Cell type-specific DNA methylation analysis of the prefrontal cortex of patients with schizophrenia

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Epigenetics reflects complex interactions between genes and the environment and plays a role in long-lasting gene expression changes. Therefore, unraveling epigenetic landscape of brain cells from patients with psychiatric disorders will contribute to understanding the pathophysiology of these disorders.[1] We performed promoter-wide DNA methylation analysis of the prefrontal cortex of patients with schizophrenia (N = 35, Table S1). Brain cells were separated into neuronal and nonneuronal nuclei by NeuN-based nuclei sorting.[2] Methylated DNA was collected using the MBD2B/3L, which does not bind hydroxymethylcytosine and enables the analysis of densely methylated regions in the genome. DNA methylation profiles were obtained with promoter tiling arrays covering 25 500 human promoters. Differentially methylated regions (DMRs) were identified by using a deposited control dataset (N = 35, GSE137921), which had been obtained by the same experimental procedure. This study was approved by the ethics committees of the participating institutes (the Research Ethics Committee of Kumamoto University, the Research Ethics Committee of the Faculty of Medicine of The University of Tokyo, the Ethical Review Board of Juntendo University, and the Wako 1st Research Ethics Committee of RIKEN). This study was conformed to the provisions of the Declaration of Helsinki. The experimental procedures are described in Appendix S1.

We identified 91 DMRs and 69 DMR-associated genes in nonneurons and 74 DMRs and 59 DMR-associated genes in neurons (Tables S2 and S3). We found that 59.4% of nonneuronal and 69.5% of neuronal DMR-associated genes were shared between the two cell types (Fig. 1a). The DMR with the most significant change in neurons was located in the *CHGA* promoter region and showed hypermethylation. This DMR was also identified and showed the most significant change in nonneurons. *CHGA* encodes chromogranin A, a neuroendocrine protein located in the vesicles of neurons. The level of chromogranin A was reported to be reduced in the cerebrospinal fluid and prefrontal cortex in schizophrenia.[3,4] The DMR with the second highest score in neurons was found in the *LINGO1* promoter region, and showed hypermethylation. This DMR was also identified and showed the fifth highest change in nonneurons. LINGO1 plays a role in myelination and neurite outgrowth, and the disturbance of LINGO1 signaling has been implicated in the pathophysiology of schizophrenia.[5] Gene ontology analysis using both neuronal and nonneuronal DMR-associated genes revealed the enrichment of potassium channel-related terms (FDR corrected $P < 0.05$) (Fig. 1b). In addition, enrichment of the Ras/Rho signaling and glucocorticoid receptor signaling pathways was detected (nominal $P < 0.05$).

We previously performed a microarray-based gene expression analysis of the same brain region in the same subjects.[6] By utilizing the dataset we assessed the gene expression status of DMR-associated genes. We found that the expression levels of 25 probes, which covered 19 DMR-associated genes, showed reliable gene expression values. Among them, five probes for three genes (*ATP2B2*, *NCOA2*, and *PEG10*) showed significantly altered expression (Welch's t-test, nominal $P < 0.05$) (Fig. 1c). *ATP2B2* encodes plasma membrane calcium ATPase isoform 2 and *de novo* damaging variants in this gene were identified in autism.[7] *NCOA2* encodes nuclear receptor coactivator 2 and has a histone acetyltransferase activity. *PEG10* encodes paternally imprinted gene 10 and contains two overlapping open reading frames, RF1 and RF2. *PEG10* was also identified as a differentially expressed gene in schizophrenia in a large-scale transcriptomics study.[8] The hypermethylated DMRs in schizophrenia are located in the 3'-UTR of *PEG10* and may affect the regulation of isoform variations.

We then assessed whether the DMRs were enriched in genome-wide association study (GWAS) loci of psychiatric disorders by promoter-based random sampling analysis (Appendix S1). We previously found that neuronal DMRs in bipolar disorder (BD) were significantly enriched in the GWAS loci of BD, but nonneuronal DMRs were depleted from the GWAS loci of schizophrenia.[9] In this analysis, we found no significant enrichment or depletion of the DMRs in the GWAS loci of any psychiatric disorders ($P > 0.05$). To increase sensitivity, we defined the DMRs with a relaxed threshold ($P < 10^{-5}$), yielding 525 neuronal and 958 nonneuronal DMRs. Similar to the DMRs in BD, we found significant depletion of the nonneuronal DMRs from the schizophrenia GWAS loci[10] ($P = 0.0354$), whereas no enrichment or depletion in the neuronal DMRs (Figs 1d and S1). These results suggest that the DMRs, especially the nonneuronal DMRs, and the GWAS loci of schizophrenia may have different spatiotemporal roles in the brain.

In summary, we identified DMRs of the prefrontal cortex of patients with schizophrenia. The DMRs reported in this study will be useful for understanding the pathophysiology of schizophrenia.

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Disclosure statement

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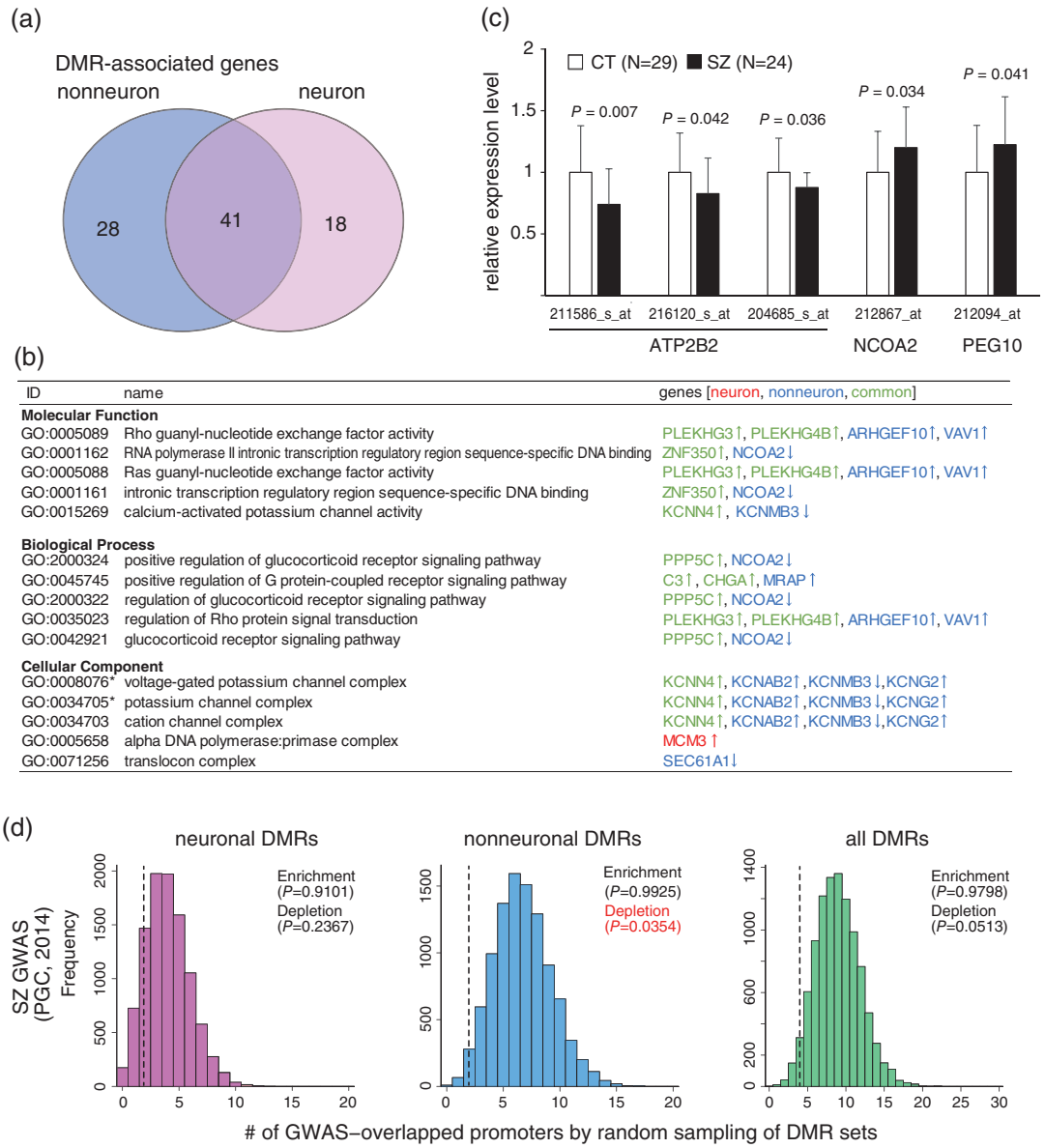

Fig.1 Differentially methylated regions (DMRs) in schizophrenia. (a) Venn diagram of DMR-associated genes. (b) Gene ontology analysis of DMR-associated genes. The top five terms in each category are shown. * indicates FDR-corrected $P < 0.05$. Green, blue, and red indicate common, nonneuronal, and neuronal DMR-associated genes, respectively. Arrows show the direction of change in schizophrenia. See Table S4 for details. c. Relative expression levels of DMR-associated genes. Gene expression levels were measured in a previous microarray study (Appendix S1). Subjects with low brain pH were omitted from data analysis. Bars indicate standard deviations of the means. CT, control; SZ, schizophrenia. d. Enrichment and depletion between the DMRs and GWAS loci, assessed by random sampling. The frequency was based on 10,000 random samplings of DMR sets. See Fig. S1 for the results for other GWAS loci. *P*-values in red indicate the significant depletion of DMRs.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1 Detailed description of methods.

Figure S1 Results of enrichment analysis.

Table S1 Summary of the subject information.

Table S2 List of neuronal DMRs.

Table S3 List of nonneuronal DMRs.

Table S4 Results of gene ontology analysis.

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White matter volume not associated with hallucinations in clinical high risk and first-episode psychosis: A voxel-based morphometry study

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Hallucinations are considered one of the primary symptoms of psychosis; however, their neural basis remains unclear. Voxel-based morphometry (VBM)[1] studies have associated hallucinations with gray matter (GM) volume reduction in the superior temporal gyrus in schizophrenia.[2] Although hallucinations are associated with brain connectivity,[3] few studies have investigated their relationship with white matter (WM) volume. Moreover, these studies have shown inconsistent results.[4,5] The chronicity of illness and long-term medications for schizophrenia may limit the interpretation of previous findings.[2] Thus, hallucination studies in clinical high risk for psychosis (CHR) and first-episode psychosis (FEP) are required, with minimal effects of chronicity of illness and medication. However, no VBM study has examined the relationship between hallucinations and WM volume in CHR and FEP. Therefore, we aimed to investigate the regional GM and WM volumes associated with hallucinations in CHR and FEP.

We enrolled 57 individuals with CHR, 50 individuals with FEP, and 33 healthy controls (HCs). The Comprehensive Assessment of At-Risk Mental States-Japanese version[6] was used to confirm whether ultra-high risk criteria[7] were met. Table S1 summarizes the demographic and clinical data of the patients. Hallucination severity was assessed using the Positive and Negative Syndrome Scale (PANSS)[8] P3 score. Fig. S1 shows the distribution of the severity of hallucinations. Some of those with a PANSS P3 score of 3 or higher were classified as having CHR because of the low frequency and duration of hallucinations. Magnetic resonance imaging (MRI) data were acquired with a 1.5-T Philips scanner. We examined the association of GM and WM volume with hallucination severity in the group that combined patients with CHR and FEP (CHR/FEP combined group) and performed group comparisons of GM and WM volume between the CHR/FEP combined and HC groups. Age, sex, and intracranial volume were entered as nuisance covariates for each analysis. VBM analysis was conducted using DARTEL[9] in SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) with standard smoothing of 8-mm full width at half maximum (FWHM). The initial statistical threshold was set at $P < 0.001$, uncorrected at the voxel level. The extent threshold for cluster size was set based on the expected number of voxels per cluster provided by SPM. Familywise error (FWE) correction at the cluster level ($P < 0.05$) was applied to identify significant clusters. Appendix S1 provides detailed information on these methods.

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Tohoku University Graduate School of Medicine and Tohoku University Hospital. Written informed consent was obtained from participants aged 18 years or older and from the parents of those under 18 years of age, with written assent from the participants. The anonymity of participants was preserved.

WM volume was not significantly correlated with hallucination severity in the CHR/FEP combined group after FWE correction at the cluster level. Reanalysis with 15-mm or 20-mm FWHM filtering did not show significant associations. Additionally, there was no significant difference in the GM and WM volumes between the CHR/FEP combined and HC groups. No regional GM volume abnormalities were significantly correlated with hallucination severity in the CHR/FEP combined group. Appendix S1, Figs. S2–S5, and Tables S2–S5 provide uncorrected results and detailed information regarding these results.

To the best of our knowledge, this is the first VBM study to investigate WM volume related to hallucinations in CHR and FEP. We found no significant correlation between WM volume and hallucination severity. In contrast to previous studies in schizophrenia,[2,4,5] this study examined the brain volume association of hallucinations in CHR and FEP. CHR and FEP are heterogeneous groups in the early stages of mental illness and do not necessarily convert to schizophrenia. Our results indicate that brain disturbances involved in hallucinations in CHR and FEP may be more subtle and/or inconsistent with those in schizophrenia. Diffusion tensor imaging, a neuroimaging method applied to CHR,[10] may be suitable for examining structural abnormalities in the development of hallucinations.

This study has some limitations. First, information regarding hallucinations is not detailed. Second, the cross-sectional design limited the interpretation of our findings. Third, we used an MRI scanner with a lower field strength than that of recent studies.[4] Fourth, there were