主論文の要旨

Resequencing and Association Analysis of *PTPRA*, a Possible Susceptibility Gene for Schizophrenia and Autism Spectrum Disorders

（統合失調症と自閉症スペクトラム障害の可能な感受性遺伝子
ー*PTPRA* のリシーケンスと関連解析）

名古屋大学大学院医学系研究科　細胞情報医学専攻
脳神経病態制御学講座　精神医学分野
（指導：尾崎 紀夫　教授）
邢 婧睿（XING Jingrui）

Introduction

Schizophrenia (SCZ) is a genetically heterogeneous disorder with heritability estimated at up to 80%. In recent years, research projects focusing on common variants have failed to explain the majority of the heritability of SCZ. Subsequently, great interest has been drawn to rare (minor allele frequency, MAF < 1%) missense mutations. Both SCZ and ASD are recognized as neurodevelopmental disorders, and are reported to have a major overlap of genetic risk.

The human protein tyrosine phosphatase receptor type A (*PTPRA*) gene encodes the enzyme receptor-type tyrosine-protein phosphatase alpha (RPTP-α), a member of the protein tyrosine phosphatase (PTP) family that is involved in numerous neurodevelopmental processes related to the pathogenesis of SCZ and ASD.

Multiple lines of biological evidence implicate the *PTPRA* gene in the etiology of SCZ or ASD, such as linkage studies, GWAS, copy number variation (CNV) studies, expression studies using postmortem brains as well as LCLs, and animal models.

Given the aforementioned studies suggesting the association between *PTPRA* and SCZ/ASD, we decided to sequence the exonic areas of the gene in search for rare, protein-altering mutations that may further strengthen the evidence implicating *PTPRA* as a risk gene for these neurodevelopmental disorders.

Materials and methods

Participants

Two independent sample sets were used in this study (Table 1). The first set, comprising 382 SCZ patients (mean age=53.6±14.2; male=56.5%;), was sequenced for missense rare variants, including single nucleotide polymorphisms (SNPs), small InDels and splicing site variations. The second, larger set, comprising 944 SCZ patients (mean age=50.4±15.6, male=58.7%), 336 ASD patients (mean age=19.3±10.0, male=77.1%), and 912 controls (mean age=39.1±15.9, male=44.5%), was used for association analysis of variants detected in the first phase.

All participants in this study were recruited in the Nagoya University Hospital and its associated institutes. Patients were included in the study if they (1) met DSM-5 criteria for SCZ or ASD and (2) were physically healthy. Controls were selected from the general population and had no personal or family history of psychiatric disorders. All subjects were unrelated, living in the central area of the Honshu island of Japan, and self-identified as members of the Japanese population.

Resequencing and Data Analysis

We included only coding regions and 3'UTR (exons 8-28) (Fig. 2). Genomic DNA was extracted from whole blood or saliva using QIAGEN QIAamp DNA blood kit or tissue kit. Primers covering all the target exons were designed with the Primer-BLAST tool by NCBI and tested for validity with UCSC In-Silico PCR. The Takara LA taq Kit was used for PCR amplification, and products were cleaned up with Illustra Exonuclease I and Alkaline Phosphatase. After that, Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit. Upon the initial discovery, for all variants, we used Sanger sequencing to confirm the detection. Sequenced samples were read on an Applied Biosystems 3130xL Genetic Analyzer. Mutation detection was performed with Mutation Surveyor. The mutation calls were then revalidated for confidence.

Association Analysis

Missense and 3'UTR mutations with MAF<1% were picked up for the association stage. Custom TaqMan SNP genotyping assays were designed and ordered from Applied Biosystems. Allelic discrimination analysis was performed on an ABI PRISM 7900HT Sequence Detection System. Differences in allele and genotype frequencies of the mutations were compared between SCZ patients/controls and ASD patients/controls using Fisher's exact test (one-tail), with a threshold of significance set at $p<0.05$.

Results

Mutation Screening Step

Eight rare mutations consisting of 2 missense SNPs, 4 synonymous SNPs and 2 variations located in the 3'UTR area were identified within the target exons (Table 2), 4 of which were not previously reported in dbSNP Build 139, the 1000 Genomes Project, or the NHLBI Exome Sequencing Project (ESP) Variant Server. All detected mutations were heterozygous.

Association Analysis

Two missense mutations, rs61742029, which had been previously observed only in the Han Chinese population, L59P, a novel variant, as well as the 174620_174623dupTGAT mutation were validated for association with SCZ and/or ASD in stage 2 (Table 3). Although we were unable to detect significance with our sample sets, it is worth noting that L59P was only present in the SCZ patient group.

Evolutionary Conservation Analysis

Conservation status of rs61742029 and L59P in 11 common species was investigated using Mutation Taster. Results showed that the amino acids corresponding to the mutations in RPTP-α were highly conserved among different species (Table 4).

In Silico Functional Effects Prediction

Possible functional implications brought by amino acid changes due to the 2 missense mutations were analyzed with PolyPhen-2, PMut and SIFT. (Table 5)

3'UTR Motif Prediction

174620_174623dupTGAT, a small duplication discovered in the 3'UTR area, was predicted by RegRNA 2.0 to be located within a human Musashi Binding Element (MBE).

Discussion

Main Findings

We were unable to detect a statistically significant association for any of the 3 mutations; this may be attributed partially to the low frequency of rare mutations in the population. However, according to our estimation using CaTS, the power calculator for two-stage association studies, it would require a sample size of around 25,000 cases and controls for the study to obtain possible significance. Also, L59P was only detected among SCZ patients in our sample, which infers possible connection of this mutation to the disorder. The evolutionary conservation status of the locus also indicates its biological importance.

Recent studies have discussed the limited impact of protein-coding variants detected in exome resequencing projects, attributing it partly to the fact that most associated variants alter gene expression rather than protein structure. These findings may help explain the lack of association for the 2 missense mutations we detected, while hinting that 174620_174623dupTGAT, predicted to be located within an expression-regulating element, may have a more significant effect.

Additionally, an increasing amount of evidence suggests that genetic risks for SCZ and ASD may not be conferred by the effects of individual variants alone, but also the amplifying interactions between multiple susceptibility loci. Thus it may be interesting to sequence the mutation carriers for additional related variants in future.

Limitations

Several limitations should be considered when interpreting the results of our study. The single candidate gene paradigm for a gene with less than robust ties to schizophrenia may have been one of the reasons leading to negative results. Besides, the Sanger method it employed predetermined its relatively small sample size and detection power in contrast with next generation resequencing. In addition, we did not have lymphoblastoid cell lines (LCLs) from the mutation carriers for expression analysis or blood samples from their family members for pedigree study. Therefore, we were unable to follow up the results with further biological evidence. Moreover, some potentially interesting regions of the PTPRA gene, such as the promoter, 5'UTR, and most of the intronic areas, were not sequenced (the rare intronic mutations we detected close to the exons can be viewed in the Supplemental files).

Conclusion

In conclusion, our study did not detect any rare missense mutations within the PTPRA gene in our samples that showed statistical association with SCZ or ASD. Nonetheless, some potentially interesting variants were identified that might increase the susceptibility of their carriers to the disorders. Also, our results may help provide genetic clues for the involvement of the PTPRA gene in the pathogenesis of psychiatric disorders.