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Short Communication

Genetic Contribution of BDNF Gene in Obsessive Compulsive Disorder in Pakistani Punjabi Population

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Authors' Contributions
J and MEB conceived the project, performed the lab work and wrote the article; TH and RS did lab work and data analysis; SR, HS, MW, MA and AAS helped in sample collection.

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OCD, BDNF gene, Polymorphism, Sequencing analysis, Punjabi.

ABSTRACT

This study is aimed to identify genetic variants in *BDNF* gene and their association with Obsessive Compulsive Disorder (OCD) among Pakistani Punjabi population. We recruited a total of 100 patients diagnosed with OCD following Diagnostic Statistical Manual-IV (DSM-IV) criteria and controls (n=120) were selected from the same ancestry during August 2011 to January 2014 from Sir Ganga Ram Hospital and Panjab Institute of Mental Health, Lahore. The demographic values for age, employment and marital status did not differ significantly among case and control groups. Exonic regions of *BDNF* gene were sequenced. In-silico analysis was performed to detect the possible disease causing effect of genetic variants with OCD. Sequencing of *BDNF* gene showed one change G>A at position c.196 (rs6265) resulting in a nonsynonymous change from valine to methionine. We found only one homozygous and heterozygous carrier for this polymorphism. Healthy siblings of the carrier individuals and control group were screened and found as negative for this polymorphism. In-silico analysis through mutation taster, exon splicing enhancer and polyphin2 software predicted this polymorphism as a site broken and possibly damaging. These findings suggest that BDNF polymorphism Val66Met has some possible role in OCD development in Punjabi patients.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is a glutamate neurotrophic factor that plays an important role in promotion and maintenance of cell differentiation (maturation), nerve cell survival (neurans) and cell death (Huang and Reichardt, 2001a). In the brain, the BDNF protein paly significant role in connections and communications between nerve-nerve cells (synopses) and helps in regulation of synaptic plasticity, which is important for memory and learning. BDNF is produced as pro-form that can be cleaved intracellular later in order to release mature secreted ligands. These ligands then bind to Trk family members of receptor tyrosine kinases that prop up Trk mediated differentiation and survival (Friedman and Greene, 1999). Pro-neurotrophins are active precursors, since they can be secreted and cleaved extracellularly and serve as high affinity ligands for p75 NTR, which encourages apoptosis in neurons and oligo-dendrocytes (Lee *et al*., 2001). The *BDNF* gene is located on chromosome 11p13, and it encodes pre-protein of 247 amino acids that is later cleaved to produce mature protein of 120 amino acids. The genetic polymorphisms identified in this gene have been associated with increased risk of developing psychiatric disorders such as obsessive compulsive disorder (OCD), Anxiety, eating disorder, schizophrenia, and bipolar disorder. A single nucleotide polymorphism (SNP) in the *BDNF* gene at position c.196 (G/A) has been reported which replace valine with methionine (Valine/Met, rs6265) in the region which encodes the prodomain. This change has been characterized with impairs the BDNF protein's function. Some studies supported this polymorphism with various psychiatric disorders (Hall *et al*., 2003; Hemmings *et al*., 2008), however, some previous and recent studies did not support these finding of association (Wang *et al*., 2015; Alonso *et al*., 2007; Wendland *et al*., 2007). This is controversial and unclear whether this polymorphism is related to these disorders, however, the other genetic and environmental factors are remain unknown.

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Table I.- Primers for coding region of *BDNF* gene.

Gene	Forward Primer	Reverse Primer	Temp
BDNF-1	TGCAGCTGGAGTTTATCACC	GCCGAACTTTCTGGTCCTC	F=57.80, R=59.72
BDNF-2	GCAAACATCCGAGGACAA	TGCCGTTACCCACTCACT	F=55.02, R=57.30
BDNF-3	CTCCTCTTCTCTTTCTGCTG	TCCACTATCTTCCCCTTTTA	F=57.80, R=53.70
BDNF-4	CCCTGTATCAAAAGGCCAAC	CGGCAACAAACCACAACAT	F=57.80, R=55.41

F, forward; R, reverse, Temp, melting temperature.

OCD is a fourth most common deliberate psychiatric disorder. Its worldwide prevalence is 1-3% of children and adults and equally affects both males and females (Sarvet, 2013). OCD is a mental disorder, according to the Diagnostic Manual of Mental Disorders (DSM-IV) criteria, this disease is characterized by intermittent and interfering obsessive thoughts and cyclic compulsive behaviors which reduce anxiety that is associated with obsessions. Previous studies on twin and family's genetic showed that OCD is a chronic disorder and established irrespective of sex, race, marital status, intelligence, socioeconomic status, nationality or religions (Hettema *et al*., 2001; Sampaio *et al*., 2013). Patients with OCD experience intrusive and repetitive thoughts known as obsession and the uncontrollable urge to repeatedly acts or rituals compulsions to reduce the anxiety produced by obsessional thoughts (American Psychiatric Association, 1994). Several studies revealed that OCD is associated mainly with dysregulation and less concentration of 5-HT neurotransmission, which may play a possible role in the pathogenesis of OCD (Zohar *et al*., 2000; Westenberg *et al*., 2007).

Materials and methods

Genomic DNA was extracted from the blood using a standard procedure (Sambrook and Russel, 2001). Primers were designed through Primer3 software to amplify the complete BDNF coding region (Table I). The complete coding region of *BDNF* gene was directly sequenced using ABI 3130xl automated sequencer (Applied Biosystem Inc, Foster City, CA). The sequenced data was aligned and analyzed using Blast Local search Alignment tool. Chi square (X^2) test was used for categorical variables and student's t-test was used for continuous variables. In-silico prediction analysis was performed using mutation taster, Polyphin2 and Exon splicing enhancer software.

Results and discussion

In this study, we investigated the genetic variation of OCD individuals in the Punjab ethnic group in Pakistan. we aimed at exploring the existence of possible genetic interaction between *BDNF* gene and clinical variables in samples of OCD patients (Fig 1). The demographic distribution of the both groups is illustrated in Table II. In both groups the distribution of male and female was similar. We observed one variation G>A, resulting in an amino acid change from valine to methionine at 66 amino acid position. Two patients were found homozygous for Val>Met and heterozygous for Val>Met at c.196 position in *BDNF* gene.

Table II.- Demographic characteristics of the subjects with OCD and without OCD included in the study.

	OCD	Control	p-value
Age	26.08 ± 7.6	29.6 ± 7.3	0.081
Gender			
Male	55 (55%)	70 (58%)	1.00
Female	45 (45%)	50 (41%)	1.00
Education in years	8.3±4.8	10.6±2.9	0.0002
Employment			
Employed	43 (43%)	81 (67.5%)	1.00
Unemployed	57 (57%)	39 (32.5%)	1.00
Marital status			
Unmarried	45 (45%)	76 (63.3%)	1.00
Married	55 (55%)	44 (36.6%)	1.00
Family history of OCD			
No	68 (68%)	120 (100%)	
Yes	32(32%)	0 (0%)	
Age at onset	24.2±6.2	-	
Duration of illness	8.2±5.8	-	
Mean Y-BOCS score			
Y-BOCS Obs score	12.8±2.6	-	
Y-BOCS Comp score	12.2±3.6	-	
Total	**25±6.2**	**-**	

SD, standard deviation; Y-BOCS, Yale-Brown obsessive compulsive scale; Obs, obsessions; Comp, compulsions; OCD, obsessive compulsive disorder. *P*-value was calculated through the Chi-square test. Values are Mean±SD.

In-silico analysis of this genetic variation was performed using mutation taster (http://www.mutationtaster.org/) and Exon Splicing Enhancer software (http://www.umd.be/HSF3/4DACTION/AW_QuickMutant). Resulting data

predicted it as disease causing with a site broken effect. We collected blood samples of other healthy family members of the patients observed with Val>Met polymorphism, but found as negative for this variation.

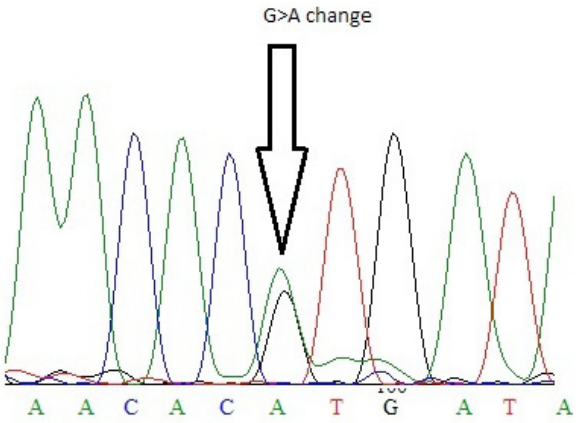

Fig. 1. The sequencing result of *BDNF* gene of OCD affected person showing the heterozygosity change from G>A.

Several studies supported the hypothesis that *BDNF* gene Val66Met genotype has been found to be associated with the clinical features of OCD in various ethnic groups in the world (Hall *et al*., 2003; Hemmings *et al*., 2008, 2013). Our results support most of previous and recent studies. BDNF Val66Met polymorphism has been extensively studied, the first study that associates Val66Met was conducted in 2003 (Hall *et al*., 2003), that study reported that the Met66 allele revealed a protective effect on OCD (Hall *et al*., 2003). Other previous studies reported Val66Met was a risk allele (Hemmings *et al*., 2008, 2013). BDNF polymorphism Val66Met has been studied in several other mental disorders such as schizophrenia, bipolar disorder, depression, drug abuse and anorexia nervosa, most of them did not associate between the Val66Met and the disease (Kanazawa *et al*., 2007; Verhagen *et al*., 2008; Brandys *et al*., 2013; Gyekis *et al*., 2013; González-Castro *et al*., 2015). Some found the association between OCD and Val66Met, showed inconsistent results (Wang *et al*., 2015; Alonso *et al*., 2007; Wendland *et al*., 2007; Zai *et al*., 2005).

It has been found that there are significant ethnic differences in BDNF Val66Met polymorphism (Pivac *et al*., 2009). Caucasian carriers with Val/Val genotype have higher anxiety scores than carriers of heterozygous Val/Met genotype or homozygous Met/Met genotype (Lang *et al*., 2005). Comparison of genotype frequency Val/Val, Val/Met and Met/Met reported as significant ethnic differences in Val66Met variation in the *BDNF* gene with a similar distribution of Val/Met alleles among Korean individuals and the most frequent genotype in Caucasian population was Val/Val (Pivac *et al*., 2009). Homozygous Val/Val (G/G) genotype is also considered as an increased risk for sporadic Alzheimer's disease (AD) and homozygous A (Met) allele with the increased risk of poorer episodic memory (Ventriglia *et al*., 2002; Egan *et al*., 2003). Also evidence that the Val66 allele might increase the risk of depression as it was specially transmitted to bipolar volunteers in families (Neves-Pereira *et al*., 2002; Sklar *et al*., 2002). However, a study conducted on Japanese population, found no association of bipolar disorder and BDNF polymorphism (Nakata *et al*., 2003).

As *BDNF* gene plays a significant role in regulating neuronal proliferation and function and participating in the synaptic plasticity (Huang and Reichardt, 2001b), many previous and recent studies found and support that its peripheral levels were down regulated in various mental disorders such as OCD (Wang *et al*., 2015), anorexia nervosa (Saito *et al*., 2009), depression (Sen *et al*., 2008) and schizophrenia (Chen *et al*., 2014). It might be suggested that the lower peripheral blood level is a common feature and might be a marker reflecting impairment of synaptic plasticity and neuronal function in various mental disorders.

Conclusion

Our finding suggests that polymorphism Val66Met (rs6265) have some possible role of OCD development in Punjabi ethnic group. Genetic association of this polymorphism can be validated by screening at large scale.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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