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Dopaminergic gene polymorphisms and cognitive function in a north Indian schizophrenia cohort

Prachi Kukshal [a,b], Venkat Chowdari Kodavali [c], Vibhuti Srivastava [d], Joel Wood [c], Lora McClain [c], Triptish Bhatia [e], A.M. Bhagwat [b], Smita Neelkanth Deshpande [e], Vishwajit Laxmikant Nimgaonkar [c,f], B.K. Thelma [a,*]

[a] Department of Genetics, University of Delhi South Campus, Benito Juarez Road, New Delhi 110 021, India
[b] C. B. Patel Research Centre, Vile Parle (West), Mumbai, India
[c] Department of Psychiatry, Western Psychiatric Institute and Clinic, University of Pittsburgh School of Medicine, 3811 O'Hara Street, Pittsburgh, PA 15213, USA
[d] Department of Translational Molecular Pathology, MD Anderson Cancer Center, 7435 Fannin Street, Houston, TX 77054, USA
[e] Department of Psychiatry, Dr. RML Hospital, New Delhi 110 001, India
[f] Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, USA

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ABSTRACT

Background: Associations of polymorphisms from dopaminergic neurotransmitter pathway genes have mostly been reported in Caucasian ancestry schizophrenia (SZ) samples. As studies investigating single SNPs with SZ have been inconsistent, more detailed analyses utilizing multiple SNPs with the diagnostic phenotype as well as cognitive function may be more informative. Therefore, these analyses were conducted in a north Indian sample.

Methods: Indian SZ case-parent trios ($n = 601$ families); unscreened controls ($n = 468$) and an independent set of 118 trio families were analyzed. Representative SNPs in the Dopamine D3 receptor (*DRD3*), dopamine transporter (*SLC6A3*), vesicular monoamine transporter 2 (*SLC18A2*), catechol-o-methyltransferase (*COMT*) and dopamine beta-hydroxylase (*DBH*) were genotyped using SNaPshot/SNPlex assays ($n = 59$ SNPs). The Trail Making Test (TMT) was administered to a subset of the sample ($n = 260$ cases and $n = 302$ parents).

Results: Eight SNPs were nominally associated with SZ in either case-control or family based analyses ($p < 0.05$, rs7631540 and rs2046496 in *DRD3*; rs363399 and rs10082463 in *SLC18A2*; rs4680, rs4646315 and rs9332377 in *COMT*). rs6271 at *DBH* was associated in both analyses. Haplotypes of *DRD3* SNPs incorporating rs7631540-rs2134655-rs3773678-rs324030-rs6280-rs905568 showed suggestive associations in both case-parent and trio samples. At *SLC18A2*, rs10082463 was nominally associated with psychomotor performance and rs363285 with executive functions using the TMT but did not withstand multiple corrections.

Conclusions: Suggestive associations with dopaminergic genes were detected in this study, but convincing links between dopaminergic polymorphisms and SZ or cognitive function were not observed.

1. Introduction

Schizophrenia (SZ) is a common, severe disorder with a lifetime prevalence of approximately 1% worldwide (Gottesman, 1982; Saha et al., 2005). Its prevalence was estimated at 4/1000 in India and likely represents an underestimate (Ganguli, 2000). Interactions between genetic and environmental etiological factors provide the most plausible explanations for the relatively high heritability estimates of 70–80% (Owen, 2002; Sullivan et al., 2003; Sullivan, 2005; Lichtenstein et al., 2009). Prior gene mapping studies have identified multiple putative susceptibility loci with genes underlying neurotransmitter pathways being implicated frequently (Allan et al., 2008; Ng et al., 2009; Seeman and Kapur, 2000; Seeman, 2002; Staddon et al., 2005; Dominguez et al., 2007; Talkowski et al., 2006, 2008; Srivastava et al., 2010). Further, neuroimaging studies in SZ patients reveal neuronal disorganization in cortical and limbic regions of the brain and increased dopamine D2 receptor binding (Keshavan et al., 2008). We have previously

* Corresponding author. Tel.: +91 11 24118201; fax: +91 11 24112761.
E-mail addresses: prachi_7@yahoo.com (P. Kukshal), thelmabk@gmail.com (B.K. Thelma).

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reported consistent associations with the gene encoding the dopamine D3 receptor (*DRD3*) and related haplotypes in Indian and US Caucasian samples (Talkowski et al., 2006). Indeed, associations at *DRD3* have been reported recently in two other independent Caucasian samples (Staddon et al., 2005; Dominguez et al., 2007), but were not replicated in a Japanese cohort (Nunokawa et al., 2010).

The dopaminergic gene associations do not feature prominently in recent genome wide association studies (GWAS) (Lencz et al., 2007; Pearson et al., 2007; Wellcome Trust Case Control Consortium, 2007; Sklar et al., 2008; Sullivan et al., 2008; Shi et al., 2009; Stefansson et al., 2008, 2009; The International Schizophrenia Consortium, 2009). On the other hand, the available genome-wide significant associations, such as human zinc finger protein 804A (*ZNF804A*), neurogranin (*NRGN*) and transcription factor 4 (*TCF4*), suggest common alleles of small effect, rare alleles of large/small effect, and complementary analysis of association signals from various genes grouped according to their interactions and pathways may contribute to some risk towards disease etiology (Jia et al., 2010). Thus pathway based candidate gene approach still holds true for genetic studies of SZ. Recently, systematic analyses of eighteen DA genes in US Caucasian samples revealed significant associations with SNPs at *DRD3*, dopamine transporter *DAT* (alias, *SLC6A3*), catechol-*O*-methyltransferase (*COMT*), dopamine beta-hydroxylase (*DBH*) and vesicular monoamine transporter 2 (*SLC18A2*). Some epistatic interactions between pairs of SNPs across these genes were also significant; these associations were replicated in a Bulgarian family based sample. Simulation studies suggested that the replicable associations were unlikely to be due to chance (Talkowski et al., 2008).

Cross population studies could help identify genuine genetic associations and enable fine mapping of disease associated loci. Such studies are infrequent in SZ research (O'Donovan et al., 2009). On the other hand, associations may not be detected consistently across ethnic groups for a number of reasons. Population structure may impact success in gene mapping studies (Novembre et al., 2008), and divergent linkage disequilibrium (LD) patterns may explain "flip-flop" associations, i.e., associations with different alleles of SNPs (Lin et al., 2007). Also, the choice of markers investigated could contribute to the inability to replicate some associations. Single marker association analyses are inefficient as they may reflect only the localized effect of an individual SNP or the polymorphism being analyzed may not be the risk variant and/or in LD with the functional variant (Johnson et al., 2001; Gabriel et al., 2002), or it may have low polymorphism information content. In such situations, approaches considering LD based/multi-marker haplotype analysis could be more informative (Akey et al., 2001; Kamatani et al., 2004; Lin et al., 2004; de Bakker et al., 2005; Dominguez et al., 2007). The multi-marker analyses may also help overcome some differences in LD structure across populations.

Against this background, we tested several SNPs from pharmacologically relevant DA pathway genes mentioned above as well as additional selected SNPs in a large Indian sample. We adopted a multi-marker analysis approach because such analyses may indicate associations even if the causative SNP is not genotyped. Though the LD structure in outbred north Indian populations is reportedly similar to Caucasian populations (Pemberton et al., 2008), the precise LD structure at the genes of interest has not been analyzed extensively in Indian samples. To maximize the chance of capturing the susceptibility locus identified in Caucasians, we employed variable-sized sliding window (VSW) analysis encompassing 2-6 SNPs. A case-control as well as family based study design was employed with the cases being common for both strategies. The dual design was utilized because each type of control has distinct advantages and shortcomings (Bacanu et al., 2000).

Dopamine is integral to cognition, learning and memory, and dysfunctions of the frontal cortical dopamine system have been implicated in both transcript and protein levels during postnatal development (Rothmond et al., 2012). Variations in *DRD2* have been associated with working memory performance (Kellendonk et al., 2006; Bertolino et al., 2010). Selective blockade of dopamine D3 receptors reverses the visual recognition memory deficit and hyperactivity produced by isolation rearing support the potential use of dopamine D3 receptor antagonists to treat schizophrenia (Watson et al., 2011). A role for *COMT* in regulating executive functions and selective attention (Barnett et al., 2007; Solis-Ortiz et al., 2010; Watson et al., 2011) has been reported. Hence we evaluate the DA gene polymorphisms in relation to cognitive functions. The A and B tasks of TMT evaluates the psychomotor and executive functions respectively. The cognitive functions measured by TMT test are highly heritable (Quinones et al., 2009) and relate mainly to executive and psychomotor functions (Bhatia et al., 2007, 2009; Quinones et al., 2009).

2. Methods

2.1. Samples

The study was approved by the Institutional Ethics Committees at Dr Ram Manohar Lohia Hospital, New Delhi, the Lok Nayak Hospital, New Delhi, Delhi University, South Campus, New Delhi and the University of Pittsburgh, Pittsburgh IRB. Written informed consent was obtained from all participants (maternal consent for neonatal samples). The Hindi version of the Diagnostic Interview for Genetic Studies (DIGS), a structured, validated diagnostic interview was administered to each patient as described (Deshpande et al., 1998; Chowdari et al., 2002; Bhatia et al., 2009). Based on this information, consensus diagnoses were established by certified psychiatrists and psychologists using DSM IV criteria. Inter-rater and inter-site diagnostic reliability was checked throughout the study and Kappa values of 0.8 or greater were aimed for (Bhatia et al., 2006).

Participants recruited in the study were confirmed to be of north Indian origin based on language/mother tongue and the geographical location for three generations and were largely drawn from Delhi and neighboring states of Uttar Pradesh, Punjab, Bihar, Haryana, Himachal Pradesh, Uttaranchal, Rajasthan, etc. They included cases, their parents and unrelated community based controls. The latter were composed of neonatal cord blood samples from live births at Lok Nayak Hospital, New Delhi. Mental Illness was evaluated among parents of schizophrenia patients using the Family Interview for Genetic studies (FIGS) (Maxwell, 1992).

2.1.1. Sample characteristics

A total of 601 case-parent trios ($n = 1800$ participants) and 468 controls were analyzed. The sample included patients diagnosed with schizophrenia using DSM IV criteria. Some of the participants ($n = 123$ families) in this study overlapped with those used in our previous genetic association studies (Talkowski et al., 2006). Another 208 samples were shared with another study (Srivastava et al., 2010). An additional 119 north Indian trio sample set was used as an independent sample for analysis of the *DRD3* associations only.

The cognitive functions of the probands, and parents were measured by administering TMT in a family to a subset of cases ($n = 260$) and their healthy parents ($n = 302$).

2.2. Genetic analysis

Genomic DNA was isolated from venous blood samples using the phenol chloroform extraction method and was quantified using