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ARTICLE

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Transcriptome-wide association study of post-trauma symptom trajectories identified *GRIN3B* as a potential biomarker for PTSD development

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Biomarkers that predict symptom trajectories after trauma can facilitate early detection or intervention for posttraumatic stress disorder (PTSD) and may also advance our understanding of its biology. Here, we aimed to identify trajectory-based biomarkers using blood transcriptomes collected in the immediate aftermath of trauma exposure. Participants were recruited from an Emergency Department in the immediate aftermath of trauma exposure and assessed for PTSD symptoms at baseline, 1, 3, 6, and 12 months. Three empirical symptom trajectories (chronic-PTSD, remitting, and resilient) were identified in 377 individuals based on longitudinal symptoms across four data points (1, 3, 6, and 12 months), using latent growth mixture modeling. Blood transcriptomes were examined for association with longitudinal symptom trajectories, followed by expression quantitative trait locus analysis. *GRIN3B* and *AMOTL1* blood mRNA levels were associated with chronic vs. resilient post-trauma symptom trajectories at a transcriptome-wide significant level ($N = 153$, FDR-corrected p value = 0.0063 and 0.0253, respectively). We identified four genetic variants that regulate mRNA blood expression levels of *GRIN3B*. Among these, *GRIN3B* rs10401454 was associated with PTSD in an independent dataset ($N = 3521$, $p = 0.04$). Examination of the BrainCloud and GTEx databases revealed that rs10401454 was associated with brain mRNA expression levels of *GRIN3B*. While further replication and validation studies are needed, our data suggest that *GRIN3B*, a glutamate ionotropic receptor NMDA type subunit-3B, may be involved in the manifestation of PTSD. In addition, the blood mRNA level of *GRIN3B* may be a promising early biomarker for the PTSD manifestation and development.

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INTRODUCTION

Posttraumatic stress disorder (PTSD) is a severe and impairing psychiatric illness occurring in susceptible individuals exposed to traumatic life experiences. PTSD is characterized by fear, avoidance, hyperarousal, hypervigilance, and reliving the trauma through intrusive memories, nightmares, and flashbacks. Early interventions have been shown to significantly reduce the prevalence of PTSD [1–3]. However, these interventions could require significant system resources, which are not always available, and might be unnecessary for resilient individuals whose symptoms may fade over time on their own. Hence, early risk biomarkers that can predict the manifestation of PTSD may facilitate both early interventions and targeted treatments, increasing positive outcomes for patients known to be at risk.

Many individuals exhibit early symptoms after experiencing a traumatic life experience, with varying degrees of severity and persistence. Only a minority of those remain highly symptomatic and manifest the full symptoms of PTSD [4]. A combination of genetic and environmental factors influence susceptibility to develop PTSD after trauma exposure. Latent class analysis has successfully identified the dissociative subtype of PTSD [5], uncovered qualitative differences between the classes, and categorized homogeneous classes of individuals based on broad characteristics not directly measurable. Moreover, longitudinal studies of psychological response to trauma have identified a

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range of outcomes over time that are not adequately captured by PTSD diagnostic criteria [6]. Thus, clusters of these symptoms over time, which can be grouped into empirical trajectories, can better reflect different outcomes (e.g., chronic-PTSD vs. resilient individuals). These symptom trajectories represent different biological dimensions and have a distinct genetic basis, facilitating the identification of biomarkers and risk genes [7]. Thus, investigating symptom trajectories leading to chronic-PTSD or resilience may play a central role in understanding this disorder. The genetic biomarkers associated with these trajectories may predict who among trauma-exposed individuals may develop PTSD [8, 9].

In human populations, blood transcriptomic profiling, which requires smaller sample sizes than traditional genome-wide association studies (GWAS), has been successfully used as a discovery tool for genes associated with PTSD [10–12]. So far, only a few blood transcriptome-wide association studies (TWAS) associated with PTSD core symptom trajectories have been reported [13]. This study design may facilitate identifying genes involved in the course of PTSD and biomarkers for early interventions.

Of note, the primary tissue of interest for the pathophysiology of PTSD is the brain, with traumatic experiences activating specific regions, including the amygdala [14, 15], hippocampus, and the prefrontal cortex [16–19]. The degree of correlation between blood and brain gene expression is difficult to establish [20]. Still, the increasing number of accessible sources, such as brain biobanks and transcriptome databases (e.g., GTEx and Brain-Cloud), has facilitated the understanding of these body–brain correlations.

The current prospective Emergency Department (ED) study recruited participants in the aftermath of trauma exposure and followed them longitudinally over 1 year. In this study, we aimed to investigate early biomarkers that could predict the course of trauma-related symptoms, using blood transcriptomes. Symptom trajectories were extrapolated using posttraumatic symptoms assessed at 1, 3, 6, and 12 months after trauma exposure [21–23]; such patterns provide more information than dichotomous PTSD case–control or individual PTSD symptom scale (PSS) analyses in the identification of genetic factors [24, 25]. Based on this approach, three distinct trajectories (chronic-PTSD, remitting, and resilient) were identified, as previously described in the papers above [21–23].

In this study, we aimed to investigate possible genes that could predict the course of trauma-related symptoms by performing a transcriptomic analysis of these empirical trajectories. Furthermore, the genes identified using this method were further investigated using several tools, including analyses of methylation loci and genetic variants. We used public databases to evaluate the direct association between genetic variants and brain gene expression. We then explored the association between these DNA variants and PTSD, using a larger independent cohort of similar demographics (the Grady Trauma Project).

MATERIALS AND METHODS

Participants

Participants were patients in the ED of Grady Memorial Hospital in Atlanta (GA) who had experienced a traumatic event within the past 24 h. Participants were included if they spoke English, were 18–65 years of age, endorsed a criterion A trauma as defined by the DSM-IV-TR [26], and provided contact information for follow-up visits. Exclusion criteria included previous hospitalization for mental health reasons, current suicidal ideation, attempted suicide in the past 3 months, current intoxication, or altered mental status during the ED visit. Informed consent was obtained from all research subjects, and all procedures were approved by the Institutional Review Board of Emory University School of Medicine and the Grady Health Systems Research Oversight Committee.

Emergency Department assessments at the time of trauma exposure

Demographic information and information about the index trauma were gathered in the ED using the Standardized Trauma Interview [27]. PTSD symptoms were assessed at baseline, 1, 3, 6, and 12 months following the ED visit. PTSD symptom severity (PSS) was measured using the 17-item self-report scale [21–23, 28, 29].

Drug and alcohol use were assessed using a ten-item version of the Drug Abuse Screening Test (DAST) [30, 31] and the Alcohol Use Disorders Identification Test (AUDIT) [30, 32], respectively. Depressive symptoms were assessed using the Beck Depression Inventory BDI [33].

Symptom trajectory

Latent growth mixture modeling (LGMM) was employed to identify PTSD symptom trajectories based on PSS total scores across 1, 3, 6, and 12 months using MPlus 7.2 [34], as previously described [21–23]. Subjects were excluded if they were missing two or more assessments, resulting in a sample size of 377. To identify the best fitting number of trajectory classes that best describe the PTSD symptom severity, we applied the LGMM, starting incrementally from one to six classes. We examined both a linear and quadratic slope to identify the best fitting trajectory shape. We used a nested model approach, testing a progressive number of classes until the model fit indices no longer favor the addition of any more classes. To determine the number of classes, we used a confluence of evidence across conventional indices, including reductions in the Bayesian Information Criterion (BIC), sample-size adjusted Bayesian Information Criterion (SSBIC), Aikaike Information Criterion (AIC) indices, and significance indicated by the Lo-Mendell-Rubin likelihood ratio test (LRT), the Vuong-Lo-Mendell-Rubin likelihood ratio test (VLRT), and the Bootstrap likelihood ratio test, along with parsimony and interpretability equally weighed consistent with recommendations from the literature[35]. We found that the best fitting LGMM model was a three-class solution (resilient, remitting, and chronic-PTSD) with a linear slope (AIC = 8,111.160, BIC = 8,158.347, SSBIC = 8,120.274, VLRT = 0.0036, LRT = 0.0046, and entropy of 83%) [23, 36].

RNA collection and RNA sequencing

Venous blood was collected in Tempus Tubes (RNA, Applied Biosystems) in the ED in the immediate aftermath of trauma exposure. After RNA extraction, mRNA libraries were created using the TrueSeq preparation kit and sequenced on the Illumina HiSeq 2000 by the Translational Genomics Research Institute (TGEN). Reads were aligned to GRCh37 using STAR v2.5.2b [37]. Gene counts were computed with FeatureCounts v1.5.1 [38], and then the counts were processed with DESeq2 [39]. The Limma-Voom R-package (version 3.6.2) [40, 41] was used for normalization, converting raw counts to log-CPM (count per million), adjusting for library sizes, and reducing heteroscedasticity. We kept genes with CPM values of 1 in at least a third of the samples (13,951 genes for the primary analysis).

Construction of weighted co-expression modules from RNA-sequencing data (WGCA)

Module eigengenes were used for summarizing clusters of highly correlated genes using weighted correlation network analyses (WGCNA) [42]. Briefly, raw RNA-sequencing data were first filtered keeping only genes with >50 reads across the samples (26,193 genes); then, the data were normalized and transformed using DESeq2 R-package [39]. Two outliers were removed by using *hclust* function. Clustering modules were defined with *Blockwisemodules* using the following parameters: soft-thresholding power 5 (chosen using pickSoftThreshold), correlation type (corType "bicor"), the network type (networkType "signed"), the type of Topological Overlap Matrix (TOMType "signed"), a relatively large minimum module size (minModuleSize 30), *p* value ratio threshold for reassigning proteins between modules (reassignThreshold 0.05), a low propensity to merge modules (mergeCutHeight 0.07), and high sensitivity to cluster splitting (deepSplit = 4). Among 26,193 genes 15,382 were assigned to 38 modules.

Cell type proportion determination

White blood cell composition was assessed to investigate whether cell type distribution was a potential confound for gene expression measures. The frequencies of PBMC cell types were estimated based on genome-wide gene expression using CIBERSORT cell type deconvolution method at the default setting [43]. Normalized expression data and the LM22 signature matrix were used as input, providing the frequencies of

22 immune cell types. Monocytes, T cell, and neutrophils were used as covariates in the analyses.

DNA genotyping

Extracted DNA from whole blood was genotyped by TGEN using the Illumina Infinium PsychArray BeadChip (v.1.0, v.1.1., and v.1.2) in three different batches. Standard quality control (QC) of the genome-wide genotyping was performed per batch with PLINK1.9 [44]: individuals with >5% missing data, and one in each pair of related individuals (cousins or a closer relation) based on identity by descent proportion were removed. Single-nucleotide polymorphisms (SNPs) with call rates <95%, minor allele frequency < 0.05, and deviation from Hardy-Weinberg proportions ($p < 1 \times 10^{-6}$ in controls and $p < 1 \times 10^{-10}$ in PTSD) were also removed. The three batches were merged, and further QC was performed. To determine population ancestry, we calculate genomic principal components (PCs) from autosomal independent genomic markers (pairwise $r^2 < 0.25$) to infer axes of ancestry and to remove outliers. One *GRIN3B* variant (rs10666583), associated with schizophrenia in previous studies [45, 46], was imputed with BC-Gene (https://bcgene.emory.edu/), using IMPUTE2 (version 2.3.0) and 1000 Genomes Phase I (December 2013, NCBI build 37) for haplotype reference.

DNA methylation

DNA was extracted from ED-collected blood by AKESOgen (http://www.akesogen.com). Genomic DNA was bisulfite converted using EZ-96 DNA Methylation Kit (Zymo Research), and DNA methylation (DNAm) levels were interrogated either with HumanMethylation450 BeadChip (>480,000 CpG sites, $N = 120$) or MethylationEPIC BeadChip (>850,000 CpG sites, $N = 36$). Hybridization and processing were performed according to manufacturer's instructions, followed by beta generation (Bead Studio) and standard QC (detection call rates <95% and an average intensity value of either <50% of the experiment-wide sample mean or <2000 arbitrary units; CpG sites with missing data for >10% of samples were excluded from analysis). Normalization was conducted using Beta Mixture Quantile Normalization [47], and ComBat was used to account for batch and positional effects [48].

Validation sample

The Grady Trauma Project is a comprehensive study of a predominantly African ancestry (AA) population of low socioeconomic status, exposed to stressful life events [49]. Similar to the ED, current PTSD symptoms were assessed using the PSS, and subjects were considered chronic-PTSD if they had a PSS score of 19 or above (third quartile cutoff) and resilient if they had a PSS of 7 or below.

DNA extraction and genome-wide genotyping using the Omni-Quad 1 M BeadChip has been described elsewhere [50]. QC was performed by using the Psychiatric Genomics Consortium PTSD Workgroup guidelines [51].

Statistical analyses

A transcriptome-wide association study (TWAS) of chronic-PTSD vs. resilient class was performed by fitting a linear model from each gene given a series of arrays using a standard function (lmfit) from the Limma R-package [40], followed by empirical Bayes moderation [52], to rank genes in order of evidence for differential expression. We included in the matrix the two-class trajectories, sex, age, baseline PTSD symptoms, cell type proportions, three ancestry PCs, DNA batch, and RNA-sequencing batch; we also added education, DAST or AUDIT score in the secondary analyses. Genes were considered associated with trajectory if they reached an FDR < 0.05. Association between GRIN3b blood mRNA level and PSS at baseline and at each subsequent follow-up assessment was examined using linear regression, adjusting for potential confounding factors.

To establish if *GRIN3B* expression levels were associated with the course of the disease, we repeated the analysis for the three-class trajectories (chronic-PTSD vs. remitting vs. resilient).

We performed a genetic association between PTSD and GRIN3B genetic variants using logistic regression in PLINK1.9 [44], assuming an additive model of association. We applied a Bonferroni correction to define statistical significance (0.05/4 variants = 0.01). To examine association between co-expression modules and the chronic vs. resilient trajectory, we performed linear regression using each eigengene as the outcome, the class trajectory as predictor, adjusting for the same covariates as mentioned above.

Methylation quantitative trait loci

Twenty *GRIN3B*-CpGs were selected as being present in both EPIC and 450 K. *GRIN3B* expression values were regressed for beta values, covarying for age, sex, three ancestry PCs, cell types, and array type. A Bonferroni corrected p value of 0.0025 (0.05/20) was used to define significance.

Expression quantitative trait loci (eQTL)

eQTL analyses were performed in R (version 3.5.2) using the package MatrixEQTL [53]. Expression levels of *GRIN3B* were regressed with the 23 variants in the gene, spanning from 974 to 1040 Kb, (including promotors, 5′ and 3′ region), assuming an additive model, and adjusting for sex, age, and the top three ancestry PCs. Variants with FDR < 0.05 were deemed eQTLs.

Brain expression eQTLs

To evaluate if the top *GRIN3B* blood eQTL variant could affect mRNA brain expression, we interrogated two publicly available databases: GTEx (https://gtexportal.org) [54] and BrainCloud (http://eqtl.brainseq.org/phase1/eqtl/) [55]. The BrainCloud used the expression values from dorsolateral prefrontal cortices (DLPFC) to identify eQTLs in a total of 412 subjects ($N = 175$ patients with schizophrenia, and $N = 237$ unaffected controls) or in DLPF-controls ($N = 237$).

RESULTS

Emergency Department study participants

In the ED cohort, a total of 377 subjects with reported post-trauma symptoms were used for inferring symptom trajectory. Participants were mostly from AA (75.5%), while more than half had some college or higher education (59.3%); the most common trauma was road accidents (70.3%), which include motor vehicle crashes and bikes or pedestrian accidents; the majority of the participants showed low to moderate baseline PTSD symptoms (PSS-median score of 6; Table S1).

Three distinct symptom trajectories emerged in the 12 months post-trauma

In the ED cohort, longitudinal trajectories of symptoms after exposure to traumatic events were identified based on the PSS score across 1, 3, 6, and 12 months [21–23, 36]. LGMM yielded three trajectories: resilient (56.2%), remitting (32.9%), and chronic-PTSD (10.9%; Fig. S1). Individuals were assigned to one of these trajectories based on their most likely class membership. The trajectory algorithm was externally validated and demonstrated ability to discriminate PTSD risk with high precision [23].

Baseline PSS was excluded from the growth curve trajectory. Overall, individuals in the chronic-PTSD class tended to have lower educational attainment, a higher percentage of sexual-assault history, and a higher representation of females (61%, Table 1) compared to the other two categories. PSS symptoms of the three-class trajectories partially overlap before the 6-month marker (Fig. S1). When considering only chronic-PTSD vs. resilient, a clear separation of symptoms was already evident after one month. Hence, we decided to perform the TWAS analyses using the two extreme phenotypes (chronic-PTSD vs. resilient).

Transcriptome-wide association study of symptom trajectory suggests that low blood *GRIN3B* mRNA expression after trauma exposure predicted resilience

To investigate blood mRNAs that could predict resilient vs. chronic symptom trajectories, we performed a transcriptome-wide differential analysis of the resilient vs. chronic-PTSD group, adjusting for potential confounding variables. Our analysis identified two genes, *GRIN3B* ($p = 4.50 \times 10^{-7}$, FDR $= 6.3 \times 10^{-3}$) and *AMOTL1* ($p = 3.63 \times 10^{-6}$, FDR = 0.0253) whose low expression after trauma predicted the resilient trajectory (Fig. 1, and Tables S2 and S3). To note, *GRIN3B* also survived a stricter Bonferroni correction adjustment (Fig. 1A). *GRIN3B*, but no *AMOLT1*, remained significant after adjusting for drug use ($N = 130$; DAST score FDR $= 6.4 \times 10^{-4}$), alcohol ($N = 126$; AUDIT

Table 1. Sociodemographic characteristics of the subset ED cohort and their prospective trajectory classes adopted for transcriptome analyses.

	Total	Chronic	Remitting	Resilience
Number of subjects (%)	224	23	71	130
Sex (% females)	50.9%	60.9%	53.5%	47.7%
Age (SD)	35.8 (12.5)	34.6 (11.2)	36.0 (13.2)	35.9 (12.4)
Race				
African ancestry	71.4%	73.9%	77.5%	67.7%
European ancestry	19.6%	13.0%	16.9%	22.3%
Other	8.9%	13.0%	5.6%	10.0%
Trauma				
No sexual assault	6.7%	0.0%	11.3%	5.4%
Sexual assault	6.3%	17.4%	7.0%	3.8%
Road accidents[a]	73.2%	69.6%	70.4%	75.4%
Gun accidents	1.8%	4.3%	1.4%	1.5%
Stabbing	1.8%	0.0%	4.2%	0.8%
Animal bites	1.3%	4.3%	1.4%	0.8%
Other	8.9%	4.3%	4.2%	12.3%
Education				
Some HS or less	14.3%	30.4%	15.5%	10.8%
HS graduate	27.2%	34.8%	26.8%	26.2%
AA/some college	39.3%	30.4%	39.4%	40.8%
BS/BA	15.2%	4.3%	14.1%	17.7%
Master/Ph.D.	4.0%	0.0%	4.2%	4.6%
Baseline PTSD symptoms (SD)	9.3 (9.9)	19.7 (12.7)	12.1 (9.14)	5.92 (7.7)

There were no significant differences in gender, age, or class trajectory representation between this subgroup and the entire ED cohort.
[a]Road accidents includes motor vehicles crashes, and pedestrians and bike accidents.

score FDR $= 2.0 \times 10^{-4}$), or education ($N = 153$, FDR $= 0.017$). Depression and PTSD are often comorbid; in our datasets, they were high correlated at baseline ($n = 147$, correlation $= 0.6$; p value $= 1.7 \times 10^{-15}$); consequently, we did not include depression in our model. A closer examination of *AMOLT1* mRNA levels in the chronic and resilience trajectories revealed a small difference in the median mRNA level (Fig. 1B). We found that the median mRNA level of *GRIN3B* was significantly higher in the chronic compared to the resilience trajectory (Fig. 1C).

We then evaluated the difference in GRIN3B and AMOLT1 expression levels in the three trajectories. We observed a gradual decrease of GRIN3B mRNA expression ($p = 1.04 \times 10^{-5}$; $t = -4.5$) from chronic to remitting to resilient categories (Fig. S2A). In contrast, AMOLT1 expression did not declined steadily ($t = -2.7$; $p = 0.01$; Fig. S2B).

Finally, we examined the association between baseline *GRIN3B* expression level and PSS score at baseline and each subsequent follow-up assessment. We found that baseline *GRIN3B* expression level was significantly associated with PSS score at 1, 3, 6, and 12-month follow-up, but not at baseline (Table S4).

Using the transcriptomic profile and weighted gene correlation network analysis, we identified 38 gene co-expression modules. The number of genes in each module ranged between 52 and 2362. Neither GRIN3B nor AMOLT1 was a member of these modules. We did not observe a significant association between the module eigengenes and class trajectory (chronic vs. resilient; Table S5).

DNA methylation loci associated with blood expression levels

As DNAm in specific genomic loci can affect mRNA expression, we evaluated whether DNAm can explain the observed decrease in *GRIN3B* mRNA levels in resilience. In a subset of patients ($N = 151$), we identified 20 CpG sites in the *GRINB3* gene using EPIC and 450 BeadChip data. After regressing beta values with mRNA expression levels, we found four CpGs sites located in the genic and intragenic region of *GRINB3* (Fig. 2A), whose DNAm levels significantly increased with higher gene expression ($p < 0.0025$; Fig. 2B). None of these four loci was associated with class trajectory (chronic $N = 14$; resilience $N = 56$), possibly due to limited statistical power.

SNP variants in *GRIN3B* associated with blood expression levels (eQTLs)

We next assessed if any genomic locus in the *GRIN3B* gene could explain the change in blood mRNA levels. Among the 23 identified GWAS variants, four SNPs (rs10401454, rs8109756, rs4806908, and rs2240158) were associated with significant change in blood expression (FDR < 0.05; Table 2 and Fig. 1A–C).

A *GRIN3B* SNP associated with PTSD phenotype in the validation dataset

To fully evaluate if any genetic eQTL variants identified in the ED cohort were associated with developing PTSD, we used the GTP cohort, a cross-sectional dataset of similar ancestry (Table S6 displays the GTP demographics).

Out of these four eQTLs, rs10401454 was nominally significantly associated with PTSD case–control phenotypes, based on PSS symptoms ($N = 3519$, $p = 0.036$, OR $= 1.13$) with the minor allele (GG) predicting resilience (Table S7 and Fig. 3); a similar result was obtained after adjusting for DAST score ($N = 1176$, $p = 0.045$, OR $= 1.24$). The direction of association was consistent with the initial ED findings, as *GRIN3B* expression was lower in the resilient class than the other two classes ($p = 1.04 \times 10^{-5}$; $t = -4.5$; Fig. S2). Similarly, rs10401454 minor allele was associated with lower expression levels in the ED cohort, and low PTSD symptoms in the GTP cohort. rs10401454 was not

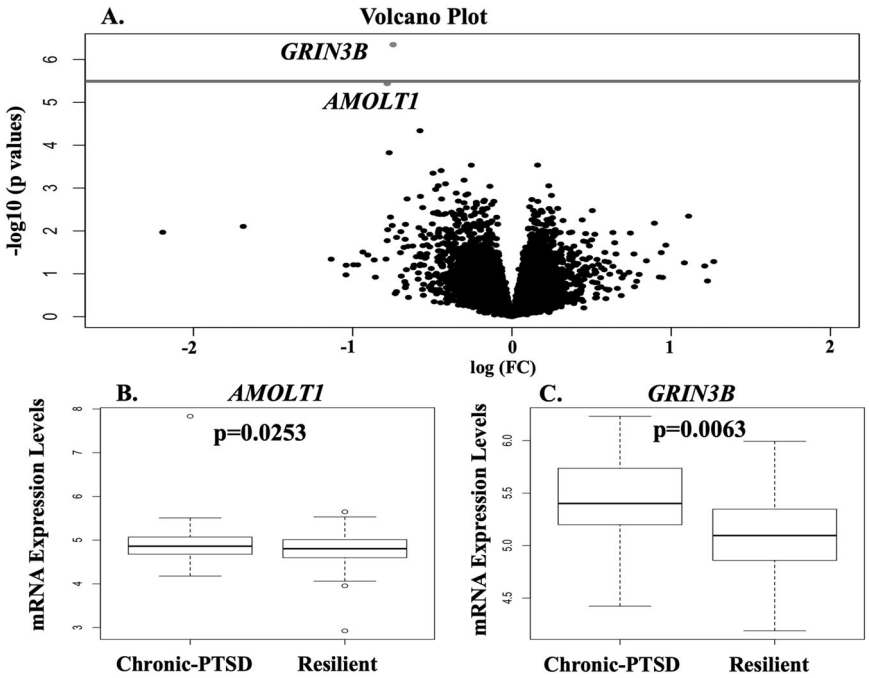

Fig. 1 Blood mRNA expressions after trauma predict resilience. A Volcano plot showing changes in gene expressions in resilient vs. chronic trajectories. The horizontal axis of the volcano plot is effect size (log2 foldchange) for the differentially expressed genes. The vertical axis is the negative of $\log_{10}$ of the *p* values. Each dot represents a gene, with red dots showing genes reaching an FDR-corrected *p* value of 0.05, and with black dots representing genes with similar expression levels in resilience and chronic clusters. The blue horizontal blue line indicates a strict Bonferroni correction *p* values threshold [$p = 3.58 \times 10^{-6}$ (0.05/13,951 tests). **B**, **C** Box and whiskers plots of blood mRNA expressions for **B** *AMOTL1* and **C** *GRIN3B* in resilient vs. chronic-PTSD trajectory. *Y*-axis show gene expression values. The box represents their quartiles; whiskers indicate the variability outside the upper and lower quartiles; the horizontal line represents the median.

associated with resilience in the ED cohort (Table S8), possibly due to limited statistical power.

GRIN3B rs10401454 associated with mRNA expression in the brain

To further corroborate the relevance of rs10401454 for gene expression in the brain, we resorted to the *BrainCloud* and the *GTEx* databases. In the *BrainCloud* dataset, we found that rs10401454 was an eQTL in DLPFC group (FDR $= 6.5 \times 10^{-7}$, beta $= -0.04$) and in the DLPFC controls (FDR $= 2.8 \times 10^{-4}$, beta $= -0.04$; Fig. S3A, B). In GTEx, rs10401454 genotypes affected mRNA levels expressed in the brain cortex ($p = 2 \times 10^{-10}$) and brain frontal cortex BA9 ($p = 6.2 \times 10^{-6}$), with the homozygote for the minor allele (GG) having less expression than CG and CC genotypes (Fig. S3C, D). The patterns of expression observed in BrainCloud and GTEx were consistent with the one in the ED-blood TWAS (Fig. 2C). Notably, in the GTEx, this SNP is also an eQTL in whole blood ($p = 1.2 \times 10^{-10}$) and several other tissues, including brain cerebellum and basal ganglia with an *m* value > 0.9 in 39 out 49 tissues (Fig. S4).

DISCUSSION

Identifying novel biomarkers of PTSD risk may facilitate early intervention, mitigating both individual suffering and public health concerns. Blood biomarkers could be particularly advantageous, being an objective measure for future symptom manifestation, and when interview for PSS symptoms and trauma type might not be feasible in the aftermath of a trauma exposure. In our study, using transcriptomes profiled in the immediate aftermath of trauma exposure, we observed that the expression levels of GRIN3B at baseline predicted resilience vs. PTSD. One eQTL variant in GRIN3B was also nominally associated with PTSD in a larger independent cohort of similar backgrounds.

Trajectory modeling analyses previously led to important findings in complex genetic traits, including psychiatric diseases (e.g., PTSD) [6, 56]. Thus, we utilized empirical trajectories, a best LGMM fitting model with a three-class solution, which statistically describes symptom progression over 12 months after trauma, as the primary outcome for gene discovery rather than diagnostic categories [23]. In prior studies, these trajectory membership showed a significant association with plasma proinflammatory tumor necrosis factor α and interferon-γ [21], with increased skin conductance response among subjects in the chronic trajectory [22] and emotion dysregulation [36].

The characteristics of the chronic-PTSD trajectory are in concordance with several other studies, in which female patients with low education were associated with a higher likelihood of developing PTSD [57]. Furthermore, in the ED, most sexual-assault victims were clustering in the chronic-PTSD category, suggesting that PTSD risk varies by type of trauma. Indeed, sexual assaults (e.g., rape) associate with both the highest probability of developing PTSD, and a longer duration of PTSD symptoms [58, 59]. The trajectory also indicates that PSS symptoms could predict outcomes, but a clear distinction among trajectories emerged after 6 months.

The TWAS of class trajectories in our study identified *GRIN3B* baseline mRNA expression as informative of PTSD course and outcomes.

We observed a significant gradual decrease of *GRIN3B* mRNA levels in chronic-PTSD vs. recovery vs. resilient trajectory, indicating a steady reduction of expression with the disease's etiology. Although the sample size is limited, the finding seems well-founded for several reasons: (a) adding either alcohol or drug use, or education, did not change the results (b) we observed a dose–response in the three-trajectory analysis. Contrary, *AMOL-T1*analyses did not show analogous results.

GRIN3B encodes a subunit (NR3B) of an *N*-methyl-D-aspartate (NMDA) receptor, a glutamate receptor, that is widely distributed

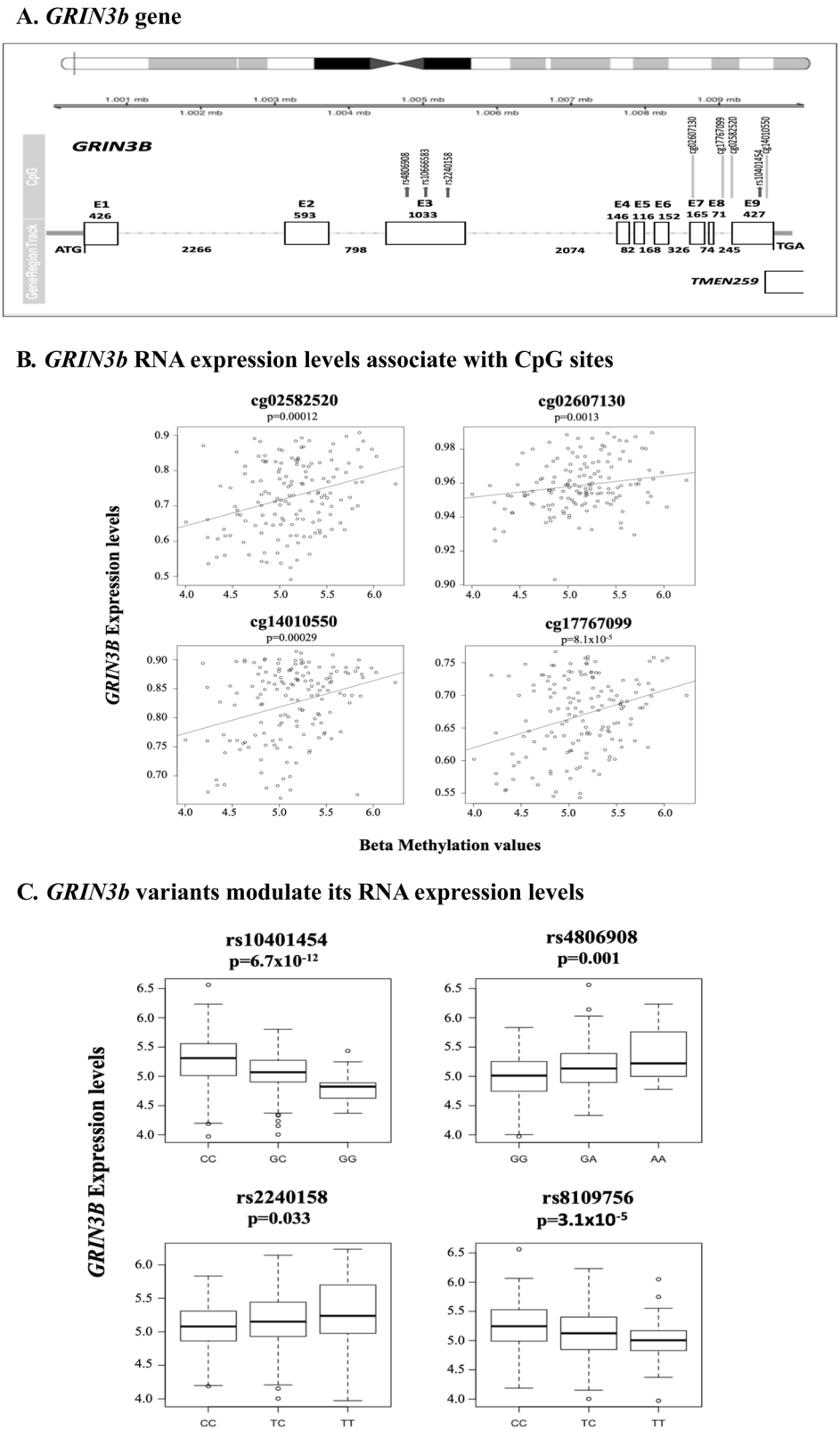

Fig. 2 GRIN3B gene: its RNA expression levels are modulated by CpG loci and DNA variants. A Schematic representation of the *GRIN3B* gene and its localization on: chromosome 19 (top of the figure). Each rectangle represents an exon. Numbers above the rectangles are the base pair of each exon; intronic base pair numbers are also indicated. Four variants (rs4806908, rs10666583, rs2240158, and rs1041454) and their localization within the gene and exon are shown; rs8109756 is located near the *CNN2* gene and not shown in the figures. None of these SNPs are in LD with each other, despite the close localization. The CpG methylation loci and their localization in the gene are also shown. **B** Four CpG sites are positive correlated with *GRIN3b* expression levels. *Y*-axis shows gene expression values; *X*-axis represents beta-methylation values. The red line indicates the predicted regression line. **C** Box and whisker plots of *GRIN3b* blood RNA expression levels changing with the genotype of the four different variants. *Y*-axis shows the mRNA expression levels at baseline; *X*-axis shows the three different genotypes.

Table 2. Genetic variants associated with mRNA expression levels (eQTLs).

SNPs	Gene	Beta	*p* Value	FDR	Location
rs10401454	*GRIN3B*	−0.23	2.90×10^{-13}	6.7×10^{-12}	Exon missense variant
rs8109756	*CNN2*	−0.14	2.71×10^{-6}	3.1×10^{-5}	Intron
rs4806908	*GRIN3B*	0.19	0.0001	0.001	Exon synonymous variant
rs2240158	*GRIN3B*	0.08	0.0058	0.033	Exon missense variant

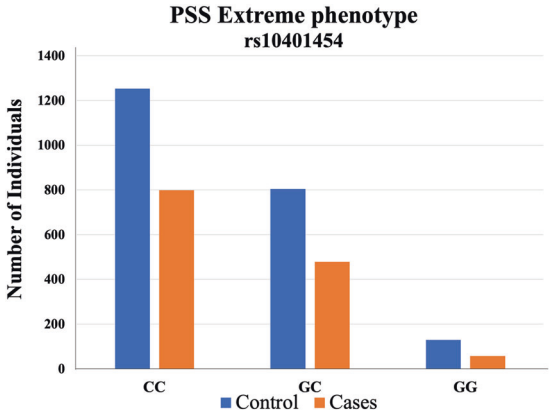

Fig. 3 GRIN3B Genetic Association with PTSD symptoms. Bar plot of number of cases (PSS ≥ 19) and controls (PSS ≤ 7) for *GRIN3B* genotype (CC, GC, and GG). The *Y*-axis represents the number of subjects.

in neurons throughout the central nervous system (CNS). In rodents, exposure to stress causes an increase in glutamate release, the excitatory neurotransmitter for NMDA receptors, with associated increased NMDAR expression in brain regions linked to emotional behavior [60, 61], including fear conditioning [62]. In a mouse model, we did not find a significant correlation between blood and amygdala expression level of GRIN3B [20]. Nevertheless, we cannot rule out significant correlations of GRIN3B level between blood and other brain regions implicated in PTSD, such as hippocampus or ventral medial prefrontal cortex. Furthermore, we cannot exclude the possibility of an indirect effect of GRIN3B through the blood immune system as leukocytes express functional NMDA receptors [63], which might be involved in the brain–body signal transmission and given the role of immune system in the etiology of PTSD [64–66]. Finally, the aim of the study was to identify peripheral biomarker for manifestation of PTSD symptoms. Thus, NMDA receptors are particularly interesting in the pathophysiology of PTSD. They also seem to be involved in plastic changes within the CNS responsible for learning and memory, development, and synapse formation [67]. Interestingly, NMDA receptor antagonists (e.g., ketamine) have been used to reduce symptom severity in patients with chronic-PTSD [68, 69]. This accumulating evidence in human and animal models of NMDA receptors' role in PTSD suggests the GRIN3B gene as an exciting candidate for predicting PTSD course shortly after trauma.

In human populations, GRIN3B has been linked to opioid addiction and schizophrenia [45, 46, 70–72], although expression of GRIN3B mRNA was not associated with computer game addiction [73]. Chronic opioid abuse, but also abstinence and methadone treatment led to the significant upregulation of GRIN3B mRNA [71]. Intriguingly, the downregulation of mRNA expression may have been caused by methadone's ability to antagonize NMDA receptors [71]. In our cohort, we observed an upregulation of GRIN3B expression in subjects with future chronic-PTSD symptoms. Furthermore, we observed a stronger association after including a variable for drug use, suggesting that addiction may contribute to GRIN3B expression changes and possibly increased PTSD risk. While no association was observed between GRIN3b expression levels and PSS symptoms at baseline, GRIN3b expression was associated with PSS symptoms during the follow-up visits, with the highest significant observed at 6 months. As a corollary, some NMDA antagonists, such as ketamine, may downregulate GRIN3B expression levels, indicating a point of intervention to improve outcome and disease course in post-trauma subjects with higher GRIN3B levels.

Similarly, expression of glutamate receptor subunits in peripheral blood was also shown to be altered by sex, pregnancy, or depression during pregnancy, with the lowest *GRIN3B* mRNA levels in healthy pregnant women [74]. Healthy volunteers evaluated for working memory showed no difference in NMDA receptor expression, denoting a different role of NMDARs in stressful situations (e.g., trauma exposure) [75].

Our results are not in contrast with previous PTSD transcriptome studies, in which immune and inflammatory genes and pathways had been identified, as we examined the blood transcriptomic profiles collected at the time of trauma exposure, which can be different from transcriptomic profiles from postmortem brain tissue [76] or from blood [12] at the time of having PTSD.

To explore possible mechanisms that may account for significant differences in *GRIN3B* expression in PTSD trajectories, we evaluated DNAm loci and genetic variants. Four CpG sites were hypermethylated, and associated with increased mRNA blood levels. Higher levels of *GRIN3B* methylation levels in mice brain had been associated with increased mRNA levels [77]. Furthermore, a locus located ~10 kb upstream of the *GRIN3B* gene was found to be hypomethylated in the brains of schizophrenic and major psychosis [78]. Notably, often gene promotors are hypomethylated, while intragenic and coding regions are hypermethylated [77, 79], both of which mechanisms may result in increased mRNA expression. Four variants (two missense mutations, a synonymous SNP, and an intronic variant) in the *GRIN3B* gene were associated with baseline blood expression levels of *GRIN3B*. While how these variants regulated mRNA expression is currently unknown, we surmise that their genomic locations could be cryptic transcription factors binding loci.

We then investigated if any of the *GRIN3B* variants could be a risk factor for PTSD in the GTP cohort [49]. rs10401454 was associated with PTSD case–control extreme phenotype. The exon 9 location of this SNP is particularly intriguing, as *GRIN3B*-Exon 9 encodes two putative Ca^{2+}/calmodulin-dependent kinase sites, important regulating the calcium-permeable channel and activation/deactivation of the NMDA receptor. High Ca^{2+} permeability of NMDA receptors also play a critical role in neurodevelopment, synaptic plasticity, and neuronal death, suggesting that variations in this exon, such as rs10401454, may confer particular vulnerability and pathological consequences.

Using data from two eQTL brain databases, we observed that rs10401454 regulates mRNA expression in the brain cortex, a region of the brain that is important for the pathophysiology of PTSD [80, 81]. Furthermore, in the GTEx dataset, rs10401454 seemed to regulate several other tissues, implying that this variant could ubiquitously affect expression levels in different organs/tissue.

Genetic variants in *GRIN3B* have been associated with an increased risk of developing schizophrenia [45, 46, 72], auditory mismatch negativity [70], and heroin addiction [82]. Specifically,

the *GRIN3B* SNP rs2240158 was associated with heroin addiction in a Han Chinese population [82] and mismatch negativity in healthy subjects [70]. Our study found that this variant was a *GRIN3B* eQTL, was associated with trajectories in the ED cohort, but not with PTSD phenotype in the GTP cohort. A four-base pair insertion variant, rs10666583, has been associated with increased schizophrenia risk in two independent studies [45, 46]. This same null variant was associated with extreme case–control phenotype in the GTP cohort (Table S5). Given that PTSD shares genetic risk factors with other psychiatric disorders, including schizophrenia, [83–85], the same variants may contribute to increased risk across diseases.

The major limitation of this study is the lack of an independent cohort with a similar study design to replicate our findings. We hope to replicate and expand these results in the future, using the AURORA study [86], which has been enrolling discharge ED subjects after trauma exposures, and followed longitudinally with a target sample size of over 5000 samples. Another limitation of this study was the relatively modest sample size in the transcriptome analysis, although in line with other studies [10, 65, 66, 87–89]. On the other hand, this work relied on growth curve trajectories, which may represent separate genetic clusters less heterogeneous than the classical PTSD phenotype, and more suitable for gene discovery. The ED also offers a primary point of contact for individuals immediately after exposure to trauma, providing an ideal foundation to (i) evaluate subjects longitudinally, (ii) assess PTSD symptoms shortly after the trauma, and (iii) appraise early individual PTSD risk factors, which may be used toward systematic prevention. Although, given the study designed, we could not analyze the patient's blood prior to the trauma exposure, we did not observe any association of GRIN3b levels at baseline with PSS symptoms, inferring a possible direct or indirect involvement of GRIN3b gene in the etiology of PTSD.

In summary, the current study provides evidence that peripheral gene expression signatures following trauma are informative of PTSD key clinical features and outcomes. Specifically, *GRIN3B* expression seems to predict post-trauma symptom trajectory and might be a promising early biomarker for PTSD manifestation and outcome, and a possible intervention and prevention point.

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AUTHOR CONTRIBUTIONS

A.L. performed the statistical analyses, analyzed and interpreted the data, and contributed to writing the manuscript. K.S. modeled the growth curve trajectories based on longitudinal PSS symptoms, helped with the statistical analyses, and contributed to editing the manuscript. I.G.-L. modeled the growth curve trajectories based on longitudinal PSS symptoms and contributed to the writing of the manuscript. N.P.D. inferred the cell type proportion, performed statistical analyses, and contributed to the manuscript's writing. S.K. performed the methylation analyses and contributed to the manuscript's writing. A.K.S. led the methylation analyses, including collection and processing of the samples, and contributed to the manuscript's writing. A.J.M. helped to design the experiment, recruit the patients, and edit the manuscript. R.R. and M.H. were involved in processing the blood for RNA analyses, including QC and statistical analyses, and they contributed to the editing of the manuscript. G.G. helped with the expression analyses, and contributed to the manuscript's editing. S.W. helped to design the experiment, recruit the patients, and write the manuscript. F.G. and P.D.H. helped to design the experiment, recruit the patients, and edit the manuscript. C.B.N. helped to design the experiment, and write the manuscript. T.J., J.L.M.-K., J.S.S., and V.M. helped to design the experiment, recruit the patients, and write the manuscript. B.O.R. helped to design the experiment and write the manuscript. A.P.W. and K.J.R. obtained funding, helped to design the experiment, analyze and interpret the data, supervise other contributors, write the initial manuscript, and edit the final manuscript. E.G. contributed to analyses and writing.

COMPETING INTERESTS

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