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THE ASSOCIATION OF *CATECHOL-O-METHYL-TRANSFERASE* AND *INTERLEUKIN 6* GENE POLYMORPHISMS WITH POSTTRAUMATIC STRESS DISORDER

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SUMMARY

Background: *Posttraumatic stress disorder (PTSD) is a disorder that occurs in some people who have experienced a severe traumatic event. Several genetic studies suggest that gene encoding proteins of catechol-O-methyl-transferase (COMT) may be relevant for the pathogenesis of PTSD. Some researchers suggested that the elevation of interleukin-6 (IL6) correlates with major depression and PTSD. The aim of this study was to investigate whether the single nucleotide polymorphisms COMT rs4680 (Val158Met) and IL6 rs1800795 are associated with PTSD and contribute to the severity of PTSD symptoms.*

Subjects and methods: *This study comprised 747 participants that experienced war between 1991 and 1999 in the South Eastern Europe conflicts. COMT rs4680 (Val158Met) and IL6 rs1800795 genotypes were determined in 719 participants (369 with and 350 without PTSD). The Mini International Neuropsychiatric Interview (M.I.N.I.), the Clinician Administrated PTSD Scale (CAPS) questionnaire and the Brief Symptom Inventory (BSI) were used for data collection.*

Results: *Regarding the COMT gene polymorphism, the results of the regression analyses for BSI total score were significant in the lifetime PTSD group in the dominant (P=0.031) and the additive allelic model (P=0.047). Regarding the IL6 gene, a significant difference was found for the recessive model predicting CAPS total score in the lifetime PTSD group (P=0.048), and indicated an association between the C allele and higher CAPS scores. n the allelic, genotypic and rezessive model, the results for BSI total score were significant in the lifetime PTSD group (P=0.033, P=0.028 and P=0.009), suggesting a correlation of the C allele with higher BSI scores*

Conclusion: *Although our nominally significant results did not withstand correction for multiple tests they may support a relevance of the COMT (Val158Met) and IL6 rs1800795 polymorphism for aspects of PTSD in war traumatized individuals.*

Key words: *war trauma - PTSD - COMT - IL6 - gene polymorphism*

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a consequence of severe traumatic experiences such as torture, war, and genocide (American Psychiatric Association 2000). PTSD etiology is considered to be multifactorial with an interaction of environmental traumatic factors and genetic factors (Agani et al. 2010). Epidemiological studies worldwide have documented a high rate of traumatic events including life-threatening accidents, rape,

combat, physical violence, witnessing the death or injury of others and natural disasters (Gillespie et al. 2009). In their study, Neuner et al. (2004) reported that prevalence rates of PTSD in survivors of civil war was 30-40%, and in further studies it is estimated to be around 35%, in people who experienced the war in Bosnia and Herzegovina and 25% in people who experienced the war in Kosovo (Priebe et al. 2010, Lopes et al. 2003). There are individual differences regarding the ability to cope with excessive stress. Therefore, while some people exposed to traumatic events do not develop PTSD, others do develop PTSD symptoms. Twin studies have shown that the development of PTSD following a trauma has a heritability of up to 30-40%. Several genetic components for PTSD have been proposed, including biologic pathways involving the hypothalamic-pituitary-adrenocortical axis (HPAA), the locus coeruleus/noradrenergic system, and the limbic systems (Broekman et al. 2007, Koenen 2007). Interestingly, a method using a cumulative risk score showed that carriers of four or more risk alleles of the candidate genes FK506 - binding protein 5 (FKBP5) (rs9470080), catechol-O-methyltransferase (COMT) (rs4680), and cholinergic receptor nicotinic alpha-5 (CHRNA5; rs16969968) conferred around 7 times the risk of PTSD (Boscarino et al. 2011). COMT is an enzyme that plays a key role in inactivating catecholamine neurotransmitters (dopamine, epinephrine, norepinephrine), their metabolites, catechol estrogens and catechol drugs via methylation. In humans, the gene coding for COMT is located on 22q11.21 (Grossman et al. 1992). The rs4680 functional variant (Val158Met polymorphism) of the COMT gene is the most widely studied COMT single nucleotide polymorphism (SNP), and because of its role in catecholamine regulation, it has garnered great interest from researchers studying psychiatric conditions ranging from PTSD (Kolassa et al. 2010) to obsessive-compulsive disorder (Pooley et al. 2007) and schizophrenia (Egan et al. 2001). A substitution of valine (Val) by methionine (Met) is associated with lower enzyme activity and a subsequent slower catalysis of catecholamines. The difference in activity between Val/Val and Met/Met genotypes is three - to four fold and Val/Met genotypes showing an intermediate activity (Malhotra et al. 2002).

Recently it has been verified that Interleukin 6 (IL6) is not involved only in the immune response, but it is also linked to disorders like depression and anxiety. It has an important role also in adult neurogenesis (Deverman & Patterson 2009). Neurogenesisis altered in some neuropathological situations such as stroke, mechanical damage, status epilepticus and neurodegenerative diseases like Alzheimer and Parkinson. A harmful effect of inflammation has been suggested in all of these diseases (Ekdahl et al. 2003, Quintana et al. 2009, Whitney et al. 2009). The IL6 family of cytokines recruits the glycoprotein gp130 for signaling (Kishimoto et al. 1995). Epinephrine and norepinephrine modulate the release of cytokines through α- and β-adrenoceptors on immune cells (Hasko & Szabo 1998). In contrast, acetylcholine inhibits the release of TNF, IL1, IL6 and IL18, from endotoxin-activated human macrophages (Borovikova et al. 2000). Pro-inflammatory cytokines such as TNF, IL1, and IL6 can increase in prolonged stressful situations, such as anxiety disorders. Higher levels of stimulated TNF and IL6 were reported in PTSD patients (Rohleder et al. 2004). In their study, Baker et al. (2001) have pointed out that IL6 is significantly elevated in patients and correlates with major depression and PTSD. IL6 is also related to schizophrenia, poor emotional responsiveness manifested with hallucinations, paranoid and mental deterioration and delusions (Erta, Quiantana & Hidalgo 2012). SNPs, such as rs1800796, influence IL6 expression (IL6 plasma levels are increasedin G allele carriers). Several studies have shown that depression and PTSD are associated with a smaller volume of the hippocampus, which exhibits a strong sensitivity to stress and the response to cytokines (Kasai et al. 2008, MacQueen & Frodl 2010). The role of IL6 in these relationships is unclear. Many authors investigated the relationship between inflammatory markers and PTSD and it has been reported that psychological stress in humans is associated with increased secretion of IL6 (Maes et al. 1999, Carpenter et al. 2010). Passos et al. (2015) propose that trauma induces chronic low-grade inflammation.

The aim of this study was to investigate the association of the COMT rs4680 (Val158Met) and the IL6 rs1800796 polymorphism with the development of PTSD and their contribution to symptom severity.

SUBJECTS AND METHODS

Subjects

In this study 719 (mean age 49.4±7.9; 487 males and 232 females) participants were recruited between 2013 and 2015 at five psychiatric research centers, located in the countries of ex-Yugoslavia, where the population has experienced war-related trauma between 1991 and 1999: Zagreb in Croatia (1991-1992), Sarajevo, Tuzla and Mostar in Bosnia and Herzegovina (1992-1995) and Prishtina in the Republic of Kosovo (1998-1999). The inclusion criteria were that participants should not be younger than 16 years of age at the time of trauma and not older than 65 years of age at time of recruitment. Exclusion criteria were: intellectual disability (MMSE<25), organic and brain trauma related disorders, epilepsy, psychotic disorders, addiction disorders except smoking, oncological illnesses, medication known to affect methylation status, e.g, valproic acid, 1st and 2nd degree relation to an already recruited person. Interviews were performed by medical personnel (psychiatrists, psychologists or psychiatric residents) after trainings. Further details on study design, process of recruitment, assessment instruments, inclusion and exclusion criteria, blood collection and transportation, DNA extraction of SEE PTSD study have been described by Džubur Kulenović et al. (2016).

For analyses participants were divided into three experimental groups, depending on the presence of PTSD. The first group included 218 patients (mean age 50.1±6.7; 157 males and 61 females) who have current PTSD. The second group constitutes of 151 participants (mean age 49.5±8.2; 98 males and 53 females), who experienced lifetime PTSD, and the third group comprised 350 (mean age 48.8±8.5; 232 males and 118 females) healthy volunteers who did not develop PTSD.

Ethical Votes

The study was approved by the local ethics committees. The information and consent form were designed by the Sarajevo center and translated into local language. All participants were informed and gave their written consent according to the principles of the declaration of Helsinki (WMA 2013).

Psychometric Instruments

Using the Structured Clinical Interview - Mini International Neuropsychiatric Interview (M.I.N.I.) we assessed the presence or absence of PTSD symptoms. To make a categorical PTSD diagnosis and to assess the severity of PTSD symptoms we used the Clinician Administrated PTSD Scale (CAPS) (Blake et al. 1995). And finally Brief Symptom Inventory (BSI) (Derogatis & Melisaratos 1983) was used for the assessment of psychological symptoms.

Molecular Analyses

Molecular analysis were performed at the Laboratory of Functional Genomics, Department of Psychiatry, Psychosomatics and Psychotherapy in Würzburg. Genomic DNA was isolated from frozen venous EDTA-blood using the FlexiGene DNA Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions and stored until genotyping at -80°C.

Genotyping of the COMT rs4680 (Val158Met) SNP was accomplished using standard PCR procedures modified from a previously published protocol (Egan et al. 2001); primers were 5′-GGGGCCTACTGTGGCTACTC-3′(forward) and 5′-TTTTTCCAGGTCTGACAACG-3′ (reverse). Briefly, PCR reactions were performed in a reaction volume of 25 ml, including approximately 45-65 ng of template genomic DNA, 0.4 mM of each primer, 0.1 mM of each dNTP, 1.5 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl (pH9), 0.01% Tween 20 and 0.5 U of Taq DNA polymerase. Cycler conditions were: 5 min denaturation at 94°C, followed by 35 cycles with 45 s at 94°C, 45 s at 58°C and 45 s at 72°C and a final extension step of 5 min at 72°C. PCR products were digested with NlaIII (3 h at 37°C; fragment sizes: wild-type G1947, 114 bp; 1947A variant, 96 and 13 bp) and subsequently visualized on a 4% agarose gel. G1947 corresponds to the high-activity Val158 allele; 1947A codes for the low-activity Met variant (Ehlis et al. 2007).

The IL6 SNP rs1800795 was genotyped following the described procedure: The respective gene region carrying the rs1800795 polymorphism was amplified from genomic DNA by PCR with following oligonucleotide primers F: 5'- ACTCAGTTCAGAACATCTTTGGT-3' and R: 5'- TTCTCTTTCGTTCCCGGTGG-3' in a 25 µl reaction volume containing 45-65 ng genomic DNA, 0.4 mM of each primer, 0.1 mM of each nucleotide, 1.5 mM $MgCL_2$ and 0.3 U Taq^{TM} DNA polymerase. Cycler conditions were: 5 min denaturation at 95°C, followed by 35 cycles with 45 s at 95°C, 45 s at 62.5°C and 45 s at 72°C and a final extension step of 5 min at 72°C. The resulting PCR fragments were digested with the restriction endonuclease SfaNI (NEB, Frankfurt a. Main, Germany) which results in differentially sized fragments representing the respective genotype. The fragments were separated in a 4% agarose gel by electrophoresis and visualized with ethidium bromide. Fragment lengths and resulting genotypes were determined by two independent investigators blinded for diagnosis.

Statistical analyses

Statistics were performed using PLINK 1.9. Both of the analyzed SNPs were polymorphous (minor allele frequency>30%), reached a minimal genotyping call rate of 99% and did not deviate from Hardy-Weinberg equilibrium ($p>0.1$). Logistic regression was used for case-control analyses. Within the two groups of patients, i.e. individuals with lifetime or current PTSD, linear regression was carried out individually for analyses on CAPS and BSI scores. The following models were tested in all phenotypes: additive allelic, dominant and recessive (all based on the minor allele), as well as the genotypic model. The significance level was Bonferroni adjusted for 23 variants that were analyzed in total within the entire project ($\alpha=0.002$) (Džubur Kulenović et al. 2016).

RESULTS

In order to characterize the role of COMT and IL6 on PTSD, two well investigated SNPs rs4680 and rs1800795 were subject to a case-control analysis in altogether 719 participants. Additonally linear regression analyses were performed with genotypes predicting the total CAPS and BSI scores for current and lifetime PTSD patients separately.

Catechol-o-methyl-transferase (COMT) gene

Allele and genotype distributions of the COMT polymorphism rs4680 in the PTSD group and controls are shown in Table 1. There was no significant difference between the PTSD group and controls in allele and genotype distributions of the COMT rs4680 variant ($P_{all}>0.05$). Also no significant difference was found in severity of PTSD symptoms (CAPS total) between allele and genotype groups neither in the lifetime PTSD group nor in patients with current PTSD diagnoses ($P_{all}>0.05$).

Table 1. Association results of COMT rs4680, along with genotype- and allele counts, for individuals in analysis, CAPS and BSI means and standard deviations (SD), as well as nominal P-values of regression analyses

COMT rs4680	Allelic Model		Genotypic Model			Dominant Model		Recessive Model	
	A	G	AA	AG	GG	AA/AG	GG	AA	AG/GG
Controls	344	354	87	170	92	344	354	87	262
$PTSD_{lifetime}$	141	159	34	73	43	107	43	34	116
$PTSD_{current}$	209	215	47	115	50	209	215	47	165
$P_{case\text{-}control}$-value	0.722			0.642		0.384		0.423	
$CAPS_{lifetime}$ (mean±SD)	67.4±16.6	66.5±18.5	68.8±14.4	66.1±18.2	66.8±18.7	67.0±17.2	66.8±18.7	68.8±14.4	66.8±18.7
P_{CAPS}-value	0.668			0.771		0.971		0.489	
$CAPS_{current}$ (mean±SD)	79.5±20.0	79.1±21.6	80.0±18.3	79.0±21.3	79.3±21.9	79.3±20.5	79.3±21.9	80.0±18.3	79.3±21.9
P_{CAPS}-value	0.835			0.969		0.932		0.802	
$BSI_{lifetime}$ (mean±SD)	78.7±53.0	68.0±45.0	80.2±54.9	77.4±51.2	59.6±36.8	78.2±52.4	59.6±36.8	80.2±54.9	59.6±36.8
P_{BSI}-value	*0.047*			0.089		*0.031*		0.305	
$BSI_{current}$ (mean±SD)	115.7±46.6	110.7±46.0	121.1±45.9	111.3±46.6	110.0±45.2	114.2±46.6	110.0±45.2	121.1±45.9	110.0±45.2
P_{BSI}-value	0.262			0.418		0.618		0.189	

PTSD - posttraumatic stress disorder; CAPS - Clinician Administered PTSD Scale; BSI - Brief Symptom Inventory; COMT - catechol-O-methyl-transferase; *Italics indicates p≤0.05*

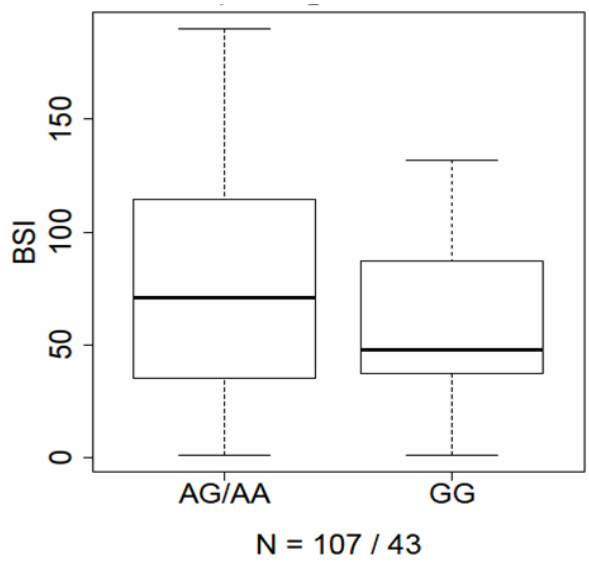

Figure 1. The distribution of the Brief Symptom Inventory (BSI) total score according to catechol-O-methyl-transferase (COMT) genotypes in the dominant model (P=0.031) within the lifetime PTSD group

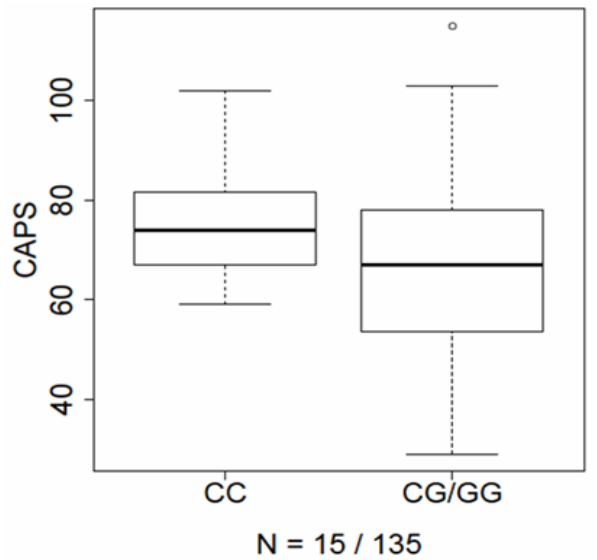

Figure 2. The distribution of the total score of posttraumatic stress disorder (CAPS) according to interleukin 6 genotypes in the recessive model (P=0.048) within the lifetime PTSD group

However, regression analyses on the BSI total score reached not in the current PTSD subgroup (P_{all}>0.05), but in the lifetime PTSD group nominal significance in the dominant (P=0.031, β=19.34, SE=8.88) (Table 1 and Figure 1) and allelic (P=0.047, β=11.46, SE=5.72) (Table 1) model. These results indicate an association between the minor (A) allele and higher BSI scores (Table 1). However, none of the nominal associations withstood Bonferroni correction for multiple testing.

Intereleukin6 gene

The allele genotype distribution of the IL6 SNP rs1800795 in the PTSD group and controls are given in Table 2. No significant difference was found regarding IL6 SNP rs1800795 allele and genotype distributions between the PTSD group and controls (P>0.05). In contrast, the recessive model predicting CAPS total score in the lifetime PTSD group was nominally significant (P=0.048, β=9.52, SE=4.78) and indicated an association between the minor (C) allele and higher CAPS scores (Table 2 and Figure 2). Also, the allelic (P=0.033, β=13.38, SE=6.21), genotypic (P=0.028) and recessive (P=0.009, β=35.63, SE=13.53) (Figure 3) model predicting BSI total score in the lifetime PTSD group were nominally significant, each indicating again an association between the minor (C) allele and higher BSI scores (Table 2). This results could not be replicated within the current PTSD subgroup, where regression analysis reached in none of the calculated models any significance for the total CAPS and BSI scores (P_{all}>0.05) (Table 2). None of the detected nominal significant associations remained significant after correction for multiple tests.

Table 2. Association results of IL6 rs1800795, along with genotype- and allele counts, for individuals in analysis, CAPS and BSI means and standard deviations (SD), as well as nominal P-values of regression analyses

IL6 rs1800795	Allelic Model		Genotypic Model			Dominant Model		Recessive Model	
	C	G	CC	CG	GG	CC/CG	GG	CC	CG/GG
Controls	244	452	40	164	144	204	144	40	308
$PTSD_{lifetime}$	97	203	15	67	68	82	68	15	135
$PTSD_{current}$	152	282	21	110	86	131	86	21	196
$P_{case\text{-}control}$-value	0.643		0.765			0.875		0.465	
$CAPS_{lifetime}$ (mean±SD)	69.3±17.0	65.3±17.8	75.5±11.0	66.7±18.1	63.2±17.1	68.4±17.6	63.2±17.1	75.5±11.0	66.0±17.6
P_{CAPS}-value	0.087		0.123			0.288		*0.048*	
$CAPS_{current}$ (mean±SD)	78.9±20.6	79.4±20.9	78.9±20.4	78.9±20.7	79.8±21.0	78.9±20.7	79.8±21.0	78.9±20.4	79.3±20.9
P_{CAPS}-value	0.734		0.931			0.705		0.919	
$BSI_{lifetime}$ (mean±SD)	78.2±52.4	70.2±46.9	105.1±55.7	71.1±48.3	70.7±45.3	74.5±50.8	70.7±45.3	105.1±55.7	70.0±47.4
P_{BSI}-value	*0.033*		*0.028*			0.214		*0.009*	
$BSI_{current}$ (mean±SD)	112.4±44.8	112.9±46.9	123.0±42.3	108.3±45.1	115.9±47.8	110.7±45.0	115.9±47.8	123.0±42.3	111.6±46.4
P_{BSI}-value	0.821		0.263			0.349		0.299	

PTSD - posttraumatic stress disorder; CAPS - Clinician Administered PTSD Scale; BSI - Brief Symptom Inventory; IL6 - interleukin 6; *Italics indicates p≤0.05*

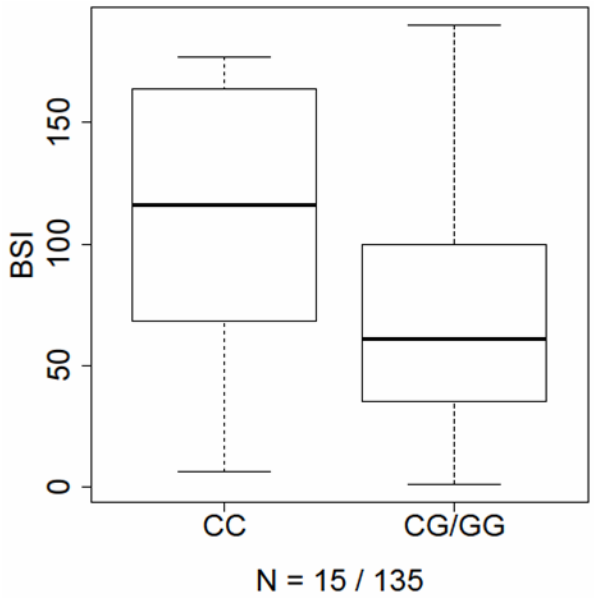

Figure 3. The distribution of the Brief Symptom Inventory (BSI) total values according to interleukin 6 genotypes in the recessive model (P=0.009) within the lifetime PTSD group

DISCUSSION

Many ongoing analyses are trying to identify novel gene candidates through genome-wide association studies (GWAS) and other powerful genomic approaches in PTSD. Although our goal is to understand the COMT and IL6 gene pathways that are associated with PTSD, this study examined particularly how those genes act on the development of the disorder and the severity of psychopathological symptoms. In our study there was no significant difference between the PTSD group and controls in genotype distributions of the COMT gene. The results of the regression analyses for the total CAPS scores were not significant in allelic and dominant models for the current and lifetime PTSD patient subgroups. However, our results suggest an association of the minor (A) allele of rs4680 and elevated BSI scores in patients with remitted/lifetime PTSD. A significant association between one or more copies of the Met158 allele and PTSD has been reported (Valente et al. 2011), and in addition a gene-environment interaction between the Met158 allele and the number of traumatic event types in predicting PTSD (Kolassa et al. 2010). While our results do not show an association of the polymorphism with PTSD itself, they are consistent with the results of the study of Norrholm et al. (2013) who examined the intermediate phenotype of fear inhibition in PTSD and they found that individuals with Met/Met genotype demonstrated impaired fear inhibition, which may be mediated by higher methylation in the COMT promoter region. It means that regulation of COMT and subsequent catecholamine neurotransmitter cascades may be an important factor in fear processing for patients with PTSD. Despite the strong influence of genotype on COMT activity, the relationship between the Val158Met polymorphism and behavior, psychiatric disorders, and cognition has been found to be moderate and inconsistent (Baekken et al. 2008, Barnett et al. 2008). The low-activity Met allele has been associated with higher incidence of major depression (Ohara et al. 1998a) and with reduced generalized anxiety across adolescence (Olsson et al. 2007). No significant association of COMT genotypes and anxiety disorder was found in the study of Ohara et al. (1998b), but several studies are in line with our study where the association of COMT gene polymorphisms with anxiety was found (Benjamin et al. 2000, Kolassa et al. 2010, Hettema et al. 2006). Lonsdorf et al. (2010) found that Val carriers (Val/Val or Val/Met) endorsed more anxiety and depressive symptoms than Met homozygotes, which correlates with the findings in our study. The Val

allele has been associated with higher level of phobic anxiety (McGrath et al. 2004), but other studies indicate the Met allele is associated with anxiety traits (Eley et al. 2003, Hoth et al. 2006). Some studies have identified sex differences in COMT activity. A meta-analysis of twenty seven studies found a sex specific association across anxiety traits, such that Val-carriers had higher anxiety scores than Met homozygotes only among males (Lee & Prescott 2014). An association of panic disorder with the Val158 allele in women has been reported (Domschke et al. 2004).

There is growing evidence of a relationship between inflammatory markers, such as IL6 and PTSD. Some studies found a positive relationship between IL6 and PTSD (Maes et al. 1999). But the question is whether IL6 is elevated only at the onset of PTSD symptomatology, or the inflammation is related to the specific key components that define PTSD. In our study a nominally significant difference was found between the genotypes of the IL6 SNP rs1800795 regarding the total CAPS and total BSI scores, in the group of patients with lifetime PTSD. In fact, homozygous C allele carriers had higher CAPS and BSI scores results than heterozygous individuals and homozygous G allele carriers.

Studies investigating inflammatory markers in PTSD have yielded controversial results. It is well known that the levels of inflammatory markers depend on the severity and duration of illness, the presence of comorbid major depressive disorders, and the use of psychotropic medication. IL6 levels remained increased in the PTSD group and were positively associated with a severity of illness. For most inflammatory markers, study heterogeneity was reported to be high. Ekdahl et al. (2003) in his study reported that inflammation is implicated in the etiology and pathophysiology of several brain pathologies such as: major depression, Alzheimer's disease, and post-stroke depression. We have also found that in the recessive model for the minor allele (C), a significant result was obtained for total CAPS and for BSI. Also some authors suggest that small-study effects may contribute to an over estimation of the association between PTSD and IL6, (Maes et al. 1999), but because the sample composition was highly correlated with depression and medication status, it is hard to tell if medication, comorbid major depressive disorder, or research bias truly predicts the observed effect size. Future genetic studies in various psychiatric disorder such as PTSD, depression or psychosis might explore more of the dual role of the IL6 gene in health and disease states.

Some limitations of this study are the heterogenity of the PTSD and control group with regard to different population origins (countries) and type and duration of trauma (war). It may also be that the small groups of patients, when separated into lifetime PTSD and current PTSD reduce the statistical power.

CONCLUSIONS

In conclusion, results from this study support the notion that the Val158Met COMT polymorphism as well as interleukin-6 genetic variation contribute to the genetic susceptibility to PTSD. Identification of PTSD biological pathways strengthens the hope of progress in the mechanistic understanding of a model psychiatric disorder and allows for the development of targeted treatments and interventions (Lynn et al. 2014).

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Contribution of individual authors:

Each author has actively participated in the international research project (see Acknowledgments) and, therefore, has substantially contributed to the development and publication of this manuscript.

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