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A missense mutation in the *ITGA8* gene, a cell adhesion molecule gene, is associated with schizophrenia in Japanese female patients

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ABSTRACT

Background: Cell adhesion molecules (CAMs) play pivotal role in the development of the central nervous system (CNS) and have also been reported to play role in the pathophysiology of schizophrenia. Missense mutations in the CAMs genes might alter the binding of their ligands, increasing the vulnerability to develop schizophrenia.

Methods: We selected 15 missense mutations in the CAMs genes of the CNS reported in the Kyoto Encyclopedia of Genes and Genomes (KEGG) and examined the association between these mutations and schizophrenia in 278 patients and 284 control subjects (first batch). We also genotyped the positive single nucleotide polymorphisms (SNPs) in 567 patients and 710 control subjects (second batch) and in 635 patients and 639 control subjects (replication samples).

Results: Genotypic and allelic distributions of rs2298033 in the *ITGA8* gene between the schizophrenia and control groups were significantly different in the first batch ($p = 0.005$ and 0.007, respectively). Gender-based analysis revealed that the allelic and genotypic distributions of rs2298033 in the *ITGA8* were significantly different between the schizophrenia and control groups among females in both batches ($p = 0.010$, 0.011 and 0.0086, 0.010, respectively) but not among males. Combine analysis of rs2298033 with the replication samples revealed a more significant differences ($p = 0.0032$; 0.0035 in the overall subjects and $p = 0.0024$; 0.0025 in the female subjects, respectively). The significant differences for rs2802808 of the *NFASC* gene were only observed in the female subgroup of the first batch.

Conclusion: These results suggest that the *ITGA8* gene might have gender-specific roles in the development of schizophrenia. Further replication and functional studies are required to confirm these findings.

1. Introduction

Schizophrenia is a common disorder caused by both genetic diathesis and environmental factors, but its etiology is still unclear. Thus, diagnosis and treatment of schizophrenia are based only on clinical assessment of symptoms and the course of the disorder. Modern treatments of schizophrenia are far from being able to cure the disorder and just relieve the symptoms. Therefore, understanding the pathophysiological processes underlying schizophrenia is considered to be essential for the development of a reliable treatment for schizophrenia (Gaur et al., 2008).

Changes in groups of molecules such as adhesion molecules, cytoskeletal proteins, neurotrophins, and cell signaling molecules have been observed in the brains of schizophrenia patients (Gaur et al., 2008; Maynard et al., 2001). Moreover, the genetic component of schizophrenia is reported to involve single nucleotide polymorphism (SNPs) that are not distributed randomly across the genome but are distributed across genes that share a common biological function or pathway (Lips et al., 2011). Therefore, rather than focusing on specific susceptibility loci, the study of schizophrenia should be broadened to collections of neuronal phenotypes (Costa et al., 2003), such as cell adhesion molecules (CAMs) in the central nervous system (CNS).

CAMs play an important role in the maintenance and modulation of synaptogenic activity within neuronal circuitries (Giagtzoglou et al., 2009; Kriebel et al., 2011) and have been reported to be important in the pathophysiology of schizophrenia (Lips et al., 2011). They also play an important role in axonal/dendritic growth, synapse formation and plasticity, and neurotransmission (Corvin, 2010; Goh et al., 2008;

Abbreviations: CAM, cell adhesion molecule; CNS, central nervous system; GWAS, genome wide association study; HWE, Hardy Weinberg equilibrium; LD, linkage disequilibrium; LTP, long term potentiation; SNP, single nucleotide polymorphism.

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Moresco et al., 2005; Myers and Gomez, 2011; Nakamoto et al., 2004; O'Dushlaine et al., 2011; Robertson et al., 2006). Schizophrenia is a neurodevelopmental disorder that involves aberrant brain wiring or disconnectivity due to synaptogenic alterations (Chan et al., 2010a; Corvin, 2010; Hildebrandt et al., 2009; Honer, 1999; Jones and Murray, 1991; Kirov et al., 2005; Maynard et al., 2001). The behavioral disturbances found in schizophrenia involve developmental disorders not only in neurons, but also in glial cells (Jones and Murray, 1991). Specific cell adhesion interactions between neurons, glial cell, and extra cellular matrices are critical for the appropriate migration and placement of cortical neurons (Stanco et al., 2009). Given their role in synaptogenesis, cortical placement, and neurotransmission, CAMs might play a role in the pathophysiology of schizophrenia.

The CAMs pathway reported in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Supplementary Fig. 1) has been reported to be associated with schizophrenia and bipolar disorders susceptibility in genome-wide association study (GWAS) populations (Corvin, 2010; O'Dushlaine et al., 2011). Amino acid changes in these CAMs might affect the protein function and contribute to the risk of developing psychiatric and neurologic disorders. In the present study, we examined the association between missense mutations in CAM genes in the CNS and schizophrenia.

2. Materials and methods

2.1. Subjects

This study was approved by the Ethical Committee for Genetic Studies of Kobe University Graduate School of Medicine and the Ethics Committee of Genetics at the Niigata University School of Medicine. Informed consent was obtained from all participants for this study. All participants were of Japanese descent and were recruited in the Kobe city area or Niigata area, Japan.

Kobe sample is consisted of two batches of subjects. The first schizophrenia group consisted of 278 unrelated patients (124 males; mean age ± SD, 48.4 ± 10.5 years; 154 females: 51.7 ± 12.6 years). The first control group consisted of 284 unrelated healthy volunteers (123 males; mean age ± SD, 44.0 ± 15.6 years; 161 females: 51.2 ± 15.5 years). There were no significant differences in the gender and age distributions between the schizophrenia and the control groups ($\chi^2 = 0.096$, $p = 0.757$ and $t = 1.793$, $df = 560$, $p = 0.074$; respectively). Two SNPs which showed significant association with schizophrenia in the pilot study were then genotyped in the second batch of subjects consisted of 567 schizophrenic patients (296 males; mean age ± SD, 53.4 ± 13.5 years; 271 females; mean age ± SD, 54.3 ± 15.0 years) and 710 controls (334 males; mean age ± SD, 52.5 ± 18.8 years; 376 females; mean age ± SD, 54.3 ± 15.0 years). There were no significant differences in the gender and age distribution between the schizophrenia and the control groups ($\chi^2 = 3.4$, $p = 0.067$ and $t = 0.163$, $df = 1246$, $p = 0.871$; respectively).

Our two batches of sample are not completely independent. Some patients recruited in the first batch are also recruited in the second batch. Therefore we replicated our experiments in another independent group of samples recruited in Niigata area, Japan. The group consisted of 635 schizophrenic patients (345 males; mean age ± SD, 39.8 ± 13.4 years; 290 females; mean age ± SD, 39.7 ± 14.5 years) and 639 controls (341 males; mean age ± SD, 36.7 ± 9.5 years; 298 females; mean age ± SD, 40.2 ± 11.8 years). The gender proportion was not significantly different ($\chi^2 = 0.12$, $p = 0.730$), although the age distribution was slightly different ($t = 2.027$, $df = 1266$, $p = 0.043$).

Psychiatric assessment was conducted in each participant as previously described (Watanabe et al., 2006; Yoshida et al., 2012). In brief, the patients were diagnosed by at least two psychiatrists according to the DSM-IV criteria for schizophrenia on the basis of unstructured interviews and reviews of their medical records. None of the control subjects had present, past or family (up to first degree relatives) histories of psychiatric disorders or substance abuse (excluding nicotine dependence). All control subjects were interviewed and were screened for psychiatric disorders based on an unstructured interview by a psychiatrist.

2.2. SNPs selection and genotyping

First we identified neural adhesion molecule gene in KEGG and consulted NCBI dbSNP (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp) to identify any missense mutations they carried. Among 30 neural adhesion molecule genes identified in KEGG, 14 carried missense mutations according to NCBI dbSNP (Supplementary Table 1). We then selected 15 missense mutations with minor allele frequencies of more than 3% in the Japanese population (based on NCBI dbSNP database) and conducted an association study using our samples of schizophrenic patients and control subjects.

For genotype determination, peripheral blood was drawn from the subjects and the leukocyte DNA was extracted. We used TaqMan assays (Applied Biosystems, Foster City, CA, U.S.A.) for genotyping. We selected pre-designed Taqman SNP genotyping assays from the Applied Biosystems database (http://www.appliedbiosystem.com) for all 15 SNPs examined. Genotyping was performed according to the protocol recommended by manufacturer.

2.3. Data analysis

Genotype distributions were examined for Hardy–Weinberg equilibrium (HWE) and the SNPs were examined for linkage disequilibrium (LD) with Haploview v 4.2 software (Barret et al., 2005) (http://www.broad.mit.edu/mpg/haploview/). Haploview was also used to determine allelic/haplotypic frequencies, as well as an association between SNPs or haplotypes and schizophrenia. Permutation tests based on 10,000 replications were performed to calculate corrected P values of allelic or haplotypic analyses for multiple comparisons by the Haploview software, if necessary. Genotype-based association was tested with Cochran–Armitage test for trend. Odd ratios were calculated with the minor allele regarded as the risk allele. Statistical significance was defined at $p<0.05$. Power analysis was calculated with the program PS v2.1.31 (Dupont and Plummer, 1998).

3. Results

A nominally significant difference was observed for both genotypic and allelic distributions of rs2298033 in the *ITGA8* gene between the schizophrenia and control groups ($Z = 2.8$, $p = 0.005$ and $\chi^2 = 7.32$, $p = 0.007$, respectively) (Table 1). Gender based analysis revealed only female population showed a difference ($Z = 2.6$, $p = 0.010$ and $\chi^2 = 6.54$, $p = 0.011$, respectively; $OR = 0.500$; 95% $CI = 0.292–0.857$). Gender-based analysis also revealed a significant difference in both genotypic and allelic distributions of rs2802808 in the *NFASC* gene in the female population ($Z = 2.0$, $p = 0.044$ and $\chi^2 = 4.5$, $p = 0.034$, respectively) which was not observed in the analyses of all subjects (Table 2). However, the observed differences did not withstand correction for multiple comparisons. The genotypic and allelic distributions of the other 13 SNPs examined were not significantly different between the control and schizophrenia groups, although these negative results might be due to a lack of power in the pilot study. The distributions of all 15 SNPs examined were in HWE for both the schizophrenia and control groups.

In the second batch of subjects, the genotypic and allelic distributions of rs2298033 remained significantly different in both overall subjects ($Z = 2.6$, $p = 0.0086$ and $\chi^2 = 6.6$, $p = 0.010$, respectively) and the female subgroup ($Z = 2.5$, $p = 0.0115$ and $\chi^2 = 6.4$, $p = 0.0114$, respectively), but not the genotypic and allelic distributions of rs2802808. The differences withstood correction for multiple comparisons. Although