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Transcriptional control of monocyte gene expression in post-traumatic stress disorder

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Abstract. Post-traumatic stress disorder (PTSD) confers an increased risk for disorders with an inflammatory etiology. PTSD-related dysregulation of the sympathetic nervous system (SNS) and hypothalamic-pituitary adrenal (HPA) axis and associated alterations in inflammatory activity may contribute to this increased risk. However, little is known about convergent SNS, HPA and inflammatory signaling at the level of the immune cell transcriptome in PTSD. To explore such signaling, we examined the prevalence of specific transcription factor binding motifs in the promoter regions of differentially expressed genes in monocytes from individuals with PTSD and matched controls. Participants included 49 men (24 PTSD+ and 25 trauma-exposed controls) and 18 women (10 PTSD+ and 8 controls). Men with PTSD showed up-regulation of target genes for the NF-κB/Rel family of transcription factors, which convey inflammatory signals, up-regulation of target genes for CREB/ATF transcription factors, which convey adrenergic signals from the SNS, and down-regulation of target genes for the glucocorticoid receptor, which conveys glucocorticoid signals from the HPA axis. Women with PTSD also showed significant up-regulation of target genes for NF-κB and non-significant down-regulation of target genes for GR, but significant down-regulation of target genes for CREB/ATF. Altered transcriptional control of monocyte gene expression could contribute to exaggerated inflammatory activity in PTSD.

1. Introduction

Post-traumatic stress disorder (PTSD) is associated with substantially increased risk for chronic diseases such as neurodegenerative disorders, cardiovascular disease, diabetes, asthma, and arthritis [8,10,49, 52,59]. Importantly, this PTSD-related increased risk for disease does not appear to be accounted for by potential confounds and mediators such as family history, smoking, obesity, alcohol dependence, or depression [5]. PTSD also confers more than two-fold higher risk for early mortality [5,7], and the increased risk for early mortality associated with PTSD is comparable to that of more traditional disease indicators such as elevated white blood cell count and erythrocyte sedimentation rate [6]. Although the biological underpinnings of PTSD are poorly understood, dysregulation of immune system processes could partially mediate PTSD-related increased risk for disease.

Elevated inflammatory activity is increasingly recognized as a potential pathway to PTSD-related increased risk for physical disease. Diseases that are more common in individuals with PTSD share an inflammatory etiology [4,59], and preliminary evidence indicates that PTSD is associated with increased levels of pro-inflammatory proteins including cytokines and C-reactive protein [22,53,57]. Moreover, a large body of research indicates that PTSD is associated with dysregulation of two systems that are known to influence inflammatory activity, specifically the sympathetic nervous system (SNS) and the hypothalamic-pituitary adrenal (HPA) axis [27,50,51]. However, the precise nature of this dysregulation remains incompletely un-

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derstood with some studies reporting up-regulation, some down-regulation and some equivalent levels of SNS and HPA axis activity in individuals with PTSD compared with controls [23,40,44,56,63].

Most, but not all, studies have documented increased SNS activity in PTSD, as indexed by increased sympathetic and decreased parasympathetic tone and elevated levels of peripheral catecholamines [40,56,65]. A more complex story has emerged regarding HPA axis activity. PTSD has generally been associated with elevated levels of corticotrophin-releasing hormone (CRH), indicating increased activation of the HPA axis [25,38], but with lower or equivalent levels of the glucocorticoid hormone cortisol, indicating decreased activation of the HPA axis [37,64]. A widely validated explanation for these conflicting findings is found in studies reporting enhanced negative feedback inhibition by cortisol in individuals with PTSD [38,43,62]. This enhanced negative feedback inhibition is thought to be mediated through higher responsiveness or sensitivity of glucocorticoid receptors (GR) [60], and is consistent with the observed pattern of elevated CRH in combination with lower cortisol. This theoretical model also underlines the importance of examining signaling pathways involved in regulating the activity of cells, including immune cells, in PTSD.

The activity of immune cells is mediated by transcription factors that bind to DNA and regulate the expression of genes. The nuclear factor-κB (NF-κB)/Rel family of transcription factors plays a crucial role in regulating the expression of genes involved in the inflammatory response by transmitting receptor signals to the nucleus [1,21,41]. The first class of NF-kB/Rel transcription factors includes NF-κB1 (p50) and NF-κB2 (p52), which can only regulate transcription when they form dimers with members of the second class of NF-κB/Rel transcription factors. The second class includes RelA (p65), RelB and c-Rel, which can directly activate transcription of many genes, including those involved in inflammatory activity. NF-κB/Rel transcription factors are activated by viruses, gram-negative bacteria and inflammatory cytokines [58], and also in response to psychological stress [2]. Inflammatory activity is additionally subject to regulation by catecholamines and glucocorticoids, which bind to adrenergic receptors and GR on immune cells respectively [15,16]. Such binding is followed by signaling to the transcriptome by associated transcription factors including cyclic adenosine monophosphate response element binding protein (CREB) and activating transcription factor (ATF), which convey adrenergic (and other) signals, and GR, which conveys glucocorticoid signals. Observed elevations in inflammatory activity in PTSD may thus be due to altered signaling by NF-κB/Rel, CREB/ATF and GR in immune cells. However, little is known about alterations in these transcriptional control pathways in PTSD.

In the present study, we analyzed monocyte gene expression data obtained from patients with PTSD and control participants using the Transcription Element Listeining System (TELiS) [13]. Monocytes were chosen for these gene expression analyses because they are influenced by psychosocial factors [19,31], bear receptors for catecholamines and glucocorticoids [20], and respond to immune challenges by producing inflammatory proteins [33]. TELiS permits examination of the promoter regions of differentially expressed genes for enrichment with transcription factor binding motifs (TFBMs) or response elements for specific transcription factors (see Fig. 1 for overview). We specifically examined if differentially expressed genes in monocytes from patients with PTSD were significantly enriched with response elements for NF-κB, CREB/ATF and GR in separate male and female samples.

2. Methods

2.1. Sample

Medically healthy participants were recruited from the Bay Area community via advertisements in public settings and from the San Francisco Veterans Affairs Medical Center. Participants included 49 men ($n = 24$ PTSD+; $n = 25$ PTSD- Control) and 18 women ($n = 10$ PTSD+; $n = 8$ PTSD- Control). Diagnoses were made by trained interviewers using structured clinical interviews. Controls were age matched and in the case of the male sample, were also matched on trauma exposure. Baseline laboratory tests included a complete blood count, serum chemistry panel, liver and thyroid function tests, serology for Hepatitis B & C, urine toxicology screen, and urine pregnancy test (if appropriate). Tests indicated that all participants were medication free, medically healthy and without current infection. Participants had normal white blood cell counts, no elevations in body temperature, negative serology for HIV and hepatitis, and no current substance use disorders. All participants provided written informed consent and the study protocol was approved by the Committee on Human Research at the University of California, San Francisco and at the San Francisco Veteran's Affairs Medical Center.

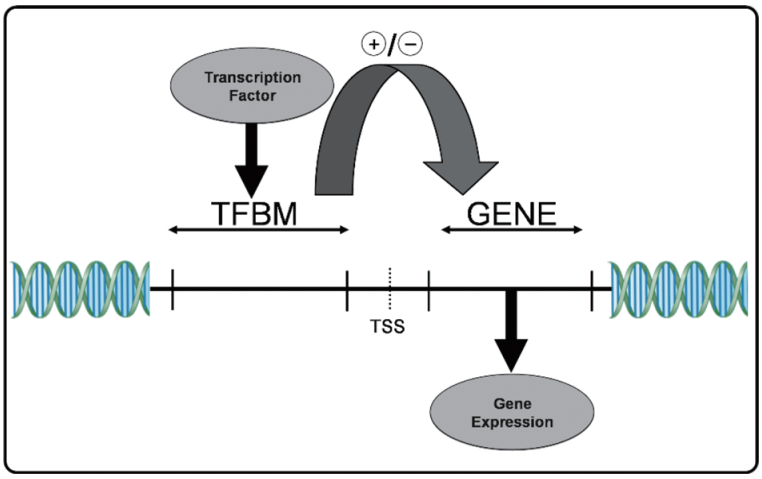

Fig. 1. Promoter regions of genes include DNA sequences called response elements or transcription factor binding motifs (TFBMs) that are specific to particular transcription factors. Binding of transcription factors to these TFBMs can inhibit or promote expression of a gene, ultimately determining the activity of the cell. The Transcription Element Listening System (TELiS) [13] uses bioinformatic techniques to examine the promoter regions of differentially expressed genes for enrichment with TFBMs for specific transcription factors of interest.

2.2. Measures

Self-report questionnaires were used to determine demographic details. A structured clinical interview was used to assess lifetime and current anxiety and mood disorders, PTSD and history of stressful life events. Interview assessments included the Structured Clinical Interview for DSM-IV, Patient edition (SCID-P) to assess mood disorders, substance use disorders, and anxiety disorders [17], and the Clinician Administered PTSD Scale (CAPS) to assess trauma exposure as well as presence and severity of PTSD [3]. Trained interviewers diagnosed all patients and weekly case consensus meetings were used to confirm diagnoses.

2.3. Monocyte gene expression analysis

For monocyte gene expression analysis, sixty milliliters of blood was drawn into cell preparation tubes (Becton Dickinson) and centrifuged to enrich for monocytes by magnetic separation using MACS CD14 microbeads (Miltenyi Biotech, Auburn, CA). Total RNA was isolated from CD14+ monocytes using Qiagen RNeasy Micro Kit (Qiagen, Valencia, CA) following the manufacturer's protocol and stored at −70C. The purity and concentration of the RNA was determined with spectrophotometer, and the integrity of the RNA was determined with an RNA picochip on an Agilent Bioanalyzer 2100 (Agilent Technologies Inc, Palo Alto, CA). CodeLink Human Whole Genome BioArrays were used to identify genes differentially expressed in CD14+ monocytes between participants with PTSD and controls [48]. The BioArrays have a total of 54,841 probes of which 83% of the represented genes are clearly annotated (based on unique UniGene IDs), 39% well annotated (categorized to a gene ontology) and 53% have been mapped to specific chromosome locations. One half microgram of total RNA isolated from CD14+ monocytes was amplified and labeled with biotin-11-UTP (Perkin Elmer, Boston, MA) using the CodeLink iExpress iAmplify Kit (AMI). Ten micrograms of fragmented cRNA were hybridized to microarrays using the procedures suggested by the manufacturer. The arrays were scanned on an Axon GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA). Image analysis and data extraction were performed by CodeLink Expression Software Kit v4.1 (AMI). Following standardization and normalization of the raw data using loess normalization in R/BioConductor, differentially expressed genes from the PTSD and control subjects were evaluated for fold change.

2.4. Transcriptional control pathways analysis

The GeneSpring GX 7.3 software package (Agilent, Santa Clara, CA) together with the Bioconductor [18] suite of R packages [54] were used for microarray data analysis [39]. To examine if differential monocyte gene expression in participants with PTSD might stem from differences in upstream inflammatory, adrenergic or glucocorticoid transcriptional activity, we performed bioinformatics analysis of promot-

er DNA sequences using TELIS software (http://www.telis.ucla.edu/) [13]. Differentially expressed genes were identified as those that were significantly different between participants with PTSD and controls on Student's t-test ($p < 0.05$) with at least 30% difference in levels of expression. Using the transcriptional shift analysis variant of TELiS, we examined differential expression of genes bearing response elements for specific response elements included in the TRANSFAC database [26]. Analyses for the NF-κB/Rel family of transcription factors were focused on response elements for NF-κB/Rel transcription factors that can independently convey pro-inflammatory signals, specifically V$NFKAPPAB65_01 for RelA (p65) and V$CREL_01 for c-Rel. Analyses for the CREB/ATF family of transcription factors were focused on V$CREB_01 for CREB-1 and V$ATF_01 for ATF-1, which are elements responsive to adrenergic signals from catecholamines [45]. Analyses for GR were focused on V$GR_Q6, a response element for GR-α and GR-β. In exploratory analyses, we examined differential expression of genes bearing response elements for other NF-κB/Rel and CREB/ATF transcription factors. Analyses used aggregate indices that had been pooled across 9 different technical specifications involving variations of promoter length and TFBM match stringency. Differential representation of TFBMs was tested using an independent sample t test with Welch's correction for heteroscedasticity.

3. Results

3.1. Male sample characteristics

The sample of men with PTSD (PTSD+) and control participants were comparable with regard to age (PTSD+ M Age = 30, $SD = 6$; Control M Age = 30, $SD = 6$; $p = 0.85$) and marital status (PTSD+ 50% single, 25% married and 25% divorced; Control 56% single, 32% married and 12% divorced; $p = 0.32$). Overall, the male sample was ethnically heterogeneous, including African American ($n = 5$), Asian ($n = 4$), Caucasian ($n = 26$), Hispanic ($n = 8$), Middle Eastern ($n = 2$), Pacific Islander ($n = 2$), and mixed race ($n = 2$) participants in both PTSD+ and control groups. There were no significant group differences in ethnicity ($p = 0.68$). More detailed demographic information can be found in [39]. Trauma exposure and PTSD-related information is presented in Table 1 where it can be seen that men with PTSD and control participants had been exposed to similar categories of trauma. Further, it can be seen that men with PTSD had significantly higher current scores on the CAPS than control participants. All control participants were negative for a lifetime diagnosis of PTSD.

4. Male transcriptional control pathways

To examine if differentially expressed genes were significantly enriched with specific response elements of interest, we used TELiS to search for NF-κB, CREB/ATF and glucocorticoid binding motifs in the promoter regions of differentially expressed genes. Using a cutoff point of 1.3 fold difference in gene expression levels, 13 up-regulated and 53 down-regulated genes were included in analyses comparing male participants with PTSD with age- and sex-matched controls (Supplementary Table 1 and cf. [39]).

Among men with PTSD, there was significant up-regulation of genes with response elements for the NF-κB/Rel transcription factors, RelA (1.55-fold difference in V$NFKAPPAB65_01, SE = 0.22, $p = 0.01$) and c-Rel (1.42-fold difference in V$CREL_01, SE = 0.13, $p = 0.004$). Second, there was significant up-regulation of genes with response elements for CREB-1 (1.50-fold difference in V$CREB_01, SE = 0.18, $p = 0.006$) and ATF-1 (1.79-fold difference in V$ATF_01, SE = 0.29, $p = 0.005$). Finally, there was significant down-regulation of genes with response elements for GR-α and GR-β (0.73-fold difference in V$GR_Q6, SE = 0.06, $p = 0.01$). Figure 2a illustrates these findings.

In exploratory analyses, we also found significant up-regulation of genes with response elements for NF-κB, NF-κB1, NF-κB2 (1.35-fold difference in V$NFKB_C, SE = 0.15, $p = 0.04$) and up-regulation of genes with response elements for three additional CREB/ATF transcription factors (2.46-fold difference in V$CREBP1CJUN_01, SE = 0.56, $p = 0.002$; 1.84-fold difference in V$CREB_Q4, SE = 0.40, $p = 0.01$; 1.38-fold difference in V$CREB_Q2, SE = 0.17, $p = 0.02$).

5. Female sample characteristics

Female PTSD+ participants were age-matched with control participants. The final sample of female PTSD+ and control participants were comparable with regard to age (PTSD+ M Age = 30, $SD = 7$; Control M Age = 27, $SD = 6$; $p = 0.33$) and non-significantly

Table 1
Clinical characteristics of male and female samples

	Male			Female		
	PTSD+ ($N = 24$)	Control ($N = 25$)	p	PTSD+ ($N = 10$)	Control ($N = 8$)	p
Trauma Exposure	0					
Combat	16 (67)	12 (48)		0	0	
Physical abuse/assault	6 (25)	5 (20)		6 (60)	0	
Sexual abuse/assault	1 (4)	0		4 (40)	1 (12)	
Accident	0	2 (8)		0	1 (12)	
Robbery	1 (4)	2 (8)		0	0	
Other	0	4 (16)	0.25	0	0	0.04*
Current CAPS M (SD)	57 (15)	4 (6)		64 (18)	2 (3)	
Intrusion	15 (5)	1 (3)		16 (6)	0	
Avoidance	19 (7)	1 (2)		26 (11)	0	
Hyperarousal	22 (7)	2 (3)	0.000*	21 (4)	2 (3)	0.001*
Lifetime CAPS M (SD)	n/a	8 (10)		n/a	13 (18)	
Intrusion		4 (6)			9 (13)	
Avoidance		1 (2)			0	
Hyperarousal		3 (1)	n/a		4 (4)	n/a

Notes. n/a indicates not applicable and * denotes statistical significance at $p < 0.05$. P values are based on Student's t-tests for continuous data and on Mann Whitney U tests for categorical data. For trauma exposure, numbers refer to n (%). The mean current and lifetime CAPS scores for females are based on only two participants who had experienced a traumatic event fulfilling Criterion A of the CAPS measure.

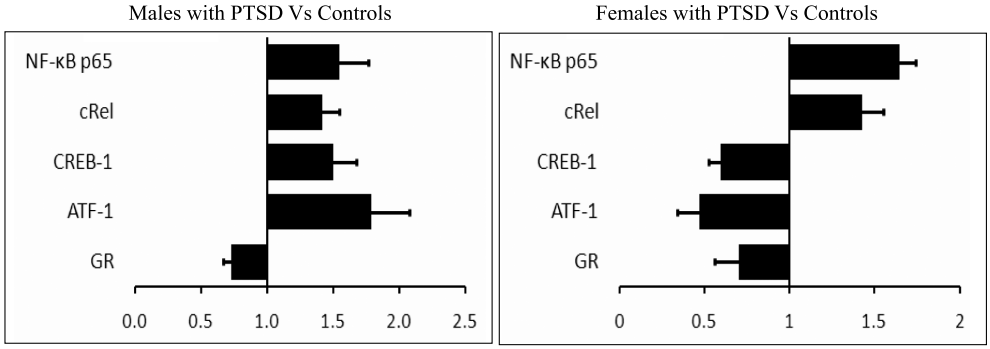

Fig. 2. Fold-difference in the prevalence of response elements for NF-κB/Rel, CREB/ATF and GR in the promoter regions of genes differentially expressed in men and women with PTSD compared with controls. Results indicate significant up-regulation of genes with response elements for NF-κB/Rel and CREB/ATF and significant down-regulation of genes with response elements for GR in men with PTSD compared with age-, sex- and trauma-matched controls ($p < 0.05$; Fig. 2a). Results also indicate significant up-regulation of genes with response elements for NF-κB/Rel, but significant down-regulation of genes with response elements for CREB/ATF in women with PTSD compared with age- and sex-matched controls ($p < 0.05$). Finally, genes with response elements for GR tended to be down-regulated in women with PTSD, but this effect was not significant ($p = 0.11$; Fig. 2b).

different on marital status (PTSD+ 70% single, 20% married and 10% divorced; Control 75% single and 25% married; $p = 0.57$). Both groups were ethnically heterogeneous, including African American, Asian and White participants in the PTSD+ group and Asian and White participants in the control groups. There were no significant group differences in ethnicity ($p = 0.67$). More detailed demographic information can be found in [39]. Trauma exposure and PTSD-related information is presented in Table 1 where it can be seen that only one control participants had been exposed to traumatic events of sufficient severity to fulfill Criterion A1 and A2 for assessment of current PTSD symptoms. No control participants fulfilled diagnostic criteria for current or lifetime PTSD.

6. Female transcriptional control pathways

Using a cutoff point of 1.3 fold difference in gene expression levels, 36 up-regulated and 16 down-regulated genes were included in analyses comparing female par-

ticipants with PTSD with age- and sex-matched controls (Supplementary Table 2 and cf. [39]).

Among women with PTSD, there was significant up-regulation of genes with response elements for the NF-κB/Rel family of transcription factors, RelA (1.65-fold difference in V$NFKAPPAB65_01, SE = 0.10, $p < 0.001$) and c-Rel (1.43-fold difference in V$CREL_01, SE = 0.13, $p = 0.008$). Second, there was significant down-regulation of genes with response elements for CREB-1 (0.60-fold difference in V$CREB_01, SE = 0.07, $p = 0.002$) and ATF-1 (0.47-fold difference in V$ATF_01, SE = 0.13, $p = 0.002$). Although there were no significant differences in expression of genes bearing response elements for GR, expression of such genes tended to be down-regulated in women with PTSD compared with controls (0.70-fold difference in V$GR_Q6, SE = 0.14, $p = 0.11$). Figure 2b illustrates these findings.

In exploratory analyses, we also found significant up-regulation of genes with response elements for NF-κB1 (2.06-fold difference in V$NFKAPPAB50_01, SE = 0.30, $p = 0.01$; 1.38-fold difference in V$NFKAPPAB_01, SE = 0.18, $p = 0.04$). Second, there was significant down-regulation of genes with response elements for additional CREB/ATF transcription factors (0.39-fold difference in V$CREBP1_Q2; SE = 0.11, $p = 0.005$).

7. Discussion

The present study represents the first demonstration of altered transcriptional control of immune cell gene expression in PTSD. Compared with trauma-exposed controls, men with PTSD had significant up-regulation of target genes for NF-κB and CREB/ATF and significant down-regulation of target genes for GR. These results indicate increased inflammatory and adrenergic signaling in conjunction with decreased glucocorticoid signaling in men with PTSD. Comparing our small sample of women with PTSD with the mixed sample of trauma-exposed and non-trauma-exposed controls, we found significant up-regulation of target genes for NF-κB, but significant down-regulation of target genes for CREB/ATF and non-significant down-regulation of target genes for GR. These results indicate increased inflammatory and decreased adrenergic signaling in women with trauma exposure and PTSD. Differences in CREB/ATF signaling in males versus females could be due to gender effects on these systems or due to differences in characteristics of our control groups. Overall, our results indicate that PTSD is associated with altered transcriptional control of monocyte gene expression, specifically with increased inflammatory signaling that could plausibly contribute to increased physical disease risk in PTSD.

Recent demonstrations of elevated inflammatory activity in PTSD have highlighted inflammatory activity as a potential mechanism accounting for increased physical disease risk [22,53,57]. However, not all studies have reported elevated inflammatory activity in individuals with PTSD [24]. Further, some of the largest studies that have demonstrated this association have included control participants who were not trauma exposed and the samples have included pooled groups of men and women with PTSD [22,53]. Primary analysis of the specific genes differentially expressed in the present sample was not suggestive of elevated inflammatory activity in men with PTSD [39]. However, the present analysis indicates that both men and women with PTSD demonstrate up-regulation of target genes for the transcription factors RelA and c-Rel from the NF-κB family, indicating elevated pro-inflammatory signaling to monocytes in these samples with PTSD. Given matching for trauma exposure in the male sample, the data also provide preliminary evidence that PTSD is associated with increased inflammatory signaling over and above trauma exposure, at least in men. Inflammatory activity has been causally implicated in the development of chronic diseases including cardiovascular disease, autoimmune disorders and cancer, as well as neurodegenerative diseases and some forms of major depressive disorder [9,14,32,36,47]. Thus, elevated inflammatory activity in PTSD could increase risk for both physical and mental disorders.

The finding that target genes for CREB/ATF were up-regulated in men with PTSD compared with trauma-exposed controls is in line with a large body of research indicating increased SNS activation in PTSD [40,65]. Our data showing increased CREB/ATF signaling in men with PTSD is consistent with prior observations of increased SNS activity and elevated levels of circulating catecholamines [56]. However, we found that target genes for CREB/ATF were significantly down-regulated in women with trauma exposure and PTSD, indicating decreased CREB/ATF signaling in monocytes in the female sample. Sex hormone differences may influence stress effects on CREB and thereby influence the expression of target genes for CREB/ATF [28,29,55]. However, it is also possible that differences in trauma exposure between the male and female control groups contributed to the findings.

Regardless of why differences in expression of target genes for CREB/ATF occurred in the male and female samples, the difference in CREB/ATF signaling between the two samples may have important implications for the regulation of inflammatory activity in PTSD. Previous research indicates that a mixed sample of men and women with trauma exposure and PTSD demonstrated elevation of pro-inflammatory cytokines as well as elevation of levels of the anti-inflammatory cytokine interleukin-10 (IL-10), but it was not clear if both male and female participants showed this effect [22]. Binding of CREB/ATF in monocytes may contribute to this up-regulation of IL-10 production [45, 46]. Because IL-10 has immunosuppressive and anti-inflammatory properties [35], this process could attenuate stress-related inflammatory activity and reduce risk for inflammatory disease. Thus, increased CREB/ATF signaling may contribute to regulating PTSD-related increases in inflammatory activity.

Our analysis indicated significant down-regulation of target genes for GR in men with PTSD and non-significant down-regulation of target genes for GR in women with trauma exposure and PTSD. As an index of lower glucocorticoid signaling to the monocyte transcriptome in PTSD, this finding is in line with a substantial body of research showing lower circulating levels of cortisol in PTSD [37,60,63,67]. However, an almost equally large body of previous research supported the hypothesis of enhanced negative feedback or enhanced GR sensitivity in PTSD [42,61,66,68]. While it is difficult to draw firm conclusions about such GR sensitivity without knowing the level of circulating cortisol in our participants, our data are not supportive of greater GR sensitivity on monocytes in PTSD.

The present paper highlights the utility of analyzing gene expression data at multiple levels. In our previous analysis of the present data, we were interested in the expression of individual genes and gene ontologies and therefore applied a stringent threshold, examining only genes differentially expressed $\geqslant 50\%$ between groups. The results showed a general decrease in expression of all genes in monocytes in the male sample, and no evidence of increased inflammation [39]. In the present study, we were less interested in individual gene probes and more interested in upstream patterns of transcriptional control by a limited number of *a priori* selected specific transcription factors involved in inflammatory, adrenergic and glucocorticoid signaling. Thus, in the present research, we used a more permissive cutoff and included genes differentially expressed $\geqslant 30\%$ between groups. While the earlier analysis indicated a general pattern of down-regulation of genes in monocytes, the present analysis indicates a propensity towards pro-inflammatory signaling in both men and women with PTSD. Further research will be necessary to shed light on how such patterns of gene expression and signaling are manifest at the level of circulating proteins. Taken together, these data underscore the importance of decisions regarding stringency levels in high-throughput data analytic techniques, and additionally highlight the value of employing analysis at different levels in research examining the influence of psychosocial factors on biological mediators of disease [11].

The major limitations of our study relate to our female sample and include the small sample size as well as the mixed sample of trauma exposed and unexposed controls. We consider data on this female sample preliminary and in need of replication. However, findings in relation to our male sample must also be interpreted in the context of some limitations. First, while males with PTSD were matched with controls for trauma exposure, it is difficult to conclude with certainty that the men with PTSD and controls experienced trauma equivalent in severity or duration. Second, the present analysis would benefit from the availability of data on circulating levels of the various proteins of interest to our analysis – specifically levels of pro- and anti-inflammatory cytokines, catecholamines and cortisol – in both male and female samples. Third, while we believe that the focus on a homogenous population of immune cells is a major strength of our research, the present findings may not apply to other immune cell populations. Overall, the results suggest a research agenda to address questions unanswered by this study. First, replication of the present research in a larger sample of women with PTSD and age-, sex- and trauma-matched controls would help to shed light on the relative impact of sex and trauma exposure on the divergent CREB/ATF signaling patterns observed between male and female samples in our study. Second, the relationship between severity of PTSD and signaling to immune cells could be addressed by conducting prospective studies that involve repeated assessments of PTSD symptom severity or non-pharmacological treatment of PTSD to avoid medication confounds. Another potentially worthwhile avenue of research is to examine *in vitro* adrenergic and glucocorticoid regulation of gene expression in monocytes from individuals with and without PTSD.

To our knowledge, the present analysis provides the first ever demonstration of altered transcriptional control of monocyte gene expression in PTSD. Our da-

ta suggest that men with PTSD have increased NF-κB signaling, increased CREB/ATF signaling and reduced GR signaling to the monocyte transcriptome. Our data additionally suggest that women with trauma exposure and PTSD have increased NF-κB signaling and decreased CREB/ATF signaling to the monocyte transcriptome. These findings contribute to a growing body of literature showing altered transcriptional control pathways in association with chronic psychological stress and psychiatric symptoms [12,30,34]. The findings also shed light on how the complex patterns of biological dysregulation in PTSD are manifest at the level of the monocyte. In sum, the data provide evidence that altered transcription factor signaling mechanisms in monocytes could contribute to elevated inflammatory activity in PTSD.

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Supplementary material

Supplementary data can be found on:
www.aoifeodonovan.net

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