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Gene expression and methylation signatures of *MAN2C1* are associated with PTSD

Monica Uddin[a,*], Sandro Galea[b], Shun-Chiao Chang[c], Allison E. Aiello[a], Derek E. Wildman[d], Regina de los Santos[a] and Karestan C. Koenen[c]
[a]*Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI, USA*
[b]*Department of Epidemiology, Mailman School of Public Health, Columbia University, NY, USA*
[c]*Departments of Society, Human Development, and Health and Epidemiology, Harvard School of Public Health, Boston, MA, USA and the Center on the Developing Child, Harvard University, Cambridge, MA, USA*
[d]*Center for Molecular Medicine and Genetics, Wayne State University School of Medicine, Detroit, MI, USA*

Abstract. As potential regulators of DNA accessibility and activity, epigenetic modifications offer a mechanism by which the environment can moderate the effects of genes. To date, however, there have been relatively few studies assessing epigenetic modifications associated with post-traumatic stress disorder (PTSD). Here we investigate PTSD-associated methylation differences in 33 genes previously shown to differ in whole blood-derived gene expression levels between those with vs. without the disorder. Drawing on DNA samples similarly obtained from whole blood in 100 individuals, 23 with and 77 without lifetime PTSD, we used methylation microarray data to assess whether these 33 candidate genes showed epigenetic signatures indicative of increased risk for, or resilience to, PTSD. Logistic regression analyses were performed to assess the main and interacting effects of candidate genes' methylation values and number of potentially traumatic events (PTEs), adjusting for age and other covariates. Results revealed that only one candidate gene – *MAN2C1* – showed a significant methylation x PTE interaction, such that those with both higher *MAN2C1* methylation and greater exposure to PTEs showed a marked increase in risk of lifetime PTSD (OR 4.35, 95% CI: 1.07, 17.77, $p = 0.04$). These results indicate that *MAN2C1* methylation levels modify cumulative traumatic burden on risk of PTSD, and suggest that both gene expression and epigenetic changes at specific loci are associated with this disorder.

Keywords: Epigenetics, psychiatric epidemiology, trauma, interaction, genomics

1. Introduction

The term "epigenetics" refers to the regulation of genetic functions mediated through mechanisms that are independent of DNA sequences. Although multiple types of epigenetic modifications have been identified [1], all involve chemical modifications that regulate chromatin structure and/or DNA accessibility, which in turn alters the transcriptional activity of the surrounding loci. Epigenetic factors have been the focus of increasing interest among those interested in studying mental illness [2], including post-traumatic stress disorder (PTSD) [3–5]. This interest may be due in part to the relatively few genes that have been conclusively shown to contribute to risk of common mood-anxiety disorders (e.g. [6,7]), and the mounting evidence that epigenetic marks can change in response to external experiences [8,9]. Indeed, as potential regulators of DNA accessibility and activity, epigenetic factors may, through influences on gene expression, offer one mechanism by which the environment can moderate the effects of genes [10]. In addition, epigenetic mechanisms may be involved in the mediation of some gene-environment (G × E) interactions, such that individuals with particular genotype/epigenotype combinations may be especially inclined toward mental illness in the presence of specific environmental exposures. For example, numerous studies investigating variation in the serotonin transporter promoter (*5HTTLPR*) locus have

*Corresponding author: Monica Uddin, PhD, Assistant Research Scientist, Dept. of Epidemiology, University of Michigan School of Public Health, 1415 Washington Heights, Ann Arbor, MI, 48109, USA. Tel.: +1 734 647 9859; E-mail: uddinm@umich.edu.

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demonstrated that carriers of the short allele are more susceptible to depression following exposure to stressful life experiences (reviewed in [11]). The s allele, in turn, has recently been associated with increased depressive symptoms in adolescents, but only among individuals who also showed relatively high methylation levels at this locus [12]. More broadly, in the case of PTSD, epigenetic effects offer a plausible way in which an environmental exposure (i.e. potentially traumatic event, PTE) may modify biological substrates (i.e. gene expression) in a manner that may increase risk of an adverse psychopathological outcome [4].

Although epigenetic studies of mental illness have been growing in number in recent years (reviewed in [2]), studies focused specifically on PTSD are still few in number. Animal models of PTSD have identified epigenetic differences that can discern rats with PTSD-like vs. non-PTSD-like behaviors, using a validated PTSD rat model [13]. In this model, rats are exposed to a predator stimulus (cat scent carried on soiled litter) which serves as the PTE; seven days following the stress exposure, animals are classified as exhibiting PTSD-like and non-PTSD-like behavior according to their performance on behavioral tests (elevated pulse-maze and acoustic startle response [14]). Assessment of methylation changes in brain tissue following this stress paradigm identified some loci (e.g. *Dlgap2*) that showed PTSD-associated changes in both methylation and gene expression patterns in postmortem hippocampal tissue [15]. Interestingly, in this study a significant correlation was observed between *Dlgap2* gene expression and the degree of behavioral stress responses in individual rats, suggesting that this locus may contribute to the molecular substrate of traumatic stress adaptation in these animals [15]. Another recent study focusing on humans found that those with lifetime PTSD were distinguished by methylation profiles suggesting an upregulation of genes involved in immune system-related genes, and a relative downregulation of genes involved in neurogenesis and the startle response [16]. In the latter, human-based case, however, the reported PTSD-associated epigenetic differences were only hypothesized to be associated with downstream differences in gene expression.

A small but growing literature has provided evidence for gene expression patterns that distinguish between those with vs. without PTSD. The majority of these microarray-based studies have assessed gene expression changes in RNA derived from either peripheral blood mononuclear cells (PBMCs) or whole blood. The earliest work assessed PTSD-associated gene expression signatures among trauma survivors admitted to the emergency room immediately following a traumatic event [17]. Bioinformatic functional analyses of transcripts that were differentially expressed ($n = 656$) between the two groups showed a reduced expression of transcriptional enhancers, distinct expression signatures of transcripts involved in immune activation, and a significant enrichment of genes that encode neural and endocrine proteins [17]. A more recent study used a custom-made "stress/immune" cDNA microarray to assess expression levels of 384 genes among PTSD-affected and –unaffected individuals using RNA obtained from whole blood [18]. Of note in this study, all of the PTSD-affected individuals had been exposed to the same traumatic event almost 20 years prior to testing – the Ramstein air show catastrophe of 1989 – yet typical PTSD symptoms persisted in this group. Analyses showed a total of 19 differentially expressed transcripts, five and 14 of which were up- and down-regulated, respectively [18]. The majority of down-regulated transcripts (which were the focus of the study) were associated with immune functions or with reactive oxygen species. Most recently, Yehuda and colleagues reported whole blood-derived gene expression levels among PTSD-affected and –unaffected individuals who had had exposure to the 9/11 attack on New York City [19]. Differential expression was detected in 16 distinct genes, several of which are involved in signal transduction, brain and immune cell function and HPA axis activity. Of note, although several genes in this study had previously been linked to PTSD and/or stress-related outcomes (e.g. *FKBP5* [20, 21], *MHC Class II* [22]), the gene showing the largest difference in expression was mannosidase, alpha, class 2C, member 1 (*MAN2C1*), a locus that had not previously been linked to PTSD.

Collectively, these few early studies suggest that investigations of genome-wide gene expression and epigenetic differences associated with PTSD may help to shed light on the molecular underpinnings of this disorder. Among these early studies, however, there are as yet no reports of *both* gene expression and epigenetic signatures that characterize PTSD in humans. Here we build on the extant literature that reports results from large-scale, gene expression and epigenetic investigations of PTSD in humans. More specifically, we assess whether genes previously identified as showing PTSD-associated gene expression patterns may also show epigenetic signatures indicative of increased risk for, or resilience to, PTSD. The goal of this work was to conduct a secondary data analysis of the extant PTSD-

associated genomic data in order to build a platform for future studies that assess both gene expression and epigenetic differences associated with PTSD from the same individuals, in the same tissues. Since epigenetic variation occurs in a tissue-specific fashion [23], we focused on the two studies that assessed gene expression in peripheral whole blood [18,19], consistent with the starting material of PTSD-associated epigenetic signatures reported in a previous recent study [16]. Furthermore, we focused our analyses on the potential interaction between methylation values of these candidate genes and cumulative traumatic burden, in order to investigate more fully the complex ways in which internal (molecular) and external (PTE) factors may modify risk for this mental illness and result in phenotypic variation following trauma exposure [24].

2. Methods and materials

2.1. Participants

This study is based on samples obtained from participants in the Detroit Neighborhood Health Study (DNHS). The DNHS is a study of adults, 18 years or older, from the Detroit population. A probability sample of 1,547 households within the city limits of Detroit was initially chosen and one individual per household was then randomly selected for a telephone interview. Participants were administered a 40-minute assessment which included questions on exposure to traumatic events, socio-demographic and behavioral characteristics, and a standardized assessment of post-traumatic stress disorder (PTSD). A subsample of eligible participants during wave 1 of the study consented to provide blood specimens by way of venipuncture ($n = 501$). Wave one samples were collected principally to assess immune function, and preservation of RNA for gene expression testing was not included in wave 1 biospecimen collection efforts. For the purposes of this study, 100 of these 501 participants were selected midway through the wave 1 sample collection effort in order to conduct exploratory, pilot testing of epigenetic profiles associated with mental illness in community-based settings.

Wave 1 survey participants were representative of the Detroit population in terms of age, gender, race, income, and educational attainment [25]. The 100 participants in the current study were similar to the full sample on these key sociodemographic characteristics but were, on average, slightly younger than individuals in the full survey sample (45.3 vs. 50.6 years, $p = 0.003$). Since our subsample of 100 participants for this study was selected specifically according to presence of mental illness, the prevalence of lifetime PTSD is higher in our study sample (23%) than in the full survey sample (14.4%). The DNHS was approved by the Institutional Review Board at the University of Michigan. More details regarding this study can be found in [16].

2.2. Assessment of lifetime post-traumatic stress disorder

Individual assessment of PTSD symptoms was conducted using the PTSD checklist (PCL-C) [26], a 17-item self-report measure of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) [27] PTSD symptoms, augmented by additional questions about duration, timing, and impairment or disability due to the symptoms. Participants were initially asked to identify PTEs that they experienced in the past from a list of 19 events that had previously been implemented in an earlier epidemiologic study to assess PTSD in the Detroit area [28], and an additional question that allowed the participant to briefly describe any other extraordinarily stressful situation or event. We then asked those participants who had experienced at least one traumatic event to choose which one they considered to be the worst. Participants rated each of the 17 PTSD symptoms on a scale indicating the degree to which the respondent had been bothered by a particular symptom as a result of this trauma from 1 (not at all) to 5 (extremely) in reference to this event. An additional PTSD section assessed symptoms based on a randomly chosen traumatic event (excluding the worst event) for those participants who had experienced more than one PTE. Respondents were considered affected by lifetime PTSD if all six DSM-IV criteria were met in reference to either the worst or the random event.

To validate our identification of PTSD obtained from the telephone interview responses, we conducted clinical in-person interviews among a random subsample of 51 participants. A licensed clinician conducted one-hour clinical interviews after obtaining signed consent from participants, utilizing the Clinician-Administered PTSD Scale for DSM-IV (CAPS) for PTSD. The counselor was blinded to the information obtained from the participants during the telephone interview. Participants received $50 for participation in this part of the study. Analysis of data from the in-person interviews showed that the PCL-C used during the telephone interviews had excellent internal consistency and high

concordance. The PCL-C yielded a Cronbach coefficient alpha (α) of 0.93. Using clustering scoring based on DSM-IV criteria (i.e. to be a case, the participant's symptoms had to meet all six criteria), the instrument had a sensitivity (SE) of 0.24, specificity (SP) of 0.97, positive predictive value (PPV) of 0.80, negative predictive value (NPV) 0.72, and an area under the ROC curve (AUC) of 0.76. Low sensitivity values imply that our survey-based PTSD prevalence estimates are conservative. Importantly, the high specificity insures our PTSD group is made of true cases. All 100 individuals included in this pilot study were exposed to at least one PTE; among these, 23 were PTSD-affected and 77 were –unaffected.

2.3. Assessment of additional demographic and behavioral information

Additional demographic and behavioral information including age, race/ethnicity, drinking and smoking was assessed during the telephone portion of the DNHS survey. Socioeconomic status (SES) was assessed via self-report according to whether participants had attained high school equivalency or greater (high SES) or less than high school (low SES). Venipuncture specimens were obtained from consenting participants by trained phlebotomists during home visits, at which time the use of any medication was noted on a specimen collection log. Peripheral blood mononuclear cells (PBMCs) were isolated via Ficoll-gradient centrifugation of whole blood and re-suspended in 6ml of freezing medium, 1ml of which was loaded into a packed cell volume (PCV) tube and centrifuged the tube at 2400RCF for 1 minute. PCV tubes were then assessed for PBMC count using the TPP Easy Read Ruler from MIDSCI (St. Louis, MO) and following manufacturer's recommended protocols.

2.4. Microarray analyses

Bisulfite conversion of whole blood-derived DNA samples was performed using the EZ-96 DNA methylation kit from Zymo Research (Orange, CA). One microgram (μg) of each sample (including controls) was subjected to bisulfite conversion following manufacturer's recommended protocol. Experimental controls included replicates for two samples to assess variation throughout the experimental process (i.e. from initial bisulfite conversion through microarray analysis), as well as one sample of completely unmethylated and completely methylated human DNA, commercially available through Zymo Research, in each of the two 96 well plates used in the bisulfite conversion step. All control replicates were placed on separate microarray chips, and the remaining samples were assigned to microarray chips at random, without regard to PTSD status. Bisulfite converted DNA samples were subjected to methylation profiling via the humanmethylation27 (HM27) DNA Analysis BeadChip by Illumina following the manufacturer's recommended protocol. Using this platform, methylation levels were determined for 27,578 CpG dinucleotides spanning 14,495 genes in each of the 100 test samples. The resulting data were background normalized using Bead Studio. Correlation coefficients of the two replicated samples were 0.81 and 0.89, respectively. The average beta value for the methylated controls was 0.93 and the correlation 0.96. The average beta value and correlation of unmethylated controls were 0.17 and 0.98, respectively. Methylation microarray data were validated via pyrosequencing and DNA sequencing of a subset of individuals tested on the original microarray and are reported in detail elsewhere [16].

2.5. Analyses

We assembled a list of the genes ($n = 33$; Table 1) identified as significantly differentially expressed between those with vs. without PTSD in whole blood based on two previous studies [18,19]. Genes were included in the list if they showed PTSD-associated differences in gene expression in either of the two studies [18,19] and were also represented on the HM27 BeadChip. We then tested for potential main and interaction effects between methylation at CpG sites associated with these 33 loci and number of potentially traumatic events on risk of PTSD. Data were analyzed using R v2.10.0 and SAS v9.2. Initially, bivariate associations were assessed for PTSD and each of the variables of interest. All tests were two-tailed and evaluated at the 5% significance level. Main effects and interaction effects models were then fitted to establish the relationships of the predictors to PTSD. PTSD diagnosis was based on the DSM-IV criteria as implemented by the PCL-C (and additional questions) as described in the previous section; the predicted probability of lifetime PTSD was then modeled using logistic regression using the following equation:

$$\text{logit}[P(Y_i = 1)] = \alpha + \beta' X_i + \gamma PTE_i * \text{Methylation}_i$$

Table 1
Genes investigated in the current study

Gene name	Reference sequence*	Expression level in those with vs. without PTSD	Reference paper
MANNOSIDASE, ALPHA, CLASS 2C, MEMBER 1	NM_006715	Higher	[18]
DEAD (ASP-GLU-ALA-ASP) BOX POLYPEPTIDE 17	NM_006386	Higher	[18]
N-ACYLSPHINGOSINE AMIDOHYDROLASE (ACID CERAMIDASE) 1	NM_177924	Lower	[18]
FK506 BINDING PROTEIN 5	NM_004117	Lower	[18]
CYCLIN D3	NM_001760	Lower	[18]
NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE	NM_012343	Lower	[18]
CHROMOSOME 2 OPEN READING FRAME 34	NM_024766	Lower	[18]
BRIDGING INTEGRATOR 1	NM_004305	Lower	[18]
SIGNAL TRANSDUCER ACTIVATOR OF TRANSCRIPTION 5B	NM_012448	Lower	[18]
TIA1 CYTOTOXIC GRANULE-ASSOC RNA BINDING PROTEIN	NM_022037	Lower	[18]
CGG TRIPLET REPEAT BINDING PROTEIN 1	NM_003663	Lower	[18]
ENDOPLASMIC OXIDOREDUCTIN-1-LIKE PROTEIN	NM_014584	Lower	[18]
METHYLTRANSFERASE LIKE 7A	NM_014033	Lower	[18]
RIBOSOMAL PROTEIN S6 KINASE, 90KDA, POLYPEPTIDE 5	NM_004755	Lower	[18]
INSULIN-LIKE GROWTH FACTOR 2	NM_000612	Higher	[17]
CXCR1 CHEMOKINE (C-X-C MOTIF) RECEPTOR 1 (*IL-8 RECEPTOR ALPHA*)	NM_000634	Higher	[17]
INTEGRIN BETA 4	NM_001005619	Higher	[17]
SOLUTE CARRIER FAMILY 1 (HIGH-AFFINITY ASPARTATE/GLUTAMATE TRANSPORTER), MEMBER 6	NM_005071	Higher	[17]
LYSOPHOSPHATIDIC ACID RECEPTOR 2 (*EDG4*)	NM_004720	Higher	[17]
COLONY-STIMULATING FACTOR 2 RECEPTOR, BETA, LOW-AFFINITY (GRANULOCYTE-MACROPHAGE)	NM_000395	Lower	[17]
THIOREDOXIN REDUCTASE 1 (*TXR 1*)	NM_182729	Lower	[17]
INTERLEUKIN 18 (INTERFERON-GAMMA-INDUCING FACTOR) (*IL-18*)	NM_001562	Lower	[17]
CHEMOKINE (C-C MOTIF) RECEPTOR 1	NM_001295	Lower	[17]
PHOSPHOGLYCERATE DEHYRDROGENASE (*3-PGDH*)	NM_006623	Lower	[17]
CASEIN KINASE 1, GAMMA 3	NM_004384	Lower	[17]
CASPASE 2, APOPTOSIS-RELATED CYSTEINE PEPTIDASE	NM_001224	Lower	[17]
SUPEROXIDE DISMUTASE 1, SOLUBLE (*SOD 1*)	NM_000454	Lower	[17]
CD247 MOLECULE (*CD 3Z*)	NM_000734	Lower	[17]
INTERLEUKIN 16 (LYMPHOCYTE CHEMOATTRACTANT FACTOR) (*IL-16*)	NM_004513	Lower	[17]
SPHINGOSINE-1-PHOSPHATE RECEPTOR 1 (*EDG1*)	NM_001400	Lower	[17]
G-PROTEIN-COUPLED RECEPTOR 65	NM_003608	Lower	[17]
CALNEXIN	NM_001746	Lower	[17]
CD 81 MOLECULE	NM_004356	Lower	[17]

If the official gene name currently differs from that presented in the original paper, the earlier name (or symbol) is provided parenthetically, in italic font.
*Where more than one RefSeq applies to a single gene, only one representative RefSeq is shown (corresponding to the RefSeq that was analyzed in this study).

Where i represents an individual, Y is lifetime PTSD, α is the intercept, β is a vector of regression coefficients, and X is a vector of covariates for each individual, γ is the regression coefficient of the interaction term, PTE*Methylation represents the interaction between number of PTEs and Methylation level at a CpG site corresponding to one of the 33 candidate genes. Continuous variables such as age, PBMC count, number of potentially traumatic events (PTE), and methylation beta values were centered to the mean. All predictor variables were maintained in all the models for consistency. Estimated coefficients were evaluated at $\alpha = 0.05$.

3. Results

Among the 33 loci identified as showing differential gene expression in whole blood between those with vs. without PTSD [18,19], only one demonstrated a significant ($p = 0.04$) methylation x PTE interaction on risk of PTSD: mannosidase, alpha, class 2C, member 1(*MAN2C1*), which showed this effect at CpG site cg04008455 on the HM27 BeadChip. Table 2 presents the descriptive statistics and bivariate associations for the whole sample and for those with vs. without lifetime PTSD for the variables included in our final *MAN2C1* model. Table 2a classifies the 19 PTEs assessed by the study survey into four main trauma types and provides

Table 2
Descriptive statistics and bivariate comparisons of participants with vs. without lifetime PTSD

			PTSD				
			Without ($n = 77$)		With ($n = 23$)		Test
	n/mean	%/sd	n/mean	%/sd	n/mean	%/sd	p
MAN2C1 methylation beta value	0.38	0.15	0.37	0.15	0.39	0.13	0.59
Number of Potentially traumatic events	6.04	3.60	5.58	3.48	7.57	3.65	**0.02**
Age	45.32	16.78	44.91	17.29	46.70	15.19	0.66
High SES	86	86.0	67	87.01	19	82.61	0.73
Female	60	60.0	45	58.44	15	65.22	0.56
Race = White	14	14.0	10	12.99	4	17.39	0.79*
Race = Black	79	79.0	62	80.52	17	73.91	–
Race = Other	7	7.0	5	6.49	2	8.70	–
Ever Drink	77	77.0	60	77.92	17	73.91	0.69
Ever Smoke	58	58.0	41	53.25	17	73.91	0.08
PBMC	23.34	8.05	23.35	8.31	23.29	7.25	0.97
Any Medication	48	48.0	36	46.75	12	52.17	0.65

*global 2×3 chi-square test.

Table 2a
Descriptive statistics and bivariate comparisons of trauma type among those with vs. without lifetime PTSD

			PTSD				
			Without ($n = 77$)		With ($n = 23$)		Test
	n/mean	%/sd	n/mean	%/sd	n/mean	%/sd	p
Number of Potentially traumatic events	6.04	3.60	5.58	3.48	7.57	3.65	**0.02**
Assaultive violence	68	68.0	48	62.3	20	87.0	**0.04**
Other injury or shocking event	79	79.0	58	75.3	21	91.3	0.14
Learning about traumas to others	73	73.0	54	70.1	19	82.6	0.29
Sudden, unexpected death of close friend or relative	88	88.0	66	85.7	22	95.7	0.29

information regarding their prevalence in the overall sample, and among those with vs. without lifetime PTSD. The average age of the study sample was approximately 45 years, a majority of participants were female (60%), African-american (79%) and had attained high school equivalency or greater (86%). When analyzed according to PTSD status, those with vs. without the disorder differed significantly on the number of PTEs to which they had been exposed, with those with PTSD showing a higher average number of PTEs than those without (7.57 vs. 5.58; $p = 0.02$). In addition, a greater proportion of participants with PTSD reported having ever smoked (73.91% vs. 53.25%), although this difference did not reach statistical significance ($p = 0.08$). The remaining variables included in the final model did not differ appreciably between the two groups (Table 1).

Table 3 presents the results of our logistic regression main effects and interaction models focused on *MAN2C1*. In the main effects model, having ever smoked significantly increased risk of PTSD (OR 5.18, 95% CI: 1.26, 21.26, $p = 0.02$). In addition, exposure to greater numbers of PTEs was associated with a significant increase risk of PTSD, (OR = 1.21, 95% CI: 1.03, 1.42, $p = 0.02$), mirroring the results observed in bivariate associations (Table 1). In contrast, having ever drank appeared to protect against PTSD (OR 0.22, 95% CI: 0.05, 0.97, $p = 0.05$). No other factors showed a significant association with risk of PTSD in the main effects model (Table 2). Interaction models showed similar significant associations on risk of PTSD as main effects model. In addition, the interaction term was significant: those with both higher *MAN2C1* methylation beta values and greater exposure to PTEs showed a marked increase in risk of lifetime PTSD (OR 4.35, 95% CI: 1.07, 17.77, $p = 0.04$), confirming that methylation levels at this locus modify cumulative traumatic burden on risk of lifetime PTSD. These results are depicted graphically in Fig. 1.

4. Discussion

The goal of this work was to assess epigenetic signatures of PTSD among loci that had previously been implicated in gene expression differences associated with this disorder. Our results demonstrate that among the 33 loci identified in previous work as being associated with PTSD based on gene expression levels assessed in peripheral whole blood, only one – *MAN2C1* –

Table 3
Effect of *MAN2C1* methylation and number of traumatic events on risk of lifetime PTSD

	Main effects model				Interaction effect model			
	OR	95% CI		p	OR	95% CI		p
MAN2C1 methylation beta value	1.71	0.03	90.38	0.79	0.31	0.00	32.38	0.62
PTE	1.21	1.03	1.42	**0.02**	1.25	1.03	1.52	**0.02**
Age	1.00	0.96	1.03	0.79	0.99	0.95	1.03	0.62
High SES	0.81	0.19	3.43	0.78	0.83	0.19	3.66	0.81
Female	1.99	0.59	6.66	0.27	1.81	0.50	6.63	0.37
Race = Black	0.38	0.07	2.00	0.25	0.31	0.05	1.89	0.20
Race = Other	0.26	0.02	3.17	0.29	0.17	0.01	3.08	0.23
Ever Drink	0.22	0.05	0.97	**0.05**	0.22	0.05	0.94	**0.04**
Ever Smoke	5.18	1.26	21.26	**0.02**	4.84	1.14	20.54	**0.03**
PBMC	0.98	0.91	1.05	0.56	0.98	0.92	1.05	0.59
Any Medication	0.79	0.25	2.51	0.69	0.95	0.29	3.08	0.93
MAN2C1* PTE	–	–	–	–	4.35	1.07	17.77	**0.04**

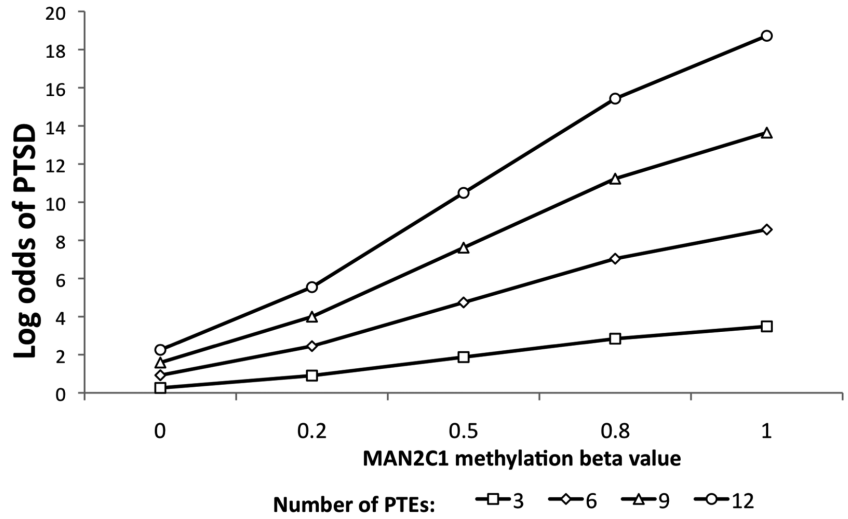

Fig. 1. Risk of lifetime PTSD according to *MAN2C1* methylation beta value and number of potentially traumatic events (PTEs). Shown are the log odds of lifetime PTSD given 3, 6, 9, and 12 PTEs across increasing methylation values for *MAN2C1*.

showed evidence of a significant interaction with number of potentially traumatic events in predicting risk of PTSD. Notably, this interaction was detected for the locus showing the greatest fold change with respect to differential expression between those with vs. without PTSD in an earlier study (Fig. 2; [19]); yet prior to that work, the gene had not been implicated in PTSD etiology. Few studies exist that focus specifically on *MAN2C1*. Nevertheless, the locus is known to be involved in cancer progression, as its expression appears to modulate tumor growth in *in vivo* [29,30] experiments. In addition, both *in vitro* [31,32] and *in vivo* [32] work has implicated *MAN2C1* in T-cell functioning, and yeast two hybrid assays have shown that it interacts with an immune-system related gene, HLA-B associated transcript 2 (*BAT2*) [33]. Both gene expression [17–19] and methylation [16] studies of PTSD have indicated that dysregulation in immune system related genes in peripheral blood are associated with this disorder. Together with these earlier gene expression results, our study provides additional, specific evidence of this immune-related gene's importance in developing PTSD, and suggests more generally that both gene expression and epigenetic changes at specific loci are associated with this disorder.

Our detection of a significant interaction between *MAN2C1* methylation and participants' exposure to potentially traumatic events adds to a small but growing literature examining the joint and interacting ways in which molecular factors and cumulative traumatic burden may modify risk for PTSD. Recent work by Kolassa and colleagues [34,35] has focused on survivors of the Rwandan genocide and risk for PTSD in light of genetic variation at two distinct loci: the serotonin

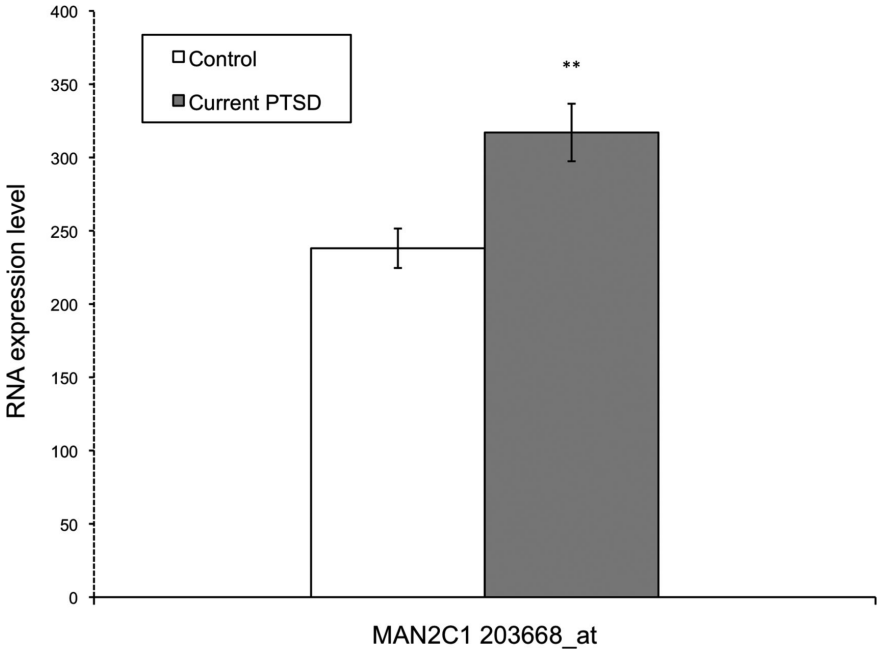

Fig. 2. Gene expression values of *MAN2C1* among those with ($n = 15$) vs. without ($n = 20$) PTSD in [19]. Figure is adapted from data presented in Table 2 of [19]. $^{**} = p < 0.01$ PTSD vs. control.

transporter (*SLC6A4*) locus [34] and the catechol-O-methyltransferase (COMT) locus [35]. Although the prevalence of PTSD approached 100% when traumatic exposure reached extreme levels in both studies, those homozygous for the short *SLC6A4* promoter allele showed 100% probability of PTSD independent of number of traumatic events [34]; and those homozygous for the Met *COMT* allele showed a high risk of PTSD even with small traumatic loads [35]. These results suggest that molecular variation can moderate risk for PTSD among those exposed to similar numbers of traumatic events. Results presented here add a new dimension to these findings, in that the molecular variation under consideration is methylation level, rather than genotype: at low levels of *MAN2C1* methylation, there appears to be little variation in risk for PTSD among those exposed to lower vs. higher numbers of PTEs (Fig. 1); at higher *MAN2C1* methylation levels, however, the risk of PTSD is exponentially higher among those with greater numbers of PTEs. Still unknown is the role that both genetic and gene expression variation play in moderating risk of PTSD at this locus. Future work considering the combined and interacting effects of genetic, epigenetic, and gene expression variation on risk for PTSD are warranted.

It is notable that the remaining 32 loci implicated in the two gene expression studies examined in this work did not demonstrate significant methylation x PTE interactions in predicting risk of PTSD. These results raise the possibility that epigenetic modifications may not play a substantial role in the molecular biology of PTSD; however, since power may have been an issue in this study (discussed further in the limitations section below), additional work with larger samples will be required to evaluate this hypothesis. Equally likely is the possibility that we may have missed assessing methylation at CpG sites relevant to gene function by relying on CpG sites represented on the HM27 BeadChip. More specifically, within any single gene, methylation of some CpG sites may be unimportant to gene function and thus lack relevance to the phenotype under investigation. For example, in the focused study of *SLC6A4* methylation differences associated with depressive symptoms discussed in the introduction, *in vitro* expression analyses showed that partial methylation of just four targeted sites within the promoter region of this gene was adequate to reduce gene expression to levels obtained through complete methylation of the entire promoter region [12]. These results suggest that methylation of the remaining CpG sites in the promoter is unrelated to gene function. Notably, in this example, there was no association observed between the four gene expression-relevant CpG sites and depression [12], emphasizing the complexity of pinpointing

gene-expression relevant epigenetic signatures that are also relevant to complex mental illness.

Furthermore, epigenetic regulation of gene expression is a complex biological phenomenon that is still being worked out in basic biological studies. In general, methylation and gene expression are typically believed to show an inverse correlation, such that increased methylation corresponds to decreased gene expression and vice-versa. Recent work, however, has demonstrated a more complicated picture. A genome-wide assessment of quantitative trait loci (QTL) that assessed SNP variation in association with either methylation or gene expression QTLs showed that, in contrast to the classical prediction of an inverse correlation between methylation and gene expression patterns at specific loci, almost half of the significant methQTLs and expression QTLs correlated in the same direction [36]; however, when joint meth-and expression QTLs involved a methylation site falling within a CpG island – which occurred in only a minority of cases – the classical inverse pattern was observed [36]. On a more clinically relevant note, the genome-wide scan of methylation differences in the rat model of PTSD discussed in the introduction identified four total genes that showed PTSD-associated methylation differences [15]; however, only one–*Dlgap2* –showed altered gene expression between rats with vs. without PTSD-like phenotypes. Thus, just as some loci show PTSD-associated methylation, but not gene expression, differences, the converse is also true: there are likely to be genes that show PTSD-associate gene expression, but not methylation, differences.

Our study must be interpreted in light of a number of limitations. First, although we were able to identify PTSD-associated epigenetic signatures in genes previously implicated in PTSD dysregulation via gene expression studies, the ideal study would pair gene expression and methylation (or other epigenetic) data from the same individuals, in order to more robustly assess the correspondance between gene expression and methylation data with respect to PTSD. Studies to address this issue are currently underway. Second, in relying upon previous studies of gene expression data, we recognize that the analytic approaches used to identify differentially expressed genes differed between studies, such that the candidate gene list we used to investigate PTSD-associated epigenetic differences was arrived at by heterogeneous methods. In addition, by constraining our inquiry only to genes that have been implicated in whole blood-derived gene expression studies, we are undoubtedly missing other epigenetic signatures relevant to this disorder that have been implicated in other work (i.e. genotype-based candidate gene studies in humans and animal models of PTSD, e.g. [6,15]). We thus consider our approach to be only an initial, limited assessment of epigenetic signatures associated with this disorder. Third, although one of the two gene expression studies was genome-wide [19], the epigenetic study with which it was paired in this work assessed methylation levels at only 14,500 genes – the largest number available to assess via microarray to date, but not completely genome-wide in coverage. As such, we could not analyse some loci that were represented on the HG U133 Plus2 Array but not on the HM27 Beadchip (e.g. *HLA-DRB1*, *TMEM167A*). Fourth, although we included 100 individuals with and without lifetime PTSD – greater than the number assessed in either of the gene expression studies – we were likely underpowered to detect methylation x PTE interactions at some of the candidate genes tested in this study. Although the optimal sample size for epigenomic studies is currently unknown [37], our own estimates suggest that our power to detect an interaction in this study was roughly 0.24, far lower than the typical study ideal of 0.8 or greater. Finally, although we did detect a significant interaction for *MAN2C1*, the p value did not meet the criteria for statistical significance when accounting for our multiple comparisons across genes. Nevertheless, the p value far exceeds the more liberal p-value cutoffs ($p < 0.2$) sometimes adopted for G x E interactions (e.g. [38]). Replication studies are clearly needed in order to address these several study limitations.

Despite these limitations, our work takes a first step towards demonstrating that coordinated gene expression and epigenetic changes may offer potential molecular signatures of increased risk for and resilience to this disorder. Our primary result stemming directly from the analyses conducted in this work – that *MAN2C1* methylation level modifies traumatic burden to predict risk of lifetime PTSD – also helps to shed light on the biological correlates of individual differences, thereby helping to explain why some people develop PTSD in the face of exposure to PTEs, while others do not [4, 24]. Ongoing work assessing genotype, gene expression, and methylation differences between individuals with vs. without this disorder should provide a more robust characterization of the molecular variation underlying phenotypic variation following PTE exposure at *MAN2C1* and other loci.

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