Is there a Link Between Anxiety, Depressive Disorders and 5HT 2A Receptor Gene Polymorphism? – Study from A Conflict Area, India-controlled Kashmir

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ABSTRACT

BACKGROUND: 5 HT-2A receptor gene polymorphisms are associated with various psychiatric disorders, including anxiety and depression. However, no study has investigated 5HT 2A gene polymorphisms in anxiety and depression in a distressed society such as a conflict area. To study 5HT-2A -1438 A/G single nucleotide polymorphism in depression and anxiety disorders in India-controlled Kashmir.

METHODS: Thirty patients with depression disorders, 31 patients with anxiety disorders, and 40 unrelated healthy volunteers (controls) were studied in a case-control study design. Polymorphism was determined using polymerase chain reaction and agarose gel electrophoresis after digestion with the restriction enzyme MspI. Genotypes and allele frequencies were compared using chi square and Fischer's exact tests, and p value of <0.05 was considered to be statistically significant.

RESULTS: The mean±standard deviation of age for the anxiety, depression and control groups was 33.5±11.7, 32.4 ± 9.9 and 29.7 ± 10.1 respectively and was not statistically significant (p= 0.32). In the anxiety group, GG genotype (41.9 %) was significantly more prevalent (p= 0.045) than the control group (17.5%). Similarly, depression group had higher prevalence of GG genotype (50%) (p=0.004) than the control group (17.5%). Comparison of allelic frequencies found a statistically significant excess of G allele in the depressive group (68.3%) as compared with the control group (36.2%) (P ≤0.0001, OR=3.8 (1.9 to 7.7).

CONCLUSION: In a conflict area, we found that the -1438 A/G single nucleotide polymorphism in the promoter region of 5HT-2A gene was associated with anxiety and depressive disorders. Our findings confirm the role of this locus in psychiatric disorders and further show that the association remains robust in a distressed society.

Keywords: Anxiety; Depressive Disorders; 5HT 2A Receptor Gene Polymorphism

INTRODUCTION

Anxiety and depressive disorders result from a complex interaction between an individual's genetic endowment and environmental factors [1, 2]. The influence of environment is difficult to evaluate in a genetic association study and is usually a confounding factor [3]. Molecular cloning has identified 7 major types of 5-hyrdoxytryptamine (5HT) receptors [4] and the roles of these receptors in psychiatric disorders have gained prominence over the last two decades [4]. 5HT2A receptor is the main excitatory G protein-coupled receptor (GPCR) of 5HT and mediates it action via G protein q (Gq) through phospholipase C (PLC) and protein kinase C (PKC), leading to the release of calcium [4]. 5HT2A receptor gene is located on chromosome 13q14-q21 and is widely expressed in most 5HT terminals in CNS as well as in megakaryocytes/platelets and monocytes [4, 5].
Studies have suggested that -1438 A/G single nucleotide polymorphism is associated with psychiatric disorders, especially anxiety and

depressive disorders [3, 5, 6]. 5HT2A exerts antidepressant effects in humans and modulates antidepressant effects in animal models of anxiety and depression [2, 5]. Various studies have shown an association of -1438 A/G polymorphism with depressive and some anxiety disorders [5, 6, 7]. However, the association of this variant with anxiety and depression in a conflict area has not been studied. In the present study, we use a case-control study design to investigate the association of 5HT2A -1438 A/G polymorphism in anxiety and depressive disorders in a conflict area.

METHODS AND MATERIALS

Setting: The study was conducted in the Postgraduate Department of Psychiatry, Institute of Mental Health and Neurosciences, Kashmir. A total of 101 unrelated individuals (30 with major depression, 31 with anxiety, and 40 healthy volunteers) were enrolled in the study from March 2012 to April 2013. The diagnoses of major depression and anxiety were made based on DSM IV TR criteria. Individuals with anxiety disorder consisted of those with panic disorder (n=17), posttraumatic stress disorders (n=5), generalized anxiety disorder (n=5) and obsessive compulsive disorders (n=4). Bipolar depression was excluded on the basis of history and mental status examination. The inclusion criteria included patients above 18 years of age and patients willing to participate in the study, by means of informed consent. The exclusion criteria included depressive and anxiety disorders due to general medical conditions and due to psychoactive substance use.
All patients underwent detailed physical and mental status examination clinically. Data were meticulously recorded on a specially designed proforma. Observer rating scales (HAM-D and HAM-A) were used to assess anxiety and depression severity [8, 9].

DNA Extraction: For genotyping, DNA was extracted from a portion of whole blood using GENEI Genomic Extraction Kit supplied by the Messer's Bangalore GENEI, India.

Determination of 1438 A/G Polymorphism: Polymerase chain reaction was carried out with primers 5' AAC CAA CTT ATT TCC TAC CAC–3' and 5' AAG CTG CAA GGT AGC AAC AGC-3' (T Tencomnao *et al* 2010) [10]. PCR was performed in 20 ul mixture containing about 50 ng genome DNA, 10 pmol of each primer, Tris-HCL, pH=8, 100 uMdNTP's, 1U of Taq Polymerase using Tech-gene–Thermal cycler (UK). The amplification was carried out in the following way; after an initial incubation at 95 degree Celsius for 10 minutes, 30 cycles of denaturation at 94 degree Celsius for 1 minute, an annealing step at 65 degree Celsius for 1 minute, followed by an extension step at 72 degree Celsius for 1 minute were performed and final incubation at 72 degree Celsius for 10 minutes was done. After a 3% agarose gel electrophoresis, the PCR products were stained with ethidium bromide and then bands were observed under UV light (figure 1). Allelic size was determined by the comparison of bands with size standards after electrophoresis in polyacramide gel, followed by silver staining, 3 genotypes i.e. GG, AA and AG were observed . Fragment A had 468 base pairs and G had two bands of 244 and 224 bp. Randomised selected DNA samples were subjected to direct sequencing to validate the genotype.

Statistical Analysis: Genotypes and allele frequencies were compared using analysis of variance (ANOVA), chi square and Fisher exact tests, as appropriate and a p value of <0.05 was considered to be significant. The quantitative data were analysed using one way analysis of variance (ANOVA) and two sample independent t-tests.

RESULTS

The mean±SD age of anxiety, depression and control group was 33.5± 11.7, 32.4 ± 9.9 and 29.7 ± 10.1 respectively (p= 0.32). The mean HAM-A score was significantly higher in the anxiety group than in the group with depression (28.2 vs. 16.7; p=0.001) whereas mean HAM-D of anxiety group was lower than the depression group (15 vs. 25.1; p=0.001) (Table 1).
The type of disorder i.e. anxiety and depressive disorder were compared with controls with respect to genotypic distribution and allelic frequency (Table 1 and 2). Patients suffering from anxiety had significantly higher GG genotype (41.9%; p= 0.045) than control (17.5 %). Similarly, depression group had significantly higher GG genotype (50 %; p=0.004) than control (17.5%). Comparison of allelic frequency found an excess of G allele in depression group (68.3%) compared with control (36.2%) and was found to be statistically significant ($p \leq 0.0001$, OR=3.8; 95%CI = 1.9 to 7.7). Allelic frequency was also significantly higher in anxiety disorder

Table 1: Characteristics of the studied groups

	Anxiety group (n=31)	Depression group (n=30)	Control group (n=40)	P –value
Age (Mean ± SD)	33.5± 11.7	32.4 ± 9.9	29.7 ± 10.1	0.318
Male	15(48.4)	17 (56.7)	26(65)	Missing
Female	16(51.6)	13(43.3)	14(35)	Missing
HAM-A	28.2	16.7	-	0.001
HAM-D	15.0	25.1	-	0.001
Genotypes				
AA	7 (22.6)	4(13.3)	18(45)	0.045a
AG	7 (22.6)	11(36.7)	15(37.5)	0.004[b]
GG	13(41.9)	15(50)	7 (17.5)	

Values within parenthesis are percentages. a = Anxiety group vs Control group; b = Depression group vs Control group

Table 2: Allelic frequency of the 1438 A/G gene in anxiety and depressive disorders

Group	G	A	p value	OR (95% CI)
Anxiety	37(59.7)	25(40.3)	0.007*a	2.6(1.32 to 5.1)
Depressive	41 (68.3)	19 (31.7)	≤ 0.001*b	3.8 (1.9 to 7.7)
Control	29(36.2)	51 (63.7)	------	----------

Values within parenthesis are percentages. a = Anxiety group vs Control group; b = Depression group vs Control group

(59.7%) compared to control (36.2%) (p= 0.007, OR= 2.6, 95%CI = 1.3 to 5.1).

DISCUSSION

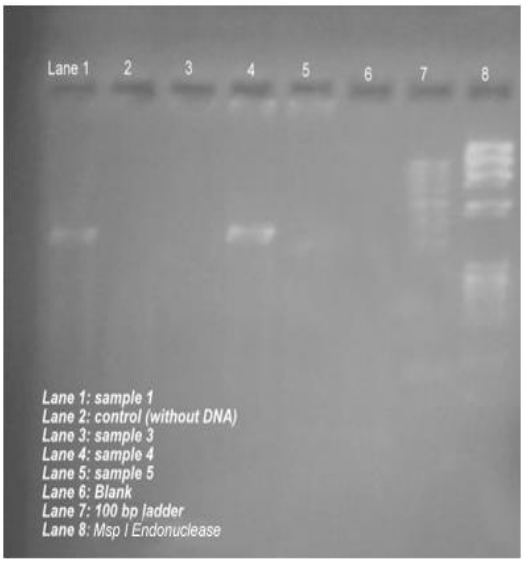
Psychological as well as psychiatric disorders are on the rise in Kashmir for the past two decades [11, 12]. There have been periods of insurgency and political turmoil, which have increased mental health problems [11]. Genetic, biological, psychological and social factors are postulated to be the etiological factors responsible for the emergence of anxiety and depressive disorders [5]. While the association of environmental factors with psychiatric disorders have been examined in this setting, the role of 5HT2A receptor gene polymorphism, in particular with anxiety and depressive disorders has not been studied [13].

Major depressive disorders are the second major cause of disease burden worldwide [10] and genetic factors contribute approximately 40% towards the risk of major depressive disorders [14]. 5HT2A receptor gene has been implicated in a wide variety of psychiatric disorders [5, 10]. Some studies have found strong association of major depressive disorder and anxiety disorders with 5HT2A while others failed to show any association [5, 6, 14, 15]. Some authors suggest that the inconsistent results may be due to ethnic variations among the populations studied [15].

To the best of our knowledge, this is the first study that investigates the association of depressive and anxiety disorders, with 5HT2A -1438 A/G gene in a conflict area. The mean anxiety and depression scores were significantly higher in the anxiety and depressive groups than the control group (table 1), thus supporting the hypothesis that anxiety and depression are two faces of the same disorder [16, 17]. This may also explain why drugs that act on 5HT2A receptors are effective in reducing both anxiety and depressive features [3, 18].

Our findings suggest that GG genotype (50%) and the frequency of G allele (68%) was associated with major depressive disorders and with anxiety disorders. Similar findings were reported by Choi et al who found that the frequency of G allele in Korean population suffering from depression approximately was 49% [5]. Our findings are also supported by Ono et al (2001) and Nakamura et al (1999), who

Figure 1: Determination of 1438 A/G Polymorphism



found higher prevalence of GG genotype in the range of 46-48% in individuals with depression [19, 20]. However our findings are in contrast with Tencomnao T et al (2010), who reported no association of G allele (19.7%) or GG genotype (56%) with major depressive disorder [10]. These differences of genotype in ethnic variation were explained on the basis of differences in linkage disequilibrium between genetic markers and dissimilarities in allelic frequencies [5].

Similarly, our finding that GG genotype and G allele are associated with anxiety is in agreement with several other studies [7], who report a strong association of G alleles with various anxiety disorders [6, 7, 16]. The proposed mechanism through which 5HT2A receptor acts in the development of depression and anxiety disorders is by facilitating GABA release in the amygdala and facilitating GABA-mediated synaptic transmission in the basolateral amygdala (BLA) and thus inducing anxiolytic action. Long standing stresses, one of the causative factors for anxiety disorders decrease 5HT 2A receptor signaling [18]. Antidepressants are effective in anxiety disorders because of their antagonism of the 5HT 2A receptor action [21, 22, 23] These drugs can be helpful in people, who have anxiety disorders with comorbid depression or vice versa. Our study is a first of its kind conducted in the North-Indian subcontinent mountainous valley of Kashmir, where susceptibility towards such disorders is high, due to ongoing conflict in the area [11, 12]. During the last two decades, people of the valley have witnessed extreme violence which further magnified the effect of genetic predisposition. Further large samples are needed to study understand the role of these genetic variants on depression and anxiety disorders.

CONCLUSION

In summary, we found that the -1438 A/G single nucleotide polymorphism in the promoter region of 5HT-2A gene was associated with anxiety and depressive disorders in a conflict area. Our findings confirm the role of this locus in psychiatric disorders and further show that the association remains robust in a distressed society.

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