Aims and objectives

Primary open-angle glaucoma (POAG), a progressive optic neuropathy characterized by irreversible loss of retinal ganglion cells (RGCs) that causes defects in the visual field, is a leading cause of blindness worldwide. Deterioration of the visual field in patients with POAG typically progresses from the periphery towards the fovea according to the distribution of vulnerable RGCs.

Animal experiments and human postmortem studies in glaucoma have suggested widespread degeneration of the lateral geniculate body (LGB) and primary visual cortex as well as RGCs [1-4]. Recent magnetic resonance (MR) imaging studies have revealed decreased volume in the primary visual cortex, secondary visual cortex, LGB, and optic nerve in patients with POAG [5-9]. Furthermore, degeneration of the optic nerve, optic chiasm, and optic radiation has been shown by diffusion tensor imaging (DTI), which indicates white matter integrity by measuring molecular diffusion and is highly sensitive in detecting axonal injury [10]. Altogether, these previous findings suggest involvement of the entire visual pathway in human POAG.

Voxel-based morphometry (VBM) is an objective automated technique that demonstrates changes in brain volume by providing a probabilistic measure of local gray matter (GM) density throughout the brain without a priori identification of a region of interest [11]. Several MR imaging studies of structural changes in the brain that occur in POAG have used VBM. These studies have demonstrated decreases or increases in focal GM density associated with POAG, most consistently in the visual cortex [5-8]. However, the correlation between alteration in the volume of cortical GM associated with POAG and clinical disease stage has not been sufficiently evaluated using VBM.

We aimed to examine possible changes in cortical GM using VBM and evaluate their relationship with the severity of defects in the visual field.

Furthermore, we used visual field measurements to chart changes in visual sensitivity associated with POAG.
Methods and materials

Subjects

This study was approved by the local institutional review board and is in compliance with the Declaration of Helsinki.

Participants were 25 consecutive patients with POAG (17 men, 8 women, aged 44 to 82 years [63.9 ± 9.57]) recruited from the patient population of the Department of Ophthalmology of Tohoku University Hospital in Sendai, Japan and 19 healthy female volunteer controls (aged 50 to 70 years [59.3 ± 4.75]) with no history of oculopathy or disease of the central nervous system (CNS). Patients were diagnosed clinically through assessment of the angle of the open anterior chamber or identification of defects in the visual field typical of glaucoma, optic disc cupping, or elevated intraocular pressure (IOP). We did not include patients with other ophthalmic disease that might affect the visual field and those with history of any CNS disorder, including cerebrovascular and neurodegenerative diseases (Table 1).

Visual field measurements

Visual fields were recorded using the Humphrey Field Analyzer (Carl Zeiss Meditec, Dublin, CA, USA) running the 30-2 Swedish interactive threshold algorithm (SITA) fast program, a standard method for examining the central 30° of the visual field. In this type of measurement, the subject faces a white illuminated sphere on which points of light of varying intensities are briefly flashed, fixates on a set of centrally positioned points during the measurement, and responds to stimuli as they are presented one by one on a grid in the central 30° of the visual field when he or she perceives the flash. Sensitivity at each location in the visual field is determined by changing the intensity of the flash on subsequent presentations. Each eye is measured independently; one eye is covered while the other is tested.

We calculated mean sensitivity deviation maps in 2 steps for each group (patient and controls) to assess the relationship between reduction in visual field sensitivity and changes in the density of cortical GM. First, we created a combined visual field map for each subject by taking the largest sensitivity deviation from the left and right eyes' monocular measurements at each position. Second, we obtained an average binocular sensitivity deviation map by averaging the combined maps from all subjects in a group.

Magnetic resonance imaging
High resolution MR examinations were performed with a 3-tesla scanner (Achieva Intera 3T; Philips Healthcare, Quasar Dual, The Netherlands) with an 8-element head coil. A 3-dimensional magnetization-prepared rapid acquisition gradient echo (MPRAGE) structural image was acquired for each subject of the group with POAG using a $T_1$-weighted magnetization sequence (repetition time [TR], 8.70 ms; echo time [TE], 3.1 ms; 8° flip angle; field of view [FOV], 240 × 162 × 190 mm; and voxel size, 0.9 × 0.9 × 0.9 mm$^3$) and for each control volunteer (TR, 6.5 ms; TE, 3 ms; FOV, 240 × 162 × 240; and voxel size, 1 × 1 × 1 mm$^3$).

**Voxel-based morphometric analysis**

We performed voxel-based morphometric analysis [10], which is part of the SPM8 statistical parametric mapping software (Wellcome Department Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Voxel-based morphometry statistically assesses local changes in GM density between groups of anatomical scans. The brain images were registered to the space template of the International Consortium for Brain Mapping. Used DARTEL, a standard segmentation protocol in SPM8, to classify each voxel as one of 3 different tissues#gray matter, white matter, or cerebrospinal fluid (CSF). Non-brain voxels and voxels with GM density value less than 0.2 were excluded from statistical analysis. Finally, the images resulting from the segmentation were smoothed with a Gaussian kernel of 8-mm full-width at half-maximum. For statistical analysis, we defined one contrast that compared GM density between the group with POAG and controls. For more strict statistics, we set statistical levels at family-wise error ($P < 0.05$) and added the subjects’ ages and sex as a covariate to the analysis as an additional measure to eliminate any potential effect from them.

**Volume-of-interest-based analysis**

In addition to the voxel-based morphometric analysis, we performed an anatomical volume-of-interest (VOI) analysis, configuring the VOI at the place at which VBM indicated clustering of consistently statistically significant voxels. Both the patient and control groups, we calculated mean GM density values from the identical VOI in each patient. We used t-test to compare average GM density values between the POAG and control groups, with $P < 0.05$ considered statistically significant. Next, we used Spearman’s nonparametric correlation test to calculate correlation between these values and the mean sensitivity deviation of the right and left visual fields, with $P < 0.05$ considered statistically significant.
### Baseline patient demographics

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<tr>
<td><strong>Age (years)</strong></td>
<td>63.9 ± 9.57</td>
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<tr>
<td><strong>Male/Female</strong></td>
<td>17/8</td>
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<td><strong>Best-corrected acuity (logMAR)</strong></td>
<td>0.043 ± 0.27</td>
<td>0.155 ± 0.34</td>
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<td><strong>Intraocular pressure (mmHg)</strong></td>
<td>13.4 ± 3.3</td>
<td>12.8 ± 3.8</td>
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<td><strong>Visual field MD (dB)</strong></td>
<td>-9.24 ± 8.5</td>
<td>-10.6 ± 9.0</td>
<td>0.36</td>
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MD, mean deviation

**Table 1:** Baseline patient demographics

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Results

Figure 1 shows a mean binocular sensitivity deviation map of the group of patients with POAG that indicates reduced mean sensitivity of the visual field in the periphery that is predominant in the upper hemifield and relative sparing of sensitivity in the macular region.

Figure 2 shows a comparison of GM density between the POAG and control groups using VBM that indicates significant reduction in the density of cortical GM in the inferior lip of the calcarine sulci as lingual gyrus, and the tectum, paracentral lobule, frontal base, and inferior surface of cerebellum.

Figure 3 shows the results of an additional VOI analysis that confirms the results of VBM. Average GM density in the VOI is plotted for each participant group, and mean GM density is more greatly reduced in the POAG group (0.40 +/- 0.06 SD) than controls (0.59 +/- 0.04 SD) ($P < 0.001$).

Figure 4 shows the relationship between mean GM density in the VOI of the visual cortex and the severity of visual field defects in both the right and left eyes in patients with POAG. No significant correlation was noted between GM density of the visual cortex and MD (left eye, $r = -0.12$; right $r = 0.24$). Mean GM density remained relatively fixed irrespective of the severity of visual field defects.
Fig. 1: Figure 1 shows a mean binocular sensitivity deviation map of the group of patients with POAG that indicates reduced mean sensitivity of the visual field in the periphery that is predominant in the upper hemifield and relative sparing of sensitivity in the macular region.

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**Fig. 2:** Figure 2 shows a comparison of GM density between the POAG and control groups using VBM that indicates significant reduction in the density of cortical GM in the inferior lip of the calcarine sulci as lingual gyrus, and the tectum, paracentral lobule, frontal base, and inferior surface of cerebellum.
**Fig. 3:** Figure 3 shows the results of an additional VOI analysis that confirms the results of VBM. Average GM density in the VOI is plotted for each participant group, and mean GM density is more greatly reduced in the POAG group (0.40 +/- 0.06 SD) than controls (0.59 +/- 0.04 SD) (P < 0.001).

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**Fig. 4:** Figure 4 shows the relationship between mean GM density in the VOI of the visual cortex and the severity of visual field defects in both the right and left eyes in patients with POAG. No significant correlation was noted between GM density of the visual cortex and MD (left eye, $r = -0.12$; right $r = 0.24$). Mean GM density remained relatively fixed irrespective of the severity of visual field defects.

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Conclusion

Discussion

Our primary study finding is that visual field defects associated with POAG can lead to retinotopically specific reduction in GM density in the visual cortex. In patients with POAG, visual field deterioration typically progresses from the periphery towards the fovea. When field defects occur in both eyes and overlap, the retinotopic organization of the visual cortex [11, 12] prevents stimulation of the corresponding cortical parts that may lead to changes in the cortical structure [8, 13, 14]. Anatomically, stimulation from the distribution of RGCs in the foveal region primarily affects the area near the posterior pole of the occipital cortex, and their distribution in the more periphery of the visual field greatly affects the anterior part in the occipital cortex. Furthermore, the upper hemifield of the visual field corresponds with the inferior lip of the calcarine sulci, lingual gyrus, and the lower hemifield of the visual field corresponds with the superior lip, precuneus gyrus in the primary visual cortex.

In our study, the mean visual field sensitivity map of patients with POAG showed predominant loss of sensitivity in the periphery of the upper visual hemifield with relative sparing of sensitivity in the macular region (Figure 1). Our VBM results revealed significant reduction in GM in the anterior part of the inferior lip of the visual cortex that corresponded with the approximate projections of visual field defects in POAG, results compatible with findings of previous studies with VBM [5-8]. Therefore, we speculate that this area could be relatively specific to POAG and that the degree of reduction in GM density in the corresponding area could be an objective and quantitative biomarker with clinical great utility for the diagnosis of POAG.

Our VBM results also showed statistically significant regions in the tectum, paracentral lobule, frontal base, and inferior surface of the cerebellum. Our findings in the frontal base and inferior surface of the cerebellum might be explained by spatial blurring artifact or a problem in the segmentation process caused by limitations in MR imaging acquisition between the POAG and control groups in our study. The tectum deeply affects visual function, which connects with the LGB via the brachium of the superior colliculum. POAG might influence the function and structure of the tectum, but the location of the tectum at the edge of the brain tissue could result in technical artifact as previously mentioned. The clinical significance of reduction in GM density in the precentral lobule is not clear.

Gupta and colleagues reported significant reduction in the volume of the LGB in patients with glaucoma in both postmortem histological and MR imaging examinations [15, 16]. Nevertheless, our VBM result could not indicate atrophy of the LGB. We speculate that because of the small structure of the LGB, GM density values of the voxels corresponding
with atrophic LGB on each tissue probability map were too low for VBM to include areas for statistical analysis.

VOI study detected prominent GM density loss in the visual cortex in patients with POAG compared to controls. However, our additional study revealed no significant correlation between GM density values of the visual cortex and visual field defects among the subjects with POAG (Figure 3). GM density values varied little and remained relatively constant irrespective of the severity of visual field deterioration.

Kerrigan-Baumrind and associates reported statistically significant visual field abnormalities when the death of RGCs exceeded 25 to 35% in the corresponding retinal location [17], and Harwerth's group related systematic sensitivity and neural loss only after the death of about 50% of RGCs (Figure 5 [18]). Because the relationship between loss of visual sensitivity and loss of RGC# is logarithmic, subjective loss of visual sensitivity in the preclinical stage requires the loss of a relatively large proportion of RGC # (40 to 50%), but the decreasing ratio of RGCs must be much smaller after the loss of visual sensitivity becomes obvious in the clinical stage (Figure 5 [18]). Suppose that neuronal degeneration of the central visual pathway was synchronous with or followed RGCs degeneration# in the preclinical period, most alteration in the structure of the visual cortex might be finished at the point of clinical diagnosis using perimeter testing; at the clinical stage, its alteration slope might be gentle or bottomed out after exposure of the loss of visual sensitivity. Yu's group confirmed this hypothesis when they reported positive correlation of the thickness of both the bilateral visual cortex and retinal nerve fiber layer (RNFL), which can be detected with optic coherent tomography (OCT) [19]. The demonstration by all our participants with POAG of various degrees of clinically significant loss of visual sensitivity by perimeter testing does not contradict our findings of relatively low and consistent GM density values of the visual cortex irrespective of the severity of visual sensitivity loss. Our results indicated a possibility that early neuronal degeneration associated with POAG pathogenesis could be detected earlier by MR imaging than clinical perimetry testing.

Our study has some limitations. The sample size is rather small and does not include subjects without visual sensitivity loss. We did not examine the correlation between the GM density value of the primary visual cortex and RNFL thickness, and differences in spatial blurring artifact or the technical process of segmentation caused by variation in MR imaging acquisition between patients and controls may have affected the results of VBM. Further investigation is needed.

**Conclusions**

Our findings indicate that VBM may be sufficiently sensitive to detect cortical alterations in POAG and that the measurement has great potential for clinical application. Combined
with sophisticated image analysis including VBM and VOI analysis, MR imaging may become an objective and quantitative biomarker tool for early diagnosis of POAG.
Fig. 5: Figure 5, cited from Harwerth et al. IOVS 1999 ref No 18 showed that the relationship between loss of visual sensitivity and loss of retinal ganglion cells is logarithmic.

References


11. Dougherty RF, Koch VM, Brewer AA, Fischer B, Modersitzki J, Wandell BA. Visual field representations and locations of visual areas V1/2/3 in human visual cortex. J Vis...


