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ORIGINAL ARTICLE

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SYNGR1 is associated with schizophrenia and bipolar disorder in southern India

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Abstract Chromosome 22q11–13 is one of the most consistent linkage regions for schizophrenia (SCZ) and bipolar disorder (BPAD). The *SYNGR1* gene, which is associated with presynaptic vesicles in neuronal cells, is located on 22q13.1. We have previously identified a novel nonsense mutation in the *SYNGR1* gene in a SCZ pedigree. In the present study, a detailed analysis of this gene was performed in a case–control cohort (198 BPAD, 193 SCZ and 107 controls from southern India) to test for association with SCZ and BPAD. Sequence analysis of all exonic and flanking intronic regions of the *SYNGR1* gene in 198 BPAD and 193 SCZ cases revealed a novel mutation Lsy99Glu (in one BPAD patient) and two other novel common polymorphisms [synonymous single nucleotide polymorphism (SNP—Ser97Ser) and an Asn ins/del] in the *SYNGR1* gene. We also validated 9 out of 14 dbSNPs in our population. Case–control analysis revealed allelic ($P = 0.028$–0.00007) association of five polymorphisms with SCZ and/or BPAD cases. Further, 3-SNP (with LD block 1 SNPs) and 2-SNP (with LD block 2 SNPs) haplotype analyses did not show any association with either SCZ or BPAD. Our results support *SYNGR1* as a probable susceptibility gene for SCZ and BPAD. Also, the observed association of *SYNGR1* with both SCZ and BPAD suggests the likely involvement of a common pathway in the etiology of these disorders.

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Introduction

Schizophrenia (SCZ) and bipolar disorder (BPAD) are complex neuropsychiatric disorders with a lifetime prevalence rate of ~1% worldwide (Torrey 1987; Bland 1997). The etiology of these disorders is still not evident; however, various family, twin and adoption studies have suggested a strong genetic component underlying the pathogenesis of these disorders (Berrettini 2004). Among the various chromosomal regions that have shown evidence of linkage with these disorders, 22q11–13 is consistently one of the most extensively studied regions (Coon et al. 1994; Pulver et al. 1994; Gill et al. 1996; Papolos et al. 1996; Bassett and Chow 1999; Schwab and Wildenauer 1999; Kelsoe et al. 2001; Jorgensen et al. 2002). Our group has also reported association of a novel CAG repeat marker (22CH3) on 22q13.2 with both SCZ and BPAD in the southern Indian population (Saleem et al. 2001). Taken together, these studies provide clues regarding the existence of potential susceptibility genes for SCZ and BPAD on 22q11–13.

Synaptogyrin 1 (*SYNGR1*), which encodes an integral membrane protein associated with presynaptic vesicles in neuronal cells (Baumert et al. 1990; Stenius et al. 1995), is one of the interesting candidate genes on 22q13.1. *SYNGR1* is ~36 kb long, with six exons and three alternative transcripts (Kedra et al. 1998). We previously reported a novel nonsense mutation (Trp27Ter) in this gene in a SCZ pedigree during screening of *SYNGR1* exons in 20 SCZ probands (Verma et al. 2004). Genotyping of 144 schizophrenics and 186 ethnically matched normal controls failed to find this mutation in any other individual. However, due to the limitation of the screening sample size used, this does not preclude the existence of other associated variations in the same gene. Further, reduced levels of *SYNGR1*

transcripts have been observed in SCZ cases in a microarray study (Mirnics et al. 2000), and double knockout mice for synaptophysin 1/synaptogyrin 1 show severely reduced short-term and long-term synaptic plasticity (Janz et al. 1999). All these findings support the notion that *SYNGR1* is a potential candidate gene for SCZ/BPAD susceptibility.

To test the hypothesis that the *SYNGR1* gene increases susceptibility to SCZ/BPAD, we evaluated sequence variations in this gene in a cohort of SCZ/BPAD patients using a case–control association approach. Our study suggests the likely involvement of *SYNGR1* as susceptibility gene for SCZ and BPAD.

Materials and methods

Study population

Case–control analysis was performed in 198 BPAD (105 males and 93 females; mean age at onset: 20.76 ± 7.38 years, mean age at assessment: 28.95 ± 10.79 years), 193 SCZ (116 males and 77 females; mean age at onset: 22.53 ± 7.69 years, mean age at assessment: 29.8 ± 9.31 years) and 107 matched controls (70 males and 37 females; mean age: 29.39 ± 9.09 years). All bipolar subjects analyzed in this study were BPAD I cases with severe disease phenotype and substantially early age at onset. All patients were of southern Indian origin and were recruited from the clinical services of the National Institute of Mental Health and Neuroscience (NIMHANS), Bangalore, India. This research was approved by the institutional review board of NIMHANS, and all participants gave written consent for interview as well as sampling. Diagnosis was made according to DSM IV criteria for both SCZ and BPAD cases. The final best estimate diagnosis was established on the basis of a structured interview of all patients by experienced psychiatrists using SCAN (Schedules for Clinical Assessment in Neuropsychiatry; Wing et al. 1990) and OPCRIT 3.1 (Operational Criteria for Psychotic illness; McGuffin et al. 1991). Additional information was obtained through examination of hospital records and interviews with family members of the probands. Control individuals were also recruited from the same location (Bangalore) and ethnicity (southern Indian origin) with due consent. In addition, control subjects were further interviewed in order to exclude any family history of psychiatric illness. The case–control sample set analyzed in this study is ethnically quite homogenous, being of south Indian origin. Further details of the study cohort are described elsewhere (Verma et al. 2005).

Detection of novel sequence variations in the *SYNGR1* gene

Blood samples were obtained from all the subjects and genomic DNA was isolated using a modification of a salting out procedure (Miller et al. 1988). Novel variations were identified through bi-directional sequencing of all exons and flanking intronic sequences of the *SYNGR1* gene (covering 5,576 bp in six amplicons with sizes ranging from 553 to 1,057 bp) except the 3′ untranslated region of exon 6 in 198 BPAD and 193 SCZ cases (primers and conditions for PCR reactions are given in Supplementary Table 1). The products of sequencing reactions were further electrophoresed and analyzed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Wellelsley, MA). The sequences thus obtained were aligned with the NCBI database reference sequences (Build 34) using "Clustal X" multiple alignment software (version 1.810) installed on an irix platform (Thompson et al. 1997).

Validation of database single nucleotide polymorphisms located in the remaining unsequenced regions of the *SYNGR1* gene

We further analyzed the involvement of unscreened regions of this gene in conferring predisposition to SCZ and BPAD. We attempted to validate 14 dbSNPs (single nucleotide polymorphisms; http://www.ncbi.nlm.nih.gov/SNP) spanning almost the entire gene by sequencing ten control samples; 9 out of these 14 SNPs were observed to be polymorphic in our population. SNPs rs12185894, rs11912071, rs12484294, rs5757644 and rs7289425 were absent in our screening sample set.

Genotyping of *SYNGR1* polymorphisms

We genotyped 11 *SYNGR1* polymorphisms (2 novel and 9 validated dbSNPs) in the case–control sample set using SNaPSHOT assay (PE Biosystems, Foster City, CA). Primers used for genotyping *SYNGR1* polymorphisms are given in Supplementary Table 2.

Statistical methods

Linkage disequilibrium and haplotype construction from case–control data

Pairwise linkage disequilibrium (LD) between these 11 polymorphisms was calculated from genotype data of the case–control population cohort with Haploview software ver 2.05 (Barrett et al. 2005) using confidence-interval model to define LD blocks. 3-SNP haplotypes (using block 1 SNPs) and 2-SNP haplotypes (using block 2 SNPs) in the same case–control sample set were constructed using the program PHASE ver 2.1 (Stephens et al. 2001).

Case–control association

Allelic association for *SYNGR1* polymorphisms with SCZ/BPAD was performed by Fisher's exact test.

Genotypic association was calculated for these polymorphisms using chi-square test. Genotypes were also tested for Hardy–Weinberg equilibrium as a quality check. Global haplotypic association in our case–control data set was calculated using the PHASE program, and association of individual haplotypes was calculated by 2×2 chi-square test using "best reconstructed" haplotype counts from PHASE; odds ratios were also calculated.

Considering the exploratory nature of the analyses performed in the present study, as well as the limited sample size, we considered $P < 0.05$ as a cut-off value for statistical significance. Also, in this study, we have analyzed multiple linked SNPs and their haplotypes in different datasets, hence performing a Bonferroni correction for multiple testing might result in an overly conservative test. Therefore, we have reported the uncorrected *P*-values for *SYNGR1* analyses.

In silico analysis

In our analysis, an intronic SNP rs715505 showed significant association with SCZ and BPAD. An intronic SNP may alter splicing or modify transcription factor binding sites. We evaluated the effect of this SNP on RNA splicing by using NetGene2 ver2.4 software (Brunak et al. 1991) and also tested for the modification of transcription factor binding sites using TFSEARCH ver1.3 (Heinemeyer et al. 1998).

Results

SYNGR1 sequence variation analysis

SYNGR1 is a potential candidate gene for both SCZ and BPAD. To test our hypothesis that *SYNGR1* sequence variations increase susceptibility to SCZ/BPAD, we sequenced exonic and flanking intronic sequences of the *SYNGR1* gene as a first step in our study.

Sequence analysis revealed a novel rare mutation (Lys99Glu) in exon 3 of the *SYNGR1* gene in one BPAD patient (absent in 107 controls). The other two novel sequence variations, including a synonymous SNP [referred to as SNP8 (Ser97Ser)] in exon 3 and an ins/del polymorphism of AAC, coding for the amino acid Asn, at amino acid position 203 in exon 6, were observed with minor allele frequencies of ~0.09 and ~0.16, respectively.

We also validated 9 out of 14 dbSNPs in our population. Genotypes of all *SYNGR1* gene sequence variations were in Hardy–Weinberg equilibrium (Supplementary Table 3). The details of *SYNGR1* sequence variations genotyped in this study are given in Table 1.

LD relationships among polymorphisms of the *SYNGR1* gene

Pairwise LD analysis of *SYNGR1* polymorphisms revealed two LD blocks: block 1 (2,199 bp) comprises three polymorphisms in intron 1 while block 2 (362 bp) consists of two SNPs in intron 2 (Fig. 1). The details of pairwise LD values (D' and r^2) for these polymorphisms are given in Supplementary Table 4. Using this LD block structure information, we selected SNPs within LD blocks 1 and 2 for further haplotype analysis.

Case–control association analysis of *SYNGR1* polymorphisms

The case–control analysis revealed significant allelic association of rs909685 and rs715505 with both SCZ ($P = 0.007$; $P = 0.00007$) and BPAD ($P = 0.028$;

Table 1 *SYNGR1* gene sequence variations and association with schizophrenia/bipolar disorder (SCZ/BPAD). F_{allele} reported for minor alleles; P_{allele} and $P_{genotype}$ refer to allelic and genotypic association of *SYNGR1* variations with SCZ/BPAD, respectively. Significant *P*-values < 0.05 are marked in bold; *P*-values > 0.05 are considered not significant

Lab ID	dbSNP ID	Location	Position (bp)[a]	Variant[b]	F_{allele}			P_{allele}		$P_{genotype}$	
					SCZ	BPAD	Controls	SCZ	BPAD	SCZ	BPAD
SNP1	rs909685	Intron 1	1,656	T/A	0.53	0.51	0.42	**0.007**	**0.028**	**0.025**	0.1
SNP2	rs715505	Intron 1	5,236	G/C	0.45	0.39	0.29	**0.00007**	**0.007**	**0.0004**	**0.029**
SNP3	rs2010904	Intron 1	6,976	G/A	0.1	0.16	0.09	0.5	**0.015**	0.9	0.09
SNP4	rs760741	Intron 1	7,251	T/C	0.1	0.14	0.09	0.5	0.06	0.9	0.2
SNP5	rs760742	Intron 1	9,175	T/G	0.17	0.23	0.22	0.9	0.4	0.3	0.9
SNP6	rs739363	Intron 2	14,868	A/C	0.34	0.36	0.4	0.9	0.9	0.8	0.5
SNP7	rs2012906	Intron 2	15,230	G/T	0.32	0.36	0.37	0.9	0.7	0.4	0.8
SNP8	NR[c]	Exon3	24,497	C/T	0.07	0.12	0.07	0.6	0.07	0.9	0.3
SNP9	rs4821888	Intron 3	24,582	G/A	0.1	0.15	0.11	0.7	0.1	0.9	0.4
SNP10	rs6001566	Intron5	28,433	G/A	0.17	0.21	0.24	**0.025**	0.8	0.1	0.7
ins/del	NR[c]	Exon 6	31,808	ins/del	0.13	0.21	0.14	0.7	**0.018**	0.7	0.08

[a]Position of each polymorphism is relative to the ATG start site (taking A as +1) of the *SYNGR1* gene
[b]Variants are listed with the major allele first
[c]Not reported in the dbSNP database

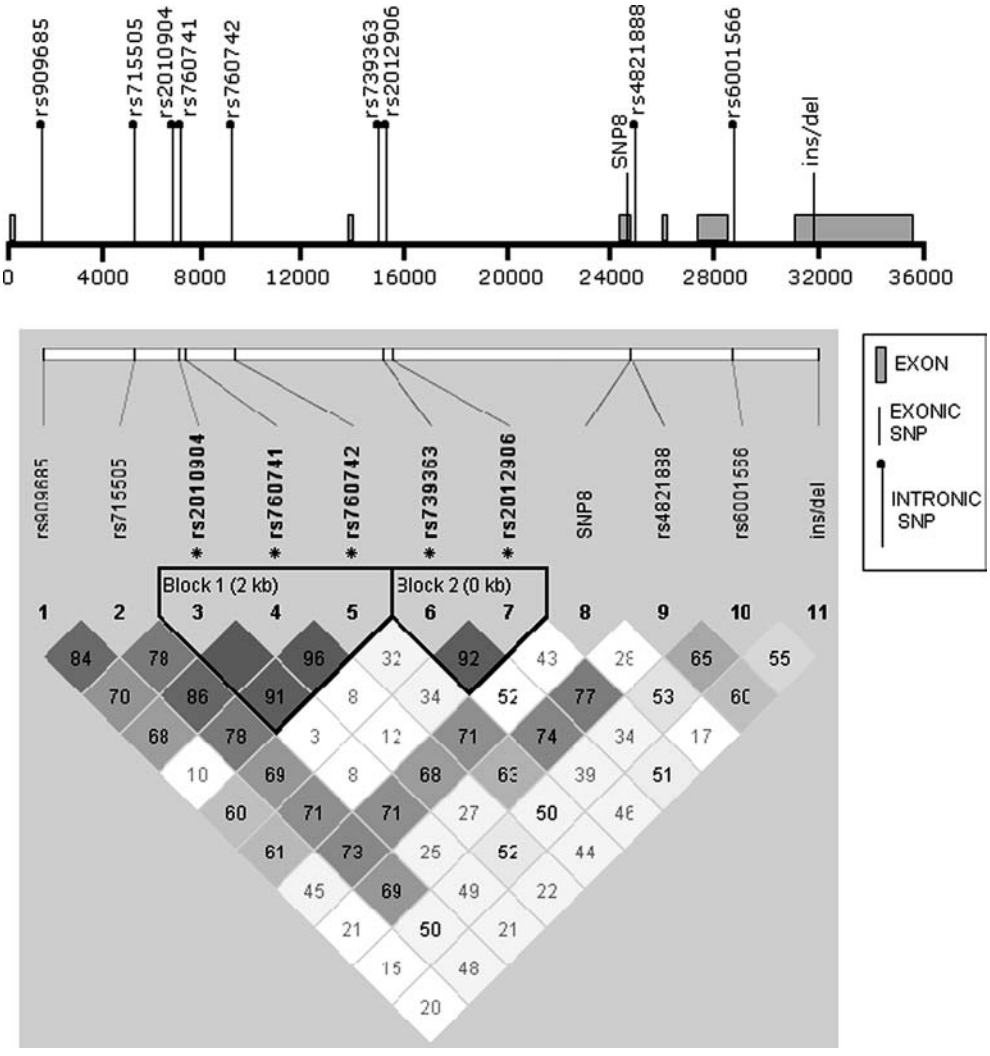

Fig. 1 Linkage disequilibrium (LD) block structure across the *SYNGR1* gene. *Top* Schematic representation of the *SYNGR1* gene, showing 11 polymorphisms [2 novel and 9 validated single nucleotide polymorphisms (SNPs)] spanning almost the entire gene. *Bottom* Haploview (ver 2.05) LD plot output, where each square (containing D' values) represents a pairwise LD relationship between two SNPs. *Gray squares* Statistically significant LD between pairs of SNPs as measured by the D' statistic, with the intensity of the grey color increasing with higher values of D' up to a maximum of 1; *white squares* pairwise D' values of <1 with no statistically significant evidence of LD. The blocks generated using the "confidence interval" algorithm of Haploview are marked. Two LD blocks were observed in this gene: block 1, covering 2,199 bp comprising three SNPs; and block 2 of 362 bp comprising two SNPs. Polymorphisms marked with *asterisks* within LD blocks 1 and 2 were selected for further haplotype analysis

$P=0.007$), while rs2010904 (block 1 SNP) and the ins/del polymorphism were associated only with BPAD ($P=0.015$; $P=0.018$), and rs6001566 only with SCZ ($P=0.025$) (Table 1). SNP rs909685 showed genotypic association only with SCZ ($P=0.025$), whereas rs715505 showed significant genotypic association with both SCZ ($P=0.0004$) and BPAD ($P=0.029$) (Table 1).

Furthermore, 3-SNP haplotype (with block 1 SNPs) and 2-SNP haplotype (with block 2 SNPs) analyses of the *SYNGR1* gene did not show any association with either SCZ or BPAD. However, specific haplotype association analyses showed that "HAP 2 (GTG)" (from 3-SNP haplotypes) was over-represented in controls as compared to SCZ cases ($P=0.046$) whereas "HAP 3 (CG)" (from 2-SNP haplotypes) was over-represented in controls as compared to BPAD cases ($P=0.034$) (Supplementary Table 5).

Discussion

SYNGR1, a gene located on 22q13.1, encodes a protein associated with presynaptic vesicles in neuronal cells, and hence is a potential candidate gene for both SCZ and BPAD. In this study, we evaluated the association of *SYNGR1* variations with SCZ and BPAD.

In this study, we found a Lys99Glu mutation in one BPAD patient. This mutation lies in the cytoplasmic loop between transmembrane domains 2 and 3 of the SYNGR1 protein. Experimental evidence from *Caenorhabditis elegans* synaptogyrin 1, and comparative sequence analysis of rat-synaptogyrin (rat-syngr) vs rat-cellugyrin (rat-cgyr) protein, suggests the importance of this cytoplasmic domain in the synaptic localization of this protein (Zhao and Nonet 2001). Hence, the Lys99-Glu mutation might affect the selective localization of SYNGR1 protein to synaptic vesicles. We have also identified two common exonic variations including an ins/del polymorphism located in exon 6. According to experimental analysis on *C. elegans* synaptogyrin 1, this ins/del polymorphism lies within the signaling sequence for synaptic localization of synaptogyrin 1 protein (Zhao and Nonet 2001), thereby suggesting a functional consequence of this polymorphism. This ins/del polymorphism also showed association with BPAD in our case–control analysis.

We further evaluated association of nine validated dbSNPs with SCZ and BPAD. Case–control analysis showed allelic association of rs909685, rs715505 with both SCZ and BPAD, while rs2010904 was associated only with BPAD and rs6001566 only with SCZ. SNPs rs909685 and rs715505 also showed genotypic association with SCZ and/or BPAD. Further 3-SNP and 2-SNP haplotype analyses did not show any association with either SCZ or BPAD. It thus appears that the association of the *SYNGR1* gene with SCZ and BPAD is due mainly to the individual allelic/genotypic association of intronic SNP rs715505.

To date, many of the polymorphisms associated with increased risk of complex disorders are not amino acid polymorphisms. For example, the variation at *CTLA4* implicated in type 1 diabetes appears to affect splicing, while variation at the *INS* VNTR, *CAPN10*, and *HNF4A* loci may affect gene expression (Permutt et al. 2005). Several lines of evidence reporting the functional importance of intronic polymorphisms in the etiology of different complex disorders are now available (Cox et al. 2004). An in vivo expression study (Baier et al. 2000) has reported significantly lower (53% reduction) levels of *CAPN10* mRNA in the skeletal muscle of individuals homozygous for the G allele of an intronic SNP-43. Until recently, effective experimental methods to evaluate the functional significance of synonymous and non-coding polymorphisms were not available (Knight et al. 2003; Rebbeck et al. 2004). In our present study, significant association was observed for intronic SNP rs715505 with both SCZ and BPAD. In silico analysis of splice site prediction or alteration of transcription factor binding sites did not show any significant effect of this SNP on these two biological processes. Further, experimental evidence will be required to evaluate the functional role of this polymorphism in affecting expression of the *SYNGR1* gene. However, SNP rs715505 was present only in 39.1% (BPAD) to 45.1% (SCZ) of cases, so rs715505 cannot explain the entire genetic susceptibility component for SCZ and BPAD in our cohort.

In the present study, we found significant association of the *SYNGR1* gene with both SCZ and BPAD, hence suggesting the involvement of the *SYNGR1* gene in a common pathway towards the pathogenesis of these two disorders. Our earlier study showed the presence of a rare nonsense mutation in a SCZ family (Verma et al. 2004). In our present study, we observed another rare mutation in a BPAD subject along with some common polymorphisms associated with SCZ/BPAD. It thus appears that a combination of rare mutations and common sequence variations of the *SYNGR1* gene might contribute as susceptibility factors for SCZ and BPAD, as would be expected given the complex mode of inheritance and heterogeneity of these disorders. Further, screening of another larger cohort of SCZ/BPAD and control individuals, especially around the rs715505 in intron 1 of the *SYNGR1* gene, may be useful in validating our results and finding other important functional sequence variations that contribute to the etiology of SCZ and BPAD.

Considering the role of *SYNGR1* in presynaptic pathways, any defects in presynaptic genes may lead to severe consequences in terms of neuronal function. Genes involved in presynaptic pathways are thus attractive candidates for SCZ and BPAD.

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