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RESEARCH ARTICLE

ASSOCIATION OF STIN2 VNTR POLYMORPHISM OF *SLC6A4* GENE WITH BIPOLAR DISORDER

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SUMMARY

In the present study, an association between STin2 VNTR polymorphism in *SLC6A4* gene and bipolar disorder (BD) in population of North Coastal Andhra Pradesh has been analysed and polymorphism was typed in 150 BD patients and 150 age and sex matched healthy controls using the polymerase chain reaction (PCR). A significant association between STin2 VNTR polymorphism of *SLC6A4* gene and BD has been observed. The frequency of 12 repeat allele is more when compared to the 10 repeat allele in both cases and controls. Considering the frequency of 10 repeat allele alone, it is higher in cases when compared to controls.

Keywords: Bipolar disorder, North coastal Andhra Pradesh, serotonin transporter, VNTR polymorphism.

INTRODUCTION

Bipolar disorder also known as manic depressive illness or mental illness, is a life threatening psychiatric disorder. It is characterized by fluctuations in mood states of mania, hypomania, mixed episode and depression with a prevalence of around 1% (Fajutrao et al. 2009). The disorder is equally prevalent in both men and women (Gold 1998, Maier et al. 2005). In India, a prevalence of 0.6% was reported in males and 0.4% in females (Murthy 2017). The disorder is generally observed in 18−30 y age group (Maier et al. 2005). Kawa et al. (2005) reported lack of gender difference in age at onset and BD but some studies recorded a later age at onset in females (Arnold 2003, Robb et al. 1998, Viguera et al. 2001). BD is the sixth leading cause of disability (Murray & Lopez 1997), imposing a significant economic burden to the society because of its expensive treatment (Hirschfeld & Vornik 2005). It is associated with premature mortality (Hayes et al. 2015) and mortality rate is double the value that is observed in general population (Petterson 1977, Vestergaard & Aagaard 1991, Weeke & Vaeth 1986). It is a multifactorial disorder caused by several contributing factors like genetic and environmental factors, structure of brain, substance use etc.

Serotonin (5HT) is synthesized in enterochromaffin cells of intestine, blood platelets and serotonergic neurons of brain. It is an intermediate product of tryptophan metabolism and is a monoamine neurotransmitter in central nervous system. Abnormality in this neurotransmitter is associated with development of BD and other psychiatric disorders like depression, anxiety, schizophrenia, mania and aggression. Neurotransmission of 5HT is controlled by synchronized action of

various 5HT related proteins like serotonin autoreceptors, tryptophan hydroxylase, monoamine oxidase and serotonin transporter (Watanabe et al. 2011). Genetic polymorphisms in these proteins, have been widely studied in association with BD.

Serotonin transporter (SERT or 5HTT) is a monoamine transporter protein that transports 5HT from synaptic cleft to pre-synaptic neurons and recycles it in a sodium dependent manner. It is encoded by *SLC6A4* (the solute carrier family 6, member 4) gene and is located on long arm of chromosome 17q11.1-17q12. 5HTT is a key protein in 5HT pathway (Mendlewicz et al. 2004) and plays an important role in cognitive process like memory and behaviours like aggression, impulsivity, nutrient intake, sleep, appetite and personality traits (Goldman et al. 2010, Mazzanti et al. 1998). It is the major site of action for various antidepressants (Lesch. 2001, Lotrich et al. 2001) like selective serotonin reuptake inhibitors as they reduce the reuptake of 5HT from synaptic cleft (Goldman et al. 2010, Mendlewicz et al. 2004), and thus acts as a major key in serotonergic neurotransmission (Amara & Kuhar 1993).

SLC6A4 gene has been associated with seasonal affective disorder, autism, severe alcoholism, psychotic symptomatology in neuroleptic free schizophrenics and schizophrenia, suicidal behaviour and anxiety disorders (Bellivier et al. 2000, Hranilovic et al. 2000, Klauck et al. 1997, Lesch et al. 1996, Malhotra et al. 1998, Rosenthal et al. 1998, Sander et al. 1997, Serretti et al. 1999) and major depressive disorder (Bellivier et al. 1997, Collier et al. 1996, Rees et al. 1997). This gene has been reported as a strong candidate gene for BD (Muller-Oerlinghausen et al. 2002).

One of the most commonly studied functional polymorphisms in *SLC6A4* gene is the 17 bp variable number of tandem repeat, named STin2 VNTR located in the second intron of the gene. This polymorphism involves two major alleles namely, STin2.10 and STin2.12 corresponding to 10 and 12-repeat units respectively (Lesch et al. 1994). Number of repeats determine the transcriptional activity and the 12 repeat allele (STin2.12) is reported to have highest expression (Fiskerstrand et al. 1999, MacKenzie & Quinn 1999).

This polymorphism is reported to be associated with major depression (Ogilvie et al., 1996), autistic disorder (Cook et al.1997), suicidal behavior (Hranilovic et al. 2003), anxiety (Evans et al. 1997) and BD (Bellivier et al. 1997, Collier et al. 1996, Craddock et al. 1996, Kirov et al. 1999, Liu et al.1999, Rees et al. 1997, Sun et al. 2004)

MATERIALS AND METHODS

The patient group consisted of 150 BD cases (104 males and 46 females) who were recruited from Government Hospital for Mental Care, Visakhapatnam during October 2015 to July 2017. The patients were aged from 18 to 70 y and were psychiatrically assessed and diagnosed with bipolar mania by trained psychologists in accordance with ICD 10 manual. Information regarding age, sex, life style factors like smoking habits and alcohol consumption was recorded for both cases and controls and that regarding the disease, like age at onset, family history etc. was collected from the cases. Individuals who are having mental retardation or using intravenous drugs and those who are related to the cases, were excluded from the study.

Blood sample was collected from each individual, after obtaining informed consent. Genomic DNA was extracted from whole blood using salting out method. PCR amplification was performed for the VNTR region of the second intron of the *SLC6A4* gene using forward and reverse primers – FP 5' GGTCAGTATCACAGGCTGCGAGTAG 3'and RP 5' TGTTCCTAG TCTTACGCCAGTGAAG 3' as described by Vijayan et al. (2009). Thermal cycling was carried out with an initial denaturation step at 98° C for 10 min followed by 40 cycles at 98° C (10 sec), 63° C (30 sec), and 72° C (1 min) and a final extension step at 72° C for 10 min.

OBSERVATIONS

PCR products were visualized by using 2.5% agarose gels which yielded three genotypes- 12/12 (299 bp), 10/10 (265 bp) and 12/10 (299 bp and 265 bp). 10 and 12 repeat alleles were observed in study population (Fig.1). The distribution of genotype and allele frequency of the STin2 VNTR polymorphism of *SLC6A4* gene among cases and controls is presented in Table 1. The genotype distribution for the control group was in Hardy–Weinberg equilibrium ($\chi^2 = 0.02$). When the genotype distribution was compared between cases and controls, homozygosity for the STin10 allele was found to be significantly more in the cases ($P = 0.000$; odds ratio = 3.10, 95% CI = 1.78−5.41) whereas heterozygosity was overrepresented in control population ($P = 0.000$; odds ratio = 5.75, 95% CI = 2.97−11.13). Regarding allele frequency distribution, the STin10 allele was observed more in cases as compared to controls ($P = 0.21$; odds ratio = 1.23, 95% CI = 0.88−1.70). Gender based distribution of the polymorphism revealed that both males and females were significantly associated with the disorder ($p = < 0.05$).

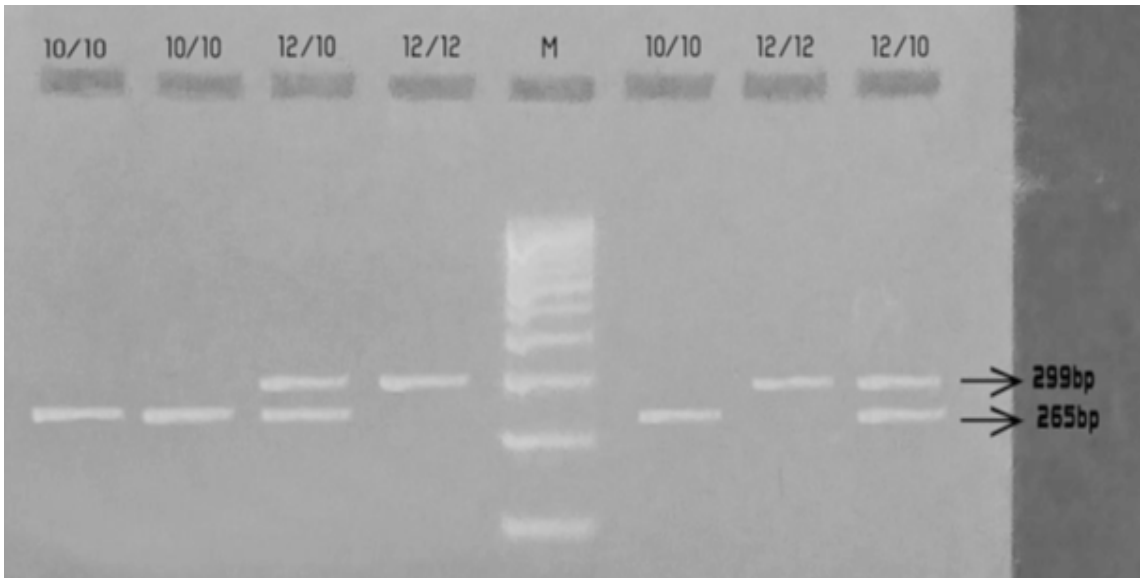

Fig. 1: Agarose gel showing bands of STin2 VNTR of *SLC6A4* gene (M–100 bp DNA ladder).

TABLE 1: Genotype distribution and allele frequency for the STin2 VNTR polymorphism of *SLC6A4* gene among BD cases and controls.

Polymorphism STin2 VNTR	Cases (%) n = 150	Controls (%) n = 150	Odds ratio	95% CI	P-value
Genotype					
12/12	70 (46.7)	56 (37.3)			
12/10	29 (19.3)	72 (48.0)	5.75	2.97–11.13	0.000
10/10	51 (34.0)	22 (14.7)	3.10	1.78 – 5.41	0.000
Allele					
12	169 (0.56)	184 (0.61)			
10	131 (0.44)	116 (0.39)	1.23	0.88 –1.70	0.21

DISCUSSION

Several studies were conducted to find the association between STin2 VNTR and BD. Negative association was reported by Gutierrez et al. (1998), Hoehe et al. (1998), Stober et al. (1996) and Yen et al. (2003), while positive association was reported by some studies (Bellivier et al. 1997, Collier et al. 1996, Craddock et al. 1996, Liu et al. 1999, Rees et al. 1997, Sun et al. 2004). The present study reports positive association of STin2 VNTR polymorphism with BD which is similar to the preceding global studies which reported positive association. However, the present study is contrary to that of Saleem et al. (2000) who reported negative association of this polymorphism with BD in Indian population. The possible reason for the difference between these two Indian studies is that the sample size of Saleem et al. (2000) is only 50. However, controls were ethnically matched.

All the populations worldwide including the Indians showed high frequency of 12 repeat allele in both cases and controls compared to 10 repeat allele. Gender stratification results also report high frequency of 12 repeat allele than 10 repeat allele in both cases and controls. We found that the STin10 allele was significantly more frequent among the BD cases than among the controls. Same trend is noticed in the data reported by Mellerup et al. (2001) in Danish population, Gutierrezz et al. (1998) in Spanish population, Hoehe et al. (1998) in French and German Caucasians and Stober et al. (1996) in German population.The present study supports Lasky-Su et al.'s (2005) metaanalysis (includes 11 studies with 2292 controls and 1357 cases) which reported no association between affective disorders which include unipolar and bipolar disorders and an increase in the number of tandem repeats.

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