Association analysis of the DISC1 gene with schizophrenia in the Japanese population and DISC1 immunoreactivity in the postmortem brain

Woraphat Ratta-apha

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学位論文の内容要旨

Association analysis of the DISC1 gene with schizophrenia in the Japanese population and DISC1 immunoreactivity in the postmortem brain

日本人における統合失調症と DISC1 遺伝子の関連研究と

死後脳における DISC1 蛋白発現解析

神戸大学大学院医学研究科医科学専攻

精神医学

（指導教員：曽良一郎 教授）

Woraphat Ratta-apha

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Introduction

The Disrupted-in-Schizophrenia 1 (DISC1) gene plays a role in the regulation of neural development, including progenitor cell proliferation, the assembly of centrosome and microtubule networks, and synaptic signaling. Although previous evidence from genetic association and biological studies has implicated the DISC1 in the pathophysiology of schizophrenia, the data are inconsistent.

One of the most common missense variants of DISC1 is the Ser704Cys SNP (rs821616). It has been reported to be associated with schizophrenia in the Chinese Han population, but it has not been associated with schizophrenia in Japanese populations. This genetic variant reportedly accounts for many phenotypes caused by alterations of brain structure and function, for example, a reduction of the posterior prefrontal cortex, hippocampus, cingular cortex, and posterior gyrus.

The purpose of this study was to examine whether there is a relationship between DISC1 gene polymorphisms and schizophrenia in the Japanese population. We conducted an association study of the DISC1 gene in schizophrenic subjects and performed a meta-analysis of the Ser704Cys variant in the Japanese population. Furthermore, we examined the DISC1 immunoreactivity in the prefrontal cortex of postmortem brains isolated from schizophrenic subjects and non-psychiatric control subjects.

Materials and methods

To perform an association analysis, we first genotyped the rs821616 SNP of the DISC1 gene. After the rs821616 SNP showed nominally significant association with schizophrenia, we confirmed this result by genotyping the rs821621 SNP, which has a perfect

linkage disequilibrium (LD) with the rs821616 SNP. To detect a potential genetic association between a haplotype block that included the rs821616 SNP and schizophrenia, the rs1772702, rs1754603, and rs821624 SNPs (tagging SNPs) were selected using the Haploview software Tagger algorithm. We examined the association of these SNPs with schizophrenia in 503 schizophrenic patients and in 511 healthy subjects.

We included all studies analyzing the association between the Ser704Cys variant and schizophrenia in the Japanese population for the meta-analyses. We then added the data from this study and performed a meta-analysis.

For the DISC1 expression analysis, we performed immunoblotting using postmortem prefrontal cortex brain samples that were obtained from 24 control and 14 schizophrenic patients.

This study was conducted with the approval of the ethical committee for genetic studies of the Kobe University Graduate School of Medicine. Informed consent was obtained from all genotyped participants and from close relatives of the subjects whose brain tissue was used for the postmortem studies.

Results

The five selected SNPs were in LD with each other ($D' = 0.82$-0.97). The genotypic distribution and allelic frequency of the rs821616 and rs821621 SNPs were significantly different between the schizophrenic and control groups (rs821616: genotypic $p = 0.010$, $\chi^2 = 6.46$, and allelic $p = 0.011$; and rs821621: genotypic $p = 0.019$, $\chi^2 = 5.471$, and allelic $p = 0.019$). However, the differences between these two SNPs did not withstand correction for multiple comparisons. In the haplotype analysis, the schizophrenic group exhibited a nominally significant association between two and three markers [rs1772702-rs1754603-rs821616] in the LD block.

According to the meta-analysis of the Ser704Cys DISC1 variant, four association

studies examining Japanese populations were identified in a literature search (Hashimoto et al., 2006; Hotta et al., 2011; Kinoshita et al., 2012; Zhang et al., 2005). Significant heterogeneity among the studies was detected in the pool data (Q = 15.953, *df* = 4, and *p* = 0.003). A random-effects model analysis showed no significant association between the allelic frequencies (Number of T alleles in schizophrenia = 708, total number = 1,651, pool OR = 0.97, 95% CI = 0.78 – 1.21, Z-value = -0.268, and p value = 0.789).

To analyze protein expression, we measured the DISC1 immunoreactivity, and normalized it to β–actin expression levels. The protein bands of DISC1 and β–actin were detected at 91 and 43 kilodalton, respectively. After normalization to β–actin expression, the DISC1 expression in the schizophrenic subjects was significantly reduced compared with the control subjects (t = 3.920, df = 36, and $p < 0.001$). However, DISC1 immunoreactivity was not correlated with the age (*Pearson r* = 0.048 and p = 0.773), brain pH (*Pearson r* = - 0.221 and p = 0.182), or the postmortem interval (*Pearson r* = 0.044 and p = 0.792). To rule out potential confounding factors, we used a one-way ANCOVA in the subsequent analyses. When normalized to β–actin levels, DISC1 protein expression levels in the schizophrenic groups were also reduced compared with the control group [$F(1,32)$ = 7.816 and p = 0.009]. However, no differences in DISC1 immunoreactivity were detected in the Ser704Cys variant after genotypes (AA, AT, and TT) or alleles (Ser-allele or Cys-allele carrier) were set as independent variables {[$F(1,29)$ = 0.002 and p = 0.964], and [$F(1,30)$ = 0.016 and p = 0.900], respectively}.

Discussion

This study demonstrates that DISC1 immunoreactivity in the postmortem brain was significantly reduced in the schizophrenic group compared with the control group, even though we were not able to identify an association between the DISC1 gene and

schizophrenia after performing a meta-analysis of the missense mutation rs821616 (Ser704Cys) SNP.

Because many factors affect protein stability and gene expression in brain tissue, our findings should be carefully interpreted. In addition, previous studies have reported that DISC1 has many isoforms, including many that may still be unknown. Not all of the DISC1 protein bands were detected by western blot analyses. Furthermore, DISC1 protein expression is relatively low in the brain with high background levels and is difficult to detect using the available antibodies. Some researchers have suggested that using reliable pairs of DISC1 antibodies for immuno-precipitation followed by western blot analysis would improve the ability to detect DISC1 protein in brain samples.

Several limitations should be concerned. 1) The small sample size of the association study. 2) We did not have complete clinical case histories for the subjects, thus it was not clear whether psychotropic medications or other factors might have affected DISC1 expression. 3) This study investigated only the Ser704Cys variant and neighboring SNPs; therefore, the expression and function of additional variants should be examined in schizophrenia. 4) The immunoreactivity of DISC1 was examined only in prefrontal cortex specimens. The other areas of the brain that are involved in the pathophysiology of schizophrenia should also be examined. 5) The use of postmortem brain tissue for performing immunoreactivity and quantifying protein expression levels can be complicated by various confounding factors, for instance, the sample quality may have been affected by degradation during the postmortem interval.

In conclusion, these data provide evidence that the functional genetic variants of DISC1 do not underlie the pathophysiology of schizophrenia in the Japanese population. Because the cause of reduced DISC1 expression in schizophrenia is unclear, further studies examining DISC1 expression and association studies including a larger sample size will better reveal the role of DISC1 in schizophrenia.