Acta Psychiatrica Scandinavica

Acta Psychiatr Scand 2018: 137: 148–156

DOI: 10.1111/acps.12843




# Trauma exposure interacts with the genetic risk of bipolar disorder in alcohol misuse of US soldiers

Polimanti R, Kaufman J, Zhao H, Kranzler HR, Ursano RJ, Kessler RC, Stein MB, Gelernter J. Trauma exposure interacts with the genetic risk of bipolar disorder in alcohol misuse of US soldiers.

**Objective:** To investigate whether trauma exposure moderates the genetic correlation between substance use disorders and psychiatric disorders, we tested whether trauma exposure modifies the association of genetic risks for mental disorders with alcohol misuse and nicotine dependence (ND) symptoms.
**Methods:** High-resolution polygenic risk scores (PRSs) were calculated for 10 732 US Army soldiers (8346 trauma-exposed and 2386 trauma-unexposed) based on genome-wide association studies of bipolar disorder (BD), major depressive disorder, and schizophrenia.
**Results:** The main finding was a significant BD PRS-by-trauma interaction with respect to alcohol misuse ($P = 6.07 \times 10^{-3}$). We observed a positive correlation between BD PRS and alcohol misuse in trauma-*exposed* soldiers ($r = 0.029$, $P = 7.5 \times 10^{-3}$) and a negative correlation in trauma-*unexposed* soldiers ($r = -0.071$, $P = 5.61 \times 10^{-4}$). Consistent (nominally significant) result with concordant effect, directions were observed in the schizophrenia PRS-by-trauma interaction analysis. The variants included in the BD PRS-by-trauma interaction showed significant enrichments for gene ontologies related to high voltage-gated calcium channel activity (GO:0008331, $P = 1.51 \times 10^{-5}$; GO:1990454, $P = 4.49 \times 10^{-6}$; GO:0030315, $P = 2.07 \times 10^{-6}$) and for Beta1/Beta2 adrenergic receptor signaling pathways ($P = 2.61 \times 10^{-4}$).
**Conclusions:** These results indicate that the genetic overlap between alcohol misuse and BD is significantly moderated by trauma exposure. This provides molecular insight into the complex mechanisms that link substance abuse, psychiatric disorders, and trauma exposure.

**R. Polimanti[1], J. Kaufman[2,3], H. Zhao[4,5], H. R. Kranzler[6], R. J. Ursano[7], R. C. Kessler[8], M. B. Stein[9,10], J. Gelernter[1,5,11]**

[1]Department of Psychiatry, Yale School of Medicine and VA CT Healthcare Center, West Haven, CT, [2]Center for Child and Family Traumatic Stress, Kennedy Krieger Institute, Baltimore, MD, [3]Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD, [4]Department of Biostatistics, Yale University School of Public Health, New Haven, CT, [5]Department of Genetics, Yale University School of Medicine, New Haven, CT, [6]Department of Psychiatry, University of Pennsylvania School of Medicine and VISN 4 MIRECC, Crescenz VAMC, Philadelphia, PA, [7]Center for the Study of Traumatic Stress, Department of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD, [8]Department of Health Care Policy, Harvard Medical School, Boston, MA, [9]Departments of Psychiatry and of Family Medicine and Public Health, University of California San Diego, La Jolla, CA, [10]VA San Diego Healthcare System, San Diego, CA, and [11]Department of Neuroscience, Yale University School of Medicine, New Haven, CT, USA

Key words: alcohol; bipolar disorder; genetic epidemiology; trauma

Joel Gelernter, Yale University School of Medicine, Department of Psychiatry, VA CT 116A2, 950 Campbell Avenue, West Haven, CT 06516, USA.
E-mail: joel.gelernter@yale.edu

Accepted for publication November 21, 2017

**Significant outcomes**

- There is a gene-by-trauma crossing interaction between bipolar disorders on alcohol misuse with a positive correlation in trauma-exposed soldiers and a negative correlation in trauma-unexposed soldiers.
- The variants included in the gene-by-trauma interaction showed significant enrichments for gene ontologies related to high voltage-gated calcium channel activity and for Beta1/Beta2 adrenergic receptor signaling pathways.

**Limitations**

- Gene-by-environment genome-wide interaction studies of cohorts with larger sample sizes are needed to understand better how trauma exposure affects genome regulation in determining psychiatric disorders.
- Experimental studies are required to confirm our hypothesis regarding the role of L-type calcium channels and beta adrenergic receptors signaling as molecular targets for the treatment of alcohol misuse.

## Introduction

A predisposition to psychiatric disorders—either one trait in an individual or a comorbid set of disorders—is related to the complex interplay of multiple genetic and environmental factors. Recent studies conducted using large genome-wide datasets showed a strong genetic overlap among psychiatric disorders (1, 2), providing molecular validation to underpin the psychiatric comorbidity observed. Exposure to traumatic life events is probably the most investigated environmental factor in psychiatry, and there is consistent evidence that trauma exposure is associated with an increased risk of mental illness and with worse outcomes in psychiatric patients (3, 4). The role of trauma exposure in the comorbidity between substance use disorders and other psychiatric disorders is particularly evident: Subjects with mental illness exposed to traumatic events are more likely to be affected by substance use disorders than unexposed psychiatric patients (5, 6). These observations strongly suggest that the trauma exposure affects the phenotypic expression of human genetic variation, moderating the predisposition to mental illness comorbidity.

To date, however, there is only limited information from gene-by-trauma investigations to inform the biological nature of these comorbidities. Previous studies that focused on candidate stress-response genes, such as *SLC6A4**5-HTTLPR, *PER1,* and *FKBP5* (7–9), show evidence of pleiotropy (10), but these studies have not examined predictors of comorbidity. Recently, we conducted a genome-wide gene-by-trauma interaction study of alcohol misuse in more than 24 000 individuals, identifying *PRKG1* as a risk locus (11). Although the role of this gene in trauma response is strongly supported by studies conducted in animal models (12, 13), variation at this locus explains only a small fraction of the polygenic architecture contributing to the mental health consequences of trauma exposure. Genome-wide investigations, that is, genome-wide association studies (GWAS) and gene-by-environment genome-wide interaction studies (GEWIS), could help to uncover the genetic basis of complex traits, but very large sample sizes ($N > 100\ 000$) may be needed to identify numerous risk loci. However, it is also possible to use the summary statistics from large GWAS to investigate the genetic correlation among complex traits (14). In particular, high-resolution polygenic risk scores (PRSs) derived from large GWAS can identify phenotypes that share genetic vulnerability and PRSs derived from psychiatric disorders were able to predict substance use disorders in independent cohorts (15–17). A PRS approach can be used to test the effect of trauma exposure in the genetic predisposition to comorbidity between substance abuse disorders and other psychiatric illnesses, providing information regarding the mechanisms by which traumatic events affect the phenotypic expression of human variation on a genome-wide basis.

The aim of this study was to investigate whether trauma exposure modifies the association of genetic risks for mental disorders with alcohol misuse and nicotine dependence (ND) symptoms. To do this, we calculated high-resolution PRS for US soldiers of European descent from the Army Study to Assess Risk and Resilience in Servicemembers (STARRS) (18) using summary statistics from large genome-wide association studies of bipolar disorder (BD) (19), major depressive disorder (MDD) (20), and schizophrenia (21) conducted by the Psychiatric Genomics Consortium (PGC). Then, we tested whether trauma exposure moderates the correlation of alcohol and nicotine use disorders with psychiatric PRS. We focused this investigation only on these three psychiatric disorders to avoid reducing statistical power due to multiple testing comparison correction.

## Material and methods

### Participants

The Army STARRS project is the largest study of mental health risk and resilience ever conducted in military personnel (18). Subjects investigated in this study were selected from the participants in

the STARRS. All subjects gave written informed consent to participate in procedures that were approved by the Human Subjects Committees of all collaborating clinical institutions. Two study populations were included in the STARRS Initiative: the New Soldier Study (NSS), which includes soldiers at the start of their basic training at one of three Army installations; and the Pre-Post Deployment Study (PPDS), which collected data from US Army soldiers in three brigade combat teams prior to their deployment to Afghanistan. For PPDS subjects, we considered the information reported at time 0 (within approximately six weeks prior to deployment). Detailed information about the design and conduct of Army STARRS is available in a previous report (18). STARRS participants were assessed using a self-administered questionnaire, including a computerized version of the Composite International Diagnostic Interview Screening Scales (CIDI-SC) (22). From the CIDI-SC assessment, we extracted information regarding lifetime trauma exposure, alcohol misuse, and ND symptom count.

### Assessment of clinical characteristics

Lifetime trauma exposure was defined as the presence of at least one of the following experiences: serious physical assault; sexual assault or rape; witnessing someone being seriously injured or killed; discovering or handling a dead body; a life-threatening illness or injury; a disaster; any other experience that put the subject at risk of death or serious injury; murder of a close friend or relative; suicide of a close friend or relative; combat death of a close friend or relative; or accidental death of a close friend or relative. Further details on the trauma assessments were reported previously (11, 23).

A dimensional measure of alcohol misuse was derived by summing responses to 13 items that assessed frequency and consequences of alcohol use including the array of alcohol misuse symptoms: (i) Frequency of Drinking; (ii) Frequency of Binge Drinking; (iii) Drinking Interfered with Responsibility; (iv) Drinking Caused an Argument; (v) Drinking Resulted in Someone Getting Hurt; (vi) Out of Control Drinking; (vii) Arrested Due to Drinking; (viii) Worried to Not Be Able to Drink; (ix) Worried About Drinking; (x) Feel a Need to Cut Down; (xi) Feel Annoyed by People Who Mention Drinking; (xii) Feel Guilty About Drinking; (xiii) Drink First Thing in the Morning. Respondents rated each symptom on a 5-point frequency scale that ranged from "never" through "every or nearly every day." As for our other genetic studies of substance use disorders (24–27), we included only subjects who reported having ever consumed an alcoholic drink (i.e., alcohol exposed) in the alcohol misuse analysis. Further details on the alcohol misuse scale were reported in our previous studies (11, 28).

A dimensional scale of ND was derived by summing five ND symptoms: (i) "Could not stop or cut down tobacco use"; (ii) "Stop tobacco use and had physical symptoms"; (iii) "Tobacco use caused physical symptoms"; (iv) "Continue tobacco use with physical symptoms"; (v) "Developed tobacco tolerance". In the ND symptoms analysis, we included only subjects who reported having ever smoked at least 100 cigarettes lifetime (i.e., nicotine exposed).

The NSS and PPDS samples underwent genotyping using the Illumina OmniExpress and Exome array with additional custom content or the Illumina PsychChip array. Methods for genotyping, imputation, ancestry assignment, and principal component (PC) analysis were described previously (23, 29). We used imputed genotypes to maximize a consistent SNP panel between the training and testing sets. In our analysis, we included imputed SNPs with minor allele frequency $\geq 5\%$, high imputation quality (genotype call probability $\geq 0.8$), and missingness per marker $\leq 1\%$. After applying these quality control criteria, we obtained information on more than 4 800 000 variants.

### Data analysis

We conducted cross-phenotype PRS analyses using PRSice software (30) available at http://prsice.info/. For polygenic profile scoring, we used summary statistics generated from the GWAS of BD (19), MDD (20), and schizophrenia (21) conducted by the PGC (available at https://www.med.unc.edu/pgc/results-and-downloads) considering multiple association *P*-value thresholds (PT) for SNP inclusion in our high-resolution analysis. The PRSs were calculated after using a *P*-value-informed clumping with a linkage disequilibrium (LD) cutoff of $r^2 = 0.3$ within a 500-kb window, and excluding the major histocompatibility complex (MHC) region of the genome because of its complex LD structure. Because the subjects in the GWAS that were used as sources for summary statistics were of European descent, we restricted our analysis to Army STARRS participants of European ancestry. The PRSs that were generated were fitted in regression models with adjustments for age, sex, and the top 10 within-ancestry PCs to calculate Nagelkerke's R as the figure of merit for prediction ability. Before being entered in the regression

model, alcohol misuse and ND symptom count were normalized using appropriate Box-Cox power transformations. The PRS-by-trauma interaction test compared the difference between regression coefficients in the trauma-exposed subjects with the trauma-unexposed subjects. Due the correlation within the phenotypes investigated, within the PRS generated, and within the psychiatric disorders considered, we considered a significance threshold of $P = 8.33 \times 10^{-3}$, accounting for the number of phenotypes and PRS tested, in accordance with what was recently proposed by other analyses (29, 31).

Finally, we conducted gene ontology (GO) and pathway enrichment analyses of the PRS-by-trauma interaction results that survived correction for multiple testing. Specifically, we verified the trauma interaction of the variants included in the significant PRS and the SNPs with concordant direction with PRS-by-trauma interaction were included in the enrichment analysis. We considered the variants with effect interaction concordant with the PRS results, because these are driving the interaction and, thereby, they should be enriched for the molecular mechanisms involved. The variants were mapped to the corresponding genes using the Ensembl Variant Effect Predictor (32), and then, the loci were entered in the enrichment analysis conducted using the PANTHER v11.1 Overrepresentation Test (release 20160715; Reference List: Homo Sapiens) (33). Bonferroni correction was applied to the enrichment results for multiple testing. We also verified whether variants included in the significant PRS showed gene–trauma interactions that survived correction for multiple testing accounting for the number of variants included in the PRS. Due to the exploratory nature of this analysis, FDR $q < 0.1$ was applied to the single-variant association analysis for multiple testing correction.

## Results

We investigated how exposure to traumatic events interacted with genetic predisposition to psychiatric disorders (BD, MDD, and schizophrenia) with respect to alcohol misuse and ND symptoms. The study was conducted in participants of European descent from the Army STARRS cohort. The alcohol-misuse investigation included a total of 10 732 individuals (8346 trauma-exposed and 2386 trauma-unexposed); the ND symptom analysis included 6132 subjects (4938 trauma-exposed and 1194 trauma-unexposed). The analyses were conducted separately for the two phenotypic outcomes; thus, the sample sizes are differed due to the definitions of exposure of alcohol or nicotine exposed. Details regarding the characteristics of the two samples are reported in Table 1.

Table 2 summarizes the top findings observed in our PRS-by-trauma interaction analysis. Detailed results are reported in Table S1. The main result was a significant PRS-by-trauma interaction for BD PRS on alcohol misuse (top PT = $10^{-5}$, $z = 2.74$, $P = 6.07 \times 10^{-3}$; Fig. 1). Considering the top results in the two groups tested, we observed a *positive* correlation between BD PRS and alcohol misuse in *trauma-exposed* soldiers (top PT = 0.3, $r = 0.029$, $P = 7.5 \times 10^{-3}$) and a *negative* correlation in *trauma-unexposed* soldiers (top PT = $10^{-5}$, $r = -0.071$, $P = 5.61 \times 10^{-4}$). The top-PT results varied between the groups tested. This is attributable to the high-resolution PRS approach applied, which permits maximization of value of available GWAS summary statistics to generate PRS (30). The distribution of the BD PRS in trauma-exposed and trauma-unexposed individuals is reported in Fig. S1; there is no association between BD PRS and trauma exposure (Table S2). A nominal replication of the BD result with consistent effect directions was observed in the SCZ PRS analysis: PRS-by-trauma interaction top PT = $10^{-4}$, $z = 2.46$, $P = 0.014$; trauma-exposed top PT = $10^{-3}$, $r = 0.025$, $P = 0.023$; trauma-unexposed top PT = $10^{-4}$, $r = -0.040$, $P = 0.052$.

Considering the top PT for the PRS-by-trauma interaction (i.e., PT = $10^{-5}$), we verified the direction of trauma interaction of the variants included in the PRS with respect to alcohol misuse (Table S3). The variants with gene-by-trauma interaction direction for alcohol misuse concordant with the PRS-by-trauma interaction showed significant enrichments for multiple GOs: *GO:0008331~high voltage-gated calcium channel activity* (fold enrichment > 100, $P = 1.51 \times 10^{-5}$); GO:1990454~*L-type voltage-gated calcium channel complex* (fold enrichment > 100, $P = 4.49 \times 10^{-6}$); GO:0030315~*T-tubule* (fold enrichment > 100, $P = 2.07 \times 10^{-6}$). We also observed significant enrichment for the Beta1/Beta2 adrenergic

Table 1. Sample characteristics for alcohol misuse and nicotine dependence symptoms

| Characteristics | Sample–alcohol misuse $N = 10\ 732$ | Sample–nicotine dependence symptoms $N = 6132$ |
|---|---|---|
| Age years, mean (SD) | 23.1 (5.3) | 22.9 (4.9) |
| Sex, women *n* (%) | 1109 (10) | 514 (8) |
| Trauma, exposed *n* (%) | 8346 (78) | 4938 (80) |

Table 2. Top correlations (Nagelkerke's R, *P* value) of psychiatric Polygenic Risk Scores (PRSs) with alcohol misuse and nicotine dependence symptoms in trauma-exposed and trauma-unexposed subjects. PRS-by-trauma interaction (PRS × Trauma) results (*Z* score, *P* value) are also reported. Full results are reported in Table S1

| | Alcohol misuse | | | Nicotine dependence symptoms | | |
|---|---|---|---|---|---|---|
| PRS | Trauma-exposed | Trauma-unexposed | PRS×Trauma | Trauma-exposed | Trauma-unexposed | PRSxTrauma |
| BD | 0.029, $7.5 \times 10^{-3}$ | −0.071, $5.61 \times 10^{-4}$ | 2.74, $6.07 \times 10^{-3}$ | 0.029, 0.045 | −0.039, 0.183 | 1.15, 0.249 |
| MDD | 0.014, 0.193 | 0.045, 0.029 | −2.29, 0.022 | 0.042, $3.18 \times 10^{-3}$ | −0.026, 0.373 | 2.12, 0.034 |
| SCZ | 0.025, 0.023 | −0.040, 0.052 | 2.46, 0.014 | 0.030, 0.032 | 0.034, 0.241 | 1.45, 0.146 |

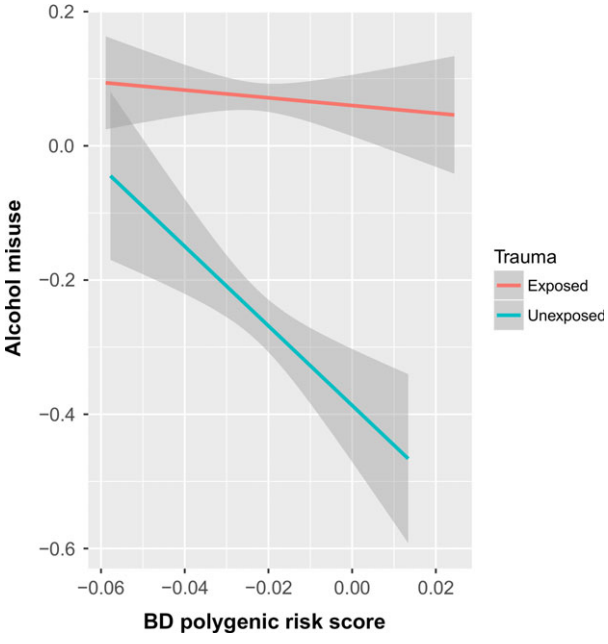


*Fig. 1.* Relationship between alcohol misuse and BD PRS in trauma-exposed and trauma-unexposed individuals. The results reported for both groups are those related to the top PT in the PRSxTrauma analysis (i.e., PT = $10^{-5}$). [Colour figure can be viewed at wileyonlinelibrary.com]

receptor signaling pathways (PANTHER Pathway IDs: P04377 and P04378; fold enrichment = 82.9, $P = 2.61 \times 10^{-4}$). No enrichment was observed for the variants with non-concordant interaction directions. Among the variants included in the significant BD PRS (PT = $10^{-5}$), *AKAP13* rs12902447 showed a trauma exposure interaction with respect to alcohol misuse concordant with the PRS-by-trauma interaction that survived multiple testing correction ($P = 3.31 \times 10^{-3}$, false discovery rate $q < 0.1$). Nominal trauma interactions ($P < 0.05$) were observed for MDD and SCZ PRS on alcohol misuse. For ND symptoms, no PRS-by-trauma interaction survived Bonferroni correction (Table S1). However, we observed a nominally significant PRS-by-trauma interaction ($P = 0.034$), where MDD PRS showed a significant positive correlation with ND symptoms in trauma-exposed soldiers ($r = 0.041$, $P = 3.18 \times 10^{-3}$).

## Discussion

Exposure to traumatic events is associated with a wide range of deleterious health outcomes, and these negative consequences are related to different molecular mechanisms. Among these mechanisms, we can count changes in DNA regulation that affect the pathways and processes by which genetic variation is translated into behavioral phenotypes (10, 34). These effects should be particularly evident for psychiatric traits, where the role of traumatic experiences in molding future experiences and behaviors is widely recognized. Although single loci have been identified as moderators of the response to traumatic experiences with respect to psychiatric disorders (7–9), evidence that demonstrates effects of trauma exposure on a genome-wide basis is still lacking. For complex traits, such as those considered in this study, that is, substance dependence and psychiatric disorders, we anticipate a level of complexity that cannot be well described on the basis of the actions of one or a few genetic loci. We expect the genetic architecture to encompass small effects at many loci across the genome. These kinds of effects can be modeled by means of high-resolution polygenic score analysis, the method that we used to test whether trauma exposure moderates the correlation of alcohol and nicotine use disorders with psychiatric disorders.

Our investigation identified a significant interaction effect of BD PRS with respect to alcohol misuse, when trauma exposure was considered. In trauma-exposed individuals, the BD PRS was *positively* correlated with alcohol misuse (high BD PRS was associated with high alcohol misuse). Conversely, in trauma-unexposed subjects, we observed the opposite effect: BD PRS was *negatively* correlated with alcohol misuse (high BD PRS was associated with low alcohol misuse). These results indicate that there is genetic overlap between BD and alcohol misuse, but exposure to traumatic events changes the direction of the genetic correlation. A nominal replication with consistent effect direction of the BD results was

also present in the SCZ PRS analysis. The difference of significance may be due to variability in the statistical power of the GWAS used to generate the PRS. It could also be attributable to a true biological difference between the traits and their relationships to trauma and alcohol.

Exposure to traumatic events is associated with a greater risk of psychiatric disorders (3, 4), including alcohol use disorders and BD (6, 35). It is also known that BD patients exposed to traumatic events are more likely to have a co-occurring alcohol use disorder (36). These data indicate that there are multiple interactive processes linking trauma, genetics, BD, and alcohol misuse. Trauma exposure is a known risk factor for BD, and patients with the disorder (i.e., those with high genetic liability for BD) are often trauma-exposed. In this scenario, the observed epidemiological correlation between BD and alcohol use disorder is driven by the effect of trauma on the genetics of BD. Thus, in individuals with high genetic liability for BD who do not develop the disease because of lack of trauma exposure, their genetic predisposition to BD would be protective from alcohol misuse. That is—genetic BD predisposition that is unfulfilled with respect to BP because of lack of deleterious environmental events, may play out as protective with respect to alcohol dependence. This scenario may explain the apparently counterintuitive protective association between BD PRS and alcohol misuse in trauma-unexposed subjects. As mentioned, this is just one possible scenario to explain the results; other potential explanations may involve a more complex interplay among trauma exposure, BD, and alcohol misuse.

To understand the biological meaning of our result, we conducted GO and pathway enrichment analyses. We observed a strong enrichment for GOs related to L-type voltage-gated calcium channel activity. The role of calcium signaling has long been implicated in BD (37) and GWAS confirmed its involvement (19). L-type calcium channel antagonists have also been used to treat BD for over 30 years, albeit with inconsistent results (38). The activity of L-type calcium channels is also known to be involved in moderating alcohol consumption: A recent *in vivo* study demonstrated that central Cav1.2 channels mediate alcohol-seeking behaviors (39). Pregabalin, a calcium channel subunit ligand, has shown promise in the treatment of drug and alcohol withdrawal symptoms (40). These data support the results of our enrichment analysis, in which L-type voltage-gated calcium channel activity appeared to be a mechanism involved in both alcohol misuse and BD. Furthermore, multiple lines of evidence indicate that this system is involved in stress response. In rats, blockade of L-type calcium channels enhances stress-induced impairment of memory retrieval (41). In patients affected by calcium channelopathy (i.e., carriers of calcium channel gene mutations), psychological or physical stress is one of the triggers associated with transient neurological impairments (42). Recent genome-wide investigations of trauma interaction and post-traumatic stress disorder identified calcium signaling as one of the mechanisms involved (11, 43). Accordingly, our current results expand the previously identified BD-associated mechanisms, showing trauma exposure and voltage-gated calcium channel activity could be one of the gene mechanism–environment interactions at the basis of BD pathophysiology.

Our pathway enrichment analysis identified Beta1/Beta2 adrenergic receptor signaling pathways. This is consistent with the recognized role of L-type calcium channels in the fight-or-flight stress responses, which are triggered by a signaling pathway involving β-adrenergic receptors together with cyclic adenosine monophosphate, and protein kinase A (PKA) (44). With respect to substance abuse, β-adrenergic receptors seem to mediate the stressor-induced reinstatement of extinguished drug-induced conditioned place preference in mice (45). This experimental evidence is consistent with our results that alcohol misuse and BD share genetic mechanisms related to L-type calcium channel activity, and trauma exposure is a powerful moderator of this overlap. Further confirmation of the relevance of the mechanisms identified by our study is provided by the identification of *AKAP13* rs12902447 as a potential locus involved in gene-by-trauma interaction. This gene is included in A-kinase anchoring proteins (AKAPs), which bind PKA subunits regulating synaptic plasticity and memory consolidation (46). The inhibition of PKA anchoring to A-kinase anchoring proteins impairs consolidation and facilitates extinction of contextual fear memories (47).

No PRS-by-trauma interaction was observed for ND symptoms. This could be due to the smaller sample size available for this trait. However, we found that MDD PRS was positively correlated with ND symptoms in trauma-exposed soldiers with a nominally significant PRS-by-trauma interaction. This is in line with previous studies indicating that shared genetic and environmental factors and interactive mechanisms underlie the association of smoking with depression (48). Our data suggest that trauma exposure may be one of the interactive factors involved in this comorbidity, but further investigation using larger samples is needed to confirm this preliminary result.

In conclusion, our results show that the genetic overlap between alcohol misuse and BD is significantly moderated by trauma exposure. This provides insight into the complex mechanisms that link substance abuse, psychiatric disorders, and trauma exposure. In particular, we identified specific molecular pathways involving L-type calcium channels, beta adrenergic receptors signaling, and A-kinase anchoring proteins. We hypothesize that these are shared mechanisms in alcohol misuse and BD and that there is moderation via the exposure to traumatic events. Our study suggests that, in individuals exposed to trauma, drugs targeting calcium signaling (38, 40) could be efficacious in reducing their risk of developing alcohol use disorder. However, the findings presented in this study may be specific to the cohort investigated, which is a military mostly-male sample, and may be not generalizable to cohorts with different characteristics. Further studies will be needed to follow up the current hypotheses and test them in other population groups. In particular, longitudinal studies could help to dissect how the time of trauma exposure interacts with genetic predisposition to psychiatric disorders in determining psychiatric and behavioral traits.

## Acknowledgements

This study was funded by National Institutes of Health grants R21 AA024404, RC2 DA028909, R01 DA12690, R01 DA12849, R01 DA18432, R01 AA11330, R01 AA017535, P50 AA012870, U01 MH109532, and the VISN1 and VISN4 MIRECCs. Army STARRS was sponsored by the Department of the Army and funded under cooperative agreement number U01 MH087981 (2009-2015) with the U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Mental Health (NIH/NIMH). Subsequently, STARRS-LS was sponsored and funded by the Department of Defense (USUHS grant number HU0001-15-2-0004). The contents are solely the responsibility of the authors and do not necessarily represent the views of the Department of Health and Human Services, NIMH, or the Department of the Army, or the Department of Defense.

The Army STARRS Team consists of Co-Principal Investigators: Robert J. Ursano, MD (Uniformed Services University of the Health Sciences) and Murray B. Stein, MD, MPH (University of California San Diego and VA San Diego Healthcare System). Site Principal Investigators: Steven Heeringa, PhD (University of Michigan), James Wagner, PhD (University of Michigan), and Ronald C. Kessler, PhD (Harvard Medical School). Army liaison/consultant: Kenneth Cox, MD, MPH (US Army Public Health Center). Other team members: Pablo A. Aliaga, MA (Uniformed Services University of the Health Sciences); COL David M. Benedek, MD (Uniformed Services University of the Health Sciences); Laura Campbell-Sills, PhD (University of California San Diego); Carol S. Fullerton, PhD (Uniformed Services University of the Health Sciences); Nancy Gebler, MA (University of Michigan); Robert K. Gifford, PhD (Uniformed Services University of the Health Sciences); Paul E. Hurwitz, MPH (Uniformed Services University of the Health Sciences); Sonia Jain, PhD (University of California San Diego); Tzu-Cheg Kao, PhD (Uniformed Services University of the Health Sciences); Lisa Lewandowski-Romps, PhD (University of Michigan); Holly Herberman Mash, PhD (Uniformed Services University of the Health Sciences); James E. McCarroll, PhD, MPH (Uniformed Services University of the Health Sciences); James A. Naifeh, PhD (Uniformed Services University of the Health Sciences); Tsz Hin Hinz Ng, MPH (Uniformed Services University of the Health Sciences); Matthew K. Nock, PhD (Harvard University); Nancy A. Sampson, BA (Harvard Medical School); CDR Patcho Santiago, MD, MPH (Uniformed Services University of the Health Sciences); LTC Gary H. Wynn, MD (Uniformed Services University of the Health Sciences); and Alan M. Zaslavsky, PhD (Harvard Medical School).

## Declaration of interest

Dr. Stein has in the last three years been a consultant for Actelion Pharmaceuticals, Healthcare Management Technologies, Janssen, Neurocrine, Pfizer, Resilience Therapeutics, Tonix Pharmaceuticals, and Oxeia Biopharmaceuticals. Dr. Kaufman has provided consultation to Pfizer and Merck Pharmaceutical Company to train investigators to assess bipolar disorder in youth. Dr. Kranzler has been an advisory board member, consultant, or CME speaker for Indivior, Lundbeck, and Otsuka. He is also a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which is supported by AbbVie, Alkermes, Ethypharm, Indivior, Lilly, Lundbeck, Pfizer, and XenoPort. In the past 3 years, Dr. Kessler received support for his epidemiological studies from Sanofi Aventis; was a consultant for Johnson & Johnson Wellness and Prevention, Shire, Takeda; and served on an advisory board for the Johnson & Johnson Services Inc. Lake Nona Life Project. Kessler is a co-owner of DataStat, Inc., a market research firm that carries out healthcare research. The other authors reported no biomedical financial interests or potential conflict of interests.

## References

1. Cross-Disorder Group Of The Psychiatric Genomics Consortium, Lee SH, Ripke S, Neale BM et al. Genetic relationship between five psychiatric disorders estimated from genome-wide SNPs. Nat Genet 2013;**45**:984–994.
2. Bulik-Sullivan B, Finucane HK, Anttila V et al. An atlas of genetic correlations across human diseases and traits. Nat Genet 2015;**47**:1236–1241.
3. Howlett JR, Stein MB. Prevention of trauma and stressor-related disorders: a review. Neuropsychopharmacology 2016;**41**:357–369.
4. Stoddard FJ Jr. Outcomes of traumatic exposure. Child Adolesc Psychiatr Clin N Am 2014;**23**:243–256, viii.
5. Maguire C, McCusker CG, Meenagh C, Mulholland C, Shannon C. Effects of trauma on bipolar disorder: the mediational role of interpersonal difficulties and alcohol dependence. Bipolar Disord 2008;**10**:293–302.
6. Simmons S, Suarez L. Substance abuse and trauma. Child Adolesc Psychiatr Clin N Am 2016;**25**:723–734.
7. Lieberman R, Armeli S, Scott DM, Kranzler HR, Tennen H, Covault J. FKBP5 genotype interacts with early life trauma to predict heavy drinking in college students. Am J Med Genet B Neuropsychiatr Genet 2016;**171**:879–887.
8. Baranger DA, Ifrah C, Prather AA et al. PER1 rs3027172 genotype interacts with early life stress to predict problematic alcohol use, but not reward-related ventral striatum activity. Front Psychol 2016;**7**:464.

9. Kaufman J, Yang BZ, Douglas-Palumberi H et al. Genetic and environmental predictors of early alcohol use. Biol Psychiatry 2007;**61**:1228–1234.
10. Montalvo-Ortiz JL, Gelernter J, Hudziak J, Kaufman J. RDoC and translational perspectives on the genetics of trauma-related psychiatric disorders. Am J Med Genet B Neuropsychiatr Genet 2016;**171B**:81–91.
11. Polimanti R, Kaufman J, Zhao H et al. A genome-wide gene-by-trauma interaction study of alcohol misuse in two independent cohorts identifies PRKG1 as a risk locus. Mol Psychiatry 2017; https://doi.org/10.1038/mp.2017.24 [Epub ahead of print].
12. Urquhart-Cronish M, Sokolowski MB. Gene-environment interplay in Drosophila melanogaster: chronic nutritional deprivation in larval life affects adult fecal output. J Insect Physiol 2014;**69**:95–100.
13. Burns JG, Svetec N, Rowe L et al. Gene-environment interplay in Drosophila melanogaster: chronic food deprivation in early life affects adult exploratory and fitness traits. Proc Natl Acad Sci USA 2012;**109**(Suppl 2):17239–17244.
14. Pasaniuc B, Price AL. Dissecting the genetics of complex traits using summary association statistics. Nat Rev Genet 2017;**18**:117–127.
15. Hartz S, Horton AC, Oehlert M et al. Association between substance use disorder and polygenic liability to schizophrenia. bioRxiv 2017;**82**:709–715.
16. Polimanti R, Agrawal A, Gelernter J. Schizophrenia and substance use comorbidity: a genome-wide perspective. Genome Med 2017;**9**:25.
17. Reginsson GW, Ingason A, Euesden J et al. Polygenic risk scores for schizophrenia and bipolar disorder associate with addiction. Addict Biol 2017; https://doi.org/10.1111/adb.12496 [Epub ahead of print].
18. Ursano RJ, Colpe LJ, Heeringa SG et al. The Army study to assess risk and resilience in servicemembers (Army STARRS). Psychiatry 2014;**77**:107–119.
19. Psychiatric Gwas Consortium Bipolar Disorder Working Group. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nat Genet 2011;**43**:977–983.
20. Major Depressive Disorder Working Group Of The Psychiatric Gwas Consortium, Ripke S, Wray NR, Lewis CM et al. A mega-analysis of genome-wide association studies for major depressive disorder. Mol Psychiatry 2013;**18**:497–511.
21. Schizophrenia Working Group Of The Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014;**511**:421–427.
22. Kessler RC, Ustun TB. The world mental health (WMH) survey initiative version of the world health organization (WHO) composite international diagnostic interview (CIDI). Int J Methods Psychiatr Res 2004;**13**:93–121.
23. Stein MB, Chen CY, Ursano RJ et al. Genome-wide association studies of posttraumatic stress disorder in 2 Cohorts of US army soldiers. JAMA Psychiatry 2016;**73**:695–704.
24. Polimanti R, Zhao H, Farrer LA, Kranzler HR, Gelernter J. Ancestry-specific and sex-specific risk alleles identified in a genome-wide gene-by-alcohol dependence interaction study of risky sexual behaviors. Am J Med Genet B Neuropsychiatr Genet 2017;**174**:846–853.
25. Polimanti R, Meda SA, Pearlson GD et al. S100A10 identified in a genome-wide gene x cannabis dependence interaction analysis of risky sexual behaviours. J Psychiatry Neurosci 2017;**42**:252–261.
26. Polimanti R, Wang Q, Meda SA et al. The interplay between risky sexual behaviors and alcohol dependence: genome-wide association and neuroimaging support for LHPP as a risk gene. Neuropsychopharmacology 2017;**42**:598–605.
27. Polimanti R, Zhang H, Smith AH et al. Genome-wide association study of body mass index in subjects with alcohol dependence. Addict Biol 2017;**22**:535–549.
28. Stein MB, Campbell-Sills L, Gelernter J et al. Alcohol misuse and co-occurring mental disorders among new soldiers in the U.S. army. Alcohol Clin Exp Res 2017;**41**:139–148.
29. Polimanti R, Chen CY, Ursano RJ et al. Cross-phenotype polygenic risk score analysis of persistent post-concussive symptoms in U.S. army soldiers with deployment-acquired traumatic brain injury. J Neurotrauma 2016;**34**:781–789.
30. Euesden J, Lewis CM, O'Reilly PF. PRSice: polygenic risk score software. Bioinformatics 2015;**31**:1466–1468.
31. Simonti CN, Vernot B, Bastarache L et al. The phenotypic legacy of admixture between modern humans and Neandertals. Science 2016;**351**:737–741.
32. McLaren W, Gil L, Hunt SE et al. The ensembl variant effect predictor. Genome Biol 2016;**17**:122.
33. Mi H, Huang X, Muruganujan A et al. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res 2017;**45**:D183–D189.
34. Turecki G, Ota VK, Belangero SI, Jackowski A, Kaufman J. Early life adversity, genomic plasticity, and psychopathology. Lancet Psychiatry 2014;**1**:461–466.
35. Aas M, Henry C, Andreassen OA, Bellivier F, Melle I, Etain B. The role of childhood trauma in bipolar disorders. Int J Bipolar Disord 2016;**4**:2.
36. Agnew-Blais J, Danese A. Childhood maltreatment and unfavourable clinical outcomes in bipolar disorder: a systematic review and meta-analysis. Lancet Psychiatry 2016;**3**:342–349.
37. Carman JS, Wyatt RJ. Calcium: bivalent cation in the bivalent psychoses. Biol Psychiatry 1979;**14**:295–336.
38. Cipriani A, Saunders K, Attenburrow MJ et al. A systematic review of calcium channel antagonists in bipolar disorder and some considerations for their future development. Mol Psychiatry 2016;**21**:1324–1332.
39. Uhrig S, Vandael D, Marcantoni A et al. Differential roles for L-type calcium channel subtypes in alcohol dependence. Neuropsychopharmacology 2017;**42**:1058–1069.
40. Freynhagen R, Backonja M, Schug S et al. Pregabalin for the treatment of drug and alcohol withdrawal symptoms: a comprehensive review. CNS Drugs 2016;**30**:1191–1200.
41. Rashidy-Pour A, Vafaei AA, Taherian AA et al. Verapamil enhances acute stress or glucocorticoid-induced deficits in retrieval of long-term memory in rats. Behav Brain Res 2009;**203**:76–80.
42. Raike RS, Weisz C, Hoebeek FE et al. Stress, caffeine and ethanol trigger transient neurological dysfunction through shared mechanisms in a mouse calcium channelopathy. Neurobiol Dis 2013;**50**:151–159.
43. Polimanti R, Amstadter AB, Stein MB et al. A putative causal relationship between genetically determined female body shape and post-traumatic stress disorder. Genome Med 2017;**9**:99.
44. Qian H, Patriarchi T, Price JL et al. Phosphorylation of Ser 1928 mediates the enhanced activity of the L-type Ca2 + channel Cav1.2 by the beta2-adrenergic receptor in neurons. Sci Signal 2017;**10**:1–11.

45. Vranjkovic O, Hang S, Baker DA, Mantsch JR. beta-adrenergic receptor mediation of stress-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: roles for beta1 and beta2 adrenergic receptors. J Pharmacol Exp Ther 2012;**342**:541–551.
46. Moita MA, Lamprecht R, Nader K, Ledoux JE. A-kinase anchoring proteins in amygdala are involved in auditory fear memory. Nat Neurosci 2002;**5**:837–838.
47. Nijholt IM, Ostroveanu A, Scheper WA et al. Inhibition of PKA anchoring to A-kinase anchoring proteins impairs consolidation and facilitates extinction of contextual fear memories. Neurobiol Learn Mem 2008;**90**:223–229.
48. Keskitalo-Vuokko K, Korhonen T, Kaprio J. Gene-environment interactions between depressive symptoms and smoking quantity. Twin Res Hum Genet 2016;**19**: 322–329.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Distribution of the BD PRS (PT = $10^{-5}$) in subjects exposed and unexposed to trauma.

**Table S1.** Results of the PRSxTrauma interaction study of Alcohol Misuse and ND symptoms.

**Table S2.** Association results between BD PRS and trauma exposure.

**Table S3.** Gene-by-Trauma interaction (GxT) results for Alcohol Misuseof the variants included in the BD PRS (PT = $10^{-5}$).