Pakistan J. Zool., vol. 49(5), pp 1921-1924, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.5.sc4

Short Communication

Association between the *COMT* Gene and Obsessive Compulsive Disorder: A Case-Control Study

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ABSTRACT

This study aimed to find out the genetic variations in *Catechol O Methyl transferase* (*COMT*) gene and association of these genes with obsessive compulsive disorder (OCD) in the Pakistani Patients. We selected OCD patients (n=100) following the Diagnostic Statistical Manual-IV (DSM-IV) criteria and controls (n=120) from Sir Ganga Ram Hospital and Panjab Institute of Mental Health, Lahore from August 2011 to January 2014. During the sample collection the factors like age/period, employment status and marital status were considered after informed consent. We found one single base change G>C at c.745 resulting in a nonsynonymous change p.E249Q in the COMT gene. In-silico analysis predicted it to be damaging and disease causing. Screening of case and control group data showed no deviation from Hardy-Weinberg equilibrium with *p*-value 0.879 and 0.32, respectively. There was no significant difference in age (*p*-value 0.081), employment status (*p*-value 0.34) and matrimonial status (*p*-value 0.28) but there was a significant difference in their education (*p*-value 0.0002). The prevalence of C allele was 13.5% in disease, 5.83% in controls and found significant with *p*-value 0.007. These findings suggest that c.G745C in *COMT* gene has a significant association and a possible role in the OCD development in Pakistani patients.

Article Information
Received 09 August 2016
Revised 14 October 2016
Accepted 01 December 2016
Available online 25 August 2017

Authors' Contributions
Javeria and MEB conceived the project, Javeria, AN and AW performed lab work. Javeria, AA and MW wrote the article. AA, SAS and MA analysed the data. SR helped in sampling. MEB supervised the project.

Key words
COMT gene, Genetic variant, OCD, Nonsynonymous, Pakistani patients.

Obsessive compulsive disorder (OCD) is an heterozygous psychiatric disorder. Its worldwide prevalence is 1-3% population (Kessler *et al*., 2005; Hasler *et al*., 2006; Karno *et al*., 1988; Weissman *et al*., 1994). It is distinguished by intermittent and interfering obsessive feelings and cyclic compulsive behaviors ascociated with obsessions which reduce anxiety. Family case studies and twin studies showed that OCD is a genetic disorder (Rasmussen and Tsuang, 1986; Hettema *et al*., 2001).

Catechol O Methyl transferase (COMT) is an enzyme that degrades catecholamine *i.e.* epinephrine, dopamine and norepinephrine. COMT protein encoded by the *COMT* gene in human (Karno *et al*., 1988) was discovered by Axelrod (1957). Many drugs target COMT to change its activity and make accessibility of catecholeamines when the regulation of catecholeamines is disturbed in different diseases (Tai and Wu, 2002; Muller, 2015).

Catecholamine (dopamine, adrenaline and noradrenalin) are key hormones and neurotransmitters that play key role in the regularization of physiological processes. Degradation of catecholamines occurs either through O-methylation by COMT or by de-amination with the help of monoamine oxidase (MAO). Disregulation of noreadrenergic pathways are involved in mood disorder. Some scientists did not find association of the *COMT* variants with OCD (Umehara *et al*., 2015; Sampaio *et al*., 2015). A common functional polymorphism rs4680 is reported at codon 158 in *COMT* gene, where a nucleotide transition from G to A causes a change in the amino acid sequence from Val to Met in the COMT protein. This rs4680 is considered as associated with OCD in male and female patients (Alsobrook *et al*., 2002; Karayiorgou *et al*., 1999; Katerberg *et al*., 2010; Pooley *et al*., 2007). The present study is aimed at investigating the association of *COMT* gene in Pakistani OCD patients.

Materials and methods

The conduct of this study was approved by the Ethics Committee of the University of Veterinary and Animal Sciences, Lahore. Blood samples of OCD patients (n=100) were collected from Sir Ganga Ram Hospital and Punjab Institute of Mental Health, Lahore. All the patients were given written informed consent for participation in this study.

The demographic distribution of both control and diseased groups are mentioned in Table I. All patients met criteria for either a current (South Africa) or a lifetime (Netherlands) diagnosis of OCD or subclinical OCD according to DSM-IV criteria (First *et al*., 2002). Diagnoses were established using the Structured Clinical Interview for Axis I disorders (SCID-I/P) (First *et al*.,

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0030-9923/2017/0005-1921 $ 9.00/0

2002) or the Mini International Neuropsychiatric Interview (MINI) version 5.0.0. (Sheehan *et al*., 1998). We enrolled unrelated healthy controls (n=120) without any family history of psychiatric disorder. DSM-IV criteria (American Interview for Axis I disorders (SCID-I/P) (First *et al*., 2002) was used for the diagnosis of OCD symptoms and severity of OCD was measured by Y-BOCS scale (Goodman *et al*., 1989a, b).

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

	Case Group	Control Group	*p*-value
Age (yr)	26.08 ± 7.6	29.6 ± 7.3	0.081
Gender			
Male	55 (55%)	70 (58%)	1.000
Female	45 (45%)	50 (41%)	1.000
Education in years	8.3±4.8	10.6±2.9	0.0002
Employment			
Employed	43 (43%)	81 (67.5%)	1.000
Unemployed	57 (57%)	39 (32.5%)	1.000
Marital status			
Unmarried	45 (45%)	76 (63.3%)	1.000
Married	55 (55%)	44 (36.6%)	1.000
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	-	

Comp, compulsions; Obs, obsessions; OCD, obsessive compulsive disorder; Mean±SD, Mean ± standard deviation; Y-BOCS, yale-brown obsessive compulsive scale.

For amplification of *COMT*, genomic DNA was extracted from leukocytes through standard protocol (Sambrook and Russel, 2001). Primers designed from Primer 3 software to amplify all the coding regions of *COMT* gene (Table II). Amplification used SuperScript III platinum Taq Polymerase (Invitrogen Carlsbad. CA), dNTPs, $MgCl_2$ (Fermantas brand). Amplified product was sequenced using ABI3130xl automated sequencer (Applied Biosystem Inc, Foster City, CA). Sequence was aligned for nucleotide change detection.

Sequencing data was aligned using NCBI BLAST tool. Allelic and genotypic frequency distribution was calculated for Hardy-Weinberg Equilibrium. Chi-square (X^2) test was used for categorical variables and Student's t-test for continuous variables. *In-silico* analysis was performed to predict the possible effect of sequence change through mutation tester, polyphin2 and exon splicing enhancer.

Table II.- Primers used for coding region of *COMT* gene.

Gene	Primer used	Tm
COMT-1	F: GGGGCTACTTGTGGCTAGA	F: 57.9
	R: CAGTTCCCATCCAGATTCC	R: 57.8
COMT-2	F: GGAAGGGGCTCAGGTAT	F: 54.8
	R: AGAGTGGACATGTGCTCAG	R: 54.5
COMT-3	F: GGAGGAGCACAGAGCAC	F: 55.0
	R: TGGGGTGATAACAGCTTC	R: 54.3
COMT-4	F: GAGGTGAAATACCCCTCCAG	F: 58.4
	R: TCAGTGAACGTGGTGTGAAC	R: 58.1
COMT-5	F: TGTCATCCCAGAACCCTA	F: 54.6
	R: ATCTCCACCCACCACAG	R: 54.8
COMT-6	F: TAGTGAGGAGCACCCATC	F: 54.3
	R: GCCAGTGTTAGTAAAGAAGTCA	R: 54.0

F, forward; R, reverse, Tm, melting termperature.

Table III.- Allele frequency distribution calculation using chi-square test.

***COMT* gene**	**GG**	**GC**	**CC**	**HWE *p*-value**	**Sig. level**
Case group (n=100)	75	23	2	0.879	Not significant
Control group (n=120)	107	12	1	0.32	Not significant

COMT, *Catechol O Methyl transferase*; n, number; sig., significance.

Results

The aim of this study was to explore the genetic variations in the *COMT* gene among Pakistani OCD patients. The demographic distribution of the both OCD and control groups is illustrated in Table I and showed no significant difference in age (*p*-value 0.081), employment status (*p*-value 0.34) and marital status (*p*-value 0.28) while it was significant (*p*-value 0.0002) for their education. We found one single base change G>C at c.745 in the *COMT* gene resulting in a nonsynonymous change E249Q. In OCD group, sequencing result for the C allele showed homozygosity in two samples (n=2) and heterozygosity in twenty three samples (n=23) while the control group showed homozygosity in one sample (n=1) and heterozygosity in twelve samples (n=12). Allele frequency distribution for the OCD group (*p*-value=0. 87) and for the control group (*p*-value=0.325) calculated through Chi-Square test was found as nonsignificant. Our data is in accordance with the Hardy-Weinberg equilibrium (Table III). Variation was tested to find the association of the C allele with the case group using online fisher exact test (http://www.socscistatistics.com/tests/fisher/Default2.aspx). Our results were found significant with *p*-value 0.0078 (Table IV) revealing that this genetic variation (G>C) has some effective role in the development of OCD.

In-silico analysis performed through mutation taster (http://www.mutationtaster.org/cgi-bin/MutationTaster/MutationTaster69.cgi), exon splicing enhancer software and polyphin2 softwares. This change is predicted to be as an alternate splicing site in the gene. This effect on splicing site can lead to the formation of truncated protein or wrong gene product which then may lead to disease and prove damaging.

Table IV.- Association analysis of c.G745C of *COMT* gene with OCD using fisher exact test.

***COMT* gene**	**G**	**C**	***p*-value**	**Sig. level**
Case group (n=100)	173	27	0.0078	Significant
Control group (n=120)	226	14		

Discussion

In the present study, we found a significant association between genetic variant c.G745C and OCD in Pakistani patients with *p*-value 0.00786. A functional genetic variant in the *COMT* gene changes the amino acid valine to methionine at 158 position. This change catalyzes the dopamine up to four times than the rate of methionine (Lachman *et al*., 1996b). Scientists observed low expression of *COMT* gene in OCD patients studied through real time quantitative reverse transcription reaction (Wang *et al*., 2009).

The *COMT* gene was found to be a risk factor for schizophrenia (Harrison and Weinberger, 2005). Previously Lachman *et al*. (1996b) observed a functional genetic variant G>A (rs4680) in the *COMT* gene associated with OCD and some other anxiety related traits like extraversion and neuroticism (Stein *et al*., 2005). Some other studies did not find association of rs4680 with psychiatric disorders (Henderson *et al*., 2000). Different genetic variations in the *COMT* gene have been found to be associated with several personality traits, *i.e.* aggression and avoidance (Rujescu *et al*., 2003; Stein *et al*., 2005). In a case–control study, rs4680 was found in the *COMT* gene in association with some psychotic symptoms and schizophrenia (Pooley *et al*., 2007; Caspi *et al*., 2005) and in emotional processing (Lelli-Chiesa *et al*., 2011).

The most studied polymorphism for OCD in the *COMT* gene is rs4680 (G>A). The rs4680 lowers the expression level of *COMT* gene in Bipolar spectrum disorder and in Velo-cardio-facial-syndrome (VCFS) observed in US population (Lachman *et al*., 1996a). This low enzyme activity increases the susceptibility of different psychiatric disorders (Shifman *et al*., 2004). Several studies suggested that the *COMT* gene has a significant role in the causation of neuro-psychiatric disorders (Tsankova *et al*., 2007; Hemmings and Stein, 2006).

In our sequencing data, we found a nonsynonymous mutation at position c.745 of *COMT* gene that changed the protein structure by changing the amino acid from glutamic acid to glutamine at p.249 postion. This genetic variant has been observed in 98 patients. Allele frequency distribution followed the Hardy-Weinberg equilibrium among diseased (*p*-values 0.0879) and control group (*p*-value=0.032). *In-silico* analysis showed this genetic variation as splice site is broken and possibly causes disease. *COMT* gene is found to be associated with OCD with *p*-value 0.049 (Alsobrook *et al*., 2002). Meta-analysis of anxiety related traits has showed p.Val158Met in the *COMT* gene associated with the higher neuroticism (*p*-value 0.03) in Caucasian population and higher harm avoidance (*p*-value 0.004) in Asian population (Lee and Prescott, 2014). Significant association of *COMT* genetic variant Val158Met (*p*-value 0.002) with OCD has been observed in a case-control study. The same group performed a meta analysis for case-control data and found the association of the *COMT* gene with OCD (*p*-value 0.001) (Pooley *et al*., 2007). Genetic aspects of rs4680 is associated (*p*-value=0.01) with bipolar disorder, mood disorder, anxiety related disorder and OCD (Massat *et al*., 2011; Stein *et al*., 2005; Katerberg *et al*., 2010). The genetic variant c.G745C found in *COMT* gene has association with OCD in Pakistani patients compared to the normal from the same ancestory.

Conclusion

Our analysis showed that *COMT* genetic variation c.G745C contribute significantly with OCD. This study provides significant evidence for an association between C allele with Pakistani OCD patients. This study could be helpful for investigation of genetic aspects of other psychiatric disorders in Pakistani population.

Acknowledgements

Higher Education Commission of Pakistan is highly acknowledged for providing funds for this study.

Conflict of interest statement

We declare that we have no conflict of interest.

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