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

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Abstract

Background: The PTPRA gene, which encodes the protein RPTP-α, is critical to neurodevelopment. Previous linkage studies, genome-wide association studies, controlled expression analyses and animal models support an association with both schizophrenia and autism spectrum disorders, both of which share a substantial portion of genetic risks.

Methods: We sequenced the protein-encoding areas of the PTPRA gene for single nucleotide polymorphisms or small insertions/deletions (InDel) in 382 schizophrenia patients. To validate their association with the disorders, rare (minor allele frequency <1%) missense mutations as well as one InDel in the 3’UTR region were then genotyped in another independent sample set comprising 944 schizophrenia patients, 336 autism spectrum disorders patients, and 912 healthy controls.

Results: Eight rare mutations, including 3 novel variants, were identified during the mutation-screening phase. In the following association analysis, L59P, one of the two missense mutations, was only observed among patients of schizophrenia. Additionally, a novel duplication in the 3’UTR region, 174620_174623dupTGAT, was predicted to be located within a Musashi Binding Element.

Major Conclusions: No evidence was seen for the association of rare, missense mutations in the PTPRA gene with schizophrenia or autism spectrum disorders; however, we did find some rare variants with possibly damaging effects that may increase the susceptibility of carriers to the disorders.

Introduction

Schizophrenia (SCZ) is a genetically heterogeneous disorder with heritability estimated at up to 80% [1]. In recent years, although research projects such as large-scale genome-wide association studies (GWAS) have focused on common variants, they have failed to explain the majority of the heritability of SCZ [2,3]. Subsequently, great interest has been drawn to rare (minor allele frequency, MAF <1%) missense mutations as potentially important contributing factors to the ‘missing heritability’ [4,5].

The concept of Autism Spectrum Disorders (ASD) has been defined in the newly released Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) to include previous diagnoses of autistic disorder, Asperger’s syndrome and PDD-NOS ( pervasive developmental disorders not otherwise specified) [6]. Both SCZ and ASD are recognized as neurodevelopmental disorders, and are reported to have a major overlap of genetic risk, especially from de novo, deleterious mutations, [7–10] although further research concerning implicated loci and/or genetic risk factors (i.e., copy number variants [CNV], insertion/deletions, and single nucleotide variants) is required.
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 such as myelination, radial neuronal migration, cortical cytoarchitecture formation and oligodendrocyte differentiation [11–14]. Moreover, RPTP-α is also functionally involved in the neuregulin 1 (NRG1) signaling pathway, which regulates neurodevelopment as well as glutamatergic and gamma-aminobutyric acid–ergic neurotransmission [15–17]. The NRG1 gene, together with two other genes in the same pathway—ERBB4, which encodes a downstream tyrosine kinase receptor[16–19], and PTPRZ1, which encodes an ERBB4-associated protein tyrosine phosphatase[19]—have been reported by some studies to be associated with SCZ [20–22].

Multiple lines of biological evidence implicate the PTPRA gene in the etiology of SCZ or ASD. Previous linkage studies conducted in 270 Irish high-density families (p = .0382) and an inbred, Arab Israeli pedigree of 24 members (LOD score = 2.56 at 9.53 cM) have pointed to the area that harbors the gene [23,24]. A GWAS comprising 575 cases and 564 controls of the Japanese ethnicity showed an association between polymorphisms within the PTPRA gene and SCZ (best uncorrected p = .002), albeit not at the level of genome-wide significance [25]. This result was followed by a replication study of 890 cases and 829 controls, which further confirmed the association (p = .04, p = .0008 for pooled analysis of first and second stages) [26]. Patients carrying copy number variations (CNVs) within the gene have been reported to suffer from autism, or have delayed language and speech development or stereotypical behaviors [27]. Reduced PTPRA expression levels have been observed in postmortem brains from patients with SCZ when compared to brains from healthy controls (13% decrease; p = .018). In the same study, a significant difference in the expression of mRNA levels of one alternative splicing variant when compared to brains from healthy controls (13% decrease; p = .018). In the same study, a significant difference in the expression of mRNA levels of one alternative splicing variant was observed in postmortem brains from patients with SCZ (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 (first-degree relatives only based on the subject’s interview). The selection was based on the following: (1) questionnaire responses from the subjects themselves during the sample inclusion step; or (2) an unstructured diagnostic interview conducted by an experienced psychiatrist during the blood collection step. All subjects were unrelated, living in the central area of the Honshu island of Japan, and self-identified as members of the Japanese population. The Ethics Committees of the Nagoya University Graduate School of Medicine approved this study. Written informed consent was obtained from all participants. In addition, the patients’ capacity to consent was confirmed by a family member when needed. Individuals with a legal measure of reduced capacity were excluded.

Resequencing and Association Analysis of the PTPRA Gene

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.

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Resequencing and Data Analysis

The human PTPRA gene is located at Chromosome 20: 2,844,830–3,019,320 and has a total of 28 exons (Ensembl release 73; Genome assembly: GRCh37; Transcript: ENST00000380393) (Fig. 1). 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 (QIAGEN Ltd, Hilden, Germany). Primers for 10 amplicons ranging from lengths of 700 to 3000 bps covering all the target exons were designed with the Primer-BLAST tool by NCBI [http://www.ncbi.nlm.nih.gov/tools/primer-blast/] and tested for validity with UCSC In-Silico PCR (http://genome.ucsc.edu/cgi-bin/hgPcr). The Takara LA taq Kit (Takara Bio Inc., Shiga, Japan) was used for PCR amplification, and products were cleaned up with Illustra Exonuclease I and Alkaline Phosphatase (GE Healthcare & Life Science, Little Chalfont, United Kingdom). After that, Sanger sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, United States). Upon the initial discovery, for all variants, we used Sanger sequencing to confirm the detection.

Table 1. Profiles of participants in the resequencing and association sample sets.

|                     | Schizophrenia | Association Study | ASD | Control | Total |
|---------------------|---------------|-------------------|-----|---------|-------|
| **Total**           | 382           | 944               | 336 | 912     | 2192  |
| **Male**            | 216 (56.5%)   | 554 (58.7%)       | 259 (77.1%) | 406 (44.5%) | 1037 (47.3%) | 1253 (48.7%) |
| **Female**          | 166 (43.5%)   | 369 (39.1%)       | 77 (22.9%) | 503 (55.2%) | 1131 (51.6%) | 1297 (50.4%) |
| **Mean Age (years)**| 53.6 ± 14.2   | 50.4 ± 15.6       | 19.3 ± 10.0 | 39.1 ± 15.9 | 44.9 ± 18.7 | 42.3 ± 18.7 |

Note: Some samples in the association study group were not identified by sex.
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Sequenced samples were read on an Applied Biosystems 3130xL Genetic Analyzer. Mutation detection was performed with Mutation Surveyor (Softgenetics, State College, PA, USA). 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. Due to the altering effects that splice site variants have on the structure of mRNAs, and consequently the production of the protein, [36,37] they were also included in the association analysis if they met the MAF criteria.

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 (Applied Biosystems, Foster City, California, United States). 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 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 and PMut. According to the results, the mutation L59P, which was only observed in schizophrenia patients, was predicted to be mostly benign, while rs61742029 showed a high probability of pathogenicity in PolyPhen-2.

**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), an evolutionarily conserved region shown to affect neural cell differentiation through its mRNA translation regulator properties.

**Clinical Information of the Carriers of Mutation L59P and 174620_174623dupTGAT**

The patient carrying the PTPRA L59P mutation was a male diagnosed with SCZ at the age of 19. The patient was born in 1947 had a normal course of development during childhood. In early 1966, he started to suffer from auditory hallucinations, and soon withdrew into an indoor lifestyle. His family reported him being irritated when visited, as well as behaving improperly in public. He was promptly diagnosed and admitted to a psychiatry ward in the same year, and spent the rest of his life living in a hospital. A remarkable improvement was observed in his positive symptoms after admission and administration of antipsychotic drugs; however, he remained secluded, hardly communicating with people around him. At the time of his enrollment in the study, he was 162 cm tall.
and weighed 48 kg. No comorbid physical or mental illnesses were present. He had 3 children, among whom, one daughter had a mild intellectual disability at the age of 27, while he was enrolled in high school. He had a normal conception and birth, born to a 28-year-old father and 27-year-old mother. His father died when he was 3. Delayed intellectual development was observed since his childhood, with reports of illiteracy, hyperactivity, poor concentration and low performance at school. He subsequently dropped out of high school in his first year and started attending a technical school. After graduation, not being able maintain a steady job frequently. He presented at onset with hallucinations, persecutory delusions, and psychomotor excitement, and was subjected to involuntary commitment due to harmful behavior to others as a result of his delusions. At the time of recruitment, he was 61 years old, with a chronic condition of hypertension, and was subjected to involuntary commitment due to poor insight and lack of adherence to treatment. At the time of recruitment, she was 61 years old, with a chronic condition of hypothyroidism. She died in 2012 at the age of 62.

The patient carrying the L59P mutation was a female diagnosed with SCZ at the age of 34. No childhood development abnormalities were reported, but she was noted to have a history of irritability/aggressive tendencies in high school. Since onset, she had experienced auditory hallucinations and persecutory delusions, as well as continued irritability and aggression. Despite the efficacy of antipsychotic drugs on her positive symptoms, the patient suffered numerous relapses throughout her course of illness due to poor insight and lack of adherence to treatment. At the time of recruitment, she was 61 years old, with a chronic condition of diabetes and no comorbid mental conditions. She died in 2012 at the age of 62.

Table 3. Association analysis results of two rare missense mutations and one 3'UTR variant.

| Mutation | Genotype Counts (Resequencing) | Genotype Counts (Association) | P Value b |
|----------|---------------------------------|-------------------------------|-----------|
|          | 171999G>GA, 673V>VI             | 0/3 | 0/2 | 0/4 | 0.3276 | 0.2829 |
|          | 101281T>TC, 59L>L/P             | 0/2 | 0/0 | 0/1 | 1.0000 | 1.0000 |
|          | 174620_174623dupTGAT           | 0/1 | 0/0 | 0/1 | 0.4914 | 1.0000 |

Notes:
a: Homozygote of minor allele/heterozygote/homozygote of major allele.
b: Calculated using Fisher’s exact test, one-tailed.
Ctrl: healthy controls.
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Main Findings

In this study, we sequenced the encoding regions, splicing sites, and 3'UTR region of the PTPRA gene in 382 SCZ patients using the Sanger sequencing method, and discovered 8 rare variants. We then conducted association analysis in a much larger sample set for the 2 rare, missense mutations and one 3'UTR InDel identified during the mutation-screening phase in order to investigate their relationship with SCZ and/or ASD.

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 [http://www.sph.umich.edu/csg/abecasis/CaTS/], it would require a sample size of around 25,000 cases and controls for the study to obtain possible significance [39,40]. 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. [10]

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 [41–44]. Thus it may be interesting to sequence the mutation carriers for additional related variants in future.

Discussion

To our knowledge, this is the first study that systematically screened all coding regions and 3'UTR of the PTPRA gene for rare variants in SCZ patients and assessed the association of identified mutations in such a study with SCZ/ASD.
Table 4. Evolutionary conservation information for rs61742029 and L59P

| Mutation | Species          | Match          | Gene             | AA       | Alignment                  |
|----------|------------------|----------------|-------------------|----------|----------------------------|
| L59P     | Human            | —              | ENST00000380393   | 59       | K T S N P T S S L T S     |
|          | Mutant           | Not conserved  | —                 | 59       | K T S N P T S S L T S *P  |
|          | P. Troglodytes   | All identical | ENSPTRG000000033879 | 59       | K T S N P T S S L T S     |
|          | M. Mulatta       | All identical | ENSMMUG00000005878 | 59       | K T S N P T S S L T S     |
|          | F. Catus         | All identical | ENSFCAG00000019232 | 59       | K T S S P A S S V T S     |
|          | M. Musculus      | All identical | ENSMUSG00000027303 | 59       | K T S N S T S S V I S     |
|          | G. Gallus        | All identical | ENSGALG00000015995 | 56       | N V S - - - S P M T T     |
|          | T. Rubripes      | All identical | ENSTRUG00000014770 | 99       | P T P S P A S D G T L     |
|          | D. rerio         | Not conserved  | ENSDARG00000001769 | 101      | P P V V P P A V P I *P  |
|          | D. Melanogaster  | No homologue   | —                 | N/A      |                            |
|          | C. Elegans       | No alignment   | C09D8.1           | N/A      |                            |
|          | X. Tropicalis    | All conserved  | ENSXETG00000017982 | 71       | T T A P F T T T T R A     |
| rs61742029| Human            | mutated        | —                 | 664      | L K K E E E C E S Y T     |
|          |                 | all conserved  |                   | 664      | S Y T                      |
|          | P. Troglodytes   | all identical | ENSPTRG000000033879 | 673      | L K K E E E C E S Y T     |
|          | M. Mulatta       | all identical | ENSMMUG00000005878 | 673      | L K K E E E C E S Y T     |
|          | F. Catus         | all identical | ENSFCAG00000019232 | 674      | L K K E E E C E S Y T     |
|          | M. Musculus      | all identical | ENSMUSG00000027303 | 700      | L K K E E E C E S Y T     |
|          | G. Gallus        | all identical | ENSGALG00000015995 | 680      | L K K E E E C E S Y T     |
|          | T. Rubripes      | all identical | ENSTRUG00000014770 | 710      | Y T                        |

*Marks the position of the amino acid change due to mutation.
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areas, were not sequenced (the rare intronic mutations we detected close to the exons can be viewed in Table S1).

Conclusion
In conclusion, our study did not detect any rare missense mutations within the \textit{PTPRA} gene in those 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 \textit{PTPRA} gene in the pathogenesis of psychiatric disorders.

Supporting Information

Table S1 Rare intronic mutations identified during the resequencing stage. \textsuperscript{a}: Based on NCBI build 37.1. \textsuperscript{b}: Based on NCBI Reference Sequence NC_000020.10. All mutations are heterozygous.

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\textbf{Author Contributions}

Conceived and designed the experiments: JX BA MI NI NO. Performed the experiments: JX CW HK YT. Analyzed the data: JX SK AY YN TK IK BA NO. Contributed reagents/materials/analysis tools: JX YU TO BA MI YN TK IK BA NO. Wrote the paper: JX SK AY YN TK MB IK YU TO BA NO.

\textbf{Table S1} Rare intronic mutations identified during the resequencing stage. \textsuperscript{a}: Based on NCBI build 37.1. \textsuperscript{b}: Based on NCBI Reference Sequence NC_000020.10. All mutations are heterozygous.
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