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Association of *BDNF* and *SERT* gene polymorphisms with depression among Pakistani population

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Abstract: Brain-derived neurotrophic factor (BDNF) and serotonin transporter (SERT) is implicated in the adverse life events which lead to depression. The variation in genetic make-up of *BDNF* (*Val66Met*) and *SERT* (*5'-HTTLPR*) are potential biomarkers in the development of neuropsychiatric disorders including depression. The purpose of this study was to investigate the correlation of functional polymorphisms of *BDNF* and *SERT* genes with depression among Pakistani population. A total of 373 participants (204 cases with depressive episodes and 169 healthy controls) with age between 14 and 65yrs were recruited from Pakistani population. *BDNF* and *SERT* gene polymorphisms were genotyped using PCR-RFLP analysis. The result showed that lack of association of *Val66Met* (χ^2: 3.596, p>0.05) and *5'-HTTLPR* (χ^2: 0.634, p>0.05) gene polymorphisms were found with depression. However, *SERT* 'SL' (OR: 1.150, 95%CI: 0.601-2.201) and *BDNF* 'AA' (OR: 1.651, 95%CI: 0.585-4.660) and 'GA' (OR: 2.279, 95%CI: 0.825-6.298) genotypes might be a risk genotypes for depression. Hence, it is concluded that the functional *BDNF* (*Val66Met*) and *SERT* (*5'-HTTLPR*) gene polymorphisms may not be associated with depression. Replication studies on these polymorphisms with large sample size are needed.

Keywords: *BDNF, SERT,* polymorphism, depression, Pakistan

INTRODUCTION

Depression is a major contributor to global burden results from genetic and environmental factors. The major symptoms of a depressed individual will be grief and melancholy, lack of attention, underweight/obesity, insomnia/hypersomnia, agitation, feeling of unimportance, poor/noconcentration, suicidal ideation and decrease/increase appetite. In Pakistan, the prevalence of depression is about 22% among the elder population (Hammad *et al.*, 2006) and 34% in general population (Mirza and Jenkins, 2004). Many genes contribute to the regulation of depression among which *BDNF* and *SERT* genes are more important.

BDNF is a neurotrophin and a nerve growth factor which is extensively present in the brain of human mostly in the hippocampus and cerebral cortex. It plays an integral role in the control of growth, development, differentiation and survival of neurons in the brain (Mattson, 2008). It also involves in the control of serotonergic, dopaminergic, glutamatergic and GABA-ergic neurons and applies for a substantialrolein the neurogenesis and neuroplasticity (Numakawa *et al.*, 2010). The impaired expression of BDNF induces the anomalies in the synaptic plasticity which may result in the anatomical and functional strife (Sen *et al.*, 2008). So, BDNF is a candidate molecule which produces adverse effects on brain upon exposure to early life stresses and increases the risk of major depression. The gene which encodes the *BDNF* resides at the short arm of chromosome 11p13 which consist of numerous exons and one exon encoding the pro-BDNF protein. The regulation of *BDNF* gene promoters is specific to the tissues and its activity (Aid *et al.*, 2007). It was previously documented that localized synthesis and transportation of BDNF occurs indendrites which could regulate the BDNF activities (Chen *et al.*, 2008b).

Functional polymorphism of *BDNF* gene (rs6265) is a G to A variation present at 196 positionin the5'pro-region of the *BDNF* gene which results in a valine (Val) to methionine (Met) replacement at codon 66. The pro-BDNF protein binds with a trafficking protein called "sortilin" which is obligatory for the effective BDNF sorting. This contact of sortilin with the BDNF is decreased in presence of Met allele resulting in the decreases trafficking and release of Met-BDNF. A Met allele also affects the intercellular signaling and BDNF secretion by establishing homodimers and heterodimers which are

less effectively sorted and released from neurons (Chen *et al.*, 2008b). This will result in the accretion of BDNF in neurons and short falls of BDNF in the synapses (Egan *et al.*, 2003). Thus, Met allele decreases the BDNF activity (Egan *et al.*, 2003) and lowers BDNF serum levels (Ozan *et al.*, 2010) and seems to be related with the stress and depression (Zhao *et al.*, 2018, Youssef *et al.*, 2017) as compared to the Val allele, even though all studies do not agree (Chen *et al.*, 2008a, Iga *et al.*, 2007). Moreover, Met allele increases the risk of suicides (Iga *et al.*, 2007, Schenkel *et al.*, 2010), especially among depressive patients (Sarchiapone *et al.*, 2008) and those who exposed to early life stresses (Pregelj *et al.*, 2011).

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The serotonin transporter (SERT)/5-HT transporter (5-HTT) is responsible to control the serotonergic transmission in the brain and peripheral nervous system which contributes to the development of depression. The gene which encodes the serotonin transporter is also known as [the solute carrier family 6, member 4 (SLC6A4) gene] and is situated at chromosome 17q11.1-12. The gene is 31 kb long and consists of 14 exons (Ramamoorthy *et al.*, 1993). The polymorphisms in the *SERT* (*5-HTTLPR*) promoter (*SLC6A4*) are associated with depression (Ho *et al.*, 2013). The promoter region of *SERT* gene consists of short (S) or long (L) tandem repeats of a base-pair sequence. The 'S' allele was assumed to be associated with the increased susceptibility to stressful life events. 'S' allele decreases the expression of 5-HTT by reducing the efficiency of serotonin promoter during transcription which results in the shortfall in the expression of *SERT* mRNA and binding capacity of SERT than 'L' allele (Lesch *et al.*, 1994). A study showed that SS genotype individuals supposed to reveal maximum chances of occurrence of depressive episodes with stressful life events as compared to the LL genotype (Kendler *et al.*, 2005). Children with SS genotype and history of maltreatment showed greater symptoms of depression and suicidal ideation than those with the LL genotype (Cicchetti *et al.*, 2010). However, the aim of present investigation was to explore the association of *BDNF* (*Val66Met*) and *SERT* (*5-HTTLPR*) gene polymorphisms with depression among Pakistani population.

MATERIALS AND METHODS

Selection of study participants

This study enrolled the 373 participants with age range of 14-65years including 204 patients with depressive episode were selected from tertiary hospitals of Karachi, Pakistan in coordination with experienced psychiatrists and 169 non depressive subjects randomly selected from the same geographical region of Pakistan. The diagnosis of depression was ascertained according to the DSMIV (Diagnostic and Statistical Manual of Mental Disorders IV) criteria. All participants with diseases other than depression and those who used an antidepressant for more than 3 years were excluded from the study. All participants were of Asian origin and they were inborn Pakistani population. A written informed consent was also taken from all participants before contributing to the study and their anonymity was maintained. The study has also followed the guidelines of Helsinki Declaration and was approved by Ethical Review Board with approval number (IRB-178/DUHS-10). A pretested self-structured questionnaire was also given to all participants to collect the information.

DNA Isolation

Three (3) cc of blood was collected from all participants. Genomic DNA was extracted using kit method (Cat # A5081 Wizard Genomic DNA purification Kit, Promega, USA).

BDNF gene amplification and genotyping

BDNF (rs6265) genotyping was performed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. The primers specific for amplification of *BDNF* gene had the following sequence: F 5'-ACTCTGGAGAGCGTGAATGG-3' and R 5'-ACTACTGAGCATCACCCTGGA-3' (Mata *et al.*, 2010). A 20 ml of PCR mixture contained10ml of 2x green master mix (Promega, USA), 2µl of 150ng of genomic DNA and 0.5µl of 12pmole of each primer. Amplification conditions were: initial denaturation at 94°C for 5mins, 40 cycles of 94°C for 30secs, 62°C for 30secs, 72°C for 1min and final elongation at 72°C for 7mins. PCR was executed in an automated thermal cycler (Veriti, Applied Biosystem, USA). The amplified product was digested with 5 Units of the Eco72I enzymeat 37°C (Fermentas, USA). Digested fragments were then separated on 2.5% agarose gel electrophoresis with the voltage set at 80 V and finally visualized by GelDoc (UVP) Vision works LS software (version 7.1). The undigested product size was 197 bp (allele A, Val) whereas the digested bands were 124 and 73 bases long (Allele G, Met).

SERT gene amplification and genotyping

Genotyping of 5-HTTLPR was performed using primer specific PCR.A 20 ml of PCR mixture contained10ml of 2x green master mix (Promega, USA), 2µl of 150ng of genomic DNA and 0.5µM of each oligonucleotide forward (F 5'-GGCGTTGCCGCTCTGAATGC-3') and reverse (R 5'-GAGGGACTGAGCTGGACAACCAC-3') primers were used (Wells *et al.*, 2010). The reaction was run on PCR thermal cycler using cycling parameters 5mins incubation at 94°C, followed by the 40 cycles of 94 °C for 30secs, 60°C for 30secs, 72°C for 1min followed by the 7mins final extension step of 72°C. The resulting products were fractionated on a 2.5% agarose gel yielding the 484bp short product (S-allele) and 528bp long product (L-allele). The amplified products were then visualized using GelDoc (UVP) Vision works LS software (version 7.1).

STATISTICAL ANALYSIS

Data were statistically analyzed using IBM-SPSS version 22 software. The genotype frequency of BDNF and SERT SNPs was tested for Hardy-Weinberg equilibrium (HWE) in non-depressive control using online HWE calculator (www.dr-petrek.eu/documents/HWE.xls). The association test was done using Pearson's $\chi 2$ test. Odds ratios (ORs) and their 95% confidence interval (95% CI) were calculated to identify the risk of depression. The level of significance was employed for comparison at p-value <0.05.

Table 1: Characteristics of study participants

Risk factors	Cases (N)	%	Control (N)	%	χ2 (p-value)
Depression stages					
Mild	10	4.9	-	-	-
Moderate	188	92.16			
Severe	6	2.94			
Total	204				
Age of onset of depression					
16 to 25	74	36.27	-	-	-
26 to 45	105	51.47			
46 to 65	25	12.25			
Total	204				
Duration of depression					
less than 1 Month	6	2.94	-	-	-
less than 1 Year	49	24.02			
1-5 Years	102	50			
greater than 5 Years	47	23.04			
Total	204				
Marital status					
Single	74	36.2	63	37.2	0.016 (0.9)
Married	130	63.7	106	62.7	
Total	204		169		
Sadness					
No	0	0	130	76.9	239.799 (0.000)
Yes	204	100	39	23	
Total	204		169		
Lack of enjoyment					
No	0	0	136	80.4	258.37 (0.000)
Yes	204	100	33	19.5	
Total	204		169		
Fatigue					
No	50	24.5	156	92.3	171.82 (0.000)
Yes	154	75.4	13	7.6	
Total	204		169		
Sleeplessness					
No	32	15.6	149	88.1	194.391 (0.000)
Yes	172	84.3	20	11.8	
Total	204		169		
Lack of concentration					
No	0	0	141	83.4	273.642 (0.000)
Yes	204	100	28	16.5	
Total	204		169		
Lack of confidence					
No	0	0	3	1.7	3.651 (0.05)
Yes	204	100	166	98.2	
Total	204		169		
Worthlessness					
No	168	82.3	161	95.2	14.813 (0.000)
Yes	36	17.6	8	4.7	
Total	204		169		
Pessimistic ideas					
No	168	82.3	152	89.9	4.366 (0.037)
Yes	36	17.6	17	10.1	
Total	204		169		
Suicidal ideas					
No	10	4.9	164	97	315.293 (0.000)
Yes	194	95.1	5	3	
Total	204		169		

Countinue.....

Anxiety					
No	0	0	153	90.5	313.127 (0.000)
Yes	204	100	16	9.5	
Total	204		169		
Possessiveness					
No	111	54.4	169	100	102.634 (0.000)
Yes	93	45.5	0	0	
Total	204		169		

Table 2: Association of BDNF and SERT gene polymorphisms on the risk of depression

Polymorphisms	Genotypes	Cases	Controls	$\chi 2$ (p-value)	*OR	(95% CI)
		N (%)	N (%)			
BDNF (Val66Met)	GG	6 (4.4)	13 (8.4)	3.596 (0.166)	1	Reference
	GA	81 (60)	77 (50.3)		2.279	(0.825-6.298)
	AA	48 (35.5)	63 (41.1)		1.651	(0.585-4.660)
	Total	135	153			
	Alleles					
	G	93 (34.4)	103 (33.6)	0.039 (0.843)	1	Reference
	A	177 (65.5)	203 (66.3)		0.966	(0.684-1.364)
	Total	270	306			
SERT (5'-HTTLPR)	LL	30 (16.5)	21 (17.3)	0.634 (0.728)	1	Reference
	SL	92 (50.8)	56 (46.2)		1.150	(0.601-2.201)
	SS	59 (32.5)	44 (36.6)		0.939	(0.475-1.854)
	Total	181	121			
	Alleles					
	L	152 (41.9)	98 (40.4)	0.133(0.715)	1	Reference
	S	210 (58.1)	144 (59.5)		0.940	(0.675-1.309)
	Total	362	242			

Among 204 cases and 169 controls, 181 cases and 153 controls DNA were successfully amplified. *ORs and their 95% CI were calculated by logistic regression analysis.

Table 3: Association of risk factors, genotyping and depression

Parameters	BDNF				SERT			
	AA		GA		SS		SL	
	Controls/ Cases	Odds Ratio (95% CI)	Controls/ Cases	Odds Ratio (95% CI)	Controls/ Cases	Odds Ratio (95% CI)	Controls/ Cases	Odds Ratio (95% CI)
Marital status								
Married	37/30	1	47/49	1	29/34	1	35/59	1
Unmarried	26/18	0.85 (0.39-1.84)	30/32	1.02 (0.54-1.94)	15/25	1.42(0.63-3.19)	21/33	0.93 (0.47-1.86)
Sadness								
No	52/0	1	53/0	1	36/0	1	42/0	1
Yes	11/48	7E9 (0.00-N.C)	24/81	5E9 (0.00-N.C)	8/59	1.19E10 (0.00-N.C)	14/92	1.0E10 (0.00-N.C)
Lack of enjoyment								
No	53/0	1	57/0	1	36/0	1	47/0	1
Yes	10/48	7E9 (0.00-N.C)	20/81	6E9 (0.00-N.C)	8/59	1.19E10(0.00-N.C)	9/92	1.65E10 (0.00-N.C)
Fatigue								
No	59/10	1	71/23	1	42/12	1	51/23	1
Yes	04/38	56.05 (16.39-91.58)*	6/-58	29.84 (11.39-78.17)*	2/47	82.25 (17.393-388.9)*	5/69	30.60 (10.89-85.93)*
Sleeplessness								
No	54/15	1	68/9	1	40/8	1	51/16	1
Yes	09/34	14.57 (5.68-37.33)*	9/72	60.44 (22.65-161.30)*	4/51	63.75 (17.91-226.91)*	5/76	48.45 (16.70-140.54)*

Countinue.....

Lack of concentration								
No	55/0	1	61/0	1	40/0	1	45/0	1
Yes	08/48	9E9 (0.00-N.C)	16/81	8E9 (0.00-N.C)	4/59	2.3E10 (0.00-N.C)	11/92	1.3E10 (0.00-N.C)
Lack of confidence								
No	01/0	1	1/0	1	1/0	1	1/0	1
Yes	62/48	1E8 (0.00-N.C)	76/81	1E9 (0.00-N.C)	43/59	2E9 (0.00-N.C)	55/92	2E9 (0.00-N.C)
Worthlessness								
No	60/45	1	73/65	1	43/50	1	55/74	1
Yes	3/3	1.33 (0.26-6.91)	4/16	4.49 (1.43-14.12)*	1/9	7.74 (0.94-63.57)	1/18	13.37 (1.73-103.26)*
Pessimistic ideas								
No	56/45	1	69/65	1	39/50	1	51/74	1
Yes	7/3	0.53 (0.13-2.18)	8/16	2.12 (0.85-5.29)	5/9	1.40 (0.43-4.52)	2/18	2.48 (0.87-7.11)
Suicidal ideas								
No	60/1	1	75/3	1	42/2	1	56/5	1
Yes	3/47	940.00 (94.70-9330.3)*	2/78	975.00 (158.44-5999.85)*	2/57	598.50 (80.99-4422.70)*	0/87	1.80E10 (0.00-N.C)
Anxiety								
No	57/0	1	67/0	1	41/0	1	52/0	1
Yes	6/48	1.292E+10 (0.00-N.C)	10/81	1.309E10 (0.00-N.C)	3/59	3.177E10 (0.00-N.C)	4/92	3.71E10 (0.00-N.C)
Obsessive								
No	63/34	1	77/52	1	44/32	1	56/47	1
Yes	0/14	2E9 (0.000-N.C)	0/29	2E9 (0.000-N.C)	0/27	2E9 (0.000-N.C)	0/45	1E9 (0.000-N.C)

GG genotype of BDNF and LL genotype of SERT gene is taken as the reference category. ORs and their 95% CI were calculated using Multiple regression analysis. *p<0.05 is considered as significant

RESULTS

Demographics and clinical characteristics

Most of the patients had moderate depression (92.16%) and the age of onset of depression was between 16 and 45 years. Significant differences was found in depressive symptoms among cases and controls including sadness (χ2: 239.799, p<0.01), lack of enjoyment (χ2: 258.370, p<0.01), fatigue (χ2: 171.820, p<0.01), sleeplessness (χ2: 194.391, p<0.01), lack of concentration (χ2: 273.642 , p<0.01), lack of confidence (χ2: 3.651, p=0.05), worthlessness (χ2: 14.813, p<0.01), pessimistic ideas (χ2: 4.366, p<0.05), suicidal ideas (χ2: 315.293, P<0.01), anxiety (χ2: 313.127, P<0.01) and possessiveness (χ2: 102.634, P<0.01) (table 1).

BDNF (Val66Met) and SERT (5'-HTTLPR) gene polymorphisms

Genotype frequencies of *BDNF* (χ^2: 2.463, p-value>0.05) and *SERT* (χ^2: 0.19, p-value>0.05) were in HWE for non-depressive controls. No association of *BDNF* (χ2: 3.596, P>0.05) and *SERT* (χ2: 0.634, P>0.05) gene polymorphisms were found for genotypes. However, the risk of depression was increased with *BDNF* AA (OR: 1.651, CI: 0.585-4.660) and GA (OR: 2.279, 95% CI: 0.825-6.298) genotypes. Similarly, SL (OR: 1.150, 95% CI: 0.601-2.201) genotype of *SERT* was also associated with depression risk (table 2).

Fatigue, sleeplessness, worthlessness and suicidal ideation are common among those individual having *BDNF* AA and GA and *SERT* gene SS and SL genotypes suggesting *BDNF* Met allele and short variant of *SERT* are associated with the suicidal ideation and other depression associated traits (table 3).

DISCUSSION

Depression is one of the psychiatric problems which vary with environments, sex and ages. Now a day, depression is a disease having etiological variability including behavioral, environmental, psychosocial and genetic variations. Genetics of an individual may play a pivotal role in the onset of depression. However, this study focused on the genetic basis of depression among Pakistani population.

It had been observed that sadness, lack of enjoyment, fatigue, sleeplessness, lack of concentration, lack of confidence, worthlessness, pessimistic ideas, suicidal ideas, anxiety and possessiveness are significantly associated with the risk of depression. However, marital

status is not associated with depression which is consistent with other studies (Dienemann *et al.*, 2000, Inandi *et al.*, 2002, Campbell, 2002).

The present study found a lack of association of *BDNF* (*Val66Met*) polymorphism with depression among Pakistani population which is in agreement with the other study (Grzywacz *et al.*, 2010). A study revealed that *BDNF* mutation might play a role in the contribution to the geriatric depression with early life stressors that are neglected (Hwang *et al.*, 2006). However, *BDNF* (*Val66Met*) gene polymorphism in association with early life stress produces symptoms of major depression (Youssef *et al.*, 2018, Ribeiro *et al.*, 2007). The present study also suggested that *BDNF* AA (Met allele) and GA genotypes were at increased risk of having depression. A recent study was examined the relationship of various aspects of learning and cognitive flexibility with *BDNF* (*Val66Met*) polymorphism in a mouse model and found that *BDNF* Met alleles carriers are more subtle to the environmental variation (Vandenberg *et al.*, 2018). In a meta-analysis, the gender stratification analyses have shown significant effects of Met allele among men with major depressive disorders (Verhagen *et al.*, 2008).

In this study, lack of association of *SERT* genotype was found with depression among Pakistani population which is not in agreement with previous studies (Lesch *et al.*, 1996, Grabe *et al.*, 2005). A study reported that SS genotype was significantly revealed more depressive symptoms as compared to the SL or LL genotypes among children with a history of having no proper treatment (Kaufman *et al.*, 2005). Other studies documented that SS genotype carrier experiences maximum probability regarding adverse life events and the onset of depression (Caspi *et al.*, 2003). Moreover, a meta-analysis showed that the subjects carrying S allele reported having a greater sensitivity to stress (Karg *et al.*, 2011). *SERT* gene is also associated with the post stroke depression among stroke survivor (Kohen *et al.*, 2008). It also modifies the risk of depressive episodes among patients with irritable bowl syndrome (Jarrett *et al.*, 2007). However, it was previously confirmed a significant interaction of S allele indicating a higher mental susceptibility to social stresses and chronic diseases (Grabe *et al.*, 2005).

The present study also explores that fatigue, sleeplessness, worthlessness and suicidal ideation are more common among those individual having *BDNF* AA and GA and *SERT* gene SS and SL genotypes suggesting *BDNF* Met allele and short variant of *SERT* are associated with the suicidal ideation and other depression associated traits. A meta-analysis suggested that *BDNF* Met allele carriers were associated with the history of having suicidal attempts (Zai *et al.*, 2012). Generally, it seems that anomalies in the *BDNF* signaling pathway may work as an important biological risk predictor in the etiology and pathogenesis of suicide (Dwivedi, 2010). Another study showed that *BDNF* Met allele carriers are more susceptible towards the sleeplessness i.e. prolonged wakefulness (Grant *et al.*, 2018). Furthermore, there is a close relationship of the *5-HTTLPR* polymorphic region and chronic fatigue syndrome (CFS) (Narita *et al.*, 2003) and suicidal attempts (de Medeiros Alves *et al.*, 2015), however, 'L' allele were at higher risk for CFS (Narita *et al.*, 2003) and suicide (de Medeiros Alves *et al.*, 2015). A study also documented that S allele carriers of the 5-*HTTLPR* were statistically significant in the patients suffering from insomnia (Deuschle *et al.*, 2010).

A relatively modest sample size is the major limitation of the study which can be overcome by recruiting the large samples. Serum BDNF and 5'-HTT levels can also be estimated to get better results.

CONCLUSION

BDNF (*Val66Met*) and *SERT* (*5'-HTTLPR* or *SLC6A4*) gene polymorphisms may not associate with depression susceptibility among the population of Pakistan. However, *BDNF* AA and GA genotypes along with the *SERT* SL genotype might develop the risk of depression. However, further investigations are needed in this area with a large sample size in order to draw definitive conclusions about the relationship of *BDNF* (*Val66Met*) and *SERT* (*5'-HTTLPR*) gene polymorphisms with depressive disorders and also determine their relationship with the depression associated traits.

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