Genome-wide Association Studies of Posttraumatic Stress Disorder in 2 Cohorts of US Army Soldiers

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>10.1001/jamapsychiatry.2016.0350</td>
</tr>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:33839674">http://nrs.harvard.edu/urn-3:HUL.InstRepos:33839674</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
Genome-wide Association Studies of Posttraumatic Stress Disorder in 2 Cohorts of US Army Soldiers

Murray B. Stein, MD, MPH; Chia-Yen Chen, ScD; Robert J. Ursano, MD; Tianxi Cai, ScD; Joel Gelernter, MD; Steven G. Heeringa, PhD; Sonia Jain, PhD; Kevin P. Jensen, PhD; Adam X. Malhofer, MS; Colter Mitchell, PhD; Caroline M. Nievergelt, PhD; Matthew K. Nock, PhD; Benjamin M. Neale, PhD; Renato Polimanti, PhD; Stephan Ripke, MD; Xiaoying Sun, MS; Michael L. Thomas, PhD; Qian Wang, PhD; Erin B. Ware, PhD; Susan Borja, PhD; Ronald C. Kessler, PhD; Jordan W. Smoller, MD, ScD; for the Army Study to Assess Risk and Resilience in Servicemembers (STARRS) Collaborators

**IMPORTANCE**  Posttraumatic stress disorder (PTSD) is a prevalent, serious public health concern, particularly in the military. The identification of genetic risk factors for PTSD may provide important insights into the biological foundation of vulnerability and comorbidity.

**OBJECTIVE**  To discover genetic loci associated with the lifetime risk for PTSD in 2 cohorts from the Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS).

**DESIGN, SETTING, AND PARTICIPANTS**  Two coordinated genome-wide association studies of mental health in the US military contributed participants. The New Soldier Study (NSS) included 3167 unique participants with PTSD and 4607 trauma-exposed control individuals; the Pre/Post Deployment Study (PPDS) included 947 unique participants with PTSD and 4969 trauma-exposed controls. The NSS data were collected from February 1, 2011, to November 30, 2012; the PPDS data, from January 9 to April 30, 2012. The primary analysis compared lifetime DSM-IV PTSD cases with trauma-exposed controls without lifetime PTSD. Data were analyzed from March 18 to December 27, 2015.

**MAIN OUTCOMES AND MEASURES**  Association analyses for PTSD used logistic regression models within each of 3 ancestral groups (European, African, and Latino American) by study, followed by meta-analysis. Heritability and genetic correlation and pleiotropy with other psychiatric and immune-related disorders were estimated.

**RESULTS**  The NSS population was 80.7% male (6277 of 7774 participants; mean [SD] age, 20.9 [3.3] years); the PPDS population, 94.4% male (5583 of 5916 participants; mean [SD] age, 26.5 [6.0] years). A genome-wide significant locus was found in ANKRD55 on chromosome 5 (rs159572; odds ratio [OR], 1.62; 95% CI, 1.37-1.92; \( P = 2.34 \times 10^{-8} \)) and persisted after adjustment for cumulative trauma exposure (adjusted OR, 1.64; 95% CI, 1.39-1.95; \( P = 1.18 \times 10^{-8} \)) in the African American samples from the NSS. A genome-wide significant locus was also found in or near ZNF626 on chromosome 19 (rs11085374; OR, 0.77; 95% CI, 0.70-0.85; \( P = 4.59 \times 10^{-8} \)) in the European American samples from the NSS. Similar results were not found for either single-nucleotide polymorphism in the corresponding ancestry group from the PPDS sample, in other ancestral groups, or in transancestral meta-analyses. Single-nucleotide polymorphism–based heritability was nonsignificant, and no significant genetic correlations were observed between PTSD and 6 mental disorders or 9 immune-related disorders. Significant evidence of pleiotropy was observed between PTSD and rheumatoid arthritis and, to a lesser extent, psoriasis.

**CONCLUSIONS AND RELEVANCE**  In the largest genome-wide association study of PTSD to date, involving a US military sample, limited evidence of association for specific loci was found. Further efforts are needed to replicate the genome-wide significant association with ANKRD55—associated in prior research with several autoimmune and inflammatory disorders—and to clarify the nature of the genetic overlap observed between PTSD and rheumatoid arthritis and psoriasis.

Published online May 11, 2016. Corrected on July 6, 2016.

Author Affiliations: Author affiliations are listed at the end of this article.

Group Information: The members of the Army STARRS Collaborative Team are listed at the end of the article.

Corresponding Author: Murray B. Stein, MD, MPH, Department of Psychiatry, University of California, San Diego (Mail Code 0855), 9500 Gilman Dr, La Jolla, CA 92039 (mstein@ucsd.edu).
Posttraumatic stress disorder (PTSD) is a common consequence of exposure to extreme, life-threatening events.\textsuperscript{1,2} Posttraumatic stress disorder is also frequently associated with other mental health problems such as major depressive disorder,\textsuperscript{3} substance abuse,\textsuperscript{4} and suicidality\textsuperscript{5,6} with other adverse health sequelae such as obesity,\textsuperscript{7} cardiovascular disease,\textsuperscript{8-10} and type 2 diabetes mellitus\textsuperscript{11,12}; and with immune-related disorders, such as rheumatoid arthritis.\textsuperscript{13}

Although most Americans (50%-85%) experience traumatic events during their lifetime, the lifetime prevalence of PTSD is approximately 7%,\textsuperscript{14} suggesting differential vulnerability to the disorder. Rates of trauma exposure and PTSD are higher among US military personnel and veterans,\textsuperscript{15} particularly those exposed to combat.\textsuperscript{16,17} Much of the research on the risk for PTSD has focused on the differential impact of type,\textsuperscript{18} frequency, duration, and consequences (eg, extent of physical injury) of trauma exposures.\textsuperscript{19} Pretrauma risk factors, including personality characteristics and early life experiences, have also been scrutinized extensively,\textsuperscript{18,20,21} as have posttrauma factors, such as social support.\textsuperscript{22}

Twin studies have long established that genetic variation contributes to risk for PTSD symptoms, with heritability estimates in the range of 0.28 to 0.46.\textsuperscript{23-26} Genetic association studies have focused on a limited set of candidate genes and have been largely underpowered to detect loci of modest effect.\textsuperscript{27} More recently, several genome-wide association studies (GWAS) of PTSD have been reported in civilian\textsuperscript{28,29} and military or veteran\textsuperscript{30-33} samples, yielding several genome-wide significant associations that have yet to be widely replicated.

The present investigation uses data from the Army Study to Assess Risk and Resilience in Servicemembers (Army STARRS),\textsuperscript{34} a large, coordinated set of study components intended to improve understanding of suicide, PTSD, and related mental health risk and resilience in the US Army. Blood samples for DNA were provided by participants in the following 2 components of Army STARRS: a study of new soldiers during their first week of basic training (New Soldier Study [NSS]), and a study of 3 brigade combat teams before their deployment to Afghanistan (Pre/Post Deployment Study [PPDS]). Each of these studies has, to our knowledge, a larger PTSD-affected sample than any previously published genetic study of PTSD. We report herein results from within-ancestral group and within-study genome-wide analyses, followed by meta-analyses across studies.

### Methods

#### Participants
Detailed information about the design and conduct of Army STARRS is available in a separate report\textsuperscript{34} and in the eMethods in the Supplement. The recruitment, consent, human participant, and data protection procedures were approved by the Uniformed Services University of the Health Sciences, Harvard University, University of Michigan, and University of California, San Diego. Written informed consent was obtained from all participants.

#### Key Points

**Question** Are genome-wide significant risk loci associated with posttraumatic stress disorder (PTSD) in a US military sample?

**Finding** In a genome-wide association study, 2 genome-wide significant loci were associated with PTSD in a cohort of new soldiers, but these findings were not replicated in a separate sample of soldiers. We also found significant evidence of genetic pleiotropy between PTSD and rheumatoid arthritis and psoriasis.

**Meaning** These findings should encourage further study of the association of the risk for immune-related disorders and PTSD.

### New Soldier Study
The NSS was performed among new soldiers at the start of their basic training at 1 of 3 army installations from February 1, 2011, to November 30, 2012. Of 39784 NSS participants who completed the computerized, self-administered questionnaire, 33088 (83.2%) provided blood samples. Genotyping was conducted on samples from the first half of the cohort, from which 7999 samples were selected based on phenotype and case-control status (NSS1). When the remaining half of the cohort collection was completed, we selected an informative subset of 2835 samples for genotyping (NSS2) (eMethods in the Supplement).

#### Pre/Post Deployment Survey
The PPDS is a multiple-wave panel survey that collected baseline data (time 0) from US Army soldiers in 3 brigade combat teams from January 9 to April 30, 2012, within approximately 6 weeks of their deployment to Afghanistan. Seven thousand nine hundred twenty-seven PPDS soldiers with eligible self-administered questionnaire responses underwent genotyping for the GWAS.

#### Demographics and Case-Control Status
The population, sex, and age composition of our analyzed sample of cases and controls is shown in Table 1. Most of the participants were male, and we analyzed male and female participants together. A total of 3167 unique PTSD cases and 4607 trauma-exposed controls from NSS1 and NSS2 as well as 947 unique PTSD cases and 4969 trauma-exposed controls from PPDS entered the statistical analysis.

#### Measures
The self-administered questionnaire included a computerized version of the Composite International Diagnostic Interview Screening Scales\textsuperscript{35} and a screening version of the PTSD Checklist (PCL) (range, 17-85, with higher scores indicating worse symptoms).\textsuperscript{36} Trauma exposure was assessed from answers pertaining to childhood, adulthood civilian, and, for PPDS participants, military traumatic events (eMethods in the Supplement). A diagnosis of PTSD was assigned using multiple imputation methods that relied on the PCL and Composite International Diagnostic Interview Screening Scales data; our clinical reappraisal study found satisfactory concordance with independent clinical diagnoses based on blinded Struc-
GWAS of Posttraumatic Stress Disorder in the US Army

Table 1. Ancestry, Sex, and Age Distributions in the Case-Control Samples*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NS1 Participants</th>
<th>NS2 Participants</th>
<th>All NSS Participants</th>
<th>PPDS Participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>All</td>
<td>Case</td>
</tr>
<tr>
<td>Ancestry, No. of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European American</td>
<td>1245</td>
<td>2291</td>
<td>3536</td>
<td>895</td>
</tr>
<tr>
<td>African American</td>
<td>306</td>
<td>664</td>
<td>970</td>
<td>191</td>
</tr>
<tr>
<td>Latino American</td>
<td>306</td>
<td>697</td>
<td>1003</td>
<td>224</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>1446</td>
<td>(77.8)</td>
<td>3052</td>
<td>(83.5)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>20.7</td>
<td>(3.0)</td>
<td>21.3</td>
<td>(3.5)</td>
</tr>
<tr>
<td>Total No. of</td>
<td>1857</td>
<td>3652</td>
<td>5509</td>
<td>1310</td>
</tr>
</tbody>
</table>

Abbreviations: NSS, New Soldier Study; PPDS, Pre/Post Deployment Study.

* Cases with posttraumatic stress disorder (PTSD) are identified through information provided by the PTSD Checklist and Composite International Diagnostic Interview Screening Scales. Controls were exposed to at least 1 nondeployment trauma (NSS1, NSS2, and PPDS) or deployment trauma (PPDS). Cohorts are described in the Participants subsection of the Methods section.

tured Clinical Interviews for DSM-IV (area under the curve, 0.70–0.79; χ, 0.4–0.6).37

DNA Collection and Genotyping

Whole-blood samples were shipped to Rutgers University Cell and DNA Repository, where they were frozen for later DNA extraction using standard methods. The NS1 and PPDS samples underwent genotyping using the OmniExpress and Exome array (Illumina) with additional custom content. The NS2 samples were genotyped on the PsychChip array (Illumina) (eMethods in the Supplement). Imputation, population assignment, and principal component analysis are described in the eMethods and eFigures 1 to 5 in the Supplement.

Statistical Analysis

Data were analyzed from March 18 to December 27, 2015. Lifetime PTSD cases and controls (ie, individuals without lifetime PTSD) reporting at least 1 traumatic event were included in the association analyses. We used PLINK (version 1.9)38 to perform association tests on imputed single-nucleotide polymorphism (SNP) dosage with logistic regression adjusted for the top 10 within-population principal components. The meta-analysis of NS1 and NS2 is the primary analysis. The analysis of the PPDS sample is our internal attempt at replication analysis. We sought external replication with other published military-relevant data sets (described in the Results section below).

Single-nucleotide polymorphism–based heritability was estimated using the GCTA (genome-wide complex trait analysis) software tool.39 We tested the genetic correlation (proportion of variance that phenotypes share owing to genetic causes, which considers only causal variants with the same directionality of effects) and pleiotropy (effect of the same gene on multiple phenotypes, which considers causal variants with the same and opposite effects) of PTSD in all European American samples with psychiatric disorders (including schizophrenia, bipolar disorder, attention-deficit/hyperactivity disorder, major depressive disorder, autism spectrum disorder, and a cross-disorder phenotype44) and with immune-related disorders (including Crohn disease,45 ulcerative colitis,46 multiple sclerosis,47 psoriasis,48 rheumatoid arthritis,49 systemic lupus erythematosus,50 celiac disease,51 primary biliary cirrhosis,52 and type 1 diabetes mellitus53) using linkage disequilibrium (LD) score regression54 and the GPA (Genetic Analysis Incorporating Pleiotropy and Annotation) R package,55 respectively.

We followed up the significant pleiotropic outcomes with enrichment analysis using DEPICT (Data-driven Expression Prioritized Integration for Complex Traits), version 156 and DAVID (Database for Annotation, Visualization and Integrated Discovery), version 6.7.57 Further details are available in the eMethods in the Supplement.

Results

Genome-wide Association Analyses

The NS1 population was 80.7% male (6277 of 7774 participants; mean [SD] age, 20.9 [3.3] years); the PPDS population, 94.4% male (5583 of 5916 participants; mean [SD] age, 26.5 [6.0] years). The λGC and the QQ plot showed negligible inflation of association P values in the NS (meta-analysis of NS1 and NS2) and PPDS samples (eFigure 6 in the Supplement). An SNP on chromosome 19 was significantly associated with PTSD in the NS results in European American samples (rs11085374; odds ratio [OR], 0.77; 95% CI, 0.70–0.85; P = 4.59 × 10−6). A SNP on chromosome 5 was significantly associated with PTSD in the NS results in African American samples (rs159572; OR, 1.62; 95% CI, 1.37–1.92; P = 2.34 × 10−6) and persisted after adjustment for cumulative trauma exposure (adjusted OR, 1.64; 95% CI, 1.39–1.95; P = 1.18 × 10−6). We did not find similar results for either SNP in the corresponding ancestry groups from the PPDS sample. The individual study and meta-analysis results are presented in Table 2, and the Manhattan plots in NSS African American and European American samples are shown in Figure 1. We further created
No significant associations were observed in the Latino NSS or PPDS samples or in any of the transethnic meta-analyses. Adjustment for lifetime trauma exposure slightly strengthened the genome-wide significant associations for the 2 lead SNPs (eTable 1 in the Supplement), whereas simultaneous adjustment for lifetime trauma exposure, sex, and age slightly attenuated the associations (eTable 2 and eFigure 8 in the Supplement).

The top SNP, rs159572 (eFigure 7A in the Supplement), on chromosome 5 is intronic to the ankyrin repeat domain 55 gene (ANKRD55 [NCBI Entrez Gene 79722]), and multiple other SNPs in this region were in LD with this SNP. ANKRD55 has been associated with several autoimmune diseases, including multiple sclerosis, type 2 diabetes mellitus, celiac disease, and rheumatoid arthritis. The top SNP, rs11085374, on chromosome 19 is located near the zinc finger protein 626 gene (ZNF626 [NCBI Entrez Gene 199777]). We found minimal LD between this SNP and surrounding SNPs (eFigure 7B in the Supplement), and no other SNPs in the region showed evidence of association.

### Meta-analysis With Other Military Data Sets
We performed a meta-analysis of the results for SNP rs159572 on chromosome 5 between 3 GWAS of African American military samples, including data reported herein from Army STARRS (the current analysis), the Marine Resiliency Study, and a recently published genetic study of Iraq-Afghanistan US veterans (Figure 2). The results were directionally consistent in the Army STARRS NSS and PPDS samples as well as the Marine Resiliency Study but not in the PTSD veteran GWAS (OR for meta-analysis, 1.17; 95% CI, 1.05-1.31).

### Alternate Phenotypic Characterization
To examine the robustness of our findings, we tested for association of the top 2 SNPs at the chromosome 19 and 5 loci with an alternate phenotypic characterization of PTSD; all participants were administered the 17-item Patient Health Questionnaire Depression Scale (PHQ-17).

### Table 2. Genome-wide Significant Loci in the NSS1, NSS2, and PPDS Individual Analyses and Meta-analyses From the Standard GWAS Analysis

<table>
<thead>
<tr>
<th>Population</th>
<th>Study Sample</th>
<th>FRQ</th>
<th>INFO</th>
<th>P Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr 5/SNP rs159572*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European American NSS1</td>
<td>0.75</td>
<td>1.00</td>
<td>.93</td>
<td>1.00 (0.89-1.12)</td>
<td></td>
</tr>
<tr>
<td>NSS2</td>
<td>0.73</td>
<td>0.98</td>
<td>.045</td>
<td>0.84 (0.71-0.997)</td>
<td></td>
</tr>
<tr>
<td>NSS-MA NA</td>
<td>NA</td>
<td>.23</td>
<td>0.94 (0.86-1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPDS</td>
<td>0.73</td>
<td>1.00</td>
<td>.36</td>
<td>0.94 (0.82-1.07)</td>
<td></td>
</tr>
<tr>
<td>All-MA NA</td>
<td>NA</td>
<td>.13</td>
<td>0.94 (0.87-1.02)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American NSS1</td>
<td>0.46</td>
<td>1.04</td>
<td>4.91 × 10⁻⁶</td>
<td>1.57 (1.29-1.91)</td>
<td></td>
</tr>
<tr>
<td>NSS2</td>
<td>0.44</td>
<td>0.95</td>
<td>1.16 × 10⁻³</td>
<td>1.74 (1.25-2.43)</td>
<td></td>
</tr>
<tr>
<td>NSS-MAb NA</td>
<td>NA</td>
<td>2.34 × 10⁻⁸</td>
<td>1.62 (1.37-1.92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPDS</td>
<td>0.47</td>
<td>1.03</td>
<td>.28</td>
<td>1.18 (0.87-1.60)</td>
<td></td>
</tr>
<tr>
<td>All-MA NA</td>
<td>NA</td>
<td>6.13 × 10⁻⁶</td>
<td>1.51 (1.30-1.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latino American NSS1</td>
<td>0.68</td>
<td>1.03</td>
<td>.52</td>
<td>1.07 (0.87-1.31)</td>
<td></td>
</tr>
<tr>
<td>NSS2</td>
<td>0.67</td>
<td>0.96</td>
<td>.21</td>
<td>0.82 (0.60-1.12)</td>
<td></td>
</tr>
<tr>
<td>NSS-MA NA</td>
<td>NA</td>
<td>.88</td>
<td>0.99 (0.83-1.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPDS</td>
<td>0.69</td>
<td>1.09</td>
<td>.07</td>
<td>0.81 (0.65-1.02)</td>
<td></td>
</tr>
<tr>
<td>All-MA NA</td>
<td>NA</td>
<td>.23</td>
<td>0.92 (0.80-1.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chr 19/SNP rs11085374c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European American NSS1</td>
<td>0.32</td>
<td>0.98</td>
<td>3.67 × 10⁻⁶</td>
<td>0.77 (0.69-0.86)</td>
<td></td>
</tr>
<tr>
<td>NSS2</td>
<td>0.30</td>
<td>0.81</td>
<td>3.62 × 10⁻³</td>
<td>0.77 (0.65-0.92)</td>
<td></td>
</tr>
<tr>
<td>NSS-MAb NA</td>
<td>NA</td>
<td>4.59 × 10⁻⁸</td>
<td>0.77 (0.70-0.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPDS</td>
<td>0.31</td>
<td>0.97</td>
<td>.50</td>
<td>1.05 (0.91-1.21)</td>
<td></td>
</tr>
<tr>
<td>All-MA NA</td>
<td>NA</td>
<td>5.40 × 10⁻⁸</td>
<td>0.86 (0.80-0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American NSS1</td>
<td>0.51</td>
<td>0.90</td>
<td>.63</td>
<td>0.95 (0.77-1.17)</td>
<td></td>
</tr>
<tr>
<td>NSS2</td>
<td>0.50</td>
<td>0.67</td>
<td>.62</td>
<td>1.10 (0.75-1.61)</td>
<td></td>
</tr>
<tr>
<td>NSS-MAb NA</td>
<td>NA</td>
<td>.50</td>
<td>0.98 (0.82-1.18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPDS</td>
<td>0.51</td>
<td>0.85</td>
<td>.79</td>
<td>0.96 (0.68-1.34)</td>
<td></td>
</tr>
<tr>
<td>All-MA NA</td>
<td>NA</td>
<td>.77</td>
<td>0.98 (0.83-1.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latino American NSS1</td>
<td>0.27</td>
<td>0.92</td>
<td>.08</td>
<td>1.23 (0.98-1.54)</td>
<td></td>
</tr>
<tr>
<td>NSS2</td>
<td>0.25</td>
<td>0.76</td>
<td>.65</td>
<td>1.09 (0.74-1.61)</td>
<td></td>
</tr>
<tr>
<td>NSS-MAb NA</td>
<td>NA</td>
<td>.08</td>
<td>1.19 (0.98-1.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPDS</td>
<td>0.26</td>
<td>0.93</td>
<td>.34</td>
<td>0.87 (0.66-1.16)</td>
<td></td>
</tr>
<tr>
<td>All-MA NA</td>
<td>NA</td>
<td>.38</td>
<td>1.08 (0.92-1.26)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** All-MA, meta-analysis of NSS1, NSS2, and PPDS results; Chr, chromosome; FRQ, allele frequency; GWAS, genome-wide association study; INFO, imputation quality score; NA, not applicable; NNS, New Soldier Study; NSS-MA, meta-analysis of NSS1 and NSS2 results; OR, odds ratio; PPDS, Pre/Post Deployment Study; SNP, single-nucleotide polymorphism.

* Position 55507046 is in NCBI Build 37/UCSC hg19 coordinates. The nearest gene is ANKRD55; FRQs and ORs shown are for allele A, and allele C is the reference allele.

b Indicates genome-wide significance (P < 5 × 10⁻⁸).

c Position 20906220 is in NCBI Build 37/UCSC hg19 coordinates. The nearest gene is ZNF626; FRQs and ORs shown are for allele A, and allele T is the reference allele.
Participants in the respective ancestral groups were included. For this purpose, we chose a dimensional measure of lifetime worst PTSD severity from a 6-item version of the PCL (PCL-6) (range, 6-30, with higher scores indicating worse symptoms) that has been used in other published Army STARRS research reports. Among European American participants, the number of risk alleles (0-2) for rs11085374 was nominally significantly associated with lifetime PCL-6 severity in the NSS1 (P = .007) and NSS2 (P < .001) but not PPDS (P = .82) samples. Among African American participants, the number of risk alleles (0-2) for rs159572 was nominally significantly associated with lifetime PCL-6 severity in the NSS1 (P = .002) and NSS2 (P = .03) but not PPDS (P = .42) samples.

**Heritability of Lifetime PTSD Phenotype**

We estimated SNP-based heritability (\(h^2_g\)) using the GCTA software tool\(^{39}\) in European American samples for the NSS1, NSS2, PPDS, and all cohorts pooled together. We found no significant \(h^2_g\) estimates in overall (\(h^2_g = 0.062\) [SE, 0.049]; P = .10) or sex-specific analyses (eTable 3 in the Supplement).

**Pleiotropy and Genetic Correlation**

We tested the pleiotropy shared by PTSD and 6 psychiatric disorders and 9 immune-related disorders in the European American samples (Table 3). Significant pleiotropy was observed for PTSD and rheumatoid arthritis (\(P = 3.04 \times 10^{-7}\)) and psoriasis (\(P = 2.41 \times 10^{-3}\)). No significant pleiotropy was observed between PTSD and the other psychiatric disorders tested. No sig-
significant genetic correlations were found in the same data sets (eTable 4 in the Supplement).

To further characterize the observed pleiotropy, we performed an enrichment analysis of SNPs with pleiotropy posterior probability of greater than 0.5. For pleiotropy for PTSD and rheumatoid arthritis, we observed several significant enrichments for medical subject headings for tissue and cell type annotations (eTable 5 in the Supplement) and gene ontology terms (eTable 6 in the Supplement) related to several immune systems and functions. No enrichment was present for pleiotropy for PTSD and psoriasis. Finally, we estimated that the probability for an SNP associated with PTSD to be a central nervous system SNP is 2.28 (SE, 0.24) times the probability for an SNP not associated with PTSD to be a central nervous system SNP. Similarly, the enrichment ratio for immune-related expression quantitative trait loci in PTSD is 2.36 (SE, 0.27).

Discussion

This study is, to our knowledge, the largest GWAS of PTSD conducted to date. Because the study is reflective of the US Army, the composition of the samples was ethnically diverse, obligating us to conduct initial association analyses within ancestral groups and then to attempt transancestral meta-analyses. We found no genome-wide significant loci at the level of the transancestral meta-analyses but found 2 genome-wide significant loci, one each in the African American and European American samples from the NSS.

In the African American NSS sample, we observed genome-wide significant association with PTSD for SNPs on chromosome 5 in ANKRDS55. Inclusion of data from African American participants from additional military cohorts continued to yield a significant result of the meta-analysis at this locus, albeit attenuated compared with that of the NSS alone (Figure 2). This gene, whose function is currently unknown, has been reported to be associated with a range of autoimmune and inflammatory disorders, including multiple sclerosis, type 2 diabetes mellitus, celiac disease, and rheumatoid arthritis.

We also found evidence of significant pleiotropy between PTSD and 2 immune-related disorders, namely, rheumatoid arthritis and, to a lesser extent, psoriasis. We may have seen this genetic overlap in European American participants (whereas the ANKRDS55 association finding was in African American individuals) because human populations can present ancestry-specific risk alleles in the context of similar underlying biological mechanisms of disease predisposition.

These novel findings are consistent with recent reports of pleiotropy between other mental disorders, such as schizophrenia, and immune disorders, such as rheumatoid arthritis and multiple sclerosis. In the context of new evidence that schizophrenia involves allelic variation in the major histocompatibility complex, these observations suggest that intensive scrutiny of immune factors, and perhaps in particular of complement component 4, should be the subject of further study in other mental disorders, such as PTSD.

A hypothetical immune-related or inflammatory cause of PTSD has, in fact, gained some empirical support. To further characterize the observed pleiotropy, we performed an enrichment analysis of SNPs with pleiotropy posterior probability of greater than 0.5. For pleiotropy for PTSD and rheumatoid arthritis, we observed several significant enrichments for medical subject headings for tissue and cell type annotations (eTable 5 in the Supplement) and gene ontology terms (eTable 6 in the Supplement) related to several immune systems and functions. No enrichment was present for pleiotropy for PTSD and psoriasis. Finally, we estimated that the probability for an SNP associated with PTSD to be a central nervous system SNP is 2.28 (SE, 0.24) times the probability for an SNP not associated with PTSD to be a central nervous system SNP. Similarly, the enrichment ratio for immune-related expression quantitative trait loci in PTSD is 2.36 (SE, 0.27).

Discussion

This study is, to our knowledge, the largest GWAS of PTSD conducted to date. Because the study is reflective of the US Army, the composition of the samples was ethnically diverse, obligating us to conduct initial association analyses within ancestral groups and then to attempt transancestral meta-analyses. We found no genome-wide significant loci at the level of the transancestral meta-analyses but found 2 genome-wide significant loci, one each in the African American and European American samples from the NSS.

In the African American NSS sample, we observed genome-wide significant association with PTSD for SNPs on chromosome 5 in ANKRDS55. Inclusion of data from African American participants from additional military cohorts continued to yield a significant result of the meta-analysis at this locus, albeit attenuated compared with that of the NSS alone (Figure 2). This gene, whose function is currently unknown, has been reported to be associated with a range of autoimmune and inflammatory disorders, including multiple sclerosis, type 2 diabetes mellitus, celiac disease, and rheumatoid arthritis.

We also found evidence of significant pleiotropy between PTSD and 2 immune-related disorders, namely, rheumatoid arthritis and, to a lesser extent, psoriasis. We may have seen this genetic overlap in European American participants (whereas the ANKRDS55 association finding was in African American individuals) because human populations can present ancestry-specific risk alleles in the context of similar underlying biological mechanisms of disease predisposition.

These novel findings are consistent with recent reports of pleiotropy between other mental disorders, such as schizophrenia, and immune disorders, such as rheumatoid arthritis and multiple sclerosis. In the context of new evidence that schizophrenia involves allelic variation in the major histocompatibility complex, these observations suggest that intensive scrutiny of immune factors, and perhaps in particular of complement component 4, should be the subject of further study in other mental disorders, such as PTSD.

A hypothetical immune-related or inflammatory cause of PTSD has, in fact, gained some empirical support. Two recent studies have found elevated levels of the inflammatory biomarker C-reactive protein in individuals at risk for PTSD or with PTSD. Other studies have found abnormal cytokine regulation or other evidence of a proinflammatory milieu in PTSD. Posttraumatic stress disorder is itself highly comorbid with several of the disorders associated with ANKRDS55, including type 2 diabetes mellitus and rheumatoid arthritis. Moreover, a recent epidemiologic study of Iraq and Afghanistan military veterans found PTSD to be associated with a broad range of autoimmune disorders, including inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis. Further research is needed to determine whether variation in ANKRDS55—or other genes contributing to the observed pleiotropy accounts for these associations. Why the association of ANKRDS55 with PTSD is apparently restricted to African American individuals should also be determined, although this restriction may be explained by differing LD block structure and increased minor allele frequency.

We also detected in the European American NSS samples 1 genome-wide significant SNP on chromosome 19 near ZNF626, a gene thought to be involved in the regulation of RNA transcription. The regional plot showed no other associated SNPs in LD with this result. This finding may represent a spurious association, but it also may reflect a lack of nearby variants in LD with the index SNP, which requires further study.

Genome-wide significant results from the NSS were not replicated in the PPDS. The PPDS sample was smaller and distinct in important ways from the NSS sample. Participants in the NSS were younger (mostly 18-20 years of age), and their trauma exposure and resultant PTSD were entirely premilitary. In contrast, PPDS participants were older, their mean nonmilitary trauma exposure was higher than in the NSS (reflect-
ing the accrual of traumatic exposures over time), and many participants had additionally experienced deployment-related traumas. This finding of consistent results in identically ascertained samples (ie, NSI1 and NSI2) but inconsistent results in a second military sample with different rates and types of trauma exposure serves as a reminder of the challenges this field will face in working across and combining data sets that include individuals with heterogeneous trauma experiences.78

In this regard, adjustment for trauma exposure tended to increase the statistical significance of genome-wide or near-genome-wide significant SNPs. We know, however, that certain types of trauma have higher conditional risks for PTSD than others.13 Therefore, adjustment for trauma based on tallying exposure to different trauma types—without taking into account differential conditional risks for PTSD for certain trauma types—might inadequately model these relationships. Our results underscore the need for additional work to determine the appropriate metrics for trauma exposure and the optimal functional forms for modeling these outcomes in genetic data sets. For example, when these effects should be modeled by covarying for trauma exposure or when interactions—with overall trauma severity or with specific trauma types—should be considered remains unclear. Well-powered gene- and environment-wide interaction studies may be especially illuminating given observations that the interaction of PTSD susceptibility genes with early trauma (eg, childhood maltreatment) exposure may be paramount.79

The cross-phenotype LD score regression results failed to provide evidence of genetic correlation between PTSD and the other mental disorders examined. Previous studies have reported evidence of shared genetic risk between PTSD and bipolar disorder.31,80 Clinical and genetic epidemiologic studies have found considerable comorbidity—at least some of which is explained by shared genetic vulnerability.26,81,82—between PTSD and major depressive disorder and attention-deficit/hyperactivity disorder.83,84 Insufficient power is a possible explanation for our study’s failure to find evidence of a shared genetic risk across these disorders. However, enrichment analysis suggested that risk variants for PTSD aggregate in many of the same biological pathways shared with other neuropsychiatric disorders, notably those involved in immune regulation.85

Our results should be interpreted in light of several additional limitations. First, samples sizes—especially within ancestral groups—are likely to be insufficiently powered to detect loci of modest effect. Given our total sample size, we would have 80% power to detect associations for SNPs with 20% minor allele frequency with an OR of 1.2 or higher. Second, the genetic correlation of the risk for trauma exposure with risk for PTSD is well established.24,46,87 Therefore, although exclusion of control individuals with no trauma exposure should have improved our power to detect PTSD risk loci given trauma exposure, it may have reduced our ability to detect loci that contribute to PTSD by increasing the risk for trauma. Third, our finding of no apparent heritability emanating from the GCTA analyses may be owing to insufficient power. Fourth, the use of the GPA package to detect pleiotropy is quite novel, and here-tofore unappreciated limitations in this approach may exist.

Conclusions

We found no genome-wide significant evidence that transcended ancestry and replicated across studies. We did, however, find genome-wide significant evidence of an association of ANKR55, a gene previously associated with inflammatory and immune disorders, with PTSD in African American participants. This association was observed in a pre-military PTSD sample (NSS), not replicated in a mixed pre-military and military PTSD sample (PPDS), but showed similar effect size and directionality in an independent sample of Marines with PTSD (Marine Resiliency Study).31 This association is small in magnitude and, even if replicated, would be of no obvious clinical utility at present. Its value may lie, however, in eventual elucidation of the nature of PTSD and its association with other illnesses. The finding of pleiotropy between PTSD and rheumatoid arthritis and psoriasis should further motivate the study of immune-related factors in PTSD, their potential contribution to comorbidity with inflammatory disorders, and a possible role for anti-inflammatory treatments in PTSD.88

ARTICLE INFORMATION

Correction: This article was corrected on July 6, 2016, to fix 2 typographical errors in the Abstract.

Submitted for Publication: October 10, 2015; final revision received February 6, 2016; accepted February 9, 2016.


Author Affiliations: Department of Psychiatry, University of California, San Diego (UCSD), La Jolla (Stein, Malhofer, Nievergelt, Thomas); Department of Family Medicine and Public Health, UCSD, La Jolla (Stein, Jain, Sun); Psychiatry Service, Veterans Affairs San Diego Healthcare System, San Diego, California (Stein); Department of Psychiatry, Massachusetts General Hospital and Harvard Medical School, Boston (Chen, Smoller); Stanley Center for Psychiatric Research, Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge (Chen, Neale, Ripke, Smoller); Department of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, Maryland (Ursano); Harvard T H Chan School of Public Health, Boston, Massachusetts (Cai); Department of Psychiatry, Yale University, New Haven, Connecticut (Gelernter, Jensen, Polimanti, Wang); Department of Genetics, Yale University, New Haven, Connecticut (Gelernter, Polimanti, Wang); Department of Neuropsychology, Yale University, New Haven, Connecticut (Gelernter, Polimanti, Wang); Institute for Social Research, University of Michigan, Ann Arbor (Heeringa, Mitchell, Ware); Department of Psychology, Harvard University, Cambridge, Massachusetts (Nock); currently with Department of Computational Biology and Bioinformatics, Graduate School of Arts and Sciences, Yale University, New Haven, Connecticut (Wang); National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland (Borja); Department of Health Care Policy, Harvard Medical School, Boston, Massachusetts (Kessler).

Author Contributions: Dr Stein had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Stein, Chen, Ursano, Gelernter, Heeringa, Jain, Mitchell, Nock, Borja, Kessler, Smoller.

Acquisition, analysis, or interpretation of data: Stein, Chen, Ursano, Gelernter, Heeringa, Jain, Jensen, Malhofer, Mitchell, Nievergelt, Neale, Polimanti, Ripke, Sun, Thomas, Wang, Ware, Borja, Kessler, Smoller.

Drafting of the manuscript: Stein, Chen, Ursano, Mitchell, Nievergelt, Wang, Smoller.

Critical revision of the manuscript for important intellectual content: Stein, Chen, Ursano, Cai, Gelernter, Heeringa, Jain, Jensen, Malhofer, Mitchell, Nievergelt, Neale, Polimanti, Ripke, Sun, Thomas, Wang, Ware, Borja, Kessler, Smoller.

JAMA Psychiatry July 2016 Volume 73, Number 7 701

Copyright 2016 American Medical Association. All rights reserved.
GWAS of Posttraumatic Stress Disorder in the US Army


Obtained funding: Stein, Ursano, Gelernter, Heeringa, Nock, Kessler. Administrative, technical, or material support: Ursano, Heeringa, Mitchell, Thomas, Borja, Kessler. Study supervision: Ursano, Gelernter, Mitchell, Nievergelt, Smoller.

Conflict of Interest Disclosures: Dr Stein reports serving as a consultant for Healthcare Management Technologies, Actelion, Dart Neuroscience, Janssen, Oxeia Biopharmaceuticals, Pfizer, Resilience Therapeutics, and Toxin Pharmaceuticals in the last 3 years. Dr Kessler reports serving as a consultant for Hoffman-La Roche, Inc, Johnson & Johnson Wellness and Prevention, and Sanofi Groupe in the last 3 years; serving on advisory boards for Manserte Corporation, Plus One Health Management, Lake Nona Institute, and US Preventive Medicine, and ownership of a 25% share in DataStat, Inc. Dr Smoller reports serving as an unpaid member of the Scientific Advisory Board of PsyBrain, Inc. No other disclosures were reported.

Funding/Support: This study was sponsored by the Department of the Army and supported by cooperative agreement U01MH087981 from the US Department of Health and Human Services, National Institutes of Health, National Institute of Mental Health (NIMH) (Dr Stein and Ursano). The GPA analyses were supported by grant ROI DA12690 from the National Institute of Drug Abuse (Dr Gelernter).

Role of the Funder/Sponsor: The funding sources 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.

Group Information: The Army STARRS Collaborative Team consists of coprincipal investigators Robert J. Ursano, MD (Uniformed Services University of the Health Sciences) and Murray B. Stein, MD, MPH (University of California, San Diego [UCSD] and Veterans Affairs San Diego Healthcare System); site principal investigators Steven G. Heeringa, PhD (University of Michigan) and Ronald C. Kessler, PhD (Harvard Medical School); NIMH collaborating scientists Lisa J. Colpe, PhD, MPH, and Michael Schoenbaum, PhD; Army liaisons/consultants COL Steven Cersovsky, MD, MPH (US Army Public Health Center [USAPHC]), and Kenneth Cox, MD, MPH (USAPHC). Other team members include Pablo A. Aliaia, MA (Uniformed Services University of the Health Sciences), COL David M. Benedek, MD (Uniformed Services University of the Health Sciences), K. Nikki Benedeis, MA (Uniformed Services University of the Health Sciences), Paul D. Bilese, PhD (University of South Carolina), Susan Borja, PhD (NIMH), Evelyn J. Bromet, PhD (Stony Brook University School of Medicine), Gregory G. Brown, PhD (UCSD), Christina Buckley, BA (Uniformed Services University of the Health Sciences), Laura Campbell-Sills, PhD (UCSD), Tianci Cai, ScD (Harvard T. Chan School of Public Health), Chia-Yen Chen, ScD (Massachusetts General Hospital), Catherine L. Dempsey, PhD, MPH (Uniformed Services University of the Health Sciences), Julie O. Denenberg, MA, UCSD, Carol S.


