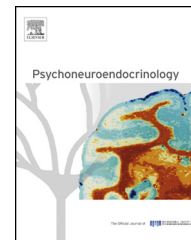




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Genomic predictors of combat stress vulnerability and resilience in U.S. Marines: A genome-wide association study across multiple ancestries implicates *PRTFDC1* as a potential PTSD gene

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Received 2 May 2014; received in revised form 10 October 2014; accepted 16 October 2014

KEYWORDS

GWAS;
Meta-analysis;
PTSD;
Ancestry;
Polygenic risk score;

Summary

Background: Research on the etiology of post-traumatic stress disorder (PTSD) has rapidly matured, moving from candidate gene studies to interrogation of the entire human genome in genome-wide association studies (GWAS). Here we present the results of a GWAS performed on samples from combat-exposed U.S. Marines and Sailors from the Marine Resiliency Study (MRS) scheduled for deployment to Iraq and/or Afghanistan. The MRS is a large,

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<http://dx.doi.org/10.1016/j.psyneuen.2014.10.017>

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Please cite this article in press as: Nievergelt, C.M., et al., Genomic predictors of combat stress vulnerability and resilience in U.S. Marines: A genome-wide association study across multiple ancestries implicates *PRTFDC1* as a potential PTSD gene. *Psychoneuroendocrinology* (2014), <http://dx.doi.org/10.1016/j.psyneuen.2014.10.017>

Trauma;
GxE;
Bipolar disorder;
Pleiotropy

prospective study with longitudinal follow-up designed to identify risk and resiliency factors for combat-induced stress-related symptoms. Previously implicated PTSD risk loci from the literature and polygenic risk scores across psychiatric disorders were also evaluated in the MRS cohort.

Methods: Participants ($N = 3494$) were assessed using the Clinician-Administered PTSD Scale and diagnosed using the *DSM-IV* diagnostic criterion. Subjects with partial and/or full PTSD diagnosis were called cases, all other subjects were designated controls, and study-wide maximum CAPS scores were used for longitudinal assessments. Genomic DNA was genotyped on the Illumina HumanOmniExpressExome array. Individual genetic ancestry was determined by supervised cluster analysis for subjects of European, African, Hispanic/Native American, and other descent. To test for association of SNPs with PTSD, logistic regressions were performed within each ancestry group and results were combined in meta-analyses. Measures of childhood and adult trauma were included to test for gene-by-environment (GxE) interactions. Polygenic risk scores from the Psychiatric Genomic Consortium were used for major depressive disorder (MDD), bipolar disorder (BPD), and schizophrenia (SCZ).

Results: The array produced >800 K directly genotyped and >21 M imputed markers in 3494 unrelated, trauma-exposed males, of which 940 were diagnosed with partial or full PTSD. The GWAS meta-analysis identified the phosphoribosyl transferase domain containing 1 gene (*PRTFDC1*) as a genome-wide significant PTSD locus (rs6482463; OR = 1.47, SE = 0.06, $p = 2.04 \times 10^{-9}$), with a similar effect across ancestry groups. Association of *PRTFDC1* with PTSD in an independent military cohort showed some evidence for replication. Loci with suggestive evidence of association ($n = 25$ genes, $p < 5 \times 10^{-6}$) further implicated genes related to immune response and the ubiquitin system, but these findings remain to be replicated in larger GWASs. A replication analysis of 25 putative PTSD genes from the literature found nominally significant SNPs for the majority of these genes, but associations did not remain significant after correction for multiple comparison. A cross-disorder analysis of polygenic risk scores from GWASs of BPD, MDD, and SCZ found that PTSD diagnosis was associated with risk scores of BPD, but not with MDD or SCZ.

Conclusions: This first multi-ethnic/racial GWAS of PTSD highlights the potential to increase power through meta-analyses across ancestry groups. We found evidence for *PRTFDC1* as a potential novel PTSD gene, a finding that awaits further replication. Our findings indicate that the genetic architecture of PTSD may be determined by many SNPs with small effects, and overlap with other neuropsychiatric disorders, consistent with current findings from large GWAS of other psychiatric disorders.

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1. Introduction

Post-traumatic stress disorder (PTSD) is an anxiety disorder and unique in that exposure to an environmental event (Criterion-A traumatic event; APA, 2000) is a necessary condition for diagnosis. Lifetime prevalence is ~8% in adult Americans (Kessler et al., 1995; Kilpatrick et al., 2013) and is especially high among those exposed to combat, with values ranging from 6% to 31% as reported in a recent review of studies on US combat veterans (Richardson et al., 2010). A large number of demographic and environmental factors and their interactions contribute to PTSD susceptibility, including female gender, age, existence of previous mental health issues, early life stress, as well as severity, duration and number of traumatic incidents, and other factors such as lack of social support (Zoladz and Diamond, 2013). Notably, there are race/ethnic differences in traumatic event exposure, in type of event, age at exposure, as well as the development of PTSD given a specific trauma, with African Americans having somewhat higher risks than whites and Asians (Roberts et al., 2010).

In addition, individual differences in heritable factors affect the risk to develop PTSD. Twin studies indicate that PTSD is moderately heritable, with genetic factors explaining a substantial proportion (30–46%) of vulnerability

to PTSD (reviewed e.g. in Wolf et al., 2013). Remaining variance is attributable to the non-shared environment, including trauma encountered during war zone deployments. For some, combat exposure acts as a catalyst that augments the impact of hereditary and environmental contributions to PTSD (Wolf et al., 2013).

A large proportion of the genetic liability for PTSD is also shared with other mental disorders such as anxiety and panic disorder (Goenjian et al., 2008), major depressive disorder (MDD) (Fu et al., 2007; Sartor et al., 2012), and substance use (Xian et al., 2000), hence genes that confer risk for PTSD may also influence risk for other psychiatric disorders and vice versa (Nugent et al., 2008). Such pleiotropic effects have been demonstrated across several psychiatric disorders (Solovieff et al., 2013). For example, a recent study that examined schizophrenia (SCZ), bipolar disorder (BPD), MDD, and attention-deficit/hyperactivity disorder (ADHD) found that SNP-based heritability ranged from 17 to 29% within disorders. Genetic correlations between disorders were also observed with highest associations between SCZ and BPD, and moderate correlations between SCZ and MDD, BPD and MDD, and ADHD and MDD (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Cross-Disorder Group of the Psychiatric Genomics Consortium and Genetic Risk Outcome of Psychosis Consortium, 2013).

Until recently, the genetic contribution to PTSD has been investigated largely via candidate gene association studies (reviewed in [Almli et al., 2014](#); [Amstadter et al., 2009](#); [Norrholm and Ressler, 2009](#)). Most research has focused on: (1) the hypothalamic–pituitary–adrenal (HPA) axis, (2) the ascending brainstem locus coeruleus noradrenergic system, and (3) the limbic amygdalar frontal pathway mediating fear processing. Among the over 25 PTSD candidate genes currently reported ([Amstadter et al., 2009, 2011](#); [Binder et al., 2008](#); [Boscarino et al., 2011](#); [Cao et al., 2013](#); [Comings et al., 1996](#); [Dragan and Oniszczenko, 2009](#); [Gillespie et al., 2013](#); [Goenjian et al., 2012](#); [Grabe et al., 2009](#); [Guffanti et al., 2013](#); [Hauer et al., 2011](#); [Kolassa et al., 2010](#); [Logue et al., 2013a,b](#); [Lyons et al., 2013](#); [Mellman et al., 2009](#); [Nelson et al., 2009](#); [Ressler et al., 2011](#); [Segman et al., 2002](#); [Solovieff et al., 2014](#); [Voisey et al., 2010](#); [Wilker et al., 2013](#); [Xie et al., 2013](#)), promising findings include associations of PTSD symptoms with the serotonin transporter gene (*SERT*, *SLC6A4*) ([Xie et al., 2009](#)), which is linked to depression and anxiety disorders, as well as differential acquisition of conditioned fear and increased amygdala excitability in humans. In addition, *FKBP5*, a co-chaperone of the glucocorticoid receptor involved in the HPA axis, has a significant interaction with severity of child abuse in the prediction of adult PTSD symptoms, indicating a gene by environment (GxE) interaction ([Binder et al., 2008](#)). Interestingly, the ankyrin-3 gene (*ANK3*), a known BPD and SCZ gene, was nominally associated with PTSD ([Logue et al., 2013b](#)). Although candidate gene studies have not conclusively identified a genetic basis of PTSD, and await replication in independent studies, they suggest a likely polygenic contribution to PTSD development, where a substantial overall genetic effect aggregates over many common variants which individually contribute only minimal effects, further complicated by complex GxE interactions. These findings are in line with the genetic architecture of many psychiatric disorders investigated to date.

To date, only 3 GWASs in PTSD have been published with results implicating several novel loci. The first study on European American (EA) military veterans and their intimate partners identified the retinoid-related orphan receptor alpha (*RORA*) as a potential PTSD gene ([Logue et al., 2013a](#)). The second study, including EAs recruited for substance abuse, identified the Toll-like 1 gene (*TLL1*) ([Xie et al., 2013](#)), and the third, a study in primarily African American women, implicated a lincRNA (*LINC01090*, alias *AC068718.1*) as a risk factor for PTSD ([Guffanti et al., 2013](#)).

In this study we present results from a GWAS on PTSD in the Marine Resiliency Study (MRS), including 3494 trauma-exposed participants. The MRS is a well-characterized, prospective study of Marines and Sailors scheduled for combat deployment to Iraq or Afghanistan, with longitudinal follow-up to track the effect of combat stress ([Baker et al., 2012](#)). This young, all-male military cohort is among the largest and most homogenous of PTSD studies available and presents a unique resource to test mechanisms of risk that mediate the link between stressor exposure and outcome, or that moderate or synergize with exposure to mitigate or exacerbate its effect over time. We performed the first GWAS across ancestral groups, including

subjects of European, African, Hispanic/Native American, and other ancestries. In addition, we attempted to replicate significant associations in 25 putative PTSD genes from the literature, and tested for main effects and GxE interactions in the MRS. Lastly, we tested for a genetic overlap of PTSD with other psychiatric disorders using polygenic risk profiles from Psychiatric Genomics Consortium (PGC) BPD, MDD, and SCZ GWAS ([Purcell et al., 2009](#)).

2. Methods

2.1. Study subjects

Participants were recruited from two studies including military personnel: (1) the Marine Resiliency Study, a prospective PTSD study with longitudinal follow-up (pre- and post-exposure to combat stress) of U.S. Marines bound for deployment to Iraq or Afghanistan ([Baker et al., 2012](#)) (here referred to as MRS-I), and (2) the Marine Resiliency Study-II (MRS-II), which followed a very similar protocol. The protocols were approved by the University of California – San Diego Institutional Review Board, and all participants provided written informed consent to participate. Subjects with available genotypes included a total of 3494 unrelated males (MRS-I: $N = 2376$; MRS-II: $N = 1118$) from 6 different battalions. Based on self-reported race and ethnicity, the cohort was racially 85.5% white and was ethnically 75.5% non-Hispanic. Participant age ranged from 18 to 48 years, with a mean of 23.1 years. Descriptive statistics of the cohort are shown in [Table 1](#).

2.2. Phenotype assessments

Details of phenotype assessments are described in Supplemental methods. In brief, participants were assessed for PTSD diagnosis up to 3 times, once before deployment and 3 and/or 6 month post deployment. Post-traumatic stress (PTS) symptoms were assessed using a structured diagnostic interview, the Clinician Administered PTSD Scale (CAPS), and PTSD diagnosis followed the *DSM-IV* criteria for partial and full PTSD. All participants ($N = 3494$) included in this study met the *DSM-IV* criteria A1 event; 38% of them had 2 assessments and 39% had 3 assessments, respectively. For participants assessed at multiple timepoints (i.e. pre- and post-deployments; $N = 2689$), the timepoint with the highest CAPS score was used (54% of the CAPS came from pre-deployment, and 46% from post-deployment assessments). Participants meeting criteria for partial or full PTSD diagnosis were designated as cases ($N = 940$, including 324 with a full PTSD diagnosis), all other participants were designated controls ($N = 2554$). Childhood trauma was assessed in 3385 subjects using a modified version of the Childhood Trauma Questionnaire Short Form (CTQ), and general lifetime trauma was assessed at the time of CAPS assessment in 3494 participants using the Life Events Checklist (LEC), a self-report inventory that inquires about exposure to 16 different potentially traumatic events known to increase risk for PTSD.

Table 1 Descriptive statistics for the Marine Resiliency GWAS cohorts (MRS) studied based on PTSD case versus control status.

	All	MRS-I	MRS-II	PTSD	Controls	<i>p</i> -Value ^a
Number of Subjects	3494	2376	1118	940	2554	
Age, mean (\pm SD)	23.1 (3.4)	23.3 (3.5)	22.6 (3.0)	23.0 (3.0)	23.2 (3.5)	0.98
Range	18–48	18–48	18–43	18–38	18–48	
Self reported race						
White	85.5%	84.6%	87.5%	84.1%	86.1%	0.23
African American	4.4%	4.5%	4.1%	4.4%	4.4%	
Other	10.0%	10.8%	8.4%	11.5%	9.5%	
Self reported ethnicity						
Hispanic	24.5%	23.3%	26.2%	25.9%	23.6%	0.16
Non-Hispanic	75.5%	76.7%	73.8%	74.1%	76.4%	
CTQ, mean (\pm SD)	39.6 (13.5)	40.3 (13.8)	38.0 (12.3)	44.3 (12.8)	37.8 (12.3)	$<2.2 \times 10^{-16}$
Range	25.0–107.5	25.0–106.5	25.0–107.5	25.0–106.5	25.0–107.5	
LEC, mean (\pm SD)	6.9 (3.5)	6.7 (3.5)	5.8 (3.3)	8.2 (3.4)	5.7 (3.3)	$<2.2 \times 10^{-16}$
Range	0–16	0–16	0–16	0–16	0–16	
Prior deployment	78%	78%	78%	83%	76%	1.4×10^{-5}

^a *p*-Values (PTSD versus Controls) based on Wilcoxon tests (chi-square tests for Race and Ethnicity). CTQ, childhood trauma questionnaire; LEC: life events checklist.

2.3. DNA sample preparation, genotyping, and quality control

Details of sample preparation and genotyping procedures are given in Supplemental methods. In brief, genomic DNA was prepared from blood leukocytes and prepared for genotyping. GWAS-I: genotyping for MRS-I was carried out by Illumina (<http://www.illumina.com/>) using the HumanOmniExpressExome (HOEE) array with 951,117 loci and resulted in a high initial locus success rate and overall data quality. Additional data cleaning was performed in PLINK v1.07 (Purcell et al., 2007), using standard procedures (Anderson et al., 2010). SNPs were excluded if the call rate was $<95\%$, if they violated Hardy Weinberg Equilibrium ($p < 1 \times 10^{-6}$), or if they showed plate effects (p -value $< 1 \times 10^{-8}$ for any one plate or $< 1 \times 10^{-4}$ for two or more plates). After removal of problematic DNA samples and markers, the final dataset included 851,541 markers genotyped in 2548 subjects. GWAS-II: a second GWAS for MRS-II samples was carried out by RUCDR (<http://www.rucdr.org>) using the HOEE array with 967,537 loci and identical data quality procedures were applied. Genotypes ($N = 849,099$ SNPs) of 1471 GWAS-II subjects and 23 duplicates (subjects in common with GWAS-I) were then merged with GWAS-I. Array effects were identified by comparing SNP allele frequency variation between GWAS-I and GWAS-II using a chi-squared association test and 132 SNPs with p -values $< 5 \times 10^{-8}$ were removed. Reproducibility including 23 replicate pairs (subjects genotyped in both GWAS-I and GWAS-II) was $>99.99\%$. Ancestry was distributed equally across GWAS-I and GWAS-II (chi-squared = 3.50, $df = 3$, $p > 0.32$; Supplemental Fig. 1), but there were more PTSD cases in GWAS-I compared to GWAS-II (chi-squared = 29.07, $df = 1$, $p < 6.98 \times 10^{-8}$); a covariate for array was included in the association analyses (see below). The final dataset included 888,113 markers genotyped in 3494 MRS participants (and 525 samples unrelated to this study).

2.4. Genotype imputations

Imputations were performed using the default parameters in IMPUTE2 v2.2.2, using 1000 Genomes Phase 1 integrated variant set haplotypes for the autosomes and the interim set for the X chromosome (see Nievergelt et al., 2014 for details). In brief, prior to imputation, genetic markers that failed Hardy–Weinberg equilibrium ($p < 5 \times 10^{-4}$), or had exceedingly rare alternative alleles (minor allele frequency MAF < 0.005) were excluded. Next, genomes were divided into approximately 5 Mb segments, and phasing and imputed genotypes were calculated for each. Imputed markers with low imputation quality values ($\text{Info} \leq 0.5$) were dropped. A total of 21,692,209 variants were imputed across the two genotyping arrays, resulting in a total of 21,693,469 genotyped and imputed markers for association analyses.

2.5. Ancestry assessment and control for genetic background heterogeneity

Ancestry was determined using genetic information as described in Nievergelt et al. (2013). In brief, genotypes of 1783 ancestry-informative markers (AIMs) were used to determine a subject's ancestry at the continental level for the 7 geographic regions Africa, Middle East, Europe, Central/South Asia, East Asia, Americas, and Oceania. Ancestry estimates were determined using STRUCTURE v2.3.2.1 (Falush et al., 2003) at $K = 7$, including prior population information of the HGDP reference set (Li et al., 2008). To preserve power for the GWAS and reduce type I errors due to population stratification, we aimed to place subjects into large, homogenous groups (European-Americans, EA, $N = 2179$) and groups with simple one-way admixture (African-Americans, AA, $N = 205$; Hispanic and Native Americans, HNA, $N = 640$). All other subjects, including 50 East

Asians, were grouped as Others ($N = 470$) (see Supplemental Fig. 1 for details).

GWAS was performed separately in each of the 4 main ancestral groups. To control for additional genetic background heterogeneity within the 4 ancestral groups, and varying degrees of EA admixture within the HNAs, AAs and others, a principal component analysis (PCA) implemented in EIGENSTRAT (Price et al., 2006) was performed based on 10,000 random, autosomal SNPs separately for each of the 4 groups. Scree plots (data not shown) of the Eigenvalues of the principal components (PC's) indicated that the first five PC's substantially accounted for genetic variability within EA (0.69% cumulative of 5 PC's), AA (6.70% for 5 PC's), HNA (2.81% for 5 PC's), and Others (8.44% for 5 PC's), respectively and were included as covariates in the association analyses.

2.6. Statistical analyses

To test for association of SNPs (at a minor allele frequency $MAF > 0.01$, $N = 10,446,675$ SNPs) with PTSD status logistic regressions were performed in PLINK for each of the 4 ancestry groups, including battalion, GWAS platform, and the first 5 PC's as covariates. Alleles were coded additively in the GWAS and alternative genetic models were tested post hoc for top hits. To account for uncertainty in SNP imputation, SNP dosages were used rather than allele calls. Resulting p -values were adjusted using genomic control (GC) to correct for genome wide inflation and significance was declared at $p < 5 \times 10^{-8}$. A fixed-effects meta-analysis across ancestry groups was performed based on GC corrected standard errors (SE) using the inverse-variance weighted method in METAL (Willer et al., 2010). Regional association plots were constructed using LocusZoom (Pruim et al., 2010), using the 1000 Genomes project Europeans as reference population and R^2 as the measure for linkage disequilibrium (LD).

Candidate gene analyses: associations for single gene analyses selected from the literature are reported at a nominal p -value of 0.05. Gene-wide significance was estimated using the set-based permutations in PLINK with default parameters. Gene by environment (GxE) interactions were calculated using a robust SE method (Voorman et al., 2011) as implemented in the R-package rms (Harrell, 2014).

Polygenic risk score analyses: risk score analyses were performed in EA MRS participants based on data downloaded from the PGC website for bipolar disorder (BPD), major depressive disorder (MDD), and Schizophrenia (SCZ). LD-pruned SNP sets for the 3 disorders were filtered at varying p -value thresholds (P_T) (at $p < 0.01$, < 0.05 , < 0.10 , < 0.20 , < 0.30 , < 0.40 , and < 0.50). A risk score for each MRS participant was computed by the number of risk alleles weighted by the log of the odds ratios (ORs). To test if the polygenic risk scores for these disorders could predict PTSD status in MRS, logistic regressions with the specific SNP sets were performed, including battalion, GWAS platform, and the first 5 PC's as covariates.

Power calculations for the association analysis were performed using the case-control module for discrete traits (Purcell et al., 2003) at $D' = 1$ and parameters derived from the MRS.

2.7. VA replication sample

GWAS hits in the discovery sample were tested for replication in an independent cohort including 491 VA samples. Sample ascertainment, characterization, genotyping, and data cleaning methods used have been described elsewhere in detail (Logue et al., 2013a). Briefly, the sample is a subset of a cohort of military veterans and their intimate partners ascertained from two studies performed at U.S. Department of Veterans Affairs (VA) medical centers. All participants were assessed using the CAPs with excellent inter-rater reliability ($\kappa = 0.87$). Genotyping was performed using the Illumina HumanOmni2.5-8 array and samples were excluded if they had a call rate of $< 95\%$ or if their reported sex did not match their inferred sex based on X-chromosome genotypes. Only white non-Hispanic subjects (based on a STRUCTURE (Falush et al., 2003; Pritchard et al., 2000) analysis of 10,000 markers) with a DSM-IV defined PTSD Criterion-A traumatic event were included in the analysis. The sample analyzed includes 491 white non-Hispanic veterans and their intimate partners including 313 lifetime-PTSD cases and 178 trauma-exposed controls. Association between the SNP and lifetime PTSD was tested using PLINK (v. 1.07). First, the sample was analyzed using a logistic model adjusting for the top 3 PC's computed in EIGENSTRAT based on 10,000 randomly chosen markers.

3. Results

Meta-analysis of GWASs with PTSD in subjects of European (EA), African (AA), Hispanic/Native American (HNA), and other descents. Genome-wide association studies for PTSD were performed with genotypes of 2179 EA's, 640 HNA's, 205 AA's, and 470 subjects of other or mixed ancestral descent. The genomic control (GC) inflation factor lambda was close to 1.0 in all analyses (see Supplemental Fig. 2 for QQ-plots). GC-corrected p -values were combined in a meta-analysis and resulted in a genome-wide significant association for a SNP in the phosphoribosyl transferase domain containing 1 gene (rs6482463 in *PRTFDC1*; $OR = 1.47$, $SE = 0.06$, $p = 2.04 \times 10^{-9}$) (Fig. 1A and Supplemental Table 1A). *PRTFDC1* is a 104 kb long gene on chromosome 10, including 9 exons. The top SNP rs6482463 (imputed based on the genotyped proxy SNP rs6482463, $R^2 = 0.995$, imputation info score = 0.99) is located in a ~40 kb LD-block spanning most of intron 3 (Fig. 1B). An analysis of the large EA subgroup identified a different SNP (rs2148269, imputed) as top hit in this gene. SNP rs2148269 is located in the same LD-block as rs6482463 ($R^2 = 0.27$) (see Supplemental Fig. 3A and B for the EA Manhattan and regional association plots). However, the meta-analysis top SNP rs6482463 shows consistent odds ratios (OR) across all 4 ancestry groups, and a test for heterogeneity between studies was not significant ($p = 0.9$ for Cochran's Q; Table 2A). Given the parameters from the meta-analysis of rs6482463 ($MAF = 0.27$, relative risk = 1.324), a power calculation indicated that the study was sufficiently powered (~80%) to detect an effect size of this magnitude ($OR = 1.47$).

Replication of the *PRTFDC1* association with PTSD was attempted in an independent military cohort (VA replication

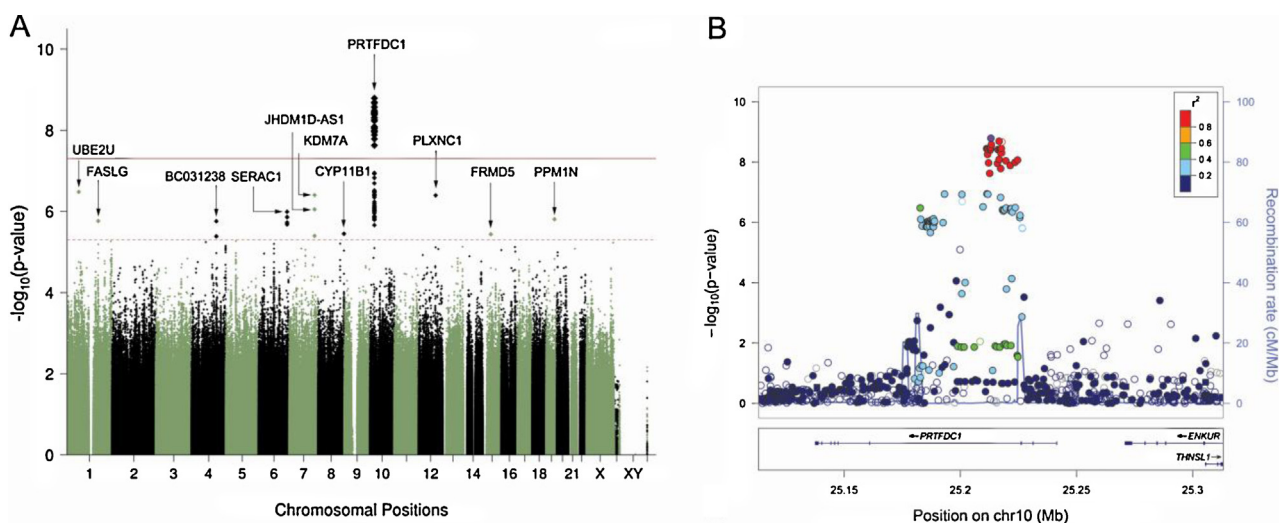


Figure 1 (A) Manhattan plot of genome-wide association results for PTSD from a meta-analysis of subjects from mixed ancestries. The red line represents genome-wide significance at $p < 5 \times 10^{-8}$ and the dashed line represents suggestive evidence for association at $p < 5 \times 10^{-6}$. (B) Regional association plot, showing significant regions in *PRTFDC1* on chromosome 10. Results are reported for the most significant SNP rs6482463 from the meta-analysis. The color of each circle is based on R^2 with rs6482463 and recombination rates are based on European reference subjects from the 1000 Genomes Project.

sample). The imputed SNP rs6482463 was not available, but rs1033962 (a SNP 3678 bp apart) was genotyped in both MRS and NCPTS. Associations for rs1033962 in the MRS meta-analysis were slightly less strong than for the top SNP rs6482463 ($p = 4.93 \times 10^{-9}$; Table 2B). Association of rs1033962 in the smaller NCPTS replication study was not significant ($N = 491$; $p = 0.14$). However, the direction of the effect of the A allele was consistent with MRS, and a meta-analysis of MRS and NCPTS showed no heterogeneity (Cochran $p = 0.91$) and further decreased the p -value to 2.06×10^{-9} .

We also explored alternative statistical models for *PRTFDC1* associations with PTSD, extending from the basic model with an additively coded SNP effect, and the covariates battalion, GWAS platform, and 5 PC's for population stratification. Compared to the additive model ($p = 2.04 \times 10^{-9}$), recessive and dominant genetic models did not show stronger effects for rs6482463 ($p = 2.03 \times 10^{-3}$ or $p = 3.2 \times 10^{-9}$, respectively). In addition, we tested the effects of age, different types of traumas (CTQ, LEC, and prior deployments; see Table 1), and GxE interactions on PTSD status (Supplemental Table 1B). Age and the 3 types

Table 2 Meta-analyses of *PRTFDC1* associations with PTSD for (A) the most significant imputed SNP rs6482463 in four Marine Resiliency Study (MRS) ancestry groups, and (B) for the genotyped SNP rs1033962 in MRS and an independent replication sample from the National Center for PTSD/Boston (NCPTS).

Study	Ancestry	A1	A2	MAF	N subjects	OR	SE	P	Q
(A) Association analysis for rs6482463									
MRS	EA	A	G	0.22	2179	1.41	0.08	2.98×10^{-5}	
	AA	A	G	0.46	205	1.49	0.26	0.118	
	HNA	A	G	0.31	640	1.58	0.14	1.25×10^{-3}	
	OTH	A	G	0.31	470	1.55	0.18	0.012	
Meta		A	G	—	3494	1.47	0.06	2.04×10^{-9}	0.90
(B) Association analysis for rs1033962									
MRS	EA	A	G	0.22	2179	1.40	0.08	4.48×10^{-5}	
	AA	A	G	0.47	205	1.45	0.25	0.148	
	HNA	A	G	0.31	640	1.57	0.14	1.37×10^{-3}	
	OTH	A	G	0.31	470	1.52	0.17	0.016	
Meta	All	A	G	—	3494	1.45	0.06	4.93×10^{-9}	0.90
NCPTS	EA	A	G	0.21	491	1.28	0.17	0.144	
Meta	All	A	G	—	3985	1.43	0.06	2.06×10^{-9}	0.91

MAF, minor allele frequency for A1 allele; OR, odds ratio; SE, standard error of the mean; Q, p -value for Cochran's Q statistic; meta, inverse-variance weighted meta-analysis; EA, European American; AA, African American; HNA, Hispanic and Native American descent; OTH, other.

Please cite this article in press as: Nievergelt, C.M., et al., Genomic predictors of combat stress vulnerability and resilience in U.S. Marines: A genome-wide association study across multiple ancestries implicates *PRTFDC1* as a potential PTSD gene. *Psychoneuroendocrinology* (2014), <http://dx.doi.org/10.1016/j.psyneuen.2014.10.017>

of trauma significantly predicted PTSD in univariate analyses ($p < 0.05$ in all cases), and explained between 2.8% (age) and 14.9% (LEC) of the variability (% VE). Adding these predictors to the top SNP rs6482463 slightly decreased the p -values for the SNP effect for models including SNP plus age, CTQ, or prior deployment, respectively. Tests for GxE interactions using the LEC, CTQ or prior deployment were not significant ($p > 0.05$ in all cases). Finally, a cumulative model including SNP, age, LEC, CTQ and prior deployment (plus the standard covariates battalion, GWAS platform, and 5 PC's for ancestry) was most significant in predicting PTSD status ($p = 4.07 \times 10^{-94}$) and explained ~20% of the variance (Supplemental Table 1B).

In addition to the genome-wide significant association with *PRTFDC1*, SNPs in 26 genes met the threshold for suggestive evidence of association ($p < 5 \times 10^{-6}$), including SNPs in 10 genes from the meta-analysis (Fig. 1A) and 15 genes in specific ancestry groups. A summary for the top SNPs per gene are shown in Table 3 (see also extended data in Supplemental Table 2). As expected based on the size of the subsets, most of the associations meeting suggestive evidence were found in the largest EA subgroup (see also Manhattan plot for EA in Supplemental Fig. 2). There was considerable heterogeneity across the 4 ancestral groups in regards to the effect of the top SNPs. The direction of the effects across the 26 genes was consistent only for 7 of the top SNPs, and Cochran's Q value showed significant heterogeneity across studies for 12 associations, including all 4 of the SNPs meeting suggestive evidence in the AAs and both SNPs meeting suggestive evidence in the HNA's. No SNP met suggestive evidence for association in the 470 subjects of 'other' descent.

In addition to testing for a main SNP effect on PTSD diagnosis we also tested for an interaction of childhood trauma (CTQ) and the top SNPs listed in Table 3 (GxE interaction). Six SNPs showed nominally significant GxE interactions in one or more ancestry groups. However, none of them remained significant after correction for multiple comparisons (at a threshold of $p < 0.002$ for 26 tests performed).

3.1. Comparison of PTSD genes reported in the literature with results from the MRS GWAS

We compared the results from the MRS association analyses in the EA and AA subgroups for 25 genes with significant association with PTSD for either a main SNP effect and/or a significant GxE interaction previously reported in the literature (see Table 4 for EA and Supplemental Table 3 for AA). Most of the genes were identified in candidate gene studies, but *LINC01090* (Guffanti et al., 2013), *RORA* (Logue et al., 2013a), and *TLL1* (Xie et al., 2013) came from recent GWASs, thus meeting the stricter level for genome-wide significance in the original studies. We first investigated the specific SNP reported in the literature and found that none of the reported SNPs were nominally significant in the MRS EA or AA subgroups. Next we tested all available SNPs within the 25 genes for association with PTSD. The number of SNPs per gene available in the MRS GWAS ranged from 11 SNPs in *RGS2* to 1976 SNPs in *ANK3*. With the exception of *APOE*, all genes included

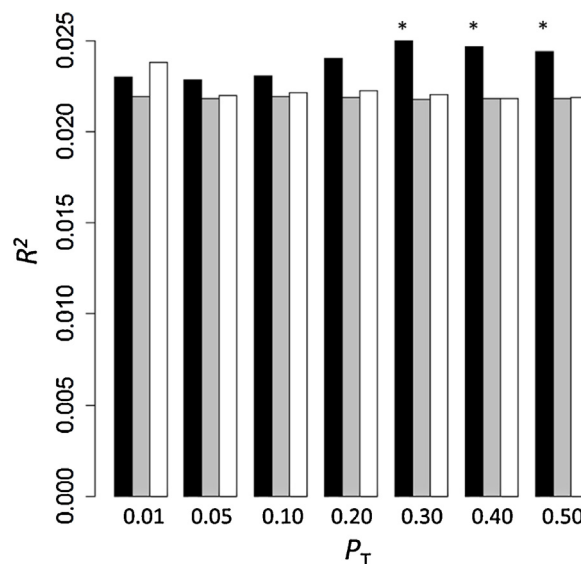


Figure 2 Polygenic risk score profiling in European American subjects, using discovery sets from GWAS on bipolar disorder (BPD, black bars), major depressive disorder (MDD, gray bars), and schizophrenia (SCZ, white bars) from the Psychiatric Genomic Consortium (PGC). The x-axis shows results at seven p -value thresholds ($P_T = 0.01, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50$). The y-axis shows the Nagelkerke pseudo R^2 , the proportion of variance in PTSD case-control status explained by the risk score profile. * indicates nominal significance at $p < 0.05$.

at least one nominally significant SNP in the EA and/or AA subgroup. However, after controlling for multiple comparisons at the gene level (not yet considering the number of genes tested), none of these associations remained significant.

Significant GxE interactions were reported for SNPs in 7 genes (Table 4), predominantly including childhood trauma as the environmental factor. We did not replicate a GxE effect in these 7 genes in the MRS ($p > 0.05$). GxE interactions in 4 other genes without a reported GxE in the original studies were nominally significant in MRS, but did not meet the threshold after correction for multiple comparisons ($p < 0.002$ for 25 genes tested).

3.2. Association of cross-disorder polygenic risk scores in the MRS PTSD GWAS

We also tested for a genetic overlap of PTSD with bipolar disorder (BPD), major depressive disorder (MDD), and schizophrenia (SCZ) using polygenic risk scores. These scores, an aggregate of many SNPs with small individual effects retrieved from large PGC GWAS studies, were used at different p -value thresholds (P_T), ranging from 0.01 to 0.5 (Fig. 2). We found that the polygenic risk scores for BPD explained a significant proportion of phenotypic variance in the MRS for $P_T = 0.3$ (Nagelkerke $R^2 = 0.025$, $p = 0.028$), $P_T = 0.4$ ($R^2 = 0.025$, $p = 0.037$), and $P_T = 0.5$ ($R^2 = 0.024$, $p = 0.047$). Polygenic risk scores from GWAS of SCZ and MDD did not significantly predict PTSD in the MRS.

Table 3 Top hits from genome-wide association studies with PTSD in subjects of European (EA), African (AA), Hispanic/Native American (HNA), and other descents, and meta-analysis across ancestry groups.

SNP	CHR	Gene	Location	Allele 1/2	EA		AA		HNA		Other		Meta-analysis			
					P_{main}	P_{GxE}	P_{main}	P_{GxE}	P_{main}	P_{GxE}	P_{main}	P_{GxE}	Q	OR	P_{main}	Direction
rs138384996	1	UBE2U	Downstream	G/A	1.6E-05	0.08	0.686	0.999	0.11	0.17	0.022	0.16	0.82	0.24	4.1E-07	-+---
rs4916008*	1	JAK1	Intron	C/T	4.0E-06	1.00	0.49	0.26	0.58	0.34	0.90	0.06	0.033	1.40	2.6E-04	+++-
rs74939664	1	LPHN2	Downstream	T/C	2.0E-06	0.33	0.66	0.05	0.43	0.81	0.27	0.13	0.06	1.73	3.5E-05	++++
rs2312236*	1	POGK	Upstream	T/C	0.99	0.47	0.94	0.30	2.9E-06	0.93	0.11	0.62	4.4E-05	1.13	0.204	+--+
rs6681010	1	FASLG	Downstream	G/A	0.001	0.66	0.010	0.06	0.19	0.74	0.08	0.27	0.83	0.46	2.0E-06	-----
rs4511180	1	PTPRV	Exon	A/G	0.16	0.37	7.7E-07	0.81	0.65	0.98	0.30	0.80	2.8E-05	1.12	0.049	+++
rs3100127	1	LGR6	Upstream	C/A	0.14	0.26	1.3E-06	0.58	0.55	0.91	0.19	0.81	2.5E-05	0.89	0.05	-----+
rs10737854	1	RGS7	Intron	G/A	4.6E-06	0.53	0.93	0.96	0.83	0.11	0.036	0.82	0.07	0.78	1.7E-05	-+++
rs187093517	2	UBE2E3	Upstream	A/G	2.8E-07	0.61	0.26	0.72	0.84	0.29	0.77	0.73	0.038	0.51	1.1E-05	-+++
rs62275374	4	LRPAP1	Upstream	G/A	4.5E-06	0.006	0.88	0.76	0.13	0.27	0.62	0.95	0.004	0.73	1.3E-03	---++
rs1380630	4	BC031238	Upstream	T/C	1.8E-05	0.54	1.00	0.55	0.21	0.14	0.029	0.49	0.56	0.69	1.9E-06	-+---
rs10457838	6	UST	Intron	C/T	0.004	0.65	2.2E-06	0.83	0.84	1.00	0.27	0.53	4.3E-06	0.88	0.039	-+---
rs115028822	6	SERAC1	Intron	C/A	1.6E-04	0.44	0.09	0.42	0.08	0.09	0.08	0.23	0.99	0.45	1.6E-06	-----
rs79485117	7	KDM7A	Intron	C/T	1.5E-05	0.92	0.36	0.027	0.14	0.94	0.036	0.19	0.89	0.58	4.4E-07	-----
rs2471320*	7	JHDM1D-AS1	Downstream	T/C	2.0E-05	0.69	0.80	0.003	0.22	0.18	0.19	0.16	0.96	0.55	4.2E-06	-----
rs2616978	8	CSMD1	Intron	T/C	0.12	0.11	0.014	0.050	2.8E-06	0.77	0.40	0.87	1.0E-06	1.04	0.46	-+++
rs142570922	8	CYP11B1	Upstream	A/C	2.2E-04	0.27	0.82	0.82	0.003	0.036	0.15	0.027	0.40	1.34	3.8E-06	++++
rs10511822	9	LINGO2	Intron	C/T	3.1E-06	0.15	0.80	0.77	0.79	0.94	0.73	0.38	0.05	0.73	1.6E-04	-----+
rs58649573	9	LHX2	Downstream	T/C	0.37	0.70	9.0E-07	0.25	0.18	0.06	0.94	0.77	8.4E-06	1.04	0.48	-+++
rs2148269	10	PRTFDC1	Intron	A/G	4.6E-06	0.90	0.93	0.66	0.007	0.74	0.653	0.36	0.25	0.69	8.0E-07	-----
rs6482463	10	PRTFDC1	Intron	G/A	3.0E-05	0.98	0.12	0.73	0.001	0.91	0.012	0.30	0.90	0.68	2.0E-09	-----
rs73220799	12	PLXNC1	Upstream	C/T	4.3E-06	0.91	0.046	0.09	0.47	0.05	0.12	0.38	0.48	1.96	4.4E-07	++++
rs9545302*	13	LINCO1080	Downstream	A/C	1.4E-06	0.88	0.22	0.92	0.66	0.46	0.40	0.79	6.3E-04	0.84	0.004	-+++
rs78826942	15	FRMD5	Intron	T/C	3.1E-05	0.18	0.14	0.34	0.024	0.69	0.60	0.18	0.32	0.52	3.8E-06	-+---
rs148952004	19	PPM1N	Intron	A/G	3.8E-06	0.87	0.38	0.57	0.10	0.21	0.75	0.020	0.56	0.23	1.8E-06	-----
rs199563271	20	PTPRT	Intron	CACAT/C	1.4E-06	0.24	0.65	0.020	0.82	0.24	0.85	0.59	0.028	0.47	1.4E-04	-+---
rs6528940	X	MAGEC1	Downstream	T/C	1.3E-06	0.09	0.28	0.68	0.033	0.23	0.44	0.49	1.1E-04	1.13	0.003	+--+

Gene by environment (GxE) analyses are based on Childhood trauma. p -Values for the main analysis (P_{main}) in bold meet suggestive ($p < 5 \times 10^{-06}$) or genome-wide significance ($p < 5 \times 10^{-08}$). GxE interaction p -values (P_{GxE}) and Q values (p -value for Cochran's Q statistic) in bold meet nominal significance ($p < 0.05$). * Genotyped SNPs, all other SNPs listed are imputed.

Table 4 PTSD association analysis of SNPs in 25 putative PTSD genes from published PTSD candidate gene and genome-wide association studies in 2179 MRS subjects of European descent.

Gene	Reported in literature						MRS GWAS				
	Study	Ancestry	Reported SNP	P/P _{GxE}	P	P _{GxE}	N SNPs	Top SNP	P	P _{GxE}	P _{gene}
<i>ADCYAP1R1 (PAC1)</i>	Ressler et al. (2011)	AA	rs2267735	Y/–	0.66	0.25	65	rs6968349	0.017	0.84	0.47
<i>ANK3</i>	Logue et al. (2013a,b)	EA	rs9804190	Y/N	0.93	0.76	1976	rs139604943	0.008	0.85	0.53
<i>APOE</i>	Lyons et al. (2013)	EA	rs429358, rs7412 ^a	Y/Y	0.96	0.27	27	rs1081105	0.05	0.036	1
<i>CHRNA5</i>	Boscarino et al. (2011)	EU	rs16969968	Y/Y	0.22	0.67	72	rs518425	0.07	0.61	1
<i>COMT</i>	Kolassa et al. (2010)	AA	rs4680	N/Y	0.53	0.55	116	rs174686	0.049	0.64	1
<i>CRHR1</i>	Amstadter et al. (2011)	Other	rs12944712	Y/–	0.41	0.52	1034	rs116897693	0.023	0.63	0.24
<i>DRD2</i>	Comings et al. (1996)	Other	rs1800497	Y/–	0.06	0.93	177	rs75924850	0.021	0.48	0.74
<i>DRD4</i>	Dragan and Oniszczenko (2009)	EU	VNTR	Y/N	NA	NA	14	rs4987059	0.10	0.53	1
<i>DTNBP1</i>	Voisey et al. (2010)	EA	rs9370822	Y/–	0.90	0.004	346	rs116647843	0.06	0.82	0.86
<i>FKBP5</i>	Binder et al. (2008)	AA	rs9296158	N/Y	0.15	0.32	239	rs9366890	0.015	0.23	0.19
<i>GABRA2</i>	Nelson et al. (2009)	Other	rs279836	N/Y	0.36	0.13	255	rs148139959	0.024	0.16	1
<i>HTR2A</i>	Mellman et al. (2009)	AA	rs6311	Y/N	0.88	0.17	232	rs6314	0.049	0.20	0.79
<i>LINC01090 (AC068718.1)^c</i>	Guffanti et al. (2013)	AA, EA	rs10170218	Y/–	1.00	0.93	1582	rs6759539	0.002	0.28	0.27
<i>NR3C1</i>	Hauer et al. (2011)	EA	rs41423247	Y/–	0.73	0.86	162	rs79590198	0.09	0.022	1
<i>RGS2</i>	Amstadter et al. (2009)	EA	rs4606	N/Y	0.49	0.45	11	rs141129523	0.022	0.23	0.08
<i>RORA^c</i>	Logue et al. (2013a)	EA, AA	rs8042149	Y/N	0.99	0.71	1706	rs12442490	0.003	0.65	0.58
<i>SLC18A2</i>	Solovieff et al. (2014)	EA, AA	rs363276	Y/–	0.16	0.52	107	rs363238	0.022	0.34	0.48
<i>SLC6A3 (DAT1)</i>	Segman et al. (2002)	EA	VNTR	Y/–	NA	NA	86	rs144782362	0.008	0.37	0.33
<i>SLC6A4 (SERT)</i>	Grabe et al. (2009)	EU	rs4795541, rs25531 ^b	Y/Y	NA	NA	43	rs28914827	0.14	0.54	1
<i>SRD5A2</i>	Gillespie et al. (2013)	AA	rs523349	Y/N	0.11	0.69	137	rs77929608	0.049	0.39	0.65
<i>STMN1</i>	Cao et al. (2013)	Other	rs182455	Y/–	0.51	0.66	32	rs4659395	0.09	0.23	1
<i>TLL1^c</i>	Xie et al. (2013)	EA, AA	rs6812849	Y/–	0.46	0.24	434	rs113712660	0.017	0.19	0.62
<i>TPH1</i>	Goenjian et al. (2012)	EA	rs2108977	Y/–	0.67	0.58	42	rs544437	0.25	0.75	1
<i>TPH2</i>	Goenjian et al. (2012)	EA	rs11178997	Y/–	0.46	0.32	250	rs183063707	0.017	0.13	0.73
<i>WWC1 (KIBRA)</i>	Wilker et al. (2013)	AA	rs10038727	Y/N	0.99	0.44	469	rs17551315	0.001	0.14	0.10

Gene by environment (GxE) analyses are based on Childhood trauma. Nominally significant *p*-values (*p* < 0.05) are bolded and gene-set *p*-values are corrected for the number of SNPs tested per gene. P/P_{GxE}: indicates if the study reported a significant main effect (P: yes/no) or a significant gene by environment interaction (P_{GxE}: yes/no). P_{gene}: set-based empirical *p*-values for each gene, corrected for the number of SNPs (N SNPs) tested per gene. Top SNP: best result for the tested MRS SNPs.

^a rs429358, rs7412 (ϵ_2 , ϵ_3 , ϵ_4).

^b rs4795541 (L/S) + rs25531 (L_A/L_G)

^c Genome-wide significant genes from published GWA studies.

4. Discussion

We present the first multi-ethnic GWAS of PTSD to date, including subjects of European, African, Native American/Hispanic, and other ancestry, typically found in U.S. military cohorts. Participants were recruited from the MRS, a large, prospectively assessed cohort of Marines and Sailors with index deployments to Iraq or Afghanistan (Baker et al., 2012). This all-male study included 3494 subjects exposed to a *DSM-IV* criteria A1 traumatic event and represents one of the largest and most homogenous PTSD GWAS to date. Due to the military culture and training of the participants we did not require the endorsement of the A2 criteria i.e. that the traumatic experience is accompanied by intense fear, helplessness, or horror. However, removal of A2 from the *DSM-IV* criterion set does not seem to substantially increase the number of people who qualify for PTSD diagnosis (Karam et al., 2010), and A2 has been dropped entirely in the new *DSM-V* PTSD definition.

The GWAS meta-analysis across ancestry groups identified the phosphoribosyl transferase domain containing 1 gene (*PRTFDC1*) as a potential PTSD gene meeting genome-wide significance. This finding was supported by a smaller, independent VA cohort including 491 EA veterans and their intimate partners with 313 lifetime-PTSD cases (Logue et al., 2013a). *PRTFDC1* is a ~100 kb long gene located on chromosome 10p12. It encodes the phosphoribosyltransferase domain-containing protein 1, a relatively small protein with highest expression in the brain. *PRTFDC1* belongs to the purine/pyrimidine phosphoribosyltransferase family and is a paralog of *HPRT1*, but may have lost its ancestral *HPRT* activity (Keebaugh et al., 2007). However, *PRTFDC1* has been reported as a possible tumor-suppressor gene that is frequently silenced by aberrant promoter hypermethylation (Suzuki et al., 2007). To our knowledge *PRTFDC1* has not yet been implicated in GWAS of PTSD or other psychiatric disorders and its potential role in the etiology of PTSD remains to be determined.

As expected from a meta-analysis across ancestries, the *PRTFDC1* top hit from the meta-analysis was a SNP with a similar effect across multiple ancestry groups. This SNP (rs6482463) is located in a ~40 kb LD block spanning most of intron 3. The GWAS for the largest subgroup, including 2179 EAs, identified a different top hit in the same LD block, complicating a functional analysis of these findings. However, based on the UCSD genome browser annotations the whole region of the LD block shows enrichment in H3K27Ac and H3K4Me3 histone marks, indicative of high transcriptional activity (see Supplemental Fig. 4).

A hallmark of PTSD association studies are frequent findings of GxE interactions, where the effect of a gene on PTSD risk is exaggerated in the presence of a high trauma burden (Koenen et al., 2008). For example, this has been found for childhood trauma (Binder et al., 2008) as well as adult trauma such as combat exposure (Lyons et al., 2013). The thoroughly characterized MRS includes pre- and post-combat exposure trauma assessments, allowing for detailed testing of GxE interactions. We found that, while the overall model to predict PTSD status improved when we included trauma exposure into the model (from a model with baseline covariates and the SNP alone explaining ~4% of the variability to the complete model including trauma exposure

explaining a cumulative ~20% of the variability), GxE interactions for childhood trauma, adult life events, or previous combat deployments were not significant. Since our cohorts experienced a relatively large trauma burden, with significantly more trauma of all types reported by participants diagnosed with PTSD compared to Marines with low PTS symptoms (see Table 1), we conclude that power in MRS was similar to other studies that reported significant interactions. However, GxE interactions have been difficult to replicate and have a high potential to be false positives (Duncan and Keller, 2011). Recent methods based on model-robust estimates of standard errors are promising, especially in the context of genome-wide GxE analyses (Voorman et al., 2011).

In addition to the genome-wide significant *PRTFDC1* we found SNPs in 25 genes with suggestive evidence for association with PTSD. These results stem from specific ancestry groups and/or from the meta-analysis across groups. A comparison of findings between the different ancestry groups is limited by the much smaller size of the non-EA subgroups. Interesting genes with suggestive evidence for association include *CSMD1*, a gene previously implicated in large GWAS of other psychiatric disorders (Schizophrenia Psychiatric Genome-Wide Association Study, 2011), genes (*JAK1*, *FASLG*) related to immune response, a pathway that has previously been implicated for PTSD by GWAS (Guffanti et al., 2013) as well as gene expression studies (Glatt et al., 2013), and genes (*UBE2E3*, *UBE2U*) from the ubiquitin system, which has been implicated in the etiology of schizophrenia and bipolar disorder (Bousman et al., 2010). Before conclusions can be drawn however these genes must be replicated in larger GWASs and meta-analyses currently planned by the PGC PTSD working group (Koenen et al., 2013).

We also compiled a list of genes that have been reported in the literature to be significantly associated with PTSD, either showing a main effect for the genetic marker, and/or a significant GxE interaction (Amstadter et al., 2009, 2011; Binder et al., 2008; Boscarino et al., 2011; Cao et al., 2013; Comings et al., 1996; Dragan and Oniszczenko, 2009; Gillespie et al., 2013; Goenjian et al., 2012; Grabe et al., 2009; Guffanti et al., 2013; Hauer et al., 2011; Kolassa et al., 2010; Logue et al., 2013a,b; Lyons et al., 2013; Mellman et al., 2009; Nelson et al., 2009; Ressler et al., 2011; Segman et al., 2002; Solovieff et al., 2014; Voisey et al., 2010; Wilker et al., 2013; Xie et al., 2013). Since most studies were performed in subjects of either European or African descent, we used these specific ancestry groups for comparison with MRS. We found that most of the 25 candidate genes showed nominally significant associations in MRS for at least one of the SNPs tested. However, none of these results remained significant after appropriate Bonferroni corrections. Comparing the number of PTSD cases and overall study sizes between MRS and other studies indicated that we were adequately powered to detect many of the reported effects at least for the EA studies. A similar, well-powered study recently failed to replicate findings for 20 PTSD candidate genes after appropriate adjusting for multiple testing (Solovieff et al., 2014). This lack of replication may be due to a relatively large heterogeneity between PTSD studies, which are complicated by the requirement of exposure to a traumatic event, leading to potential differences in type, timing of, and time since trauma, and the observed GxE interactions.

However, it has been demonstrated that reports from candidate gene association studies (Sullivan, 2007), and especially GxE interactions (Duncan and Keller, 2011), have a high false discovery rate and a robust replication of findings is now a policy required by many journals.

In regards to our inability to replicate previous findings from GWASs, which met the stringent genome-wide significant thresholds, power calculations indicated that MRS was sufficiently powered for a replication of rs8042149 in *RORA* (Logue et al., 2013a) for EA's (OR 2.1 in original study and 1.22 in MRS; data not shown). However, the association of rs6812849 in *TLL1* (Xie et al., 2013) was originally detected in a larger study, and rs10170218 in *LINC01090* (Guffanti et al., 2013) was originally found in an all-female AA cohort, which was also larger than the all-male MRS AA cohort, and MRS findings for these genes remain inconclusive.

On the other hand, the large MRS GWAS was able to replicate a recent finding from a candidate gene study including 300 genes (Solovieff et al., 2014) that demonstrated for the first time the existence of common SNPs between PTSD severity and bipolar disorder based on cross-disorder polygenic risk score analyses. We used the standard polygenic scoring approach (Purcell et al., 2009) with results from the PGC for MDD, BP, and SCZ (Cross-Disorder Group of the Psychiatric Genomics Consortium and Genetic Risk Outcome of Psychosis Consortium, 2013) and found that PTSD diagnosis was predicted by risk scores derived from BPD, but not from MDD or SCZ. Our results for BPD reached significance at p -value thresholds >0.3 from the original GWAS, similar to the PTSD candidate gene study (Solovieff et al., 2014). Pleiotropic effects across a range of psychiatric disorders have recently been reported (Cross-Disorder Group and Genetic Risk Outcome, 2013) and provide exciting new insights into the genetic architecture of PTSD and other psychopathologies.

Power analyses for the population-based MRS cohort GWAS indicated increased power using a broad definition for PTSD, including 616 subjects with partial, and 324 subjects with a full *DSM-IV* based diagnosis (data not shown), compared to confining the sample to subjects with full PTSD diagnosis only. For example, the smaller size of the full PTSD case group would diminish the significance of our top finding for rs6482463 in *PRTFDC1* (OR = 1.47, SE = 0.06, $p = 2.04 \times 10^{-9}$) to below genome-wide significance, despite similar effect size (OR = 1.46, SE = 0.096, $p = 7.64 \times 10^{-5}$). As an alternative to using a specific disease cut-off we have considered quantitative analyses of PTSD symptoms. However, population-based studies require careful consideration of PTSD symptom distributions (e.g. CAPS symptoms in MRS are best characterized by a zero-inflated negative binomial distribution; Yurgil et al., 2014), which may lead to increased rates of false positives if not modeled appropriately. The broad PTSD definition used in this study may potentially limit a direct comparison with findings from other PTSD studies. In addition, our findings stem from a very homogenous all-male military cohort and generalizability into other population groups may be limited.

In summary, this first multi-ethnic PTSD GWAS highlights the potential to increase power of GWAS through meta-analyses of multi-ethnic association analyses for SNPs with consistent effects across ancestries. We found evidence for *PRTFDC1* as a novel PTSD gene, a finding

that awaits further replication. And lastly, the genetic architecture of PTSD may be determined by many SNPs with small effects, and overlap with other neuropsychiatric disorders, consistent with current findings from large GWAS of other psychiatric disorders, suggesting that genetic contributions to psychiatric disorders may not completely map to present diagnostic categories (Cross-Disorder Group of the Psychiatric Genomics Consortium and Genetic Risk Outcome of Psychosis Consortium, 2013).

Conflict of interest

None declared.

Acknowledgments

CMN is supported by NIH grants 1 R01MH093500, 1 U01MH092758, and by the Marine Corps and Navy Bureau of Medicine and Surgery (BUMED). VA Health Services and Development (HSR&D), the Marine Corps, and BUMED provided funding for MRS-I and MRS-II (DGB, NJS, DTO, MAG, VBR, MRS Team). The VA dataset was supported by NIH grant R01MH079806 and VA grant 5I01CX000431-02 to MWM.

We acknowledge the MRS-I and MRS-II Investigators William P. Nash (Boston VA Research Institute), Brett T. Litz (Veterans Affairs Boston Healthcare System, Boston, Massachusetts;), Paul S. Hammer (Navy Medicine Information Systems Support, San Antonio, Texas), Gerald E. Larson (Naval Health Research Center, San Diego, California), Jennifer J. Vasterling (Veterans Affairs Boston Healthcare System, Boston, Massachusetts and Boston University), and Jennifer A. Webb-Murphy (Naval Center for Combat & Operational Stress Control, San Diego, California), and special assistance from members of VA Center of Excellence for Stress and Mental Health, VA San Diego Research and Fiscal Services, and the 1st Marine Division and Navy Medicine at 29 Palms and at Camp Pendleton.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.psyneuen.2014.10.017>.

References

- Almli, L.M., et al., 2014. Genetic approaches to understanding post-traumatic stress disorder. *Int. J. Neuropsychopharmacol.* 17, 355–370.
- Amstadter, A.B., et al., 2009. Variant in *RGS2* moderates posttraumatic stress symptoms following potentially traumatic event exposure. *J. Anxiety Disord.* 23, 369–373.
- Amstadter, A.B., et al., 2011. Corticotrophin-releasing hormone type 1 receptor gene (*CRHR1*) variants predict posttraumatic stress disorder onset and course in pediatric injury patients. *Dis. Markers* 30, 89–99.
- Anderson, C.A., et al., 2010. Data quality control in genetic case-control association studies. *Nat. Protoc.* 5, 1564–1573.
- APA, 2000. Section 309.81: Posttraumatic Stress Disorder, Diagnostic and Statistical Manual of Mental Disorders: *DSM-IV-TR*, fourth ed. American Psychiatric Association, Washington, DC.

- Baker, D.G., et al., 2012. Predictors of risk and resilience for posttraumatic stress disorder among ground combat Marines: methods of the Marine Resiliency Study. *Prev. Chronic. Dis.* 9, E97.
- Binder, E.B., et al., 2008. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* 299, 1291–1305.
- Boscarino, J.A., et al., 2011. Association of FKBP5, COMT and CHRNA5 polymorphisms with PTSD among outpatients at risk for PTSD. *Psychiatry Res.* 188, 173–174.
- Bousman, C.A., et al., 2010. Preliminary evidence of ubiquitin proteasome system dysregulation in schizophrenia and bipolar disorder: convergent pathway analysis findings from two independent samples. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 153B, 494–502.
- Cao, C., et al., 2013. Stathmin genotype is associated with re-experiencing symptoms of posttraumatic stress disorder in Chinese earthquake survivors. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 44, 296–300.
- Comings, D.E., et al., 1996. Dopamine D2 receptor (DRD2) gene and susceptibility to posttraumatic stress disorder: a study and replication. *Biol. Psychiatry* 40, 368–372.
- Cross-Disorder Group of the Psychiatric Genomics Consortium, Genetic Risk Outcome of Psychosis Consortium, 2013. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. *Lancet* 381, 1371–1379.
- Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. *Nat. Genet.* 45, 984–994.
- Dragan, W.L., Oniszczenko, W., 2009. The association between dopamine D4 receptor exon III polymorphism and intensity of PTSD symptoms among flood survivors. *Anxiety Stress Coping* 22, 483–495.
- Duncan, L.E., Keller, M.C., 2011. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *Am. J. Psychiatry* 168, 1041–1049.
- Falush, D., et al., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164, 1567–1587.
- Fu, Q., et al., 2007. Differential etiology of posttraumatic stress disorder with conduct disorder and major depression in male veterans. *Biol. Psychiatry* 62, 1088–1094.
- Gillespie, C.F., et al., 2013. Sex dependent influence of a functional polymorphism in steroid 5-alpha-reductase type 2 (SRD5A2) on post-traumatic stress symptoms. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 162B, 283–292.
- Glatt, S.J., et al., 2013. Blood-based gene-expression predictors of PTSD risk and resilience among deployed marines: a pilot study. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 162B, 313–326.
- Goenjian, A.K., et al., 2012. Association of TPH1, TPH2, and 5HTTLPR with PTSD and depressive symptoms. *J. Affect. Disord.* 140, 244–252.
- Goenjian, A.K., et al., 2008. Heritabilities of symptoms of post-traumatic stress disorder, anxiety, and depression in earthquake exposed Armenian families. *Psychiatr. Genet.* 18, 261–266.
- Grabe, H.J., et al., 2009. Serotonin transporter gene (SLC6A4) promoter polymorphisms and the susceptibility to posttraumatic stress disorder in the general population. *Am. J. Psychiatry* 166, 926–933.
- Guffanti, G., et al., 2013. Genome-wide association study implicates a novel RNA gene, the lincRNA AC068718.1, as a risk factor for post-traumatic stress disorder in women. *Psychoneuroendocrinology* 38, 3029–3038.
- Harrell Jr., F.E., 2014. rms: Regression Modeling Strategies. R Package Version 4.1-3. <http://CRAN.R-project.org/package=rms>
- Hauer, D., et al., 2011. Relationship of a common polymorphism of the glucocorticoid receptor gene to traumatic memories and posttraumatic stress disorder in patients after intensive care therapy. *Crit. Care Med.* 39, 643–650.
- Karam, E.G., et al., 2010. The role of criterion A2 in the DSM-IV diagnosis of posttraumatic stress disorder. *Biol. Psychiatry* 68, 465–473.
- Keebaugh, A.C., et al., 2007. Gene duplication and inactivation in the HPRT gene family. *Genomics* 89, 134–142.
- Kessler, R.C., et al., 1995. Posttraumatic stress disorder in the National Comorbidity Survey. *Arch. Gen. Psychiatry* 52, 1048–1060.
- Kilpatrick, D.G., et al., 2013. National estimates of exposure to traumatic events and PTSD prevalence using DSM-IV and DSM-5 criteria. *J. Trauma. Stress* 26, 537–547.
- Koenen, K.C., et al., 2013. From candidate genes to genome-wide association: the challenges and promise of posttraumatic stress disorder genetic studies. *Biol. Psychiatry* 74, 634–636.
- Koenen, K.C., et al., 2008. Gene–environment interaction in post-traumatic stress disorder: review, strategy and new directions for future research. *Eur. Arch. Psychiatry Clin. Neurosci.* 258, 82–96.
- Kolassa, I.T., et al., 2010. The risk of posttraumatic stress disorder after trauma depends on traumatic load and the catechol-o-methyltransferase Val(158)Met polymorphism. *Biol. Psychiatry* 67, 304–308.
- Li, J.Z., et al., 2008. Worldwide human relationships inferred from genome-wide patterns of variation. *Science* 319, 1100–1104.
- Logue, M.W., et al., 2013a. 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* 18, 937–942.
- Logue, M.W., et al., 2013b. The ankyrin-3 gene is associated with posttraumatic stress disorder and externalizing comorbidity. *Psychoneuroendocrinology* 38, 2249–2257.
- Lyons, M.J., et al., 2013. Gene–environment interaction of ApoE genotype and combat exposure on PTSD. *Am. J. Med. Genet. B: Neuropsychiatr. Genet.* 162B, 762–769.
- Mellman, T.A., et al., 2009. Serotonin polymorphisms and post-traumatic stress disorder in a trauma exposed African American population. *Depress. Anxiety* 26, 993–997.
- Nelson, E.C., et al., 2009. Association of childhood trauma exposure and GABRA2 polymorphisms with risk of posttraumatic stress disorder in adults. *Mol. Psychiatry* 14, 234–235.
- Nievergelt, C.M., et al., 2013. Inference of human continental origin and admixture proportions using a highly discriminative ancestry informative 41-SNP panel. *Investig. Genet.* 4, 13.
- Nievergelt, C.M., et al., 2014. Chip-based direct genotyping of coding variants in genome wide association studies: utility, issues and prospects. *Gene* 540, 104–109.
- Norrholm, S.D., Ressler, K.J., 2009. Genetics of anxiety and trauma-related disorders. *Neuroscience* 164, 272–287.
- Nugent, N.R., et al., 2008. Genetics of post-traumatic stress disorder: informing clinical conceptualizations and promoting future research. *Am. J. Med. Genet. C Semin. Med. Genet.* 148, 127–132.
- Price, A.L., et al., 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet.* 38, 904–909.
- Pritchard, J.K., et al., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.
- Pruim, R.J., et al., 2010. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 26, 2336–2337.
- Purcell, S., et al., 2003. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 19, 149–150.

- Purcell, S., et al., 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81, 559–575.
- Purcell, S.M., et al., 2009. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460, 748–752.
- Ressler, K.J., et al., 2011. Post-traumatic stress disorder is associated with PACAP and the PAC1 receptor. *Nature* 470, 492–497.
- Richardson, L.K., et al., 2010. Prevalence estimates of combat-related post-traumatic stress disorder: critical review. *Aust. N.Z. J. Psychiatry* 44, 4–19.
- Roberts, A.L., et al., 2010. Race/ethnic differences in exposure to traumatic events, development of post-traumatic stress disorder, and treatment-seeking for post-traumatic stress disorder in the United States. *Psychol. Med.*, 1–13.
- Sartor, C.E., et al., 2012. Common heritable contributions to low-risk trauma, high-risk trauma, posttraumatic stress disorder, and major depression. *Arch. Gen. Psychiatry* 69, 293–299.
- Schizophrenia Psychiatric Genome-Wide, 2011. Genome-wide association study identifies five new schizophrenia loci. *Nat. Genet.* 43, 969–976.
- Segman, R.H., et al., 2002. Association between the dopamine transporter gene and posttraumatic stress disorder. *Mol. Psychiatry* 7, 903–907.
- Solovieff, N., et al., 2013. Pleiotropy in complex traits: challenges and strategies. *Nat. Rev. Genet.* 14, 483–495.
- Solovieff, N., et al., 2014. Genetic association analysis of 300 genes identifies a risk haplotype in SLC18A2 for post-traumatic stress disorder in two independent samples. *Neuropsychopharmacology* 39, 1872–1879.
- Sullivan, P.F., 2007. Spurious genetic associations. *Biol. Psychiatry* 61, 1121–1126.
- Suzuki, E., et al., 2007. *PRTFDC1*, a possible tumor-suppressor gene, is frequently silenced in oral squamous-cell carcinomas by aberrant promoter hypermethylation. *Oncogene* 26, 7921–7932.
- Voisey, J., et al., 2010. A polymorphism in the dysbindin gene (*DTNBP1*) associated with multiple psychiatric disorders including schizophrenia. *Behav. Brain Funct.* 6, 41.
- Voorman, A., et al., 2011. Behavior of QQ-plots and genomic control in studies of gene–environment interaction. *PLoS ONE* 6, e19416.
- Wilker, S., et al., 2013. The role of memory-related gene *WWC1* (*KIBRA*) in lifetime posttraumatic stress disorder: evidence from two independent samples from African conflict regions. *Biol. Psychiatry* 74, 664–671.
- Willer, C.J., et al., 2010. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26, 2190–2191.
- Wolf, E.J., et al., 2013. Combat exposure severity as a moderator of genetic and environmental liability to post-traumatic stress disorder. *Psychol. Med.*, 1–11.
- Xian, H., et al., 2000. Genetic and environmental influences on post-traumatic stress disorder, alcohol and drug dependence in twin pairs. *Drug Alcohol Depend.* 61, 95–102.
- Xie, P., et al., 2009. 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* 66, 1201–1209.
- Xie, P., et al., 2013. Genome-wide association study identifies new susceptibility loci for posttraumatic stress disorder. *Biol. Psychiatry* 74, 656–663.
- Yurgil, K.A., et al., 2014. Association between traumatic brain injury and risk of posttraumatic stress disorder in active-duty marines. *JAMA psychiatry* 71, 149–157.
- Zoladz, P.R., Diamond, D.M., 2013. Current status on behavioral and biological markers of PTSD: a search for clarity in a conflicting literature. *Neurosci. Biobehav. Rev.* 37, 860–895.