GENETIC SUSCEPTIBILITY TO POSTTRAUMATIC STRESS DISORDER: ANALYSES OF THE OXYTOCIN RECEPTOR, RETINOIC ACID RECEPTOR-RELATED ORPHAN RECEPTOR A AND CANNABINOID RECEPTOR 1 GENES

Sabina Kučukalić1*, Elma Ferić Bojić2*, Romana Babić3, Esmina Avdibegović4, Dragana Bačić2, Ferid Agani5, Miro Jakovljević5, Ahdžadžić5, Alma Bravo Mehmedbašić6*, Eminica Šabić Džananović1, Damir Marjanović2, Nermisna Kravic4, Marko Pavlović4, Branka Aukst Margetić6, Nenad Jakšić6, Ana Cima Franc5, Dusko Rudan5, Spende Haxhibeqiri5, Aferdita Goci Uka9, Blerina Hoxha9, Valde Haxhibeqiri10, Mirnesa Muminović Umihan11, Osman Sinanović12, Nada Božina13, Christiane Ziegler14, Christiane Wolf15, Bodo Warrings15, Katharina Domschke14, Jürgen Deckert15 & Alma Đzubur Kulenović1

1Department of Psychiatry, Clinical Center University Sarajevo, Sarajevo, Bosnia and Herzegovina
2Department of Genetics and Bioengineering, International Burch University, Sarajevo, Bosnia and Herzegovina
3Department of Psychiatry, University Clinical Center of Mostar, Bosnia and Herzegovina
4Department of Psychiatry, University Clinical Center of Tuzla, Bosnia and Herzegovina
5Faculty of Medicine, University Hasan Prishtina, Prishtina, Kosovo
6Department of Psychiatry, University Hospital Centre Zagreb, Zagreb, Croatia
7Department of Psychiatry, University Hospital Centre Sestre Milosrdnice, Zagreb, Croatia
8Institute of Kosovo Forensic Psychiatry, University Clinical Center of Kosovo, Prishtina, Kosovo
9Department of Psychiatry, University Hospital Centre Zagreb, Zagreb, Croatia
10Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia
11Department of Psychiatry and Psychotherapy, University Hospital Freiburg, Freiburg, Germany
12Department of Psychiatry, Psychosomatics and Psychotherapy, Center of Mental Health, University Hospital Wurzburg, Wurzburg, Germany

*Authors contributed equally to the text

received: 1.2.2019; revised: 9.5.2019; accepted: 21.5.2019

SUMMARY

Background: Exposure to life-threatening events is common and everyone will most likely experience this type of trauma during their lifetime. Reactions to these events are highly heterogeneous and seems to be influenced by genes as well. Some individuals will develop posttraumatic stress disorder (PTSD), while others will not. In this study, our aim was to analyze the correlation between single nucleotide polymorphisms (SNPs) within the oxytocin receptor (OXTR) gene (rs53576 and rs2254298), the RAR-related orphan receptor A (RORA) gene (rs8042149) and the cannabinoid receptor 1 (CNR1) gene (rs1049353) and PTSD. All candidate genes have been previously associated with stress related disorders and the reaction to traumatic events.

Subjects and methods: Participants (N=719) have been exposed to war-related trauma during the war in South-Eastern Europe (Bosnia and Herzegovina, Croatia and Kosovo). We correlated the presence and absence of current and lifetime PTSD as well as PTSD severity (Clinician Administered PTSD scale (CAPS)) and current psychopathology (Brief Symptom Inventory (BSI) score) with the mentioned SNPs. DNA was isolated from whole blood and genotyped for OXTR rs2254298 and rs53576 following previously published protocols, for RORA rs8042149 via PCR-RFLP and CNR1 rs1049353 via KASP.

Results: Nominally significant results were found for OXTR rs53576 in connection with the CAPS and BSI scores within lifetime PTSD patients. The additive allelic model indicated that G allele carriers achieved lower CAPS (p=0.0090) and BSI (p=0.0408) scores than participants carrying one or two copies of the A allele. These results did not withstand correction for multiple tests. No significant results were observed for OXTR rs2254298, RORA rs8042149 and CNR1 rs1049353 although the results for RORA showed a slight tendency that rs8042149 may influence the level of BSI scores in current PTSD patients.

Conclusions: This study points to a role of the OXTR gene in PTSD and the related psychopathology following war related trauma.

Key words: posttraumatic stress disorder - oxytocin receptor gene - RAR-related orphan receptor A - cannabinoid receptor 1
INTRODUCTION

Posttraumatic stress disorder (PTSD) is a common and very often chronic disorder that can develop following exposure to a life-threatening or traumatic event. Trauma is an essential part of the diagnosis of PTSD, and approximately 70% of the population will experience at least one traumatic event during their lifetime (Benjet et al. 2015). An estimated 10% of exposed individuals will develop PTSD symptoms (Kessler et al. 2013), which include the following:

- Symptoms of re-experiencing in the form of intrusive thoughts, nightmares, flashbacks, emotional distress and physical reactivity to traumatic reminders (criterion B).
- Symptoms of avoidance, which include avoiding traumatic reminders that trigger symptoms of re-experiencing (criterion C).
- Alterations in mood and cognition, which include a variety of symptoms such as dissociative amnesia, persistent negative beliefs about oneself and the environment, diminished interest in usual activities and affective changes (criterion D).
- Symptoms of hyperarousal that can be experienced as aggressive behavior including auto and hetero-aggression, sleep disturbance, impulsivity and cognitive deficits (criterion E).

Furthermore, symptoms have to be experienced for at least a month and cause a related change in functionality. Long term mental health consequences of war are observed in survivors even after decades and they still present a severe mental health burden. Priebe et al. (2010) found that PTSD prevalence in a sample of 640 participants from Bosnia and Herzegovina was 35.4%, which was the highest of all countries that were investigated (Croatia, Kosovo, Serbia and Republic of Macedonia). However, the key question of why some individuals develop symptoms of trauma related disorders following a traumatic event and why some do not still remains unsolved.

The effects of many genes on the development and persistence of anxiety and stress-related disorders are now widely studied. An example is the oxytocin receptor (OXTR) gene, which seems to be an important genetic variable (Gottschalk & Domschke 2016). A large body of evidence links oxytocin (OXT) to stress regulation, and it has been found that the plasma levels of oxytocin seem to increase as a response to stress stimuli (Neumann et al. 2000, Onaka 2004). The first discoveries in humans revealed that plasma levels of oxytocin increased following exposure to uncontrollable noise and several types of psychosocial stressors (Sanders et al. 1990, Olff et al. 2013). The hypothesis is that during stressful stimuli or situations, oxytocin release serves to dampen physiological stress levels. The higher the basal levels of oxytocin, the lower the norepinephrine levels, blood pressure and heart rate in response to stress (Light et al. 2004). The probable stress regulation mechanism of oxytocin appears to be related to functional changes of the amygdala (Viviani et al. 2011). In support of these findings, neuroimaging studies have shown that intranasal administration of oxytocin lowered amygdala activity probably by enhancing amygdala-prefrontal cortex connectivity (Domes et al. 2007, Sripada et al. 2012).

The effects after oxytocin administration are important for the autonomic stress response (Koch et al. 2016), amygdala reactivity and anxiety levels, as well as beneficially impact socio-emotional processes and behavior (Preece et al. 2014). Furthermore, the allelic status seems to play an important role in determining behavior patterns after stress, meaning that carrying different alleles such as the G allele of rs53576 increased prosociality (Kogan et al. 2011) and empathy (Rodrigues et al. 2009). This means that different allele carriers respond and behave differently after major traumatic events.

Furthermore, studies have shown amygdala size differences between PTSD and non-PTSD groups, with the PTSD cohort having smaller amygdala volumes (Morey et al. 2012). OXTR rs2254298 A homozygotes were found to have smaller volumes of corticolimbic system structures such as the amygdala, anterior cingulate cortex and the posterior brainstem than carriers of the A allele (Furman et al. 2011). Also, the OXTR rs2254298 A allele was positively correlated with bilateral amygdala volume. The same correlation was seen in the A/A genotype in rs53576, whereby significant association was observed (Wang et al. 2014). Allele-load-dependent changes in the hypothalamus are considered to be the oxytonergic “core” of the brain. This means that carrying a risk allele (rs53576 A allele) leads to anatomical and functional interaction change in structures that is a central fear and anxiety regulator (Tost et al. 2010). The association between OXTR polymorphisms and amygdala volume could be a potential explanation of the complex and very often severe psychopathology related to stress (Furman et al. 2011).

Studies have concluded that OXTR variants such as rs53576 play an important role in attachment styles and consequently influence anxiety levels as shown in studies of probands with social anxiety and PTSD (Notzon et al. 2016, Sippel et al. 2017). As such, the interaction between attachment styles and SNPs could present a promising factor that may contribute to vulnerability to PTSD (Sippel et al. 2017). Early traumatic experiences seem to influence the oxytocin system later in life, and the main consequences are seen in changes in attachment styles which become insecure and present a major risk factor for development of mental health disorders (Olff et al. 2013).
The Retinoic Acid Receptor-Related Orphan Receptor Alpha (RORA) gene is also an intriguing target to investigate in the context of trauma. RORA, whose function is complex and still poorly understood, belongs to the nuclear hormone receptors 1 subtype (NR1). The encoded protein seems to be related to brain development, neuroprotection and the regulation of circadian rhythms. RORA is widely expressed in cortical and subcortical neurons, where it protects them against oxidative stress and inflammation. These seem to be related to a possible mechanism linked to the effects of traumatic stress on the brain.

The correlation of RORA polymorphisms and traumatic stress was first evaluated a few years ago in genome-wide association study conducted by Logue et al. (2013). The study was conducted on a population of white non-Hispanic participants and their spouses and demonstrated a significant association between lifetime PTSD diagnosis and RORA rs8042149. Probands with low trauma exposure who are homozygous for the high-risk allele (G) have exhibited higher PTSD symptoms compared to carriers of the low risk allele (A). Another study demonstrated that the RORA gene was associated with higher PTSD symptoms, and this association was more pronounced in persons previously exposed to child abuse (Lowe et al. 2015). The main effects were observed in chronically elevated symptoms, which lead to the conclusion that RORA might influence the maintenance of subthreshold symptoms, causing patients not to remit on time. The underlying mechanism still seems to be more complex, but some promising results do exist. Miller et al. (2013) concluded that RORA might play a role in protection against neurodegeneration after oxidative stress. RORA has also been correlated with regulation of circadian rhythms and steroid hormone levels, both of which are important for the PTSD symptom spectrum (Germain 2013). The same mechanism was observed in another study, which included acute stressors only (Amstadter et al. 2013). The association after acute stressors was the same, meaning that carrying the G allele presented a risk factor for developing higher PTSD symptoms. Since the heritability of PTSD was mainly evaluated for chronic symptoms, this study underlines the assumption that the development of PTSD is genetically influenced. Yet other studies suggest that RORA might play an important role in general psychopathology, possibly through its relationship with other vulnerability factors such as neuroticism (Kim et al. 2017).

Several studies have investigated the association between PTSD, major depressive disorder (MDD) and generalized anxiety disorder (GAD) and the cannabinoid system (Fani et al. 2012, Lindstrom et al. 2011, Sveen et al. 2009). Neumeister et al. (2013) have found that PTSD is associated with an increased cannabinoid receptor type 1 (CB1) availability in an amygdala-hippocampal-cortico-strialatal neural circuit as well as in brain regions outside this circuit, suggesting a greater brain-wide CB1 receptor availability in individuals with PTSD relative to controls with and without of trauma histories exposure. Increased availability of the CB1 receptor in the amygdala was associated with increased attentional bias to threat, as well as symptoms associated with increased severity of trauma-related threat in humans with a broad dimensional spectrum of trauma-related psychopathology (Pietrzak et al. 2014). Another significant finding was that the relation between CB1 receptor availability in the amygdala and severity of threat symptomatology is controlled by attentional bias to it (Pietrzak et al. 2014).

Given the aforementioned and the numerous investigations of the mentioned gene variants within the realm of psychiatry, we set out to investigate the possible association between PTSD and OXTR rs2254298 and rs53576, RORA rs8042149 and CNR1 rs1049353 (1359 G/A).

**SUBJECTS AND METHODS**

**Subjects**

Participants were recruited between 2013 and 2015 at five research centers (Sarajevo, Prishtina, Tuzla, Zagreb and Mostar) in the context of a Stability Pact for the South Eastern Europe (SEE) collaborative research study “Molecular Mechanisms of Posttraumatic Stress Disorder”, supported by DAAD (Deutscher Akademischer Austausch Dienst). Methods regarding recruitment, diagnostic assessment, inclusion and exclusion criteria, as well as sample size and gender distribution were previously described (Dzubur Kulenovic et al. 2016). Most of the volunteers have experienced traumatic events related to war and ethnic cleansing in the time frame from 1991-1999, while some of them had not experienced any trauma or never had symptoms of PTSD. Therefore the experimental sample of a total of 719 volunteers (mean age 49.4±7.9; 232 females and 487 males) was divided into three major groups (Table 1).

The experimental group comprised 218 participants with current PTSD (mean age 50.1±6.7; 61 females and 157 males), 151 participants with lifetime PTSD (mean age 49.5±8.2; 53 females and 98 males), and 350 participants with no diagnosable PTSD (mean age 48.8±8.5; 118 females and 232 males) (Dzubur Kulenovic et al. 2016).

| Table 1. Participant Groups According to Trauma |
|-----------------------------------------------|
| Group 1 | Group 2 | Group 3 |
| Trauma + | Trauma + | Trauma +/- |
| Current PTSD | Recovered or Lifetime PTSD | No PTSD in life course |
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. Categorization of PTSD symptoms on current and lifetime was conducted using the Clinician Administered PTSD Scale (CAPS) (Blake et al. 1995), while general psychiatric symptoms were measured with the Brief Symptom Inventory (BSI) (Derogatis & Melisaratos 1983).

Molecular Analyses

Genomic DNA was isolated from frozen venous blood according to manufacturer’s instructions using the FlexiGene DNA Kit (Qiagen, Hilden, Germany). It was stored at -80°C until genotyping at the Laboratory of Functional Genomics in Würzburg.

OXTR rs2254298 genotyping was conducted following published protocols with minor modifications (Wu et al. 2005). DNA was first amplified by PCR in a 25 µl reaction volume containing 45-65 ng genomic DNA, 0.4 mM of each primer (F: 5’-GCCCCACACCGAUTTCAGC-3’ and R: 5’-GTGGCCCTTTTCTCTGGC-3’), 0.1 mM of each nucleotide, 1.5 mM MgCl2 and 0.3 U TaqTM DNA polymerase under the following cycler conditions: 5 min denaturation at 95°C, followed by 35 cycles of 45 s at 95°C, 45 s at 66.4°C and 45 s at 72°C and a final extension step of 5 min at 72°C. PCR fragments were digested with the restriction endonuclease NlaIII (NEB, Frankfurt a. Main, Germany) for 3h at 37°C, which results in differentially sized fragments representing the respective genotypes. The fragments were separated on a 3% agarose gel by electrophoresis and visualized with ethidium bromide. Fragment lengths and resulting genotypes were determined by two independent investigators blinded for diagnosis.

CNR1 rs1049353 was genotyped using a custom designed KASPTM genotyping assay (LGc, Berlin, Germany). A PCR reaction including an end-point fluorescent read-out was done according to manufacturers’ instructions in a CFX384 Touch Cycler (Biorad, Munich, Germany). Genotype analysis was performed using the CFX Manager Software.

Statistical Analyses

Statistics were performed using PLINK 1.9. All SNPs were polymorphous (minor allele frequency≥10%), reached a minimal genotyping call rate of 98% and did not deviate from Hardy-Weinberg equilibrium (p≥0.1). Logistic regression was used for case-control analyses by testing all patients of either subgroup, lifetime or current PTSD, in combination versus the control individuals. Within the two groups of patients, linear regression was carried out individually for analyses on CAPS and BSI scores. The additive allelic and the genotypic models were tested in all phenotypes. The significance level was Bonferroni adjusted for 23 variants that were analyzed in total within the entire project (α=0.002).

RESULTS

Oxytocin Receptor

Significant associations for OXTR rs53576 were not found for the categorical phenotype of PTSD, but nominally significant results were obtained when correlated with CAPS scores within the lifetime PTSD group (Pallelic=0.0090; P dominant=0.0173 and P genotypic=0.0309; Table 2 and Figure 1), consistent with the minor (A) allele increasing the risk for developing more severe PTSD symptoms. With regard to the BSI score, the association with OXTR rs53576 was nominally significant within the lifetime PTSD group in the allelic model (p=0.0408; Table 2 and Figure 2). The genotypic model did not yield any significant results (p≥0.05; Table 2).

However, these nominal significant results for OXTR rs53576 and CAPS and BSI scores could not be replicated in patients with current PTSD symptoms and did not withstand correction for multiple tests. For OXTR rs2254298, no significant associations were found at all (p≥0.05).
Table 2. OXTR rs53576 associations, along with genotype and allele counts, for participants with lifetime and current PTSD symptoms and controls, lifetime CAPS and BSI means and standard deviations, as well as nominal p-values

| Allelic Model | OXTR_rs53576 | Allelic Model | OXTR_rs53576 | Allelic Model | OXTR_rs53576 | Allelic Model | OXTR_rs53576 |
|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|
| A             | 214          | G             | 484          | AA            | 37           | AG            | 140          | GG            | 172           | AA/AG         | 177           | GG            |
| B             | 218          | C             | 311          | AG            | 14           | GG            | 97           | GG            | 107           | AA/AG         | 111           | GG            |
| Controls      | 214          | G             | 484          | AA            | 37           | AG            | 140          | GG            | 172           | AA/AG         | 177           | GG            |
| PTSD_lifetime | 82           | A             | 218          | 13            | 56           | 81            | 69           | 81            | 0.2961        | 0.3128        | 0.2627        |                |
| PTSD_current  | 125          | A             | 311          | 14            | 97           | 107           | 111          | 107           |                |                |                |                |
| CAPS_lifetime (mean±SD) | 71.3±17.4 | 65.3±17.4 | 75.5±18.9 | 69.5±16.3 | 63.8±17.5 | 70.6±17.0 | 63.8±17.5 |
| BSI_current (mean±SD)  | 82.4±51.4 | 70.0±48.1 | 94.3±54.6 | 77.2±49.1 | 67.5±47.5 | 80.3±50.6 | 67.5±47.5 |

CAPS = Clinician Administered PTSD Scale; BSI = Brief Symptom Inventory; OXTR = Oxytocin Receptor; SD = standard deviation; Italics indicates p ≤ 0.05

Figure 1. Distribution of lifetime CAPS values according to genotypes in the additive allelic model for OXTR rs53576 (p=0.0090)

Figure 2. Distribution of lifetime BSI values according to OXTR rs53576 genotypes in the additive allelic model (p=0.0408).

Retinoic Acid Receptor-Related Orphan Receptor Alpha and Cannabinoid Receptor Type 1

Nominally significant observations were not found for RORA rs8042149 and for CNR1 rs1049353 in any of the models (p_all≥0.05). However, RORA rs8042149 showed a slight but not nominally significant (p=0.057) influence on BSI within the current PTSD patient subgroup in the recessive model. Carriers of at least one copy of the G allele demonstrated higher scores than T allele homozygotes.

DISCUSSION

The objective of this study was to examine the correlation between OXTR rs53376 and rs2254298, RORA rs8042149 and CNR1 rs1049353 and the development of trauma- and stress-related symptoms and general psychopathology. These genes have been previously associated with stress reactivity and possible psycho-pathological changes and PTSD symptoms. Nominally significant results were only found for OXTR rs53576, which modified the severity of PTSD symptoms and psychopathology in correlation with the allelic status. Specifically, the major allele (G) seems to be a protective factor for the development of PTSD symptoms and the related psychopathology as carriers of the mentioned G allele had lower CAPS and BSI scores. On the other hand, the A allele seems to aggravate symptom severity, resulting in higher CAPS and BSI scores. Changes have only been observed in the PTSD lifetime (remitted) group, and this may imply that OXTR modulates the reaction and the recovery from stress.

Our results are similar to what was found previously, namely that carrying the A allele leads to numerous deficits in social functioning, especially in terms of empathy (Gong et al. 2017), attachment (Sippel et al. 2017), lower self-esteem and negative affect in males (Lucht et al. 2009). Considering the definition and clinical presentation of PTSD, these parameters can be
correlated with the symptoms of the subjects used within this research and possibly suggest that they indirectly influence the course of illness. In a sample of 2163 US war veterans, Sippel et al. (2017) found that the A allele of rs53576 was related to PTSD lifetime symptoms especially in conjunction with insecure attachment styles. The actual connection of insecure attachment to responsiveness to social support has been observed before (Ditzen et al. 2008). Furthermore, Chen & Johnson (2012) found that individuals carrying the G allele had lower cortisol levels in stress situations only if they received social support. This effect was not found in an environment without social support, suggesting that this genetic variation impacts the degree in which social support buffers the consequences of stress.

Sociality is important for PTSD recovery in general. A meta-analysis reported that this SNP seemed to be related to interpersonal sociality (Li et al. 2015). Again, G homozygotes seem to be more sociable than A homozygotes in terms of general sociality but not sociality in close relationships. If social support is perceived in the right way and if a person behaves prosocial following trauma, this seems to be at least the “easier” way to recovery (Maercker & Hecker 2016). It seems that the OXTR gene may modulate the social behavior reactions after trauma. Since the research group in the present study mostly involved probands who had or have chronic PTSD, it is plausible that the level of sociality and the perception of social support as individual factors could lead to poorer symptom recovery. This has been proposed in previous studies (Laffaye et al. 2008, Beck et al. 2009).

Earlier studies connected OXTR rs53576 with psychological resources not only in correlation with stress but as impacts on stress response as well (Saphire-Bernstein et al. 2011). In this study, G allele carriers benefited from their allelic state in the way that they reported higher scores for psychological resources such as mastery, self-esteem, optimism and depression. In experimental environments, carriers of the A allele were more sensitive to stress situations and they were less adept to the reaction to it (Rodrigues et al. 2009). It is evident that most studies confirm OXTR’s influence on the reaction to stress and point to the fact that this most probably leads to differences in responses on a social, behavioral and emotional level. Therefore, it may be proposed that OXTR does not necessarily reduce stress, but that it rather influences the reaction to it by shaping sociality, empathy, self-esteem, optimism and other social behaviors.

Although our results may suggest that the G allele of RORA rs8042149 might possibly be associated with higher BSI scores, no significant results were observed with RORA rs8042149, and this could be due to the fact that no detailed analyses regarding symptom clusters have been done. Previous studies have shown that specific symptom clusters seem to be related to genetic variations in this SNP, however these could be symp-toms such as anhedonia, negative affect and dysphoria – rather those related to fear – that have previously been affected by this polymorphism. In previous studies, RORA, specifically rs8042149, has been associated with PTSD following acute (Amstadter et al. 2013) and long term stress exposure (Logue et al. 2010). Studies have shown that RORA mainly influences the persistence of subthreshold and chronically elevated symptoms (Lowe et al. 2015). However, this model seems to be more complex as other factors like comorbid psychiatric disorders and childhood trauma influence the gene expression. Most of the studies consider RORA to be a gene that presents a general risk factor for psychopathology development after distress, rather than an isolated candidate PTSD gene. As previous research has demonstrated, it seems that a dynamic interplay between genetic and environmental factors exists.

Although CNR1 has been proposed to be involved in contextual fear learning and been found associated with affective and anxiety phenotypes, we could not observe any association with any of our phenotypes studied. This argues against a major role for CNR1 rs1049353 in the pathogenesis of or recovery from PTSD.

There are several limitations to this study. The major limitation is the relatively small sample size, which, when divided into three groups becomes even lower. This lack of statistical power may explain why the observed OXTR finding did not withstand Bonferroni correction and why the result for RORA did not pass the threshold defining a nominal significance. Therefore, these findings have to be considered preliminary and replication in larger cohorts is required. This study also did not allow to control for other related or comorbid mental disorders such as depression (Saphire-Bernstein et al. 2011), autism (Wu et al. 2005) or social anxiety (Notzon et al. 2016). Similarly, we did not control for the influence of earlier traumatic events, especially those dating back to childhood, although they have been reported to influence the adult oxytocin (Smearman et al. 2016) as well as cannabinoid systems.

CONCLUSIONS

Our data provide tentative evidence for a role of OXTR genetic variation in PTSD. However, given the limitations of single-gene approaches and that all socio-emotional behaviors are influenced by multiple genes, future research e.g. using genome- and epigenome-wide approaches in larger cohorts should address how these genes may interact with others involved in social and emotional processing.

**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.
Acknowledgements:

We thank all the participants and their families without whose idealistic and enthusiastic support the study would not have been possible. We also would like to thank at Sarajevo: the Association of Women Victims of War and Bakira Hasetic; the Association of Physically Handicapped, Zilko Buljugga, Zoran Budimlija, MD, PhD, Jasminka Krehic, MD, PhD, Elvira Sabinovic, RSN and Subhija Gusic; in Kosovo: Feride Rushiti, MD, Selvije Izeti, MSc, Vjosa Devaja, MD, Melita Kalabić, MD from Kosovo Rehabilitation Center for Trauma Survivors- KCRT; Emirjeta Kumnova, Vepore Shehu from Medica Kosova; Zahrine Podrimagu Komšić from the Association of Political Prisoners, Kadire Tahiraj from the Center for Promotion of Women’s Rights; Arberëre Ulaj, MD, Teuta Haxhiu, MD and Drita Gashi, MD, for their assistance in recruiting and interviewing participants; at Zagreb: Mirna Mavracic, Zoran Bradas, Zrinka Mirkovic and Maja Mezak Herceg for technical assistance in drawing blood and extracting DNA; at Tuzla: the staff of the Department of Transfusion of University Clinical Center of Tuzla, and the staff of the Department of Psychiatry, in particular Emina Hujdur, Medin Omerasiev and Avdo Saksulic, MD for technical support and Maja Brkic and Sandra Zornic for their assistance in data collection; at Würzburg: Carola Gagel for technical assistance with extracting DNA. Thanks are highly deserved by and gratefully extended to Peter Riederer as spiritus rector who brought the consortium together. The study was funded by the DAAD program Stability Pact for South Eastern Europe and supported by the DFG-funded RTG 1253 (speaker Pauli) as well as the DFG-funded CRC-TRR58 (projects C02 Dombschke, Deckert, and Z02 Deckert, Domschke).

Conflict of interest: None to declare.

References

1. American Psychiatric Association: Diagnostic and statistical manual of mental disorders (5th ed.). Washington, DC, 2013
2. Amstadter AB, Sumner JA, Acierno R, Ruggiero KJ, Koenen KC, Kilpatrick D et al.: Support for association of RORA variant and post traumatic stress symptoms in a population-based study of hurricane exposed adults. Molecular psychiatry 2013; 18:1148
3. Beck JG, Grant DM, Clapp JD, Paloy SA: Understanding the interpersonal impact of trauma: Contributions of PTSD and depression. Journal of Anxiety Disorders 2009; 23:443-450
4. Benjet C, Bromet E, Karam EG, Grant DM, Clapp JD, Palyo SA: Understanding the association for facets of neuroticism. Journal of human psychiatria Danubina, 2019; Vol. 31, No. 2, pp 219-226
5. Benjet C, Bromet E, Karam EG, Kessler RC, McLaughlin KA, Ruscio AM et al.: The epidemiology of traumatic event exposure worldwide: Results from the World Mental Health Survey Consortium. Psychological Medicine 2015; 46:1–17
6. Blake DD, Weathers FW, Nagy LM, Kaloupek DG: The development of a Clinician-Administered PTSD Scale. Journal of Traumatic Stress 1995; 8:19189–19192
7. Domes G, Heinrichs M, Glascher J, Buchel C, Braus DF, Herpertz SC: Oxytocin attenuates amygdala responses to emotional faces regardless of valence. Biol. Psychiatry 2007; 62:1187-1190
8. Dzubur Kulenovic A et al.: Molecular Mechanisms of Posttraumatic Stress Disorder (PTSD) as a Basis for Individualized and Personalized Therapy: Rationale, Design and Methods of the South Eastern Europe (SEE)-PTSD Study. Psychiatr Danub 2016; 28:154-163
9. Fani N, Tone EB, Pihler J, Norholm SD, Bradley B, Ressler KJ et al.: Attention bias toward threat is associated with exaggerated fear expression and impaired extinction in PTSD. Psychological Medicine 2012; 42:533-543
10. Furman DJ, Chen MC, Gatlib HH: Variant in oxytocin receptor gene is associated with amygdala volume. Psychoneuroendocrinology 2011; 36:891-897
11. Germain A: Sleep disturbances as the hallmark of PTSD: where are we now? American Journal of Psychiatry 2013; 170:372-382
12. Gottschalk MG & Dombschke K: Novel developments in genetic and epigenetic mechanisms of anxiety. Current Opinion in Psychiatry 2016; 29:32-38
13. Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU: Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. International Journal of Methods in Psychiatric Research 2012; 21:169–184
14. Kim SE, Kim HN, Yun YJ, Heo SG, Cho J, Kwon MJ et al.: Meta-analysis of genome-wide SNP-and pathway-based associations for facets of neuroticism. Journal of human genetics 2017; 62:903
15. Koch SBJ, van Zuiden M, Nawijn L, Frijling JL, Veltman DJ, Off M: Intranasal oxytocin normalizes amygdala functional connectivity in posttraumatic stress disorder. Neuropsychopharmacology 2016; 41:2041–2051
16. Logue MW, Baldwin C, Guffanti G, Melista E, Wolf EJ, Reardon AF et al.: A genome-wide association study of the retinoid-related orphan receptor A and cannabinoid receptor 1 genes. Psychiatria Danubina 2019; Vol. 31, No. 2, pp 219-226
orphan receptor alpha (RORA) gene as a significant risk locus. Molecular psychiatry 2013; 18:937
25. Lowe SR, Meyers JL, Galea S, Aiello AE, Uddin M, Wildman DE et al.: RORA and posttraumatic stress trajectories: main effects and interactions with childhood physical abuse history. Brain and behavior 2015; 5
26. Lucht MJ, Barnow S, Sonnenfeld C, Rosenberger A, Grabe HJ, Schroeder W et al.: Associations between the oxytocin receptor gene (OXTR) and affect, loneliness and intelligence in normal subjects. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33:860-866
27. Maercker A & Hecker T: Broadening perspectives on trauma and recovery: A socio-interpersonal view of PTSD. European Journal of Psychotraumatology 2016; 7:29103
28. Miller MW, Wolf EJ, Logue MW & Baldwin CT: The retinoid-related orphan receptor alpha (RORA) gene and fear-related psychopathology. Journal of affective disorders 2013; 151:702-708
29. Morey RA, Gold AL, LaBar KS, Beall SK, Brown VM, Haswell CC et al.: Amygdala volume changes in post-traumatic stress disorder in a large case-controlled veterans group. Arch Gen Psychiatry 2012; 69:1169-78
30. Neumann ID, Kromer SA, Toschi N, Ebner K: Brain oxytocin inhibits the (re)activity of the hypothalamic-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain region. Regul Pept 2000; 96:31-38
31. Neumeister A, Normandin MD, Pietrzak RH, Piomelli D, Zheng MQ, Gajarz-Anton A et al.: Elevated brain cannabinoid CB1 receptor availability in post-traumatic stress disorder: a positron emission tomography study. Molecular Psychiatry 2013; 18:1034-1040
32. Notzon S, Domshcheke K, Holitschke K, Ziegler C, Arolt V, Pauli P et al.: Attachment style and oxytocin receptor gene variation interact in influencing social anxiety. The World Journal of Biological Psychiatry 2016; 17:76-83
33. Offl M, Frijling JL, Kuhansky LD, Bradley B, Ellenbogen MA, Cardoso C: The role of oxytocin in social bonding, stress regulation and mental health: an update on the moderating effects of context and interindividual differences. Psychoneuroendocrinology 2013; 38:1883-94
34. Onaka T: Neural pathways controlling central and peripheral oxytocin release during stress. Journal of neuroendocrinology 2004; 16:308-312
35. Pietrzak RH, Huang Y, Corsi-Travali S, Zheng MQ, Lin SF, Henry S et al.: Cannabinoid type 1 receptor availability in the amygdala mediates threat processing in trauma survivors. Neuropsychopharmacology 2014; 39:2519-2528
36. Preckel K, Scheele D, Kendrick KM, Maier W, Hurrelmann R: Oxytocin facilitates social approach behavior in women. Frontiers in Behavioral Neuroscience 2014; 8:191
37. Priest E, Bogie M, Ajdukovic D, Franciskovic T, Galeazzi GM, Kucukali A et al.: Mental disorders following war in the Balkans: a study in 5 countries. Archives of General Psychiatry 2010; 67:518-528
38. Rodrigues SM, Saslow LR, Garcia N, John OP, Kelmer D: Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. P Nat Acad Sci USA 2009; 106:21437–21441
39. Sanders G, Freilicher J, Lightman SL: Psychological stress of exposure to uncontrollable noise increases plasma oxytocin in high emotionality women. Psychoneuroendocrinology 1990; 15:47-58
40. Saphire-Bernstein S, Way BM, Kim HS, Sherman DK & Taylor SE: Oxytocin receptor gene (OXTR) is related to psychological resources. Proceedings of the National Academy of Sciences 2011; 108:15118-15122
41. Sippel LM, Han S, Watkins LE, Harpaz-Rotem I, Southwick SM, Krystal JH et al.: Oxytocin receptor gene polymorphisms, attachment, and PTSD: Results from the national health and resilience in veterans study. Journal of psychiatric research 2017; 94:139-147
42. Smaerman EL, Ahml LM, Comely KN, Brody GH, Sales JM, Bradley B et al.: Oxytocin receptor genetic and epigenetic variations: association with child abuse and adult psychiatric symptoms. Child Development 2016; 87:122-134
43. Sripada CH, Phan KL, Labuschagne I, Welsh R, Nathan PJ, Wood AG: Oxytocin enhances resting-state connectivity between amygdala and medial frontal cortex. Int J Neuropsychopharmacol 2012; 16:255-260
44. Sreen J, Dyster-Aas J & Willebrand M: Attentional bias and symptoms of posttraumatic stress disorder one year after burn injury. The Journal of Nervous and Mental Disease 2009; 197:850-855
45. Test H, Kolachana B, Hakimi S, Lemaitre H, Verchinski BA, Mattay VS et al.: A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and hypothalamic-limbic structure and function. Proceedings of the National Academy of Sciences 2010; 107:13936-13941
46. Viviani D, Charlet A, van den Burg E, Robinet C, Hurnn N, Abatis M et al.: Oxytocin selectively gates fear responses through distinct outputs from the central amygdala. Science 2011; 333:104-107
47. Wang J, Qin W, Liu B, Zhou Y, Wang D, Zhang Y et al.: Neural mechanisms of oxytocin receptor gene mediating anxiety-related temperament. Brain Struct Funct 2014; 219:1543-1554
48. Wu S, Jia M, Ruan Y, Liu J, Guo Y, Shuang M et al.: Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. Biological Psychiatry 2005; 58:74-77
49. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. Jama 2013; 310:2191
50. Ziegler C, Dannlowski U, Bräuer D, Stevens S, Laeger I, Wittmann H et al.: Oxytocin receptor gene methylation: converging multilevel evidence for a role in social anxiety. Neuropsychopharmacology 2015; 40:1528