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

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Abstract

Background—Associations of polymorphisms from dopaminergic neurotransmitter pathway genes have 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. The 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.

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

Keywords

Schizophrenia; Dopamine genes; SNPs; association; Haplotypes; cognition

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 & Kapur, 2000; Seeman, 2002; Staddon et al., 2005; Dominguez et al., 2007; Talkowski et al., 2006; Talkowski et al., 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 etal., 2008). We have previously 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 dopamine 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; Stefansson et al., 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). On the other hand, the choice of markers investigated could also 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 the *DRD2* gene 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; Bhatia et al., 2009; Quinones et al., 2009).

METHODS

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).

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).

Genetic Analysis

Genomic DNA was isolated from venous blood samples using the phenol chloroform extraction method and was quantified using the picogreen method. A total of 59 SNPs were analyzed. They were distributed across five DA genes, namely *DRD3 (*3q13.3), *SLC6A3* (alias *DAT*, 5p15.3), *SLC18A2* (alias *VMAT2*, 10q25), *COMT* (22q11.21) and *DBH* (9q34). A list of all the SNPs is provided in Supplementary Table I. Of these, 46 SNPs were obtained from a earlier report on dopamine genes in schizophrenia (Talkowski et al., 2008). These included the four commonly investigated exonic SNPs (rs6280 from *DRD3*, rs4680 from *COMT*, and rs1108580 and rs6271 from *DBH)*. We also included 13 additional SNPs from the HapMap phase II release in this study to achieve more comprehensive coverage. SNPs were assayed using either SnaPshot (Mansour et al., 2005) or SNPlex assays, ABI Biosystems (Tobler et al., 2005) with CEPH samples as positive controls.

Statistical Analysis

The power to detect associations in the study cohort was evaluated using Quanto software (Gauderman, 2006; http://hydra.usc.edu/GxE), assuming an additive model and a disease frequency of 1%. Hardy Weinberg equilibrium (HWE) was examined for each SNP using PLINK (Purcell et al., 2007; http://pngu.mgh.harvard.edu/purcell/plink/). Only the SNPs conforming to HWE ($p > 0.01$) were included in the association analyses. LD values ($r^2>0.8$) were estimated for the genotyped data using the Tagger algorithm in Haploview version 4.1 (Barrett et al., 2005; http://www.broad.mit.edu/mpg/haploview/). Transmission distortion in families was assessed using FBAT software (Laird et al., 2000). The Armitage Trends test (SAS software) was used for case-control analysis. Sliding window haplotypes for 2-6 SNPs were generated for both cases-control and family based data using UNPHASED 3.1.5 (Dudbridge, 2008). A global or omnibus test of haplotype-based association was performed in UNPHASED. This tests log-likelihood ratios under a log-linear model for global p values. Since both common and rare variants and their haplotypes have been reported for association with schizophrenia (Li et al., 2005; Agim et al., 2013) and also in age related macular degeneration (AMD) (Raychaudhuari et al. 2011) the haplotypes frequency cutoff was set at 1% for haplotypes analysis. In the first step, all the markers were analyzed using the default Davidon-Fletcher-Powell (DFP) method in UNPHASED. All the p-values till this step were subjected to multiple corrections and haplotypes that retained significance were reassessed using Nelder & Mead's (NM) method in PLINK (Minor Haplotype ≥1%) for likelihood maximization, since DFP can sometimes converge to error-prone solutions (www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/). There were 505 tests in all, so an alpha value of 0.0001 (0.05/505) or lower was considered

to be significant. PLINK was used to estimate the actual haplotypes and frequencies, since TDT-like counts of transmitted and untransmitted alleles/haplotypes and resulting TDT statistic are not available in UNPHASED. The p-values for individual marker associations and sliding window haplotypes were graphed separately for case-control and family based data for each gene using Graphical Assessment of Sliding P-values (GrASP v.0.82 beta) to present and assess p-values from multiple tests (Mathias et al., 2006; http://research.nhgri.nih.gov/GrASP/).

Linear regression analyses were conducted for both A and B task separately using the Statistical Package for Social Sciences (SPSS Version 16) to test associations with cognitive variables. Since the parent group was older in comparison with the patients, the cognitive scores were adjusted for age. The dependent variables, namely Task A and Task B scores evaluated separately as the dependent variables, with genotypes for individual SNPs as the predictor variables, with gender and diagnosis as covariates.

RESULTS

Quality Control

Over all, more than, 90% genotype data was available for each of the markers in the entire sample set (601 case-parent trios and 468 controls). All markers were in Hardy-Weinberg equilibrium (p>0.01). Four SNPs (rs2134655, rs324030, rs6280 and rs905568 in *DRD3*) were genotyped twice (with different methods) in 123 families reported in the present and a previously published study (Talkowski et al., 2006) and two other SNPs (rs1108580 in DBH and rs4680 in COMT) were common for 208 samples analyzed in another study (Srivastava et al., 2010). The overall genotyping call discordance rate for these SNPs was 3.3%. For the discrepant genotypes, the genotypes generated by SNPlex methodology were checked using Sanger sequencing and were found to be correct.

Linkage disequilibrium (LD)

Using a cutoff value of $r^2 > 0.8$, eight out of eleven tag SNPs for *COMT*, 15 out of 18 tag SNPs for *DBH*, six out of seven tag SNPs for *DRD3*, nine out of ten tag SNPs for *SLC18A2* and 13 tag SNPs for *SLC6A3* were determined to be non-redundant. These SNPs (n=51, see below) were used for multi-marker/ haplotype analysis tests. Pattern of LD for *DRD3* for the north Indian controls used in this study, as well as all Phase II hapmap populations (http://www.hapmap.org/) is provided in Supplementary Figure I. The patterns of LD for all the five genes were generally similar in the Indian and Caucasian datasets (Basu et al., 2003).

Single SNP associations

Allelic associations were observed with four SNPs namely rs363399 C/T (p = 0.05) in *SLC18A2*, rs6271C/T (p = 0.004) in *DBH* and rs4680 A/G (p = 0.05) [OR(95%CI) 1.19[1.0 −1.42]; rs9332377 T/C (p = 0.02) [OR (95%CI) 1.34[1.06-1.71], both in *COMT,* in case-control analysis. Following family based analysis, allelic associations were observed with five SNPs (rs7631540 and rs2046496 in *DRD3*; rs10082463 in *SLC18A2*; rs4646315 in *COMT* and rs6271 in *DBH).* Thus, for rs6271 in *DBH*, nominal associations were noted with both types of analyses. Trends for association were observed at six other SNPs. SNPs with nominally significant associations (uncorrected p<0.05) or trends of association (p<0.1) are presented in Table I, but none remained associated after corrections for multiple comparisons. Allele frequencies for all the analyzed SNPs are presented in Supplementary Table I.

Multi-marker associations

Following sliding window analysis, several trends were observed for global p-values using UNPHASED (Table II, Supplementary Table II). However, only family based haplotype associations at *DRD3* remained significant following corrections for multiple comparisons (Table II). They included 2, 3, 4, 5 and 6 SNP haplotypes beginning with rs7631540, a SNP that was also associated with SZ when analyzed individually (Tables II). For each of the associated windows, estimated haplotype frequencies, the number of transmissions and TDT statistics generated using PLINK and significant ($p<0.05$) transmissions for each of the windows are presented in Supplementary Table III. To evaluate consistency in nomenclature of SNPs, in this disease associated gene, selected control individuals (n=50) were sequenced across the following SNPs: rs7631540, rs2134655, rs3773678, rs324030, rs6280 and rs905568. Rs2046496 was not sequenced as it is tagged to rs7631540 (r2= 0.99) which is further in the regulatory 3′ region.

Analysis of additional independent trio sample (n=119) for DRD3

In view of the highly significant haplotypic associations of *DRD3* markers in family analysis, the results were further tested in a smaller family sample set available to us from the same population. Though no significant associations with individual SNPs were observed, associations in the 2, 3 SNP windows comprising the 3′ region SNPs was observed, (Table II).

Comparison with published analyses of Caucasian samples

Comparison of results from this study with that reported for two US Caucasian samples in a prior study (Talkowski et al., 2008) showed 22 SNPs which were associated in either of the populations but none of them showed consistent association in the Caucasian and Indian populations (Supplementary Table IV). However, only with four SNPs namely rs2134655 and rs324030 in *DRD3*; rs403636 in *SLC6A3* and rs363338 in *SLC18A2,* associated in Caucasian samples, a trend towards association in the Indian samples was observed (Supplementary Table IV). Notably except for rs363338, the other three markers showed association with the opposite allele (Supplementary Table IV).

Power of the study

For the trio, as well as the case-control sample, the power exceeds 80% for an odds ratio of 1.4 if the SNP frequency is between 20-50%.

Cognitive analyses

Taking all the associated SNPs from table I as covariates and Task A and B as outcome variables separately, rs363285 (p=0.025) from *SLC18A2* was associated with Task B of TMT. We also analyzed SNPs having a $p<0.05$ in only family data for cognitive analysis. These included rs2046496 (in LD to rs7631540) from *DRD3*; rs6271 from *DBH*; rs10082463 from *SLC18A2* and rs4646315 from C*OMT*. Only rs10082463 (p=0.027) from *SLC18A2* showed significant association with Task A (Supplementary Table V), but was not significant following multiple corrections.

DISCUSSION

We conducted case-control and family based association analyses of key dopamine genes in a north Indian SZ sample set. Family based analyses overcome artifacts related to population sub-structure as they have an inherent correction for the confounding effects of population stratification since the cases are compared to family based controls (Seltman et al., 2001; Lange et al., 2008). On the other hand, case-control analyses can have greater power in

certain settings (Bacanu et al., 2000). Therefore, associations detected with each method can be credible, provided replications are available and plausible function can be attributed. Following individual analysis of each of 59 SNPs, significant associations were noted in both case-control and family based analyses at rs6271 (+1603C→T), a relatively infrequent non-synonymous SNP located in exon 11 of *DBH* which encodes a non-conservative amino acid change (arg535cys). In view of the low frequency of the minor allele at this SNP, there are relatively few informative transmissions from heterozygous parents, thus diminishing power for the TDT analysis (supplementary Table I). Hence the results should be interpreted with caution. In the same vein, a marginal association with rs4680, an exonic SNP at *COMT* was noted in our case-control sample and has also been reported by several other investigators (Handoko et al., 2005;Nunokawa et al., 2010;Liao et al., 2009; Okochi et al., 2009; Bhakta et al., 2012; Singh et al., 2012). Apart from these exonic variants, nominally significant SNP associations were also noted at all the other genes tested, except at *SLC6A3* (Table I) in either case-control or family based analysis. A larger Indian sample may be required for confirmation.

Occasionally, distinct genetic associations of single SNPs may not be observed but subtle contributions by a combination or a set of SNPs may be found using haplotype based analysis (Haig, 2011). Though a potential disadvantage of this strategy is increased number of multiple tests resulting in type II error (Hunter & Kraft, 2007), haplotype based analyses in north Indian samples are important since in this study, a “best guess selection” was made for tag SNPs based on available data from Hapmap CEU populations (Pemberton et al., 2008; Indian Genome Variation Consortium, 2009; Reich et al., 2009). Using the multi-SNP sliding window approach, notable haplotype based association was observed at *DRD3* (Figure 1). Since rare alleles and haplotypes have shown association with Schizophrenia (Li et al., 2005; Agim et al., 2013) the rare haplotypes frequency was set at 1% for the global haplotypes. Haplotype windows beginning rs3773678, rs2134655 and rs7631540 were significantly associated, suggesting the presence of risk factors in the 3′ region of this gene. Four of the five associated haplotype windows comprised rs6280, an exonic SNP previously nominated as a SZ risk factor (Jonsson et al., 2003; Ma et al., 2008; Hwang et al., 2010; Utsunomiya et al., 2012) was observed for *DRD3*. However, none of the associated haplotypes commenced with rs6280 (Table II). Since we used HapMap Caucasian data to select tag SNPs, it is possible that there may be some key SNP present in the Indian sample, which were excluded due to population differences. Nevertheless, our results are credible because we used a family based approach that is not prone to artifacts following sub-structure. We also applied conservative corrections for multiple comparisons, and similar results were obtained by an independent NM method of analysis. Furthermore, similar trends of haplotype based associations were observed in another smaller, independent sample ascertained identically (n=119 trio families, Table II). The pooled (n=719, 601+119) family data for *DRD3* showed similar associations in all sliding window sizes (1-6), comprising the 3′ SNPs. The 2 and 3 SNP windows starting with rs7631540 showed stronger association (P= 0.0001; p= 0.004 respectively), but the association is weaker in the 4 SNP window after pooling the families for DRD3 (Table II).

Analogous haplotype based associations have been reported at *DRD3* in a Spanish Galician sample (Dominguez et al., 2007). This group reported a 3-SNP haplotype comprising rs7631540 as being the most significantly associated after corrections in case-control analysis. Notably, nominally significant associations with haplotypes comprising rs2134655 & rs7631540 were also noted in our family sample set. Based on case-control analyses, Costas and colleagues recently proposed an operative positive selection for a common *DRD3* haplotype in three independent schizophrenia samples of European origin (Costas et al., 2009). They also concluded that this particular “common but protective haplotype in Europeans” is at intermediate frequencies in other populations, being at the lowest in Sub-

Saharan African populations. Significant association was not observed in an updated meta analysis (Nunokawa et al., 2010) for the haplotype T–T–T–G for the SNPs rs7631540–rs1486012–rs2134655–rs963468 reported by Costas and colleagues but found it less frequently in patients than in control subjects (26.5% vs. 28.6%). Differences between our sample and the European samples may reflect population differences or sample selection.

Of the 59 SNPs studied here, comparable association data were available for 46 SNPs, from our prior analyses for US and Bulgarian (Caucasian) samples (Talkowski et al., 2008). Single SNP associations reported in US sample were not detected in the Indian samples, apart from suggestive trends for rs2134655 and rs324030 at *DRD3;* rs403636 at *SLC6A3* and rs363338 at *SLC18A2.* Indeed, different alleles were over-represented among cases in the Indian and the Caucasian data sets for the first three SNPs (Supplementary Table IV). These differences were confirmed by resequencing rs7631540, rs2134655, rs3773678, rs324030, rs6280 and rs905568 at *DRD3* in 100 chromosomes in our Indian control samples. Such differences and the lack of consistent cross-national replication may indicate a lack of significant SZ risk variants at these loci. Factors such as differences in power and specific differences in LD structure may also contribute to the inconsistency. Other variables such as age dependent associations or subtle differences in allele frequencies could also impact on replicability (Lasky-Su et al., 2008; Liu et al., 2008; Greene et al., 2009). It is also possible that as yet undetected common or rare alleles confer risk for SZ, but were not genotyped in any of the populations. This possibility is supported by our haplotype based analyses at *DRD3* and locus overlap observed with reports from other populations (Staddon et al., 2005; Dominguez et al., 2007; Costas et al., 2009).

While the influence of dopamine on cognition is clearly complex (Takahashi et al., 2008) and may not provide a complete account for the full range of cognitive deficits in schizophrenia, the importance of dopamine to cognition has been demonstrated by studies in which dopamine levels and activity were modulated using different types of pharmacological agents (Condray & Yao, 2011). Administration of amphetamine, improves cognitive performance working memory, attention, and language production processes (Barch & Carter, 2005) and was associated with the significant changes in regional cerebral blood flow (rCBF) among schizophrenia patients receiving antipsychotic medication (Daniel et al., 1991). Administration of the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) to healthy volunteers produced more focused activation of semantic memory, reflected in reduced indirect priming (Kischka et al., 1996), and increased the rate of learning and long-term retention of an artificial vocabulary (Knecht et al., 2004).

Previously *COMT* Val-Met polymorphism has most widely been studied in relation to cognitive functions (Alfimova et al., 2007; Rosa et al., 2010; Solis-Ortiz et al., 2010) with mixed results. On the other hand, an exonic SNP at *DRD3* (rs6280) has been reported to be associated with executive function, but only in individuals with psychoses (Bombin et al., 2008). This association was not observed in our sample. Only two SNPs, namely rs363285 and rs10082463 of *SLC18A2* showed significant association in our samples with task B and A respectively. This may be partly due to the relatively small sample. Another SNP rs363227 from *SLC18A2* which was not associated in our sample set has been shown to be associated for Psychotic disorder in a Dutch population and their unaffected siblings (p=0.04). SNP rs10082463 is in LD to rs363227 (D'0.87; r^2=0.61).

In conclusion, we report on a systematic analysis of SNPS at five DA genes with SZ. An exonic SNP at DBH (rs6271) was nominally in both the case-control and family based analyses in our population and several others were nominally significant. We also report on suggestive haplotype based associations at DRD3 in our ethnically distinct population, which is in consonance with other independent studies. Two SNPs (rs10082463 &

rs363285) of *SLC18A2* were nominally associated with cognition. None of the associations remained significant following corrections for multiple comparisons except a DRD3 2-Marker haplotypes (rs324030-rs6280; p=0.00004). Analysis of larger samples is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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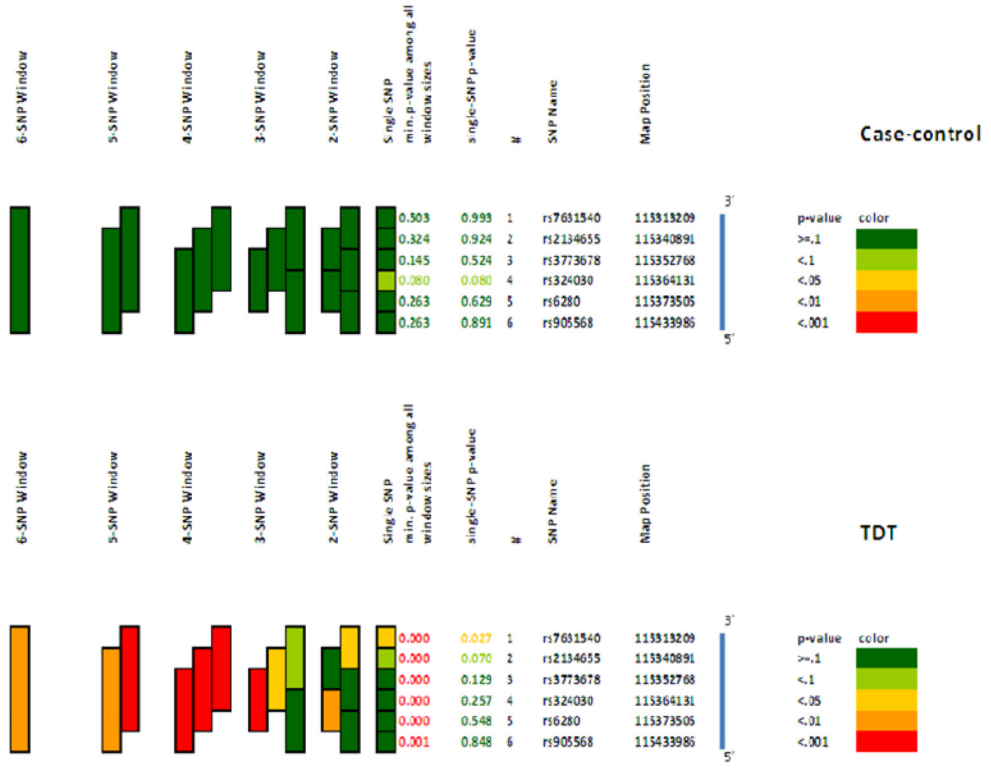

Figure I.

Haplotype based associations at DRD3 illustrated using Graphical Assessment of sliding p-values (GrASP)

Table I

SNPs in dopamine genes with suggestive associations in family based and case-control analyses

Chr	GENE	SNP	position	N	F_ca	F_co	Z1	P1	Z2	P2
3	DRD3	rs7631540	115313209	C	0.555	0.554	0.052	0.959	2.216	0.027
3	DRD3	rs2046496	115317621	C	0.557	0.553	0.165	0.868	2.765	0.006
3	DRD3	rs2134655	115340891	G	0.760	0.758	0.097	0.923	1.812	0.070
3	DRD3	rs324030	115364131	C	0.616	0.653	−1.722	0.084	−1.134	0.257
5	SLC6A3	rs403636	1491354	G	0.876	0.848	1.872	0.058	−0.187	0.852
9	DBH	rs6271	135512095	C	0.998	0.988	2.686	0.004	2.309	0.021
10	SLC18A2	rs363399	118998861	T	0.677	0.633	2.036	0.047	1.837	0.066
10	SLC18A2	rs363338	118999379	T	0.599	0.560	1.798	0.075	1.249	0.212
10	SLC18A2	rs10082463	119011397	A	0.810	0.841	−1.864	0.067	−2.085	0.037
10	SLC18A2	rs363285	119029149	A	0.844	0.817	1.627	0.093	−0.260	0.795
22	COMT	rs4680	18331271	G	0.543	0.586	−1.966	0.049	0.785	0.433
22	COMT	rs4646315	18331897	G	0.766	0.753	0.685	0.483	2.756	0.006
22	COMT	rs9332377	18335692	C	0.824	0.863	−2.447	0.016	−1.025	0.305

N: major allele in north Indian cohort

F_ca, F_co: frequency of cases and controls respectively

Z1, Z2: z scores for case-control comparisons and family based associations, respectively

P1, P2: p values for case-control comparisons and family based associations, respectively

Position: Nucleotide location based on NCBI build 36.3

TABLE II

Sliding window based haplotypes of *DRD3* markers showing significant associations in Case-control and TDT analyses

			C-C(601/468)						Trio (n=601)						Addl. Trio (n=118)						Pooled Trio (n=719)					
#	Map Information	SNP	p,df=1*	2 – mhap , df=3	3 – mhap, p(df)	4 – mhap , p(df)	5 – mhap , p(df)	6 - mhap, p(df)	,df=1*	2 – mhap (df)	3 – mhap , p(df)	4 – mhap , p(df)	5 – mhap , p(df)	6 – mhap , p(df)	,df=1*	2 – mhap df=3	3 - mhap, p(df)	4 - mhap, p(df)	5 - mhap, p(df)	6 - mhap, p(df)	,df=1*	2 – mhap df=3	3 - mhap, p(df)	4 - mhap, p(df)	5 - mhap, p(df)	6 - mhap, p(df)
DRD3																										
1 M	115313209	rs7631540	0.99	0.93(2)	0.554)	0.44(7)	0.60(9)	0.47(13)	0.03	0.01(2)	0.06(4)	6×10^{-4}(8)	5×10^{-7}(13) **	0.008(17)	0.93	4×10^{-3}(3)	0.03(5)	0.21(9)	0.20(10)	0.37(18)	0.04	1×10^{-4}(3) **	0.004(5)	0.23(8)	2×10^{-4}(17)	0.01(21)
2 M	115340891	rs2134655	0.92	0.83(2)	0.26(4)	0.31(5)	0.40(11)		0.07	0.11(2)	0.03(5)	1×10^{-4}(9) **	0.007(13)		0.62	0.20(3)	0.50(6)	0.41(8)	0.36(15)		0.07	0.12(2)	0.46(5)	0.13(11)	0.14(18)	
3 M	115352768	rs3773678	0.52	0.14(3)	0.16(4)	0.40(9)			0.13	0.28(3)	1×10^{-5}(6) **	7×10^{-4}(12) **			0.83	0.89(3)	0.07(7)	0.25(11)			0.15	0.38(3)	0.14(7)	0.09(13)		
4 M	115364131	rs324030	0.08	0.29(3)	0.31(6)				0.26	4×10^{-3}(3)	0.13(6)				0.77	0.006(3)	0.03(7)				0.25	0.17(3)	0.30(7)			
5 M	115373505	rs6280	0.63	0.85(3)					0.55	0.82(3)					0.57	0.15(3)					0.75	0.56(3)				
6 M	115433986	rs905568	0.89						0.85						0.18						0.67					

C-C: case-control

Trio: family consisting of one proband and two parents

Addl. Trio: a replicate set of trio families

Pooled Trio: total of trios and addition trios

2 – mhap: two marker haplotypes generated using UNPHASED. P-values given; 3-mhap: three marker haplotypes generated using UNPHASED. P-values (degree of freedom) given. Similarly, mhap-3, -4, -5 and -6 denote haplotypes incorporating the respective number of SNPs. Haplotypes with a frequency lower than 1% were not included in the analysis.

* p values based on analysis of individual SNPs (see Table 1).

** p values significant after Bonferroni correction of global p value for the respective haplotype (alpha value= 0.0001 for 505 tests)