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Genome-wide Association Study of Posttraumatic Stress Disorder in a Cohort of Iraq- Afghanistan era Veterans

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Abstract

Background—Posttraumatic stress disorder (PTSD) is a psychiatric disorder that can develop after experiencing traumatic events. A genome-wide association study (GWAS) design was used to identify genetic risk factors for PTSD within a multi-racial sample primarily composed of U.S. veterans.

Methods—Participants were recruited at multiple medical centers, and structured interviews were used to establish diagnoses. Genotypes were generated using three Illumina platforms and imputed with global reference data to create a common set of SNPs. SNPs that increased risk for PTSD were identified with logistic regression, while controlling for gender, trauma severity, and population substructure. Analyses were run separately in non-Hispanic black (NHB; n=949) and

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non-Hispanic white (NHW; n=759) participants. Meta-analysis was used to combine results from the two subsets.

Results—SNPs within several interesting candidate genes were nominally significant. Within the NHB subset, the most significant genes were *UNC13C* and *DSCAM*. Within the NHW subset, the most significant genes were *TBC1D2*, *SDC2* and *PCDH7*. In addition, *PRKG1* and *DDX60L* were identified through meta-analysis. The top genes for the three analyses have been previously implicated in neurologic processes consistent with a role in PTSD. Pathway analysis of the top genes identified alternative splicing as the top GO term in all three analyses (FDR $q < 3.5 \times 10^{-5}$).

Limitations—No individual SNPs met genome-wide significance in the analyses.

Conclusions—This multi-racial PTSD GWAS identified biologically plausible candidate genes and suggests that post-transcriptional regulation may be important to the pathology of PTSD; however, replication of these findings is needed.

Keywords

Posttraumatic stress disorder; Combat exposure; Genome-wide association study; Meta-analysis; Gene*environment interaction

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a complex psychiatric disorder that can develop following exposure to traumatic events (Kessler et al., 2005). Individuals with PTSD are at increased risk for substance use disorders, major depressive disorder, occupational and interpersonal impairment, physical illness, and early mortality (Del Gaizo et al., 2011; Flood et al., 2010; Greenberg et al., 1999). PTSD is relatively common, with an estimated lifetime prevalence of 6.8% in the general population (Kessler et al., 2005; Kilpatrick et al., 2013). In the United States (US), public awareness of PTSD has dramatically increased as a result of its high prevalence in Iraq/Afghanistan era veterans; a recent meta-analysis suggests the prevalence rate is 23% among Operations Enduring Freedom and Iraq Freedom (OEF/OIF) veterans (Fulton, 2015). The increased awareness of PTSD among US veterans has created a sense of urgency in the search for underlying etiologic mechanisms. The societal costs of PTSD extend well beyond the specific symptoms of the disorder. For example, individuals with PTSD receive twice the non-mental health care as individuals without the disorder (Cohen et al., 2010). A recent report by the Congressional Budget Office estimates that the Veteran's Administration (VA) alone spent \$2 billion in 2010 to treat veterans with PTSD (Office, 2012). Taken together, these individual, societal and financial costs provide compelling evidence that understanding and treating PTSD should be a healthcare priority.

However, dissecting the etiology of PTSD has been challenging. It is expected that multiple gene*environment interactions underlie the pathology. Exposure to traumatic events is a necessary, but not sufficient, environmental risk factor for developing PTSD. That is, most individuals are exposed to at least one traumatic event over the course of their lifetimes (Breslau and Kessler, 2001), but as described above, only a fraction of individuals will subsequently develop PTSD (Kessler et al., 2005). The additional variability in PTSD risk is expected to arise from genetic susceptibility. Indeed, between 30% and 70% of the variance

for PTSD risk is estimated to be attributed to genetic factors by family and twin heritability studies (Kremen et al., 2012; Lyons et al., 1993; Sack et al., 1995; Sartor et al., 2011; Stein et al., 2002; True et al., 1993; Yehuda et al., 2001). To identify the specific genetic risk factors contributing to PTSD, many candidate gene association studies have been performed in human cohorts on genes in neurobiological pathways, including the hypothalamic-pituitary-adrenal (HPA) axis, the locus coeruleus-noradrenergic system, monoamine and neuroendocrine metabolism, and the limbic-frontal system (Cornelis et al., 2010; Kim et al., 2013; Logue et al., 2013b; Lu et al., 2008; Lyons et al., 2013; Norrholm and Ressler, 2009; Pitman et al., 2012; Uddin et al., 2011; Valente et al., 2011; Wang et al., 2011; Wolf et al., 2013; Yehuda et al., 2011). More recently, the emphasis in PTSD genetics has been on genome-wide association studies (GWAS). Thus far, four GWAS for PTSD have been published (Guffanti et al., 2013; Logue et al., 2013a; Nievergelt et al., 2015; Xie et al., 2013). The effect sizes of these GWAS hits on PTSD risk were relatively large for complex genetic risk factors, with odds ratios of the associated SNPs ranging from 1.4 to 3.7. However, despite these putatively large genetic effects, each of the previously published GWAS studies identified different genes as the top hits and none of the top hits (SNPs with p -values $<10^{-5}$) overlapped. There are several plausible explanations for the lack of replication across these studies. Two of the studies (Logue et al., 2013a; Nievergelt et al., 2015) examined veteran populations whose primary type of traumatic exposure was combat, whereas the other studies (Guffanti et al., 2013; Xie et al., 2013) focused on civilian populations. One study (Xie et al., 2013) examined both non-Hispanic black (NHB) and non-Hispanic white (NHW) individuals, while others analyzed a multi-racial group (Nievergelt et al., 2015) or focused on women (Guffanti et al., 2013). Thus, heterogeneity of results could be due to differences in trauma type, race and/or gender. Additionally, the likely polygenic and gene*environment interactions underlying PTSD pathology contributed further to the heterogeneity of results. The only GWAS hit that has demonstrated some degree of replication was the RORA gene. This locus was originally identified by GWAS (Logue et al., 2013a), and subsequently some evidence of association was identified by replication in two other studies (Guffanti et al., 2014; Nievergelt et al., 2015), one of which included the current data set (Guffanti et al., 2014).

Objective of the Current Study

The purpose of the present study was to continue the search for genetic risk factors for PTSD by conducting a GWAS in a large sample comprised primarily of Iraq/Afghanistan veterans. Because there was approximately equal representation from non-Hispanic White (NHW) and non-Hispanic Black (NHB) individuals in the sample, we performed population specific analyses, and a meta-analysis combining the two subsets.

METHODS

Study participants

Participants included 1929 Iraq/Afghanistan-era veterans from the Veterans Affairs Mid-Atlantic Mental Illness Research, Education and Clinical Center (MIRECC) Study of Post-Deployment Mental Health (described in detail in (Calhoun et al., 2010) as well as 383 community civilians and all-era veterans enrolled in other trauma research studies at the

Durham VA Medical Center and Duke University Medical Center who consented to donate a blood sample for genetic analysis (total eligible $n=2312$). IRB approval was obtained prior to all of these studies, and all participants provided informed consent prior to their study participation. The research was conducted in a manner consistent with the Helsinki Declaration of 1989. The current analyses were limited to NHB ($n=949$) and NHW ($n=759$) participants from these studies who consented to the genetic aspects of the respective studies and had genetic data available for analysis at the time of the present study.

Phenotypic Measures

The Structured Clinical Interview for DSM-IV Disorders (SCID; (First, 1994) was used to diagnose PTSD in the MIRECC sample of Iraq/Afghanistan-era veterans, whereas the Clinician-Administered PTSD Scale (CAPS; (Blake et al., 1995) was used to diagnose PTSD among the cohort of community civilians and all-era veterans. Both interviews have strong psychometric properties, including good inter-rater reliability (Lobbestael et al., 2011; Weathers et al., 2001). Interviewers in all studies received extensive training and on-going supervision from clinical psychologists with expertise in the assessment and treatment of PTSD. In a limited number of cases ($n=99$), SCID or CAPS diagnostic data was not available for participants who were otherwise eligible to participate. In these cases, the Davidson Trauma Scale (DTS; (Davidson et al., 1997) was employed to categorize participants as either cases (DTS ≥ 75) or controls (DTS ≤ 24). These cutoffs were chosen in order to maximize the identification of true cases (positive predictive value of a score of 75 or greater = 0.96) and true non-cases (negative predictive value of 24 or less = 0.96; (McDonald et al., 2009)). The DTS is a 17-item self-report measure of DSM-IV-TR PTSD symptoms with strong psychometric properties, including excellent diagnostic efficiency (Davidson et al., 1997; McDonald et al., 2014). Trauma exposure was measured with the Traumatic Life Events Questionnaire (TLEQ; (Kubany et al., 2000), which is a 23-item self-report measure that assesses a wide range of possible traumatic experiences (e.g., abuse, natural disasters, combat exposure). When participants endorse a traumatic event on the TLEQ, they are also asked if the event caused them intense, fear, helplessness, or horror (i.e., Criterion A2 for a DSM-IV PTSD diagnosis). In addition, participants are asked to provide the number of times that each endorsed event occurred (never, once, twice, three times, four times, five times, or 6 or more times) which enables a total traumatic events score to be calculated. To qualify as “trauma-exposed” in the current analyses (which were based on DSM-IV-TR criteria), participants had to endorse the PTSD Criterion A2 (i.e., fear, helplessness, or horror) from DSM-IV-TR in response to at least one of the traumatic events they endorsed on the TLEQ. Participant characteristics by race are provided in Table 1.

Genotyping

DNA was extracted from whole blood using the Puregene system (Gentra Systems, Minneapolis, MN). Whole-genome genotyping data was generated in three different batches using three different platforms. A total of 2312 samples were genotyped: 587 samples with the Illumina HumanHap650 Beadchip, 545 samples with the Illumina Human1M-Duo Beadchip, and 1180 samples with the Illumina HumanOmni2.5 Beadchip (Illumina, San Diego, CA). Centre d’Etude du Polymorphisme Humain (CEPH) samples and masked sample

duplicates were included as controls. Each batch was analyzed using the GenomeStudio software (Illumina, San Diego, CA) and subsequently passed through QC pipelines separately. Samples were required to have a call rate > 98% (n=7 samples excluded) and no gender discrepancies (n=7 samples excluded). Identity by state analysis was performed using PLINK (Purcell et al., 2007) to identify duplicate individuals (n=6 samples excluded) and those related > 50% (n=16 samples excluded). To reduce effects of population stratification, participants who did not self-report as either NHW or NHB were removed (n=113). Additionally, principal components analysis (PCA) was run using the smartpca program from the software package EIGENSOFT (Patterson et al., 2006) in order to identify remaining outliers (n=22 excluded). Finally, the percentage of European admixture in the NHB samples was assessed using the linkage model in STRUCTURE (Falush et al., 2003) and those with European ancestry > 80% were removed (n=2). As such, 2139 samples passed initial genotyping quality control checks. Probes were required to have a call rate > 97% and Hardy-Weinberg Equilibrium (HWE) p-values > 10^{-6} in controls.

Genetic Imputation

Because our samples were genotyped on three different beadchips, we utilized data from The 1000 Genomes Project (www.1000genomes.org) to impute missing genotypes across the three data sets and obtain a concordant set of probes in the total data set. Each of the three data sets corresponding to the different chips were imputed separately and then merged to create a final concordant data set. A global reference panel was used to increase imputation accuracy by allowing the software to create a unique reference panel for each individual. Samples were first pre-phased using SHAPEIT (Delaneau et al., 2012) and genotypes imputed using IMPUTE2 (Howie et al., 2009). Because we used allele calls rather than allele probabilities in our analyses, imputed probes with certainty < 90% were zeroed out for specific individuals and were subsequently removed from the entire data set if the call rate was < 97% in all samples. Imputed probes were also removed if HWE p-values were < 10^{-6} in controls or if the minor allele frequency (MAF) was < 1%. These metrics were assessed in PLINK for the NHB and NHW samples separately. The average imputation accuracy, which is calculated by masking known probes and comparing the imputed genotype against the true genotype, was 98.2%. After all quality control steps, 5,616,481 probes remained in the NHB subset and 5,016,226 probes remained in the NHW subset.

Statistical Analysis

Logistic regression was run separately in the NHB and NHW subsets using PLINK. An additive genetic model was used to test for increased risk for current PTSD among all trauma exposed subjects. Therefore, participants who were not trauma exposed (i.e., had not endorsed Criterion A2 on the TLEQ) were excluded from the analyses (n=54). Also, in order to more accurately study genetic influences on chronic PTSD, those subjects without current PTSD, but with a history of lifetime PTSD (n=287) were excluded from the analyses so that participants with current PTSD were only compared to participants without history of any PTSD. Covariates included gender, trauma severity and principal components to control for population stratification. Trauma severity was defined as the total number of traumatic events reported on the TLEQ, regardless of whether the events caused fear, helplessness, or horror in the participant. As described above, PCA was run on the NHB and NHW subsets

separately and scree plots were used to determine the appropriate number of principal components needed to adequately control for substructure in each subset. Three principle components (PCs) were deemed necessary for the NHB subset, and six PCs were needed to control for the substructure in the NHW subset. After all exclusions (missing clinical data (n=90) and genotype QC) were applied, a total of 949 NHB and 759 NHW study participants were available for the GWAS analyses.

Meta-analysis was performed using METAL (Willer et al., 2010). False discovery rate (FDR) q-values were generated using PROC MULTTEST in SAS version 9.4 (SAS Systems, Cary, NC). SNPs with nominal p-values < 0.001 that also fell within a gene were subjected to pathway analysis using functional annotation tools in DAVID version 6.7 (Huang da et al., 2009a, b).

RESULTS

The frequency of PTSD in this data set was 40.57% among the NHB subset and 42.82% among the NHW subset. The NHW subset had a higher proportion of males and smokers, was significantly younger, had a greater percentage of veterans (although both were over 85% veteran), and subsequently, had a higher percentage of combat exposure compared with the NHB subset ($p < 0.0001$). While the number of traumatic event categories endorsed did not differ between the two racial groups, the total number of traumatic events experienced was higher among the NHW compared to the NHB subset (20.76 average events for the NHW compared to 18.27 average events for the NHB, $p = 0.0001$). For this reason, we included the total number of traumatic events as a covariate in our analyses. Lifetime substance abuse or dependency and lifetime alcohol abuse or dependency did not significantly differ between the NHB and NHW subsets.

GWAS was initially performed separately for the NHB and NHW subsets of our data set. Among the NHB subset of the study participants, the most significantly associated SNP was rs10768747, located in an intergenic region on chromosome 11 ($p = 4.68 \times 10^{-6}$; Figure 1, Table 2). The next most significantly associated SNPs located within a gene were rs73419609 ($p = 5.68 \times 10^{-6}$; Table 2), an intronic SNP located within the *Unc-13 Homolog C (C. Elegans) (UNC13C)* gene on chromosome 15 and rs77290333 ($p = 1.40 \times 10^{-5}$; Odds Ratio (OR) = 1.89), an intronic SNP located in *Down Syndrome Cell Adhesion Molecule (DSCAM)* on chromosome 21 (Figure 1). Other intergenic associations with p-values less than 10^{-5} were located on chromosomes 5 and 7 (Figure 1, Table 2). In the NHW subset of the study participants, the most significantly associated SNP was rs7866350 ($p = 1.1 \times 10^{-6}$; Figure 2, Table 2), an intronic SNP located within the *TBC1 Domain Family, Member 2 (TBC1D2)* gene on chromosome 9. The next most significant gene that was implicated in the NHW was the *Syndecan 2 (SDC2)* gene on chromosome 8 (rs2437772, $p = 6.36 \times 10^{-6}$) (Figure 2, Table 2). For the meta-analysis, only one genomic region on chromosome 15 was associated at the $p < 10^{-5}$ threshold; this region is annotated as encoding *AK092087*, a non-coding RNA (rs12232346; $p = 2.1 \times 10^{-6}$; Figure 3, Table 2). The most significant genes which were implicated in the meta-analysis included *protein kinase, cGMP-dependent, type I (PRKG1)* on chromosome 10 (rs10762479, $p = 1.67 \times 10^{-5}$) and *DEAD box polypeptide 60-like (DDX60L)* on chromosome 4 (rs10002308, $p = 1.67 \times 10^{-5}$) (Figure 3, Table 2).

Because PTSD develops only after experiencing a traumatic event, we explored whether the top SNPs from the GWAS interacted significantly with either exposure to childhood trauma or the total number of traumatic events (Table 2). We did not examine interactions with the presence or absence of adult trauma because there were too few individuals who had not experienced an adult trauma. For example, in Table 1, most of the sample had combat exposure. We observed very little evidence for gene*environment interactions with childhood trauma. Among the NHB, we observed nominally significant interactions with childhood trauma for *UNC13C* ($p=0.049$) and *PRKG1* ($p=0.037$) (Table 2). Among the NHW, we observed a nominally significant interaction with childhood trauma for *DDX60L* ($p=0.046$; Table 2). When we examined interaction with the total number of traumatic events, rs17504106 in the NHB subset was nominally significant ($p=0.02$; Table 2). Additionally, the interaction between the total number of traumatic events and *DDX60L* in the NHW subset was nominally significant ($p=0.01$), consistent with the interaction observation with childhood trauma (Table 2).

As noted in the Introduction section, several other GWAS studies for PTSD have been previously published. None of the most significant associations for our study overlapped with the most significant associations in the previously published studies. However, for comparison to the previous work, we provide the results of the associations in our study for these specific SNPs in Table 3. The only genes for which we observed any nominal evidence for association in our data set were *RORA* ($p=0.04$, NHW) and *TLL* ($p=0.04$, meta-analysis). These results reaffirm that PTSD is genetically complex, with many genetic risk factors.

We next performed pathway analysis in order to determine if there was evidence for consistency in the biologic pathways implicated in our three analyses since the top SNPs and genes did not appear to overlap. Table 4 describes the results of the pathway analysis for genes across the three analyses (NHB, NHW and meta-analysis) containing a SNP that was associated with risk for PTSD with a nominal p -value < 0.001 . Despite the inconsistency in the specific genes that were implicated in the GWAS analyses, there was excellent concordance across the analyses for the top pathways implicated by these genes. Alternative splicing was the most significant GO term that was enriched in all three analyses (Table 4) and was highly significant. Similarly, “splice variant” was the next most significantly enriched GO term. The third most significant term was “Immunoglobulin I-set”, which is likely unrelated to the enrichment of genes involved in splicing, but may suggest that the immune system plays an important role in PTSD risk.

DISCUSSION

Using a GWAS approach, the present study identified several putative candidate genes that may contribute to PTSD risk. Among the NHB subset of our sample, the most significant SNPs that fell within genes were located in the *UNC13C* and *DSCAM* genes. *UNC13C*, located on chromosome 15, belongs to the *UNC13* gene family which is homologous to the *C. elegans UNC13* (Brose et al., 1995). This gene family is highly expressed in brain, and is involved in presynaptic vesicle priming (Augustin et al., 1999; Augustin et al., 2001). Most functional work on these proteins has been performed in model organisms. Thus, the

specific role for this gene in the human PTSD phenotype is unknown. However, a significant reduction in *Unc13C* expression has been observed in a mouse knockout of *Vesicular Glutamate Transporter 2 (Vglut2)*, which also exhibits a strong anxiolytic phenotype (Rajagopalan et al., 2014). Reduced levels of *Unc13C* have been associated with a model system phenotype that is consistent with PTSD. *DSCAM*, located on chromosome 21, is a neural adhesion molecule that was identified in the intellectual disability (and cardiac) critical region for Down Syndrome (Yamakawa et al., 1998). The role of vertebrate *DSCAM* has been best described in the mouse retina, where the protein seems to be facilitating proper neuronal connectivity by preventing adhesion among the same neuronal cell types (Fuerst et al., 2009; Fuerst et al., 2008). This gene could be important in PTSD, either through a developmental risk via aberrant neuronal connectivity or, in situations where neurogenesis occurs subsequent to a physical head trauma, as can occur in combat veterans. To explore the latter hypothesis, we examined whether the signal for *DSCAM* in the NHB was driven by the subset of cases with head trauma. However, the analysis restricted to cases with head trauma ($n=99$) and the analysis restricted to cases without head trauma ($n=130$) were both nominally significant (p 's < 0.003) and the effect was in the same direction, suggesting that head injury is not driving this association.

Within the NHW subset, *TBC1D2* was identified as the most significantly associated gene. *TBC1D2*, a GTP-ase activating protein, has been previously implicated in risk for multiple sclerosis, another neurologic disease (Baranzini et al., 2009; Schmied et al., 2012). *Syndecan2 (SDC2)* was the next most significantly associated gene. *SDC2* is expressed in clusters along dendritic spines of hippocampal neurons (Ethell and Yamaguchi, 1999) and has been shown to be expressed in the developing zebrafish brain during the period of rapid axonal growth (Hofmeister et al., 2013). Additionally, a translocation breakpoint just proximal to *SDC2* has been previously associated with a syndromic form of autism (Ishikawa-Brush et al., 1997). Thus, both candidate genes identified in the NHW subset of this data set have been previously implicated in other brain diseases and are reasonable candidates for PTSD pathogenesis.

Given that none of the associations in the two racial subsets met FDR-correction for genome-wide significance, the two data sets were combined in a meta-analysis to increase statistical power. By combining the two subsets, our hypothesis was that similar genetic risk factors were present in both racial groups, even though the top hits in the individual analyses were different. This hypothesis seems reasonable given recent work (Nievergelt et al., 2015) which demonstrated that the signal in the *PRTFDC1* gene revealed a similar effect across racial groups. Nonetheless, despite the increased sample size by combining the two racial subsets, we did not observe a significant increase in statistical power. This was evidenced by meta-analysis findings still failing to surpass the FDR correction threshold. However, the top hit in the meta-analysis which also fell within a gene was a SNP in *cGMP-dependent protein kinase type I alpha (PRKG1)* which is an excellent candidate gene. *PRKG1* has been implicated in auditory-cued fear memory and long-term potentiation (Ota et al., 2008; Paul et al., 2008; Paul et al., 2010) and is also involved in regulating the serotonin transporter (Steiner et al., 2009; Zhang and Rudnick, 2011). The serotonin transporter has been associated with risk for PTSD, either as a main genetic effect or as an interactive effect with

traumatic events, in several independent studies (Ahs et al., 2014; Grabe et al., 2009; Graham et al., 2013; Kilpatrick et al., 2007; Kimbrel et al., 2014; Kolassa et al., 2010; Lee et al., 2005; Mellman et al., 2009; Mercer et al., 2012; Morey et al., 2011; Murrough et al., 2011; Pietrzak et al., 2013; Wald et al., 2013; Walsh et al., 2014; Wang et al., 2011; Xie et al., 2012; Xie et al., 2009). Nonetheless, meta-analyses have not consistently demonstrated the association (Gressier et al., 2013; Navarro-Mateu et al., 2013). Epigenetic alterations of *PRKG1* have been identified in women with fibromyalgia (Menzies et al., 2013), a condition which frequently exhibits comorbid anxiety and depression. Although *PRKG1* has not previously been implicated in PTSD, it has been suggested that blocking alpha-1 adrenergic receptors could be useful in the treatment of PTSD because alpha1-ADR antagonists appear to reduce the effects of stress-induced disturbances of the cGMP pathway in Leydig cells (Stojkov et al., 2014). The next most significant finding in the meta-analysis was the *DEAD box polypeptide 60 like (DDX60L)* gene, which is an interferon response gene (Khsheibun et al., 2014). Importantly, DDX60L was found to be down-regulated in a study examining genome-wide expression changes in human lymphoblastoid cell lines after response to selective serotonin reuptake inhibitors (SSRIs; (Morag et al., 2011), which are commonly used to treat individuals with PTSD. In summary, although each of the three analyses produced different candidate genes, all of the analyses produced biologically relevant candidate genes.

We explored the possibility that our most strongly associated SNPs interacted with exposure to childhood trauma or the total number of traumatic events in order to increase risk for PTSD. Similar to another PTSD GWAS, we did not observe robust evidence for gene*environment interactions (Nievergelt et al., 2015). The strongest evidence for gene*environment interactions was observed in the NHW subset of our study with the *DDX60L* gene whereby rs10002308 significantly interacted with both childhood trauma and the total number of traumatic events. It is important to note, however, that larger sample sizes may be required to identify these higher order interactions. Moreover, we limited our examination of gene*environment interactions to those SNPs that had demonstrated evidence for a main effect. It is also possible that other SNPs significantly interact with the occurrence of traumatic events even though they did not demonstrate a nominally significant main effect. However, we would not have detected such effects in the present analysis.

When we queried this data set for evidence of association in previously reported PTSD GWAS hits, we observed very limited support for associations with those genes, similar to what has been reported previously by other PTSD GWAS studies. The only two genes that provided any evidence for association were *RORA* and *TLL* (Table 3). A more thorough evaluation of *RORA* in our data set has been previously described (Guffanti et al., 2014), however, the models and covariates that were used in that analysis were different than what has been presented in the current analysis. Because the evidence for association with *RORA* observed in independent data sets has been marginal and susceptible to the selected analytic model, a more in-depth analysis with a larger sample size is required to fully evaluate the role of this gene in PTSD. We also observed evidence for *TLL* which was originally identified by Xie and colleagues (Xie et al., 2013). There is evidence that this gene could have a functional role in PTSD pathophysiology due to its expression in relevant regions of

the brain and gene function (Xie et al., 2013). However, similar to the association with *RORA*, it may be that larger sample sizes are needed to detect this signal as we observed only nominal evidence in the current meta-analysis of NHB and NHW study participants.

We also selected the top candidate genes implicated in all three analyses for downstream pathway analysis. Although the individual top hits were inconsistent across the NHB and NHW subsets, pathway analyses demonstrated a remarkable consistency of implicated pathways in the two subsets and the meta-analysis. This suggests that the lack of consistency of specific genes identified in the two racial groups and meta-analysis may be more related to statistical power or random sampling of the different subsets rather than true etiologic differences in genetic risk factors between the two racial groups. The pathway analysis also provided useful insight into the biologic processes that may be driving genetic risk for PTSD. Specifically, alternative splicing was the top FDR-significant pathway that was identified in all three analyses. While alternative splicing is a broad category, it is likely pointing to the importance of tissue-specific gene regulation in maintaining psychiatric health following exposure to a traumatic event.

While we are encouraged by our findings, we acknowledge that there are several limitations of the present study. The most significant is that the study did not identify FDR-significant GWAS findings. This suggests that either the findings are false positives (perhaps less likely due to consistency of the pathway analysis results) or that the study was statistically under-powered. Certainly, the sample sizes in the NHB and NHW subsets are smaller than many GWAS studies of complex phenotypes. Of note, the Psychiatric Genetics Consortium (PGC) has had the most success in identifying putative genetic risk factors for schizophrenia, another complex psychiatric phenotype, where the sample size now exceeds 140,000 (Schizophrenia Working Group of the Psychiatric Genomics, 2014). These are much larger than the sample sizes utilized in the present study. Similar efforts for PTSD will greatly improve statistical power and will hopefully identify FDR-significant and replicable GWAS findings. Nonetheless, given the effect sizes of the previously published PTSD GWAS studies (OR's ranging between 1.4 and 3.7) (Guffanti et al., 2013; Logue et al., 2013a; Nievergelt et al., 2015; Xie et al., 2013), power calculations demonstrated that this study should have been reasonably powered to detect those effect sizes (data not shown). This suggests that, in addition to statistical power, there is likely heterogeneity across the different studies that contributed to the lack of replication. The heterogeneity may be due to a variety of reasons, as we suggest in the Introduction, such as genetic and traumatic exposure heterogeneity. The present study included well characterized trauma-exposed controls and at least one aspect of traumatic exposure, combat exposure, was highly prevalent within the sample. This should have reduced the heterogeneity compared to other datasets and reduced the misclassification of controls. In future analyses, it will be vitally important to characterize the phenotype and exposures with a high degree of precision.

The discussion presented has focused primarily on the most significant SNPs that were located in a gene. Nonetheless, several of the most nominally significant SNPs were not annotated within a gene. It is possible that these intergenic associations are real findings and may play a role in regulation of other genes. However, because none of our associations met FDR-significance, we focused on the associations within genes so that we could evaluate

those findings more carefully within the context of gene function and possible neurobiologic relevance to PTSD. Larger sample sizes will be required to determine whether the associations in these intergenic regions are true associations with PTSD risk.

In summary, this GWAS of primarily US Iraq/Afghanistan era veterans has identified several promising new candidate genes for PTSD and implicated a role for alternative splicing in PTSD pathophysiology. Replication of these findings in other data sets will be necessary to confirm their relevance to PTSD risk. Furthermore, functional analyses of these candidate genes, particularly with *in vivo* models, are warranted in order to better understand the etiologic mechanisms by which these genes may contribute to PTSD risk.

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HIGHLIGHTS

- GWAS for PTSD was conducted in a sample of primarily Iraq/Afghanistan veterans.
- GWAS was performed in NHB and NHW subsets, and a meta-analysis combining the two.
- Several candidate genes with biologic relevance to PTSD were nominally significant.
- Pathway analysis implicated alternative splicing in PTSD pathophysiology.
- Limited evidence for replication of previous PTSD GWAS hits was observed.

Figure 1. Manhattan plot of the genome-wide association results in the non-Hispanic black subset
Genes which contained SNPs with p-values less than 10^{-5} are labeled on the plot.

Figure 2. Manhattan plot of the genome-wide association results in the non-Hispanic white subset

Genes which contained SNPs with p-values less than 10^{-5} are labeled on the plot.

Figure 3. Manhattan plot of the genome-wide association results in the meta-analysis of the non-Hispanic black and non-Hispanic white subsets

Genes which contained SNPs with p-values less than 10^{-5} are labeled on the plot.

Table 1

Participant characteristics by ethnicity

	NHB n=949	NHW n=759	p-value
Current PTSD diagnosis (%)	40.57%	42.82%	0.3484
Gender (% Male)	69.65%	84.85%	<0.0001
Age in years (S.D.)	39.06 (9.73)	36.28 (10.44)	<0.0001
Veteran status	85.04%	93.15%	<0.0001
Combat exposure	75.92%	86.76%	<0.0001
TLEQ total sum *	18.27 (13.9)	20.76 (15.02)	0.0001
TLEQ categories *	7.27 (3.97)	7.0 (3.82)	0.6217
DTS total score	44.28 (41.07)	45.11 (41.47)	0.6819
Lifetime Major Depressive Disorder (MDD) diagnosis	40.54%	40.28%	0.92
Smoking status			<0.0001
current smoker	31.78%	34.72%	
ex-smoker	16.43%	23.69%	
never smoker	51.78%	41.59%	
Lifetime substance abuse/dependency	21.95%	18.60%	0.0983
Lifetime alcohol abuse/dependency	40.13%	44.34%	0.0899

* variable was log-transformed for analysis due to non-normality

Table 2

Main and interactive effects for the SNPs most strongly associated* with PTSD.

SNP	Chromosome	Position based on hg19	Annotation	N	Odds Ratio	Main Effect P- value	SNP* child trauma P- value	SNP* total trauma P- value
Top SNPs in NHB subset								
rs10768747	11	41820450	Intergenic	937	1.66	4.68×10^{-6}	0.16	0.50
rs17504106	5	160469639	Intergenic	938	2.97	4.72×10^{-6}	0.37	0.02
rs73419609	15	54715642	UNC13C intronic	931	0.45	5.67×10^{-6}	0.049	0.74
rs2862383	11	41824125	Intergenic	939	1.64	6.17×10^{-6}	0.15	0.50
rs834811	7	135884571	Intergenic	949	1.63	7.31×10^{-6}	0.83	0.21
Top SNPs in NHW subset								
rs7866350	9	100983826	TBC1D2 intronic	744	2.27	1.10×10^{-6}	0.51	0.15
rs1116255	13	55489005	Intergenic	759	2.19	5.46×10^{-6}	0.37	0.61
rs2437772	8	97512977	SDC2 intronic	757	0.56	6.36×10^{-6}	0.56	0.26
rs61793204	4	31290282	Intergenic	753	0.40	8.60×10^{-6}	0.86	0.82
Top SNPs in Meta-analysis								
rs12232346	15	35060463	AKO92087 intronic		++	2.14×10^{-6}	0.94 NHB 0.35 NHW	0.62 NHB 0.74 NHW
rs10762479	10	53657648	PRKG1 intronic		++	1.60×10^{-5}	0.037 NHB 0.94 NHW	0.61 NHB 0.29 NHW
rs10002308	4	169399823	DDX60L intronic		++	1.67×10^{-5}	0.39 NHB 0.046 NHW	0.88 NHB 0.01 NHW

* For the NHB and NHW subsets, results are presented for SNPs with p-values $< 10^{-5}$. For the meta-analysis, results are presented for SNPs with p-values $< 10^{-4}$.

++ In the meta-analysis, the same risk allele was identified in both ethnicities suggesting that the direction of the effect was consistent.

Table 3

Main effects of SNPs identified in previously published PTSD GWAS.

SNP	Gene	Previous Study	Previous Study P- value	NHB P- value	NHW P- value	Meta- analysis P- value
rs8042149	<i>RORA</i>	Logue et al., 2013	2.5×10^{-8}	0.81	0.04	0.32
rs6812849	<i>TLL</i>	Xie et al., 2013	2.99×10^{-7}	0.10	0.18	0.04
rs10170218	<i>LINC01090</i>	Guffanti et al., 2013	5.09×10^{-8}	0.35	0.75	0.53
rs6482463	<i>PRTFDC1</i>	Nievergelt et al., 2014	2.04×10^{-9}	0.31	0.94	0.40

Table 4

Significant results of pathway analysis of top genes implicated in PTSD GWAS.

Category	Term	NHB FDR P-value	NHW FDR P-value	Meta- analysis FDR P- value
SP_PIR_KEYWORDS	alternative splicing	7.13×10^{-11}	3.31×10^{-05}	5.94×10^{-09}
UP_SEQ_FEATURE	splice variant	1.14×10^{-09}	4.60×10^{-05}	8.62×10^{-09}
INTERPRO	IPR013098:Immunoglobulin I-set	5.29×10^{-05}	0.02	0.0028