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Genome-wide association analyses of posttraumatic stress disorder and its symptom subdomains in the Million Veteran Program

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AUTHOR CONTRIBUTIONS

M.B.S. and J.G. had primary responsibility for design of the study. M.B.S., J.G., J.C., K.R., and M.A. supervised the study and managed and organized the group. D.F.L., Z.C., F.R.W., G.A.P., and R.P. contributed to genetic and bioinformatic analyses. K.H., K.C., R.Q., Y.-L.A.H., K.R., M.A., and D.P. contributed to phenotyping and phenomic analyses. Initial manuscript was drafted by M.B.S., D.F.L., R.P., and J.G. Manuscript contributions and interpretation of results were provided by M.B.S., D.F.L. Z.C., F.R.W., G.A.P., K.H., M.J.G., D.P., R.S.D., H.Z., R.P., J.C., and J.G. The remaining authors contributed to other organizational or data processing components of the study. All authors saw, had the opportunity to comment on, and approved the final draft.

COMPETING INTERESTS STATEMENT

M.B.S. has in the past 3 years been a consultant for Actelion, Acadia Pharmaceuticals, Aptinyx, Bionomics, BioXcel Therapeutics, Clexio, EmpowerPharm, Epivario, GW Pharmaceuticals, Janssen, Jazz Pharmaceuticals, Roche/Genentech, and Oxeia Biopharmaceuticals. M.B.S. has stock options in Oxeia Biopharmaceuticals and Epivario. J.G. is named as co-inventor on PCT patent application #15/878,640 entitled: "Genotype-guided dosing of opioid agonists," filed January 24, 2018. None of the other authors declare any competing interests.

Data Availability

The GWAS summary statistics generated during and/or analyzed during the current study will be made available via dbGAP; the dbGaP accession assigned to the Million Veteran Program is phs001672.v1.p. The website is: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001672.v1.p1

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Abstract

We conducted genome-wide association analyses in over 250,000 participants of European and African ancestry from the Million Veteran Program using electronic health record-validated posttraumatic stress disorder (PTSD) diagnosis and quantitative symptom phenotypes. Applying genome-wide multiple testing correction, we identified three significant loci in European case-control analyses and 15 loci in quantitative symptom analyses. Genomic structural equation modeling indicated tight coherence of a PTSD symptom factor that shares genetic variance with a distinct internalizing (mood-anxiety-neuroticism) factor. Partitioned heritability indicated enrichment in several cortical and subcortical regions, and imputed genetically regulated gene expression in these regions was used to identify potential drug repositioning candidates. These results validate the biological coherence of the PTSD syndrome, inform its relationship to comorbid anxiety and depressive disorders, and provide new considerations for treatment.

Posttraumatic stress disorder (PTSD) is a serious mental disorder that can occur after exposure to extreme, life-threatening stress[1,2]. Although 50–85% of Americans experience traumatic events over a lifetime, most do not develop PTSD – lifetime PTSD prevalence is approximately 7%[3], suggesting differential resilience to stress and vulnerability to the disorder[4]. There is a substantial heritable basis for PTSD risk[5,6], and evidence from genome-wide association studies (GWAS) shows that PTSD, like other mental disorders[7], is highly polygenic[8–13]. PTSD symptoms vary widely among individuals, and the current DSM-5 definition permits up to 163,120 unique conformations for assembly of the disorder[14]. Recognizing that this phenotypic heterogeneity may impede the detection of genetic risk factors[15], alternate phenotypes or sub-phenotypes (e.g., re-experiencing – also known as intrusion – symptoms) that may reflect more biologically homogeneous entities have been examined[16].

The use of biobanks with relatively large numbers of PTSD cases offers the opportunity to provide unprecedented sample size and, importantly, uniformity of phenotypic and genotypic platforms[17]. This investigation was conducted within the US Veterans Affairs Million Veteran Program (MVP)[18] and included several PTSD phenotypic definitions: a validated, algorithmically-defined case-control definition using data from the electronic health record (EHR), which was subsequently meta-analyzed with the case-control PGC-PTSD GWAS[13]; and quantitative trait definitions encompassing PTSD subdomains based on

recent self-reported symptoms: re-experiencing (in an expanded sample from that previously reported[16]), avoidance, hyperarousal, and a total index of recent symptom severity (PCL-Total). These analyses were conducted separately in veterans of European and African ancestry (and in trans-ancestral meta-analyses)[19,20]. Heritability of each of these phenotypes as well as phenotypic and genetic (r_g) correlations were examined with the aim of determining coherence among them; r_g with other behavioral and health-related traits was also examined. Results for the phenotype with the largest SNP heritability estimate were used to characterize PTSD genomic architecture with partitioned heritability and transcriptome-wide analyses[21] to identify genes regulated in brain regions of greatest relevance. Genomic structural equation modeling was used to determine genetic relationships between PTSD and clinically comorbid phenotypes from the internalizing spectrum[22]: major depressive disorder, anxiety, and neuroticism.

Aims of these analyses are to provide: (i) a large, uniformly phenotyped GWAS of PTSD in military veterans; (ii) thorough exploration of sub-phenotypes; (iii) replication of key associations in other datasets; (iv) demonstration of the architecture of genetic association with other health-related phenotypes; (v) investigation of brain regions implicated, and; (vi) extension to possible drug targets. These aims were all accomplished with the overarching goal of deepening biological understanding to advance precision medicine for PTSD.

RESULTS

GWAS of algorithmically defined case-control PTSD.

We first performed GWAS of PTSD in American veterans of European (EUR) and African (AFR) ancestry, basing diagnosis on a validated VA EHR algorithm[23] that had excellent discriminative ability for lifetime PTSD cases vs. controls as determined by chart review (0.90 sensitivity, 0.97 specificity, 0.87 positive predictive value, and 0.90 negative predictive value), and substantial agreement with gold-standard Clinician-Administered PTSD Scale (CAPS) interview (90.2% agreement and $\kappa = 0.75$ (95% CI: 0.62, 0.88))[17]. GWAS analyses were carried out (on two tranches of data genotyped on the same array platform at two different times) on SNP dosages imputed from 1000 Genomes Phase 3 using logistic regression for case-control traits and linear regression for continuous traits in PLINK 2.0[24], separately by ancestry, adjusting for age, sex, and the first 10 principal components of ancestry. Meta-analysis by tranche (and later by ancestral group) was performed using METAL[25]. Combat exposure information was available for only a subset (51.2%) of the sample (Supplementary Table 1), and GWAS of that subset yielded no genome-wide significant (GWS) findings (Supplementary Table 2 shows findings at $P < 10^{-6}$). However, genetic correlation (r_g) between the categorical trait (i.e., diagnosis of) PTSD in those combat-exposed and in all subjects irrespective of combat exposure status was 0.969 (s.e. 0.049, $P = 7.64 \times 10^{-89}$), and therefore results for the latter larger, more informative, sample are presented here.

The PTSD case-control GWAS for the EUR sample included 36,301 algorithmically defined probable PTSD cases and 178,107 controls. Considering LD-independent loci ($r^2 > 0.1$), we identified three distinct GWS ($P < 5 \times 10^{-8}$) genomic risk loci (Fig. 1 (top) and Supplementary Table 3a) on Chr11:28707675, rs10767744 (MAF = 0.39, $P = 1.75 \times 10^{-10}$),

proximity mapped to *METTL15*; on Chr7:70219946, rs137999048 (MAF = 0.047, $P = 1.03 \times 10^{-8}$), proximity mapped to *AUTS2*; and on Chr7:1855531, rs7680 (MAF = 0.14, $P = 4.17 \times 10^{-8}$), proximity mapped to *MAD1L1*, respectively. Regional Manhattan plots for each region are presented in Supplementary Figure 1a–c.

The GWAS for the AFR sample included 11,920 probable PTSD cases and 39,116 controls (Extended Data Fig. 1 and Supplementary Table 3b) and identified two distinct GWS loci, one on Chr3:1259951, rs4684090 (MAF = 0.04, $P = 3.59 \times 10^{-8}$) intronic to *CNTN6*, and one on Chr20:6724577, rs112149412 (MAF = 0.02, $P = 3.19 \times 10^{-9}$) near *BMP2*. GWAS for the 48,221 cases and 217,223 controls in the trans-ancestral analysis (meta-analysis of EUR and AFR samples) (Supplementary Table 3c) identified as GWS SNPs in two of the same regions found GWS in the EUR GWAS: a different lead SNP on Chr7:1959634 (rs137944087, an indel/deletion) in moderate LD with the variant identified in the EUR sample ($r^2 = 0.38$), and a different lead SNP on Chr11:28678870 (rs10767739) in LD with the variant identified in the EUR sample ($r^2 = 0.54$).

Meta-analysis of MVP and PGC PTSD case-control GWAS.—We next conducted meta-analyses of the EUR MVP and PGC PTSD case-control GWAS[13] (Fig. 1 (bottom) and Supplementary Table 4a). The EUR meta-analysis yielded four distinct GWS loci, two of which were nearest to genes found GWS in the MVP case-control analysis (*MAD1L1* and *METTL15*) although with different lead SNPs: one new SNP (nearest to *LOC645949*) and one lead SNP closest to *PACRG*, a gene linked in a head-to-head arrangement and co-regulated with *PARK2* – a gene found GWS in PGC. There were no GWS SNPs for the AFR MVP/PGC PTSD meta-analysis, but two SNPs were GWS in the trans-ancestral meta-analysis with lead SNPs closest to *PARK2* and *MAD1L1*, respectively (Supplementary Table 4b,c).

GWAS of PTSD symptom sub-phenotypes and total symptoms.

The MVP surveys included the PTSD Checklist for DSM-IV (PCL), a widely used 17-item self-report measure of past-month PTSD symptoms covering the three DSM-IV symptom cluster criteria – re-experiencing, avoidance, and hyperarousal – and a total symptom severity score (PCL-Total) as the sum of those three sub-phenotypes[26]. GWAS with these phenotypes in the EUR sample ($n = 186{,}689$ individuals) using linear regression identified multiple independent GWS SNPs, including some that were associated with PCL-Total as well as multiple sub-domains, and others that were more strongly associated with specific sub-domains (Table 1). Overlap in risk loci for the case-control and the quantitative phenotypes in the EUR and AFR samples is shown in Figure 2. Supplementary Table 5 shows PCL-Total GWAS results in the trans-ancestral sample.

Fine-mapping and variant prioritization.—For PCL-Total, we identified 15 GWS loci in the EUR population. For the case-control phenotype, we observed three loci in the EUR population and two loci in the AFR population. Each locus that included more than 10 GWS SNPs was fine-mapped[27] to prioritize variants in each locus, defined as credible sets (Supplementary Data 1). Regions associated with PCL-Total scores had multiple variants with Combined Annotation Dependent Depletion (CADD) scores > 10 (i.e., these variants

were among the top 10% of pathogenic variants across the human genome)[28]. For example, in the region Chr3:49734229–50176259, associated with PCL-Total, there were four sub-regions with one or more exonic SNPs with CADD scores > 10. CAVIAR (Causal Variants Identification in Associated Regions)[27] fine-mapping results and CADD scores are included in Supplementary Data 1.

To understand the biological effect of SNPs associated with PTSD phenotypes, we analyzed top SNPs (at suggestive threshold $P < 5 \times 10^{-6}$) for their distinct and overlapping distribution across the four sub-phenotypes. The top SNPs for each phenotype were LD pruned to obtain independent signals. We found 87 (hyperarousal), 49 (avoidance), 62 (re-experiencing), and 36 (PCL-Total) SNPs that were non-overlapping or phenotype-specific (Supplementary Data 2). These non-overlapping SNPs were assessed for their QTL protein associations (all tissues), DNA methylation (brain tissues), and splicing (brain tissues) from the QTLbase[29]. Most of the QTL associations were observed for methylation expression. These QTL associations are shown as Venn diagrams for each phenotype (Supplementary Data 2); detailed tabular results are also shown in Supplementary Data 2.

Replication of GWAS findings.

We compared top SNP associations from the PTSD case-control and PCL-Total results against the largest available external PTSD dataset, from the PGC-PTSD[13]. For the EUR case-control phenotype, there was nominal replication for one of three SNPs: for rs7680*A nearest to *MAD1L1*, with a log(OR) of −0.0712 (s.e. 0.013, P = 4.17×10^{-8}) in MVP and a log(OR) of −0.0639 (s.e. 0.0215, $P = 0.00312$) in PGC-PTSD. For the EUR PCL-Total symptom scores, there were six of 15 possible nominal replications (Supplementary Table 6).

We applied a polygenic risk score (PRS) in EUR with MVP as the base and PGC as the target. The MVP case-control and MVP PCL-Total PRS explained approximately 0.4% ($P = 2.4 \times 10^{-74}$) and 0.7–0.8% of the variance ($P = 2.2 \times 10^{-134}$), respectively, in the PGC case-control phenotype at *P*-value thresholds (P_T) ≤ 0.05 (Extended Data Fig. 2). The low phenotypic variance explained is likely due to different characteristics of the MVP and PGC-PTSD cohorts: across three MVP hold-out PRS analyses, we observed phenotypic variance explained ranging from 4% to 5.3% ($P < 6 \times 10^{-92}$; Supplementary Table 7). Evaluating the extent to which cross-ancestral PRS were useful, we found PRS biased by ancestry, with density plots of EUR and AFR PRS being substantially different (Extended Data Fig. 3).

SNP-based heritability estimates and genetic correlations across PTSD phenotypes and with other health-related traits.

Figure 3 shows the SNP-based heritability estimates (on the left) and the phenotypic (above the diagonal) and genetic (below the diagonal) correlations in EUR between the algorithmic case-control diagnosis, and each of the four continuous PTSD symptoms (re-experiencing, avoidance, hyperarousal, and their total; and the genetic correlations for the MVP/PGC case-control meta-analysis). Genetic correlations were consistently high ($r_g > 0.9$) across all PTSD traits, indicating that the traits investigated are all informative with respect to PTSD genetics. The PCL-Total quantitative trait (95%CI SNP-h^2 = 0.08–0.10) has significantly

higher SNP-based heritability than either the MVP case-control definition (95% CI SNP-h^2 = 0.05–0.07, $P_{difference} = 1.85 \times 10^{-4}$) or the MVP/PGC case-control meta-analysis (95% CI SNP-h^2 = 0.07–0.08, $P_{difference} = 5.83 \times 10^{-3}$), and significantly larger SNP-heritability *z*-score (MVP PCL-total SNP-h^2 $z = 17.73$; MVP case-control SNP-h^2 $z = 11.62$; MVP/PGC SNP-h^2 $z = 14.80$).

In the EUR sample, we estimated genetic correlations (r_g) between the PTSD case-control and the PCL-Total score and health-related traits available from UK Biobank and the PGC (Supplementary Table 8, showing r_g for all traits with h^2 *z*-score of 4 or more). The many significant genetic correlations with both PTSD traits include positive r_g with major depression, neuroticism, and related symptoms, and negative r_g with educational attainment and cognitive performance (Fig. 4). Though the magnitudes of r_g observed with PCL-Total and case-control PTSD were highly correlated (Spearman's *rho* = 0.970, $P = 2.20 \times 10^{-16}$), ten phenotypes exhibited significantly greater r_g with PCL-Total relative to case-control PTSD (Fig. 4a).

Taken together, the higher heritability, the greater magnitude of the heritability *z*-score (indicative of a stronger polygenic signal), and the larger r_g with other health-related traits confirm that PCL-Total is similar to, but more informative than, the case-control definition (for either MVP alone or the MVP/PGC meta-analysis). Accordingly, all subsequent post-GWAS analyses are based on the more powerful PCL-Total quantitative trait dataset in the EUR sample.

Genomic relationship of PTSD and other mental disorders.

We used multi-trait conditional and joint analysis (mtCOJO)[30] to address the genetic relationship between PTSD and other major mental disorders in two ways. First, we conditioned PTSD PCL-Total on a single mental disorder; then, we conditioned PTSD PCL-Total on all eight mental disorders simultaneously: autism spectrum disorder, major depression, anorexia nervosa, anxiety (case-control), alcohol dependence, schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder[31–38]. The result of this analysis is treated as genetic signal attributable to PTSD in the absence of shared genetic liabilities of other mental disorders. PCL-Total remained highly genetically correlated with the unconditioned GWAS when conditioned on genetically correlated psychiatric disorders independently (i.e. PTSD PCL-Total conditioned on MDD) and simultaneously (i.e., PTSD PCL-Total conditioned on all eight mental disorders; Fig. 5). Conditioning on all eight mental disorder traits significantly reduced the observed-scale SNP-heritability (h^2) of PCL-Total (PCL-Total original $h^2 = 9.21\%$, $P = 1.39 \times 10^{-67}$; PCL-Total conditioned $h^2 = 4.11\%$, $P = 2.61 \times 10^{-52}$) relative to the unconditioned GWAS ($P_{difference} = 1.52 \times 10^{-13}$), but this reduction in heritability did not significantly alter associations with biological pathways or tissues associated with genetic risk for PTSD as evidenced by linear relationships between tissue and pathway enrichment effects (Extended Data Fig. 4).

Genomic structural equation modeling.

Genomic structural equation models were analyzed to answer two question: (i) do PTSD subdomains (hyperarousal, re-experiencing, and avoidance) load onto one latent factor, and

(ii) does latent factor architecture and subdomain loading change in the presence of PTSD genetic and phenotypic correlates: major depressive disorder, anxiety, and neuroticism. These traits – all part of the internalizing spectrum[22,39] – are highly phenotypically and genetically correlated with PTSD.

The three PTSD phenotypic subdomains loaded onto a single latent common factor (Supplementary Fig. 2). There were no significant differences in loading values between these PTSD subdomains, suggesting roughly equal contribution of all three to the common factor (comparative fit index (CFI) = 0.996). Next, we included PTSD genetic and phenotypic correlates from internalizing disorders – anxiety, neuroticism, and major depressive disorder (all from PGC). Genomic exploratory factor analysis (EFA) identified a two-factor model as best suited to represent the six phenotypes (i.e., PTSD subdomains and the three internalizing measures). In genomic confirmatory factor analysis (CFA) of the two-factor model, PTSD subdomains independently loaded onto factor 1 while the PTSD correlates loaded onto a second factor (CFI = 0.999) (Fig. 6). The PTSD subdomain hyperarousal loads onto both factors (loading onto factor 1 = 0.90 ± 0.05; loading onto factor 2 = 0.10 ± 0.04; correlation between factors 1 and 2 = 0.72 ± 0.03), indicating that this subdomain has a genetic correlation with the internalizing psychopathologies that is not shared by the other PTSD subdomains.

Partitioned heritability of PCL-Total.

Partitioning heritability of PCL-Total in EUR revealed 1.28-fold to 1.39-fold enrichment of SNPs associated with four GTEx cortical tissue types: cortex, frontal cortex (BA9), anterior cingulate cortex (BA24), and nucleus accumbens (FDR $q < 0.05$; Supplementary Table 9). Intronic regions had 1.29-fold enrichment (FDR $q < 0.05$). Cell-type partitioning analyses support SNP-h^2 enrichment of the frontal cortex (BA9) gene sets (tau-c = 3.42×10^{-9}, $P = 0.002$) above other annotations in the model, and frontal cortex (BA9), anterior cingulate cortex (BA24), and multiple basal ganglia (putamen, caudate, and nucleus accumbens) gene expression profiles (tau-c ranging from 1.02×10^{-9} to 3.43×10^{-9}, FDR $q < 0.05$), above that of all other genomic annotations. These regions were prioritized when considering transcriptome-wide association results to constrain interpretation of those results to the most pertinent and evidence-driven tissues[40].

Enrichment in biological tissues using transcriptome-wide analysis and colocalization.

PrediXcan-S[41] was used to correlate imputed tissue-specific genetically regulated gene expression determined by association with reference transcriptome datasets with PCL-Total results. We observed significant *negative* correlation with predicted expression of the protein product of the pseudogene *LRRC37A4P* in amygdala, substantia nigra, putamen, frontal and anterior cingulate cortex, adrenal gland, and whole blood tissues. Also noted were significant *positive* correlations with predicted expression of *CRHR1* in amygdala, hippocampus, frontal and anterior cingulate cortex, adrenal, and whole blood (although *negative* correlation was seen for nucleus accumbens); significant *positive* correlation with predicted expression of *PLEKHM1*, *ARL17A, LRRC37A2,* and *DND1P1* (all of which are co-localized on 17q21.31) in multiple brain regions including amygdala, anterior cingulate cortex, and basal ganglia; and significant *negative* correlation with predicted expression of

RBM6 in frontal cortex, hippocampus, nucleus accumbens, adrenal, and whole blood. The complete list of PrediXcan-S results is available in Supplementary Table 10. The significant genes for 13 brain tissues were then tested for shared causal loci. The coloc method[42] reports posterior probability for a pair of traits under the hypothesis (H_4) that traits are associated and share a single causal variant. The genetically regulated transcriptomic profiles of *ARL17A, LRRC37A2*, *RNF123*, *FAM212A* and *PLEKHM1* showed high probability (≥90%) of a shared causal locus (coloc H_4) with PCL-Total across multiple brain regions. *CRHR1* probability was highest (85%) for hippocampus tissue expression (Supplementary Data 2).

Drug repositioning analyses.

We selected genes significantly associated with PCL-Total in the PrediXcan-S analyses and, as recommended[40], prioritized those genes with predicted expression regulation in at least one of the four tissues identified by LDSC partitioned heritability analyses: cortex, frontal cortex, anterior cingulate, and nucleus accumbens (Fig. 7).

We imported this list of eight genes (*ARHGAP27, ARL17A, CRHR1, DND1P1, LRRC37A2, LRRC37A4P, PLEKHM1,* and *RBM6*) into the Drug Gene Interaction Database v3.0 (dgidb.genome.wustl.edu)[43] to identify interactions with available drug treatments that might indicate potential novel drug strategies for PTSD. Drug repositioning analysis was also carried out in the Connectivity Map (CMap) database (https://www.broadinstitute.org/connectivity-map-cmap) and PHAROS (https://pharos.nih.gov) for the same set of eight genes[44].

No currently druggable targets were identified for *ARHGAP27, ARL17A, DND1P1, LRRC37A2, LRRC37A4P,* or *RBM6*. *CRHR1* was identified in all databases as being a potential drug target with experimental medications available. Given the positive association between PTSD symptoms and imputed *CRHR1* expression in multiple brain regions (with the exception of nucleus accumbens) seen in our dataset, a *CRHR1* antagonist would be hypothesized to be potentially therapeutic. Another gene, *PLEKHM1*, which was significantly associated with imputed increased expression and colocalized in caudate and nucleus accumbens, was considered by CMap as highly likely to share biological effects with several classes of drugs, including dopamine receptor antagonists, acetylcholine receptor antagonists, and alpha-2 adrenergic receptor and angiotensin receptor antagonists, all of which would be predicted to reduce expression and be associated with a reduction in PTSD symptoms.

DISCUSSION

The past decade has seen a proliferation in the use and usefulness of GWAS, with the prediction – and, to date, the experience – that continued sample size growth will result in even richer findings[45]. The field of psychiatric genomics has capitalized on GWAS, with substantial gains made in the understanding of serious mental disorders such as schizophrenia, major depression, bipolar disorder[7,46], and their interrelatedness[47]. We present here a large, uniformly phenotyped and genotyped case-control GWAS of PTSD in military veterans. We augment this analysis with the GWAS of a quantitative trait

corresponding to symptom severity, which proved more genetically informative than the case-control analysis even when our case-control GWAS was meta-analyzed with the next largest PTSD case-control GWAS available, from the PGC[13].

These analyses revealed several genome-wide significant (GWS) associations with PTSD visible at the case-control level, and numerous GWS associations with various dimensions of symptom severity. When combined with imputed genetically regulated expression results and enrichment analyses, these results help to illuminate the neurobiology of PTSD and begin to uncover new avenues for therapeutic development.

This is the first study to compare heritability of binary (diagnostic) and continuous (symptom-based) phenotypes for PTSD directly. Although PTSD symptoms can have a very diverse phenotypic presentation[14], their genetic overlap is very high ($r_g > 0.9$). This is an important novel insight into the biology of PTSD. The quantitative (PCL-Total) trait – which reflected the most information – was the most heritable, and therefore the most informative for biological inference. Partitioned heritability analyses of that trait indicated overrepresentation of SNPs in frontal (BA9) and anterior cingulate cortex (BA24), consistent with prevailing neural circuit theories of PTSD pathophysiology[2] that emphasize hypofunction of these regions and their connections with limbic cortex in the regulation of emotion and extinction of fear memories[48,49]. However, these analyses also pointed to the nucleus accumbens – an important component of the reward system – as being involved in PTSD symptoms. These results suggest that more extensive study of the nucleus accumbens and reward systems in PTSD may shed further light on aspects of the syndrome (e.g., its strong association with alcohol dependence)[50,51] that are currently not well understood.

Several genes – most notably *MAD1L1* (mitotic arrest deficient 1 like 1) – were repeatedly implicated across the various conceptualizations of the PTSD phenotype. The variants in *MAD1L1* also show QTL associations with DNA methylation and splicing. *MAD1L1*, widely expressed in all tissues and thought to play a role in cell cycle control, has emerged as being GWS-associated with at least two other major mental disorders, schizophrenia[31] and bipolar disorder [38] — both of which were excluded among participants in this study but have strong genetic correlations with PTSD in MVP and other cohorts[13]. These observations and the recent finding of GWS-association with anxiety[52], suggest that *MAD1L1* may be a general risk factor for psychopathology, possibly contributing to the *p factor* thought to underlie many serious mental disorders[53].

Several other genes were discovered to be associated with PTSD and replicated in the largest available independent PTSD-informative dataset, the PGC-PTSD GWAS[13]. Included among these were *TSNARE1* (T-SNARE Domain Containing 1) and *EXD3* (Exonuclease 3’−5’ Domain Containing 3). *TSNARE1*, the product of which is involved in intracellular protein transport, has been associated with risk-taking[54], which may predispose to PTSD through increasing the likelihood of exposure to traumatic events; twin studies suggest that risk for exposure to traumatic events is partially heritable[5]. *EXD3*, the product of which is involved in nucleic acid binding, has been associated with mathematical[55] and other cognitive abilities, which have been found in our study and others to be genetically negatively correlated with PTSD and mediated by socioeconomic status[56]. The MVP/PGC case-control

meta-analyses also identified associations with *PARK2* and *PACRG*, both of which are associated with susceptibility to leprosy and to intracellular pathogens[57]. It remains to be determined to what extent these associations reflect systems or processes that underlie PTSD pathophysiology, but we now have gene candidates discovered and replicated through unbiased searches that can be further examined in relation to their putative biological relationships to PTSD and other stress- and anxiety-related conditions. (See Supplementary Note for discussion of fine-mapping, functional annotation, and CADD scores.)

Analyses adjusting for the genetic signals attributable to other major psychiatric disorders verified shared heritability with these other disorders while simultaneously confirming residual, distinct heritability for PTSD. The high r_g between PTSD symptom subdomains, which do not include overlapping items, supports the coherence of PTSD as a diagnostic construct from a biological perspective: that is, the same genetic predisposition underlies different symptoms that have previously been identified as syndromic. Genomic structural equation modeling recapitulated genetic and phenotypic correlations between PTSD subdomains; this suggests that each PTSD subdomain is largely explained by the same genetic architectures. Our model also suggests that, whereas PTSD symptoms constitute a genetically distinct and cohesive module, hyperarousal may be a relevant subdomain linking the genetic and phenotypic relationships between PTSD, anxiety, major depressive disorder, and neuroticism.

CRHR1 is in a large LD block on chromosome 17, making it difficult to discern its association with PTSD apart from other genes in that LD block. In our previous study of intrusive re-experiencing symptoms in MVP, we supported *CHRH1* as the gene with strongest association via a trans-ancestral meta-analysis[16]. We now provide additional biological evidence that *CRHR1* may be causally related to PTSD. PrediXcan-S analyses pointed to *increased* expression of *CRHR1* in amygdala, hippocampus (the structure with highest colocalization probability), frontal cortex and anterior cingulate, regions repeatedly implicated as structurally or functionally abnormal in PTSD[2]. These results must be replicated and extended to other brain regions such as ventromedial prefrontal cortex, shown to be integral to fear learning and extinction[58], processes hypothesized to be central to PTSD onset and recovery, respectively[2,59]. In concert with strong preclinical and clinical priors for involvement of CRH in stress-related disorders[60], these observations position drugs that influence *CRHR1* as strong therapeutic candidates for PTSD and related conditions. Whereas a placebo-controlled trial of a CRHR1 antagonist in 128 women with PTSD produced unimpressive results[61], our findings (albeit predominantly in men) suggest that there are potential unfulfilled opportunities with *CRHR1* antagonists for PTSD that should be further explored, taking into account individual variation in *CRHR1* – including epigenetic variation[62] – as a source of differential antagonist efficacy, in keeping with the march toward precision psychiatry[63]. Furthermore, our unexpected finding of a negative association between PTSD symptom severity and predicted *CRHR1* expression in nucleus accumbens – which suggests that an agonist might be therapeutic – requires further investigation.

Our findings also tentatively support consideration of several drug classes as therapeutic repurposing candidates for PTSD. For example, acetylcholine receptor antagonists could be

considered given their association in cMAP with *PLEKHM1*. In a recent rodent study, the muscarinic receptor antagonist, scopolamine, augmented extinction in conjunction with exposure[64] (although other studies suggest that positive allosteric modulation of M1 muscarinic activity enhances contextual fear conditioning)[65]. These results together suggest that a therapeutic role for cholinergic modulation in PTSD and other fear-related conditions, possibly in concert with exposure therapy, should be investigated. Angiotensin receptor antagonists, also identified as drug candidates through cMAP, have a strong preclinical rationale for use in PTSD[66–68] and are, in fact, currently undergoing testing in a randomized placebo-controlled trial of losartan for PTSD (ClinicalTrials.gov Identifier: NCT02709018P).

Our study has limitations. It is not currently known whether genetic risk for PTSD differs by trauma type (e.g., combat exposure vs. civilian trauma exposure) or developmental timing (e.g., childhood maltreatment vs. adult assault). Such differences could possibly underlie clinically and biologically important heterogeneity[69]. Studies of even larger sample size (which MVP will attain in the coming years) and greater granularity with regard to types and chronology of trauma exposure, will be needed to address these questions. It is also important to note that the PCL is a state, not a trait measure, and therefore reflects current – but not necessarily worst-ever lifetime – severity. Our study also reports on the largest African ancestry sample in any PTSD study to date – which we leveraged by inclusion of those individuals in our trans-ancestral meta-analyses – but we relied, out of necessity, on the European ancestry sample for the post-GWAS analyses. We found, as might have been anticipated given prior work[70], that PRS derived in the European sample did not predict well into the African sample. Nevertheless, we aspire to using novel tools in the future to make better use of the ancestral diversity in MVP[20].

We used transcriptome-wide association approaches to inform our drug repurposing inquiries. As recommended[40], we attempted to limit tissue biases inherent to these approaches by constraining our sphere of interest to brain regions that were associated with PTSD severity through our partitioned heritability analyses. Nonetheless, the drug repurposing propositions, while hypothesis-generating and intriguing, are just that. They are one piece of information that might increase interest in testing the proposed drug classes in patients with PTSD; they must be buttressed by additional preclinical models, postmortem PTSD brain studies[71], and complementary bioinformatic approaches[72] supporting their use, as well as serious consideration of their safety in this population. We also remind readers that the present analyses rested solely on GWAS, thereby limiting inquiry to common genetic variants (to MAF 0.01, which still capture significant heritable variance) and that roles for rare variants and structural variation should also be explored. Epigenetic factors almost certainly also play a role in a disorder such as PTSD[10,73], which has traumatic stress as its precursor. Many other functional genomics tools can and should be brought to bear on the study of PTSD, expanding the scope of inquiry to encompass a holistic, integrative functional genomic analysis[74] of this common, serious, and yet still poorly understood and inadequately treated neuropsychiatric disorder.

METHODS

Subjects.

All subjects are enrollees in the VA Million Veteran Program (MVP)[18]. Active users of the Veterans Health Administration healthcare system learn of MVP via an invitational mailing and/or through MVP staff while receiving clinical care with informed consent and HIPAA authorization as the only inclusion criteria. As of July 2020, > 825,000 veterans have enrolled in the program; for the current analyses, genotype data were available from approximately 375,000 participants. Individuals with EHR diagnoses of schizophrenia or bipolar disorder were excluded from participation in this study of PTSD. Research involving MVP is approved by the VA Central IRB; the current project was also approved by VA IRBs in Boston, San Diego, and West Haven.

PTSD case-control (binary) electronic health record derived phenotype.— Details on the validation and psychometric properties of this phenotype are reported in our recent publication[23]. In brief, we used manual chart review (n = 500) as the gold standard. For both the algorithm and chart review, three classifications were possible: likely PTSD, possible PTSD, or no PTSD. We used Lasso regression with cross-validation first to select statistically significant predictors of PTSD from the electronic health record (EHR) and then to generate a predicted probability score of being a PTSD case for every participant in the study population. Probability scores ranged from 0–1.00. Comparing the performance of our probabilistic approach (Lasso algorithm) to a rule-based approach (ICD algorithm), the Lasso algorithm showed modestly higher overall percent agreement with chart review compared to the ICD algorithm (80% vs. 75%), higher sensitivity (0.95 vs. 0.84), and higher overall accuracy (AUC = 0.95 vs. 0.90). For purposes of the case-control binary EHR-derived phenotype used here, we applied a 0.7 probability cut point to the Lasso results to determine final PTSD case and control status; we also selected a threshold score of 30 on the PCL from the MVP survey to minimize false negative classifications (e.g., due to an absence of PTSD screening information in the EHR). This final algorithm had a 0.96 sensitivity, 0.98 specificity, 0.91 positive predictive value, and 0.99 negative predictive value for PTSD classification in the trans-ancestral sample as determined by chart review.

PTSD symptom severity (quantitative trait) sub-phenotypes.—The second optional questionnaire, the MVP Lifestyle Survey, includes the PTSD Symptom Checklist (PCL; DSM-IV version)[26], which asks respondents to report how much they have been bothered *in the past month* by symptoms in response to stressful life experiences. The PCL has 17 items, each scored on a 5-point severity scale (1 = “Not at All” though 5 = “Extremely”). The re-experiencing (REX) symptom domain is covered by 5 items (score range 5–25), the avoidance (AVOID) domain by 7 items (score range 5–35), and the hyperarousal (HYPER) domain by 5 items (score range 5–25), yielding an overall severity score (TOTAL) for the 17 items (score range 17–85). PCL items and their distributions in European Americans and African Americans are shown in Supplementary Table 1. After accounting for missing phenotype data, the final sample size for TOTAL was 186,689 in the EUR sample and 25,318 in the AFR sample.

Genotyping, imputation and quality control.

Genotyping, imputation, and quality control within MVP has been previously described[18]. Briefly, samples were genotyped using a 723,305-SNP Affymetrix Axiom biobank array, customized for MVP. Imputation was performed with minimac3[75] using data from the 1000 Genomes Project. For post-imputation QC, SNPs with imputation INFO scores of < 0.3 or minor allele frequencies (MAF) below 0.01 were removed from analysis. For the first tranche of data, 22,183 SNPs were selected through linkage disequilibrium (LD) pruning using PLINK[24,76], and then Eigensoft[77] was used to conduct principal component analysis on 343,286 MVP samples and 2,504 1000 Genomes Project samples[78]. The reference population groups in the 1000 Genomes samples were used to define EUR (n = 241,541) and AFR (n = 61,796) groups used in these analyses. Similar methods were used in the second data tranche, which contained 108,416 new MVP samples and the same 2,504 1000 Genomes Project samples. In Tranche 2, 80,694 participants were defined as EUR and 20,584 as AFR. In this manuscript, we report results as the meta-analysis of Tranche 1 and 2 data, either for EUR and AFR separately, or as a trans-ancestral meta-analysis.

Association analyses.

GWAS analysis was carried out by logistic (for the two binary traits) or linear (for the quantitative traits) regression for each ancestry group and tranche using PLINK 2.0[24] on dosage data, covarying for age, sex, and the first 10 PCs. Meta-analysis was performed using METAL[25]. We applied a standard genome-wide multiple testing correction ($P < 5 \times 10^{-8}$). No additional multiple testing correction was applied with respect to the number of phenotypes tested due to their high genetic correlation ($r_g > 0.9$). The association results were populated and visualized using Phenogram[79]. The risk loci were enumerated using FUMA[80], and each locus containing more than 10 SNPs was fine-mapped using CADD[28] and CAVIAR[27] for PCL-Total in the EUR population only as no significant associations were observed in the AFR population, and EHR case-control phenotypes for both populations. To understand the biological effect of SNPs associated with PTSD phenotypes, we analyzed top SNPs (at suggestive threshold $P < 5 \times 10^{-6}$) for their unique and overlapping distribution across the five phenotypes. The top SNPs for each phenotype were LD pruned (r^2 = 0.2, kb = 250) to obtain independent signals and investigated for their role as quantitative trait loci for protein expression, DNA methylation, and splicing (brain tissues) from the QTLbase[29].

LD score regression (LDSC) and SNP-based heritability.

SNP-heritability was calculated using LDSC[81] on the observed-scale for continuous phenotypes and the liability scale (using prevalence = 10%) for the PTSD case-control definition. Genetic correlation was estimated between PTSD case-control, PCL-Total, and all phenotypes from UK Biobank with suitable h^2 accuracies for reliable r_g estimation (h^2 z-score ≥ 4). Heritability and genetic correlation analyses were performed using the 1000 Genomes Project European LD reference panel.

Conditional analysis with other psychiatric disorders.

Considering the extensive comorbidity between major depression and PTSD[82], we conducted conditional analysis with Multi-trait Conditional and Joint Analysis (mtCOJO)[30] using GCTA software with the MVP PCL-total symptom severity summary statistics as the primary analysis and the PGC MDD2 (excluding 23andMe due to data unavailability)[83] summary statistics to condition the analysis for depression. Additional summary statistics for autism spectrum disorder, anorexia nervosa, anxiety (case-control), alcohol dependence, schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder were obtained from https://www.med.unc.edu/pgc/results-and-downloads/.

Genomic structural equation modeling.

Genomic structural equation modeling (GenomicSEM) was performed in R using the GenomicSEM package[84]. Multivariable linkage disequilibrium matrices were created using the 1000 Genomes Project Phase 3 European reference. Exploratory factor analysis (EFA) was used to estimate the most appropriate number of latent factors represented by the psychiatric phenotypes and psychopathologies tested assuming a maximum number of latent factors equal to N_{traits}-1. Confirmatory factor analysis (CFA) was used to calculate factor loadings onto each latent factor(s). Standardized loading values are reported.

Polygenic risk score (PRS) analysis.

The PRS (Extended Data Figure 2) were calculated after using *P*-value-informed clumping with an LD cutoff of $r^2 = 0.05$ within a 500-kb window, excluding the MHC region of the genome because of its complex LD structure. The European samples of the 1000 Genomes Project were used as the LD reference panel. PRS analysis was conducted based on the GWAS summary association data using the gtx R package incorporated in PRSice v1.25 software[81]. For each PRS analysis, we calculated an approximate estimate of the explained variance from a multivariate regression model[85]. For comparison of cross-ancestry PRS (Extended Data Figure 3), we clumped summary statistics from a recent PTSD GWAS[13], applying an LD cutoff of $r^2 = 0.3$ within a 500-kb window. These clumped summary statistics were used as a base for calculating the PRS in MVP individuals of European and African ancestry, independently, using PRSice 2.0 software[86].

PrediXcan-S methods.

To perform transcriptome-wide association analysis, PrediXcan-S (also known as MetaXcan)[41] was used to impute gene expression based on the GWAS summary statistics (meta-analysis of tranche 1 and 2 EAs) of PCL-Total with the reference gene expression data of 48 tissues from GTEx Release V7. Gene-expression association with PTSD PCL-Total was performed for each tissue (13 of which are brain tissues) individually.

Colocalization analysis.

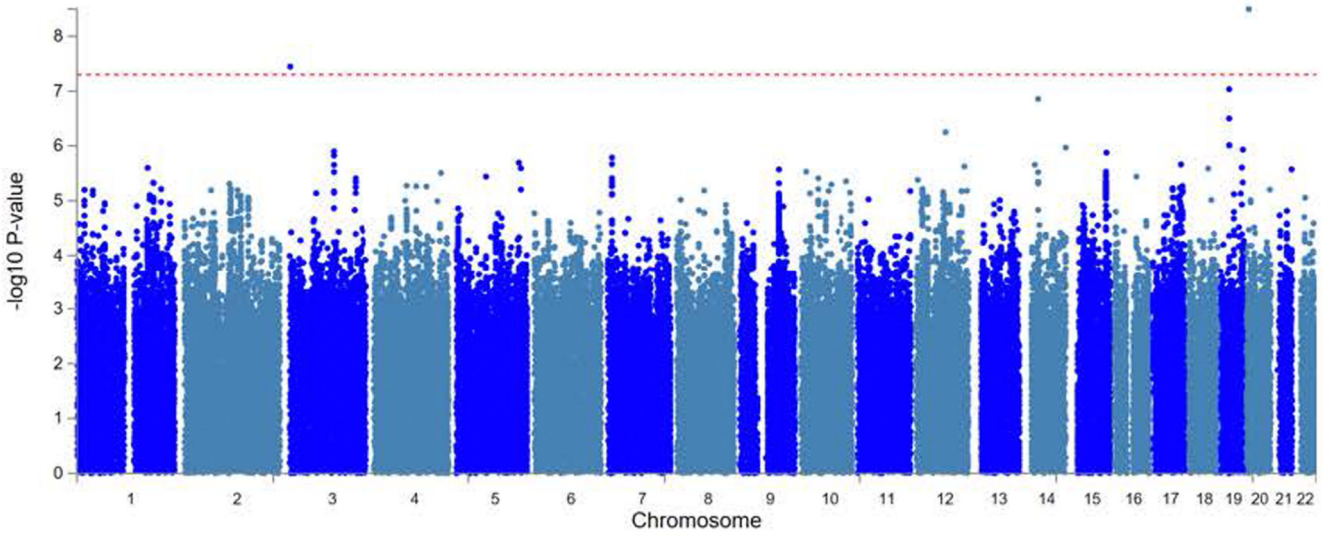
Colocalization analysis was performed using the *coloc* R package[42] for genes that were significant from the TWAS results of brain tissues with gene expression data from GTEx Release V8. The coloc.abf function was used to test for shared causal loci under four alternative hypotheses. Loci with posterior probability >90% were considered as strong

evidence for the H_4 hypothesis, i.e. both traits are associated and share a single causal variant.

Drug repositioning analysis.

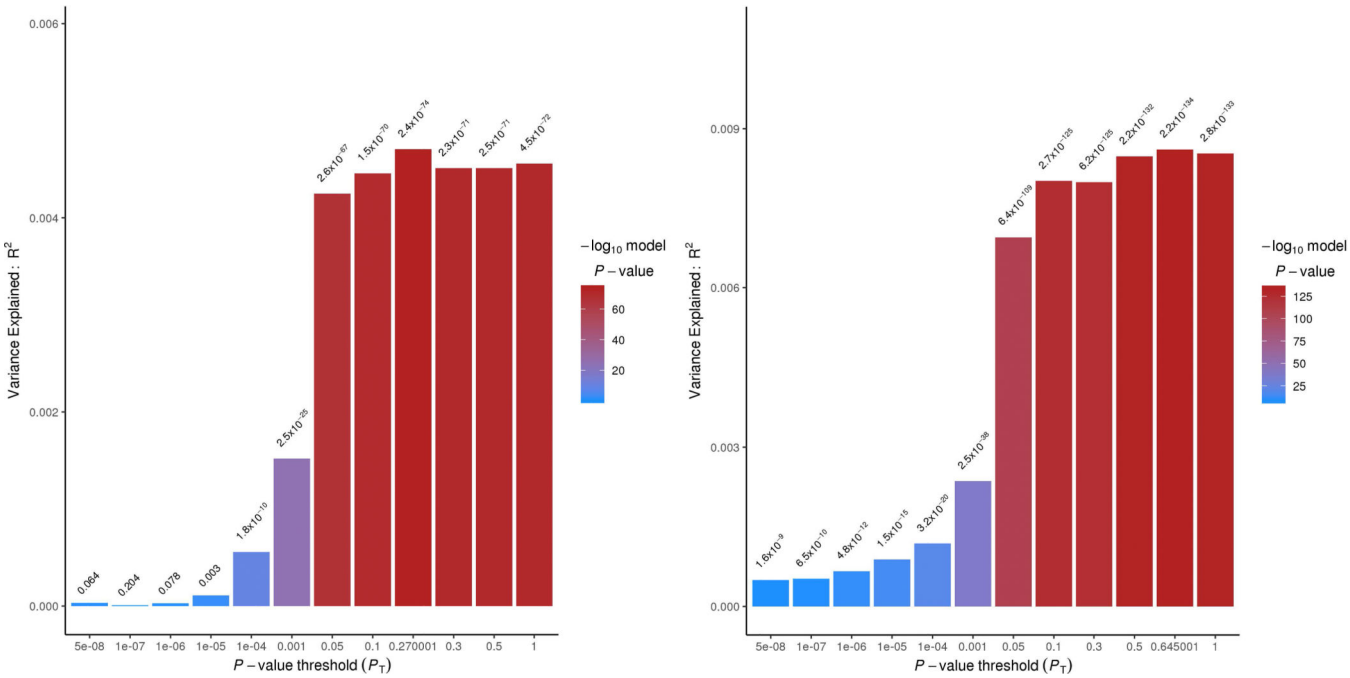
CMap (https://clue.io/cmap) provides expression similarity scores for a specific expression profile with other drug-induced transcriptional profiles, including consensus transcriptional signatures of 83 drug classes, i.e., transcriptional profiles induced by 2,837 drugs grouped into 83 drug classes. Expression similarity is evaluated by means of scores that vary from −100 to 100, with −100 the most extreme opposite expression profile and 100 the most extreme similar expression profile.

Extended Data



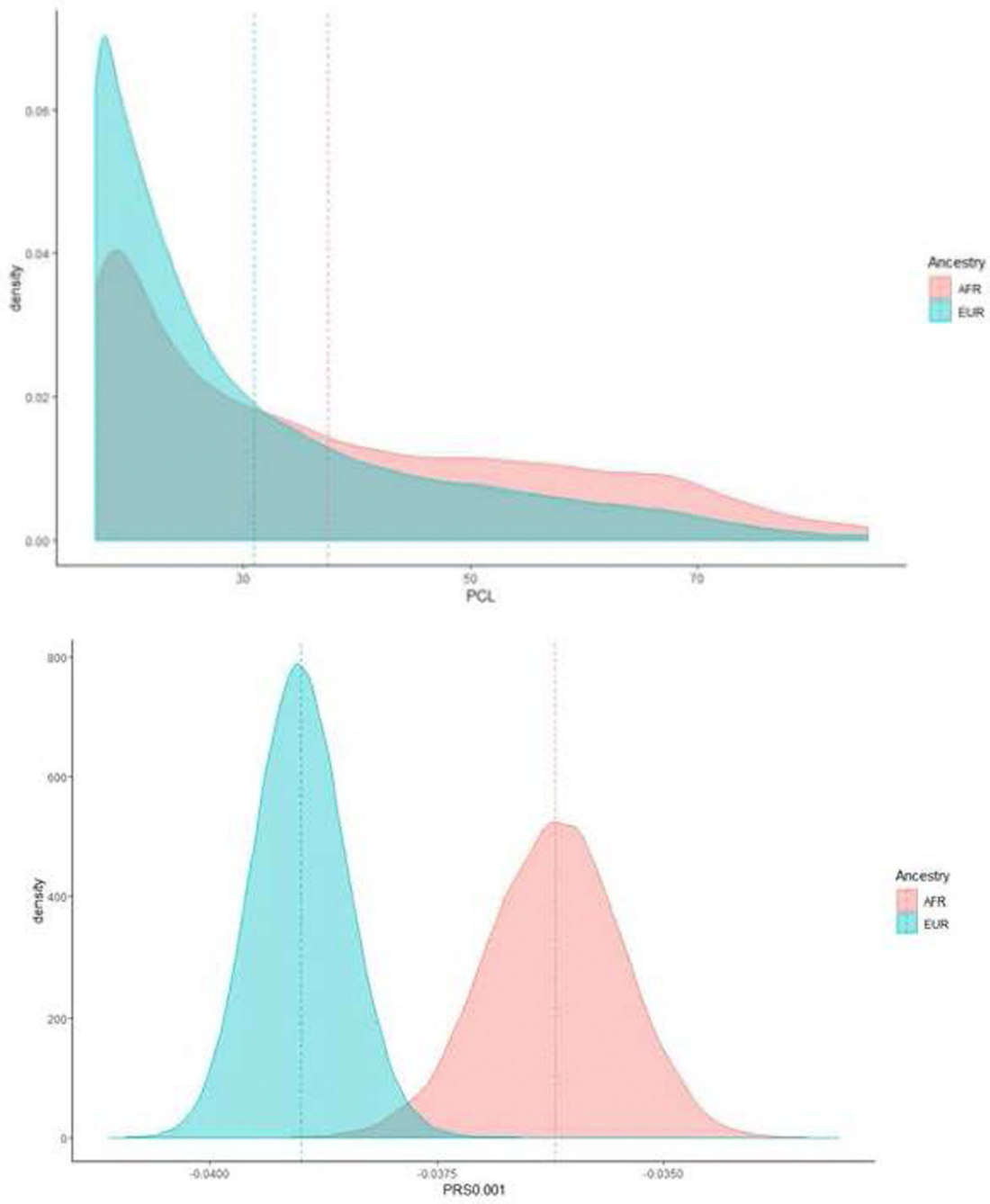
Extended Data Fig. 1. Manhattan plot of MVP AFR case-control GWAS

Horizontal red line indicates $P < 5 \times 10^{-8}$. *P*-values are uncorrected. Results are based on logistic regression.



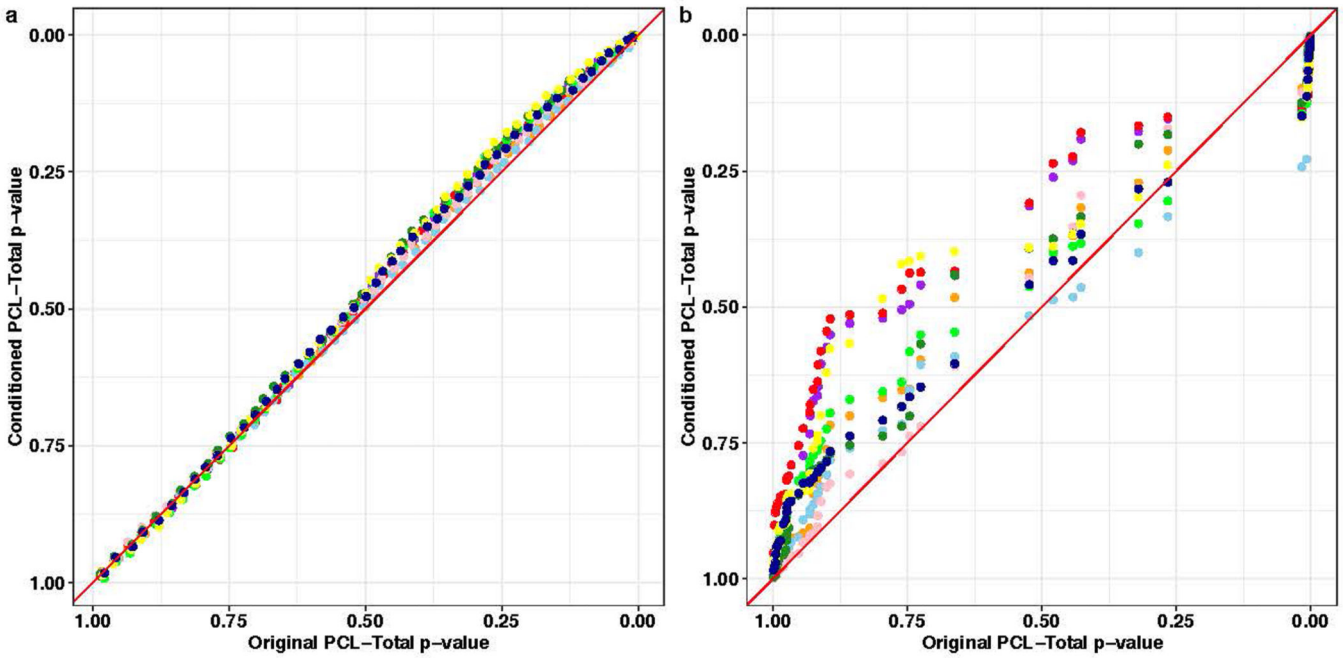
Extended Data Fig. 2. Polygenic risk scores in MVP and PGC-PTSD

Polygenic risk score (PRS) from MVP EUR case-control (left) and EUR PCL-total (right) applied to PGC-PTSD[13] case-control phenotype with varying *P*-value thresholds (PT) on the *x*-axis and explained variance (R^2) on the *y*-axis. The approximate estimate of the explained variance was calculated using a multivariate regression model. *P* values reported are two sided, and Bonferroni correction accounting for the number of *P*-value thresholds tested is $P = 2.38 \times 10^{-4}$.



Extended Data Fig. 3. Symptom and polygenic risk scores in veterans of African and European ancestry

Top shows density plot of PCL-total scores in veterans of AFR (salmon color) and EUR (teal color) ancestry. Bottom shows density plot of PRS scores (at *P*-value threshold 0.001) for MVP PCL AFR (salmon color) and MVP PCL EUR (teal color) into PGC PTSD EUR.



Extended Data Fig. 4. Gene Ontology (GO) term and GTEx tissue enrichment

a, Quantile-quantile plots between Gene Ontology (GO) term enrichment (one-sided test for positive relationship between tissue and genetic association) in original PCL-Total and conditioned PCL-Total (blue, autism spectrum disorder; purple, major depression; dark green, anorexia nervosa; light green, anxiety; pink, schizophrenia; light blue, bipolar disorder; orange, attention deficit hyperactivity disorder; red, all eight disorders simultaneously). **b**, Quantile-quantile relationship between GTEx tissue enrichment (one-sided test for positive relationship between tissue and genetic association) in original PCL-total and conditioned PCL-Total. To avoid over-plotting, enrichment *P*-values were divided into quantiles. Red diagonal lines indicate a one-to-one relationship between original and conditioned PCL-Total gene set and tissue enrichments. Two-sided tests were used to compare enrichment results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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APPENDIX

CONSORTIA

Department of Veterans Affairs Cooperative Studies Program (#575B)

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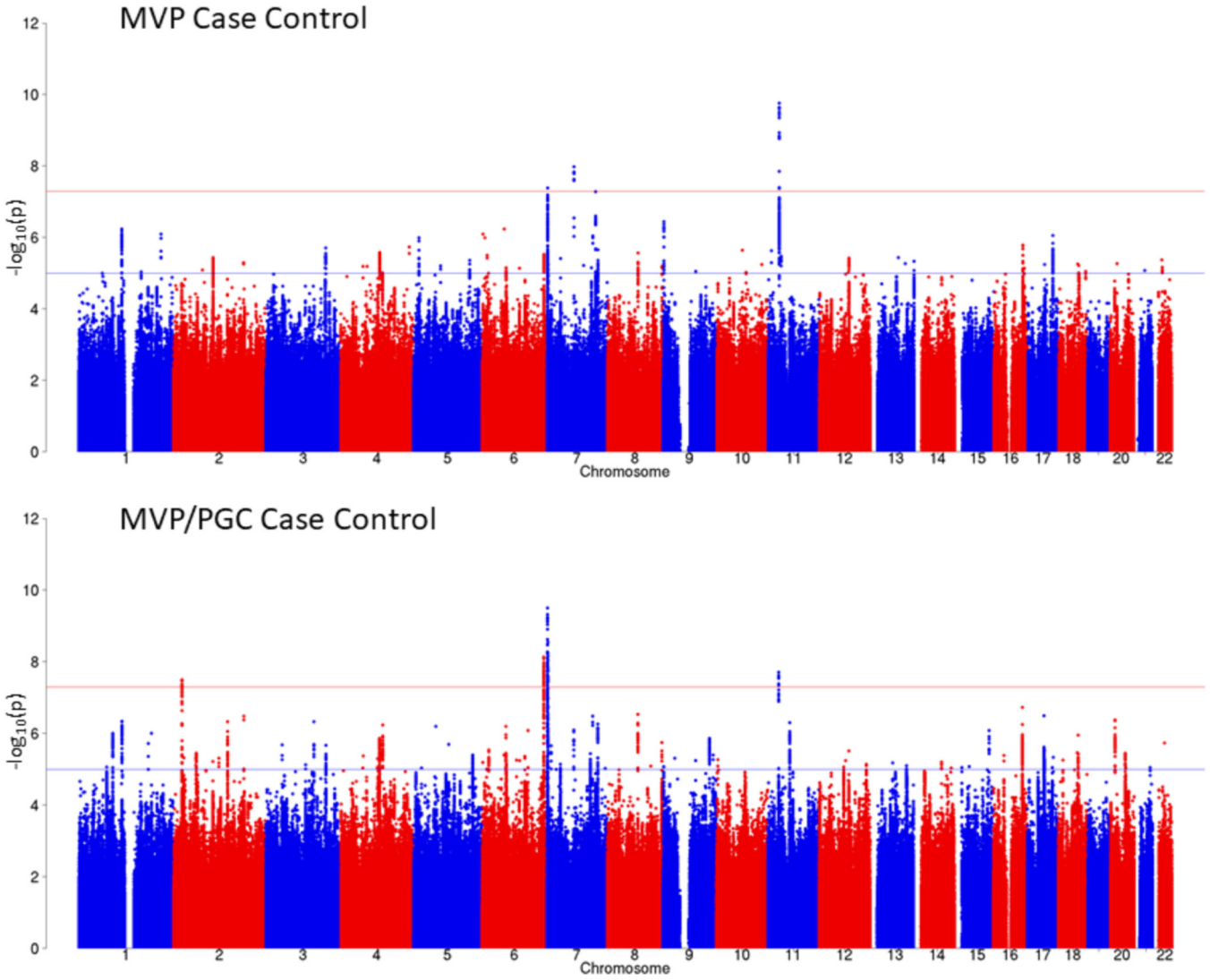


Figure 1 |. Manhattan plot for the MVP case-control GWAS (top) and for the MVP/PGC GWAS meta-analysis in EUR samples (bottom).

GWAS was performed using logistic regression, co-varying for age, sex, and the first 10 principal components of ancestry. Meta-analysis was conducted with METAL[25] using the inverse variance weighting method. Bonferroni correction was used to correct for multiple comparisons; associations with $P \leq 5 \times 10^{-8}$ (indicated by the horizontal red bar) were considered to be genome-wide significant.

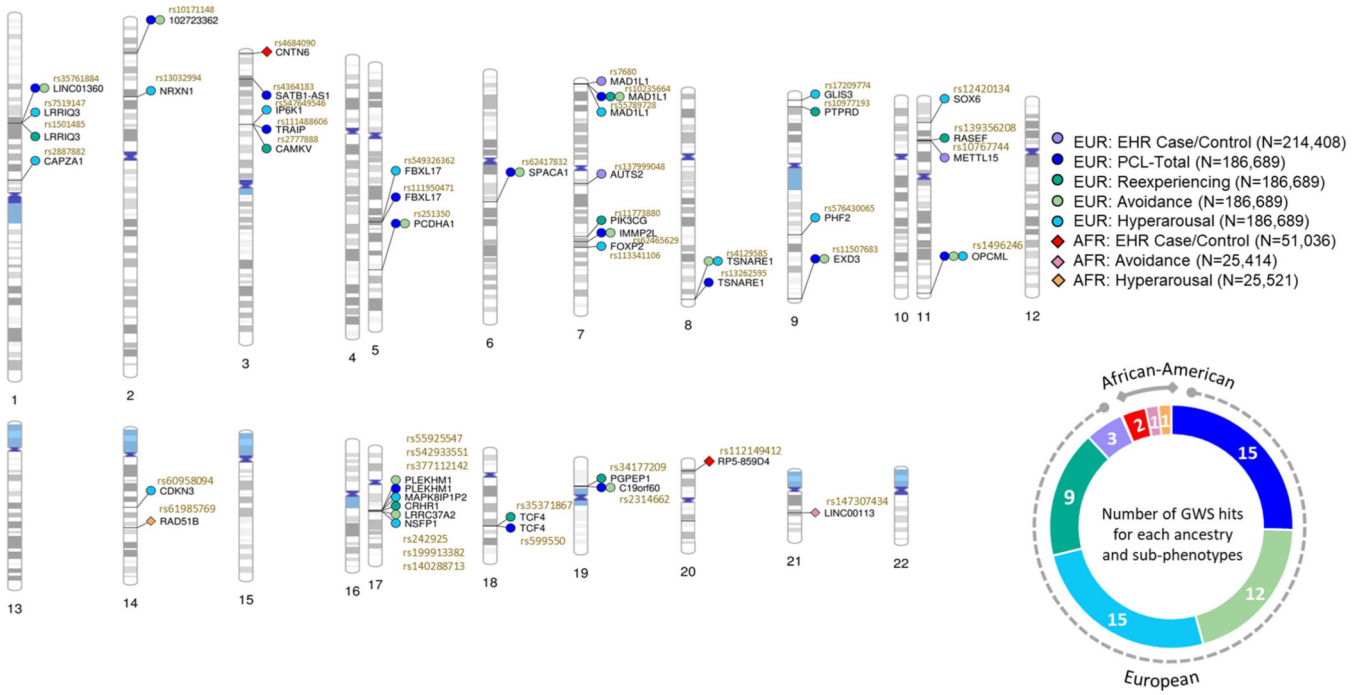
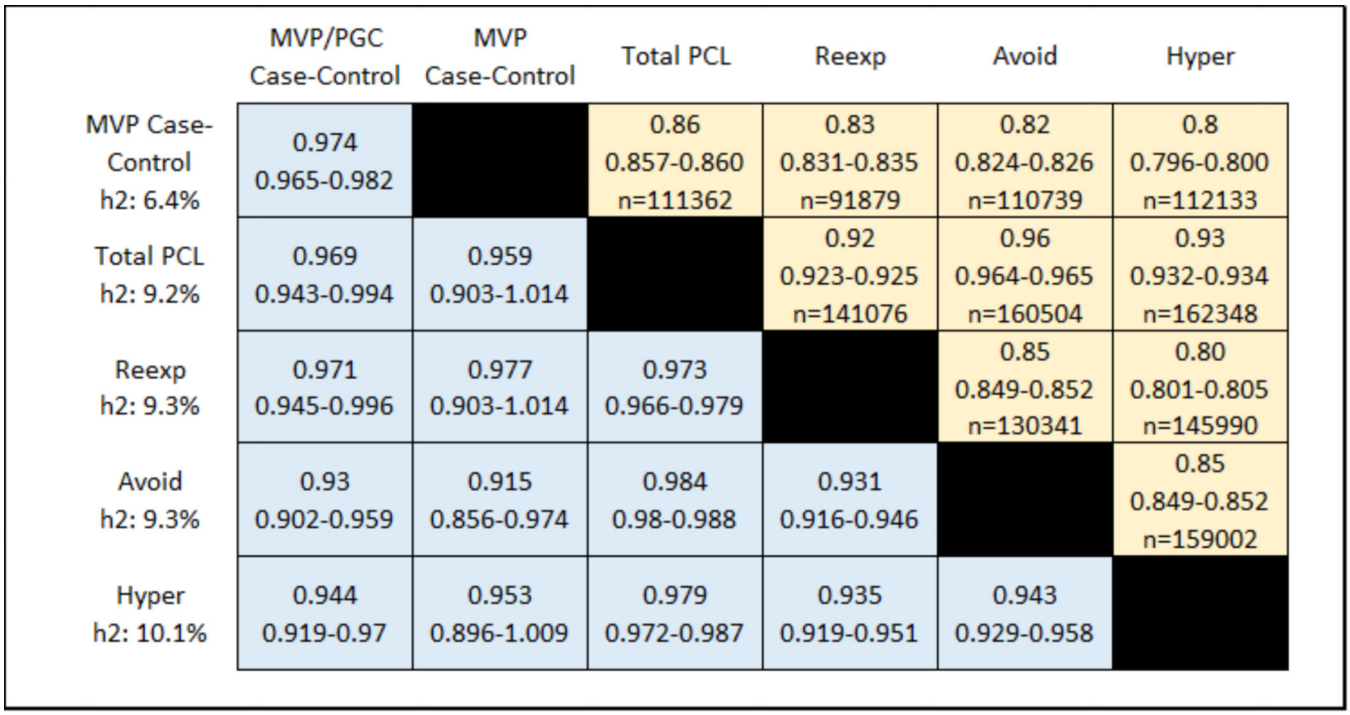


Figure 2 |. Genome-wide significant ($P < 5 \times 10^{-8}$) findings, by European (circles) and African (diamonds) ancestry, for PTSD EHR case-control, PCL-Total score and each of the PTSD subcomponents (avoidance, hyperarousal, and re-experiencing).

There were no genome-wide significant results for the African case-control and re-experiencing traits. LD independent SNPs for each phenotype and the nearest gene are labeled. The donut chart summarizes the number of hits for each phenotype in the two populations. The genes labelled are significant following regression test for a two-sided *P*-value and applied Bonferroni-threshold for multiple testing (0.05/k SNPs = $P < 5 \times 10^{-8}$).



	MVP/PGC Case-Control	MVP Case-Control	Total PCL	Reexp	Avoid	Hyper
MVP Case-Control h2: 6.4%	0.974 0.965-0.982		0.86 0.857-0.860 n=111362	0.83 0.831-0.835 n=91879	0.82 0.824-0.826 n=110739	0.8 0.796-0.800 n=112133
Total PCL h2: 9.2%	0.969 0.943-0.994	0.959 0.903-1.014		0.92 0.923-0.925 n=141076	0.96 0.964-0.965 n=160504	0.93 0.932-0.934 n=162348
Reexp h2: 9.3%	0.971 0.945-0.996	0.977 0.903-1.014	0.973 0.966-0.979		0.85 0.849-0.852 n=130341	0.80 0.801-0.805 n=145990
Avoid h2: 9.3%	0.93 0.902-0.959	0.915 0.856-0.974	0.984 0.98-0.988	0.931 0.916-0.946		0.85 0.849-0.852 n=159002
Hyper h2: 10.1%	0.944 0.919-0.97	0.953 0.896-1.009	0.979 0.972-0.987	0.935 0.919-0.951	0.943 0.929-0.958	

Figure 3 |. Phenotypic (above diagonal) and genetic (below diagonal) correlations between case-control, PCL-Total and subscale scores.

Shown are correlation point estimates, 95% CIs, and *n* (sample size). SNP-heritability (h^2) is shown in the left column. For phenotypic correlations, those for case-control are point-biserial correlations, and all others are Pearson correlations.

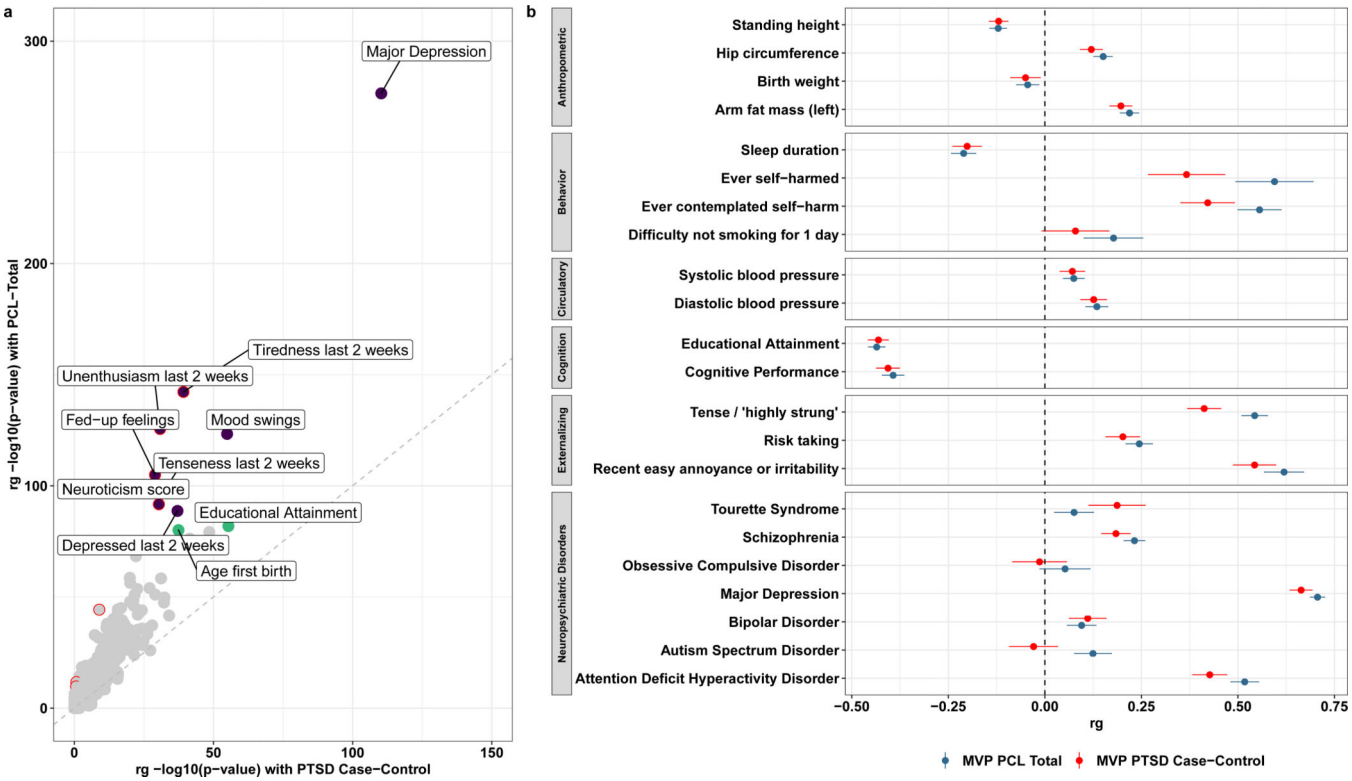


Figure 4 |. LDSC genetic correlation (r_g) analyses in EUR showing traits from UK Biobank and PGC psychiatric disorders.

a, Comparison of r_g between PTSD case-control definition and PCL-Total. The grey diagonal indicates perfect linearity between PTSD case-control and PCL-Total genetic correlates. The top ten genetic correlates of PCL-Total are labeled, with purple data points indicating positive r_g and green data points indicating negative r_g. Data points in **a** circled in red indicate significant difference in r_g magnitude between PTSD and PCL-Total. **b**, Plot showing a wide range of phenotypes and their r_g; the vertical dashed line indicates r_g equal to zero.

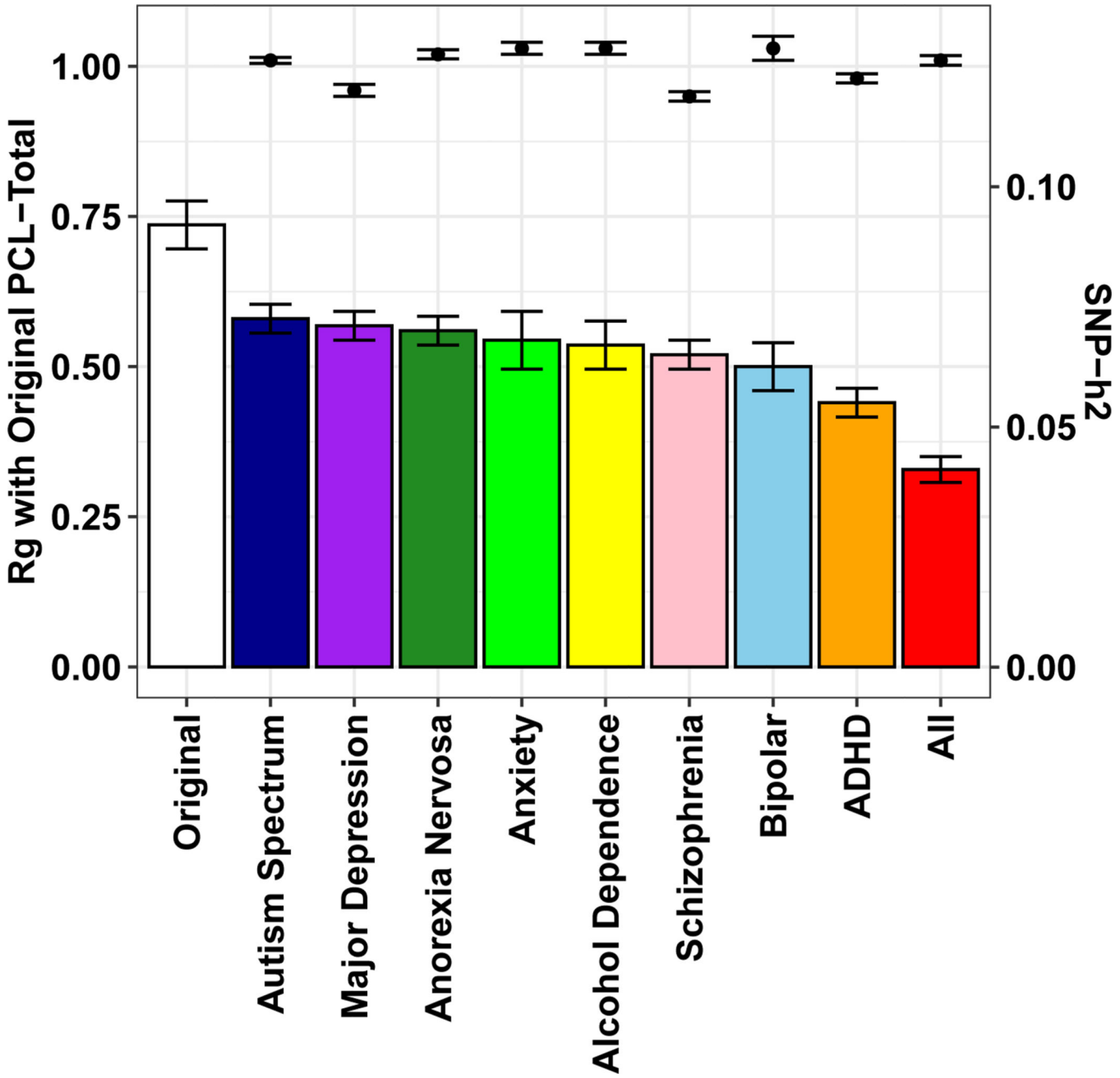


Figure 5 |.

PCL-Total SNP-heritability (SNP-h^2; bars and standard error; right *y*-axis) and genetic correlation (r_g; data points and standard error; left *y*-axis) relative to original PCL-Total (n = 186,689) after conditioning with each mental health phenotype on the *x*-axis.

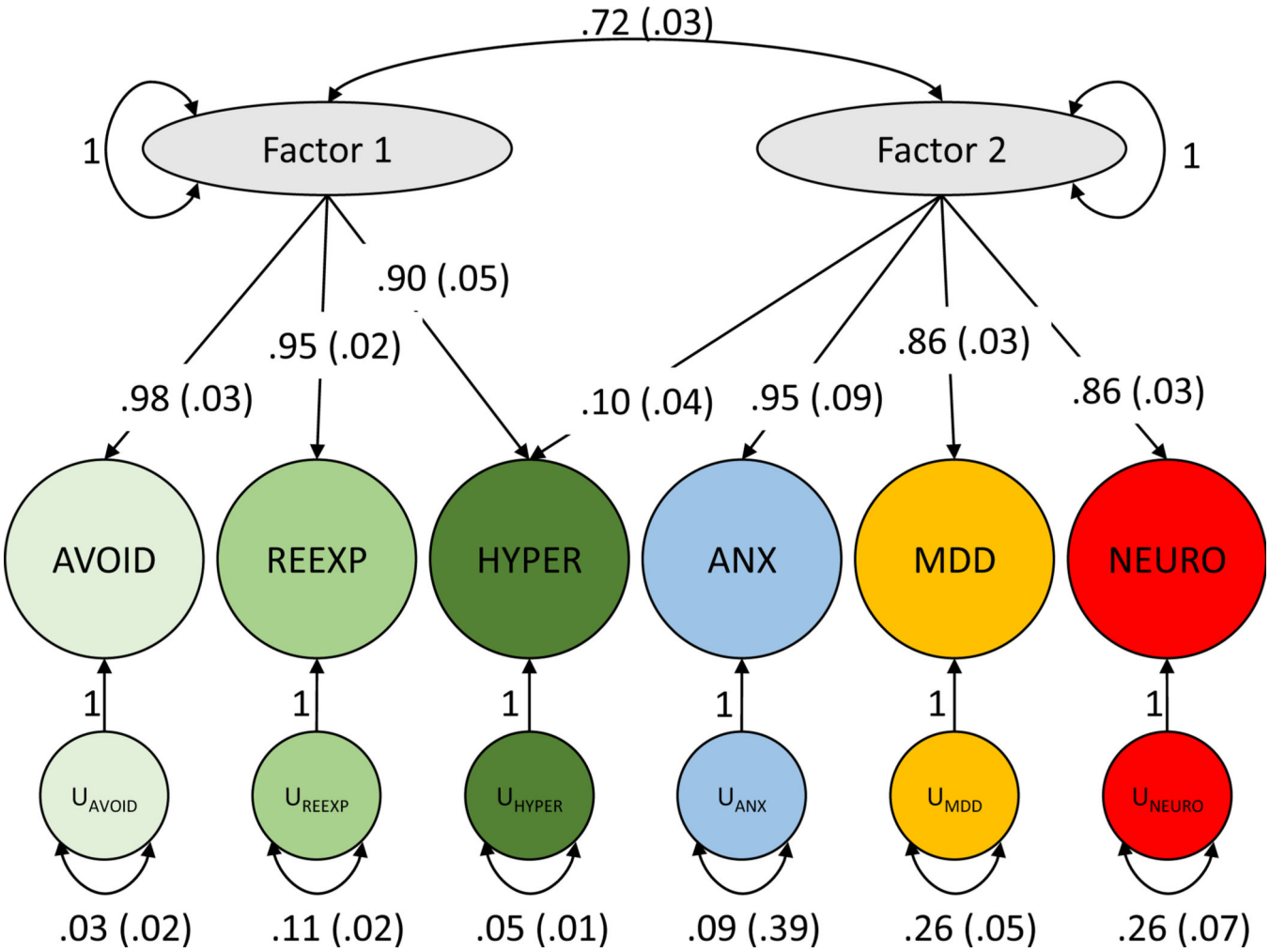


Figure 6 |. Genomic SEM model with confirmatory factor analysis indicating two correlated factors, the first consisting of PTSD symptoms and the second consisting of anxiety, major depressive disorder, and neuroticism.

Comparative fit index (CFI) = 0.999 (typically interpreted as 0.90–0.95 indicating marginal fit).

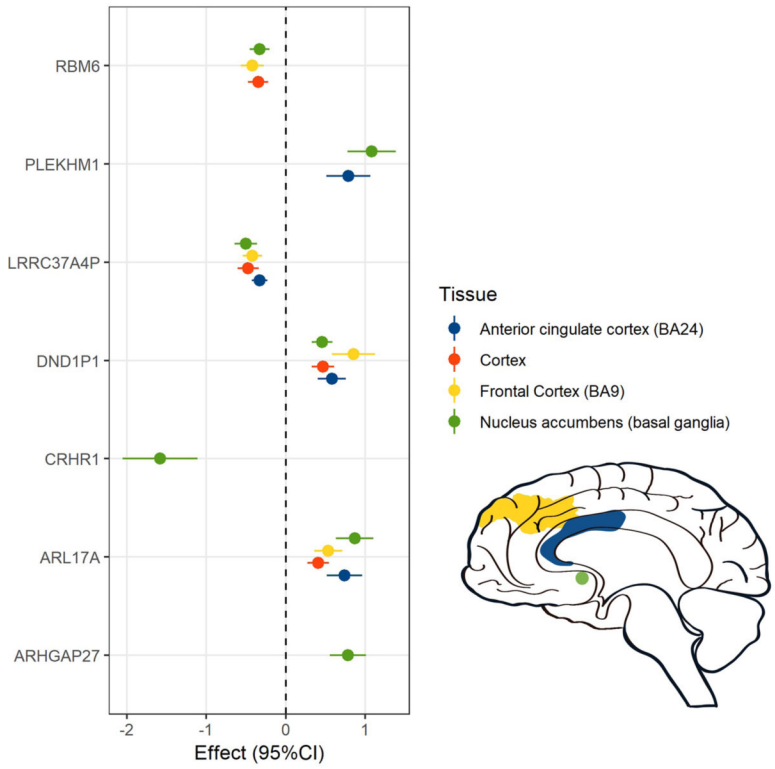


Figure 7 |. Association of genetically-regulated transcriptomic changes with PCL-Total (*n* = 186,689) in the four brain regions identified by the LDSC partitioned heritability analyses. Betas and 95% confidence intervals are reported for each association.

Table 1 |

Genome-wide significant ($P < 5 \times 10^{-8}$) findings using linear regression with lead SNPs for EUR PCL-Total and sub-phenotype GWAS analyses (n = 186,689 individuals)

	LD independent lead SNP	Chr	Effect allele	Beta	*P*	INFO score	SNP location	Nearest gene
PCL-Total	rs542933551	17	AAAAACAAAAC	0.4585	2.02E-13	0.95	43557054	*PLEKHM1*
	rs10235664	7	C	−0.3667	1.82E-11	0.93	2086814	*MAD1L1*
	rs35761884	1	C	−0.3076	3.46E-10	0.92	73787732	*LINC01360*
	rs111488606	3	CA	0.3102	1.72E-09	0.83	49864924	*TRAIP*
	rs13262595	8	G	−0.2823	2.20E-09	1.00	143316970	*TSNARE1*
	rs2314662	19	C	−0.3614	3.78E-09	0.93	18702515	*C19orf60*
	rs10171148	2	A	0.2811	5.87E-09	0.96	22466171	*LOC102723362*
	rs62465629	7	C	−0.3929	6.30E-09	0.85	110153866	*IMMP2L*
	rs1496246	11	G	0.2973	6.60E-09	0.90	133548061	*OPCML*
	rs251350	5	C	−0.2538	1.03E-08	1.12	140225137	*PCDHA1*
	rs11507683	9	T	0.4137	1.15E-08	0.96	140262424	*EXD3*
	rs599550	18	A	0.3948	1.18E-08	0.95	53252388	*TCF4*
	rs4364183	3	A	0.3043	1.22E-08	0.93	18809536	*SATB1-AS1*
	rs62417832	6	T	0.2922	2.90E-08	1.00	88640221	*SPACA1*
	rs111950471	5	TATTA	−0.2769	4.34E-08	0.98	107450098	*FBXL17*
	LD independent lead SNP	**Chr**	**Effect allele**	**Beta**	***P***	**INFO score**	**SNP location**	**Nearest gene**
Re-experiencing	rs35371867	18	A	0.1006	1.24E-10	0.97	53193027	*TCF4*
	rs2777888	3	G	0.0929	2.26E-10	0.98	49898000	*CAMKV*
	rs10235664	7	C	−0.1055	4.66E-10	0.93	2086814	*MAD1L1*
	rs242925	17	T	−0.0931	5.50E-10	0.94	43888866	*CRHR1*
	rs139356208	11	CACAAAACAAA	−0.0897	9.63E-09	0.90	28631779	*RASEF*
	rs1501485	1	G	−0.0839	1.22E-08	0.97	73995259	*LRRIQ3*
	rs11773880	7	G	−0.0977	1.97E-08	0.93	106540171	*PIK3CG*
	rs34177209	19	A	0.1205	2.34E-08	0.62	18474978	*PGPEP1*
	rs10977193	9	A	−0.0934	4.17E-08	0.96	8542019	*PTPRD*
	LD independent lead SNP	**Chr**	**Effect allele**	**Beta**	***P***	**INFO score**	**SNP location**	**Nearest gene**
Avoid-ance	rs55925547	17	C	0.1932	2.08E-13	0.98	43556807	*PLEKHM1*
	rs199913382	17	C	0.1772	1.05E-12	0.98	44625866	*LRRC37A2*
	rs35761884	1	C	−0.1388	9.72E-11	0.92	73787732	*LINC01360*
	rs251350	5	C	−0.1192	8.15E-10	1.12	140225137	*PCDHA1*
	rs4129585	8	C	−0.125	1.25E-09	1.00	143312933	*TSNARE1*
	rs2314662	19	C	−0.1599	2.74E-09	0.93	18702515	*C19orf60*
	rs62465629	7	C	−0.175	3.54E-09	0.85	110153866	*IMMP2L*
	rs62417832	6	T	0.1335	7.04E-09	1.00	88640221	*SPACA1*

	rs11507683	9	T	0.1834	7.74E-09	0.96	140262424	*EXD3*
	rs10171148	2	A	0.1211	1.07E-08	0.96	22466171	*LOC102723362*
	rs10235664	7	C	−0.1337	2.17E-08	0.93	2086814	*MAD1L1*
	rs1496246	11	G	0.1234	3.66E-08	0.90	133548061	*OPCML*
	LD independent lead SNP	**Chr**	**Effect allele**	**Beta**	***P***	**INFO score**	**SNP location**	**Nearest gene**
	rs377112142	17	CT	0.1323	3.06E-13	0.84	43663455	*MAPK8IP1P2*
	rs55789728	7	G	−0.1303	4.62E-13	0.93	2107649	*MAD1L1*
	rs576430065	9	CA	−0.1206	1.67E-11	0.78	96373697	*PHF2*
	rs140288713	17	A	0.1286	3.11E-11	0.90	44690708	*NSFP1*
	rs1496246	11	G	0.1037	1.77E-10	0.90	133548061	*OPCML*
	rs547649546	3	CA	−0.0937	1.59E-09	0.91	49789921	*IP6K1*
Hyper-arousal	rs2887882	1	T	−0.1118	1.89E-09	0.98	113170389	*CAPZA1*
	rs7519147	1	T	−0.0906	1.90E-09	0.96	73994416	*LRRIQ3*
	rs13032994	2	C	−0.0968	3.73E-09	1.00	52709559	*NRXN1*
	rs113341106	7	GC	0.0923	3.82E-09	0.93	114039998	*FOXP2*
	rs12420134	11	G	0.1229	6.45E-09	0.87	16260861	*SOX6*
	rs17209774	9	C	−0.0907	7.97E-09	0.97	4145163	*GLIS3*
	rs60958094	14	T	0.0961	1.99E-08	0.81	54711168	*CDKN3*
	rs4129585	8	C	−0.0835	2.07E-08	1.00	143312933	*TSNARE1*
	rs549326362	5	T	−0.0884	4.46E-08	0.94	107444481	*FBXL17*