and longer follow-up durations will be needed to further elucidate the relationship between the topographic characteristics of DHs and GCIPL thinning rates. Second, a causal relationship between DH and the GCIPL progression could not be established. It remains unclear whether DH is a cause or a result of GCIPL progression. If DH is a result of progression of the disease, it would be more common in eyes that are susceptible to progression. Third, patients with early-stage OAG were assessed in this study. However, DH can be detected in any stage of the disease.

### Table 3. Comparison of Mean Values of the Rates of GCIPL Thickness Change Between Eyes With DH and Eyes Without DH (Controls)

<table>
<thead>
<tr>
<th></th>
<th>Eyes With DH, ( n = 46 )</th>
<th>Non-DH Eye, ( n = 49 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of GCIPL thickness change, ( \mu m/y )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>(-0.78 \pm 0.85)</td>
<td>(-0.32 \pm 0.48)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Superior hemiretina</td>
<td>(-0.44 \pm 1.00)</td>
<td>(-0.32 \pm 0.55)</td>
<td>0.472*</td>
</tr>
<tr>
<td>Inferior hemiretina</td>
<td>(-1.00 \pm 0.94)</td>
<td>(-0.37 \pm 0.67)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Superonasal sector</td>
<td>(-0.25 \pm 1.25)</td>
<td>(-0.23 \pm 0.65)</td>
<td>0.919*</td>
</tr>
<tr>
<td>Superior sector</td>
<td>(-0.55 \pm 1.70)</td>
<td>(-0.40 \pm 0.70)</td>
<td>0.590*</td>
</tr>
<tr>
<td>Superotemporal sector</td>
<td>(-0.51 \pm 0.82)</td>
<td>(-0.36 \pm 0.75)</td>
<td>0.351*</td>
</tr>
<tr>
<td>Inferotemporal sector</td>
<td>(-1.31 \pm 1.07)</td>
<td>(-0.34 \pm 0.75)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Inferior sector</td>
<td>(-1.29 \pm 0.95)</td>
<td>(-0.43 \pm 0.99)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Inferonasal sector</td>
<td>(-0.42 \pm 2.14)</td>
<td>(-0.34 \pm 0.75)</td>
<td>0.824*</td>
</tr>
<tr>
<td>Rate of RNFL thickness change, ( \mu m/y )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>(-1.18 \pm 1.06)</td>
<td>(-0.86 \pm 0.96)</td>
<td>0.127*</td>
</tr>
<tr>
<td>Superior</td>
<td>(-1.35 \pm 1.49)</td>
<td>(-1.52 \pm 1.85)</td>
<td>0.644*</td>
</tr>
<tr>
<td>Inferior</td>
<td>(-2.18 \pm 1.99)</td>
<td>(-1.28 \pm 1.38)</td>
<td>0.012*</td>
</tr>
<tr>
<td>Rate of VF change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD, dB/y</td>
<td>(-0.19 \pm 0.67)</td>
<td>(-0.05 \pm 0.66)</td>
<td>0.309*</td>
</tr>
<tr>
<td>PSD, dB/y</td>
<td>(0.12 \pm 0.58)</td>
<td>(0.11 \pm 0.75)</td>
<td>0.918*</td>
</tr>
<tr>
<td>VFI, %/y</td>
<td>(-0.86 \pm 1.89)</td>
<td>(-0.43 \pm 1.36)</td>
<td>0.214*</td>
</tr>
<tr>
<td>Total progression of glaucoma, no. (%)†</td>
<td>24 (52.2)</td>
<td>13 (26.5)</td>
<td>0.010‡</td>
</tr>
<tr>
<td>Structural progression§</td>
<td>25 (50.0)</td>
<td>9 (18.4)</td>
<td>0.001‡</td>
</tr>
<tr>
<td>Functional progression</td>
<td></td>
<td></td>
<td>7 (14.5)</td>
</tr>
</tbody>
</table>

The data are shown as the mean and standard deviation unless otherwise indicated. Bold values indicates statistically significant with \( P \) Value ≤ 0.05.

* Independent \( t \) test.
† Combined structural and/or functional progression.
‡ \( \chi^2 \) test.
§ Determined by disc and RNFL photography.
|| Determined by GPA of Humphrey VF.

Figure 2. The GCIPL thinning rate is shown in each group. The average GCIPL thinning rate was more rapid in eyes with DH than in controls without DH. There were no differences in terms of the average GCIPL thinning rate between the other groups (A). The eyes with DH showed more rapid GCIPL thinning in the inferior hemiretina and the inferotemporal sector than fellow eyes or control eyes without DH (B, C). There were no differences in terms of GCIPL thinning rate in the global average, inferior hemiretina, or inferotemporal sector between the group with recurrent DH and the group without recurrent DH (A–C). *\( P < 0.05 \).
trend-based analysis showed that the GCIPL thicknesses measured by optical coherence tomography in eyes with localized nerve fiber layer defects. Invest Ophtalmol Vis Sci. 2011;52:1138–1144.


29. Hood DC. Improving our understanding, and detection, of glaucomatous damage: an approach based upon optical coherence tomography (OCT). *Prog Retin Eye Res*. 2017;57:46–75.


