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## Genetically regulated multi-omics study for symptom clusters of posttraumatic stress disorder highlights pleiotropy with hematologic and cardio-metabolic traits

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### <sup>8</sup>CONTRIBUTIONS

GAP designed the study, analyzed the data, and wrote the manuscript draft. KS, FRW, TWF, and CO analyzed the data and drafted the manuscript. DST provided clinical expertise for phenotype harmonization between cohorts. All the other authors provided critical feedback, context interpretation, draft revision, and editing. LKD and RP supervised the study, reviewed, and edited the manuscript.

### <sup>7</sup>DATA AVAILABILITY AND URLS

All the data is available in Supplementary files. If you need further clarification, please contact the corresponding author.

- GWAS summary statistics: [https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study\\_id=phs001672.v6.p1](https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001672.v6.p1)
- TWAS method: <http://gusevlab.org/projects/fusion/>
- Splicing and Gene Expression Weights : <http://gusevlab.org/projects/fusion/>
- Proteome weights: <https://www.synapse.org/#!/Synapse:syn23627957>
- Open Targets: <https://genetics.opentargets.org/>
- UKBB Summary Statistics : <http://www.nealelab.is/uk-biobank>
- LAVA: <https://github.com/josefin-werme/LAVA>
- CLUE: <https://clue.io/>

### <sup>10</sup>CONFLICT OF INTEREST

RP and JG are paid for their editorial work on the journal *Complex Psychiatry*. JHK has served as a scientific consultant (Individual Consultant Agreements less than \$5000 per year) to Amgen, AstraZeneca Pharmaceuticals, Bigen, Idec, MA, Biomedisyn Corporation, Forum Pharmaceuticals, Janssen Research & Development, Otsuka America Pharmaceutical, Sunovion Pharmaceuticals, Takeda Industries, and Taisho Pharmaceutical Co; is on the Scientific Advisory Board for Biohaven Pharmaceuticals, Blackthorn Therapeutics, Lohocla Research Corporation, Luc Therapeutics, Pfizer Pharmaceuticals, Tand RImaran Pharma; holds stock in Biohaven Pharmaceuticals Medical Sciences and stock options in Blackthorn Therapeutics and Luc Therapeutics; and is editor of *Biological Psychiatry* (income greater than \$10,000 per year). JG is named as an inventor on PCT patent application no. 15/878,640 entitled “Genotype-guided dosing of opioid agonists,” filed January 24, 2018. RHP is a scientific consultant to Cogstate for work that bears no relationship to the current study. The other authors report no biomedical financial interests or potential conflicts of interest.

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## Abstract

Posttraumatic stress disorder (PTSD) is a psychiatric disorder that may arise in response to severe traumatic event and is diagnosed based on three main symptom clusters (reexperiencing, avoidance, and hyperarousal) per the Diagnostic Manual of Mental Disorders (version DSM-IV-TR). In this study, we characterized the biological heterogeneity of PTSD symptom clusters by performing a multi-omics investigation integrating genetically regulated gene, splicing, and protein expression in dorsolateral prefrontal cortex tissue within a sample of US veterans enrolled in the Million Veteran Program ( $N_{\text{total}}=186,689$ ). We identified 30 genes in 19 regions across the three PTSD symptom clusters. We found nine genes to have cell-type specific expression, and over-representation of miRNA-families – miR-148, 30, and 8. Gene-drug target prioritization approach highlighted cyclooxygenase and acetylcholine compounds. Next, we tested molecular-profile based phenome-wide impact of identified genes with respect to 1678 phenotypes derived from the Electronic Health Records of the Vanderbilt University biorepository ( $N=70,439$ ). Lastly, we tested for local genetic correlation across PTSD symptom clusters which highlighted colocalization and local genetic correlation with metabolic (e.g., obesity, diabetes, vascular health) and laboratory traits (e.g., neutrophil, eosinophil, tau protein, creatinine kinase). Overall, this study finds comprehensive genomic evidence including clinical and regulatory profiles between PTSD, hematologic and cardiometabolic traits, that support comorbidities observed in epidemiologic studies of PTSD.

## Keywords

PTSD; brain; transcriptomics; metabolic; blood; expression

## 3 INTRODUCTION

Post-traumatic stress disorder (PTSD) is a psychiatric disorder that may arise in response to severely stressful events. The lifetime prevalence of PTSD in the United States is estimated at approximately 7%<sup>1,2</sup>, and more than 12% in the active US veteran population<sup>3</sup>. Twin-based studies estimated a 24–72% heritability of PTSD with strong variation depending on the characteristics of the cohort investigated<sup>4</sup>. Recent large-scale efforts from the Psychiatric Genomics Consortium and the Million Veteran Program (MVP) have identified several genetic risk loci associated with PTSD<sup>1</sup>. However, the understanding of processes underlying translation of genetic associations into pathophysiological processes remains limited due to symptomatic heterogeneity of PTSD patients and the multiplicity of implicated regulatory elements. As per the Diagnostic and Statistical Manual for Mental Disorders—Fourth Edition, Revised (DSM-IV), 17 symptoms of PTSD comprise three main clusters: reexperiencing i.e. recollection of traumatic memories, avoidance i.e. avoiding thoughts of traumatic events, and hyperarousal i.e. irritability, reactivity, or high vigilance<sup>5</sup>. Furthermore, PTSD is highly polygenic and since genetic variants can affect multiple regulatory elements, it is challenging to prioritize targets for personalized therapeutics. Neuroimaging studies indicate PTSD is associated with structural and functional changes

in the dorsolateral prefrontal cortex (dlPFC)<sup>6</sup>, which may impair learning and memory, executive cognitive functions, and emotional regulation<sup>7</sup>, and is therefore an important tissue to be investigated in the context of PTSD pathogenesis.

In the current study, we performed a characterization of PTSD symptom clusters by integrating mRNA, alternative splicing, and proteome expression of the dlPFC (N=1,280) with genome-wide association data of reexperiencing, avoidance, and hyperarousal symptoms from the MVP (N=186,689)<sup>1</sup>. We identified several novel dlPFC-specific genetically regulated loci through gene/splicing/protein-wide transcriptome-wide association studies (TWAS/SPWAS/PWAS). These loci were further investigated for their biological, and phenome-wide impact. To elucidate biological impact, we analyzed the identified genes for their role in brain cell-type specific expression, cortex-specific gene-co-expression, miRNAs, and drug targets. To assess the phenome-wide impact of identified loci, we applied two molecular-evidence based approaches. First, we performed a phenome-wide association study (PheWAS) using genetically-regulated expression (GReX) of identified genes in the electronic health records and laboratory measurements from the Vanderbilt Biobank repository (BioVU; n=70,439). Second, we performed phenome-wide colocalization analysis of the identified genes (i.e. exhibit a high probability of sharing the same causal variant between expression and phenotype). We further investigated these colocalizing traits with PTSD symptom clusters using locus-specific genetic correlation. Our findings also contribute new information on several molecular mechanisms associated with PTSD symptoms and highlight potential therapeutic targets in the context of PTSD comorbidities (Figure 1A).

## 4 SUBJECTS AND METHODS

### 4.1 GWAS of PTSD symptom clusters

We obtained authorized access to Database of Genotype and Phenotype (dbGaP) for the genome-wide statistics of PTSD symptom clusters: reexperiencing, avoidance, and hyperarousal from the MVP (dbGaP Study Accession: phs001672.v6.p1)<sup>1</sup>. This GWAS of PTSD and its quantitative symptom clusters included 186,689 US military veterans from the MVP. Genome-wide association statistics reflect results from individuals of European ancestry, inverse-variance meta-analyzed across two tranches, and reported with variant-level beta, standard errors, and p values<sup>1</sup>. We performed strict quality control using the munging procedure provided by LD-score regression (LDSC)<sup>8</sup>. Our quality control procedure resulted in a final count of 1,191,504 high-quality HapMap autosomal genetic variants.

### 4.2 Gene expression weights for dlPFC and TWAS

We obtained pretrained models of gene and splicing expression of the dlPFC fitted in the data from the Common-Mind Consortium (CMC; N=452; 5,420 expression transcript and 7,772 splicing transcripts) stored in the FUSION repository (See URLs). The quality control of RNA-seq, RNA-seq splicing, and genotype from CMC data has been previously described in detail<sup>9,10</sup>. The proteome expression weights for the dlPFC (n=376; features - 1467) were leveraged from the Religious Orders Study and Memory and Aging Project (ROSMAP)

published by Wingo et. al<sup>11</sup> (See URLs). Briefly, the expression weights were generated using FUSION's recommended pipeline described previously for the CMC data<sup>9</sup>. The transcriptome-wide association study using gene, splicing, and proteome expression models was performed using FUSION<sup>9</sup>, and genome-wide transcriptomic correlation for each of TWAS/SPWAS/PWAS across three PTSD symptom clusters was tested using RhoGE<sup>12</sup>. We applied Bonferroni correction ( $0.05/\#genes$  tested) for multiple testing correction. The genes identified through T/SP/PWAS approach as hereon referred to as PTSD-associated genes.

### 4.3 Biological characterization

**4.3.1 Brain cell-types expression**—We looked up the identified PTSD-associated genes, in the brain cell-type-specific lists from McKenzie et. al<sup>13</sup>. Briefly, the McKenzie et. al identified top genes for six brain-cell types by combining three mouse brain cell expression datasets from the cerebral cortex, somatosensory cortex and hippocampus, and primary visual cortex, and two human brain cell expression datasets from the temporal lobe.

**4.3.2 Frontal cortex-based gene co-expression and gene ontology enrichment**—To identify genes that are co-expressed with the identified genes in the frontal cortex, we used the GTEx v8 data stored within TCSBN (tissue and cancer specific biological network), a database of tissue-specific biological networks<sup>14</sup>. We extracted co-expression modules for frontal cortex, and created the minimum network (i.e., shortest path between query genes and co-expressed genes) to retain the most essential or closest genes using the *igraph* package in R. For all the genes in the network we performed an enrichment test for gene ontology of molecular function, biological processes, and cellular components. We applied false discovery rate (FDR  $q < 0.05$ ) for multiple testing correction.

**4.3.3 miRNA-gene network and miRNA family enrichment**—We mapped the PTSD-associated genes to miRNAs from the miRTarBase (hsa) using the Official Gene Id and performed enrichment of miRNA families with the miRNetR package<sup>15</sup>. FDR multiple testing correction (FDR  $q < 0.05$ ) was applied on enrichment p-values.

**4.3.4 Gene-drug assessment**—We used the Connectivity Map (CMap) Drug database to identify candidate drug targets for the PTSD-associated genes. The CMap database was accessed via the CLUE interface (See URLs), which compared the query gene list against the database of differentially expressed genes associated with various experimental perturbagens including knockdowns, overexpression, and chemical compounds. The median connectivity score ranges from  $-100$  to  $100$  indexes the similarity between the CMap-reference expression pattern and the list of query genes.

### 4.4 Phenome-wide Assessment

**4.4.1 PheWAS of genetically-predicted expression in electronic health records**—To understand the phenotypic consequences of dysregulated mRNA expression across our 30 genes of interest, we performed a phenome-wide association study (PheWAS)<sup>16</sup> using the Vanderbilt EHR and linked biobank, BioVU. Phenotypes in BioVU are represented as dichotomous traits called phecodes and they reflect a hierarchical clustering of the International Classification of Diseases 9<sup>th</sup> and 10<sup>th</sup> (ICD9/ICD10) codes.

For each phenotype we required a minimum number of 100 cases which resulted in the inclusion of 1379 phecodes in 70,439 individuals of European ancestry. We used the PheWAS package in R to perform logistic regression to identify the phecodes that are significantly associated with genetically imputed gene expression of brain tissues after adjusting for sex, age, and the top ten within-ancestry principal components from genetic data to control for population stratification (Denny et al. 2010, 2013). Multiple testing correction (FDR  $q < 0.05$ ) was applied per tissue.

**4.4.2 BioVU Biomarker LabWAS**—The lab-wide association scan (LabWAS)<sup>17</sup> permitted screening of clinical lab tests from the Vanderbilt University Medical Center EHR. For each of the 30 genes identified in the analyses, we tested the association between its predicted gene expression pattern in brain tissues and the available set of clinical labs. We applied the QualityLab cleaning pipeline<sup>17</sup> with settings to yield median age-adjusted (residual taken after regressing the cubic splines of age with 4 knots) inverse normal quantile transformed lab values (to control for skewness and non-normality). We included labs with measurements in at least 100 individuals, which resulted in 299 labs in 70,439 individuals of European ancestry. The lab tests are divided into 12 sub-categories; blood, metabolic, endocrine, kidney, immune, liver, urinary, OB/GYN, toxicology, cardiovascular, and cancer. Our analyses included the covariates age, sex, and top ten within-ancestry principal components from genetic data to adjust for genetic ancestry. Multiple testing correction (FDR  $q < 0.05$ ) was applied per tissue.

We replicated the genetically predicted PheWAS findings in an external cohort of the UK Biobank (UKBB) using PhenomeXcan<sup>18</sup> which uses complementary TWAS method, S-MultiXcan, and gene expression weights from Genotype-Tissue Expression (GTEx) consortia<sup>19</sup>. For the traits identified in BioVU cohort, we extracted phenotype associations for the same tissue and gene pairs identified in BioVU's PheWAS and LabWAS. We selected – 'Brain Cortex', 'Brain Cerebellum', and 'Brain Frontal Cortex BA9', and four genes - *LRRC37A2*, *ARHGAP27*, *RNF123*, and *WNT2B*. Two traits from BioVU ('Protein serum plasma' and 'Tau protein') were not available in PhenomeXcan/UKBB cohort, and hence were not tested. An absolute z-score of 3 (or  $p=0.0013$ ;  $0.05/8$  phenotypes reflecting Bonferroni correction) or higher was considered as evidence of replication.

**4.4.3 Colocalization of identified loci with traits**—We investigated all genes identified in this study for colocalization against all the phenotypes available in data resource-OpenTarget Genetics<sup>20</sup>. The colocalization was performed using the coloc-R package as described previously<sup>21</sup>, and the traits were considered to be colocalized when posterior probability (PP) of the hypothesis (H4) that two loci are shared under a single causal variant was  $H4 \geq 80\%$ . We performed enrichment of phenotype categories as listed within OpenTarget Genetics using hypergeometric test in two ways; i) an 'all-tissue: expression' and ii) filtered tissues using the keyword "brain". The hypergeometric test was performed for each phenotype category as ratio for colocalized/not-colocalized per category.

**4.4.4 Local genetic correlation**—We mapped 147 out of 172 colocalizing traits using the available StudyID from OpenTargets with UKBB phenotype ID (from Neal lab v2) to download their munged summary statistics (See URLs). To estimate local genetic correlation

( $r_g$ ) at 19 identified regions for 147 traits and the three PTSD symptom traits, we first estimated the heritability of the regions as recommended by Local Analysis of [co]Variant Association (LAVA) developers<sup>22</sup>. We applied an FDR multiple testing correction on heritability estimates, and subsequently performed local  $r_g$  for region:trait pairs that survived multiple testing threshold (FDR  $q < 0.05$ ).

## 5 RESULTS

### 5.1 Integrative analyses of dIPFC identifies PTSD symptom cluster-associated genes

We tested genetically regulated gene, splicing, and proteome expression of the dIPFC across the three PTSD symptoms clusters: reexperiencing, hyperarousal, and avoidance. We identified associations across our three regulatory models – TWAS, SPWAS, and PWAS, resulting in 11 unique genes for reexperiencing (6-gene expression; 7-splicing expression; 3-proteome expression), 19 unique genes for hyperarousal (7-gene expression; 9-splicing expression; 6-proteome expression), and 20 unique genes for avoidance (9-gene expression; 7-splicing expression; 7-proteome expression) (Figure 1B–D; Table S1). In total, we identified 30 unique gene associations at 19 independent genomic regions across the three PTSD symptom clusters. Seven genes (*KHK*, *CGREF1*, *RBM6*, *MAPT*, *CRHR1*, *RNF123*, *ARHGAP27*) were common to all the three PTSD symptoms, while one (*RBMX1*), seven (*EXOC6*, *CDC14B*, *CTNND1*, *SERGEF*, *CEP57*, *WNT2B*, *B3GALT1*) and nine (*HARS2*, *PDLIM2*, *TSFM*, *RAB27B*, *MAPRE3*, *NDUFA2*, *PCDHA7*, *TPM3*, *NCK1*) were distinct to Reexperiencing, Hyperarousal and Avoidance respectively (Figure 1E). We compared the genes identified from the GWAS of PTSD<sup>1</sup>, and the genes found through our TWAS/SPWAS/PWAS approach. Genes outside a 2MB window around the lead gene reported by the GWAS were considered novel. We found 17 genes that were novel relative to the GWAS of PTSD symptom clusters (Table S2). The transcriptomic correlation (RhoGE) for each of the TWAS/SPWAS/PWAS of nominally significant genes across three PTSD symptoms was  $>90\%$  ( $p < 2.92E-92$ ; Table S3), which suggests shared regulatory architectures underlying susceptibility for each cluster.

### 5.2 Biological characterization of identified genes

We performed a biological evaluation of the 30 genes for gene-brain cell types, frontal-cortex based gene co-expression, miRNA family enrichment and drug targets.

**5.2.1 Brain cell-type specific expression**—To identify if any of the observed genes were enriched for brain cell types, we explored the mouse and human single-cell datasets from McKenzie et. al<sup>13</sup>. We found nine genes that showed specificity for five cell-types; astrocytes (*CCBL2*), endothelia (*ARHGAP27*, *EXOC6*), microglia (*ARHGAP27*, *KHK*, *TPM3*), neuron (*MAPT*, *RAB27B*), and oligodendrocytes (*EXOC6*, *MAPT*, *PDLIM2*, *PIP4K2C*) (Supplementary Figure 1; Table S4).

**5.2.2 Prefrontal-cortex specific gene co-expression and gene ontology enrichment**—To understand the effect of co-expressed genes, we created a network of genes co-expressed in the frontal cortex with the identified genes and retained 22 co-expressed genes with 12 TWAS-derived genes. We tested all the genes in the network



for gene ontology and identified two cellular function processes; axon (p-value=4.04e-05) and microtubule (p-value=1.78e-04) that were FDR significant. Other nominally significant processes were neuron projection, cell projection part, and microtubule cytoskeleton among others (Figure 2A–B; Table S5).

**5.2.3 Gene-miRNA mapping and enrichment**—MiRNAs are short non-coding RNA sequences that regulate gene expression. Based on structure and function, miRNAs can be grouped into families. Using 28 genes of interest that were available in the database, we identified 51 miRNAs that interacted with > 2 genes. Enrichment analysis identified four miRNA families surviving FDR < 0.05, including 5 members within the miRNA-17 family, 3 members within the miRNA-148 family, 3 members within the miRNA-8 family, and 3 members within the miRNA-30 family. (Figure 2C–D; Table S6).

**5.2.4 Gene-drug prioritization**—To prioritize drug targets, we assessed perturbed drug-gene signatures similar to the transcriptional signatures of the identified genes, by performing a gene-drug target assessment using CMap. We found data pertaining to 11/30 genes and used the absolute median connectivity score of 90 or higher as prioritized interacting gene-drug targets. We found a total of 10 drugs with overall median connectivity score >90. These top drugs primarily include cyclooxygenase or COX-2 inhibitors, and acetylcholine receptor antagonists (Supplementary Figure 2).

### 5.3 Phenome-wide assessment of identified loci

**5.3.1 PheWAS and LabWAS of GReX in BioVU and UKBB cohorts**—We characterized the phenome-wide impact of the identified loci by analyzing clinical phenotypes in 70,439 individuals of European ancestry from BioVU cohort. We investigated GReX associations of 6 brain tissues for 1,379 clinical phenotypes and 299 laboratory measures. The PheWAS approach identified 5 phenotypes including bariatric surgery, obesity, hypertrophic cardiomyopathy, myoclonus, inflammatory bowel disease (Table S7; Figure 3A). The enrichment of phenotype categories shows a 9.3-fold increase (p-value=0.0045) for digestive processes (Figure 3B; Table S8). The LabWAS identified 12 blood traits – mean corpuscular hemoglobin concentration (MCHC), Eosinophils, tau protein, and protein-serum plasma volume (Table S9). The statistically overrepresented phenotype category was blood-related/hematological traits (7.13 log-fold enrichment; p-value=0.00027; Table S10).

We replicated these phenotypes in the UKBB cohort (N~100,000–300,000) using brain cortex, prefrontal cortex, and cerebellum-based GReX associations as per the gene:tissue pairs identified in BioVU (Table S11). Two traits from BioVU ('Protein serum plasma' and 'Tau protein') were not available in UKBB cohort. For hypertrophic cardiomyopathy identified in BioVU cohort, we found Illness of father, mother, and siblings- heart disease (*LRRC37A2*; zscore=3.1~4.1) in the UKBB cohort. For hematological traits - Erythrocyte distribution width, mean corpuscular hemoglobin concentration, platelet mean volume, identified in BioVU cohort, we found 'Red blood cell erythrocyte count' (*ARHGAP27*; z-score= 4.7~17.2), 'Raw-mean corpuscular hemoglobin' (*ARHGAP27*, *LRRC37A2*; z-score= -8.5 ~ -6), 'Mean platelet thrombocyte volume' / 'Platelet count' (*WNT2B*; z-score=

–8.7~11.15) in UKBB cohort respectively. Metabolic traits – obesity, and bariatric surgery in BioVU cohort were replicated with phenotypes – Trunk fat percentage, body fat percentage, and trunk fat mass (*ARHGAP27*, *LRRC37A2*; z-score= –5.3 ~ –3.6) in UKBB cohort. Together, these replications highlight that GReX of the identified genes with PTSD is also involved in peripheral/non-psychiatric traits.

**5.3.2 Colocalization of gene-QTL with human phenotype**—We employed the colocalization approach to assesses the probability that the association signal responsible for the predicted differences in expression and that the association signal for the trait are being caused by the same variant across a larger set of nearby cis-variants<sup>23</sup>. Colocalization is a complementary approach to GReX- based methods that aggregates several cis-variants in the region. We identified 2,457 phenotype:gene:tissue pairs with H4 probability  $\geq 80\%$  across 20 genes and 74 tissues (Figure 4A). To prioritize phenotypes, we performed an enrichment test using the OpenTarget-assigned categories and found 12 over-represented categories that survived multiple testing correction (Figure 4B). We then selected tissues that contained the term ‘Brain’ in their description, we found 489 phenotypes:tissue:gene pairs across four genes and 12 brain tissues. Here, we found four enriched categories that were the same categories as seen in all tissue analysis (Figure 4C). Overall, we identified 172 unique phenotypes and we were able to map 147 phenotypes available in UKBB to test their pleiotropic relationship with PTSD symptom clusters using local  $r_g$  (Table S12).

**5.3.3 Local genetic correlation of PTSD symptom clusters and colocalizing phenotypes**—We sought to confirm the locus-specific relationship between expression-colocalizing traits from UKBB, and PTSD symptom clusters from the MVP, we performed a local  $r_g$  analysis. We tested three PTSD symptom clusters across all 19 regions for heritability, and regions with significant heritability were tested further for local  $r_g$  within and between three PTSD symptom clusters and 147 colocalizing traits, for a total of 450 PTSD:trait pairs being investigated. We found that the highest number of correlated traits were observed in 3p21.31 (231/450), followed by 17q21.32 (107/450), and 1p13.2 (103/450) locus (Figure 5; Table S13). Using the same phenotype categories as colocalization analyses, we performed an enrichment of phenotype categories for each of the three loci (Table S14). Across the 16 phenotype categories, the 3p21.31 locus which houses three TWAS genes (*RBM6*, *RNF123*, *MON1A*) was significantly enriched for anthropometric traits (3.39 log fold enrichment; p-value:  $1.95 \times 10^{-5}$ ), and uncategorized category (2.30 log fold enrichment; p-value:  $5.29 \times 10^{-3}$ ), which contains miscellaneous phenotypes such as lifestyle factors, medication use for vascular health, and body composition traits such as impendence of leg and arm. At the 3p21.31 locus, there is a positive  $r_g$  between PTSD symptom clusters and several metabolic and blood traits (i.e., high blood pressure, cholesterol-lowering medication, BMI, illness of father, diabetes; white blood cell count; neutrophil count). The 17q21.32 locus (*ARHGAP2*, *CRHR1*, *MAPT*, *LRRC37A2*) was enriched for hematological measurement (7.25 log fold enrichment; p-value:  $3.20 \times 10^{-8}$ ), PTSD symptom clusters (8.13 log fold enrichment; p-value:  $4.95 \times 10^{-3}$ ), and biological processes (11.4 log fold enrichment; p-value:  $1.25 \times 10^{-2}$ ). The 1p13.2 locus (*WNT2B*) was enriched for anthropometric traits (5.76 log fold enrichment; p-value:  $1.55 \times 10^{-11}$ ), and bone measurements (4.77 log fold enrichment; p-value:  $7.53 \times 10^{-5}$ ).

## 6 DISCUSSION

PTSD is characterized by symptoms related to hyperarousal (hypervigilance, irritability, sleep difficulties, heightened startle response), avoidance of situations and people that serve as reminders of the trauma, and reexperiencing the traumatic event, as for example in the form of nightmares, flashbacks and/or psychosomatic episodes<sup>5</sup>. To disentangle the molecular basis of PTSD symptom clusters, we integrated the multi-tiered genetically regulated expression of gene, splicing, and protein levels, identifying 30 genes in 19 genomic regions associated with the three main PTSD symptom clusters. We identified 17 novel genes that were not identified in the previous GWAS of PTSD symptom clusters<sup>1</sup>. Four of these genes (e.g., *CCBL2*, *FURIN*, *KHK*, and *PDLIM2*) have been previously identified in gene expression studies of PTSD<sup>24–27</sup>, highlighting the importance of genetically-regulated expression in PTSD pathogenesis. *CCBL2* (also known as *KYAT3*) showed evidence of genetically predicted transcript expression, splicing, and protein expression and has previously shown specificity to astrocytes. This gene encodes a transaminase in the L-tryptophan catabolic pathway, converting kynurenine to kynurenic acid, which has antagonistic effects on glutamate receptors; dysregulation of this metabolic pathway has been implicated in a wide variety of neuropsychiatric disorders. *KHK* and *PDLIM2* genes were specific for microglia and oligodendrocyte, respectively. Variants in *FURIN* were reported to exhibit gene-environment interaction effect with lifetime trauma burden<sup>28</sup>. We identified 14 miRNAs in four miRNA families that target multiple PTSD associated genes; among these, miRNA-20a (miR-17 family), miRNA-200c (miR-8 family), and miRNA-30c (miR-30 family) were significantly dysregulated in PTSD among military combat veterans<sup>29,30</sup>. These miRNAs along with miRNA-148a (miRNA-148 family) were also identified in rodent models of PTSD<sup>31,32</sup>. The present observation of genetically regulated expression of the genes regulated by these miRNAs highlights the possibility that genetic variants could affect target binding for miRNAs which also serve as compensatory mechanisms.

To understand the impact of GReX across the clinical and laboratory phenome, we performed PheWAS and LabWAS in BioVU. The brain-tissue based GReX of the TWAS genes highlighted obesity (and related bariatric surgery), cardiomyopathy, inflammation (e.g. inflammatory bowel disease and regional enteritis), neurological (myoclonus-twitching of muscles), and several hematological traits (erythrocytes, eosinophils, mean corpuscle hemoglobin) and these findings were replicated in the larger UKBB cohort. We observed an overrepresentation of similar phenotypes using colocalization as a complementary approach (i.e., anthropometric, metabolic traits including diabetes, and cardiovascular traits including, vascular health, blood pressure medication, and hematological traits). Our local genetic correlation findings provided further support for pleiotropic associations between PTSD, metabolic and hematological traits localize in 3p21.31 and 17q21.32, respectively. Our findings also highlight some loci and genes that may exert pleiotropic effects across multiple comorbid domains.

Several epidemiologic studies identify associations between PTSD and metabolic dysfunction like diabetes, hypertension, and abnormal lipid profiles<sup>33,34</sup>, which are risk factors to cardiovascular dysfunction and failure<sup>35,36</sup>. Our hypothesis-free phenome-wide

approach provides convergent evidence and highlights loci and genes that appear to mediate risk for both PTSD symptoms and multiple associated metabolic disorder. The genes, *RBM6*, *RNF123*, *MONIA* (3p21.31), and *WNTB2* (1p13.2) have shared effects in PTSD and metabolic-traits, which have been demonstrated to have highly pleiotropic effects traits<sup>37</sup>. *RBM6* and *RNF123* were reported in GWAS and expression studies of PTSD, and cardiovascular diseases<sup>38</sup>, which may be due to association with cardiovascular-risk factors such as diabetes, and lipid metabolism<sup>39,40</sup>. *WNTB2* is associated with stress-induced neuroinflammation in PTSD<sup>41</sup> and depression<sup>42</sup>. *ARHGAP2*, *CRHR1*, *MAPT*, and *LRRC37A2* (17q21.32) are in a high LD region. These genes are known to be associated with PTSD<sup>1</sup>, but they also have functional links with metabolic traits hypothesized to guide diet-based signals via dysregulated expression in brain tissues<sup>43</sup>. Additionally, *LRRC37A2* was found to be dysregulated in CD133+ cells which are involved in vascular injury response<sup>44</sup>. While directly measured gene expression is highly influenced by environmental and technical factors, genetically regulated expression is anchored in the inherited genetic variation.

Inflammation is a well-documented common denominator between PTSD and metabolic dysfunction<sup>45</sup>. Hematological measurements such as white blood cell and platelet count are considered inflammatory markers and are often altered in PTSD patients<sup>46,47</sup>. Several studies have reported short and long-term changes in inflammatory levels as a cause and consequence of PTSD<sup>48</sup>. The drug-gene target approach further highlighted several cyclooxygenase inhibitors, including some used to treat IBD and other inflammatory disorders. Recent study using EHR data further identified role of non-psychotropic medications such as anti-inflammatory drugs with improved PTSD symptoms<sup>49</sup>. In addition, a study showed success with anticholinergics in treating reexperiencing-related traits such as PTSD-related nightmares<sup>50</sup> and an animal model showed evidence for reductions in behavioral and stress hormone responses to fear conditioning<sup>51</sup>. Metabolic syndrome also contributes to proinflammatory response<sup>52</sup>, and on the other hand, PTSD has been associated with later development of obesity<sup>53</sup>. The underlying mechanisms indicate putative role of hypothalamic/pituitary hormone signaling, which might result in uncontrolled appetite and increased cortisol contributing to central obesity<sup>54</sup>.

The drug repurposing analysis identified several drugs implicated in the treatment of stress-related response. Pterostilbene is a cyclooxygenase and FAAH inhibitor that prevents catabolism of anandamide, and endocannabinoid<sup>55-57</sup>. WH-4023 is a SRC inhibitor with an ability to reduce NMDA receptor function which exhibit ketamine-like function, and shown to reduce craving-related activation of reward circuits<sup>58,59</sup>. We also observe Carbetocin, an analogue of oxytocin which has shown involvement in reward-related functional connectivity in PTSD-affected individuals<sup>60-62</sup>. Taken together, the findings highlight complex associations between PTSD, metabolic conditions, and inflammation that have been frequently identified within the epidemiologic literature.

While our study has taken multiple investigative steps to identify biological properties, and peripheral traits associated with genes involved with PTSD symptom clusters, our study has limitations. The MVP is a military cohort, and prevalence of combat exposure that may not generalize outside this cohort. To overcome this limitation, we replicated our associations in

other cohorts such as UKBB and BioVU. Additionally, the MVP is primarily consisted of male participants, to circumvent this, we checked the identified genes against the sex-biased eQTLs from the GTEx<sup>63</sup>, and didn't find any overlap. The study was performed using summary statistics from the European ancestry, and may not translate to other ancestries, future studies with diverse ancestries and improved sample sizes are needed for better understanding. Additionally, it is possible for some of the observed effects to be mediated by unmeasured mediating variables or confounding variable effects (e.g., health-related behaviors) that are causal for our target phenotypes and strongly associated with our loci of interest. Future work may involve causal inference methods to try and elucidate specific pleiotropic model. Some of the genes identified are in high LD region and will require functional experiments to dissect gene-specific effects for possible drug development and repurposing.

In summary, our study reports comprehensive evidence including clinical and regulatory data that highlight the molecular basis of the epidemiologic associations among PTSD, inflammation, and metabolic dysfunction.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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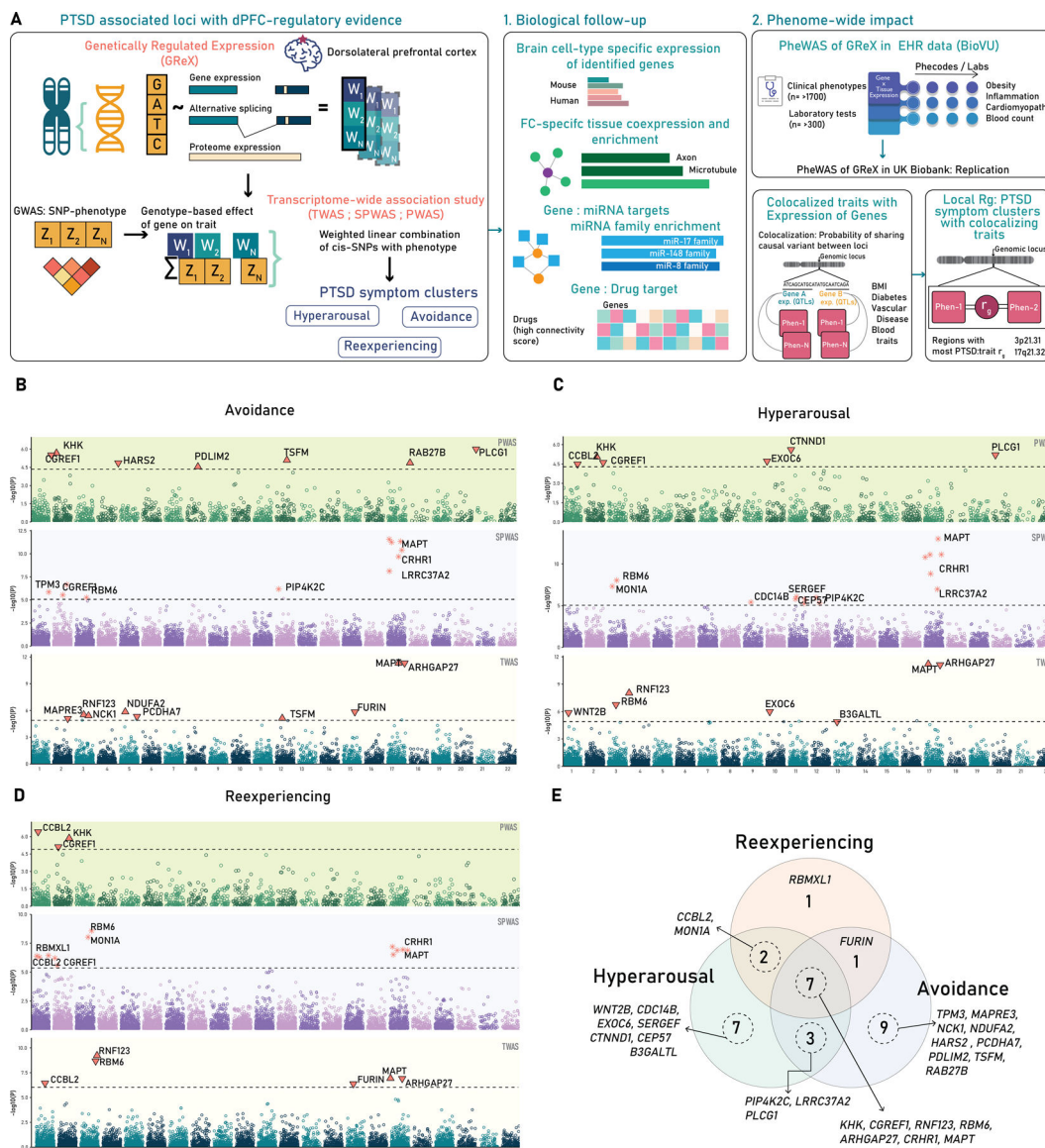
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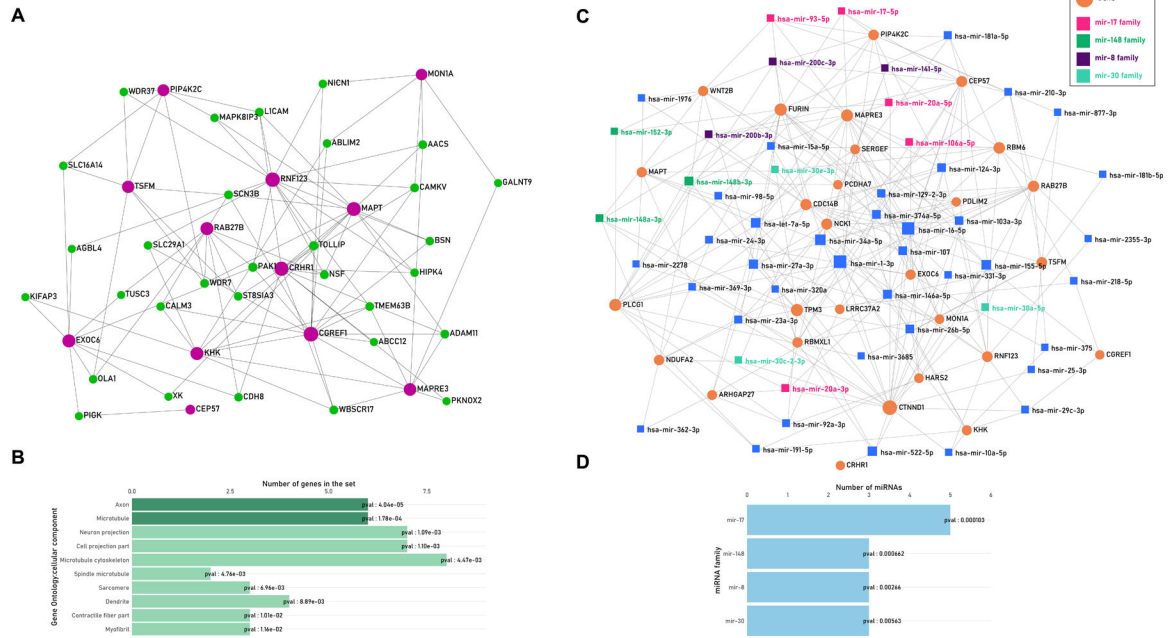


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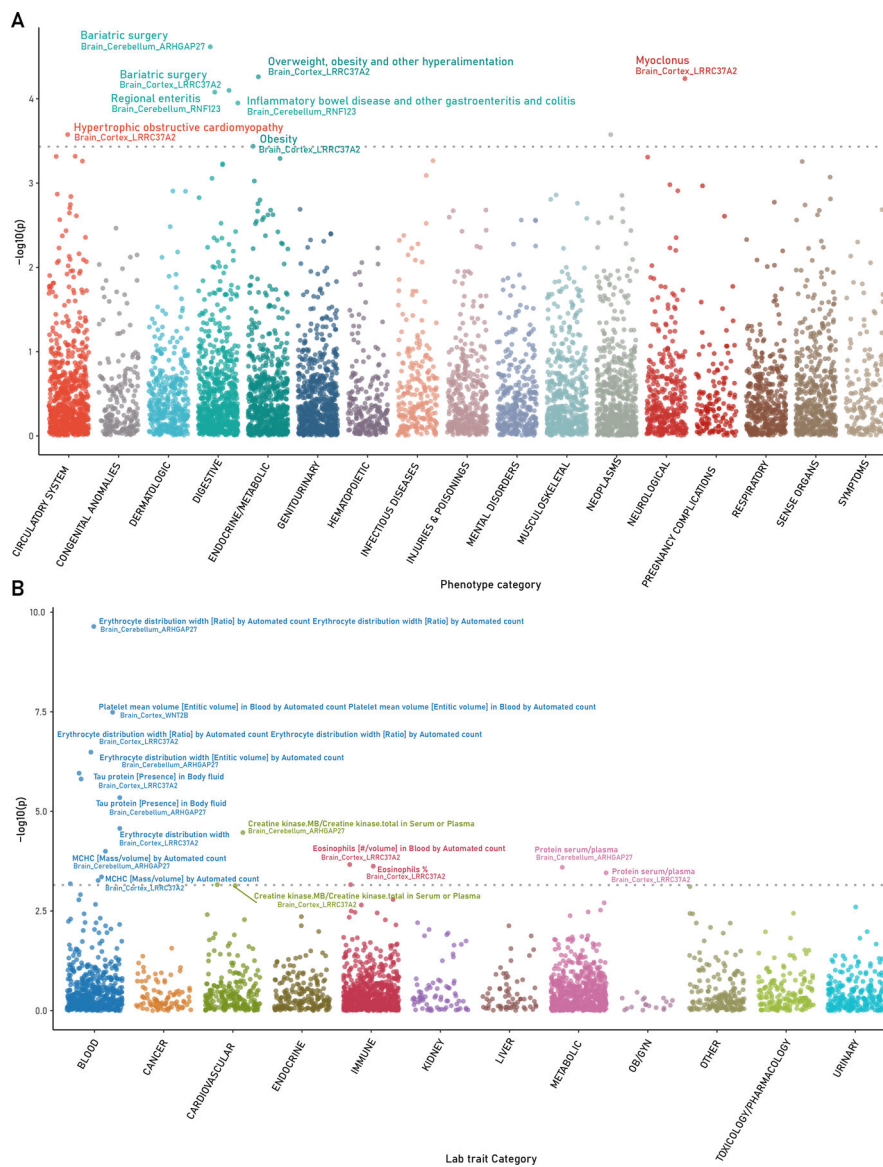


**Figure 1:**

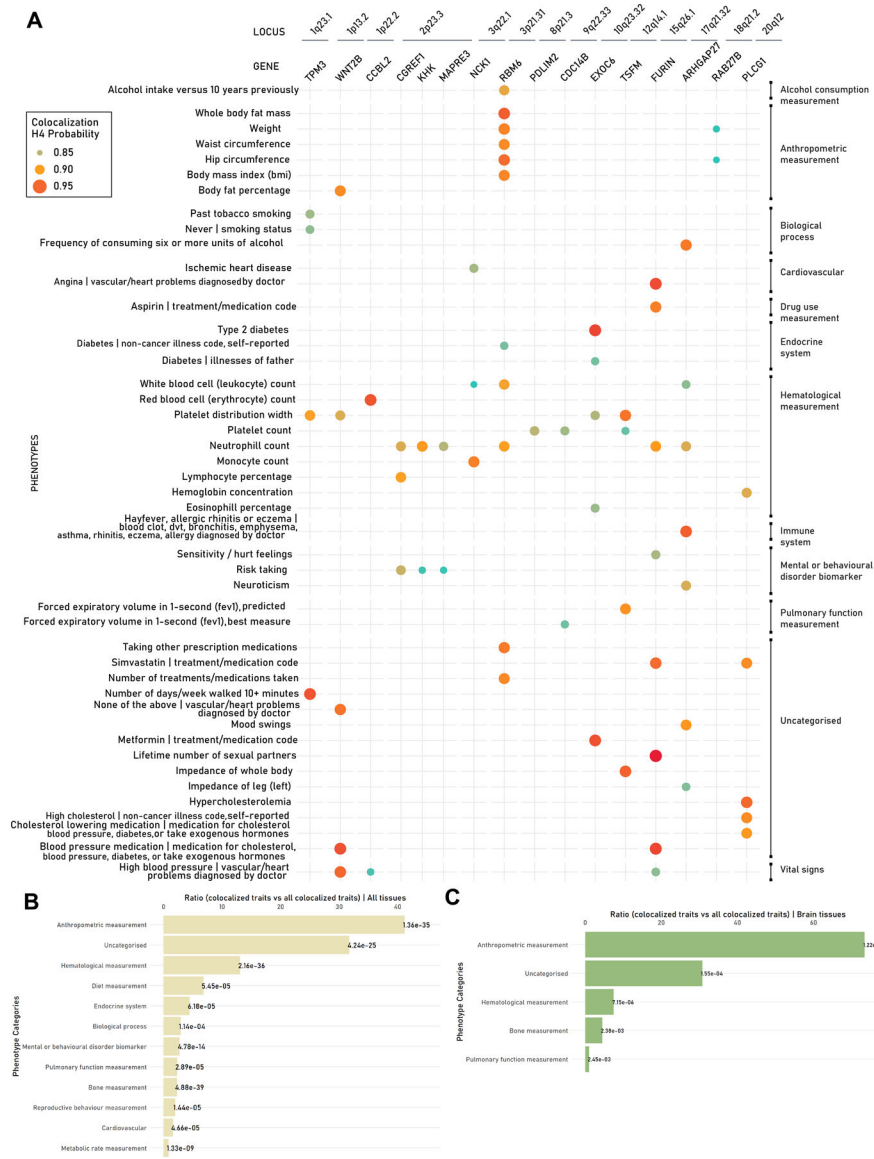
A. Study Design. A schematic of the study design showing the concept of genetically regulated expression (GRex) and applied to three PTSD symptom cluster. B-D. Three-tiered Manhattan plots of three PTSD symptoms B-Avoidance, C-Hyperarousal, D-Reexperiencing, the lowest layer is TWAS, middle is SPWAS, and top is PWAS (See text for more details). E. The Venn diagram of overlapping genes across the three PTSD symptom clusters.



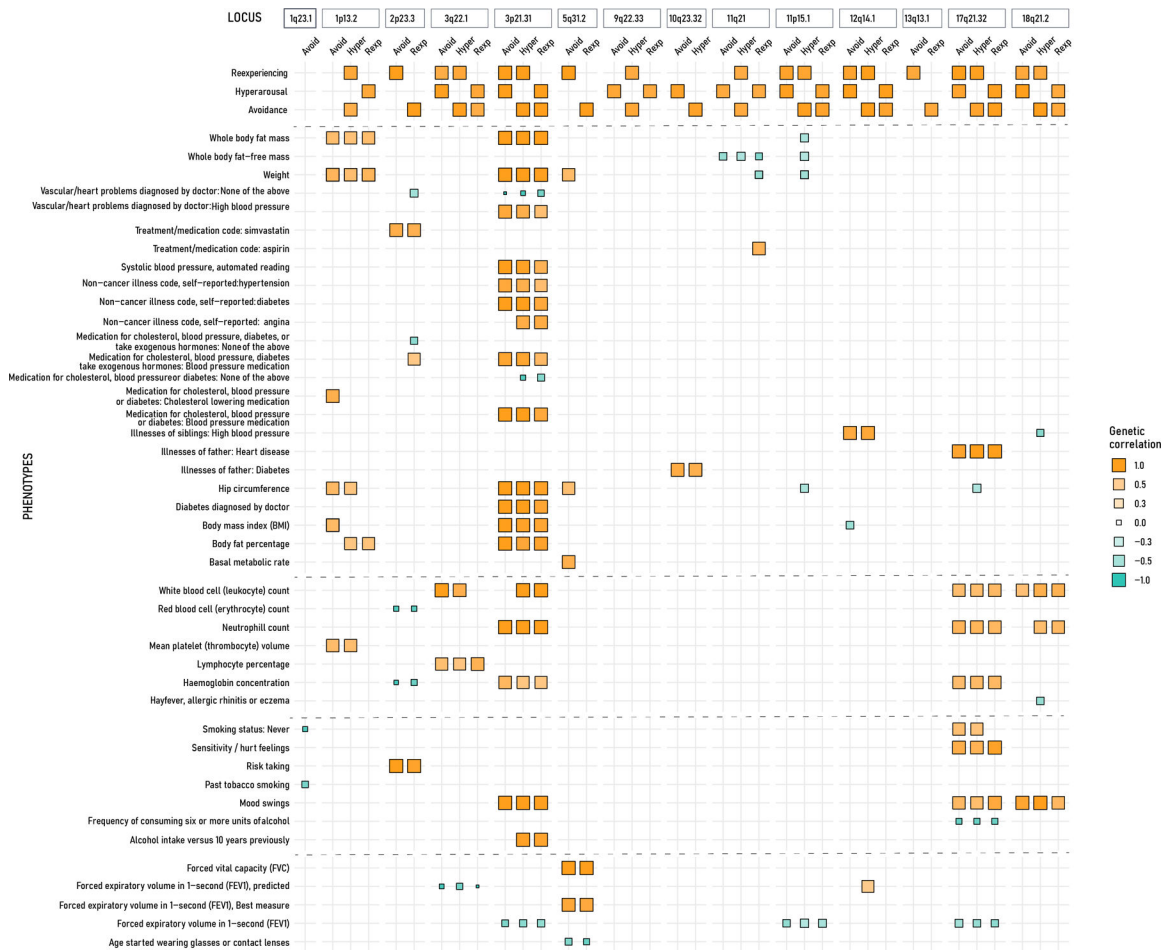
**Figure 2:**  
 A. Tissue Co-expression Network. The network of TWAS genes (purple) with prefrontal-cortex based co-expressed genes (green) B. Bar plot showing top ten gene ontology enrichment processes of all genes in the network wherein dark green processes survived FDR testing. C. MiRNA-Genes network. The network of miRNAs (orange) which map to TWAS genes (orange circles); the miRNAs belonging to overrepresented miRNA-families are color coded in the legend. D. The details of the miRNA-family enrichment are shown as bar plot, all the four miRNA families survived FDR  $q < 0.05$ .



**Figure 3:** PheWAS and LabWAS of TWAS identified genes in BioVU cohort. A. PheWAS of 1379 clinical phenotypes and B. LabWAS of 299 laboratory measures using genetically regulated expression of identified TWAS genes and brain tissues.



**Figure 4:**  
 A. Colocalization plot showing probability of having shared causal variant between gene expression (top-x axis), and phenotype (left axis), grouped by categories (right axis). B. Enrichment of Phenotype categories that survive multiple testing, comparing colocalized vs non-colocalized gene:phenotype pairs for all tissues. C. Enrichment of Phenotype categories comparing colocalized vs non-colocalized gene:phenotype pairs that survive multiple testing for brain tissues only.



**Figure 5:** Matrix plot of local genetic correlation between three PTSD symptom clusters and colocalizing traits (few presented here on the left y-axis). The x-axis shows PTSD symptoms grouped for each locus as identified in the TWAS analysis. The positive correlation is denoted in orange, and blue is negative correlation, the size of the squares is relative to genetic correlation value.