

ORIGINAL ARTICLE

Gender-specific association of *TSNAX/DISC1* locus for schizophrenia and bipolar affective disorder in South Indian population

Anjanappa Ram Murthy¹, Meera Purushottam¹, Halagur Bhoge Gowda Kiran Kumar¹, Manduva ValliKiran¹, Nithin Krishna¹, Kallahalli Jayramu Sriharsha¹, Yemmiganur Chandrashekar Janardhan Reddy¹, Saurabh Ghosh² and Sanjeev Jain¹

Genetic association studies have implicated the *TSNAX/DISC1* (disrupted in schizophrenia 1) in schizophrenia (SCZ), bipolar affective disorder (BPAD) and major depression. This study was performed to assess the possible involvement of *TSNAX/DISC1* locus in the aetiology of BPAD and SCZ in the Southern Indian population. We genotyped seven single nucleotide polymorphism (SNPs) from *TSNAX/DISC1* region in 1252 individuals (419 BPAD patients, 408 SCZ patients and 425 controls). Binary logistic regression revealed a nominal association for rs821616 in *DISC1* for BPAD and also combined cases of BPAD or SCZ, but after correcting for multiple testing, these results were non-significant. However, significant association was observed with BPAD, as well as combined cases of BPAD or SCZ, within the female subjects for the rs766288 after applying false discovery rate corrections at the 0.05 level. Two-locus analysis showed C–C (rs766288–rs2812393) as a risk combination in BPAD, and G–T (rs2812393–rs821616) as a protective combination in SCZ and combined cases of BPAD or SCZ. Female-specific associations were observed for rs766288–rs2812393, rs766288–rs821616 and rs8212393–rs821616 in two-locus analysis. Our results provide further evidence for sex-dependent effects of the *TSNAX/DISC1* locus in the aetiology of SCZ and BPAD.

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INTRODUCTION

Schizophrenia (SCZ) and bipolar affective disorder (BPAD) are severe psychiatric disorders, each affecting approximately 1% of the population worldwide.¹ Family, twin and adoption studies confirm that genetic factors have a significant role in the aetiology of these disorders.² Several putative susceptibility loci and genes have been implicated, of which the *TSNAX/DISC1* locus has shown encouraging results. The gene disrupted in schizophrenia 1 (*DISC1*) was first identified in a large Scottish family with a balanced translocation t(1, 11) (q42.2, q14.3) segregating with major mental illness, including SCZ, BPAD and recurrent major depression.^{3–5} The translin-associated factor X (*TRAX*; *TSNAX*) gene is located adjacent to the *DISC1* towards the 5' region.

Subsequently several linkage studies have observed linkage near 1q42, which harbours *DISC1*, to SCZ^{6–9} and BPAD.^{10–12} Several population-based and family-based association studies have supported the role of the *TSNAX/DISC1* locus in SCZ and/or BPAD in Caucasian^{13–25} and Asian^{26–28} populations. However, other studies have failed to replicate association between *TSNAX/DISC1*

and SCZ/BPAD.^{29–34} Recent meta-analysis found no evidence of association with SCZ with the variants in the *DISC1*.³⁵ A meta-analysis of nine European studies has supported common SCZ risk interval within *DISC1* intron 4–6 with $P = 0.002$, odds ratio = 1.27.²⁴ There is also evidence for *DISC1* being associated with poorer performance of visual attention and working memory.^{16,26,36–38} Association of *DISC1* with psychosis-related traits has been reported in Finnish population.³⁹ A genome-wide association study has shown nominal association for SCZ with the SNPs near the *DISC1* translocation break point.⁴⁰ These results support a role for *TSNAX/DISC1* genetic variation in conferring susceptibility to both SCZ and BPAD. Variation at the *TSNAX/DISC1* region thus may have a role in susceptibility to severe psychiatric illness, though the risk variants are not clearly defined, and the haplotypes vary across populations.

Several reviews of the genetic epidemiology and molecular linkage studies suggest an overlap between genetic susceptibility to SCZ and BPAD,^{41–43} indicating that these two diagnostic categories may share risk factors. The *TSNAX/DISC1* locus could be one of these

¹Department of Psychiatry, Molecular Genetics Laboratory, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India and ²Human Genetics Unit, Indian Statistical Institute (ISI), Kolkata, India

Correspondence: Professor S Jain, Department of Psychiatry, Molecular Genetics Laboratory, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore 560029, India.

E-mail: sjain.nimhans@gmail.com

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overlapping genetic susceptibility regions for psychosis. This is also supported by the observation that the translocation was found in both BPAD and SCZ phenotypes in the Scottish family, and a previous study¹³ in this region has shown association with a combined phenotype of SCZ and BPAD, along with schizoaffective disorder, SCZ spectrum diagnosis.

The full-length human DISC1 protein (854 amino acids) is predicted to consist of an N-terminal region, often referred to as the globular 'head' domain and an alpha-helical coiled-coil-containing C-terminal region.^{4,44} DISC1 protein interacts with several proteins, which are involved in neuronal migration, cytoskeleton associated movements, cell cycle, signal transduction, intracellular transport/exocytosis, golgi and central nervous system development.^{45–47} The TSNAX (290 amino acids) protein has an important role in brain development by forming brain-enriched complexes with the protein, Translin.⁴⁸ There is evidence of intergenic splicing and formation of fusion transcripts between *TSNAX* and *DISC1*. Five species of transcripts resulting from intergenic splicing have been identified, of which one encodes a novel TSNAX/DISC1 fusion protein.⁴⁹

Association between polymorphisms in *TSNAX/DISC1* and the risk of developing SCZ and BPAD in the Southern Indian population has not been evaluated before, and the present study was conducted to detect the associations reported in other populations. A case-control design was used in order to test the association of SNPs between cohorts of SCZ cases, BPAD cases, cases with a combined phenotype (SCZ or BPAD) and healthy controls. As there were previous reports^{13,15,27} of sex-specific associations, we also analysed the data based on the gender.

MATERIALS AND METHODS

Subjects

All individuals included in this study were unrelated and of Southern Indian ethnic origin. The study sample comprised of 419 patients with diagnosis of BPAD type I (225 males and 194 females; mean age at assessment: 29.02 ± 10.98 years, mean age at onset: 20.73 ± 6.96 years) and 408 patients with diagnosis of SCZ (246 males and 162 females; mean age at assessment: 29.08 ± 7.84 years, mean age at onset: 23.21 ± 7.16 years) recruited from the inpatient and outpatient services of National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, India. All patients were evaluated using the Diagnostic Interview for Genetic Studies.⁵⁰ Patients were diagnosed by at least two experienced Psychiatrists using Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) criteria.⁵¹ Control subjects ($N=425$; 243 males and 182 females mean age: 33.61 ± 11.15 years) were recruited by a standardized semi-structured interview with psychiatrists from healthy volunteers and hospital staff who had no family history of axis I disorders, in first degree relatives.⁵² Cases and controls were statistically matched for gender (Supplementary Table 1).

The study was approved by the Institute ethics committee. All participants gave written informed consent after a complete description of the study.

SNP Selection and Genotyping

Seven SNPs spanning 492 kilo bases from intron 4 of the *TSNAX* to the 3'-untranslated region of the *DISC1*, which have shown significant association with SCZ and/or BPAD in other populations, were selected for the study (Figure 1). In all, 10 ml of venous blood was collected from the participating subjects and genomic DNA was isolated using modified salting-out method.⁵³ The SNPs were genotyped in two sets using the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA, USA). PCR was carried out using an ABI 9700 (Applied Biosystems)/Eppendorf thermocycler (Eppendorf Scientific, Inc., Westbury, NY, USA). Template preparation after PCR was performed using shrimp alkaline phosphatase and exonuclease I and post-extension treatment was done using shrimp alkaline phosphatase. SNaPshot products were analysed using an ABI PRISM 377 DNA Sequencer

(Applied Biosystems). Genotypes were determined using Gene Scan v 3.1 and Genotyper v 2.1 software (Applied Biosystems). Allele scoring was performed blind to diagnosis, and two raters confirmed the scoring. The genotyping call rate was 97.8%. Conflicts in allele scoring were resolved by individual SNP genotyping.

As genetic case-control studies are known to be susceptible to population stratification, we genotyped six unlinked *Alu* polymorphisms in 100 individuals from each category ($N=300$) to assess the genetic homogeneity of our samples. *Alu* repeats are a family of short interspersed elements and are predominantly present in the non-coding regions of the primate genome. *Alu* repeats have no known biological function⁵⁴ and have been used to study the history of substitution patterns in the human genome.⁵⁵ Polymorphic *Alu* insertions have been used as markers to determine genetic distances between human sub-populations.⁵⁶ We did not observe any difference in the allelic or genotypic distribution between the samples, or between the sexes (Supplementary Table 2).

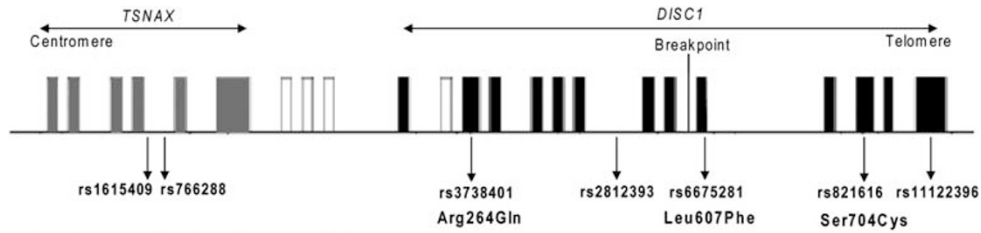
Statistical analysis

Mann-Whitney test was performed to check for age differences between cases and controls. The difference in age between cases and controls was found to be statistically significant. Binary logistic regression analysis (SPSS version 15, SPSS Inc., Chicago, IL, USA) was done with genotypes recoded into the number of alleles (0, 1 and 2, with 1 being the heterozygote) and recoded genders (1 and 2, 1 being male) considering age as a covariate. Allele and genotype frequencies were also compared using standard Chi-square (χ^2) tests. Haplotype analysis was performed using HAPLOVIEW v 4.0 (Broad Institute, Cambridge, MA, USA), which uses the expectation maximization algorithm⁵⁷ to estimate haplotype frequencies. Hardy-Weinberg equilibrium was assessed for all markers using HAPLOVIEW v 4.0. In accordance with the common variant common disease hypothesis for complex disorders, rs6675281 and rs11122396 were excluded from further analysis as the minor allele frequencies were found to be <5% in controls. The frequency of rs11122396 has been seen to be quite low (2%) also in Caucasians¹⁵ and that of rs6675281 was higher (12 to 14%) in Caucasians and Asian studies compared with our study.^{14,15,20,24,58} rs1615409 was also excluded from the analysis as it was in strong linkage disequilibrium with rs766288 (controls- $D'=0.95$, $r^2=0.606$; SCZ- $D'=0.962$, $r^2=0.562$ and BPAD- $D'=0.969$, $r^2=0.566$). The linkage disequilibrium structure for the four SNPs studied is given in Supplementary Figure 1. Association tests with p -values < 0.05 were considered to be nominally significant. We corrected for multiple tests using false discovery rate (FDR) approach.⁵⁹ We used an overall FDR of 0.05 for our association tests. As we performed tests for four SNPs, we used the FDR correction for four tests for the single-marker analyses. The corrections were done within each gender and phenotype separately. For two loci analysis, a combination of 'allele 1' in rs766288 and 'allele 2' in rs2812393 was considered the risk haplotype for regression analysis. The number of copies of the risk alleles was coded as 0, 1 or 2. For example for C-C risk combination, C/C-C/C coded as 2, C/C-C/G or C/A-C/G or C/A-C/C coded as 1 and C/C-G/G, C/A-G/G, A/A-G/G, A/A-C/C and A/A-C/G coded as 0. As there were four allelic combinations in each of the two-locus analysis, the FDR corrections were implemented for four tests. The corrections were done within each gender and phenotype separately. Power calculations were performed by using QUANTO 1.2 software (<http://hydra.usc.edu/gxe>). The power of our study sample was calculated based on the observed range of allele frequencies, the odds ratio of 1.2, prevalence of 1%, a 1:1.041 case-control ratio for BPAD and a 1:1.014 case-control ratio for SCZ under a log additive genetic model at the 0.05 significance.

RESULTS

Single-Marker analysis

SNP frequencies for cases and control groups did not deviate significantly from the Hardy-Weinberg equilibrium cutoff ($P < 0.001$). One SNP (rs821616) showed moderate significant association ($P < 0.05$) with cases compared with controls in regression analysis (Table 1). Associations were found to be significant with BPAD phenotype ($P = 0.035$) and combined phenotype of BPAD or SCZ ($P = 0.04$). However, the results did not retain statistical



Previous studies in other populations:

- rs1615409 - Hennah et al., 2003 (Finnish ; BPAD+SCZ+SA+SCZ Spectrum)
Thomson et al., 2005 (Scottish; BPAD-Males)
- rs766288 - Hennah et al., 2003 (Finnish; BPAD+SCZ+SA+SCZ Spectrum)
Thomson et al., 2005 (Scottish; BPAD-Males)
Schosser et al., 2009 (British; BPAD & MDD)
- rs3738401 - Hennah et al., 2003 (Finnish ; BPAD+SCZ+SA+SCZ Spectrum-Females)
Thomson et al., 2005 (Scottish ; BPAD-Males)
Zhang et al., 2006 (Scottish ; SCZ)
Palo et al., 2007 (Finnish; Psychotic disorder)
Song et al., 2008 (Caucasian ; SCZ)
- rs2812393 - Hodgkinson et al., 2004 (North American ; SCZ)
Thomson et al., 2005 (Scottish ; SCZ & BPAD)
- rs6675281 - Hennah et al., 2003 (Finnish ; BPAD+SCZ+SA+SCZ Spectrum)
Hodgkinson et al., 2004 (North American ; SA)
Ekelund et al., 2004 (Finish ; SCZ)
Thomson et al., 2005 (Scottish ; SCZ - Females)
- rs821616 - Callicot et al., 2005 (Caucasian ; SCZ)
Thomson et al., 2005 (Scottish ; BPAD-Females)
Qu et al., 2006 (Chinese ; SCZ)
Hashimoto et al., 2006 (Japanese ; MDD)
Palo et al., 2007 (Finnish ; Bipolar spectrum disorder)
Song et al., 2008 (Caucasian ; SCZ)
- rs11122396 - Hennah et al., 2003 (Finnish ; BPAD+SCZ+SA+SCZ Spectrum)
Thomson et al., 2005 (Scottish ; BPAD)

Inter-marker distance

- rs1615409- rs766288 (10.2 Kb)
- rs766288 - rs3738401 (136.6 Kb)
- rs3738401- rs2812393 (83.3 Kb)
- rs2812393- rs6675281 (40.4Kb)
- rs6675281- rs821616 (190.4 Kb)
- rs821616 - rs11122396 (30.6 Kb)

Figure 1 Schematic diagram of exons of *TSNAX* (grey boxes), the disrupted in Schizophrenia 1 (*DISC1*) (black boxes), and the intergenic exons (white boxes). The seven SNPs studied are shown in the context of the exonic structure of these genes. The positive reports are shown with inter-marker distance. The figure is adapted from Hennah et al. 2003.

Table 1 Logistic regression analysis—genotype + age as covariate

	P-value ^a /beta value			Controls	HWE P-value ^a		
	BPAD	SCZ	BPAD+ SCZ		BPAD	SCZ	BPAD+ SCZ
rs766288							
Gender combined	0.074/ -0.192	0.906/ -0.013	0.336/ -0.090	0.1854	0.1396	0.1792	0.0399
Females	0.004^b/ -0.483	0.03/ -0.368	0.004^b/ -0.417	0.8972	0.3421	1	0.5645
Males	0.0902/0.018	0.105/0.230	0.248/0.146	0.043	0.2669	0.05	0.0219
rs3738401							
Gender combined	0.368/0.102	0.794/ -0.030	0.520/0.065	0.0053	0.7792	0.9957	0.9283
Females	0.443/0.133	0.473/ -0.137	0.875/0.025	0.3667	0.9978	0.7332	0.9719
Males	0.0614/0.076	0.427/0.199	0.529/0.083	0.0058	0.8037	0.9973	0.7927
rs2812393							
Gender combined	0.135/ -0.158	0.093/ -0.177	0.073/ -0.164	0.1	0.0136	0.4274	0.0163
Females	0.122/ -0.245	0.026/ -0.374	0.028/ -0.312	0.6429	0.1018	0.1183	0.0165
Males	0.532/ -0.089	0.775/ -0.039	0.668/ -0.052	0.7265	0.0948	0.9193	0.3223
rs821616							
Gender combined	0.035/ -0.605	0.179/ -0.367	0.04/ -0.480	0.3521	0.678	0.2184	0.6552
Females	0.175/ -0.537	0.213/ -0.522	0.138/ -0.506	0.4544	0.9219	0.5994	0.5954
Males	0.112/ -0.670	0.579/ -0.203	0.183/ -0.429	0.302	0.4083	0.398	0.9786

Abbreviations: BPAD, bipolar affective disorder; HWE, Hardy-Weinberg equilibrium; SCZ, schizophrenia.

Genotypes recoded into the number of alleles (0, 1 and 2, with 1 being the heterozygote) and recoded genders (1 and 2, 1 being male). P value <0.05 and their corresponding beta values are shown in bold.

^aUncorrected P-value.

^bSignificant after false discovery rate corrections (n = 4).

Table 2 Results from association analysis for four-marker haplotypes in TSNAX/DISC1

Haplotype	Frequency		P-value ^a	Phenotype
	Cases	Controls		
CGGT	0.049	0.074	0.028	BPAD
CGGT	0.05	0.078	0.0189	SCZ
CGGT	0.048	0.074	0.0065	BPAD + SCZ
AGGT	0.024	0.038	0.0469	BPAD + SCZ
AGGT	0.023	0.053	0.0273	BPAD females
AGGT	0.024	0.05	0.0209	BPAD females + SCZ females
CGGT	0.054	0.086	0.0205	BPAD males + SCZ males

Abbreviations: BPAD, bipolar affective disorder; DISC1, disrupted in Schizophrenia 1; SCZ, schizophrenia.

^aUncorrected P-values; results of $P < 0.05$ are shown.

significance after multiple corrections at level 0.05 ($n = 4$). As there were reports of gender-specific effects we analysed the samples stratified by gender and significant association was found at rs766288 and rs2812393 for females (Table 1). The most significant association was found for rs766288 with BPAD females ($P = 0.004$) and combined female cases of BPAD or SCZ ($P = 0.004$), which withstood FDR corrections ($n = 4$). Moderate associations were also observed in females for rs766288 (SCZ, $P = 0.03$) and rs2812393 (SCZ, $P = 0.026$; BPAD + SCZ, $P = 0.028$). Binary logistic regression analysis for the combined sample comprising both males and females at rs766288 did not show any statistical significance after adjusting for gender (Supplementary Table 3). This could be due to the difference in allele frequencies in males (0.319) and females (0.371) at rs766288. However, there was significant evidence of genotype–gender interaction (Supplementary Table 4). Results of χ^2 tests and odds ratio is shown in Supplementary Table 5 and Supplementary Table 6.

Haplotype analysis

The haplotype tests of association were performed with all four-marker haplotypes. Haplotypes with estimated frequencies of $> 1\%$ in both cases and controls were considered for analysis. Results of haplotype analysis with $P < 0.05$ is shown in Table 2. C–G–G–T haplotype showed relatively lesser frequency in BPAD ($P = 0.028$), SCZ ($P = 0.0189$) and combined cases of BPAD or SCZ ($P = 0.0065$) and combined males of BPAD or SCZ ($P = 0.0205$) compared with controls. Haplotype A–G–G–T was also underrepresented in combined cases of BPAD or SCZ ($P = 0.0469$), BPAD females ($P = 0.0273$) and pooled females of BPAD or SCZ ($P = 0.0209$). The nominal associations found did not remain significant after multiple corrections at level 0.05.

Two-locus analysis

Two-locus analyses were performed using binary logistic regression for the three SNPs in combinations of rs766288–rs2812393, rs766288–rs821616 and rs2812393–rs821616 as we found nominal associations for these loci.

rs766288–rs2812393

Analyses of these two SNPs showed significant associations with allelic combination C–C ($P = 0.009$) in the BPAD phenotype when multiple corrections were performed for all tests within each stratified gender (combined, females, males). The regression coefficient for the C–C combination was positive indicating that this allelic combination increases the risk in BPAD. Female-specific combinations were also

Table 3 Two locus analysis of rs766288 and rs2812393

	rs766288	rs2812393	P-value ^a /beta value
BPAD (N = 419)			
	C	C	0.009/0.295
	A	G	0.412/–0.111
	C	G	0.979/0.003
	A	C	0.879/–0.038
SCZ (N = 408)			
	C	C	0.099/0.184
	A	G	0.62/–0.068
	C	G	0.46/–0.088
	A	C	0.529/0.08
Combined cases (N = 827)			
	C	C	0.016/0.236
	A	G	0.551/–0.07
	C	G	0.703/–0.039
	A	C	0.632/0.053
BPAD females (N = 194)			
	C	C	0.003/0.522
	A	G	0.598/0.096
	C	G	0.717/0.064
	A	C	0.161/–0.276
SCZ females (N = 162)			
	C	C	0.011/0.46
	A	G	0.016/–0.526
	C	G	0.626/–0.09
	A	C	0.423/–0.163
Combined female cases (N = 356)			
	C	C	0.001/0.495
	A	G	0.013/–0.488
	C	G	0.969/–0.006
	A	C	0.216/–0.212
BPAD males (N = 225)			
	C	C	0.414/0.122
	A	G	0.057/–0.389
	C	G	0.764/–0.047
	A	C	0.299/0.175
SCZ males (N = 246)			
	C	C	0.993/–0.001
	A	G	0.174/0.245
	C	G	0.639/–0.074
	A	C	0.175/0.222
Combined male cases (N = 471)			
	C	C	0.703/0.049
	A	G	0.212/0.197
	C	G	0.672/–0.058
	A	C	0.099/0.241

Abbreviations: BPAD, bipolar affective disorder; SCZ, schizophrenia. P value < 0.05 and their corresponding beta values are shown in bold.

^aUncorrected P-values; N-number of samples.

observed (Table 3). Other combinations in BPAD females (C–C, $P=0.003$, risk combination) and SCZ females (C–C, $P=0.011$, risk combination; A–G, $P=0.016$, protective combination), combined female cases (A–G, $P=0.013$, protective combination; C–C, $P=0.001$, risk combination) were found to be significantly associated after FDR corrections ($n=12$). If FDR corrections are performed only within a phenotype and gender-based analysis ($n=4$), we also obtained significance with C–C combination ($P=0.016$, risk combination) in BPAD cases.

rs766288–rs821616

Allelic combination C–A ($P=0.004$) in the female cases with combined phenotype showed significant association when multiple corrections were performed for all tests within each stratified gender (combined, females, males). A positive regression coefficient value for the C–A combination was suggestive that female cases with this allelic combination are at increased risk. Few additional combinations that were found to be significantly associated after multiple corrections ($n=12$) were A–T in BPAD females ($P=0.012$, protective combination); C–A ($P=0.014$, risk combination), C–A in SCZ females ($P=0.011$, risk combination), A–T in combined female cases ($P=0.012$, protective combination) and A–A in combined female cases ($P=0.022$, protective combination) compared with control females (Table 4). If FDR corrections are performed only within a phenotype and gender-based analysis ($n=4$), we also obtained significance with A–A ($P=0.032$, protective combination) in BPAD females. However nominal significance observed in BPAD (C–A, $P=0.024$, risk combination), cases with the combined phenotype (C–A, $P=0.019$, risk combination), SCZ males (A–A, $P=0.016$, risk combination) and males with the combined phenotype (A–A, $P=0.028$, risk combination), which did not withstand the multiple corrections.

rs2812393–rs821616

Allelic combinations of G–T in SCZ ($P=0$), combined cases of BPAD or SCZ ($P=0.001$), SCZ females ($P=0.007$) and combined females ($P=0.005$) were observed to be protective combination of alleles (Table 5). These combinations survived multiple corrections ($n=12$). In addition allelic combinations of G–T (BPAD, $P=0.017$; BPAD females, $P=0.035$; SCZ males, $P=0.02$; combined males, $P=0.039$) showed nominal significance, but did not withstand FDR corrections ($n=4$).

DISCUSSION

In the present study, we observed nominal significance in the entire sample set for a non-synonymous SNP rs821616 (Ser704Cys) with BPAD and a combined phenotype of BPAD or SCZ. However these results did not withstand multiple corrections.

At the SNP rs766288, gender-specific association was observed which remained significant after multiple corrections. Female-specific associations in *DISC1* have been reported earlier for SCZ at rs2295959 in Han Chinese²⁷ and rs821616 in a Japanese population.⁶⁰ An early study on a Finnish cohort with a broad phenotype, which included SCZ, schizoaffective disorder, SCZ spectrum diagnosis and BPAD, reported the under-transmission of a haplotype that comprised of rs751229 and rs3738401 at the 5' end of *DISC1* to affected females.¹³ HEP2 (rs1615344–rs1615409–rs766288) (C–A–C) also showed significance in combined samples of males and females and also under-transmitted when samples were stratified by gender. Interestingly rs766288 showed significance in an 8-SNP haplotype in the *TSNAX/DISC1* region of G–C–C–G–A–C–A–G (rs1630250–

Table 4 Two locus analysis of rs766288 and rs821616

	rs766288	rs821616	P-value ^a /beta value
BPAD (N = 419)			
C		A	0.024/0.241
A		A	0.667/–0.049
C		T	0.316/0.123
A		T	0.114/–0.245
SCZ (N = 408)			
C		A	0.082/0.182
A		A	0.42/0.092
C		T	0.093/–0.205
A		T	0.577/–0.084
Combined cases (N = 827)			
C		A	0.019/0.217
A		A	0.831/0.201
C		T	0.115/0.167
A		T	0.215/–0.162
BPAD females (N = 194)			
C		A	0.014/0.4
A		A	0.032/–0.38
C		T	0.835/–0.038
A		T	0.012/–0.589
SCZ females (N = 162)			
C		A	0.011/0.421
A		A	0.074/–0.331
C		T	0.307/–0.197
A		T	0.097/–0.391
Combined female cases (N = 356)			
C		A	0.004/0.412
A		A	0.022/–0.351
C		T	0.489/–0.113
A		T	0.012/–0.497
BPAD males (N = 225)			
C		A	0.413/0.177
A		A	0.187/0.199
C		T	0.244/–0.192
A		T	0.875/0.033
SCZ males (N = 246)			
C		A	0.901/0.017
A		A	0.016/0.357
C		T	0.177/–0.212
A		T	0.476/0.141
Combined male cases (N = 471)			
C		A	0.589/0.067
A		A	0.028/0.290
C		T	0.138/–0.206
A		T	0.606/0.091

Abbreviations: BPAD, bipolar affective disorder; SCZ, schizophrenia. P value < 0.05 and their corresponding beta values are shown in bold. ^aUncorrected P-values; N-number of samples.

Table 5 Two locus analysis of rs2812393 and rs821616

	rs2812393	rs821616	P-value ^a /beta value
BPAD (N = 419)			
	C	A	0.088/0.188
	G	A	0.699/0.045
	G	T	0.017/ -0.334
	C	T	0.799/ -0.035
SCZ (N = 108)			
	C	A	0.236/0.138
	G	A	0.974/ -0.004
	G	T	0/ -0.521
	C	T	0.381/ -0.199
Combined cases (N = 827)			
	C	A	0.125/0.146
	G	A	0.806/0.025
	G	T	0.001/ -0.416
	C	T	0.531/ -0.073
BPAD females (N = 194)			
	C	A	0.172/0.228
	G	A	0.729/0.061
	G	T	0.035/ -0.439
	C	T	0.752/0.063
SCZ females (N = 162)			
	C	A	0.078/0.306
	G	A	0.958/0.01
	G	T	0.007/ -0.621
	C	T	0.659/0.09
Combined female cases (N = 356)			
	C	A	0.077/ 0.265
	G	A	0.834/ 0.033
	G	T	0.005/ -0.52
	C	T	0.642/ 0.081
BPAD males (N = 225)			
	C	A	0.268/ 0.164
	G	A	0.859/ 0.027
	G	T	0.193/ -0.246
	C	T	0.537/ -0.115
SCZ males (N = 246)			
	C	A	0.931/ -0.012
	G	A	0.941/ -0.011
	G	T	0.02/ -0.451
	C	T	0.119/ -0.284
Combined male cases (N = 471)			
	C	A	0.673/ 0.053
	G	A	0.898/ 0.017
	G	T	0.039/ -0.336
	C	T	0.206/ -0.2

Abbreviations: BPAD, bipolar affective disorder; SCZ, schizophrenia.
P value < 0.05 and their corresponding beta values are shown in bold.
^aUncorrected P-values; N-number of samples.

rs1615409–rs766288–rs1025526–rs1030711–rs751229–rs1285730–rs3738401) with BPAD males, in the Scottish population.¹⁵ In our study the C allele of rs766288 was overrepresented in BPAD females (72.9%), SCZ females (71%) and combined females of BPAD or SCZ (72.1%) as compared with female controls (62.9%). Further in our study C–A (rs766288–rs821616), A–A and A–T allelic combinations showed association with female cases. C–A seems to be a risk combination while A–A and A–T are likely to be protective. The two other two-locus analysis involving rs766288 also showed C as risk haplotype. C–C (rs766288–rs2812393) allelic combination seemed to be risk factor in BPAD, which was severe in females. Our results for rs766288 are in concordance with the previous reports and suggest that C might be the risk allele.

We also observed nominal associations for rs2812393. Two-locus analysis of rs2812393–rs821616 showed G–T as protective combination of alleles in gender combined samples of SCZ, BPAD or SCZ, as well as in females. C–C (rs766288–rs2812393) appeared to be a risk combination. Thomson *et al.*¹⁵ have reported an overrepresentation of G allele of rs2812393 in a three marker haplotype C–G–T (rs2492367–rs2812393–rs1322784) in BPAD and also in BPAD female subjects from Scotland. rs766288 and rs2812393 lie in intron 4 of *TSNAX* and intron 6 of *DISC1* gene. As *TSNAX* and *DISC1* are known to form fusion transcripts, these SNPs could be involved in the formation of isoforms via alternative splicing or could be associated with some other functional variant in the region. The mechanism of sex-specific effects of these genes could be through variation in hormone responsive promoter or enhancer sequences, affecting either *TSNAX/DISC1* or other genes that interact with them.

Our results for Ser704Cys polymorphism in the complete sample set is in accordance with the report in a Caucasian sample with allele A overtransmission (71%) for SCZ.¹⁶ We also observed a relative excess of the A allele (Ser704) in cases (77.2% SCZ, 76.5% BPAD, 76.84% combined cases) compared with controls (72.2%). Discordant findings have been observed for this polymorphism with an excess of T allele in SCZ in a Chinese sample set (15.7% in cases and 10.4% in controls)²⁸ and in another Caucasian sample (33% for cases and 27% for controls).²⁰ These differences suggest that there could be allelic heterogeneity associated with the polymorphism. This SNP is in exon 11 of *DISC1*, an exon known to undergo alternative splicing and encodes a region of the protein thought to be involved in interactions with ATF4, ATF5, LIS1, GRB2 and NDEL1.⁴⁵ The Ser allele has been shown to be associated with structural and functional changes in the hippocampus including reduction in grey matter volume in Caucasian data set.¹⁶ Ser704 homozygotes have shown greater pre-frontal activation, with significant differences in the left middle and inferior frontal gyri, and in the right inferior frontal gyrus, which could be interpreted as a manifestation of less efficient pre-frontal function, with more activation required to achieve the same behavioural output.⁶¹ Ser704Cys polymorphism is also shown to be associated with altered expression of transcripts lacking exon 3 and, exon 7 and 8.⁶² The short transcripts were found to be highly expressed in the brains of patients with SCZ than in control brains, and were associated with genetic variations in the *DISC1* gene. Power analysis showed our sample set had power of 42.4% for BPAD and 42.6% SCZ for rs766288, 45.5% for BPAD, 44.1% for SCZ for rs2812393 and 40.5 and 35.4% for SCZ for rs821616 with an odds ratio of 1.2. These nominal associations observed in our sample could be a false positive finding due to inadequate statistical power. The possibility of involvement of Ser704Cys polymorphism in the susceptibility of psychosis still remains unclear.

The associations we have identified are unlikely to have arisen as a result of population stratification within our samples—firstly the allele frequencies of the analysed SNPs other than rs6675281 are congruent with the reported allele frequencies in Caucasian populations and secondly, the *Alu* markers did not show significant differences in the groups studied (Supplementary Table 2). However, these data should be interpreted with caution as there are chances of type I error.⁶³

We could not replicate the association for the other SNP studied rs3738401 with SCZ or BPAD that have been reported previously in other ethnic groups. The difference in the ethnic background of the subjects, and the study design viz sample size, sampling bias, family vs population based, and so on between studies may account for inconsistent results. These reported SNPs might not be causative, and could be in linkage disequilibrium with some other disease predisposing allele within the gene or near the gene. The SNPs studied could be associated with SCZ or BPAD in a haplotype with other polymorphisms or could be a susceptibility allele only in subgroup of the phenotype. It is evident that most of the positive studies in this region have been from Caucasian populations, while findings in the Asian population have been mixed, with largely negative reports.^{37–39}

This could be explained by the differences in the linkage disequilibrium pattern and differences in allele frequencies across populations. Alternatively, in complex diseases such as SCZ and BPAD, in which multiple loci act jointly to confer susceptibility for disease, associations of different alleles at the same locus could be attributed to a correlation with a causal polymorphism at another locus.⁶⁴ However our results for rs766288, rs2812393 and rs821616 are suggestive of the involvement of this locus in susceptibility to BPAD and SCZ.

The present study has some limitations. Firstly, the number of SNPs studied might not represent the entire region, as *TSNAX/DISC1* region comprises ~610 kb. Thus, we cannot exclude the possibility of other SNPs in the region being involved in conferring risk for BPAD and SCZ. Second, the sample size for the current analyses was relatively modest, with further reduction for gender-based comparisons, and further replication is necessary.

However, it should also be noted that the multiple corrections applied here are very conservative taking into account our study design has a directional hypothesis based on previous findings. Despite the limitations, we believe our study contributes to greater insight into the *TSNAX/DISC1* locus association with psychosis. In an era where genome-wide association study have rapidly become a standard method for disease gene discovery, there is still a need for focused candidate gene association studies in different populations, as most genome-wide association study have been with European sample sets, and limited reports are available from Asian populations,^{65–67} as there are likely to be population-specific variants.⁶⁸

In conclusion, we find significant gender-specific associations with two intronic SNPs in *TSNAX* and *DISC1*, and an exonic SNP from *DISC1*. Further replication, in larger and more diverse samples, using dense markers, may be necessary to understand the role of these genes in the pathophysiology of these disorders.

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