Original Investigation

Interaction of the *ADRB2* Gene Polymorphism With Childhood Trauma in Predicting Adult Symptoms of Posttraumatic Stress Disorder

Israel Liberzon, MD; Anthony P. King, PhD; Kerry J. Ressler, MD, PhD; Lynn M. Almli, PhD; Peng Zhang, PhD; Sean T. Ma, PhD; Gregory H. Cohen, MPH; Marijo B. Tamburrino, MD; Joseph R. Calabrese, MD; Sandro Galea, MD, MPH

IMPORTANCE Posttraumatic stress disorder (PTSD), while highly prevalent (7.6% over a lifetime), develops only in a subset of trauma-exposed individuals. Genetic risk factors in interaction with trauma exposure have been implicated in PTSD vulnerability.

OBJECTIVE To examine the association of 3755 candidate gene single-nucleotide polymorphisms with PTSD development in interaction with a history of childhood trauma.

DESIGN, SETTING, AND PARTICIPANTS Genetic association study in an Ohio National Guard longitudinal cohort (n = 810) of predominantly male soldiers of European ancestry, with replication in an independent Grady Trauma Project (Atlanta, Georgia) cohort (n = 2083) of predominantly female African American civilians.

MAIN OUTCOMES AND MEASURES Continuous measures of PTSD severity, with a modified (interview) PTSD checklist in the discovery cohort and the PTSD Symptom Scale in the replication cohort.

RESULTS Controlling for the level of lifetime adult trauma exposure, we identified the novel association of a single-nucleotide polymorphism within the promoter region of the *ADRB2* (Online Mendelian Inheritance in Man 109690) gene with PTSD symptoms in interaction with childhood trauma (rs2400707, $P = 1.02 \times 10^{-5}$, significant after correction for multiple comparisons). The rs2400707 *A* allele was associated with relative resilience to childhood adversity. An rs2400707 × childhood trauma interaction predicting adult PTSD symptoms was replicated in the independent predominantly female African American cohort.

CONCLUSIONS AND RELEVANCE Altered adrenergic and noradrenergic function has been long believed to have a key etiologic role in PTSD development; however, direct evidence of this link has been missing. The rs2400707 polymorphism has been linked to function of the adrenergic system, but, to our knowledge, this is the first study to date linking the *ADRB2* gene to PTSD or any psychiatric disorders. These findings have important implications for PTSD etiology, chronic pain, and stress-related comorbidity, as well as for both primary prevention and treatment strategies.

JAMA Psychiatry. 2014;71(10):1174-1182. doi:10.1001/jamapsychiatry.2014.999
Published online August 27, 2014.

Supplemental content at jamapsychiatry.com

Author Affiliations: Department of Psychiatry, University of Michigan, Ann Arbor (Liberzon, King, Ma); Veterans Affairs Ann Arbor Health System, Ann Arbor, Michigan (Liberzon, King); Emory University, Atlanta, Georgia (Ressler, Almli); The Johns Hopkins University, Baltimore, Maryland (Zhang); Columbia University, New York, New York (Cohen, Galea); University of Toledo, Toledo, Ohio (Tamburrino); Case Western University, Cleveland, Ohio (Calabrese).

Corresponding Author: Israel Liberzon, MD, Department of Psychiatry, University of Michigan, 4250 Plymouth Rd, Ann Arbor, MI 48105 (liberzon@med.umich.edu).

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

Posttraumatic stress disorder (PTSD) is a debilitating and highly prevalent (7.6% over a lifetime) consequence of trauma exposure.[1,2] Recent large-scale military deployments and high-profile traumatic events have contributed to greater recognition of the PTSD burden both among health professionals[3] and the general public.[4] Trauma exposure is presumed to constitute a key etiologic factor in PTSD[5]; however, only a subset of trauma-exposed individuals develops PTSD,[2] suggesting that vulnerability and resilience factors might have an important role in PTSD development.

Heritable factors and trauma exposure have been implicated in PTSD by twin and family studies,[6-8] suggesting that both genetic and environmental factors are involved; however, larger-scale efforts to identify specific genetic factors are relatively nascent. A recent review of the candidate gene studies of PTSD (approximately 40 to date) addressed 18 gene variants[9]; however, the majority (10 of 18) were based on a single, relatively small discovery cohort each. Only a few (eg, the *FKBP5* gene[10-14] and the *SLC6A4* gene[15,16]) had more than 1 positive association, showed no reported negative findings, and involved relatively large cohorts. Beyond candidate gene studies, a small genome-wide association study[17] (GWAS) identified a single-nucleotide polymorphism (SNP) within the retinoid-related orphan receptor alpha gene (*RORA*) as associated with PTSD, and a larger GWAS[18] identified SNPs near the Tolloid-like 1 gene (*TLL1*) as associated with PTSD. Given that expected variance contributed by any single genetic factor is small[19] and that the likelihood of pleiotropic and polygenic effects in psychiatric disorders is high,[20] major effort is required to both replicate the reported findings and to identify novel risk genes if the goal of identifying genetic risk factors for PTSD is to be accomplished.

Given the centrality of trauma exposure to the etiology of PTSD, a broad range of environmental exposures has been examined in this context, with early childhood trauma emerging as a risk factor linked to both the incident PTSD and the course of PTSD over time.[21,22] Importantly, childhood trauma has been shown to act in interaction with the 2 most consistently replicated PTSD risk alleles in the *SLC6A4* and *FKBP5* genes,[10,13,15,16] suggesting that gene × environment (G × E) approaches utilizing continuous measures of adult PTSD severity might be particularly useful in the study of PTSD pathogenesis.

Finally, on the physiological level, while adrenergic and noradrenergic abnormalities have long been believed to have a key etiologic role in PTSD development,[23,24] contributing to exaggerated physiological reactivity and hyperarousal symptoms,[25-27] direct evidence of genetic variance in noradrenergic and adrenergic function in PTSD has been missing. The PTSD genetic findings so far have mainly implicated serotonin, dopamine, and hypothalamic-pituitary-adrenal axis genes. To address key open questions listed above, we have conducted a candidate gene study in PTSD-relevant pathways, including the adrenergic and noradrenergic systems, using independent discovery and confirmation cohorts and G × E models.

Methods

Sample

All participants provided written informed consent for genetic association analyses approved by the institutional review boards of Veterans Affairs Ann Arbor Health System, Emory University, or Case Western University. **Table 1** lists the demographics of the discovery and replication cohorts. The discovery cohort included 810 active duty Ohio National Guard soldiers. Participants of non-European ancestry by principal components analysis (PCA) (n = 38) or with no lifetime trauma exposure (n = 57) were excluded, leaving 715 trauma-exposed soldiers. Soldiers were from the Ohio National Guard Study of Risk and Resilience,[28] a prospective longitudinal study of postdeployment psychological health (n = 2616), recruited from 6514 randomly selected Ohio National Guard members during predeployment training and assessed over 3 annual follow-ups. Demographics, military history, details of deployments, exposures, and psychiatric symptoms were assessed using computer-aided telephone interviews. In total, 72.1% of soldiers had been deployed (≤4 deployments) to combat zones, including Iraq (61.1%), Afghanistan (13.1%), and other combat zones, such as Bosnia and Somalia (8.0%), and 42% of soldiers had been exposed to military combat. Some soldiers had more than 1 deployment. The replication cohort included 2083 trauma-exposed persons, primarily African American women with low levels of income and education, enrolled in the Grady Trauma Project at Emory University.[29-31]

Assessment

The discovery cohort was assessed for exposures to 16 categories of deployment-related and 17 nondeployment adult traumatic events and to 4 categories of adverse childhood events (ACEs), including physical abuse, sexual abuse, emotional abuse, and witnessing violence between parents. Their PTSD symptoms were assessed using a 17-item Structured Interview Scale derived from the PTSD Checklist (PCL)[32] (score range, 17-85) performed as structured telephone interviews by lay interviewers using epidemiological methods (forced choice symptom severity range, 1-5). Reliability of the telephone interview was validated against the criterion standard, in-person Clinician-Administered PTSD Scale[33] interview in a clinical subsample (n = 500), demonstrating high specificity (0.92). Separate PTSD severity assessments were performed for adult lifetime deployment-related and nondeployment-related traumatic exposures. Because the discovery cohort came from a longitudinal study with up to 3 assessments per individual, the highest severity score from any available assessment was used for the highest lifetime PTSD symptom severity.

The replication cohort was assessed for exposures to 14 categories of lifetime traumatic events and ACEs, including childhood sexual, physical, and emotional abuse, using the Traumatic Events Inventory.[34,35] Their PTSD symptoms were assessed with the 17-item PTSD Symptom Scale, a self-report instrument (score range, 0-51).[36]

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

Table 1. Descriptive Characteristics of the Discovery Cohort and the Replication Cohort

Characteristic	%	
	Discovery Cohort (n = 810)	Replication Cohort (n = 2083)
Sex		
Male	84.3	30.1
Female	15.7	69.9
Age, y		
18-24	37.1	21.8
25-34	31.3	19.7
35-44	21.3	18.1
≥45	10.4	40.4
Educational level		
Did not complete high school	2.1	22.3
Completed high school or GED	20.0	41.8
Some college	38.8	23.8
College graduate	39.2	12.2
Employment		
Employed	100.0	29.9
Disability support	0.0	18.7
Unemployed	0.0	51.4
Other	0.0	0.0
Marital status		
Married	47.5	11.3
Single	45.0	75.7
Other	7.5	12.9
Frequency of types of adult trauma exposure		
Military combat	41.8	2.1
Sexual assault	8.9	10.5
Physical assault	29.0	33.8
Nonperpetrated	28.2	46.5
Frequency of types of childhood trauma exposure		
Sexual abuse	2.7	26.7
Physical abuse	13.3	21.8
Emotional abuse	14.9	19.7
No. of childhood adversity categories		
0	73.6	65.8
1	14.2	24.8
≥2	12.2	9.5
Lifetime diagnosis of PTSD	15.1	31.5
Household monthly income, $		
0-499	0.0	35.9
500-999	0.0	26.6
1000-1999	16.3	24.9
2000-3333	21.3	12.7
3334-5000	37.4	0.0
≥5000	25.4	0.0

Abbreviations: GED, general educational development; PTSD, posttraumatic stress disorder.

Genotyping

In the discovery cohort, DNA was obtained from saliva samples (Oragene; DNA Genotek Inc) and was extracted using a machine for rapid isolation (QuickGene-810; Autogen Inc), quantified using a kit (Picogreen; Thermo Fisher Scientific Inc) and gel electrophoresis, and normalized to 600 ng. Genotyping was performed at the University of Michigan DNA Sequencing Core using a custom array (Infinium; Illumina) of 3755 candidate gene SNPs covering haplotype tagging and previously reported SNPs in 295 candidate genes for PTSD (neurotransmitter, neuroendocrine, and other systems associated with PTSD) and 319 SNPs from psychiatric GWAS studies over the past 7 years.

Genome-wide genotyping of the replication cohort was performed at Emory University using a microarray (Human Omni1-Quad BeadChip; Illumina). DNA was obtained from saliva samples (Oragene) or from whole blood, extracted using a kit (Blood DNA Midi; Omega Biotek), quantified by gel electrophoresis, and normalized to 400 ng. The rs2400707 SNP in the replication cohort was imputed from the Human Omni1-Quad BeadChip using unrelated individuals from HapMap[37] phase 3 reference samples (reference data set range of chr5: 143185574-153183640 and replication cohort data set range of chr5:143192222-153181562). Imputed SNPs with an estimated $r^2 < 0.30$ between imputed and true genotypes and those with posterior probabilities less than 0.90 for the most likely genotype were excluded from subsequent analysis. The SNP rs2400707 had a posterior probability of 0.99.

Statistical Analysis

Data were analyzed with PLINK[38] and R.[39] Standard data cleaning and quality control were performed before analysis. Some SNPs were excluded owing to a genotyping call rate less than 0.95, minor allele frequency less than 0.05, or Hardy-Weinberg equilibrium deviations. Ten samples with a call rate less than 0.95 were excluded. To assess population substructure, PCA was conducted on genotype data (Eigensoft 3.0; http://helix.nih.gov/Applications/README.eigensoft), and principal components (PCs) were included in the association analyses to guard against potential bias due to population stratification. In the discovery cohort, 88 ancestry-informative SNPs and 1472 SNPs in equilibrium (linkage disequilibrium $R^2 < .30$) from data pruning (Structure; http://pritchardlab.stanford.edu/structure.html) were utilized for PCA, and the top 4 PCs were used in analyses. In the discovery cohort, the entire GWAS data were used for PCA, and 10 PCs were included in the association analysis.

Associations between SNPs and PTSD phenotype (highest reported PCL score) were evaluated using linear regression models in PLINK. Similar to previous work in PTSD examining G × E interactions,[10,29] we used a quantitative trait approach (ie, continuous measures of PTSD symptom severity) to increase sensitivity to detect potential genetic influences on the development of PTSD symptoms. Given the strong effect of environmental factors on PTSD symptoms, we tested linear models controlling for sex, lifetime adult trauma, and childhood adversity (ACEs), including interaction terms for SNP × adult trauma and SNP × ACE. The numbers of categories of childhood and adult trauma exposures were used as predictors, as previously reported in both epidemiological[22,40] and genetic association[10] studies. Because we had an a priori

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

Table 2. Single-Nucleotide Polymorphisms Showing Association With Posttraumatic Stress Disorder Symptoms in Interaction With the Level of Childhood Adversity

Chromosome	SNP	Size, base pair	Locus	Location	Position Relative to Gene	*P* Value for HWE	MAF	Allele	*P* Value	
									Raw	Corrected[a]
5	rs2400707	148 205 052	*ADRB2*	5′-Flank	−1124	.919	0.438	*G:A*	1.02×10^{-5}	.0448
5	rs2053044	148 205 372	*ADRB2*	5′-Flank	−804	>.99	0.439	*G:A*	1.37×10^{-5}	.0575
5	rs1432622	148 203 762	*ADRB2*	5′-Flank	−2414	>.99	0.440	*G:A*	1.42×10^{-5}	.0594
5	rs1432623	148 204 008	*ADRB2*	5′-Flank	−2168	>.99	0.440	*A:G*	1.42×10^{-5}	.0594
5	rs11168068	148 204 121	*ADRB2*	5′-Flank	−2055	>.99	0.440	*A:G*	1.42×10^{-5}	.0594
5	rs1042714	148 206 473	*ADRB2*	Nonsynonymous	E27Q	>.99	0.438	*G:C*	2.05×10^{-5}	.0786
5	rs11168070	148 205 927	*ADRB2*	5′-Flank	−249	>.99	0.438	*G:C*	2.11×10^{-5}	.0799
10	rs16914791	61 895 547	*ANK3*	Intron	−1416	.87	0.050	*A:G*	4.23×10^{-5}	.1305
21	rs420121	31 146 608	*GRIK1*	Intron	−80 226	.95	0.392	*T:C*	1.01×10^{-4}	.2383

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

[a] Corrected for multiple comparisons.

hypothesis regarding gene × early life environment interaction in PTSD, we focused primarily on the SNP × ACE term in analyses of the discovery cohort. Inflation factors in the discovery cohort analyses were calculated for each of the model terms of main effects of SNPs, SNP × ACE, and SNP × adult trauma interactions using ordinary least squares standard errors and with robust (heteroscedasticity consistent) standard errors using the R GWAtoolbox[41] robust function.[42] To control for multiple comparisons, we used 10 000 permutation analyses permuting the PTSD symptom severity score in the full model (containing main effects and interaction terms)[10] using the most significant *P* value of the SNP × ACE term from each permuted model as the null distribution.

Results

Discovery Cohort

Table 1 lists the characteristics of the primary (Ohio National Guard) and replication (Grady Trauma Project) cohorts. Interview data suggested that 15.1% of the discovery cohort had a lifetime diagnosis of PTSD, with a PTSD symptom severity mean (SD) PCL score of 30.4 (12.5) and a range of 17 (no symptoms endorsed) to 77. As expected, both lifetime adult trauma load (number of categories of exposures endorsed, $P < 10^{-5}$) and childhood adversity (number of categories of ACE exposures endorsed, $P < 10^{-5}$) influenced the level of PTSD symptoms, with no significant interaction between these factors (interaction term $F_{1,809} = 1.2$, $P = .25$) and a small correlation between ACEs and adult trauma exposures ($r = 0.12$).

No significant main effects of SNPs on PTSD symptoms that survived Bonferroni correction threshold for 3755 SNPs ($P = 1.33 \times 10^{-5}$, data available on request) were detected. Because we had an a priori hypothesis regarding interaction with childhood adversity, we tested SNP × ACE interactions in a linear regression model controlling for adult trauma exposure. Associations were detected for the SNP × ACE interaction term in rs2400707 (mean [SD] unstandardized estimate [B] = 3.07 [0.69], β = 0.243, $t = 4.48$, $P = 1.02 \times 10^{-5}$) and 6 other SNPs in the *ADRB2* locus in high linkage disequilibrium with rs2400707 (**Table 2**). Permutation analysis of the SNP × ACE interaction term further confirmed a significant association with rs2400707 ($P < .05$ corrected for multiple comparisons). As shown in **Figure 1**A, rs2400707 *AA* homozygotes demonstrated lower levels of PTSD symptoms in the presence of 2 or more categories of reported childhood adversity in the discovery cohort. Stratification of the sample by ACEs showed an effect of rs2400707 only in persons with 2 or more reported categories of ACEs (n = 93, mean [SD] B = −6.44 [2.08], $t = -3.10$, $P = .003$). Although our primary focus was on continuous measures of PTSD severity, a secondary analysis of binary logistic regression of PTSD caseness confirmed an rs2400707 × ACE interaction (odds ratio, 0.54; $P = 5.16 \times 10^{-4}$), indicating that the *A* allele is protective in terms of adult PTSD symptoms on categorical PTSD diagnosis.

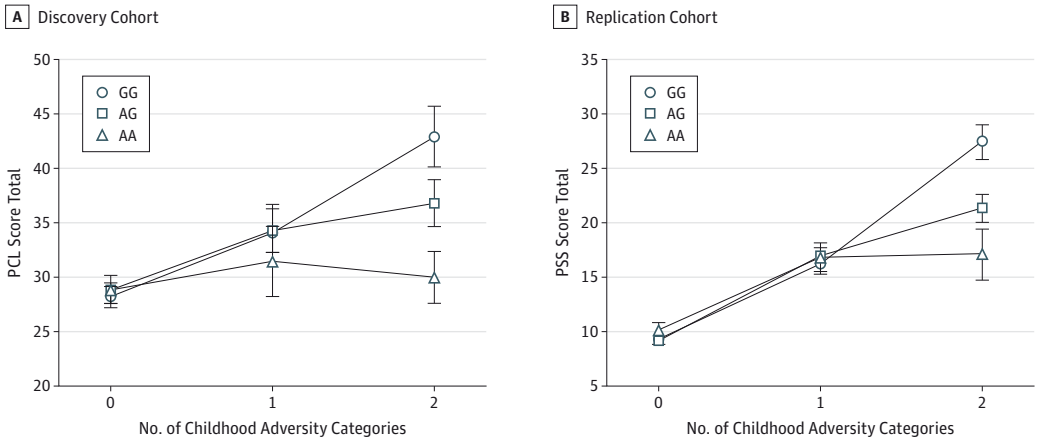
To control for potential effects of population stratification, we examined the association with the top 4 PCs from the PCA. The PCs were not significantly associated with PTSD symptom severity in a linear model ($P > .25$ for all), and addition of PCs did not alter the significant association of rs2400707 ($P = 1.11 \times 10^{-5}$). As previously discussed,[43] linear regression interaction terms (eg, SNP × ACE and SNP × adult trauma) showed apparent evidence of inflation using ordinary least squares ($\lambda = 1.51$ and $\lambda = 1.53$, respectively). This was not diminished by the inclusion of PCs ($\lambda = 1.50$ and $\lambda = 1.52$, respectively) but was substantially diminished by the use of robust standard errors ($\lambda = 0.99$ and $\lambda = 0.99$, respectively), suggesting that the apparent inflation in the ordinary least squares models is not due to population substructure.[43]

The 7 *ADRB2* SNPs associated with PTSD phenotype in interaction with childhood adversity were in strong linkage disequilibrium within a single haploblock (**Figure 2**A). Haplotype analysis in the discovery cohort showed 3 major haplotypes accounting for greater than 98% of those observed, H1 (43.6%), H2 (37.4%), and H3 (17.4%) (Figure 2B), consistent with previous findings.[44] H1 is tagged by the rs2400707 *A* allele, which was 99.7% concordant with the H1 haplotype (resolved by the MaCH 1.0 program[45]) in the discovery cohort. Loading of the H1 haplotype demonstrated a similar interaction of H1 × childhood adversity in linear regression of

Copyright 2014 American Medical Association. All rights reserved.

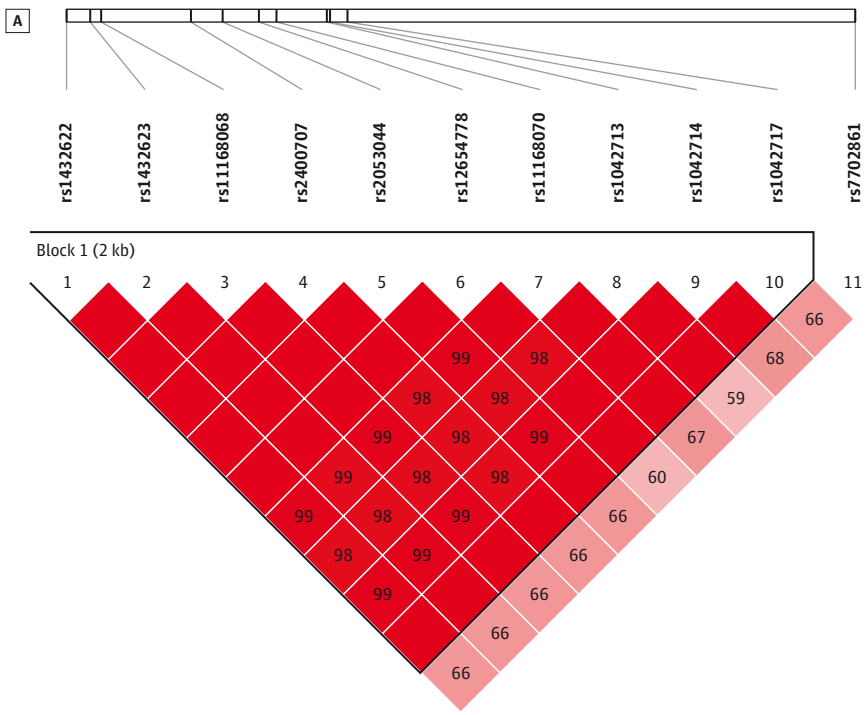
Downloaded From: https://jamanetwork.com/ on 08/22/2022

Figure 1. Interaction of *ADRB2* Single-Nucleotide Polymorphism rs2400707 With Childhood Adversity in Adult Posttraumatic Stress Disorder



Exposure to childhood adversity is coded 0 for no reported abuse (521 in the discovery cohort and 1370 in the replication cohort), 1 for 1 category reported (101 in the discovery cohort and 516 in the replication cohort), or 2 for 2 or more forms of child abuse reported (93 in the discovery cohort and 197 in the replication cohort). The PTSD Checklist (PCL) and PTSD Symptom Scale (PSS) scores are continuous measures of posttraumatic stress disorder. A, Discovery cohort (n = 715, $P = 1.02 \times 10^{-5}$ for interaction). B, Replication cohort (n = 2083, $P = .0009$ for interaction).

Figure 2. Linkage Disequilibrium and Haplotype Analysis of the *ADRB2* Locus



B

01	02	03	04	05	06	07	08	09	10	
A	G	G	A	A	G	C	G	C	G	.436
G	A	A	G	G	A	G	A	G	G	.374
G	A	A	G	G	G	G	G	G	A	.174

A, *ADRB2* locus linkage disequilibrium map demonstrating the physical location (lines) and linkage disequilibrium pattern of the selected *ADRB2* single-nucleotide polymorphisms within the region in proximity to the most significant SNP (rs2400707), a 47-kilobase region in chromosome 5. The x-axis shows the position on chromosome 5. The lower part shows a linkage disequilibrium plot generated with Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) using R^2 as the measure of linkage disequilibrium. B, Three common haplotypes in the *ADRB2* promoter region and the observed frequency in the discovery cohort.

PTSD symptoms (mean [SD] B = 3.08 [0.71], $t = 4.343$, $P = 1.61 \times 10^{-5}$) and in logistic regression of PTSD caseness (odds ratio, 0.53; $P = 6.1 \times 10^{-4}$). The next-highest SNP × ACE interactions were in SNPs in the *ANK3* and *GRIK1* genes (Table 2); however, these effects were not significant ($P > .05$) after controlling for multiple comparisons. Parameter estimates and raw and corrected P values of ACE × SNP interaction terms for all SNPs tested are listed in eTable 1 in the Supplement.

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

Replication Cohort

We confirmed the association of *ADRB2* SNP rs2400707 with PTSD symptom level (modified PTSD Symptom Scale score) and interaction with childhood adversity in our replication cohort. In total, 31.5% of the replication cohort had a lifetime diagnosis of PTSD, with a mean (SD) PTSD Symptom Scale score of 12.5 (12.2) and a range of 0 to 45. The observed frequency of the *A* allele in the replication cohort was 0.43, and the observed *AA* genotype was found in 417 (20.0%). As shown in Figure 1B, rs2400707 *AA* homozygotes showed lower levels of PTSD symptoms in the presence of 2 or more categories of reported childhood adversity in the replication cohort. The rs2407007 × ACE interaction in the association with PTSD symptoms in the Grady Trauma Project (mean [SD] B = 1.80 [0.54], t = 3.328, P = .0009) remained significant when using robust standard errors (mean [SD] B = 1.81 [0.60], t = 3.02, P = .0026) after controlling for sex and adult trauma exposure (mean [SD] B = 1.58 [0.50], t = 3.15, P = .0017) and after controlling for the 10 PCs (mean [SD] B = 1.55 [0.51], t = 3.06, P = .0022) (eTable 2 in the Supplement). Again, an effect of rs2400707 was found only in persons with 2 or more reported ACEs (n = 197, mean [SD] B = −5.34 [1.35], t = 3.95, P = .00011). All 3 subscales of PTSD symptoms showed rs2400707 × ACE interaction, including intrusive (mean [SD] B = 0.47 [0.17], t = 2.79, P = .005) and hyperarousal (mean [SD] B = 0.61 [0.20], t = 3.07, P = .002); however, the subscale for avoidance symptoms had the most robust interaction (mean [SD] B = 0.82 [0.24], t = 3.43, P = .0006).

Finally, we tested the same model (linear regression of SNP, ACEs, adult trauma, and SNP × ACE and SNP × adult trauma interaction terms) in the combined data set (adjusting symptom severity measures to the same scale) of both the discovery and replication cohorts. We found that the rs2400707 × ACE interaction term had a mean (SD) B = 0.77 (0.15) (β = 0.19, t = 5.08, $P = 3.97 \times 10^{-7}$).

Discussion

Our data provide strong evidence that *ADRB2* SNPs are associated with PTSD in male soldiers of European American ancestry and in civilian women of African American ancestry who were exposed to trauma and adverse events during childhood. To our knowledge, to date, this is the first direct evidence of the role of genetic variance in the noradrenergic system in PTSD. The association between PTSD symptom severity and rs2400707 in interaction with childhood adversity in the discovery cohort was found using robust standard errors and correction for multiple testing with permutation analyses. The same finding of rs2400707 genotype × childhood adversity interaction was confirmed in the replication cohort, and the combined data set revealed a similar effect (unadjusted $P = 3.97 \times 10^{-7}$). In concert, an association was also found for several other SNPs in the same region of the *ADRB2* gene, all in strong linkage disequilibrium with rs2400707, and haplotype analyses suggested that the H1 haplotype is protective. The genetic associations were demonstrated using a G × E model, such that individuals with different rs2400707 genotypes show differential levels of PTSD symptoms as a function of the number of types of adverse childhood exposure (Figure 1A). We found an essentially identical interaction in the replication cohort (Figure 1B). Together, these findings suggest that the *ADRB2* gene interacts with childhood adversity, constituting a vulnerability and resilience factor to the development of PTSD symptoms following adult trauma. The rs2400707 *A* homozygotes (from populations of approximately 19% of European ancestry and 20% of African ancestry [Yoruba from Ibadan, Nigeria] based on International HapMap Project[37] data) represent the most resilient group, with no increase in PTSD symptoms despite the exposure to more types of childhood adversity, while the *G* homozygotes show the greatest vulnerability, and the heterozygotes demonstrate intermediate vulnerability.

The rs2400707 SNP is located in the promoter region of the intronless *ADRB2* gene, approximately 1 kilobase (kb) upstream of the start site. It is in strong linkage disequilibrium with several other SNPs in an approximately 2-kb region spanning the promoter and part of the coding region of the *ADRB2* gene, which form a single haploblock. We found 3 major haplotypes accounting for greater than 98% of those observed in our discovery cohort, consistent with previous work.[44] The *A* allele of rs2400707 (associated with resilience) tags H1 (HapMap[37] tool TagSNP picker), whereas H2 and H3 are differentiated by rs1042713 and rs1042717. *ADRB2* haplotypes, including nonsynonymous SNPs in the coding region (rs1042713, Arg16Gly and rs1042714, Gln27Glu), have been linked to altered agonist-induced internalization of the receptor.[46] Diatchenko and colleagues[44] argued that these haplotypes are associated with altered transcription efficiency, with H1 (tagged by rs2400707 *A*) coding for low-efficiency transcription. We hypothesize that the *AA* genotype/H1H1 diplotype (resilient in the face of childhood adversity in both cohorts) represents a low-transcription variant of *ADRB2*, the gene for the β2-adrenergic receptor. Decreased β2-adrenergic receptor levels are believed to be associated with decreased sympathetic responsiveness, which could be protective against PTSD despite trauma exposure.

Physiologically, the β2-adrenergic receptor is a major transducer of the sympathetic nervous system and the fight-or-flight response. Increased adrenergic and noradrenergic function had been repeatedly invoked to explain exaggerated arousal, hypervigilance, enhanced autonomic responses, and even persistent trauma memories in PTSD,[23,26,47-49] and adrenergic β-blockers have been tried as potential early intervention and secondary prevention strategies,[50,51] with mixed results. A deletion variant in a different adrenergic system gene (*ADRA2B*), leading to decreased agonist-promoted phosphorylation and receptor desensitization, has been associated with altered memory for emotionally arousing events[47] and increased amygdala activity.[52] Molecularly, the β2-adrenergic receptor is a member of the G protein–coupled receptor superfamily and associates intracellularly with the class C L-type calcium channel $Ca_v1.2$ encoded by the *CACNA1C* gene.[53] *CACNA1C* is one of the most replicated and strongest signals for psychiatric vulnerability identified to date[53] and is associated with risk for both bipolar disorder and schizophrenia in

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

large GWAS studies.[54-59] The fact that *ADRB2* and *CACNA1C* are within the same gene network supports the idea that genetic variation within this network could affect psychiatric vulnerability. The next most significant association in interaction with ACEs in the discovery cohort was an SNP in the *ANK3* gene. While this finding did not survive multiple comparisons correction, it is intriguing in light of the recent association of *ANK3* with PTSD.[60]

To our knowledge, this is the first report of genetic risk factors for PTSD in National Guard soldiers. The question as to whether the genetic risks for PTSD development are similar in different populations that are exposed to different traumas at different periods in their lives remains to be empirically tested. While some genetic variants (eg, *ADCYAP1R1*) have been identified as risk factors for women only,[31] our findings suggest that *ADRB2* factor might be shared by men and women, African Americans and European Americans, and military and civilians. This is consistent with the idea that some genetic risk factors for PTSD might be common across populations and even shared by other stress-related disorders, such as depression.[61-63] In this context, the same *ADRB2* SNPs have been linked to risk for the development of chronic pain,[64,65] and it was previously suggested based on epidemiology and shared physiology[66] that common vulnerability factors for stress-related disorders and chronic pain must exist. More recently, evidence was also reported of shared vulnerability for pain and posttrauma psychological symptoms in the *COMT* gene.[67] Further studies will be required to examine both shared and unique genetic vulnerability factors, especially in military cohorts exposed to unique sets of traumas and stressors.

Lifetime trauma exposure was a strong predictor of PTSD symptoms, regardless of the *ADRB2* diplotype. This is expected because the severity of trauma exposure had been identified as a major risk factor for PTSD in epidemiological studies.[2,21,22] We did not observe significant interaction between genetic variance and lifetime adult trauma exposure, suggesting that genetic variance in interaction with childhood trauma alone can influence adult PTSD symptom severity. However, exposure to 2 or more different childhood traumas predicted PTSD symptoms in rs2400707 *G* carriers but not in *A* homozygotes, consistent with findings of interaction with childhood adversity in *FKBP5* (which regulates glucocorticoid regulator sensitivity).[10] Exposure to 2 or more types of childhood trauma or adversity is likely to signify a qualitatively different type of parental supervision and thus different childhood experience during critical developmental periods. Lower-efficiency transcription and thus lower expression of *ADRB2* could protect against negative biological consequences of chronic or repeated activation of adrenergic and noradrenergic systems in childhood adversity exposure.

Certain limitations should be kept in mind when considering these findings. While we have used discovery and replication cohorts and the total number of participants included in the analyses is substantial (n = 2798), it is still relatively modest compared with large-scale genetic projects involving tens of thousands. Thus, further confirmation in large cohorts of participants will be very useful. On the other hand, the G × E analyses used herein require detailed data collection with regard to PTSD symptoms and lifetime and childhood trauma exposure, the collection of which is seldom feasible in very large genetic studies. With respect to generalizability, our cohorts included both sexes, European-ancestry soldiers, and African American civilians. Still, whether the same risk factors are shared by other racial/ethnic groups, various ages, or members of different branches of the military will have to await future confirmation. Finally, physiological data will help to confirm the functional effects of *ADRB2* polymorphisms on adrenergic system function.

Conclusions

In summary, we provide evidence that *ADRB2* SNPs are strongly associated with the development of PTSD symptoms in persons with a history of childhood adversity. While additional investigations (including deep sequencing) are clearly needed to confirm the existing findings and to identify new ones, these data provide an important lead for both examining the pathogenesis of PTSD and developing specific and effective prevention and intervention strategies.

ARTICLE INFORMATION

Submitted for Publication: August 14, 2013; final revision received May 1, 2014; accepted May 8, 2014.

Published Online: August 27, 2014. doi:10.1001/jamapsychiatry.2014.999.

Author Contributions: Drs King and Liberzon contributed equally to this work. Drs King and Liberzon had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Liberzon, King, Tamburrino, Calabrese, Galea.
Acquisition, analysis, or interpretation of data: Liberzon, King, Ressler, Almli, Zhang, Ma, Cohen, Calabrese.
Drafting of the manuscript: Liberzon, King, Ressler, Almli, Tamburrino.
Critical revision of the manuscript for important intellectual content: Liberzon, King, Ressler, Zhang, Ma, Cohen, Calabrese, Galea.
Statistical analysis: King, Ressler, Almli, Zhang, Ma.
Obtained funding: Liberzon, King, Tamburrino, Calabrese, Galea.
Administrative, technical, or material support: Liberzon, King, Ressler, Cohen, Tamburrino, Calabrese, Galea.
Study supervision: Liberzon, King, Calabrese, Galea.

Conflict of Interest Disclosures: None reported.

Funding/Support: This study was supported by grant W81XWH-10-1-0579 from the Congressionally Directed Medical Research Programs, Department of Defense (Calabrese).

Role of the Funder/Sponsor: The funding source had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

1. Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB. Posttraumatic stress disorder in the National Comorbidity Survey. *Arch Gen Psychiatry*. 1995;52(12):1048-1060.

2. Breslau N. Epidemiologic studies of trauma, posttraumatic stress disorder, and other psychiatric disorders. *Can J Psychiatry*. 2002;47(10):923-929.

3. Committee on the Assessment of Ongoing Effects in the Treatment of Posttraumatic Stress Disorder, Institute of Medicine. *Treatment for Posttraumatic Stress Disorder in Military and Veteran Populations: Initial Assessment*. Washington, DC: National Academies Press; 2012.

4. Tanielian T, Jaycox LH, eds. *Invisible Wounds of War: Psychological and Cognitive Injuries, Their*

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

Consequences, and Services to Assist Recovery. Santa Monica, CA: RAND Corp; 2008.

5. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th ed. Arlington, VA: American Psychiatric Association; 2013.

6. Koenen KC, Harley R, Lyons MJ, et al. A twin registry study of familial and individual risk factors for trauma exposure and posttraumatic stress disorder. *J Nerv Ment Dis*. 2002;190(4):209-218.

7. Kremen WS, Koenen KC, Afari N, Lyons MJ. Twin studies of posttraumatic stress disorder: differentiating vulnerability factors from sequelae. *Neuropharmacology*. 2012;62(2):647-653.

8. McLeod DS, Koenen KC, Meyer JM, et al. Genetic and environmental influences on the relationship among combat exposure, posttraumatic stress disorder symptoms, and alcohol use. *J Trauma Stress*. 2001;14(2):259-275.

9. Pitman RK, Rasmusson AM, Koenen KC, et al. Biological studies of post-traumatic stress disorder. *Nat Rev Neurosci*. 2012;13(11):769-787.

10. Binder EB, Bradley RG, Liu W, et al. Association of *FKBP5* polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA*. 2008;299(11):1291-1305.

11. Boscarino JA, Erlich PM, Hoffman SN, Rukstalis M, Stewart WF. Association of FKBP5, COMT and CHRNA5 polymorphisms with PTSD among outpatients at risk for PTSD. *Psychiatry Res*. 2011; 188(1):173-174.

12. Boscarino JA, Erlich PM, Hoffman SN, Zhang X. Higher *FKBP5*, *COMT*, *CHRNA5*, and *CRHR1* allele burdens are associated with PTSD and interact with trauma exposure: implications for neuropsychiatric research and treatment. *Neuropsychiatr Dis Treat*. 2012;8:131-139.

13. Klengel T, Mehta D, Anacker C, et al. Allele-specific *FKBP5* DNA demethylation mediates gene–childhood trauma interactions. *Nat Neurosci*. 2013;16(1):33-41.

14. Mehta D, Gonik M, Klengel T, et al. Using polymorphisms in *FKBP5* to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies. *Arch Gen Psychiatry*. 2011;68(9):901-910.

15. Xie P, Kranzler HR, Farrer L, Gelernter J. Serotonin transporter *5-HTTLPR* genotype moderates the effects of childhood adversity on posttraumatic stress disorder risk: a replication study. *Am J Med Genet B Neuropsychiatr Genet*. 2012;159B(6):644-652.

16. Xie P, Kranzler HR, Poling J, et al. Interactive effect of stressful life events and the serotonin transporter *5-HTTLPR* genotype on posttraumatic stress disorder diagnosis in 2 independent populations. *Arch Gen Psychiatry*. 2009;66(11): 1201-1209.

17. Logue MW, Baldwin C, Guffanti G, et al. A genome-wide association study of post-traumatic stress disorder identifies the retinoid-related orphan receptor alpha (*RORA*) gene as a significant risk locus. *Mol Psychiatry*. 2013;18(8):937-942.

18. Xie P, Kranzler HR, Yang C, Zhao H, Farrer LA, Gelernter J. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. *Biol Psychiatry*. 2013;74(9):656-663.

19. Cichon S, Craddock N, Daly M, et al; Psychiatric GWAS Consortium Coordinating Committee. Genomewide association studies: history, rationale, and prospects for psychiatric disorders. *Am J Psychiatry*. 2009;166(5):540-556.

20. Smoller JW, Craddock N, Kendler K, et al; Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis [published correction appears in *Lancet*. 2013;381(9875):1360]. *Lancet*. 2013;381(9875):1371-1379.

21. Breslau N. Psychiatric morbidity in adult survivors of childhood trauma. *Semin Clin Neuropsychiatry*. 2002;7(2):80-88.

22. Cabrera OA, Hoge CW, Bliese PD, Castro CA, Messer SC. Childhood adversity and combat as predictors of depression and post-traumatic stress in deployed troops. *Am J Prev Med*. 2007;33(2):77-82.

23. Southwick SM, Bremner JD, Rasmusson A, Morgan CA III, Arnsten A, Charney DS. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry*. 1999;46(9):1192-1204.

24. Southwick SM, Krystal JH, Bremner JD, et al. Noradrenergic and serotonergic function in posttraumatic stress disorder. *Arch Gen Psychiatry*. 1997;54(8):749-758.

25. Strawn JR, Geracioti TD Jr. Noradrenergic dysfunction and the psychopharmacology of posttraumatic stress disorder. *Depress Anxiety*. 2008;25(3):260-271.

26. Krystal JH, Neumeister A. Noradrenergic and serotonergic mechanisms in the neurobiology of posttraumatic stress disorder and resilience. *Brain Res*. 2009;1293:13-23.

27. Southwick SM, Paige S, Morgan CA III, Bremner JD, Krystal JH, Charney DS. Neurotransmitter alterations in PTSD: catecholamines and serotonin. *Semin Clin Neuropsychiatry*. 1999;4(4):242-248.

28. Calabrese JR, Prescott M, Tamburrino M, et al. PTSD comorbidity and suicidal ideation associated with PTSD within the Ohio Army National Guard. *J Clin Psychiatry*. 2011;72(8):1072-1078.

29. Almli LM, Mercer KB, Kerley K, et al. *ADCYAP1R1* genotype associates with post-traumatic stress symptoms in highly traumatized African-American females. *Am J Med Genet B Neuropsychiatr Genet*. 2013;162B(3):262-272.

30. Gillespie CF, Bradley B, Mercer K, et al. Trauma exposure and stress-related disorders in inner city primary care patients. *Gen Hosp Psychiatry*. 2009; 31(6):505-514.

31. Ressler KJ, Mercer KB, Bradley B, et al. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor [published correction appears in *Nature*. 2011;477(7362):120]. *Nature*. 2011;470(7335):492-497.

32. Blanchard EB, Jones-Alexander J, Buckley TC, Forneris CA. Psychometric properties of the PTSD Checklist (PCL). *Behav Res Ther*. 1996;34(8):669-673.

33. Blake DD, Weathers FW, Nagy LM, et al. The development of a clinician-administered PTSD scale. *J Trauma Stress*. 1995;8(1):75-90.

34. Schwartz AC, Bradley R, Penza KM, et al. Pain medication use among patients with posttraumatic stress disorder. *Psychosomatics*. 2006;47(2):136-142.

35. Schwartz AC, Bradley RL, Sexton M, Sherry A, Ressler KJ. Posttraumatic stress disorder among African Americans in an inner city mental health clinic. *Psychiatr Serv*. 2005;56(2):212-215.

36. Foa EB, Tolin DF. Comparison of the PTSD Symptom Scale–Interview Version and the Clinician-Administered PTSD Scale. *J Trauma Stress*. 2000;13(2):181-191.

37. Thorisson GA, Smith AV, Krishnan L, Stein LD. The International HapMap Project web site. *Genome Res*. 2005;15(11):1592-1593.

38. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81(3):559-575.

39. R Core Team. R: a language and environment for statistical computing. 2014. http://www.R-project.org. Accessed July 17, 2014.

40. Dube SR, Anda RF, Felitti VJ, Chapman DP, Williamson DF, Giles WH. Childhood abuse, household dysfunction, and the risk of attempted suicide throughout the life span: findings from the Adverse Childhood Experiences Study. *JAMA*. 2001; 286(24):3089-3096.

41. Fuchsberger C, Taliun D, Pramstaller PP, Pattaro C; CKDGen Consortium. GWAtoolbox: an R package for fast quality control and handling of genome-wide association studies meta-analysis data. *Bioinformatics*. 2012;28(3):444-445.

42. Cookson JA. Package "tonymisc": functions for econometrics output. R package version 1.1.1. 2011.

43. Voorman A, Lumley T, McKnight B, Rice K. Behavior of QQ-plots and genomic control in studies of gene-environment interaction. *PLoS One*. 2011;6(5):e19416. doi:10.1371/journal.pone.0019416.

44. Diatchenko L, Anderson AD, Slade GD, et al. Three major haplotypes of the β_2 adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141B(5):449-462.

45. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR. MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol*. 2010;34(8):816-834.

46. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu Rev Pharmacol Toxicol*. 2003;43:381-411.

47. de Quervain DJ, Aerni A, Roozendaal B. Preventive effect of β-adrenoceptor blockade on glucocorticoid-induced memory retrieval deficits. *Am J Psychiatry*. 2007;164(6):967-969.

48. Krauseneck T, Padberg F, Roozendaal B, et al. A β-adrenergic antagonist reduces traumatic memories and PTSD symptoms in female but not in male patients after cardiac surgery. *Psychol Med*. 2010;40(5):861-869.

49. McFall ME, Veith RC, Murburg MM. Basal sympathoadrenal function in posttraumatic distress disorder. *Biol Psychiatry*. 1992;31(10):1050-1056.

50. Hoge EA, Worthington JJ, Nagurney JT, et al. Effect of acute posttrauma propranolol on PTSD

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022

outcome and physiological responses during script-driven imagery. *CNS Neurosci Ther*. 2012;18 (1):21-27.

51. Pitman RK, Sanders KM, Zusman RM, et al. Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol Psychiatry*. 2002;51(2):189-192.

52. Rasch B, Spalek K, Buholzer S, et al. A genetic variation of the noradrenergic system is related to differential amygdala activation during encoding of emotional memories. *Proc Natl Acad Sci U S A*. 2009;106(45):19191-19196.

53. Bhat S, Dao DT, Terrillion CE, et al. *CACNA1C* ($Ca_v1.2$) in the pathophysiology of psychiatric disease. *Prog Neurobiol*. 2012;99(1):1-14.

54. Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. *Nat Genet*. 2011;43(10):969-976.

55. Psychiatric GWAS Consortium Bipolar Disorder Working Group. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near *ODZ4*. *Nat Genet*. 2011;43(10):977-983.

56. Alsabban S, Rivera M, McGuffin P. Genome-wide searches for bipolar disorder genes. *Curr Psychiatry Rep*. 2011;13(6):522-527.

57. Lett TA, Zai CC, Tiwari AK, et al. *ANK3*, *CACNA1C* and *ZNF804A* gene variants in bipolar disorders and psychosis subphenotype. *World J Biol Psychiatry*. 2011;12(5):392-397.

58. Roussos P, Giakoumaki SG, Georgakopoulos A, Robakis NK, Bitsios P. The *CACNA1C* and *ANK3* risk alleles impact on affective personality traits and startle reactivity but not on cognition or gating in healthy males. *Bipolar Disord*. 2011;13(3):250-259.

59. Nyegaard M, Demontis D, Foldager L, et al. *CACNA1C* (rs1006737) is associated with schizophrenia. *Mol Psychiatry*. 2010;15(2):119-121.

60. Logue MW, Solovieff N, Leussis MP, et al. The ankyrin-3 gene is associated with posttraumatic stress disorder and externalizing comorbidity. *Psychoneuroendocrinology*. 2013;38(10):2249-2257.

61. Chantarujikapong SI, Scherrer JF, Xian H, et al. A twin study of generalized anxiety disorder symptoms, panic disorder symptoms and post-traumatic stress disorder in men. *Psychiatry Res*. 2001;103(2-3):133-145.

62. Tambs K, Kendler KS, Reichborn-Kjennerud T, et al. Genetic and environmental contributions to the relationship between education and anxiety disorders: a twin study. *Acta Psychiatr Scand*. 2012; 125(3):203-212.

63. Wolf EJ, Miller MW, Krueger RF, Lyons MJ, Tsuang MT, Koenen KC. Posttraumatic stress disorder and the genetic structure of comorbidity. *J Abnorm Psychol*. 2010;119(2):320-330.

64. Diatchenko L, Nackley AG, Slade GD, et al. Catechol-*O*-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. *Pain*. 2006;125(3):216-224.

65. Diatchenko L, Nackley AG, Tchivileva IE, Shabalina SA, Maixner W. Genetic architecture of human pain perception. *Trends Genet*. 2007;23(12): 605-613.

66. McLean SA, Clauw DJ, Abelson JL, Liberzon I. The development of persistent pain and psychological morbidity after motor vehicle collision: integrating the potential role of stress response systems into a biopsychosocial model. *Psychosom Med*. 2005;67(5):783-790.

67. McLean SA, Diatchenko L, Lee YM, et al. Catechol *O*-methyltransferase haplotype predicts immediate musculoskeletal neck pain and psychological symptoms after motor vehicle collision. *J Pain*. 2011;12(1):101-107.

Copyright 2014 American Medical Association. All rights reserved.

Downloaded From: https://jamanetwork.com/ on 08/22/2022