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ORIGINAL ARTICLE

Association involving serotonin transporter gene linked polymorphic region and bipolar disorder type 1 in Iranian population

Mohammad Ghadiri[1] MD, Issa Nourmohammadi[2,*] PhD, Mahdi Fasihi Ramandi[3] PhD & Ehsan Moazen Zadeh[4] MD

1 Iran Psychiatric Hospital, Iran University of Medical Sciences, Tehran, Iran
2 Department of Biochemistry, Iran University of Medical Sciences, Tehran, Iran
3 Research Center of Molecular Biology, Baqiyatallah University of Medical Sciences, Tehran, Iran
4 Mental Health Research Center, Tehran Psychiatric Institute, Iran University of Medical Sciences, Tehran, Iran

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Correspondence
Ehsan Moazen Zadeh MD, Mental Health Research Center, Tehran Psychiatric Institute, No. 1, Mansouri Street, Niayesh Avenue, Sattarkhan Boulevard, P.O. Box: 14456-13111, Tehran, Iran.
Tel: +98 21 88077892
Fax: +98 21 88077892
Email: moazenzadeh.ehsan@gmail.com

*Present address:
Department of Biology, Oklahoma City Community College, Oklahoma City, OK, USA.

Author contributions
Mohammad Ghadiri: design, sampling, data collection, final interpretation, manuscript writing. Issa Nourmohammadi: design, final interpretation, manuscript writing. Mahdi Fasihi Ramandi: design, laboratory procedures, data collection, final interpretation, manuscript writing. Ehsan Moazen Zadeh: design, data analysis, final interpretation, manuscript writing.

Research sites
Iran Psychiatric Hospital, 7th kilometer, Lashkari Expy., P.O. Box: 13989-13151, Tehran, Iran. Hazrat-e-Rasoul Akram Hospital, Niayesh Avenue, Sattarkhan Boulevard, P.O. Box: 14456-13131, Tehran, Iran.

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Abstract

Introduction: Serotonin transporter gene linked polymorphic region, also called 5HTTLPR, is a candidate in the genetics of bipolar disorder; however, the results of previous association studies are inconsistent. Several explanations have been proposed for that inconsistency; among them are the existing differences both in the genetic basis of bipolar disorder subtypes and the genetic backgrounds of the studied populations. We aimed to investigate the association of 5HTTLPR with bipolar disorder type I (BP-1) in Iranian population.

Methods: In this case–control study, 146 patients with BP-1 and 165 controls were recruited. The patients were selected through the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, 4th edition*. It was required that the patients do not have any present history of general medical conditions, substance abuse, and concurrent major psychiatric disorders. The polymorphism was evaluated by blood sampling and subsequent DNA extraction, polymerase chain reaction, and agarose gel electrophoresis. Chi-square test was used for analyzing allelic and genotype frequencies and two-tailed *P* values were obtained.

Results: The S allele was significantly more frequent in the BP-1 patients compared with the controls ($P = 0.02$, S allele odds ratio = 1.5, confidence interval 95% = 1.06–2.11).

Discussion: Our statistically significant results suggest that the role of 5HTTLPR in the pathogenesis of BP-1 needs to be clarified by further scrutiny in Iranian population and other populations of Near East.

Introduction

As a chronic and debilitating psychiatric disorder, bipolar disorder (BD) affects general population with lifetime prevalence of 1% in the world (Merikangas *et al.*, 2011) and 0.96% in Iran (Mohammadi *et al.*, 2005). Numerous lines of evidence have represented genetic susceptibility to BD, among which are 40% and 13.5% concordance rates of BD in respective monozygotic twins and dizygitoc twins (McGuffin *et al.*, 2003), and lifetime risks of 40–70% and 5–10% in respective monozygotic co-twins and first-degree relatives of a bipolar proband (Craddock and Jones, 1999). To unveil the genes that underlie BD, researchers have primarily focused on the neurotransmitter systems that are the target of medications for BD (Craddock *et al.*, 2001). Serotonin is a key neurotransmitter in the mood regulation and the etiopathology of mood disorders (Müller-Oerlinghausen *et al.*, 2002). So, many studies have investigated the variations in the serotonin transporter (5HTT) gene. Among the variations, a 44 base-pair insertion/deletion polymorphism in the promoter region of the 5HTT gene, also named the 5HTT gene linked polymorphic region (5HTTLPR), has attracted a special attention (Mynett-Johnson *et al.*, 2000). The two main allelic forms of the polymorphism influence transcription efficiency of 5HTT gene in such a way that the short variant (S) is associated with lower transcription rates comparing with the long variant (L) (Collier *et al.*, 1996; Lesch *et al.*, 1996). Primary studies reported a potential association of anxiety-related traits and mood disorders, including BD, with the S allele of 5HTTLPR (Collier *et al.*, 1996; Lesch *et al.*, 1996); however, the association issue has remained controversial. Nearly half of the conducted genetic studies, including genome-wide association studies and single nucleotide polymorphism (SNP) studies, have produced negative results (Piletz *et al.*, 2011). As well, the statistically significant results of two meta-analyses on SNP studies favor the association (Cho *et al.*, 2005; Jiang *et al.*, 2013), whereas another meta-analysis found no significant association between 5HTTLPR and BD (Seifuddin *et al.*, 2012). Putting aside the environmental factors and possible false-negative or false-positive studies, there are proposed genetic reasons for such contradictory results. One genetic explanation is that the subtypes of BD may be different in terms of association with 5HTTLPR, since the S allele was more frequent in the patients who had characteristics of bipolar disorder type 1 (BP-1) compared with the total sample incorporating both BP-1 and BP-2 patients (Ho *et al.*, 2000; Ospina-Duque *et al.*, 2000). As another genetic explanation, we can take into account the role of population stratification in the genetic association studies (Gelernter *et al.*, 1999). The fact that distributions of multiple SNPs, including 5HTTLPR, in the 5HTT gene vary significantly across populations, implies the importance of establishing genetic association studies in genetically different populations and avoiding extrapolation of the results of studied populations to unstudied ones (Murdoch *et al.*, 2013). To the best of our knowledge, there has not been any research into the association of 5HTTLPR with BD in Near Eastern populations, including Iranian population. From the historical point of view, Near Eastern populations, inclusively Arab and Persian populations, have been reciprocally subject to considerable gene flow and genetic homogeneity (Shepard and Herrera, 2006). On the other hand, the genetic heterogeneity increases when moving outwards of the Near East, which has been suggested to be mainly because of the geographical barriers on the borders of this region (Shepard and Herrera, 2006).

Based on those proposed genetic explanations for the inconsistent results of previous association studies, the objective of this preliminary study was to investigate the association between 5HTTLPR and BP-1 in Iranian population.

Methods

Subjects

In this case–control study, 146 unrelated patients with BP-1 were selected through random sampling method from the inpatients and outpatients of an academic psychiatric hospital, Tehran, Iran, 2010–2012. The patients were diagnosed by the trained psychiatrist through the Structured Clinical Interview for DSM-IV (SCID) based on *Diagnostic and Statistical Manual of Mental Disorders, 4th edition*, text revision (DMS-IV-TR). It was required for the patients not to have any present history of general medical conditions, substance abuse, and concurrent major psychiatric disorders. Controls consisted of 165 people with no history of major psychiatric disorders including mood disorders. They were selected from unrelated personnel and patients' relatives from another academic referral hospital in Tehran, Iran, 2010–2012. Afterward, they were interviewed by the trained psychiatrist and matched with the patients for age. All the subjects were Iranians.

Ethics

Written informed consent was obtained from the participants. The process was authorized by the Ethics Committee of the university in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki, Edinburgh 2000 revision).

Genotyping

Whole genomic DNA was extracted from peripheral venous blood leukocytes using the salting out method (Genomic DNA Prep Kit, SolGent Co. Ltd., Daejeon, South Korea). Using the available thermocycler (Rotor Gene 6000, Corbett Research Pty. Ltd., Sydney, Australia), the location of 5HTTLPR was amplified by polymerase chain reaction (PCR) in 30 µl of reaction solution containing 100 ng DNA, 0.2 mM each of dATP, dTTP, dCTP and dGTP, 25 pmol each of primers (Forward 5′_GGCGTTGCCGCTCTGAATGC_3′; Reverse 5′_GAGGGACTGAGCTGGACAACCAC_3′), 10 mM Tris_HCl (PH = 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 1.5 U Taq DNA polymerase. Thermal cycling was performed with the initial denaturation step for 3 minutes at 95°C, and subsequent 30 time repeated steps of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 61°C and elongation for 60 seconds at 72°C. Finally, PCR products (528 bp and 484 bp) were separated on agarose gel (2%) and detected by ethidium bromide staining under fluorescence light.

Statistical analysis

Statistical analysis was carried out using PASW 18 (IBM SPSS Statistics, IBM Co., New York, NY, USA) for mainframe analysis and STATA 12 SE (StataCorp LP, Lakeway Dr, TX, USA) for power estimations. Mean ages (±standard deviation) and gender distributions were compared by Student's *t*-test and Chi-squared test, respectively. Genotype distributions in both cases and controls were checked for Hardy Weinberg equilibrium. Between-group comparison of allelic and genotype frequencies was performed by either Chi-squared test or Fisher's exact test according to expected values. Type 1 error of 5% and two sided *P* values were the assumptions in all analyses. Odds ratios (OR) were calculated while considering L allelic and L/L genotype frequencies as reference.

Results

Calculated mean ages for patients and healthy controls were 39 ± 11 years and 43 ± 9 years, respectively, with no significant difference. Also, the gender distributions were not significantly different between the 146 patients (85 male and 61 female) and 165 controls (91 male and 74 female). Genotype distributions were in Hardy Weinberg equilibrium for BP-1 patients ($\chi^2 = 0.0005$, $df = 2$, $P = 0.99$) and healthy controls ($\chi^2 = 2.57$, $df = 2$, $P = 0.28$). Results of analysis for between group comparison of allelic and genotype distributions are presented in Table 1. Assuming the S allele as a predisposing factor for BP-1, we calculated further measures: sensitivity (57.5%) and specificity (56.4%).

Discussion

We found that the frequency of S allele was significantly higher in the BP-1 patients compared with the healthy controls, which implies the possible role of S allele in genetic etiopathology of the disorder; however, the OR for the S allele was not such a high value to favor the role of the S allele strongly (OR = 1.5, confidence interval [CI] 95% = 1.06–2.11). In addition, the L/S and the S/S genotypes were more frequent in the BP-1 patients and the ORs also favored those higher frequencies (Table 1). Although the difference in genotype distribution for the S/S genotype did not reach the significance level, the relevant ORs

Table 1. Genotype and allelic distribution of 5HTTLPR polymorphism in BP-1 patients and controls (*n* = 311)

	Controls	BP-1 patients	χ^2	*df*	*P*	OR (CI 95%)
Allelic distribution						
L	243 (74%)	190 (65%)	5.38	1	0.02	Reference
S	87 (26%)	102 (35%)				1.5 (1.06–2.11)
Genotype distribution						
L/L	93 (56.5%)	62 (42.5%)	5.99	2	0.05	Reference
L/S	57 (34.5%)	66 (45%)				1.74 (1.08–2.80)
S/S	15 (9%)	18 (12.5%)				1.80 (0.84–3.84)

5HTTLPR, 5HTT gene linked polymorphic region; BP-1, bipolar disorder type 1; CI, confidence interval; OR, odds ratio.

was relatively high (OR = 1.8, CI 95% = 0.84–3.84). In fact, the low number of people with S/S genotype in the both groups may have been the main reason for lack of significance in difference. The fact that genotype frequencies of the patients and healthy controls followed the Hardy Weinberg equilibrium makes our sampling and the obtained results more reliable. Our findings are in line with the common disease, common variant (CD/CV) hypothesis, which has been a principal hypothesis in most of recent genetic studies of BD (Iyengar and Elston, 2007; Piletz *et al.*, 2011).

The results of our study are favored by some of the previous investigations. In a meta-analysis, Cho *et al.* (2005) concluded that 5HTTLPR has a small effect on predisposition toward BD when considering this polymorphism as the only involved genetic factor. In the most recent meta-analysis, Jiang *et al.* (2013) revealed a significant association between S allele of 5HTTLPR and BD ($P = 0.005$, OR [CI 95%] = 1.10 [1.03–1.17]) with a comprehensive sample size (cases = 3778, controls = 4997) and inclusion of 20 case–control studies of European ancestry (Jiang *et al.*, 2013). Of course, the small value of ORs in that study should be taken into account when interpreting the results.

Our results are contrasted by some other investigations. A meta-analysis conducted in 2012 failed to show any significant association between the 5HTTLPR and BD ($P = 0.249$, OR [CI 95%] = 0.92 [0.80–1.06]) with inclusion of 18 studies (cases = 1809, controls = 2693) (Seifuddin *et al.*, 2012). Recent studies in American and Malaysian populations showed that allelic and genotype frequencies of 5HTTLPR are not significantly different when comparing BD patients with controls (Cosgrove *et al.*, 2012; Mohamed Saini *et al.*, 2014); however, the low number of cases and consequent inadequate powers of those studies make it difficult to infer that no association exists. In addition, the cases in those studies were consisted of both BP-1 and BP-2 patients as a pooled sample comparing with the sample of BP-1 patients in our study. In another study, Ospina-Duque *et al.* (2000) assessed 5HTTLPR inclusively in BP-1 patients of a Colombian isolate population and found no significant difference in allelic or genotype frequencies between cases and controls; however, the sample size of that study was noticeably smaller than ours and did not reach the minimally accepted value for the power (0.8), which makes it difficult to reject the hypothesis of association between 5HTTLPR and BP-1.

To explain the discrepancies in the available reports, besides the limitations on power of the samples and the pitfalls in psychiatric diagnosis, there are confirmed aspects in the literature. In one aspect, BD has a complex spectrum of symptoms that has lead to the subcategorization of BD into BP-1 and BP-2. There could be differences in the genetic basis of each subtype and even each clinical characteristic of BD in the spectrum (Ho *et al.*, 2000; Ospina-Duque *et al.*, 2000). In the other aspect, BD is a multifactorial disorder that involves a number of genes and environmental factors. In fact, environmental factors play a prominent role in pathogenesis of psychiatric disorders, which is hypothesized to be mainly executed through epigenetic mechanisms (Abdolmaleky *et al.*, 2004; Crow, 2007; Cornelis *et al.*, 2010). The variability in the genetic backgrounds of populations is also another important aspect that we should consider when pooling and comparing the results of different studies (Murdoch *et al.*, 2013). Concerning that aspect, we found that the frequencies of S allele in the populations outside the Near East were different from the frequency of S allele in our sample of controls: European population 43% (Mendlewicz *et al.*, 2004), African-American population 28% (Williams *et al.*, 2003), Indian population 50% (Vijayan *et al.*, 2009), Japanese population 80% (Gelernter *et al.*, 1997) and Iranian population 26%. Relative homogeneity among Near Eastern populations (Shepard and Herrera, 2006) and among different ethnicities living in Iran (Farjadian *et al.*, 2009) implies that the real 5HTTLPR allelic distributions in the populations of Near East may be comparable with the results of our study; however, it is a matter of further investigations.

As the limitations in our work, there are things to be mentioned: first, the power estimations for our study did not reach the appropriate level of 0.8, and due to budget limitations, it was not possible for us to collect more subjects. But, the differences in the allelic frequencies and the L/S genotype frequencies were significant. Therefore, the lack of appropriate power level achieves its importance mainly in the comparison of S/S genotype frequencies, in which the apparent difference between the two groups did not reach statistical significance. Second, we did not consider sociodemographic backgrounds of subjects in the matching process. As both of the genetic and the environmental factors play important roles in the pathogenesis of psychiatric disorders, assessing social backgrounds of BP-1 patients besides genetic analysis can be of real importance. Third, we did not consider a recently introduced SNP (rs25531) that is located in 5HTTLPR region (Ozaki *et al.*, 2003).This SNP results in three possible allelic forms: L_A, L_G and S. The transcription and consequent mRNA expression of 5HTT

gene is modulated by the newly known L_G allele as well as the previously investigated L_A allele and S allele. Considering that L_G allele and S allele influence 5HTT gene expression nearly in the same way, the effect of 5HTTLPR may be underestimated in our study if there would be abundant L_G alleles in our sample (Hu *et al*., 2006).

In conclusion, the results of our study were statistically significant when comparing the frequency of 5HTTLPR between BP-1 patients and controls; however, to elucidate the role of 5HTTLPR in etiopathology of BP-1, further research is needed with bigger sample sizes and consideration of the role of gene–environment interaction in pathogenesis of the BD. It also necessitates investigations into the association of 5HTTLPR and BP-1 in the other populations of Near East.

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