主論文の要旨

Novel rare variants in F-box protein 45 (*FBXO45*) in schizophrenia

（統合失調症に影響を与える稀な新規変異をF-box protein 45 (*FBXO45*) 遺伝子内に同定した）

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Background

Schizophrenia (SCZ) is a severe psychiatric disorder with a lifetime prevalence of around 1% and a heritability of 64%. Despite high heritability, the genetic basis of SCZ remains largely unknown despite many years of researches. The genetic architecture of SCZ has been explored through genome-wide association studies (GWAS), rare structural variant studies, and next-generation sequencing (NGS). GWAS have identified common variants with extremely small effects on risk and have clarified that heritability of SCZ cannot be explained only by such common variants. The rare variant model is supported by rare structural variant studies in which individual rare copy number variants (CNVs) with large effects increase susceptibility to SCZ. NGS analysis suggested that some SCZ cases are caused by highly penetrant de novo variants. The ubiquitin ligase F-box protein 45 (*FBXO45*) is a member of the F-box protein family and required for normal synaptogenesis, axon navigation, and neuronal migration in developing central and peripheral neurons through ubiquitylation. FBXO45 also negatively regulates neurotransmission in mature hippocampal neurons through ubiquitylation. Two proteins, FSN-1 and Fsn, which are the invertebrate homologues of FBXO45, were reported to regulate presynaptic differentiation and terminal synaptic growth through ubiquitylation proteolysis. *FBXO45* is included in the 3q29 microdeletion region that confers a significant risk for schizophrenia, as shown by rare structural variant studies. Thus, *FBXO45* is considered a prominent candidate for mediating schizophrenia pathogenesis. Here, we investigated rare, deleterious single nucleotide variants (SNVs) as well as small insertions and deletions (INDELs) in *FBXO45* that may contribute to schizophrenia susceptibility.

Method

Using Sanger sequencing, we performed mutation screening in *FBXO45* exon regions in 337 schizophrenia patients (mean age 49.3 ± 14.6 years, male/female = 200/137). Only novel missense or nonsense SNVs, splicing variants, and small (< 900 base pairs) INDELs were followed up with a genetic association study in an independent sample set of 601 schizophrenia patients (mean age 52.2 ± 15.0 years, male/female = 355/246) and 916 controls (mean age 38.9 ± 15.5 years, male/female = 386/530), a case report for assessing the clinical consequence of the mutations, a pedigree study for measuring mutation inheritance in the proband's family. In order to evaluate mutation effect on protein structure and function, we performed bioinformatics analyses including (1) localization of the protein domain with the Human Protein Reference Database (http://www.hprd.org/index_html), (2) prediction and comparison of secondary and tertiary protein structure changes with the I-TASSER algorithm and UCSF Chimera, (3) prediction of qualitatively functional effects, i.e., benign/possibly damaging/probably damaging with Polyphen-2 and PMut software, (4) sequence alignment of F-box proteins with BLAST (http://blast.ncbi.nlm.nih.gov/), and (5) evolutionary conservation with the HomoloGene database (http://www.ncbi.nlm.nih.gov/homologene/). In order to examine mutation transcriptional influence on *FBXO45* expression, we performed mRNA

expression analysis in mRNA expression sample set, comprised 50 SCZ patients (mean age 42.5 ± 11.0 years, male/female = 24/26), 52 healthy controls (mean age 41.7 ± 11.5 years old, male/female = 25/27), one SCZ patient with a rare missense mutation (R108C; 50 years old, male) detected with resequencing analysis and his mother with same mutation (77 years old female).

Result

One heterozygous, novel, and rare missense mutation (R108C) was identified in a single schizophrenia patient and in his mother. The mother of the proband who carried the R108C variant had no history of medical or mental illness, but the father suffered from Alzheimer's disease. At age 20, this patient was diagnosed with paranoid schizophrenia and carried some clinical features of 3q29 deletion phenotypes, including premorbid IQ decline. With follow-up genotyping, this mutation was not found in either the schizophrenia group (0/601) or the healthy control group (0/916). Using the combined resequencing and genotyping samples, the R108C mutation was not statistically overrepresented in SCZ patients compared to controls. Bioinformatics analyses predicted that R108C probably pathologically impacted function of the FBXO45 protein, and resulted in a reversed hydrophobic distribution in the alpha helix next to the SPRY domain. According to conservation analysis, the protein and DNA sequences of *FBXO45* in different species are highly conserved from *Caenorhabditis elegans* to mammals (over 90% identical amino acids between human and mouse). R108C was located in an evolutionarily conserved region. The relative expression of FBXO45 in SCZ case with R108C mutation was relatively low when compared to 50 schizophrenia patients and 52 healthy controls. But the relative expression level of *FBXO45* did not show a nominally significant difference among the 50 SCZ patients and 52 controls ($P = 0.36$, directional test, with the Mann-Whitney *U* test).

Conclusion

One heterozygous, novel, and rare missense mutation (R108C) in schizophrenia was found by our resequencing analysis and genetic association study. Because the R108C mutation was a very rare variant in our sample set, we could not determine its significant association with SCZ or estimate its odds ratio. A further study with a larger sample size is needed to precisely verify the odds ratio of the R108C mutation in SCZ, and reconsider an association between mutation and disease. The R108C mutation in *FBXO45* might be a rare variant in schizophrenia with modest effect because it may disrupt the structure and function of the FBXO45 protein, proved by our follow-up analyses. Our findings also suggest that *FBXO45* may be a new attractive candidate gene for schizophrenia.