

REGULAR RESEARCH ARTICLE

Monoamine Oxidase A Gene Methylation and Its Role in Posttraumatic Stress Disorder: First Evidence from the South Eastern Europe (SEE)-PTSD Study

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Significance Statement

The present study for the first time suggests increased monoamine oxidase A (MAOA) gene DNA methylation as a possible epigenetic marker/correlate of disease status and severity in a large sample of male patients with current posttraumatic stress disorder (PTSD) recruited from post-war southeastern Europe regions in Bosnia-Herzegovina, Croatia, and the Republic of Kosovo. Interestingly, this applied particularly to PTSD symptom clusters related to reexperiencing of trauma (symptom cluster B) and hyperarousal (symptom cluster D), which have been suggested to be driven primarily by the noradrenergic system. As MAOA hypermethylation has been proposed to confer decreased MAOA transcription and consequently increased noradrenergic signaling, the present data could inform future personalized treatment approaches with increased MAOA methylation in male PTSD patients, potentially indicating a beneficial use of antiadrenergic agents particularly regarding reexperiencing and hyperarousal symptoms.

Abstract

Background: Posttraumatic stress disorder is characterized by an overactive noradrenergic system conferring core posttraumatic stress disorder symptoms such as hyperarousal and reexperiencing. Monoamine oxidase A is one of the key enzymes mediating the turnover of noradrenaline. Here, DNA methylation of the monoamine oxidase A gene exon1/intron1 region was investigated for the first time regarding its role in posttraumatic stress disorder risk and severity.

Methods: Monoamine oxidase A methylation was analyzed via direct sequencing of sodium bisulfite-treated DNA extracted from blood cells in a total sample of N=652 (441 male) patients with current posttraumatic stress disorder, patients with remitted posttraumatic stress disorder, and healthy probands (comparison group) recruited at 5 centers in Bosnia-Herzegovina, Croatia, and the Republic of Kosovo. Posttraumatic stress disorder severity was measured by means of the Clinician-Administered Posttraumatic Stress Disorder Scale and its respective subscores representing distinct symptom clusters.

Results: In the male, but not the female sample, patients with current posttraumatic stress disorder displayed hypermethylation of 3 CpGs (CpG3=43656362; CpG12=43656514; CpG13=43656553, GRCh38.p2 Assembly) as compared with remitted Posttraumatic Stress Disorder patients and healthy probands. Symptom severity (Clinician-Administered Posttraumatic Stress Disorder Scale scores) in male patients with current posttraumatic stress disorder significantly correlated with monoamine oxidase A methylation. This applied particularly to symptom clusters related to reexperiencing of trauma (cluster B) and hyperarousal (cluster D).

Conclusions: The present findings suggest monoamine oxidase A gene hypermethylation, potentially resulting in enhanced noradrenergic signalling, as a disease status and severity marker of current posttraumatic stress disorder in males. If replicated, monoamine oxidase A hypermethylation might serve as a surrogate marker of a hyperadrenergic subtype of posttraumatic stress disorder guiding personalized treatment decisions on the use of antiadrenergic agents.

Keywords: MAOA, epigenetics, DNA methylation, PTSD, CAPS

Introduction

Posttraumatic stress disorder (PTSD) is a severe and disabling trauma- and stress-related disorder, which develops after experiencing or witnessing traumatic events including rape, assault, and combat. The disorder is characterized by 3 distinct symptom clusters comprising (1) the reexperiencing of symptoms in so-called flash-backs, intrusions, or nightmares, (2) avoidance behavior related to the distinct traumatic situation including emotional and social withdrawal, as well as (3) states of physical hyperarousal including insomnia, impaired concentration, and an increased startle response (American Psychiatric Association, 2013). While the lifetime prevalence of PTSD in the US-American population is estimated to be 6.8% (Kessler et al., 2005a), in populations from traumatized regions such as those involved in the war in Bosnia-Herzegovina from 1992 to 1995 or in the war in Kosovo from 1998 to 1999, point prevalence rates of PTSD are estimated at 35% or 18%, respectively (Priebe et al., 2010). Selective serotonin reuptake inhibitors (SSRIs) are considered first-line pharmacological treatment options for adult PTSD with, however, nonresponse rates of up to 40% (Stein et al., 2006).

The pathogenesis of PTSD is complex with a heritability of 30% and a considerable influence of environmental factors (True et al., 1993; Stein et al., 2002). On a neurotransmitter level, besides

the serotonin system and the hypothalamus-pituitary-adrenal-axis (HPA)-related stress system, locus coeruleus (LC)-associated noradrenergic dysfunction underlying hyperadrenergic PTSD symptoms such as excessive arousal, reexperiencing, anxiety, tachycardia, increased diastolic blood pressure, and diaphoresis have been suggested to play a crucial role in the pathogenesis of PTSD (see O'Donnell et al., 2004; Krystal and Neumeister, 2009; Berridge et al., 2012; Hendrickson and Raskind, 2016). For instance, combat-related auditory stimuli resulted in a significant activation of the sympathetic nervous system as indicated by increased plasma noradrenaline levels and higher heart rate as well as systolic blood pressure in PTSD patients (Blanchard et al., 1982). Also, noradrenaline concentrations were found to be increased in the cerebrospinal fluid of PTSD patients and correlated with total symptom ratings on the Clinician-Administered PTSD Scale (CAPS) (Geraciotti et al., 2001). The LC-associated noradrenergic system has furthermore been implicated in the overconsolidation of emotional material, particularly fear memories after traumatic exposure (O'Carroll et al., 1999; Southwick et al., 2002). Pharmacologically, challenge studies with yohimbine, an α 2-adrenoautoreceptor antagonist increasing brain norepinephrine turnover, in humans (e.g., Morgan et al., 1995; Southwick et al., 1997, 1999) and in animal models (e.g., Arnsten et al., 1999;

Gazarini et al., 2014) further support the importance of noradrenergic transmission in PTSD. Reciprocally, α_2 -receptor agonists such as clonidine and guanfacine decreasing noradrenaline transmission have been suggested to be efficacious in treatment-resistant PTSD patients, with predominant effects on hyperarousal and agitation (Kinzie and Leung, 1989; Connor et al., 2013; Belkin and Schwartz, 2015). Furthermore, the α_1 -adrenoreceptor antagonist prazosin, reducing noradrenaline effects at brain α_1 -adrenoreceptors, has been demonstrated to decrease overall PTSD symptoms, particularly distressing dreams (Ipser and Stein, 2012; De Berardis et al., 2015; Khachatryan et al., 2016). Propranolol, a β -adrenoreceptor antagonist, has received considerable attention for its therapeutic potential in PTSD when paired with behavioral therapy soon after trauma, possibly by reducing noradrenergic hyperarousal and by disrupting reconsolidation of strong fear memories (see Giustino et al., 2016).

Monoamine oxidase A (MAO-A) is one of the key enzymes mediating the turnover of biogenic amines, including noradrenaline and adrenaline, and is thus to be considered a major candidate molecule in PTSD. MAO-A is encoded by the monoamine oxidase A (MAOA) gene located on chromosome Xp11.4-p11.3. Given the inextricable interplay of genetic and environmental factors in PTSD, epigenetic processes constituting temporally dynamic, stress-responsive, and functionally highly relevant biochemical mechanisms seem to be of particular pathogenetic importance in the context of PTSD (cf. Schuebel et al., 2016; Zannas and Chrousos, 2017). While most presently available epigenetic studies in PTSD aimed at investigating epigenetic variation in genes involved in the HPA-axis or the serotonergic system (cf. Zannas et al., 2015), no data on the role of epigenetic modification of the MAOA gene in PTSD risk have been published so far.

Against the background of (1) an overactive noradrenergic system conferring key symptoms of PTSD, (2) a pivotal role of MAO-A activity in governing noradrenaline turnover, and (3) epigenetic mechanisms such as DNA methylation of cytosine bases in cytosine-guanine dinucleotides (CpGs) impacting gene transcription in interaction with environmental factors, we set out to analyze the role of MAOA DNA methylation in the pathogenesis of PTSD. Given robust evidence for an overactive noradrenergic system along with increased sympathetic activation in PTSD as reviewed above as well as for DNA methylation in gene promoter regions to result in decreased gene transcription (Suzuki and Bird, 2008; Brenet et al., 2011), we expected to discern increased MAOA gene exonI/intronI methylation, decreasing MAO-A activity and thereby increasing noradrenaline levels, to be associated with PTSD status and severity in patients with current PTSD when compared with healthy probands. Furthermore, we hypothesized that MAOA DNA methylation levels in remitted PTSD patients would be comparable with those in healthy probands.

Methods

Sample

Participants with current PTSD, remitted PTSD, and healthy probands (total N = 747, 509 male) were recruited in the context of a multicentre study at 5 centers in Bosnia-Herzegovina (Sarajevo, Mostar, Tuzla), Croatia (Zagreb), and the Republic of Kosovo (Pristina). All recruited participants were at age 16 or older when traumatized in the context of the wars in the 3 countries between 1991 and 1999. Current and lifetime PTSD diagnosis or its absence, respectively, was assessed using the PTSD section of the M.I.N.I. (M.I.N.I. 5.0.0, DSM-IV; Dzubur-Kulenovic et al., 2008; Priebe et al., 2010). Exclusion criteria were: age older than 65;

mental retardation (MMSE <25); disorders related to organic or brain trauma; diagnosis of epilepsy, psychotic disorders, alcohol or drug abuse; or oncological disorders based on clinical records and examination by experienced psychiatrists. Medication known to affect methylation status, for example, valproic acid, as well as 1st- and 2nd-degree relation to an already recruited person were further exclusion criteria. Detailed information regarding recruitment, diagnostic assessment, inclusion and exclusion criteria, as well as sample size and gender distribution is given in greater detail elsewhere (Dzubur-Kulenovic et al., 2016).

PTSD severity was measured by means of the CAPS, a structured interview assessing 17 PTSD symptoms (Blake et al., 1995). In addition, CAPS subscores representing symptom clusters B (persistent reexperiencing of trauma), C (persistent avoidance of stimuli associated with the trauma), and D (persistent symptoms of increased arousal) were used to differentiate the severity of distinct PTSD symptoms. Smoking status was ascertained for all probands. Ethical votes were obtained at all clinical centres between 2011 and 2013. Participants gave written informed consent according to the principles of the declaration of Helsinki.

Blood Sample Collection and DNA Extraction

EDTA blood was collected from 716 participants (487 male) at the respective centers and stored at -80°C until DNA extraction. DNA was isolated using a standard procedure (FlexiGene DNA Kit, QIAGEN) and stored at -80°C until further processing.

DNA Methylation Analysis and MAOA VNTR Genotyping

Analysis of MAOA DNA methylation as well as quality control of obtained methylation data were performed as described previously (Ziegler et al., 2015, 2016). Briefly, direct sequencing of bisulfite-converted DNA was used to determine DNA methylation levels at 13 CpG sites located in an amplicon spanning exon I and intron I of the MAOA gene (chromosome X, GRCh38.p2 Primary Assembly, NCBI Reference Sequence: NC_000023.11, 43656260–43656613). CpGs were numbered in analogy to previous studies on MAOA methylation in neuropsychiatric phenotypes: CpG1=43656316; CpG2=43656327; CpG3=43656362; CpG4=43656368; CpG5=43656370; CpG6=43656383; CpG7=43656386; CpG8=43656392; CpG9=43656398; CpG10=43656427; CpG11=43656432; CpG12=43656514; CpG13=43656553 (Domschke et al., 2012, 2015; Ziegler et al., 2016). Due to partly insufficient quality of DNA samples, MAOA DNA methylation results were available for statistical analyses in a final sample of 652 (441 male) participants (for sample description, see Table 1).

All participants were genotyped for the MAOA VNTR according to published protocols (Deckert et al., 1999; Reif et al., 2014; Ziegler et al., 2016). All participants were grouped into low expression (females: 2/2, 2/4, 3/3, 3/4, 3/5, 3.5, 3.5/3.5, and 3.5/4; males: 2 and 3) and high expression (females: 4/4, 4/5, 5/5; males: 4 and 5) MAOA VNTR genotype/allele groups as published elsewhere (Domschke et al., 2012, 2015; Reif et al., 2014; Ziegler et al., 2016).

Statistical Analysis

All statistical tests were performed using SPSS (version 23.0, IBM Corp). Possible categorical differences in average MAOA methylation levels between the 3 groups (current PTSD patients, remitted PTSD patients, and a comparison group of healthy probands) were analyzed by ANCOVAs with age and smoking status as

Table 1. Sample Characteristics

Characteristics	Current PTSD (N=195)	Remitted PTSD (N=136)	Healthy probands (N=321)
Age (mean±SD)	49.73 ± 6.78	49.97 ± 8.16	48.63 ± 8.62
Sex (males vs. females)	140 vs. 55	88 vs. 48	213 vs. 108
Smoking status (Smokers vs. non-smokers)	109 vs. 85 ^a	58 vs. 78	140 vs. 181
MAOA VNTR grouped 'low expression' vs. 'high expression'	72 vs. 121 ^b	51 vs. 85	135 vs. 180 ^d
CAPS score (mean±SD)	79.02 ± 21.37	66.83 ± 17.99 ^c	n.a.
Medication (yes vs. no)	151 vs. 44	71 vs. 65	33 vs. 288

Sample characteristics are shown for patients with a current PTSD diagnosis, remitted PTSD patients, and healthy probands (comparison group). MAOA VNTR genotypes were grouped into a low expression and a high expression group (for details, see Methods). Medication comprised psychotropic medication (SSRIs, SNRIs, NaSSA, Z-drugs, atypical neuroleptics, benzodiazepines). CAPS, Clinician-Administered PTSD Scale (Blake et al., 1995); n.a., not applicable. Statistics are reported in the Results.

^aOne missing value.

^bTwo missing values.

^cThree missing values.

^dSix missing values.

covariates (Philibert et al., 2008, 2010). Additionally, possible differences in DNA methylation at single CpG sites between 3 groups (current PTSD patients, remitted PTSD patients, and comparison group of healthy probands) were tested by means of MANCOVA with age and smoking status as covariates given that inter-correlations were observed amongst most of the dependent variables (CpGs 1 to 13; see Results).

Posthoc tests (Bonferroni correction, as implemented in SPSS) were performed to elucidate individual mean difference comparisons in average MAOA methylation level as well as in DNA methylation level at single CpG sites across all 3 groups. For these analyses, adjusted *P* values are reported.

All associations between dimensional measures (overall symptom severity, severity of distinct symptom clusters, MAOA methylation) were tested by means of partial correlation analyses controlled for age and smoking status (for details, see Results). Associations between categorical variables (e.g., differences in gender distribution, smoking status, grouped MAOA VNTR genotypes, and medication status between patients with current PTSD, remitted PTSD patients, and comparison group) were analyzed by means of χ^2 tests. The significance level for those analyses was set at $P < .05$.

Results

Sample Characteristics

All sample characteristics are summarized in Table 1. Patients with current PTSD, remitted PTSD patients, and healthy probands in the comparison group did not differ regarding age ($F = 1.848$, $P = .158$) and gender distribution ($\chi^2 = 2.315$, $P = .314$), but for smoking status ($\chi^2 = 9.073$, $P = .011$), with a higher number of smokers in the current PTSD group compared with remitted patients and healthy probands. Furthermore, patients with current PTSD, remitted PTSD patients, and healthy probands did not differ in MAOA VNTR genotype group distribution ($\chi^2 = 2.007$, $P = .367$). As expected, CAPS total scores were significantly higher in patients with current PTSD diagnosis than in remitted patients ($F = 29.173$, $P < .001$). Psychotropic medication (comprising SSRIs, SNRIs, NaSSA, Z-drugs, atypical neuroleptics, benzodiazepines) was more frequently used in both patient groups (current and remitted) compared with the control group ($\chi^2 = 242.105$, $P < .001$).

MAOA Methylation and PTSD Diagnosis

The cohort of 652 (441 male) participants with available DNA methylation data was stratified into a male and a female subsample given the X-chromosomal location of the MAOA gene entailing hemizyosity in male probands. Both subsamples were thus investigated separately in all further analyses.

In the male subsample, age was significantly associated with average MAOA methylation, represented by a significant positive correlation between age and average MAOA methylation ($r = 0.100$, $P = .037$). Smoking status did not influence average MAOA methylation in the entire male subgroup ($F = 1.581$, $P = .209$), in healthy probands ($F = 0.036$, $P = .849$), or in patients with a current PTSD diagnosis ($F = 0.132$, $P = .717$), but in remitted PTSD patients ($F = 9.239$, $P = .003$). Here, smoking was associated with a significantly decreased average MAOA methylation (smokers: mean ± SE: 0.1723 ± 0.0105; nonsmokers: mean ± SE: 0.2100 ± 0.0074) (cf. Philibert et al., 2008, 2010). MAOA VNTR genotype did not influence average MAOA methylation ($F = 0.264$, $P = .607$). No significant influence of psychotropic medication on average MAOA methylation was discerned in the entire male subsample ($F = 1.332$, $P = .249$), the control group ($F = 1.056$, $P = .305$), or in patients with current ($F = 0.246$, $P = .620$) or remitted PTSD ($F = 1.229$, $P = .271$). Based on these confounder analyses, all subsequent analyses were conducted with age and smoking status as covariates.

Furthermore, pairwise correlations between individual DNA methylation levels at all 13 CpG sites showed an inter-correlation that ranged between $r = 0.097$ and $r = 0.856$ and reached statistical significance ($P < .05$) for correlations between CpG1 and CpGs 2-10, 12, and 13; CpG2 and CpGs 3-10, 12, and 13; CpG 3 and CpGs 4-9, 12, and 13; CpG4 and CpGs 5-13; CpG5 and CpGs 6-13; CpG6 and CpGs 7-13; CpG7 and CpGs 8-13; CpG8 and CpGs 9-13; CpG9 and CpGs 10-13; CpG10 and CpGs 11-13; CpG11 and CpG 13; as well as CpG12 and CpG13.

On a categorical level, ANCOVA revealed no significant difference in average MAOA methylation levels between current PTSD patients, remitted PTSD patients, and healthy probands ($F = 1.546$, $P = .214$). However, patients with current PTSD exhibited descriptively higher average MAOA methylation levels compared with healthy controls and with remitted PTSD patients (Table 2).

Table 2. DNA Methylation Levels for Average MAOA Methylation as Well as for Single CpG Sites in the Male Cohort

	Current PTSD patients (N=140)	Remitted PTSD patients (N=88)	Healthy probands (N=213)	Statistics	
	Mean (SE)	Mean (SE)	Mean (SE)	F-value	P-value
Average methylation	0.2039 (0.0038)	0.1952 (0.0061)	0.1949 (0.0037)	1.546	0.214
CpG1	0.1406 (0.0102)	0.1171 (0.0129)	0.1141 (0.0072)	1.878	0.154
CpG2	0.1140 (0.0070)	0.0923 (0.0070)	0.0963 (0.0046)	2.606	0.075
CpG3	0.3059 (0.0132)	0.2531 (0.0139)	0.2708 (0.0098)	3.907	0.021
CpG4	0.0998 (0.0057)	0.1035 (0.0073)	0.1061 (0.0043)	0.549	0.578
CpG5	0.0949 (0.0046)	0.1004 (0.0071)	0.0939 (0.0041)	0.329	0.720
CpG6	0.0697 (0.0038)	0.0814 (0.0059)	0.0733 (0.0032)	1.7079	0.341
CpG7	0.0955 (0.0039)	0.1122 (0.0067)	0.1009 (0.0037)	2.073	0.127
CpG8	0.0472 (0.0029)	0.0578 (0.0044)	0.0509 (0.0024)	1.956	0.143
CpG9	0.1164 (0.0041)	0.1277 (0.0070)	0.1181 (0.0036)	1.040	0.354
CpG10	0.1061 (0.0045)	0.1270 (0.0065)	0.1188 (0.0043)	2.858	0.059
CpG11	0.1146 (0.0052)	0.1324 (0.0079)	0.1146 (0.0045)	2.430	0.089
CpG12	0.9052 (0.0091)	0.8357 (0.0154)	0.8634 (0.0096)	8.309	<0.001
CpG13	0.4378 (0.0126)	0.3953 (0.0165)	0.4102 (0.0119)	3.464	0.032

MAOA DNA methylation levels are shown for average MAOA methylation as well as for single CpG sites for current PTSD patients, remitted PTSD patients, and healthy probands (comparison group), respectively. F- and P values from (M)ANCOVAs controlled for age and smoking status. Posthoc tests (Bonferroni) were performed; P values from these tests are reported in the results section.

For single CpG sites, a statistically significant MANCOVA effect was obtained (Pillai's trace=0.119, $F=2.009$, $P=.002$). Separate univariate ANCOVAs showed significant DNA methylation differences at 3 of 13 CpG sites (Table 2) between the 3 tested groups (patients with current PTSD, remitted PTSD patients, and healthy probands).

Posthoc tests (Bonferroni correction) revealed trend-wise significant ($P_{\text{adjusted}} < .1$) to significant ($P_{\text{adjusted}} < .05$) hypermethylation in current PTSD patients compared with healthy probands at CpG3 ($P_{\text{adjusted}} = .089$), CpG12 ($P_{\text{adjusted}} = .011$), and CpG13 ($P_{\text{adjusted}} = .086$) as well as at CpG3 ($P_{\text{adjusted}} = .029$), CpG12 ($P_{\text{adjusted}} < .001$), and CpG13 ($P_{\text{adjusted}} = .058$) compared with remitted PTSD patients (Figure 1).

In the female subsample, confounder analyses revealed a significant influence of age on average MAOA methylation ($r=0.168$; $P=.015$) as well as a marginally significant difference in average MAOA methylation between smokers and nonsmokers ($F=3.689$; $P=.056$). In contrast to the male subgroup, smoking status influenced average MAOA methylation in patients with current PTSD ($F=6.610$, $P=.013$) with increased average MAOA methylation in smokers (mean \pm SE: 0.4835 ± 0.0079) compared with nonsmokers (mean \pm SE: 0.4510 ± 0.0101), but not in remitted patients ($F=0.063$, $P=.803$) or in healthy probands ($F=0.988$, $P=.323$). MAOA VNTR genotype did not influence average MAOA methylation ($F=0.615$, $P=.434$). Psychotropic medication did not influence average MAOA methylation in the entire female subsample ($F=1.019$, $P=.314$), healthy probands ($F=0.634$, $P=.428$), or in remitted patients ($F=0.226$, $P=.637$). However, within the current PTSD group, medicated patients exhibited increased average MAOA methylation (mean \pm SE: 0.4838 ± 0.0076) compared with nonmedicated patients (mean \pm SE: 0.4359 ± 0.0074 , $F=13.702$, $P=.001$). Based on these *a priori* confounder analyses, statistical tests were controlled for age, smoking status, and medication in the female subgroup.

In addition, pairwise correlations between individual DNA methylation levels at all 13 CpG sites showed an inter-correlation ranging between $r=-0.183$ and $r=0.865$ reaching statistical significance ($P < .05$) for correlations between CpG1 and CpGs 2-10, and 12; CpG2 and CpGs 3-10, and 12; CpG 3 and CpGs 4-13;

CpG4 and CpGs 5-12; CpG5 and CpGs 6-10, and 12; CpG6 and CpGs 7-10, and 12; CpG7 and CpGs 8-10, and 12; CpG8 and CpGs 9, 10, and 12; CpG9 and CpGs 10 and 12; CpG10 and CpG12; as well as CpG12 and CpG13.

On a categorical level, analyses revealed no significant differences in average MAOA methylation levels or methylation at any individual CpG site between the 3 tested groups (all $P > .05$). Thus, all following analyses were restricted to the male subsample.

MAOA Methylation and PTSD Symptom Severity

On a dimensional level, PTSD symptom severity as represented by total CAPS scores was positively correlated with average MAOA methylation ($r=0.206$, $P=.016$; Figure 1B) in male patients with current PTSD, but not in remitted patients ($r=0.182$, $P=.100$). For single CpGs, this association remained significant for CpGs 3 ($r=0.186$, $P=.033$), 12 ($r=0.306$, $P < .001$), and 13 ($r=0.277$, $P=.001$) in male patients with a current PTSD diagnosis. Again, no significant correlation was found in remitted patients (all $P > .05$), except for CpG3 ($r=0.221$; $P=.046$).

When considering CAPS subscores representing symptom clusters B (persistent reexperiencing of trauma), C (persistent avoidance of stimuli associated with the trauma), and D (persistent symptoms of increased arousal) and applying an exploratory approach, average MAOA methylation was significantly associated with cluster B scores ($r=0.279$, $P=.001$) and cluster D scores ($r=0.226$, $P=.008$), but not with cluster C scores ($r=0.071$, $P=.408$) in current PTSD patients (Figure 2). For single CpG sites, methylation at CpGs 3, 6, 8, 12, and 13 in patients with current PTSD was significantly associated with the cluster B score ($r=0.179 - 0.303$, $P < .001 - .040$); methylation at CpGs 12 and 13 with the cluster C score ($r=0.188 - 0.197$, $P=.023 - .031$), and methylation at CpGs 3, 12, and 13 with the cluster D score ($r=0.232 - 0.337$, $P < .001 - .008$). No association of average MAOA methylation and the different cluster scores could be discerned in remitted patients (all $P > .05$). For single CpG sites, however, CpG3 methylation significantly correlated with the symptom cluster D score ($r=0.237$, $P=.032$), while methylation at CpG6 was significantly associated with the symptom cluster B score ($r=0.258$, $P=.019$) in patients with remitted PTSD.

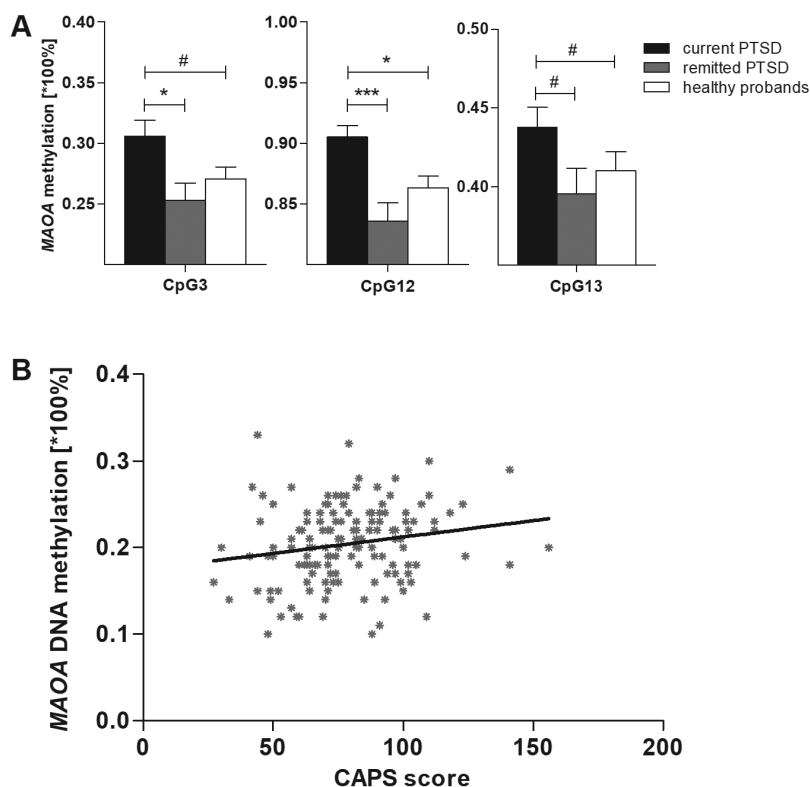


Figure 1. Association of MAOA methylation with current PTSD in male patients. (A) Association of monoamine oxidase A (MAOA) methylation at CpGs 3, 12, and 13 with current posttraumatic stress disorder (PTSD) in male patients; $^{\#}P_{\text{adjusted}} < .1$, $^*P_{\text{adjusted}} < .05$, $^{**}P_{\text{adjusted}} < .01$, $^{***}P_{\text{adjusted}} < .001$ (adjusted *P* values from posthoc comparisons [Bonferroni] are shown; for details see results section). (B) Scatter plot depicting the correlation of average MAOA methylation with PTSD severity in male patients with current PTSD as measured by the Clinician-Administered PTSD Scale (CAPS; Blake et al. (1995), $r = 0.206$, $P = .016$).

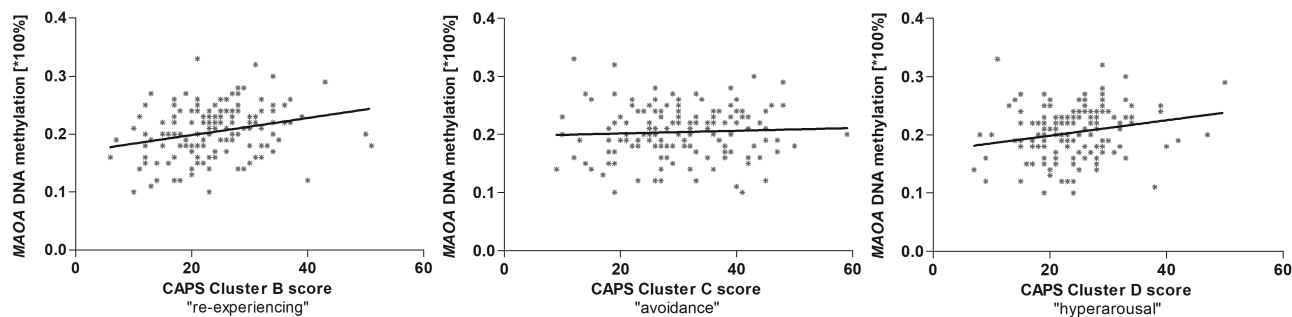


Figure 2. Association of MAOA methylation with severity of CAPS symptom clusters. Scatter plots depicting the correlation of average MAOA methylation with severity of different symptom clusters in male patients with current PTSD as measured by the Clinician-Administered PTSD Scale (CAPS; Blake et al., 1995), CAPS Cluster B score: $r = 0.279$, $P = .001$ (left); CAPS Cluster C score: $r = 0.071$, $P = .408$ (middle); CAPS Cluster D score: $r = 0.226$, $P = .008$ (right).

Discussion

The present multicenter study for the first time suggests hypermethylation of 3 CpGs (CpG3 = 43,656,362; CpG12 = 43,656,514; CpG13 = 43,656,553, GRCh38.p2 Primary Assembly) in the MAOA gene exonI/intronI region in male patients with current PTSD compared with remitted male PTSD patients and healthy male probands. This categorical finding was corroborated on a dimensional level, where PTSD symptom severity in male patients with a current PTSD diagnosis significantly correlated with MAOA methylation, that is, the higher the PTSD severity, the higher the MAOA methylation at particularly CpG sites 3, 12, and 13.

Given that increased methylation of the MAOA gene promoter has been shown to confer decreased gene expression in a functional *in vitro* assay (Checknita et al., 2015) and to correlate with diminished brain MAO-A enzyme activity in a positron

emission tomography study using [(11)C]clorgyline (Shumay et al., 2012), MAOA gene hypermethylation might indeed underlie an increased availability of catecholamines in PTSD patients conferred by a decreased monoaminergic turnover. The identified MAOA hypermethylation in male patients with current, but not remitted PTSD thus supports the hypothesis of a dysregulated noradrenergic signalling, that is, elevated sympathetic activation driven by an enhanced noradrenergic tonus, to constitute a core biological mechanism/correlate of acute PTSD (see Introduction; for review, see Southwick et al., 1997; Krystal and Neumeister, 2009; Hendrickson and Raskind, 2016). At first sight at odds with this notion, antidepressants such as SSRIs and SNRIs or monoamine oxidase inhibitors increasing serotonin and/or noradrenalin levels by either blocking monoamine presynaptic reuptake or inhibiting their degradation are successfully used to treat PTSD (e.g., Lee et al., 2016). A theory

potentially reconciling these seemingly paradoxical observations suggests that altered functioning and reactivity of noradrenergic neurons may be particularly involved in hyperarousal and reexperiencing symptoms of PTSD (O'Donnell et al., 2004). Thus, different pharmacological approaches might be applicable to different clinical phenotypes of PTSD described as (1) fear-based/hyperadrenergic, (2) dysphoric/anhedonic, (3) externalizing, or (4) dissociative phenotypes (Friedman and Bernardy, 2017). Antiadrenergic medication is predicted to be efficacious in the treatment of the fear-based/hyperadrenergic phenotype, while dysphoric/anhedonic patients might rather profit from classical antidepressant treatment augmenting serotonin and/or noradrenaline. This point of view is supported by the present results showing a significant association between MAOA gene hypermethylation and increased scores on distinct CAPS subclusters in male patients with current PTSD, that is, with cluster B (persistent reexperiencing of trauma) and cluster D (persistent symptoms of increased arousal), but not with cluster C (persistent avoidance of stimuli associated with the trauma). Thus, the present findings could contribute to a more individualized treatment of PTSD, where MAOA methylation patterns, serving as a marker of PTSD subphenotypes primarily defined by adrenergic tonus, might guide pharmacological treatment decisions in a precision medicine approach hoped to reduce nonresponse rates in PTSD. However, given that the functionality of MAOA hypermethylation in the presently implicated region is not fully elucidated yet, interpretation and clinical deductions have to be considered with caution. This is particularly true, since DNA hypermethylation in PTSD might not be MAOA gene-specific as suggested by a recently published epigenome-wide association study reporting the majority (84.5%) of 5600 differentially methylated CpG islands representing 2800 genes to be hypermethylated in male PTSD patients compared with veterans without PTSD diagnosis (Hammamieh et al., 2017).

The presently observed male-specific association of MAOA hypermethylation, assumed to confer decreased MAO-A activity, with PTSD is in line with association of less active MAOA gene variation with aggressive and impulsive behavior in males (Caspi et al., 2002; Kim-Cohen et al., 2006; Reif et al., 2007), which might correspond with the fact that presentation of PTSD symptoms in males is more often characterized by features of irritability, impulsiveness, and other “externalizing symptoms” (substance use and aggressive behavior) than in females (Kessler et al., 2005b). In anxiety and affective disorder phenotypes, however, female-specific association findings of the more active MAOA gene alleles have been reported (e.g., Deckert et al., 1999; Domschke et al., 2008; Reif et al., 2012, 2014; Ziegler et al., 2016; for review, see Howe et al., 2016). This sexually dimorphic phenomenon adds evidence to the notion of the MAOA gene functioning as a “plasticity” rather than a “risk gene” crucially dependent on sex as well as environmental constellations and clinical phenotypes (cf. Holz et al., 2016; also see Belsky et al., 2009). Future studies might thus want to further elucidate the role of MAOA gene activity in shaping sex differences in the regulation of the noradrenergic stress-response and arousal system in PTSD (cf. Bangasser et al., 2016).

The present results must be interpreted in the light of some limitations: due to the X-chromosomal location of the investigated MAOA gene, analyses were carried out separately for males and females entailing a reduction of sample size and consequently statistical power particularly in the female cohort, which might in part explain the absence of a statistically significant categorical association of MAOA methylation with PTSD diagnosis in females. Along these lines, it must be noted that

in general prevalence rates of PTSD are higher in females than in males and that female gender has emerged as a risk factor for PTSD especially in the field of civil traumatization (Bisson and Shepherd, 1995; Breslau et al., 1998; Momartin et al., 2003). However, studies explicitly involving Bosnian war survivors reported that gender did not influence PTSD rates (Mollica et al., 1999; Momartin et al., 2004; Thulesius and Hakansson, 1999). Possibly reflecting these particularly war-related epidemiological numbers and given special attendance of mental health services to war veterans at the clinical centers in Bosnia-Herzegovina, Croatia, and Kosovo, the present sample indeed comprised more male patients with a current PTSD diagnosis (71.79%) as well as more male remitted patients (64.71%) than females. Still, given that the present sample has not been recruited as a population-representative cohort, the male:female ratio in the present study might not correctly represent the actual rates of PTSD in males and females in the traumatized regions. Furthermore, antidepressant medication should be considered as a potential confounder influencing DNA methylation patterns as, for example, shown in an animal model of depression (Melas et al., 2012). In the presently studied cohort, 91.4% of patients with current PTSD, 63.6% of patients with remitted PTSD as well as some (11.7%) healthy probands without a clinical PTSD diagnosis were medicated with centrally acting agents (Table 1). However, given that no significant influence of medication on MAOA methylation could be discerned in the male composite sample or in the male healthy control sample, a major influence of medication on the present results can be excluded. Furthermore, comorbidity with neuropsychiatric disorders other than those mentioned in the methods section as exclusion criteria was not systematically documented and controlled for, which does not allow for conclusions regarding the specificity of the present findings to PTSD, particularly given previous association findings of MAOA hypomethylation with panic disorder (Domschke et al., 2012; Ziegler et al., 2016), depression (Melas et al., 2013; Melas and Forsell, 2015), nicotine and alcohol abuse (Philibert et al., 2008), or MAOA hypermethylation, respectively, with schizophrenia (Chen et al., 2012), antisocial personality disorder (Checknita et al., 2015), and borderline personality disorder (Dammann et al., 2011). Also, there is evidence for increased norepinephrine levels, as in the present context potentially conferred by MAOA hypermethylation, to be found in PTSD patients without comorbid depression only (Yehuda et al., 1998). Limitations of studying peripheral tissue DNA methylation comprise cell-type heterogeneity potentially confounding DNA methylation measures in blood and, with regard to neuropsychiatric phenotypes, the generally questionable validity of peripheral tissue as a brain biomarker (Bakulski et al., 2016). However, several lines of evidence such as positron emission tomography studies reporting peripheral methylation in leucocytes to correlate with the respective brain levels of the molecule of interest (e.g., Shumay et al., 2012; Wang et al., 2012) point to a certain comparability of blood methylation patterns with central processes and suggest peripheral tissues as potential “windows to the brain,” that is, as surrogates or proxies of central processes (cf. Gladkevich et al., 2004).

Future studies on patho(epi)genetic mechanisms of PTSD should include additional genetic as well as epigenetic factors determining monoaminergic system activity (see Domschke, 2012; Bandelow et al., 2016) and consider their crosstalk with the HPA-related (Klengel et al., 2013; Zannas and Chrousos, 2017), serotonergic and neuropeptide Y systems (cf. Krystal and Neumeister, 2009) in sufficiently powered samples to allow for a comprehensive evaluation of (epi)genetically determined neurotransmitter dysfunction in shaping risk, severity, and course of

PTSD. Longitudinal studies are urgently warranted to clarify the nature of the presently identified association, that is, whether the observed MAOA hypermethylation is a causal or a compensatory, yet insufficient mechanism (cf. Gershon and High, 2015), and to elucidate the potentially moderating influence of negative environmental factors apart from trauma and/or positive environment such as perceived social support after traumatization or coping mechanisms like general self-efficacy on the (epi)G × E driven PTSD risk (Cluver et al., 2009; Schiele et al., 2016).

In conclusion, the present study suggests MAOA hypermethylation, possibly conferring increased noradrenergic signalling, as a disease status/severity marker of PTSD in male patients. After robust replication in preferably longitudinal studies, MAOA hypermethylation might serve as an epigenetic marker within the complex risk factor constellation of PTSD. In a personalized pharmacotherapeutic approach, increased MAOA methylation in male PTSD patients might indicate the beneficial use of anti-adrenergic agents buffering increased noradrenergic signalling conferred by MAOA hypermethylation.

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Statement of interest

None.

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