

Original Article

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The neuropathological study of myelin oligodendrocyte glycoprotein in the temporal lobe of schizophrenia patients

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Abstract

Objective: Recent studies based on the neuroimaging analysis, genomic analysis and transcriptome analysis of the postmortem brain suggest that the pathogenesis of schizophrenia is related to myelin-oligodendrocyte abnormalities. However, no serious neuropathological investigation of this protein in the schizophrenic brain has yet been performed. In this study, to confirm the change in neuropathological findings due to the pathogenesis of this disease, we observed the expression of myelin-oligodendrocyte directly in the brain tissue of schizophrenia patients. **Methods:** Myelin oligodendrocyte glycoprotein (MOG) was evaluated in the cortex of the superior temporal gyrus (STG) and the hippocampus in 10 schizophrenic and nine age- and sex-matched normal control postmortem brains. **Results:** The expression of MOG was significantly lower in the middle layer of the neocortex of the STG and stratum lucidum of CA3 in the hippocampus in the long-term schizophrenic brains (patients with ≥ 30 years of illness duration) than in the age-matched controls. Furthermore, the thickness of MOG-positive fibre-like structures was significantly lower in both regions of the long-term schizophrenic brains than in the age-matched controls. **Conclusion:** These findings suggest that a long duration of illness has a marked effect on the expression of MOG in these regions, and that myelin-oligodendrocyte abnormalities in these regions may be related to the progressive pathophysiology of schizophrenia.

Significant outcomes

- Morphological alternation of myelin oligodendrocyte glycoprotein (MOG) was detected neuropathologically in the brain tissue of schizophrenia patients with a long duration of illness.
- Myelin-oligodendrocyte abnormalities might be related to the progressive pathophysiology of schizophrenia.

Limitations

- We focused only on the inferior region of the superior temporal gyrus (STG) and CA3 of the hippocampus.
- Postmortem human brain tissue may be affected by various events throughout the patient's lifetime that might hamper an accurate neuropathological observation.

Introduction

The aetiology of schizophrenia has been unclear despite intensive investigations. However, it has been shown in recent neuroimaging and molecular biological studies that schizophrenia is a disease arising from abnormal neural communication (1–3). Dysfunction of myelin-oligodendrocytes has been hypothesised to underlie the pathophysiology of this abnormal neural communication in schizophrenia patients. Myelin-oligodendrocytes are the predominant constituents of white matter (WM) and play an important role in neural transmission under schizophrenic conditions (4,5). This hypothesis was initially supported by a series of post-mortem brain studies, neuroimaging studies and genetic evidence (6). Recent diffusion tensor



imaging studies have repeatedly reported WM abnormalities in schizophrenia patients based on decreases in anisotropy indices in the WM tracts and structures (7,8). Furthermore, studies based on genome analyses have also repeatedly reported mutations of myelin-oligodendrocyte-related genes in schizophrenia patients (9). Postmortem studies of brains with this disease have demonstrated the decreased gene expression of myelin-oligodendrocyte-related proteins (e.g. *MOG*, *CNP*, *MAG*, *PLP1*, *ERBB3*) (10,11). These findings by neuroimaging and genetic studies have indicated myelin-oligodendrocyte dysfunction in this disease. However, the altered morphology of myelin-oligodendrocyte has not been unequivocally confirmed by direct observation of the brain tissue by microscopy. Therefore, it is important to examine the neuropathological appearance of those myelin-oligodendrocyte-related proteins in the postmortem brain tissues of schizophrenia patients.

With regard to oligodendrocytes, neuropathological abnormalities in oligodendrocytes have been reported not only in the WM but also in the cortex (12). However, the neuropathological appearance of myelin in the postmortem brain tissues of schizophrenia patients has not yet been examined in detail. *MOG* is a specific component of the central nervous system localised on the outermost lamellae of mature myelin (13) and its gene may be involved in the development of schizophrenia (11,14).

The superior temporal gyrus (STG) and hippocampus are regions of interest for the aetiology of schizophrenia. The STG is involved in auditory processing, including language, but it has also been implicated as a critical structure in social cognition (15,16). Neuroimaging studies have found that patients with schizophrenia have structural abnormalities in their STG (17,18). We have also confirmed the morphological changes in the STG in schizophrenia patients using postmortem brain tissue (19). In the neocortex of the STG, layer III is especially important for schizophrenia. The mean pyramidal neuron somal volume and the densities of markers of axon boutons and dendritic spines were reported to be reduced in layer III of the STG (20–22). The hippocampus is one of the most intensively studied regions of the brain (23), and CA3, a subfield in the hippocampus, is hypothesised to be involved in abnormal neural connectivity within the hippocampus in schizophrenia patients (24). Progressive morphological changes in the hippocampus (25) and STG (26) have also been shown to correlate with the illness duration (23).

Aims of the study

We neuropathologically compared the appearance of *MOG* in the STG and CA3 in the hippocampus of schizophrenia patients with the findings in control patients to clarify the myelin-oligodendrocyte dysfunction in schizophrenia patients with particular focus on the effects of the duration of illness on *MOG* appearance.

Materials and methods

Subjects

Brain specimens from 10 schizophrenia patients and nine age- and sex-matched normal control subjects among autopsy cases at Tokyo Metropolitan Matsuzawa Hospital were used for this study. The demographic details of the subjects are summarised in Table 1. Patients with ≥ 30 years of illness were defined as long-term schizophrenia patients. Control subjects were adjusted to match the long-term schizophrenia with regard to age at death (Table 2).

Table 1. Basic demographic characteristics of the subjects

| | Control | Schizophrenia | <i>p</i> value |
|----------------------------------------------------------------|------------------------|--------------------------|----------------|
| Number | 9 | 10 | |
| Sex (M/F) | 6/3 | 8/2 | 0.51 |
| Age at death (years) | 56.0 ± 13.1 | 59.4 ± 15.7 | 0.65 |
| Age at onset (years) | – | 26.1 ± 8.0 | |
| Duration of illness (years) | – | 33.3 ± 17.8 | |
| Subtype (paranoid/disorganised/ catatonic/undifferentiated) | – | 6/3/0/1 | |
| GAF scores | – | 26.0 ± 5.9 | |
| Lifetime antipsychotic dosage [‡] (g) | – | 7717 ± 5708* | |
| Cause of death (cardiac/respiratory/ other) | 1/3/5 | 1/5/4 | 0.76 |
| Brain weight (g) | 1268 ± 157 | 1358 ± 179 | 0.46 |
| PMI (hours) | 4.4 ± 4.0 [†] | 10.8 ± 14.9 [†] | 0.31 |

GAF, Global Assessment of Functioning; PMI, postmortem interval; SD, standard deviation (mean ± SD).

There were no significant differences between the control and schizophrenic subjects.

*Not known for two schizophrenic subjects.

†Not known for one schizophrenic subject.

‡Lifetime antipsychotic dosage are represented as Chlorpromazine-equivalent dose (g).

Table 2. Basic demographic characteristics of long-term schizophrenic subjects and age-matched controls

| | Age-matched control | Long-term schizophrenia | <i>p</i> value |
|----------------------------------------------------------------|---------------------|-------------------------|----------------|
| Number | 4 | 6 | |
| Sex (M/F) | 3/1 | 4/2 | 0.78 |
| Age at death (years) | 69.0 ± 2.4 | 71.3 ± 6.5 | 0.66 |
| Age at onset (years) | – | 24.7 ± 4.9 | |
| Duration of illness (years) | – | 46.7 ± 7.7 | |
| Subtype (paranoid/disorganised/ catatonic/undifferentiated) | – | 3/2/0/1 | |
| GAF scores | – | 23 ± 5.4 | |
| Lifetime antipsychotic dosage [‡] (g) | – | 12 255 ± 5242* | |
| Cause of death (cardiac/ respiratory/other) | 1/2/1 | 1/3/2 | 0.93 |
| Brain weight (g) | 1208 ± 99 | 1315 ± 170 | 0.45 |
| PMI (h) | 5.1 ± 5.3 | 5.3 ± 5.5 [†] | 0.81 |

GAF, Global Assessment of Functioning; PMI, postmortem interval; SD, standard deviation (mean ± SD).

There were no significant differences between the age-matched control and long-term schizophrenic subjects.

*Not known for two schizophrenic subjects.

†Not known for one schizophrenic subject.

‡Lifetime antipsychotic dosage are represented as Chlorpromazine-equivalent dose (g).

We confirmed the diagnosis by reviewing the clinical records to verify that the cases satisfied the DSM-IV-TR criteria for schizophrenia and that the control subjects had no evidence of psychiatric or neurological disorders. We also excluded cases with

neuropathological changes, such as neurodegenerative disorders (e.g. Alzheimer-type dementia, frontotemporal lobar degeneration, etc.), cerebrovascular diseases, brain invasion of tumours, severe malnutrition, metabolic encephalopathies and inflammatory or traumatic processes based on the records of clinicopathological conferences with several expert neuropathologists. We additionally excluded any cases with a history of alcohol or substance abuse or convulsions that might affect neuropathological changes, based on the patients' clinical records. We confirmed that all cases had a sufficient social function before onset and had completed compulsory education.

We assessed the subtype of schizophrenia using the DSM-IV-TR criteria. We assessed the clinical severity in the predominant state using the Global Assessment of Functioning (GAF) scale. We measured the total dosage of antipsychotic medication taken throughout the lifetime (lifetime antipsychotic dosage) by reviewing the clinical records.

This study was approved by the Nagoya University School of Medicine Ethical Review Board.

Brain tissue processing

In all cases, the cadavers were kept at 4°C before autopsies. All brains were extracted and fixed in 10% formalin within 48 h after death. After fixation, the brains were sectioned in the coronal plane. The brains were embedded in paraffin and cut at a thickness of 10 µm. We used each left coronal section at the level where the hippocampus, the STG and the inferior parietal lobule (IPL) were involved.

Immunohistochemistry

The prepared sections were deparaffinised in xylene and rehydrated through an ethanol gradient. Endogenous peroxidase activity was blocked with 0.3% (v/v) H₂O₂/methanol. Sections were then incubated with primary antibody against MOG (rabbit polyclonal, 1:1000; Sigma Aldrich, St. Louis, MO, USA) at 4°C overnight. After rinsing, the sections were subjected to either a streptavidin–biotin complex or an enhanced indirect immunoperoxidase method using Envision (DakoCytomation, Glostrup, Denmark). Immunoreactivity was detected using 3,3'-diaminobenzidine, and sections were counterstained with haematoxylin.

Image acquisition and morphometric analysis

Equipment and software programs

The photographs were downloaded to a PC from a digital camera (DP72; Olympus Co., Tokyo, Japan) as digital data. A morphometrical analysis was performed using the Adobe Photoshop 6.0 (Adobe Systems Inc., San Jose, CA, USA) and ImageJ 1.43u (free software developed by the NIH: <http://rsb.info.nih.gov/ij/>) software programs by one of the coauthors blinded to the diagnosis.

STG

The middle areas of the inferior region of the STG were observed (Fig. 1a). At 100× magnification, we investigated the distribution of MOG in the cortex as follows: We randomly placed a column of the neocortex 424-µm wide (from layers I to VI) (Fig. 1c). The column of the neocortex was vertically divided into three equal parts: the upper layer, the middle layer and the lower layer (Fig. 1c). The cytoarchitecture (i.e. a six-layer cortical structure) is assumed to be altered or disorganised in the cortex of the schizophrenic brain (27). Therefore, in this study, we considered it

best to estimate the distribution of the MOG in the neocortex of the STG based on equal partitions of the neocortex independent of the anatomical cortical structure with the six layers. After we removed haematoxylin dye from the image of the STG using the colour deconvolution plugin (<http://www.mecourse.com/landinig/software/cdeconv/cdeconv.html>) (Fig. 2a-i, ii), in two columns per case, we measured the total area of the MOG-positive structures via certain intensity (pixel value: 0–210) staining with anti-MOG antibody in each layer to determine the expression of MOG in each layer (Figs. 2a-iii). The ratio of the expression of MOG in each layer was calculated as follows: [(expression area of MOG in each layer)/(expression area of MOG in the whole layer)].

We next investigated the pathological changes reflecting altered distribution with greater magnification (200×). In two randomly placed 47-µm-square areas of layer III of each case (Figs. 2c-i), we extracted the MOG-positive fibre-like structures using ImageJ, based on line continuity. We traced the MOG-positive fibres manually. The sum of the length of the MOG-positive fibre-like structures and the area of the MOG-positive structures (pixel value: 0–210) was determined (Figs. 2c-iii, ii). To statistically investigate the morphological differences of myelination, we defined and calculated the thickness of MOG-positive fibre-like structures as follows: the area of MOG-positive structures/the sum of the length of MOG-positive fibre-like structures.

We also observed the IPL using the same procedure to determine whether or not the changes were region-specific in schizophrenic postmortem brains.

Hippocampus

We used specimens in which CA3 could be clearly differentiated for the morphometric analysis (Fig. 1b). At 100× magnification, in the five randomly placed 212-µm-square areas of the stratum oriens and the stratum lucidum of the CA3 of each case (Figs. 1d, e), we measured the total area of MOG-positive structures via certain intensity (pixel value: 0–210) staining with anti-MOG antibody to determine the expression of MOG and calculated the occupancy ratio of expression of MOG (Fig. 2b). At 200× magnification, using the same methods as in the STG, the thickness of MOG-positive fibre-like structures was calculated.

Statistical analyses

For the statistical analyses, we performed nonparametric tests because of our small sample size. We used the Mann–Whitney *U* test to assess the differences in the brain weights, postmortem interval (PMI) and age at death between the diagnostic groups. We used Fisher's exact test to assess the differences in sex and causes of death between the diagnostic groups. To assess the expression of MOG, we used the Mann–Whitney *U* test between the diagnostic groups. We used Spearman's rank correlation to assess how age at onset, PMI, duration of illness, brain weight, GAF scores or lifetime antipsychotic dosage affect the appearance of MOG. A value of $p < 0.05$ was considered to be statistically significant. All statistical analyses were performed using the SPSS Statistics 17.0 software program (SPSS Inc., Chicago, IL, USA).

Results

Basic demographic data

There were no significant differences in the sex, cause of death, brain weight, PMI or age at death between the control and schizophrenia patients (Tables 1, 2).

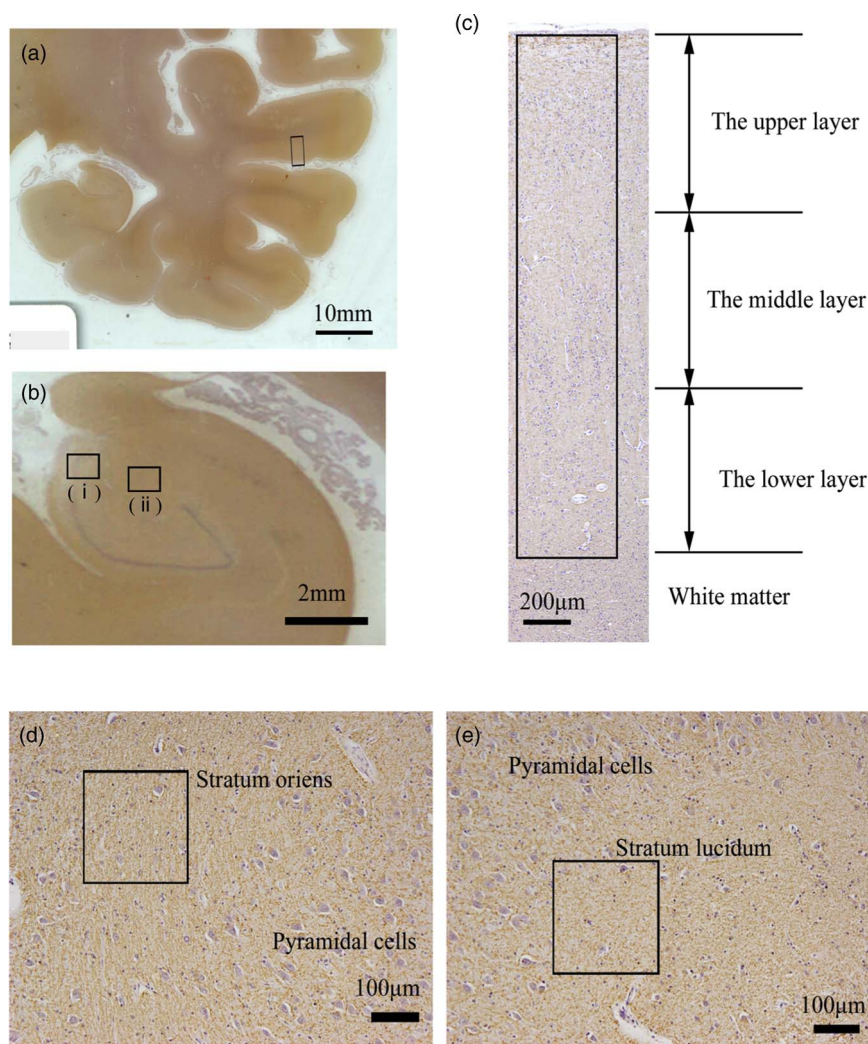


Fig. 1. Areas examined in the superior temporal gyrus (STG) and the hippocampus of postmortem brains. (a) Left coronal section of temporal lobe stained with anti-myelin oligodendrocyte glycoprotein antibody (MOG). The middle areas of the inferior region of the STG (box) were examined. (b) Left coronal section of hippocampus stained with anti-MOG antibody. The stratum oriens (i) and stratum lucidum (ii) of CA3 in the hippocampus were examined. (c) The neocortex of the middle areas of the inferior region in the STG stained with anti-MOG antibody. We randomly placed the 424- μ m-wide column (box) in the neocortex (from layers I to VI). The columns are vertically divided into three equal parts: the upper, the middle and the lower layer. (d) Stratum oriens of CA3 in the hippocampus stained with anti-MOG antibody. We randomly placed 212- μ m-square regions of interest (box). (e) Stratum lucidum of CA3 in the hippocampus stained with anti-MOG antibody. We randomly placed 212- μ m-square regions of interest (box).

MOG in the neocortex of the STG

MOG-positive structures were observed in all layers of the neocortex of the STG. Those via certain intensity (pixel value: 0–210) were detected as intensely positive structures in myelin (Fig. 2a-iv, v). In the control group, MOG was observed most abundantly in the middle layer followed by the lower layer. In contrast, in the schizophrenic group, MOG was observed most abundantly in the lower layer followed by the middle layer (total patients; Fig. 3a). However, there were no statistically significant differences in the distribution between the control and schizophrenic groups (Table 3). In the long-term schizophrenic group, MOG was also observed most abundantly in the lower layer followed by the middle layer. Furthermore, the ratio of expression of MOG in the middle layer was significantly smaller in the long-term schizophrenic group than in the age-matched controls ($p=0.03$; Table 3). In the neocortex of the IPL, there were no significant differences in the distribution between the control and schizophrenic groups or between the long-term schizophrenic groups and controls.

The middle layer was mainly equivalent to layers III and IV. At higher magnification, MOG via certain intensity (pixel value: 0–210) was observed as the myelin sheath, as distinguished from background (Figs. 2c-i, ii). The thickness of MOG-positive fibre-like structures, determined as ‘the area of MOG-positive structures/ the sum of the length of MOG-positive fibre-like structures’, was significantly smaller in layer III in the long-term schizophrenic group than in the age-matched controls ($p=0.011$; Fig. 4a).

MOG in CA3 of the hippocampus

MOG-positive structures via certain intensity (pixel value: 0–210) were detected as intensely positive structures of myelin in CA3 of hippocampus (Figs. 2b-iv, v). There were no significant differences in the occupancy ratio of expression of MOG in both the stratum oriens and the stratum lucidum between the schizophrenic and control group (Table 3; Fig. 3b). However, the occupancy ratio of expression of MOG in the stratum lucidum was significantly smaller in the long-term schizophrenic group than in the age-matched controls ($p=0.03$) (Table 3; Fig. 3d).

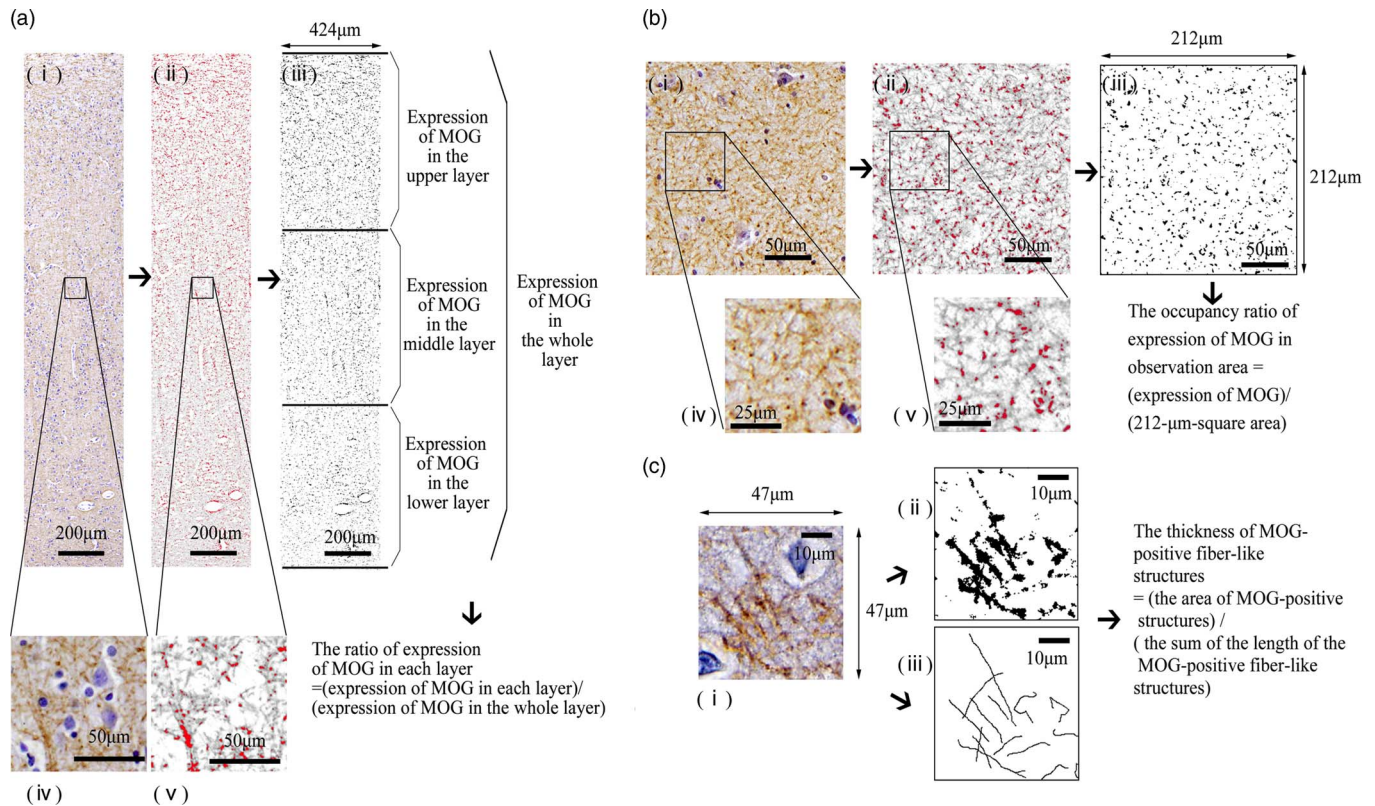


Fig. 2. A morphometric analysis of myelin oligodendrocyte glycoprotein (MOG) in each observation area. (a) A morphometric analysis of MOG in the superior temporal gyrus (STG). From the observation area (424- μ m-wide column) of the image of the STG (i, iv), we removed haematoxylin dye (ii, v) using the ImageJ software program. We measured the total area of the MOG-positive structures via certain intensity (pixel value: 0–210) staining with anti-MOG antibody [red area in (ii) and (v), black area in (iii)] in each layer to determine the expression of MOG in each layer (iii). We calculated the ratio of expression of MOG in each layer to investigate the distribution of MOG. (b) A morphometric analysis of MOG in the CA3 of the hippocampus. From the image of observation area (212- μ m-square area) of the CA3 (i, iv), we removed haematoxylin dye (ii, v) using the ImageJ software program. We measured the total area of the MOG-positive structures via certain intensity (pixel value: 0–210) staining with anti-MOG antibody [red area in (ii) and (v), black area in (iii)] in the 212- μ m-square area to determine the expression of MOG in the CA3. We calculated the occupancy ratio of expression of MOG. (c) Measurement of the thickness of MOG-positive fibre-like structures in the observation area. In 47- μ m-square areas of layer III of the neocortex in the STG (i) and stratum lucidum stained with anti-MOG antibody, we extracted the MOG-positive fibre-like structures using the ImageJ software program. The sum of the length of the MOG-positive fibre-like structures and the area of the MOG-positive structures (pixel value: 0–210) was determined (ii, iii). We calculated the thickness of MOG-positive fibre-like structures.

The thickness of MOG-positive fibre-like structures was significantly smaller in the stratum lucidum of the long-term schizophrenic group than in the age-matched controls ($p=0.011$; Fig. 4b).

Correlation analysis using clinical profile data

The age at death, PMI, duration of illness, brain weight and lifetime antipsychotic dosage were not correlated with the ratio of expression of MOG in the STG or the occupancy ratio of expression of MOG in the CA3. The GAF scores were positively correlated with the occupancy ratio of expression of MOG in the stratum lucidum of the CA3 ($r=0.89$, $p=0.001$). However, the GAF scores were not correlated with the ratio of expression of MOG of the STG and the occupancy ratio of expression of MOG in the stratum oriens of the CA3.

Discussion

MOG in the STG in schizophrenia patients

In this study, we observed a significant reduction in the distribution of MOG (i.e. the ratio of expression of MOG) in the middle layers of the neocortex of the STG in the long-term schizophrenia patients in comparison with age-matched controls. The

cytoarchitecture (i.e. six-layer cortical structure) is assumed to be altered or disorganised in the cortex of the schizophrenic brain. Although the change in the distribution of MOG in our study may be implicated in the cytoarchitectural abnormalities reported in schizophrenia patients (27), this finding might also be associated with the dysfunction of the myelin-oligodendrocytes in this region as the abundance of MOG-positive fibre-like structures in layer III of the STG of long-term schizophrenia patients was different from that in control patients. We also observed a significant reduction in the thickness in the MOG-positive fibre-like structures in layer III of the STG in long-term schizophrenia patients.

The middle layer in this study corresponded mainly to layers III and IV, which are rich in interneurons. Cerebral cortical interneuron dysfunction has also been hypothesised to be involved in the pathophysiology of schizophrenia (28), especially in layers III and IV (29,30). In addition, the loss of inhibitory input into the pyramidal neurons of layers II/III has been reported in animal models of schizophrenia (31) and postmortem brains (32). Furthermore, in the STG, the impairment of inhibitory neurotransmission (mainly GABAergic neurotransmission) is considered to play a critical role in the pathophysiology of schizophrenia (33). It is suspected that the deficit of interneuron myelination might be involved in the pathogenesis of schizophrenia (34). The findings of our study may support these phenomena; however, myelinated

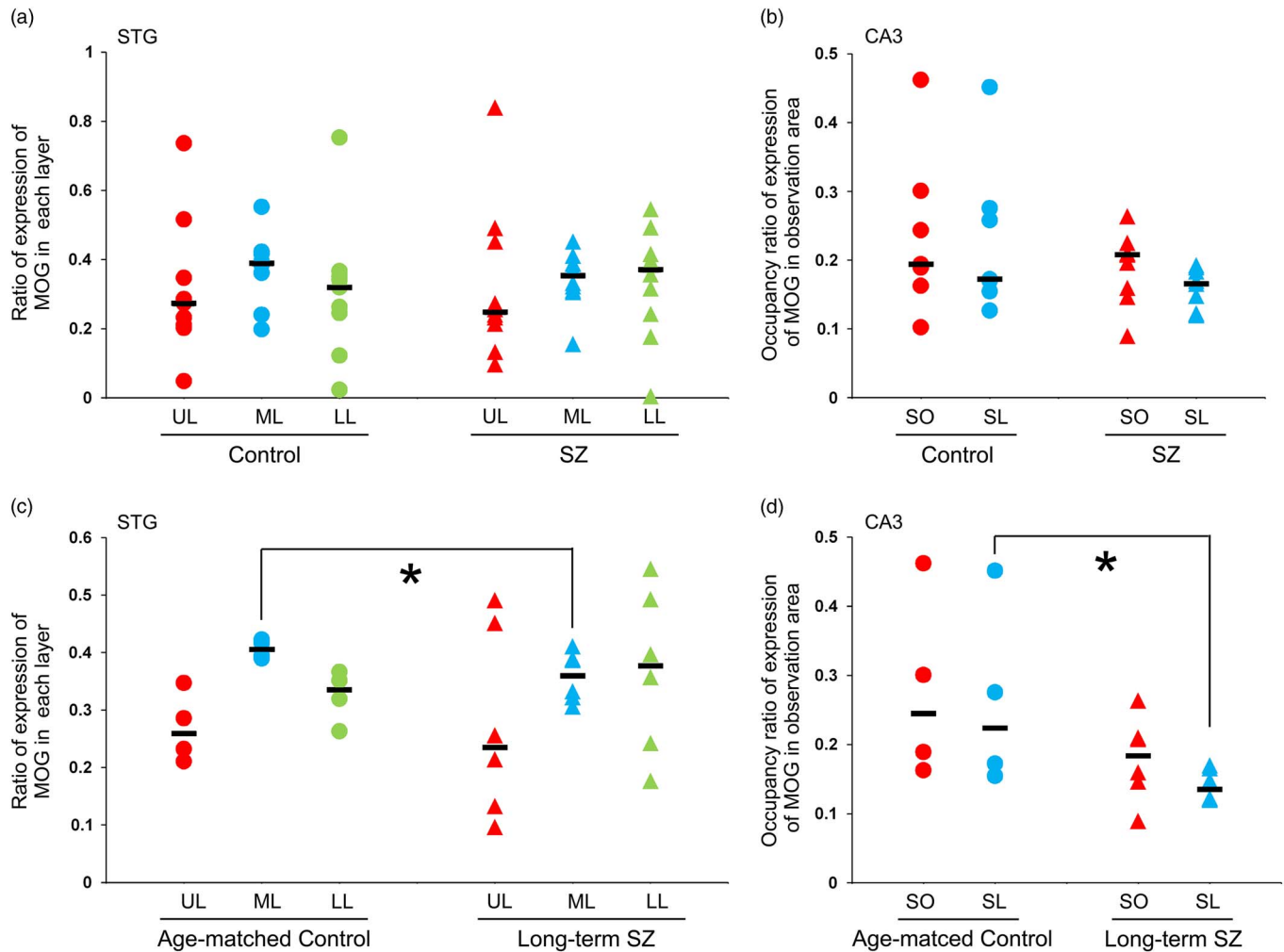


Fig. 3. The expression of myelin oligodendrocyte glycoprotein (MOG) in the neocortex of the superior temporal gyrus (STG) and the CA3 of the hippocampus. (a) The ratio of expression of MOG in each layer of the STG in schizophrenic and control subjects. (b) The occupancy ratio of expression of MOG in the CA3 in schizophrenic and control subjects. (c) The ratio of expression of MOG in each layer of the STG in the long-term schizophrenic and age-matched control subjects. The ratio of expression of MOG in the middle layer was significantly smaller in the long-term schizophrenic group than in the age-matched controls. (d) The occupancy ratio of expression of MOG in the CA3 in the long-term schizophrenic and age-matched controls. The occupancy ratio of expression of MOG in the stratum lucidum was significantly smaller in the long-term schizophrenic group than in the age-matched controls. The horizontal black lines indicate the median value. * $p < 0.05$. LL, lower layer; ML, middle layer; SL, stratum lucidum; SO, stratum oriens; SZ, schizophrenia; UL, upper layer.

axons connected to other brain regions may also be implicated in our findings (35). Although it is unclear what kind of neurons or fibres are actually involved in the decrease in the expression of MOG in the middle layer, this finding was specific to the STG, as we did not observe a significant reduction in the distribution of MOG in the neocortex of the IPL.

A few neuropathological studies have examined the myelin-oligodendrocytes in the STG of schizophrenia patients. In the cortex, Cotter et al. (36) reported no decreased glial densities, including oligodendrocytes in schizophrenia patients. With regard to WM, decreased populations of glial cells have been reported in schizophrenia patients (37). However, in these investigations, no distinction was made between oligodendrocytes and other glial cells. In contrast, in our present study, we distinguished myelin-oligodendrocyte-related proteins from other components of brain tissue, using an immunohistochemical method. To our knowledge, this is the first neuropathological study that focused on myelin-oligodendrocyte-related proteins and analysed them digitally in the STG of schizophrenia patients.

MOG in the CA3 in schizophrenia patients

In the stratum lucidum of the CA3 in long-term schizophrenia patients, the occupancy ratio of expression of MOG was lower than that in controls, although no significant differences were observed in the stratum oriens or on comparing total schizophrenia patients and controls. Furthermore, the findings from our microinvestigation of the stratum lucidum suggested that the decreased expression in the region also reflects dysfunction of myelin-oligodendrocytes.

The granule cell mossy fibre pathway projects from the dentate gyrus into the stratum lucidum, which is rich in inhibitory interneurons (38). While mossy fibre is generally thought to be unmyelinated fibre, myelination of interneurons in the hippocampus has been reported (33). Inhibitory neurotransmission impairment in the hippocampus is thought to play a critical role in the pathophysiology of schizophrenia (33). The reduction of MOG in the stratum lucidum in our study may be related to a dysfunction of inhibitory neurotransmission (38,39).

Table 3. The expression of myelin oligodendrocyte glycoprotein (MOG) in the superior temporal gyrus (STG), inferior parietal lobule (IPL) and CA3 of hippocampus

| | Control | Schizophrenia | <i>p</i> value | Age-matched control | Long-term schizophrenia | <i>p</i> value |
|------------------------------------------------------------|--------------|--------------------------|-----------------|---------------------|-------------------------|-----------------|
| Ratio of expression of MOG in each layer of the neocortex | | | | | | |
| STG | | | | | | |
| Upper layer | 0.27 ± 0.11 | 0.25 ± 0.12 | <i>p</i> = 0.87 | 0.26 ± 0.05 | 0.24 ± 0.16 | <i>p</i> = 0.83 |
| Middle layer | 0.39 ± 0.06 | 0.35 ± 0.04 | <i>p</i> = 0.33 | 0.41 ± 0.01 | 0.36 ± 0.03 | <i>p</i> = 0.03 |
| Lower layer | 0.32 ± 0.09 | 0.37 ± 0.09 | <i>p</i> = 0.41 | 0.34 ± 0.03 | 0.38 ± 0.13 | <i>p</i> = 0.52 |
| IPL | | | | | | |
| Upper layer | 0.25 ± 0.14 | 0.24 ± 0.12 | <i>p</i> = 0.68 | 0.32 ± 0.10 | 0.27 ± 0.12 | <i>p</i> = 0.29 |
| Middle layer | 0.39 ± 0.02 | 0.37 ± 0.04 | <i>p</i> = 0.51 | 0.40 ± 0.02 | 0.36 ± 0.04 | <i>p</i> = 0.29 |
| Lower layer | 0.37 ± 0.14 | 0.37 ± 0.09 | <i>p</i> = 0.46 | 0.26 ± 0.10 | 0.36 ± 0.09 | <i>p</i> = 0.09 |
| Occupancy ratio of expression of MOG in CA3 of hippocampus | | | | | | |
| CA3 | | | | | | |
| Stratum oriens | 0.20 ± 0.07* | 0.21 ± 0.03 [†] | <i>p</i> = 0.63 | 0.25 ± 0.10 | 0.19 ± 0.03 | <i>p</i> = 0.20 |
| Stratum lucidum | 0.17 ± 0.06* | 0.17 ± 0.03 [†] | <i>p</i> = 0.12 | 0.23 ± 0.10 | 0.14 ± 0.02 | <i>p</i> = 0.03 |

QD, quartile deviation
median ± QD.

*Not known for two subjects.

[†]Not known for one subject.

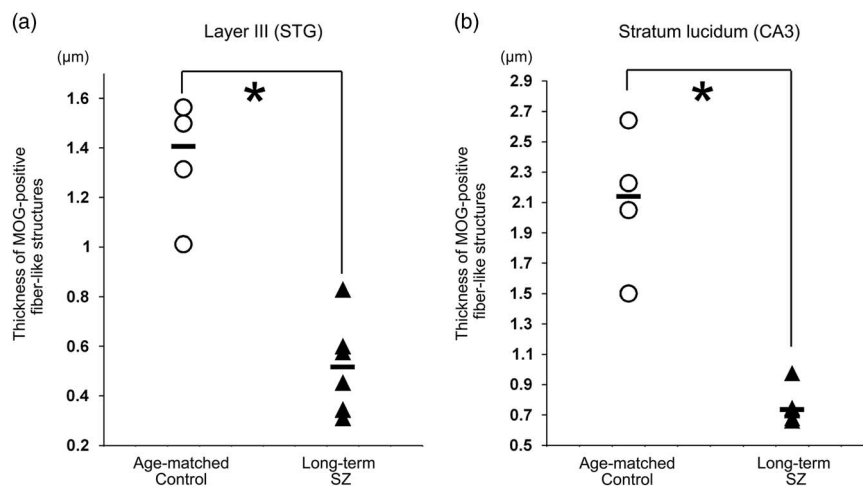


Fig. 4. The thickness of myelin oligodendrocyte glycoprotein (MOG)-positive fibre-like structures. (a) The thickness of MOG-positive fibre-like structures in layer III of the neocortex in the superior temporal gyrus (STG) in long-term schizophrenic subjects and age-matched control subjects. The thickness of MOG-positive fibre-like structures was significantly smaller in layer III of the long-term schizophrenic group than in the age-matched controls. (b) The thickness of MOG-positive fibre-like structures in the stratum lucidum of the CA3 in long-term schizophrenic subjects and age-matched control subjects. The thickness of MOG-positive fibre-like structures was significantly smaller in the stratum lucidum of the long-term schizophrenic group than in the age-matched controls. The horizontal black lines indicate the median value. **p* < 0.05. SZ, schizophrenia.

Oligodendrocyte and myelin damage and degeneration in schizophrenia patients have been shown in electron-microscopy studies of the neocortex and the CA3 of the hippocampus (40). Swelling of the periaxonal oligodendroglial process and atrophy of the inner axons found in thin myelinated fibres are the most frequently cited ultrastructural pathologies in schizophrenia patients (41). Our finding may be associated with these pathological changes.

Effects of long-term of illness

The reduction in the MOG expression is more obvious in long-term schizophrenia patients than in those with a shorter illness duration. The finding may suggest that altered myelination

is related to progressive brain changes in schizophrenia patients.

Progressive volume reductions of STG in schizophrenia patients have been frequently reported (42). In a postmortem study, we previously reported that long-term schizophrenia patients had less WM in the STG than healthy subjects, where myelin-oligodendrocytes are predominant constituents (19). Alterations in myelination might occur in the GM as well as WM in the schizophrenic brain (43). The findings of these previous macroscopic studies might be confirmed by the findings from this microscopic study.

Hippocampal volume reductions have also been reported in schizophrenia patients. Furthermore, such reductions have been

assumed to continue after illness onset (44) and correlate with illness duration (25). Reductions in not only the total hippocampal volume but also the CA3 subfield volume have been reported (45). These findings from other reports may support the assumption that altered myelination is related to the progressive brain changes in schizophrenia patients.

Effects of antipsychotics and the severity of illness

In this study, the lifetime antipsychotic dosage did not correlate with the ratio of expression of MOG in the middle layer of the neocortex in the STG or the occupancy ratio of expression of MOG in the stratum lucidum of the CA3. We assessed the equivalent chlorpromazine dose without distinguishing between the different types of antipsychotics. Notably, it has been reported that quetiapine has effects on oligodendrocyte development (46). However, typical antipsychotics – and not atypical antipsychotics – were used for all cases in the present study. On the other hand, it has also been reported that pathological changes in the myelination were not attributed to neuroleptic medication (40). These findings suggest that antipsychotics may not affect our findings.

The severity of illness may more likely affect the expression of MOG in the stratum lucidum than in other areas. Oligodendroglial abnormalities may contribute to altered connectivity with cognitive impairment in schizophrenia patients (41). It is assumed that alteration of myelination in the stratum lucidum may be closely related to the severity of schizophrenia.

Limitations

This study is suggestive, not conclusive, due to several limitations. First, this study had a small sample size because we carefully and rigidly selected brain specimens from autopsy cases that had correct and reliable diagnostic information and case information. It was difficult to use many specimens because of the rarity of such Japanese (mongoloid race) brain specimens, due to the underdeveloped status of the Psychiatric Brain Bank in Asia, including Japan. Second, we focused only on the inferior region of the STG and CA3 of the hippocampus. Therefore, future investigations should examine other regions of interest in which structural abnormalities have been reported in neuroimaging studies of schizophrenia. Third, human brains can be affected by various events throughout the lifetime, and some agonal factors may also have inflicted the tissue damage observed in the postmortem brains. Future studies should investigate brain tissue with evident biological backgrounds (i.e. genetic animal models, rare variants of schizophrenia) in order to confirm the association of the MOG appearance with the pathophysiology. Fourth, we observed morphological changes in the MOG-positive fibre-like structures two-dimensionally on glass slides. Thus, the direction and shape of the fibres may affect the detection and measurement of fibres, and our findings did not directly show the ultrastructure of myelinated fibres. While it would be better to observe these structures three-dimensionally, this currently remains a technical limitation.

Conclusion

We observed a significant decrease in the expressions of MOG in the STG and CA3 of the hippocampus in long-term schizophrenia patients compared with age-matched controls neuropathologically. These findings suggest that a long duration of illness has a marked effect on the expression of MOG in these

regions and that myelin-oligodendrocyte abnormalities in these regions may be related to the progressive pathophysiology of schizophrenia.

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Authors's Contribution. The study was conceptualised by Y.T., S.I. and N.O. The clinical data of the sample were evaluated by C.H., H.S., K.O. and K.N. Immunohistochemical staining was conducted by S.H., K.M. and J.K. The analyses were designed and conducted by TM and YT. The manuscript was written by T.M., Y.T., H.F. and S.I. All authors have approved the final manuscript.

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Conflicts of Interest. None

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