

THE ROLE OF TAQI *DRD2* (RS1800497) AND *DRD4* VNTR POLYMORPHISMS IN POSTTRAUMATIC STRESS DISORDER (PTSD)

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SUMMARY

Background: Posttraumatic stress disorder (PTSD) is a complex stress related disorder, that follows a severe traumatic experience, characterized with an intense sense of terror, fear, and helplessness. The aim of this study is to identify associations of genetic variations within candidate genes *DRD2* and *DRD4* with various PTSD related phenotypes. PTSD lifetime and PTSD current subjects were analyzed separately, each of them were analyzed in a Case/Control design, as well as regarding BSI and CAPS within cases only.

Subjects and methods: 719 (487 male, 232 female) participants who had experienced war-related trauma between 1991 and 1999 in Bosnia and Hercegovina, Kosovo and Croatia were included in the study. Sociodemographic questionnaire, Clinician Administered PTSD Scale (CAPS) and the Brief Symptom Inventory (BSI) were used to collect clinical data.

Results: The *DRD2* rs1800497 variant and a variable number tandem repeat (VNTR) located in exon three of *DRD4* were investigated for association with PTSD. In case control analyses we did not identify any significant associations. Within the PTSD current patients, we identified an association of *DRD2* rs1800497 with BSI in the genotypic and the recessive model with the T allele as the risk allele.

Conclusion: Our findings suggest that rs1800497 of *DRD2* gene is involved in pathogenesis of PTSD.

Key words: PTSD - *DRD2* rs1800497 variant - *DRD4* VNTR Exon 3 - CAPS - BSI

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a complex stress related disorder, that follows a severe traumatic experience, characterized with an intense sense of terror, fear, and helplessness (Broekman et al. 2007). The normal life of the affected people is severely

interfered by PTSD. It is estimated that 7% to 8% of the USA population (8 million adults) will have PTSD at some time point during their lives, and an even higher percentage of the gulf war and Vietnam war veterans have PTSD (Koenen et al. 2008). Various traumatic incidents can cause a PTSD, such a war, urban violence, and natural disasters like floods and earthquakes.

In contrast to the prevalence of PTSD in the US general population (Kessler et al. 2005), it is assumed to be as high as 35% in people who experienced the war in Bosnia and Herzegovina, and 25% in those who experienced the war in Kosovo (Priebe et al. 2010, Lopes Cardozo et al. 2003). The longitudinal course of PTSD is variable. Approximately 50% of persons diagnosed with PTSD will develop a chronic form of this disorder while the remaining 50% will recover. Of those individuals who will develop the chronic form of PTSD, 40% will still remain symptomatic after 10 years and ten percent will be affected even after 30 years (Kearns et al. 2012, Koenen et al. 2008, Kessler et al. 2005). Additionally, severe somatic disorders may be a long-term consequence in addition to the psychological distress (Dzubur-Kulenovic et al. 2008). That is why there is a definite need to develop new therapies for the treatment of PTSD. Research regarding the pathogenesis of PTSD contributes to an improvement of our understanding of pathways leading to the disorder. These findings may serve as a basis for the development of novel therapeutic approaches (Jakovljevic et al. 2012a). For answering the question why individuals do or do not develop PTSD following exposure to potentially psycho traumatic events, it may be useful to understand which intrapsychical and neurobiological phenomena act as either vulnerability, resilience and personal growth factors following traumatic experiences (Jakovljevic et al. 2012b).

The two most widely acknowledged contributors to the psychopathology of PTSD are genetics and stress. Despite other alternatives given to this issue, the "diathesis-stress" hypothesis has been the leading etiological model for psychiatric disorders for decades. For a broader range of psychiatric disorders, including bipolar disorder (Gilman et al. 2015) and schizophrenia (Matheson et al. 2013), stress, especially early adversity and later stressful life events, has been identified as a risk factor. However, the role of stressful environments and the physiology of stress response systems have been most closely linked to depressive, anxiety, and traumatic stress disorders (Smoller et al. 2015).

Multiple genes of the dopaminergic system are involved in the biosynthesis, transport, degradation, transmission, and signaling transduction of the neurotransmitter dopamine, such as the solute carrier family 6 member 3 (SLC6A3), the catechol-O-methyl-transferase (COMT), and the dopamine receptor D2 (DRD2) genes (Nguyen et al. 2014). In many brain processes such as reward and motivation, memory and learning, as well as fine motor control, the dopaminergic signaling system has a very high impact. (Girault et al. 2004). Abnormal dopaminergic signaling and function has been described for many neuropsychiatric disorders, such as schizophrenia and attention-deficit hyperactivity disorder (ADHD) (Kienast et al. 2006, Faraone et al. 2006).

Also, various PTSD symptoms related to attention, vigilance, arousal, and sleep are associated with a dysregulation of the dopamine system (Hamner et al. 1993). In subjects who have been exposed to traumatic events, the ability to deal with stress stimuli may be influenced by

variations of genes involved in the dopaminergic system that have an impact on dopamine synthesis, binding affinity, and signal transduction (Cornelis et al. 2010). Due to neuroimaging studies (Cubells et al. 2004), the dopaminergic system in PTSD patients may counteract or worsen the crisis response to the stressful stimuli. The enzyme dopamine beta-hydroxylase (DBH) converts dopamine to norepinephrine (Cubells et al. 2004). Continuous exposure to high stress leads to an elevated norepinephrine concentration in the cerebrospinal fluid and over activation of norepinephrine receptors. This may be associated with flashbacks and nightmares, which are frequently experienced in persons suffering from PTSD (Mason et al. 1988, Geraciotti et al. 2001).

Among dopamine receptors, D1, D2, D3, D4, and D5, that belong to the G-protein-coupled receptor family that inhibits adenyl cyclase, the DRD2 receptor is associated with pleasure and reward circuitry (Noble, 2000). Mutations of DRD2 are associated with movement disorder, myoclonus dystonia, and schizophrenia (Klein et al. 1999). The fact that dopaminergic innervations of the prefrontal cortex is highly sensitive to both, acute and chronic stress, has been shown in earlier studies (Deutch et al. 1990). Other results support the hypothesis that a defect in the central dopaminergic pathway contributes to posttraumatic symptomatology (Deutch 1995). Increased production of dopamine, caused by a polymorphism in the DRD2 receptor, which is central to the dopamine system, is associated with the increased risk of psychopathology consequent to traumatic exposure.

The 957C>T polymorphism in the DRD2 gene is suggested as one of the genetic susceptibility factors for PTSD (Joanne et al. 2009). However, in this study no control individuals who were exposed to combat situation but did not have PTSD were included. The significant association found in the study is seen as exploratory and further replication is required. The frequency of the Taq I A alleles (A1 and A2) of the DRD2 in Caucasian PTSD patients and controls was studied and results showed that the DRD2 A1 allele was associated with PTSD. This association, however, was found only for harmful PTSD drinkers. PTSD patients with the A1(+) allele consumed more alcohol than patients with the A1(-) allele (Young et al. 2002). Previously, the single nucleotide polymorphism (SNP) rs1800497, commonly also referred to as TaqI DRD2, was assigned to DRD2. Later it was found to reside in exon 8 of the adjacent gene, ankyrin repeat and kinase domain containing 1 (ANKK1), resulting in a missense variant of the encoded protein. This polymorphism is related to the regulation of DRD2 receptor density in the brain and dopamine synthesis (Neville et al. 2004). It has been linked to several neuropsychiatric disorders, such as Tourette syndrome and ADHD (Comings et al. 1996). Regarding the association of PTSD with genetic variants of DRD2, most studies focus on the SNPrs1800497. One study found that the rs12364283 polymorphism in DRD2 showed a strong association with PTSD (Voisey et al. 2009). Rs12364283 is in a merely very low linkage disequilibrium (LD) with

rs1800497 ($r^2=0.001$). Also, research showed that rs12364283 was associated with enhanced DRD2 expression. Analysis of the flanking conserved sequences of this SNP suggested that the minor C allele alters the binding sites of the putative transcription factor which up regulates DRD2 expression (Lizhuno et al. 2016). A significant association between the TaqI DRD2 polymorphism in DRD2 and PTSD has been identified (Comings et al. 1996). Absence of one of the variants of this gene (A1) is related to a greater resistance to PTSD, while the presence of this variant increases the risk for the development of this disorder. Similar results were shown by Young et al. (2002) but only in patients with a comorbidity of alcohol dependence. On the other hand, no association was found between DRD2 allele A1 and PTSD, by Gelernter et al. (1999).

The dopamine D4 receptor (DRD4) gene encodes a transmembrane protein with 7 domains, which is present in the frontal cortex, the hypothalamus, the hippocampus, and the striatum. Various polymorphisms are located within the DRD4 gene sequence. In exon 3, there is a repetitive sequence of 48 base pairs (bp) that occurs in 2 to 10 copies and belongs to the class of variable number tandem repeats (VNTR). Another VNTR comprising 12 bp is located within exon 1. Within the promoter region, the T allele of the variation -521C>T reduces the gene expression efficiency in comparison to the C allele (Aguirre-Samudio et al. 2005). A DRD4 polymorphism located in exon 3 has been linked to maladaptive stress responses and temperament traits related to PTSD (Wojciech et al. 2009). The association between the DRD4 VNTR in exon 3 and the intensity of PTSD symptoms was analyzed in a study with flood survivors. The results showed that participants with at least one copy of the long DRD4 allele (i.e. seven or eight repeats) had more intense PTSD symptoms on the Avoidance/ Numbing scale (Cohen's $f=0.22$) and the Total Scale (Cohen's $f=0.2$) of the PTSD-F than participants who were not carriers of these alleles. However, due to methodological restrictions and a small sample size, the study needs to be replicated in a larger sample (Wojciech et al. 2009).

We therefore decided to test the impact of TaqI DRD2 (rs1800497) and the DRD4 VNTR on PTSD and its psychopathology in the SEE-PTSD cohort.

SUBJECTS AND METHODS

Subjects

Of originally 747 inhabitants of South East Europe recruited between 2013 and 2015 at research centers of Bosnia and Herzegovina (Sarajevo, Tuzla and Mostar), Croatia (Zagreb) and Kosovo (Prishtina) under a Stability Pact for the SEE collaborative research study "Molecular Mechanisms of Posttraumatic Stress Disorder", supported by the DAAD (Deutscher Akademischer Austausch Dienst), 719 could be finally analyzed. Inclusion and exclusion criteria for the study, recruitment process, diagnostic assessment, sample size and gender distribution are described in detail elsewhere (Dzibur-Kulenovic et al. 2016).

In all five countries of recruitment population experienced severe war-related trauma between 1991 and 1999. As a result, many of participants developed PTSD symptoms and were for this study, in dependence on presence or absence of diagnosed current or lifetime PTSD, divided into three experimental groups. The first group consist of 218 patients (mean age 50.1 ± 6.7 ; 157 males and 61 females) with current PTSD, the second group includes 151 participants with lifetime PTSD (mean age 49.5 ± 8.2 ; 98 males and 53 females), and the last group comprised 350 healthy volunteers with no diagnosable PTSD (mean age 48.8 ± 8.5 ; 232 males and 118 females).

There were no significant differences with regard to age and gender between groups in the combined sample. Between subsamples there were gender differences due to different populations who had suffered trauma during the war; however care was taken that the control samples were gender-matched.

Ethical Votes

Ethical votes at the participating clinical centers were obtained between 2011 and 2013 on the basis of local translations of an information and consent form designed by the Würzburg center. Participants thus were informed and gave written informed consent according to the principles of the declaration of Helsinki (WMA 2013).

Psychometric Instruments

To clarify the presence or absence of PTSD symptoms, the Mini International Neuropsychiatric Interview (M.I.N.I.) was used. For categorization of PTSD symptoms within current and lifetime PTSD patients, the Clinician Adminstrated PTSD Scale (CAPS) (Blake et al. 1996) was assessed. To measure general psychiatric symptoms the Brief Symptom Inventory (BSI) (Derogatis & Melisaratos 1983) was used.

Molecular Analyses

From all participants EDTA blood was drawn for genetic and epigenetic analyses and stored at -80°C . DNA extraction was performed using the FlexiGene DNA Kit (QIAGEN, Hilden, Germany) at Zagreb (Zagreb samples), Sarajevo (Sarajevo, Tuzla and Mostar samples) and Würzburg (Prishtina samples) according to the instructions of the manufacturer. EDTA blood and DNA transport was done on dry ice. Isolated DNA was stored until genotyping at the Laboratory of Functional Genomics in Würzburg at -800C .

The TaqI DRD2 (rs1800497) variant was investigated following published protocols (see Koehler et al. 2011).

For investigation of the DRD4 variable number tandem repeat, located on exon 3, the region of interest was amplified by PCR in a 25 μl reaction volume containing 45-65 ng genomic DNA, 20 mM $(\text{NH}_4)_2\text{SO}_4$, 75 mM Tris-HCl (pH9), 0.01% Tween 20.2 μl DMSO, 0.4 mM of each primer (F: 5'-GCGACTACGTGGTCTACTCG-

3' and R: 5'-AGGACCCTCATGGCCTTG-3'), 0.1 mM of each nucleotide, 0.8 mM MgCl₂ and 0.5 U Taq DNA polymerase under the following cyclor conditions: 3 min denaturation at 95°C, followed by 30 cycles with 45 s at 95°C, 45 s at 57.2°C and 45 s at 72°C and a final extension step of 3 min at 72°C. The resulted PCR products with varying lengths, were separated on a 3% agarose gel using gel electrophoresis and visualised by ethidium bromide staining. Fragment lengths and with that the respective genotype were determined by two independent investigators blinded for diagnosis.

Statistical Analyses

Statistics were performed using PLINK 1.9. TaqI DRD2 (rs1800497) as well as the DRD4 VNTR Exon3 variant were polymorphous (minor allele frequency ≥10%), reached a minimal genotyping call rate of 98% and did not deviate from Hardy-Weinberg equilibrium after correction for multiple tests.

Logistic regression was used for case-control analyses combining both patient subgroups. 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 values according to an additive allelic, dominant and recessive (all based on the minor allele), as well as a genotypic model, respectively. The significance level was Bonferroni adjusted for 23 variants that were analyzed in total within the entire project ($\alpha=0.002$).

RESULTS

To characterize the role of dopamine receptors on the etiopathology of PTSD, we examined within this study the Taq I DRD2 (rs1800497) polymorphism and the DRD4VNTR Exon 3 variant in a classical case-control study (N=719) and analyzed additionally their influence on the two questionnaires CAPS and BSI in patients with current or lifetime PTSD symptoms.

Dopamine Receptor D2

TaqI DRD2 (rs1800497) was not associated with the categorical phenotype of PTSD ($P_{all}>0.05$), but when correlated with CAPS scores from patients with either current or lifetime PTSD symptoms our results for the recessive model, although not reaching nominal significance, pointed to possible associations ($P_{Current}=0.0504$; $P_{Lifetime}=0.0568$; Table 1) of elevated average CAPS scores and homozygosity for the minor allele (TT) in contrast to other genotypes (TC/CC). Consistently, the minor (T) allele possibly conveyed genetic risk for developing PTSD symptoms. When rs1800497 was correlated to BSI scores, not for the subjects suffering from lifetime PTSD, but for those with current PTSD symptoms, nominal significant associations could be determined for the genotypic ($P=0.0130$) as well as for the recessive model ($P=0.0037$), again with the minor (T) allele serving as the risk allele (Table 1 and Figure 1). However, none of these associations withstood Bonferroni correction for multiple testing.

Dopamine Receptor D4

Associations for the DRD4 VNTR exon 3 were found neither for the dimensional phenotypes nor for the categorical case-control study ($P_{all}>0.05$; Table 2). However, our results may suggest an influence of homozygosity for the DRD4VNTR Exon 3 short allele (SS) on higher CAPS values in patients with PTSD lifetime symptoms ($P_{allelic}=0.0768$; $P_{dominant}=0.0636$; Table 2).

DISCUSSION

As a first genetic research in PTSD of South Eastern Europe, this study is of distinct value to the genetic perspective on PTSD. All of the participants in this study were exposed to war trauma, with a prevalence of PTSD up to 35.5% in individuals from Bosnia and Herzegovina and around 18% in those who experienced the war

Table 1. Association results of DRD2 rs1800497, along with genotype- and allele counts, for participants with Lifetime and Current PTSD symptoms and controls, CAPS and BSI means and standard deviations (SD), as well as nominal p-values

DRD2	Allelic Model		Genotypic Model			Dominant Model		Recessive Model	
	T	C	TT	TC	CC	TT/TC	CC	TT	TC/CC
Controls	118	578	3	112	233	115	233	3	345
PTSD _{lifetime}	50	250	4	42	104	46	104	4	146
PTSD _{current}	75	353	5	65	144	70	144	5	209
$P_{case-control-value}$	0.9096		0.2251			0.0842		0.1107	
CAPS _{lifetime} (mean±SD)	68.6±16.8	66.6±17.8	84.0±15.0	65.6±15.4	66.8±18.2	67.2±16.2	66.8±18.2	84.0±15.0	66.5±17.4
$P_{CAPS-value}$	0.4750		0.1344			0.8979		0.0504	
CAPS _{current} (mean±SD)	80.6±22.9	78.9±20.4	96.8±13.6	78.2±23.0	79.0±19.8	79.5±23.0	79.0±19.8	96.8±13.6	78.8±20.8
$P_{CAPS-value}$	0.4906		0.1551			0.8741		0.0568	
BSI _{lifetime} (mean±SD)	73.6±48.2	73.6±49.6	65.3±37.2	75.3±49.9	73.4±49.6	74.3±49.0	73.4±49.6	65.3±37.2	73.7±49.6
$P_{BSI-value}$	0.9364		0.8984			0.8312		0.7302	
BSI _{current} (mean±SD)	118.5±48.6	111.6±45.6	172.0±17.7	110±46.5	112.1±45.5	114.5±47.8	112.1±45.5	172.0±17.7	111.3±45.7
$P_{BSI-value}$	0.2495		<i>0.0130</i>			0.7276		<i>0.0037</i>	

DRD2 - Dopamine receptor 2; PTSD - posttraumatic stress disorder; CAPS - Clinician Administered PTSD Scale; BSI - Brief Symptom Inventory; *Italics indicates p<0.05*

Table 2. Association results of DRD4 VNTR Exon3, along with genotype- and allele counts, for participants with Lifetime and Current PTSD symptoms and controls, CAPS and BSI means and standard deviations (SD), as well as nominal p-values

DRD4	Allelic Model		Genotypic Model			Dominant Model		Recessive Model	
	L	S	LL	LS	SS	LL/LS	SS	LL	LS/SS
Controls	143	551	14	115	218	129	218	14	333
PTSD _{lifetime}	57	243	2	53	95	55	95	2	148
PTSD _{current}	87	339	10	67	136	77	136	10	203
P _{case-control} -value	0.7142		0.8714			0.7064		0.6059	
CAPS _{lifetime} (mean±SD)	63.5±17.4	67.7±17.5	65.5±11.5	63.3±17.8	69.0±17.4	63.4±17.6	69.0±17.4	65.5±11.5	67.0±17.7
P _{CAPS} -value	0.0768		0.1737			0.0636		0.9070	
CAPS _{current} (mean±SD)	78.3±16.3	79.5±21.8	76.1±14.7	79.0±16.7	79.5±23.1	78.6±16.5	79.5±23.1	76.1±14.7	79.4±21.1
P _{CAPS} -value	0.6706		0.8793			0.7627		0.6320	
BSI _{lifetime} (mean±SD)	66.4±46.0	75.0±50.0	27.0±10.0	69.6±46.3	76.3±51.0	67.9±46.1	76.3±51.0	27.0±10.0	74.1±49.4
P _{BSI} -value	0.2302		0.3087			0.3395		0.1842	
BSI _{current} (mean±SD)	114.0±38.9	112.5±47.8	114.1±36.1	113.9±39.7	113.1±49.2	113.9±39.2	113.1±49.2	114.1±36.1	112.7±46.6
P _{BSI} -value	0.8992		0.9914			0.8960		0.9595	

DRD2 - Dopamine receptor 2; PTSD - posttraumatic stress disorder; CAPS - Clinician Administered PTSD Scale; BSI - Brief Symptom Inventory

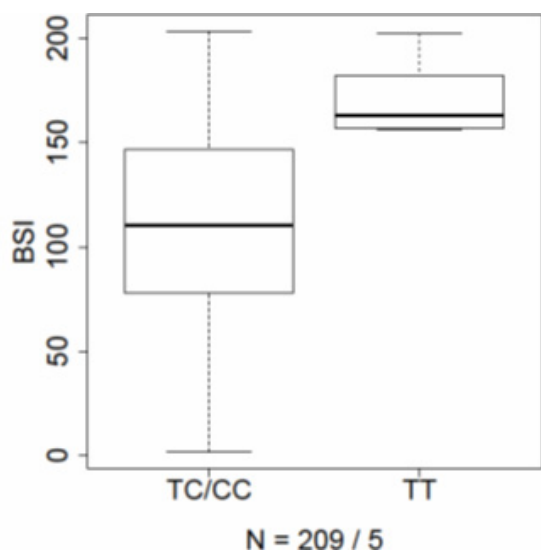


Figure 1. Distribution of BSI values in patients suffering from current PTSD symptoms according to DRD2 rs1800497 for the recessive model (TC/CC versus TT; P=0.0037)

in Kosovo and Croatia (Priebe et al. 2010, Lopes Cardozo et al. 2003). This study is another small step forward in answering the question why some individuals are susceptible while others are resilient to developing PTSD symptoms after the exposure to traumatic events, in particular with regard to the dopamine receptor genes. Previous findings showed that the DRD2 A1 allele was associated with PTSD only in the harmful PTSD drinkers (Young et al. 2002). Most studies regarding genetic variants of DRD2 so far have focused on rs1800497, as we did. In our sample, we identified just 5 individuals that are homozygous carriers of the minor allele T. Hence, the nominally significant result for causing elevated BSI scores as well as the suggestive findings regarding CAPS values in our study may be biased by a lack of statistical power. Yet, our results may support

the assumed involvement of this gene in PTSD pathogenesis as they are consistent with previous published positive studies regarding DRD2. In contrast to other studies regarding DRD2 polymorphisms, which considered only male veterans, we included also women as well as control individuals who were exposed to combat situation but did not develop PTSD in our analyses.

DRD4 and its genetic variants have been studied in association with many mental disorders like schizophrenia, ADHD, bipolar disorder, OCD with ties and in addition with behavioral traits such as novelty seeking. A possible association between DRD4 and PTSD was previously only analyzed in a sample of flood survivors and within that study methodological restrictions and a small sample size were limiting factors. Concerning the DRD4 VNTR polymorphism located in exon 3 that was related to maladaptive stress responses and temperament traits related to PTSD in previous studies, our study may point to the same direction based on a suggestive but not even nominally significant effect of homozygosity for the short allele on elevated CAPS values within the PTSD lifetime subjects. To clarify these assumptions, replication in a larger sample is required.

Limitations of the study are the relative small sample size and different trauma types among individuals in this study. Only nominally significant findings were obtained that did not withstand Bonferroni correction. Thus our findings have to be considered explorative.

CONCLUSIONS

As a conclusion, our findings may support the suggested involvement of DRD2 in PTSD, but moreover they highlight the need for larger samples to improve statistical power. More research is needed to elucidate the pathways that possibly connect the DRD2 and DRD4 polymorphisms and the risk of developing PTSD.

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Contribution of individual authors:

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