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Possible role of rare variants in Trace amine associated receptor 1 in schizophrenia

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ABSTRACT

Schizophrenia (SZ) is a chronic mental illness with behavioral abnormalities. Recent common variant based genome wide association studies and rare variant detection using next generation sequencing approaches have identified numerous variants that confer risk for SZ, but etiology remains unclear propelling continuing investigations. Using whole exome sequencing, we identified a rare heterozygous variant (c.545G > T; p.Cys182Phe) in Trace amine associated receptor 1 gene (*TAAR1* 6q23.2) in three affected members in a small SZ family. The variant predicted to be damaging by 15 prediction tools, causes breakage of a conserved disulfide bond in this G-protein-coupled receptor. On screening this intronless gene for additional variant(s) in ~800 sporadic SZ patients, we identified six rare protein altering variants (MAF < 0.001) namely p.Ser47Cys, p.Phe51Leu, p.Tyr294Ter, p.Leu295Ser in four unrelated north Indian cases (n = 475); p.Ala109Thr and p.Val250Ala in two independent Caucasian/African-American patients (n = 310). Five of these variants were also predicted to be damaging. Besides, a rare synonymous variant was observed in SZ patients. These rare variants were absent in north Indian healthy controls (n = 410) but significantly enriched in patients (p = 0.036). Conversely, three common coding SNPs (rs8192621, rs8192620 and rs8192619) and a promoter SNP (rs60266355) tested for association with SZ in the north Indian cohort were not significant (P > 0.05).
TAAR1 is a modulator of monoaminergic pathways and interacts with AKT signaling pathways. Substantial animal model based pharmacological and functional data implying its relevance in SZ are also available. However, this is the first report suggestive of the likely contribution of rare variants in this gene to SZ.

1. Introduction

Schizophrenia (SZ) is a common neuropsychiatric disorder with ~60–83% heritability (Cannon et al., 1998; Lichtenstein et al., 2009) and about 1% life time morbid risk. Evidence from twin, adoption and familial studies supports the contribution of both genetic and environmental factors (Sullivan et al., 2003), but the etiology of this complex disorder remains elusive. Positive symptoms in SZ characterized by hallucination, delusion and disorganized speech and behaviour are believed to be caused by hyperactivity in dopaminergic neurons of subcortical mesolimbic area (Abi-Dargham and Moore, 2003; Heinz et al., 2003), whereas negative symptoms (emotionless, flattened affect and apathetic behaviour/attitude) and cognitive symptoms (impaired executive functions, attention and memory) are ascribed to dopamine deficiency in the prefrontal cortex (PFC) (Abi-Dargham and Laruelle, 2005; Abi-Dargham and Moore, 2003; Heinz et al., 2003) and to some extent *N*-methyl-D-aspartate receptor (NMDAR) hypofunction (Coyle and Tsai, 2004). Though most of the cases of SZ are sporadic in occurrence, a few familial forms suggestive of major genetic component(s) are also documented. Early genome wide linkage scans had identified a number of loci on chr2q,3q,4q,5q,6q,8p,10p,10q and 13q (Riley and Kendler, 2006) linked to the disease. Subsequently, over the last two decades candidate gene based association studies have identified a large number of SNPs associated with this complex phenotype. However, difficulty in replicating these findings within and across populations has been a major limitation in the understanding of genetics and biology of SZ. Recently genome wide association studies (GWAS) have identified, over 100 risk loci (Ripke et al., 2013; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). With the advent of next generation sequencing (NGS) and whole exome sequencing (WES), several studies have identified *de novo* rare protein coding variants in SZ (Fromer et al., 2014; Girard et al., 2011; Guipponi et al., 2014; Gulsuner et al., 2013; McCarthy et al., 2014; Takata et al., 2014; Xu et al., 2011). However, most of these variants though provisionally functional are private and establishing their causative role in SZ has been

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challenging. On the other hand, a recurrent mutation or an independent mutation in phenotypically similar individuals but absent in healthy controls and/or highly penetrant rare variant(s) segregating with disease in a family may provide a strong evidence of causality.

Contrary to the common sporadic forms of SZ, a small proportion of multi member affected familial forms of the disease across ethnic groups have been observed and have been commonly used in the early genome wide linkage analysis. The powerful NGS tools have now enabled reinvestigating such families and interestingly rare variant(s) in genes such as Glutamate Receptor, Metabotropic 5 (*GRM5*) and Low Density Lipoprotein Receptor-Related Protein 1B (*LRP1B*) (Timms et al., 2013), *unc*-13 homolog B (*UNC13B*) (Egawa et al., 2016), SH3 and Multiple Ankyrin Repeat Domains 2 (*SHANK2*) and SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin (*SMARCA1*) (Homann et al., 2016) have been reported, albeit with incomplete segregation. Accordingly, in the present study, we analysed one small SZ family using the WES approach, followed by focused sequencing in two independent SZ cohorts, one of Indian and another of African-American/Caucasian ancestry. We report seven rare protein disturbing variants (MAF < 0.001) in *TAAR1*, a gene extensively investigated for pharmacological attributes in animal models (Revel et al., 2013), that could elevate risk for SZ.

2. Materials and methods

2.1. Sample recruitment

Patients with family history and receiving treatment for psychotic illnesses at Dr. RML Hospital, New Delhi were assessed using the Hindi version of the Diagnostic Interview for Genetic Studies (DIGS) and the Family Interview for Genetic Studies (FIGS) (Deshpande et al., 1998; Kukshal et al., 2013a, 2013b). Additional information was obtained from medical records and consensus diagnoses were assigned using DSM IV criteria. Unaffected members from the multimembers affected family all of north Indian origin were also recruited alongside. In addition, ~1000 SZ cases and ~1050 ethnically matched controls of north Indian origin available in the laboratory and used in a few previous genetic association studies (John et al., 2016; Kukshal et al., 2013a, 2013b) were also included in the study. Further, ~310 sporadic SZ cases mostly of African-American/Caucasian origin (henceforth referred to as American samples) recruited at Western Psychiatric Institute and Clinic (WPIC), University of Pittsburgh School of Medicine, USA were used in this study. Written informed consent was obtained from the participants and the study was approved by the institutional ethical committees of the respective institutions. Venous blood was drawn from each of the individuals for DNA isolation by the phenol chloroform method and used for genetic analysis.

2.2. Whole exome sequencing

A family comprising an affected mother with two affected and two unaffected children (Fig.1) was analysed in this study. WES of four out of five members was performed.

2.3. Exome capture and sequencing

Exome library preparations of DNA from the selected family members were performed using Agilent SureSelect Human All Exon V5 + UTR kit. Sequencing was performed in Illumina HiSeq2000, using paired-end module for 101-bp reads at Axeq Technologies, USA (http://www.axeq.com).

2.4. NGS data processing and variant calling

These were performed using standard tools and software, which are detailed in Supplementary Material under Materials and Methods.

2.5. Variant prioritization

Variants generated by WES were prioritized following the recent guidelines for identifying disease causing variants (MacArthur et al., 2014). Consistent with the goals of the study, common variants (MAF ≥ 0.001) present in public databases such as dbSNPs, 1000 genomes, ExAC browser, CG40, were removed initially. In the next step all the non-protein disturbing variants (introns, UTRs, intergenic, synonymous etc.) were removed. Keeping in mind the suggestive autosomal dominant mode of inheritance in the study family, all the homozygous variants were removed and only the heterozygous variants present in the all three affected individuals and absent in the sequenced unaffected sib in the pedigree were retained. Variants from regions with segmental duplication were also removed (Peng et al., 2013; Wang et al., 2010). Further all variants present in whole exome data of 153 control individuals of Indian ethnicity available in the laboratory were removed. In parallel, KGGSeq (Li et al., 2012) was used for prioritizing the variants as detailed above.

Prioritized variants were then checked for segregation with SZ in the unaffected member (who was not exome sequenced) by targeted sequencing. Custom-made Haloplex enrichment kit (Agilent, USA) used for the library preparation and sequencing performed on Illumina Hiseq2500 at Strand Life Sciences Pvt. Ltd., India (http://www.strandls.com). Segregating variants were further prioritized based on their functional/biological relevance or their prior implication for SZ, based on association, linkage, exome sequencing and animal studies reported in literature. All variant(s) thus identified were confirmed by

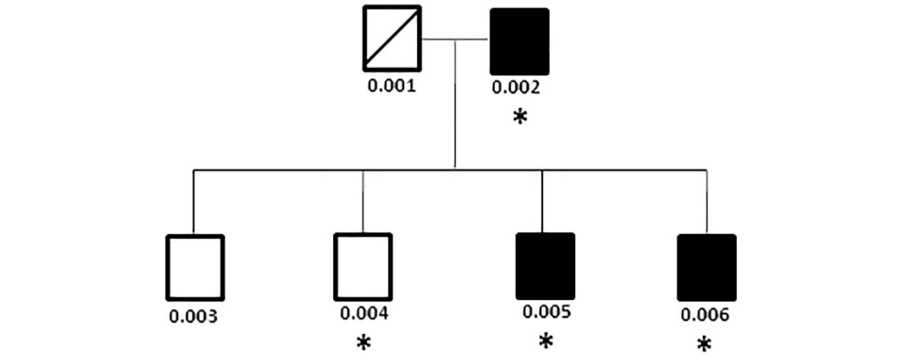

Fig. 1. Shows the pedigree of schizophrenia study family.

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