Pathophysiology of Patients with Schizophrenia: Genetic and Environmental Factors

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Schizophrenia is a debilitating disorder with a prevalence of approximately 0.5%–1% within any given population. The pathophysiology of schizophrenia involves complex genetic, environmental, and psychological etiologies. Here we summarize 26 years of research completed by the Juntendo University Schizophrenia Projects study group on the “biopsychosocial model” of schizophrenia. Clinical brain morphological abnormalities in schizophrenia were detected with magnetic resonance imaging, and these findings led to gene expression analyses of neurotransmitters. The familial aggregation pattern in schizophrenia led to the completion of genetic studies, including linkage and genome-wide analyses, and studies on environmental factors, such as nutrition, aging, stress, and inflammation. Furthermore, we developed a collaborative multicenter study that consisted of a large number of samples. This study enabled us to clearly identify the relevant pathophysiology of schizophrenia, including genetics, altered neurotransmission, brain morphology, and clinical features.

Key words: schizophrenia, genetics, environment, brain, neurotransmitter

Introduction

While completing bedside learning during my fifth year of medical school, I met a 27-year-old female patient. She was suffering from slandering auditory hallucinations that were resistant to antipsychotic medications prescribed since the onset of her condition at 17 years of age. Unfortunately, approximately 20%–30% of patients with schizophrenia have a treatment-resistant form of the disorder¹⁾. After I became aware of the existence of debilitating symptoms, such as auditory hallucinations, and the 1% prevalence rate for schizophrenia in the general population, I chose to become a psychiatrist.

Brain morphology

Schizophrenia is a debilitating disorder with a prevalence of approximately 0.5%–1% within any given population. The pathophysiology of schizophrenia involves complex genetic, environmental, and psychological etiologies. Clinically, atypical brain morphological abnormalities are often observed in patients with schizophrenia using computed tomography (CT) and magnetic resonance imaging (MRI). Previous studies showed that patients with brain morphological abnormalities typically have a severe form of the disorder that is relatively resistant to treatment²⁾. However, it was unclear if these atypical brain morphological abnormalities existed prior to symptom onset or appeared as the disorder progressed. To answer this question, I conducted an MRI study on patients

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Figure-1 A quantitative magnetic resonance imaging study revealed that patients with schizophrenia had a small prefrontal cortex (Ohnuma T, *et al.* Psychiatry Clin Neurosci, 1997; 51, partial modification³⁾)

with schizophrenia during their first episodes, prior to neuroleptic treatment. These patients were found to have small prefrontal and temporal cortices as well as a reduced hippocampus volume at symptom onset, which supports a neurodevelopmental hypothesis (Figure-1)³⁾. Thus, we could not refer to these findings as “atrophy” because the detailed neuropathological examination of the brains from individuals with schizophrenia failed to reveal any prominent neuronal loss or typical neurodegenerative changes (e.g., gliosis)^{4) 5)}.

Post-mortem brain study

To further investigate the genesis of morphological changes of the brain, including gene expression levels, I worked with the Babraham Institute (Medical Research Council, Laboratory of Molecular Neuroscience Group, Department of Neurobiology) at Cambridge University from 1996 to 1997 as a visiting scholar (Figure-2). I learned several molecular biology techniques, including Northern and Southern blots, *in-situ* hybridization, and gene

Figure-2 The Babraham Institute in Cambridge, United Kingdom

targeting in mice. I used these methods to determine the concentration and gene expression of several neurotransmitters in the prefrontal and temporal cortices of fresh-frozen post-mortem brains of patients with schizophrenia from Cambridge University Hospital. First, I investigated glutamate levels as well as the mRNA expression of

Figure-3 A model of the synaptic cleft in patients with schizophrenia (Ohnuma, *et al*: Prog Neuropsychopharmacol Biol Psychiatry, 2005; 29⁸⁾)

glutamate receptors and transporters in the brains of patients with schizophrenia and healthy controls to detect any changes in glutamatergic neurotransmission at the synaptic cleft. There was a tendency toward increased metabotropic glutamate receptors (mGluRs) and decreased excitatory amino acid transporter 2 (EAAT2) mRNA in all of the prefrontal areas examined; however, no significant differences were observed between patients with schizophrenia and controls. To better assess minimal changes in glutamatergic neurotransmission at the synaptic cleft, the “receptor/transporter ratio” (i. e., mGluRs/EAAT2 ratio) was calculated. The mGluRs/EAAT2 ratio was significantly higher in patients with schizophrenia than in controls. While measured glutamate levels using HPLC showed a non-significant decrease in post-mortem schizophrenic brain samples⁶⁾. Similar results were found for GABA⁷⁾. Overall, these data suggest that the increase in mGluRs and decrease in EAAT2 mRNA could be a compensatory mechanism for the decrease in glutamate transmission at the synaptic cleft in schizophrenic brains. However, these changes were small and not statistically significant. The “receptor/transporter ratio” combined these individual changes to produce statistically significant results (Figure-3)⁸⁾. Thus, we

hypothesized that minimal pathological changes in the prefrontal cortex, such as decreased glutamatergic and GABAergic neurotransmission in patients with schizophrenia.

Juntendo University Schizophrenia Projects

In 2003, the Juntendo University Schizophrenia Projects (JUSP) study group was established in our department. The aim of JUSP is to resolve the complicated and multifactorial pathophysiology of schizophrenia based on a biopsychosocial model (Figure-4).

1. Genetic studies

The JUSP study group completed several genetic case-controlled studies to identify genes involved in the pathophysiology of schizophrenia⁹⁾⁻¹⁸⁾. Positive findings from JUSP studies were reassessed in a large-scale genetic replication study (e.g., approximately 2,500 patients with schizophrenia and 2,500 controls) by collaborative centers in Japan¹⁹⁾⁻²²⁾. Despite their strong statistical power, these replication analyses failed to show any significant genetic associations with schizophrenia. However, these results suggested that clinically-observed familial aggregation in patients with schizophrenia cannot

Figure-4

The relationships among the studies completed under the Juntendo University Schizophrenia Projects (JUSP) study group since 2003.

be explained by the common disease-common variant hypothesis. Strong familial aggregation, which was observed in some families, is not always caused by genetic factors. Indeed, a large linkage analysis of 236 Japanese schizophrenia familial aggregation, including JUSP samples from the Japanese Schizophrenia Sib-Pair Linkage Group, supports the existence of schizophrenia susceptibility loci on chromosomes 1p, 14q, and 20p²³⁾. However, these results were not reproduced in replication studies with other ethnic populations. Thus, some environmental factors (e.g., food and stress) are likely to play a role in schizophrenia. Strong psychological sympathy can result in transmitted psychosis termed “folie à deux,” and the development of schizophrenia-like symptoms and high familial aggregation²⁴⁾. These results led our research group to complete a genome-wide association study (GWAS) while also investigating biological environmental factors.

2. Peripheral biomarker studies

We reported that hypoglutamatergic neurotransmission occurs in the brains of patients with schizophrenia. To look for biological markers of schizophrenia, we studied several excitatory amino acids involved in glutamate neurotransmission via NMDA receptors, such as endogenous glutamate, glycine, serine, and alanine. We reviewed several studies of excitatory amino acid levels in peripheral blood and our previous JUSP biomarker studies, which thoroughly investigated plasma glutamatergic amino acid levels²⁵⁾⁻²⁸⁾, to determine if any of these amino acids could be used as diagnostic, therapeutic, or symptomatic biological markers²⁹⁾. We concluded that peripheral blood levels of endogenous glycine and alanine may be symptomatic markers for schizophrenia, whereas levels of exogenous glycine and alanine may be therapeutic markers. Notably, peripheral blood levels of endogenous D-serine may correlate with its brain concentration, which suggests that this amino acid may be

Figure-5

A. The mechanism of carbonyl stress and its detoxification with vitamin B6 and glyoxalase

Reactive carbonyl compounds (RCOs) cause carbonyl stress; however, RCOs can be detoxified by degradation into lactic acid and glutathione by glyoxalase enzymes. Glyoxalase 1 and 2 (*GLO1* and *GLO2*) are the rate-limiting enzymes in this metabolic pathway. Inhibition of RCO generation and the Maillard reaction by vitamin B6 results in the suppression of AGE accumulation.

B. Serum levels of carbonyl stress markers in normal controls and patients with schizophrenia at hospital admission

B1. Pentosidine. **B2.** Pyridoxal. Fourteen patients with high pentosidine levels (>2 SD above the mean of controls, i.e., >57.6 ng/ml) are indicated with an asterisk. Values were compared using a two-tailed Mann-Whitney *U* test. Data are shown as the mean $\pm$ SD.

a useful diagnostic and therapeutic marker for schizophrenia²⁹⁾.

In 2010, a cross-sectional study of chronic schizophrenia reported altered peripheral carbonyl stress markers, including high levels of serum pentosidine that accumulates following carbonyl stress and low levels of pyridoxal (vitamin B6) that detoxifies reactive carbonyl compounds³⁰⁾. In cross-sectional and longitudinal studies, we investigated if the serum levels of these markers reflected the clinical course of the disorder³¹⁾. One hundred and thirty-seven acute-stage Japanese patients were enrolled. Among these, 53 patients were followed from the acute stage to remission. A portion of patients in the acute stage (14 cases, 10.2%) showed extremely high pentosidine levels (Figure-5). These levels were not associated with the severity of symptoms, but were associated with antipsychotic dose amounts. Pyridoxal levels were lower in schizophrenia and increased according to the clinical course of the illness (Figure-5). Furthermore, 18 patients with decreased pyridoxal levels according to the clinical course showed that the greater the decrease in pyridoxal levels, the less improvement in symptoms. Thus, extremely high pentosidine levels in a portion of patients may be caused by higher daily antipsychotic doses, whereas pyridoxal levels were lower in schizo-

phrenia and increased according to the clinical course. Patients with decreasing pyridoxal levels during the clinical course showed less improvement in symptoms. Carbonyl stress markers may also be therapeutic biological markers in some patients with schizophrenia³¹⁾. We measured glyceraldehyde-derived AGEs (Glycer-AGEs), which are highly neurotoxic, and soluble receptors for AGEs (sRAGE), which may ameliorate the effects of AGEs, to determine their potential as diagnostic, therapeutic, or clinical biological markers in patients with schizophrenia³²⁾. Glycer-AGEs were significantly higher and sRAGE levels were significantly lower in patients with acute schizophrenia compared with those in healthy controls (Figure-6). Furthermore, discriminant analyses confirmed that Glycer-AGE levels and Glycer-AGEs/sRAGE ratios can be used as diagnostic markers for schizophrenia and distinguished between patients and healthy controls in 70.0% of cases. These data highlight the potential of Glycer-AGEs, sRAGE, and their relative ratios as diagnostic markers for patients with schizophrenia³²⁾. Based on the speculation that aging stress advances inflammation, we are further investigating micro-inflammation biomarkers in patients with schizophrenia.

Figure-6 Serum levels of carbonyl stress markers at hospital admission

Glycer-AGEs levels (A), sRAGE level (B), and Glycer-AGEs/sRAGE ratio (C) were compared between patients with schizophrenia and healthy control subjects using a two-tailed Mann-Whitney *U* test. Data are presented as the mean $\pm$ SD.

Collaborative, large-scale multicenter study

1. Genome wide association study

To test the common disease-common variant hypothesis of schizophrenia, a GWAS was completed as part of our collaborative multicenter study³³⁾. Significant genome-wide differences were detected in the meta-analysis of the combined data sets (6,668 patients and 12,791 controls) from the Japanese population, including JUSP samples ($p = 3.4 \times 10^{-8}$, OR=0.87, 95%CI=0.83-0.92). Assuming a prevalence of 1%, these results suggest that in a population of 700 without risk T allele of the rs2071287 SNP in *NOTCH4*, there should be seven patients with schizophrenia. In a population of 700 with at least one risk C risk allele, there should be eight patients with schizophrenia with an OR of 1.15. Thus, genetics can weakly influence the pathophysiology of schizophrenia via common SNPs.

2. Copy number variations

Based on the etiological limitations of the common disease-common variant hypothesis for schizophrenia, genetic studies of copy number variations (CNV)s, mainly rare CNVs, were developed^{34) 35)}. Indeed, subjects with rare large deletions (>3 Mb) of the ch22q11.2 region (22q11.2 deletion syndrome) often show schizophrenia-like symptoms³⁶⁾. Relatively large CNVs (>1 Mb) that account for 50% of all CNVs in humans are considered to be *de novo* CNVs. In other words, these CNVs are not inherited and the effects of these mutations would

not be reflected in the aforementioned linkage and GWAS studies. Common CNVs (frequencies > 0.05), especially those in the ch22q11.2 region should be investigated in patients with schizophrenia. We previously completed a CNV case-control study for *COMT*³⁷⁾ and the glutathione (GSH)-related genes *GSTT1* and *GSTT2*³⁸⁾. Our second multicenter replication study included a large number of subjects (1,854 patients vs. 2,137 controls) revealed significant association between CNVs at the promoter 1 and exon 6 regions of *COMT* and the presence of schizophrenia³⁷⁾. These results highlight the importance for further CNV analyses that address specific CNV characteristics, such as *de novo*/inherited or large/small CNVs.

3. Is schizophrenia a syndrome? Biopsychosocial modeling in a subpopulation of patients with schizophrenia

A recent collaborative multicenter study reported interesting results³⁹⁾ (Figure-7). This study had three major findings: 1) the risk allele of a brain-enriched sorting nexin, *ARHGAP33* (a high-affinity receptor for brain-derived neurotrophic factor), was associated with patients with schizophrenia; 2) *ARHGAP33* knockout mice exhibited reduced synaptic *TrkB* expression, impaired spine development, and neuropsychiatric disorder-related behavioral abnormalities; and 3) the risk allele of *ARHGAP33* is associated with smaller prefrontal and temporal cortical volumes based on MRI measurements from patients with schizophrenia³⁾. Thus, this study shows the sequential effects

Figure-7 ARHGAP33 is associated with schizophrenia, and ARHGAP33 KO mice show impaired spine development

A. The locations of the SNPs analyzed in this study. **B.** and **C.** The impact of the risk T allele on grey matter volume of the left middle temporal gyrus in patients with schizophrenia. A significant cluster of the genotype effect was observed in the left middle temporal gyrus of patients with schizophrenia. The regions are identified by cross-hairs. The relative grey matter volumes were extracted from the left middle temporal gyrus, right medial frontal gyrus and right inferior temporal gyrus. * $p < 0.05$. Data are expressed as the mean $\pm$ SEM. **D.** ARHGAP33 KO mice showed a decrease in total and mature spine densities. Golgi staining of granule cells in the hippocampal dentate gyri from 12-week-old ARHGAP33 KO and WT mice. Scale bar, 10 μ m. **E.** and **F.** Z-stacks were created, and individual spines were measured (WT, $n = 33$ cells KO, $n = 29$ cells; each $n = 4$ mice; total spine density: $p = 4.4 \times 10^{-5}$; percentage of mature spines: $p = 7.7 \times 10^{-17}$; one-way ANOVA; b, c). * $p < 0.05$. The bars represent the mean value. (Nakazawa T, *et al*: Nat Commun, 2016; 7: 10594³⁹⁾)

of a genetic risk factor, including decreased neurotransmission caused by a decrease in mature spines and clinically-based morphological brain abnormalities. This study also suggests that the pathophysiology of schizophrenia, including genetics and clinical features, is influenced by this gene in some patients. The odds ratio of the risk allele at ARHGAP33 (e.g., rs231228, T allele) is 1.14, which suggests a weak genetic influence that could not account for the 1% prevalence rate for schizophrenia. Schizophrenia research is often non-specific because the diagnostic criteria for schizophrenia are wide-ranging and heterogeneous⁴⁰⁾. These criteria do not take into account the presence of auditory hallucinations, which is one of the most common symptoms of schizophrenia. Thus, it is difficult to establish differences in the pathophysiology of patients with and without auditory hallucinations. Studies on biological and psychophysiological features of schizophrenia that are not included in the diagnostic criteria can resolve the clinical features (including symptoms). Furthermore, these diagnostic limitations suggest that schizophrenia should be currently classified as a syndrome rather than a disorder.

Conclusion

The past 26 years of our research at Juntendo University was based on clinical practices and molecular biology studies. We established that the pathophysiology of schizophrenia can be influenced by genetics, altered neurotransmission, brain morphology, and clinical features. However, these results are not ready for direct translation to all patient populations, especially treatment-resistant patients. Ceaseless efforts from the JUSP study group continue to focus on schizophrenia pathophysiology in hopes of discovering new therapeutics for our patients.

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