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ORIGINAL RESEARCH ARTICLE

Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors

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Trauma survivors show marked differences in the severity and persistence of post-traumatic stress disorder (PTSD) symptoms. Early symptoms subside in most, but persist as acute and chronic PTSD in a significant minority. The underlying molecular mechanisms or outcome predictors determining these differences are not known. Molecular markers for identifying any mental disorder are currently lacking. Gene expression profiling during the triggering and development of PTSD may be informative of its onset and course. We used oligonucleotide microarrays to measure peripheral blood mononuclear cell (PBMC) gene expression of trauma survivors at the emergency room and 4 months later. Gene expression signatures at both time points distinguished survivors who met DSM-IV diagnostic criteria for PTSD at 1 and 4 months, from those who met no PTSD criterion. Expression signatures at both time points correlated with the severity of each of the three PTSD symptom clusters assessed 4 months following exposure among all survivors. Results demonstrate a general reduction in PBMCs' expression of transcription activators among psychologically affected trauma survivors. Several differentiating genes were previously described as having a role in stress response. These findings provide initial evidence that peripheral gene expression signatures following trauma identify an evolving neuropsychiatric disorder and are informative of its key clinical features and outcome. Replications in larger samples, as well as studies focusing on specific markers within the signatures discovered, are warranted to confirm and extend the diagnostic utility and pathogenetic implications of our results.
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Post-traumatic stress disorder (PTSD) is a maladaptive response to life-threatening events, consisting of a symptom triad of re-experiencing of the traumatic event, avoidance and numbing, and increased vigilance and arousal.[1] With a lifetime prevalence of 9–14%,[2,3] PTSD is a common mental disorder.[4] Many survivors exhibit PTSD symptoms at the early aftermath of traumatic events (eg 94% of rape victims[5]), with marked variability in terms of severity and persistence of each of the symptom clusters.[6] Twin data show that each cluster possesses a distinct genetic basis,[7] suggesting they represent discrete biological dimensions. Whereas early symptoms are often transient, a significant minority of survivors remains highly symptomatic exhibiting the full persisting clinical disorder,[2,8–10] marked by a disabling and unremitting longer-term course.[2,9,10] Early treatment might prevent PTSD,[11] but known risk factors[12] and early PTSD symptoms[13] do not effectively predict chronic PTSD, and therefore have limited use in guiding early treatment.

Biological alterations may underlie the onset severity and persistence of PTSD symptoms.[3,4,14] Such alterations are likely to be associated with differential gene transcription, during or after exposure to the triggering event. Acute stress exposure has been shown to induce long-term expression differences in the rat brain for cholinergic[15] and neuroendocrine genes.[16,17] While direct sampling of the brain is not possible in humans, peripheral blood cell gene expression may provide a surrogate indicator of differential response to stress and subsequent PTSD. Supporting this tenet, acute psychological stress is associated with immune activation,[18] and persistent immune alterations have been linked with chronic

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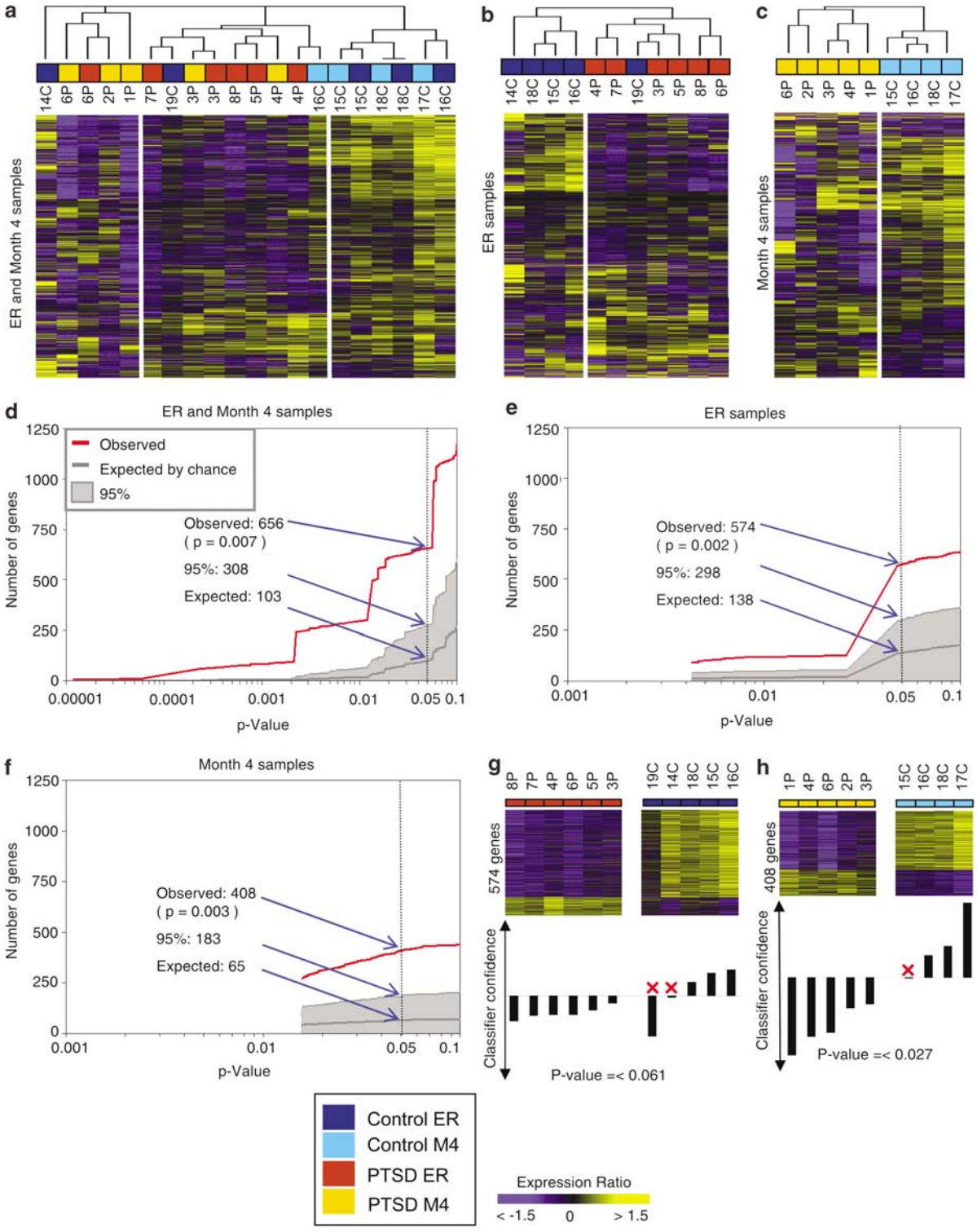
Table 1 Clinical and demographic characteristics of subjects

Variable	*Full consistent PTSD at 1 and 4 months*								*Full PTSD at 4 months*					*No PTSD criterion met at any time*						*No PTSD criterion met by month 4*				
Subject number	1P	2P	3P	4P	5P	6P	7P	8P	9P	10P	11P	12P	13P	14C	15C	16C	17C	18C	19C	20C	21C	22C	23C	24C
M1 PTSD diagnosis[a]	Y	Y	Y	Y	Y	Y	Y	Y	Pa	Pa	Pa	Pa	Pa	N	N	N	N	N	N	Pa	Pa	Pa	Pa	Pa
M4 PTSD diagnosis[b]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N	N	N	N	N	N	N
Gender	2	1	2	1	1	1	2	2	2	2	2	2	2	1	2	1	2	1	2	1	1	2	2	2
Ethnic origin[c]	2	2	2	2	2	2	1	1	2	2	2	3	2	2	1	2	2	2	3	1	2	2	2	2
Age at trauma	56	36	21.5	21	43	21	25	46	29	48	27	33	51	31	22	25	20	23	24	26	19	49	25	25
M4 IES—intrusion[d]	33	15	15	35	19	35	29	35	19	9	10	3	13	1	0	3	3	0	2	3	10	0	3	2
M4 IES—avoidance	17	14	18	34	20	18	19	16	15	14	5	6	13	1	0	0	5	0	11	1	12	0	1	11
M4 IES—arousal	24	15	21	27	23	33	17	35	11	5	14	10	8	3	1	4	5	0	0	1	7	3	1	3
M4 IES—total	74	44	54	96	62	86	65	86	45	28	29	19	34	5	1	7	13	0	13	5	29	3	5	16
Comorbid current psychiatric diagnoses[e]						4, 30, 40	4		4				30								4			
Past psychiatric diagnoses[f]													4, 30								4		4	
Type of trauma[g]	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1
Trauma severity	20	15	13	22	12	26	15	28	14	22	17	22	10	19	9	23	10	10	9	20	7	11	24	7
PBMC samples	M4	M4	ER M4	ER M4	ER	ER M4	ER	ER	M4	M4	M4	ER M4	ER	ER	ER M4	ER M4	M4	ER M4	ER	ER M4	M4	M4	M4	ER M4

[a]Clinical diagnosis 1 month after trauma exposure (Yes = full blown acute PTSD; Partial = subthreshold criteria for acute PTSD; No = no diagnostic criteria for PTSD).
[b]Clinical diagnosis 4 months after trauma exposure (Yes = full blown chronic PTSD; Partial = subthreshold criteria for chronic PTSD; No = no diagnostic criteria for PTSD).
Clinical diagnoses of consistent full acute and chronic PTSD, at 1 and 4 months, respectively.
Clinical diagnoses of chronic PTSD at 4 months, and only partial PTSD at 1 month.
No clinical criteria for PTSD have been met at any time during the 4 months after trauma.
No clinical criteria for chronic PTSD met at 4 months after trauma. At 1 month after trauma, subject met partial PTSD criteria.
[c]Ethnic origin: 1 = Jewish Ashkenazi; 2 = Jewish Sepharadic; 3 = Jewish mixed origin.
[d]IES symptoms: Raw scores, including subscores for intrusive memories, avoidance and increased arousal, and Total score. M4 = 4 months after trauma.
[e]Comorbid current psychiatric diagnoses (current structured clinical interview for axis-I DSM-IV disorders (SCID) diagnoses: 4 = major depressive disorder; 30 = obsessive–compulsive disorder; 40 = body dysmorphic disorder).
[f]Past psychiatric diagnoses (retrospective SCID diagnoses: 4 = major depressive disorder; 30 = obsessive–compulsive disorder; 40 = body dysmorphic disorder).
[g]Type of trauma: 1 = motor vehicle accident and 2 = other.

PTSD.[19,20–22] Additionally, recent microarray studies in human CNS disorders (multiple sclerosis, stroke and seizure) as well as rodent models of such disease suggest specific gene expression signatures in PBMCs.[23,24]

Microarrays allow high-throughput gene expression profiling of transcriptional reactivity. Applied to PBMCs, they may detect signatures of biological processes that underlie adaptive and pathological reactions to traumatic stress as they unfold over time.



We hypothesized that the transcriptional response of PBMCs will correlate with the development of PTSD among trauma survivors. Here, we provide initial evidence that gene expression patterns evaluated 4 months after trauma identify survivors who either persistently manifested full criteria for acute and chronic PTSD at both 1 and 4 months, respectively, or remained healthy at follow-up. Signatures measured within hours of trauma correlated with later course, and expression patterns at both early and late time points correlated with core symptom trajectories among all survivors.

Materials and methods

Subjects

Included in this study were trauma survivors who were admitted to the emergency room (ER) immediately following a traumatic event (mean time between incident and arrival = 45 ± 130 min) and who either met DSM IV[1] diagnostic criteria for acute and chronic PTSD upon prospective follow-up 1 and 4 months later or did not meet any DSM IV[1] diagnostic criterion at these time point. Subjects with partial criteria for acute PTSD at 1 month were also included for part of the analyses, if they either met full criteria for PTSD by month 4, or improved and showed no formal PTSD criteria by month 4. The study's recruitment and follow-up procedure have been published before.[13] Subjects were considered for inclusion in this study if they were between 18 and 65 years old and had just experienced a life-threatening event meeting DSM IV[1] PTSD criterion A. Subjects were not included if they had head injury, burn injury or serious physical injury, had current or lifetime history of alcohol or illicit drugs abuse, had past or present psychiatric diagnoses other than depressive or anxiety disorders, or had medical or neurological illness that could confound the assessments.

Blood samples and preliminary psychological assessments were obtained at the ER. Comprehensive assessments (see Supplementary Methods) took place 1 week, 1 month and 4 months following trauma exposure. This timing match DSM IV[1] duration requirements for diagnoses of acute PTSD (1 month) and chronic PTSD (ie 4 months).[8] Blood samples were obtained, again, at 4 months. Subjects were compared for type of trauma, trauma severity scores, age, gender, ethnic origin and psychiatric comorbidity (Supplementary Methods and Supplementary Tables A–C).

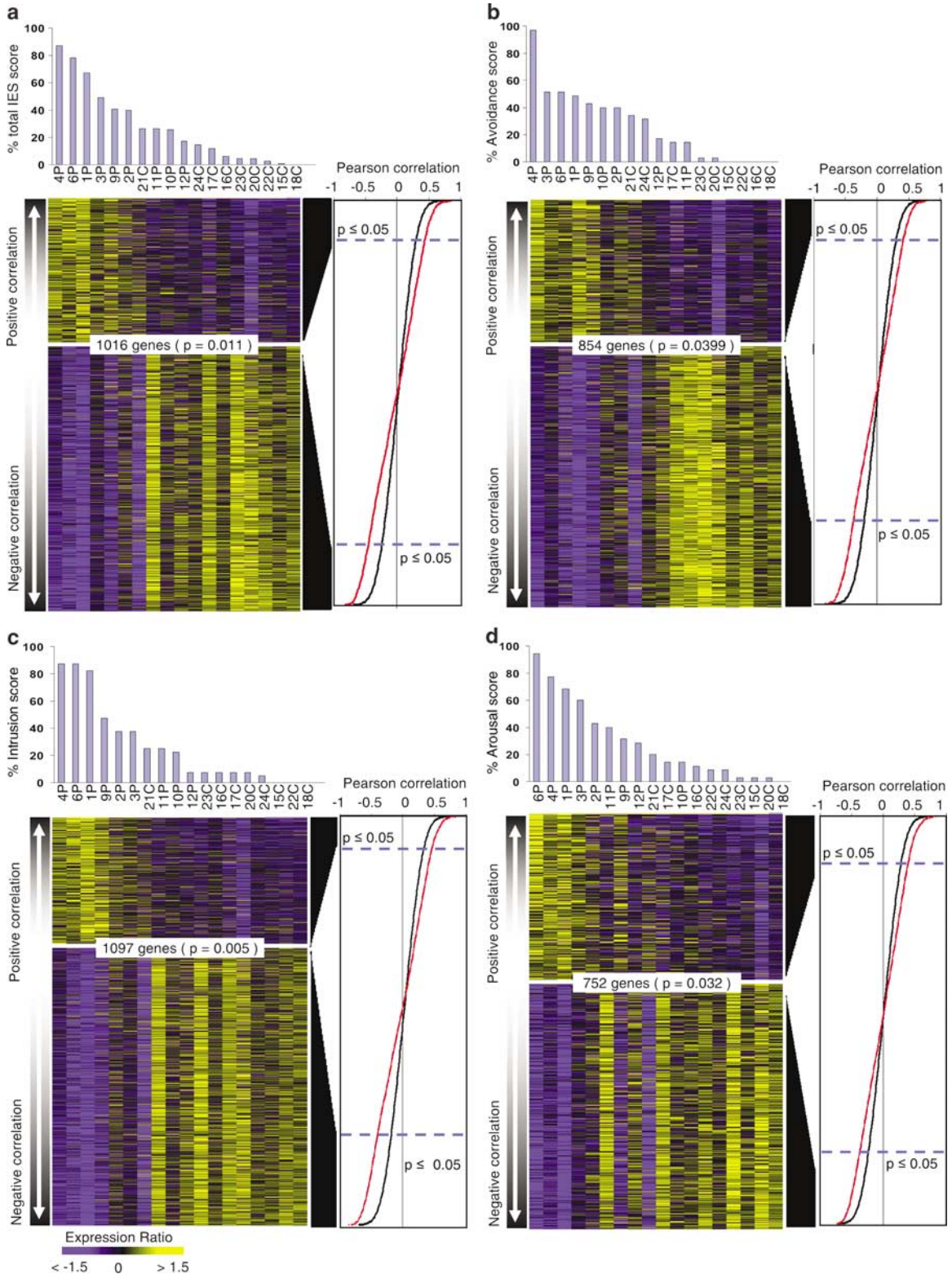
Sample preparation and microarray hybridization

Total RNA extracted from PBMCs was used for sample preparation and hybridization as recommended by the manufacturer of the arrays (Affymetrix, Santa Clara, CA, USA), except that a total of 9 μg of the cRNA was hybridized on every HG-U95A microarray (Affymetrix, Santa Clara, CA, USA).

Figure 1 Analysis of gene expression patterns of subjects with consistent PTSD phenotype. Shown are analysis results of expression profiles measured from PBMC samples obtained from subjects with consistent phenotypes at both time points (a, d) or separately at time of admission to ER (b, e and g), and 4 month after the trauma (c, f and h). (a–c) Unsupervised hierarchical clustering of expression profiles of 4512 active genes from the entire sample set (a), at ER only (b) or at 4 months after trauma (c). In each case, the dendrogram of samples built by the clustering procedure is shown on top, annotated by the subject number and an indication of the clinical condition of the subject and the time the sample was taken by colored boxes (dark blue, a control subject at ER; light blue, a control subject at month 4; brown, a PTSD subject at ER; yellow, a PTSD subject at month 4). The expression values are displayed in the bottom panel (columns, samples; rows, genes), where yellow indicates increased expression and blue indicates decreased expression relative to the average expression of the gene in all the arrays in the study. Clustering of samples from both time points (a) partially distinguishes the PTSD samples from the controls. Two clusters contain all PTSD samples, and the third cluster contains most of control samples. Clustering of ER samples (b) distinguishes almost perfectly between the classes of samples, with one misclassified sample. Clustering of samples from month 4 alone (c) distinguishes perfectly between PTSD and control samples. (d–f) Overabundance plots evaluating the statistical significance of the number of genes that are differentially expressed between PTSD and control samples. Red line, the number of differentially expressed genes that separate PTSD from control samples (*y*-axis) that were scored a given *P*-value (*x*-axis) in a consensus of three statistical scoring measures (see Materials and methods); dark gray line, the number of genes expected by chance with that *P*-value from 1000 simulations with random reshuffling of subject labels; light gray area, the range of numbers of differentially expressed genes in the 95th percentile of 1000 random simulations. The dotted vertical line indicates the *P*-value 0.05, the threshold for defining a gene as differentially expressed. Overabundance of differentially expressed genes is observed when using the data of both time points (d), only the ER samples (e) or only samples taken at the month 4 (f). (g and h) Evaluation of the supervised classification of subjects' phenotypes. Top: Expression profiles of differentially expressed genes. Labels and colored boxes indicate subjects and sample time. Bottom: Classification results from LOOCV. The procedure evaluates the classifier's prediction at each sample by removing it from the training data, selecting differentially expressed genes based on the remaining samples, training a classifier, and then applying it to the removed sample (see Materials and methods). Each bar indicates the prediction obtained for the corresponding sample, when holding it out. The sign of the outcome value indicates the predicted phenotype (positive, control; negative, PTSD), and the magnitude indicates relative confidence. Red cross marks denote misclassified examples. The *P*-value of this classification is evaluated using 1000 simulations with random reshuffling of subjects. The classification of samples at ER succeeds in nine of 11 samples (g) and at month 4 it succeeds in eight of nine samples (h).

Data analysis

Scanned output files were analyzed with Microarray Analysis Suite 5.0 software (Affymetrix). Arrays were scaled to an average intensity of 100 analyzed independently and normalized using D-chip.[25] We identified 4512 'active' transcripts as those that had at least one value between 50 and 7500, one 'Present' call by Microarray Analysis Suite 5.0 (Affymetrix, Santa Clara), and changed in at least one sample two-fold or more from the geometric mean of all samples.



All values below 20 were brought to 20 and all values above 10 000 were brought to 10 000. Expression measurements were then transformed to log (base 2) of the ratio between the expression value and the geometric mean of the gene's expression value in all samples within the same batch (for more information see Supplementary Methods).

Statistical analysis and clustering

The general approach to analysis has been outlined by us[26] and performed using the ScoreGenes package (http://compbio.cs. huji.ac.il/scoregenes/). We identified differentially expressed genes using three test statistics: TNoM, *t*-test and Info[27]; the significance of each score was determined as described previously.[26] Genes that had a *P*-value <0.05 in all three scoring methods were considered differentially expressed. We determined the significance of the number of differentially expressed genes by using a randomized permutation test with 1000 random reshuffling of subject labels. Overabundance analysis and Pearson's correlation were calculated using ScoreGenes. Clustering was performed using DoublePCluster, an agglomerative hierarchical model-based biclustering approach, and Cluster.[28] The data set used and a detailed description of the statistical methods applied are available in the Supplementary Methods.

Results

Gene expression patterns discern PTSD status

To define gene expression patterns associated with evolving PTSD symptom course, survivors of life-threatening events who did not sustain serious physical injury were prospectively followed from the time of admittance to a general hospital ER shortly after trauma. Eight subjects had persistent full diagnostic criteria for the three symptom clusters that compose PTSD at both 1 and 4 months after trauma, and six subjects met no formal clinical criterion for PTSD at any time (*consistent phenotype* subjects). For part of the analyses, we included additional five subjects who showed partial intermediate PTSD clinical criteria 1 month after trauma and full criteria at 4 months, and five subjects with partial intermediate PTSD criteria at 1 month that resolved by 4 months (*partial phenotype* subjects). Using oligonucleotide arrays (Affymetrix HU95A), we measured gene expression profiles from PBMCs sampled immediately after trauma at the ER and 4 months after the trauma (M4) from these 24 subjects. A total of 33 PBMC samples (18 M4 and 15 ER) were available for analysis (20 samples for the consistent phenotype group, see Table 1 and Supplementary Methods). After signal quantitation and normalization, we identified a set of 4512 active transcripts that are expressed and show some variance among the collected profiles (see Materials and methods).

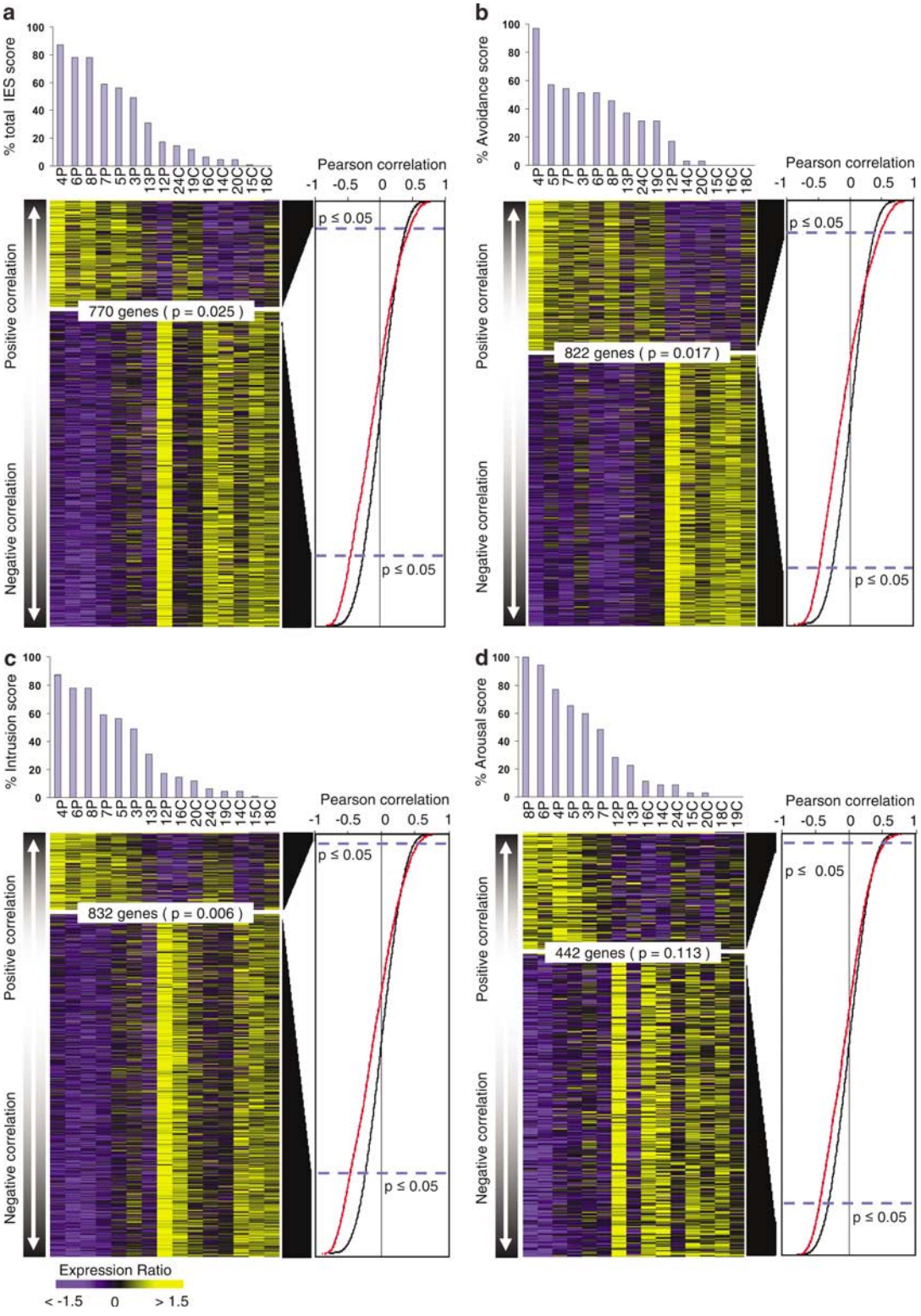
We first determined whether gene expression patterns could distinguish PTSD from control subjects. PTSD is a complex disorder showing a spectrum of severity. To get the most informative view of the core phenotype, we focused, for this comparison, on the difference between subjects exhibiting the consistent phenotype (subjects with full persistent PTSD (1–8 Table 1) and subjects showing no PTSD criteria at any time (14–19)). Subjects showing an intermediate partial phenotype were not included in this analysis. Unsupervised hierarchical clustering (performed blind to clinical diagnoses, see Materials and methods) distinguishes the clinical status at 1 and 4 months (Figure 1a). When only M4 samples were analyzed, all subjects were classified into two clusters, one containing PTSD subjects and the other control subjects (Figure 1b). A similar pattern (with one misclassified subject) is evident in clustering of samples taken at ER, hours after trauma (Figure 1c), suggesting that gene expression patterns at the immediate aftermath of trauma may be informative of the later development of the PTSD phenotype.

To better characterize the differences between PTSD and control subjects, we identified genes that are differentially expressed between the two groups. Of the 4512 active transcripts, we find a signature of 656 transcripts that are differentially expressed between PTSD and control samples (see Materials and methods and Supplementary Methods). This number is significantly larger than expected by chance ($P=0.007$, Figure 1d). Similarly, we define signatures of differentially expressed transcripts when we examine M4 samples or ER samples separately. These signatures, with 408 differential transcripts at M4 and 574 transcripts at ER, are both

Figure 2 Analysis of the correlation between gene expression from samples at month 4 and continuous IES scores, assessed 4 months after trauma. Shown is the expression of genes with significant positive or negative correlation to the Total IES score (a), Avoidance score (b), Intrusion score (c), and Arousal score (d). Each panel consists of three elements. Top: The scores (represented as the percent of the maximal score) of each of the 18 subjects who had month 4 samples. Bottom left: Expression levels of genes with significant ($P<0.05$) positive and negative correlations with the respective score. The number of correlated genes is shown together with the probability of observing such a number (or higher) computed using 1000 simulations with random reshuffling of subjects' scores. Bottom right: Correlation coefficients of all 4512 active genes with the subject score. Red line: Curve showing the Pearson correlation of each of the 4512 active genes with the subject score, when the genes are sorted in a decreasing order of correlation. Dark gray line: Curve showing the expected sorted Pearson correlations in 1000 simulations with random reshuffling of subjects' scores. (Dashed line) Threshold denoting genes with significant correlation ($P<0.05$). This analysis shows significant overabundance of genes expressed at month 4 that are correlated with the composite IES PTSD symptoms score, as well as with each of the three symptom clusters of PTSD.

significantly larger than expected by chance (Figure 1e and f).

To further explore the predictive abilities of these gene expression signatures, we used the Naïve Bayesian classifier. The leave-one-out cross validation (LOOCV) procedure was used to evaluate the classification accuracy of the classifier in either M4 samples or ER samples (see Materials and methods). The classifier was able to classify correctly eight out of nine M4 samples (Figure 1g) and nine out of 11 ER samples (Figure 1h). Evaluating the significance of these classifications compared to



randomized reshuffling of subject labels shows that the classification accuracy is significant with M4 samples ($P=0.027$), and nearly significant with ER samples ($P=0.061$). In contrast, the groups did not show statistically significant differences in type of trauma, trauma severity scores, age, gender, ethnic origin and psychiatric comorbidity (Table 1 and Supplementary Table A). Although none of the variables showed significant differences between groups, we wanted to exclude a possible confound by examining their correlation with PTSD outcome. We performed a multiple logistic regression analysis of age, gender, ethnic origin, trauma severity and comorbid psychiatric diagnoses, with consistent PTSD status as the dependent variable. None of the variables contributed to explain the variance in PTSD status.

We also compared gene expression patterns between all 13 subjects diagnosed with complete PTSD criteria by 4 months (including the five that did not consistently exhibit the complete diagnostic criteria at 1 month after trauma) against all 11 subjects who showed no formal criterion by 4 months (including the five that exhibited partial PTSD criteria at 1 month but decreased to subthreshold levels and did not meet any formal clinical criteria by 4 months). When subjects with a partial intermediate phenotype were included, comparison of M4 samples revealed 220 differentiating genes showing trend significance ($P<0.066$), and ER samples yielded nonsignificant results (data not shown). The mean age at trauma was 35 years among M4 PTSD *vs* 26 years among M4 control subjects ($P=0.05$), and mean trauma severity score was 18.15 among PTSD *vs* 13.55 among controls ($P=0.082$); gender ethnic origin and comorbidity did not show significant differences among groups (Supplementary Table B). Multiple logistic regression analysis of age, gender, ethnic origin, trauma severity and comorbid psychiatric diagnoses, with PTSD status at M4 as the dependent variable (13 chronic PTSD *vs* 11 controls), revealed that none of these variables contributed to explain the variance in PTSD M4 status.

Gene expression patterns correlate with severity of PTSD symptoms and its three symptom clusters

To investigate the persistence of symptom trajectories among all survivors, we employed an alternative continuous phenotype measure. We correlated gene expression profiles with the composite PTSD severity score, and with the severity of each of the three PTSD symptom clusters as measured by the Impact of Event Scale (IES) (see Supplementary Methods and Table 1). The analysis included the entire sample of 24 subjects (both consistent and partial phenotype subjects, and regardless of their threshold clinical designation as PTSD or control, at 1 or 4 months). Among the 18 available M4 samples, we found a significant overabundance of genes showing significant correlations ($P\leq0.05$) with the IES intensity total score (a measure of all PTSD symptoms) measured at 4 months after trauma (Figure 2a), as well as with each of the three IES PTSD symptom clusters (Figure 2b–d). Among the 15 available ER samples, we also found a significant overabundance of genes that correlated with continuous PTSD IES scores measured 4 months later (Figure 3).

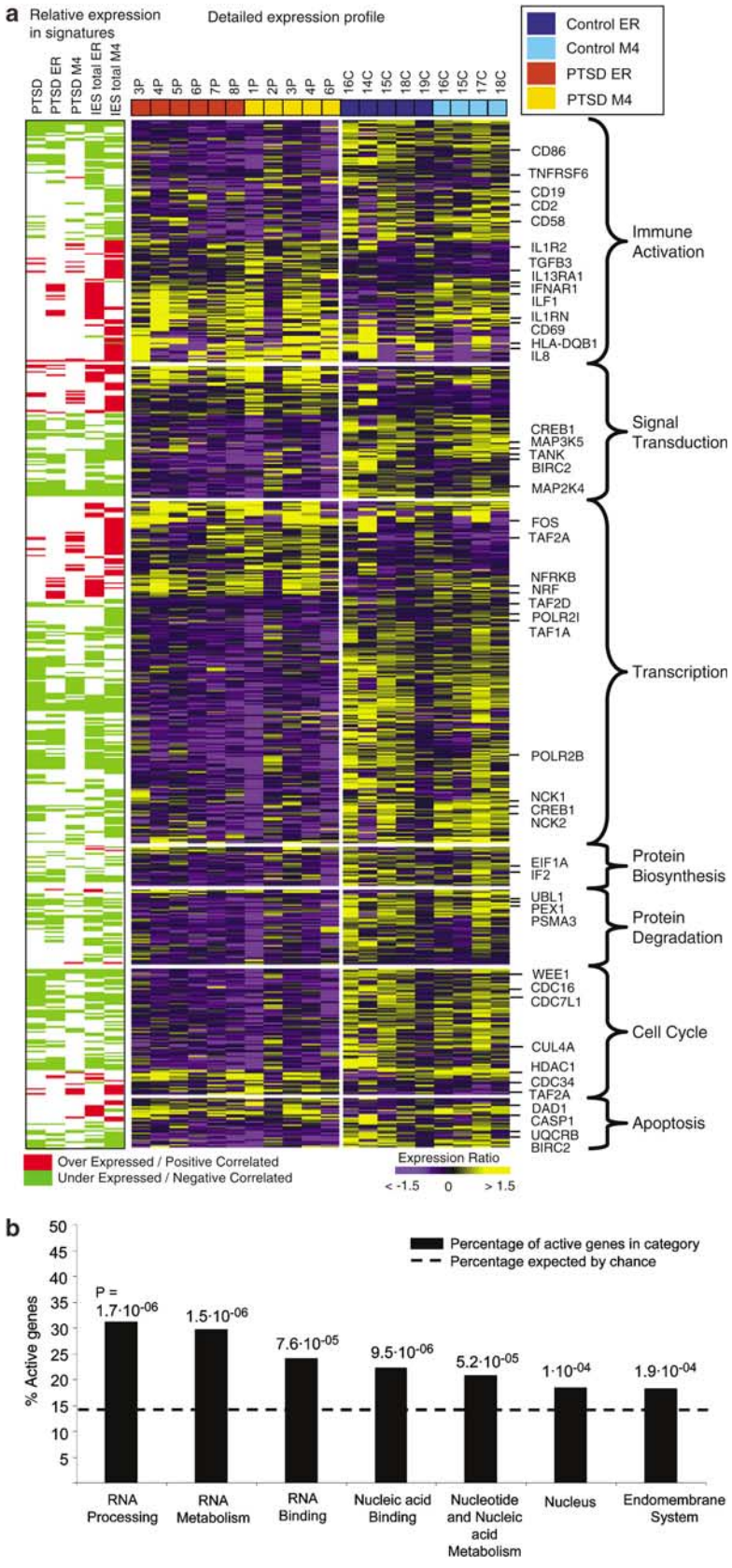
The mean M4 IES score did not show significant differences between male and female subjects, subjects of different ethnic origins or subjects with comorbid psychiatric diagnoses. There was a trend for significant correlation between M4 IES scores and trauma severity scores ($r=0.39$ $P=0.058$), and no significant correlation of IES with age (Supplementary Table C). A multiple regression analysis revealed that none of these variables contributed to explain the variance in M4 IES score.

Of the genes expressed at ER and M4 that showed significant correlation with total M4 IES scores among all survivors, 369 and 260 transcripts, respectively, overlapped with the ER and M4 informative sets of genes that separated the above consistent PTSD and control subsample. This may point to a shared biological basis between spectral PTSD symptoms and threshold defined clinical PTSD.

Affected trauma survivors show reduced expression of transcriptional enhancers and distinct immune activation

Our analyses identified signatures of differentially expressed transcripts among subjects with consistent phenotypes as well as transcripts whose expression levels correlated with PTSD IES scores among all subjects. To gain better understanding of these informative transcripts, we examined their functional classifications. We identified several functional

Figure 3 Analysis of the correlation between gene expression from samples at ER and continuous IES scores, assessed 4 months after trauma. Shown is the expression of genes with significant positive or negative correlation to the Total IES score (a), Avoidance score (b), Intrusion score (c) and Arousal score (d). Each panel consists of three elements. Top: The scores (represented as the percent of maximal score) of each of the 15 subjects who had ER samples. Bottom left: Expression levels of genes with significant ($P<0.05$) positive and negative correlations with the respective score. The number of correlated genes is shown together with the probability of observing such a number (or higher) computed using 1000 simulations with random reshuffling of subjects' scores. Bottom right: Correlation coefficients of all 4512 active genes with the subject score. Red line: Curve showing the Pearson correlation of each of the 4512 active genes with the subject score, when the genes are sorted in a decreasing order of correlation. (Dark gray line) Curve showing the expected sorted Pearson correlations in 1000 simulations with random reshuffling of subjects' scores. (Dashed line) Threshold denoting genes with significant correlation ($P<0.05$). This analysis shows significant overabundance of genes expressed at ER that are correlated with the composite IES PTSD symptoms score, as well as with two of the three symptom clusters of PTSD.


a
Relative expression in signatures
Detailed expression profile
PTSD
PTSD ER
PTSD M4
IES total ER
IES total M4
3P 4P 5P 6P 7P 8P 1P 2P 3P 4P 6P 16C 14C 15C 18C 19C 16C 15C 17C 18C
Control ER
Control M4
PTSD ER
PTSD M4
CD86
TNFRSF6
CD19
CD2
CD58
IL1R2
TGFB3
IL13RA1
IFNAR1
ILF1
IL1RN
CD69
HLA-DQB1
IL8
Immune Activation
CREB1
MAP3K5
TANK
BIRC2
MAP2K4
Signal Transduction
FOS
TAF2A
NFRKB
NRF
TAF2D
POLR2I
TAF1A
POLR2B
NCK1
CREB1
NCK2
Transcription
EIF1A
IF2
Protein Biosynthesis
UBL1
PEX1
PSMA3
Protein Degradation
WEE1
CDC16
CDC7L1
CUL4A
HDAC1
CDC34
TAF2A
Cell Cycle
DAD1
CASP1
UQCRB
BIRC2
Apoptosis
Over Expressed / Positive Correlated
Under Expressed / Negative Correlated
Expression Ratio
< -1.5 0 > 1.5
b
Percentage of active genes in category
Percentage expected by chance
% Active genes
50 45 40 35 30 25 20 15 10 5
P = 1.7·10-06
1.5·10-06
7.6·10-05
9.5·10-06
5.2·10-05
1·10-04
1.9·10-04
RNA Processing
RNA Metabolism
RNA Binding
Nucleic acid Binding
Nucleotide and Nucleic acid Metabolism
Nucleus
Endomembrane System

groups that are represented in these signatures (Figure 4a). Notably, we observe downregulation of transcripts encoding for proteins that are involved in transcriptional activation, and cell cycle and proliferation among affected subjects (eg whether defined as consistent PTSD or according to PTSD IES symptoms severity). We also observe distinct expression signatures for transcripts involved in immune activation, signal transduction and apoptosis. To attempt a quantitative analysis, we considered the annotations available through the Gene Ontology (GO) database. The percentage of GO annotation was calculated among the informative subset of genes that separate PTSD from controls, and was compared to the percentage among all 4512 active genes on the chip. Significantly increased representations ($P<0.0005$) of genes involved in RNA metabolism and processing, as well as nucleotide metabolism, was found in the consistent PTSD signature (Figure 4b). Significant increased representation of GO annotations was found also in the other signatures (Supplementary Table D).

Signatures of affected trauma survivors are significantly enriched for genes that encode for neural and endocrine proteins

To further pursue how peripheral transcriptional response may be relevant to the neuropsychiatric process, we examined to what extent differentially expressed transcripts are also expressed in primary tissues involved in the mediation of neural and endocrine reactivity to stress. We assessed the enrichment of transcripts known to be expressed in primary tissues in the signature of differentially expressed transcripts we identified above. Gene transcripts known to be expressed in brain amygdalar and hippocampal regions, and the hypothalamic-pituitary-adrenal (HPA) axis, were found to be significantly overabundant among the genes that distinguished trauma survivors with consistent PTSD (Figure 5a). For example, out of 656 differentially expressed transcripts, 533 are expressed in relevant brain and neuroendocrine regions. Significant increased representation of coexpressed genes was found also in the other signatures. Hippocampal gene enrichment was significant only among ER samples, whereas the coexpressed genes in the other tissues showed significant enrichment at both time points (Supplementary Table E).

Some of the transcripts showing differential expression patterns among affected trauma survivors play a major role in the neural and endocrine modulation of the stress response (Figure 5b). Examples include the $GABA_A$ receptor (a major brain target for neuroactive steroids), the serotonergic receptor 5-hydroxytriptamine 3 and phosphodiesterase E4A, both selective targets for drugs possessing antianxiety properties, as well as multiple genes related to endocrine response including 17 alpha and 21 hydroxylases.

Discussion

We used complementary approaches to analyze the relationship of gene expression data and PTSD. Our results converge to suggest that expression signatures in PBMCs sampled in the immediate aftermath of trauma exposure as well as 4 months later are informative of the development of PTSD, and its main symptom clusters. To the best of our knowledge, this is the first evidence that gene expression signatures contain information that may prove useful for identifying a mental disorder.

Current notion holds that detection of an informative gene transcriptional signal depends on focusing on homogeneous target cells directly involved in the disease process.[29,30] Our data reveal a robust differential signal that remains detectable despite cellular heterogeneity of PBMCs, and their apparent lack of primary involvement in the pathogenesis of PTSD. Of note, PBMCs are known to be perturbed following acute psychological stress,[18] in part through neuroendocrine and sympathetic modulation.[31] Long-term alterations in sympathetic[32] and HPA reactivity[4] have

Figure 4 Functional analysis of genes that are differentially expressed between PTSD and control subjects and genes that correlate with continuous IES scores. (a) Expression profiles of genes with selected functional roles. Shown are expression profiles (middle) for genes from selected functional categories (right) across samples from patients with consistent phenotypes (top labels and colored boxes, see legend). Yellow and blue indicate upregulation and downregulation, respectively. Genes are selected based on their participation in previously identified signatures (left). PTSD, PTSD ER and PTSD M4, genes that are differentially expressed between PTSD and control subjects when considering samples at both time points, ER only or month 4 only, respectively; IES total ER and IES total M4, transcripts expressed at ER or month 4, respectively, which significantly correlate with Total IES score at month 4. Green, the gene is downregulated in PTSD or negatively correlated with IES Total score; red, the gene is upregulated in PTSD or positively correlated with IES Total score; white, the gene does not appear in the signature (ie it is not significant under the relevant statistical test). We observe a marked overall decreased expression for genes coding for transcription enhancers, regulators of protein biosynthesis and degradation, and cell proliferation. We also observe marked differences in expression patterns among genes encoding for immune activators, regulators of signal transduction and apoptosis. (b) Enrichment of differentially expressed genes within GO categories. Shown are functional categories that are significantly enriched for differentially expressed genes (following a False Discovery Rate correction). For each category, we show the percentage of active genes in the category that are also differentially expressed between PTSD and control subjects when considering both time points. The horizontal line marks the percentage expected by chance. We observe a significant enrichment in genes encoding for RNA metabolism and processing, as well as nucleotide metabolism.

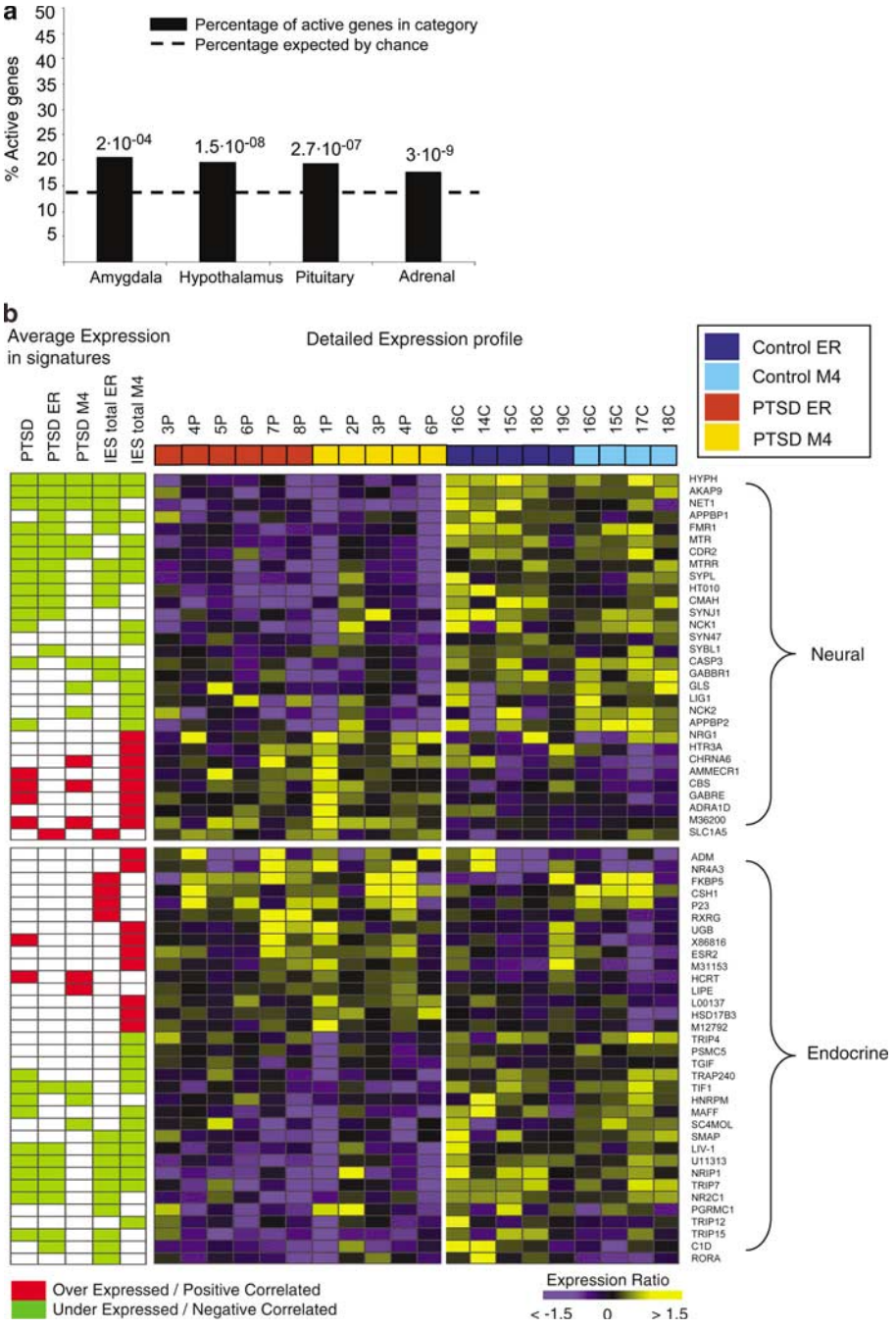


Figure 5 Analysis of genes from neural and endocrine tissues that are differentially expressed between PTSD and control subjects and genes that correlate with continuous IES scores. (a) Enrichment of differentially expressed genes within groups of genes known to be coexpressed in different brain areas. Genes expressed within each area were determined using OMIM and UniGene databases. Shown are brain areas that are significantly enriched for differentially expressed genes (following a False Discovery Rate correction). For each group of coexpressed genes, we show the percentage of active genes in the group that are also differentially expressed between PTSD and control subjects when considering both time points. The horizontal line marks the percentage expected by chance. We observe a significant enrichment in genes expressed in areas mediating stress reactivity, including the HPA axis and amygdala. (b) Expression profiles of neural and neuroenocrine genes that are known to be involved in modulation of the stress response. Shown are expression profiles (middle) for genes involved in these processes (right) across samples from patients with consistent phenotypes (top labels and colored boxes, see legend). Yellow and blue indicate upregulation and downregulation, respectively. Genes are selected based on their participation in previously identified signatures (left). PTSD, PTSD ER, and PTSD M4, genes that are differentially expressed between PTSD and control subjects when considering samples at both time points, ER only or month 4 only, respectively; IES total ER and IES total M4, transcripts expressed at ER or month 4, respectively, which significantly correlate with Total IES score at month 4. Green, the gene is downregulated in PTSD or negatively correlated with IES Total score; red, the gene is upregulated in PTSD or positively correlated with IES Total score; white, the gene does not appear in the signature (ie it is not significant under the relevant statistical test).

been described in PTSD, and suggested to impart alterations in immune modulation.[31–33] Altered white cell markers[19,20] and cytokine levels[21,22] have previously been reported in PTSD. In line with these observations, we found differential transcriptional patterns of genes encoding immune activators, as well as regulators of proliferation differentiation and demise of leukocytes, among psychologically affected trauma survivors following exposure to stress. Redistribution of white blood cells follows acute psychological trauma.[31] Distinct changes in the composition of circulating white cells among affected survivors may be an additional mechanism underlying the immediate expression changes observed here. In such case, measuring gene expression in a composite population of PBMCs will *enhance* the differences observed. Time coursed flow cytometry would allow further characterization of leukocyte composition, as well as focus on the distinctive expression changes among white blood cells subclasses.

Current practice groups survivors into those with and without clinical PTSD, by applying a severity threshold on the conglomerate score of the three PTSD symptom clusters,[1] with a consequent inherent loss of data.[34,35] Our results demonstrate that gene expression signatures in PBMCs contain information that is highly correlated with continuous symptom trajectories among all survivors regardless of threshold clinical designation. Initial PBMC gene expression signatures are informative of later clinical course. If replicated, this could have a significant potential for guiding early detection and focused early intervention among survivors of trauma. Furthermore, while it is now well accepted that gene expression patterns in cancer tissues are indicative of a patient's prognosis,[36,37] our data suggest that such information exists in the much more accessible peripheral blood.

It is unclear whether the changes observed in PBMCs are merely informative of the development of PTSD or also bear relevance to its pathogenesis.

Abnormal immune reaction to stress may play a neuromodulatory role,[33] in which case differential PBMC perturbation may directly participate in the disease process. For example, transcripts showing differential expression among affected trauma survivors in our data, such as those encoding interleukin-1-related peptides, are known to modulate hypothalamic corticotrophin-releasing factor secretion.[33]

Alternatively, PBMCs may participate in a stress-induced systemic perturbation of transcriptional events, and merely reflect pertinent processes taking place in more relevant cell populations. In support, two differentially expressed genes in post-mortem brains of subjects with bipolar disorder were also shown to be differentially expressed in lymphoblastoid cells of living subjects with the same condition.[38] Our findings may point to similar phenomena on a much larger scale.

This is particularly relevant if individual genomic variation may direct related transcriptional responses in distant cells. In this case, expression signatures among PBMCs in response to extreme psychological stress may reflect in part genomic predisposition to develop PTSD, beyond the putative participation of immune cells in this neuropsychiatric disorder. Genes showing expression differences in lymphocytes from two patients with bipolar disorder have recently been shown to constitute promising candidates for search of causative genomic polymorphisms associated with risk for the disorder, suggesting that peripheral expression differences contain pathogenetically relevant information for the neuropsychiatric process.[39] Indirect support for this notion can be found in our data in the increased proportion of genes coexpressed in the brain and endocrine tissues, as well as specific genes related to neural transduction of stress among the informative transcripts observed in PBMCs.

Our results demonstrate a general reduction in PBMC expression of transcription activators among psychologically affected trauma survivors in response to stress. This decrease may explain much of the differences in gene expression signatures observed between the PTSD and control subjects. It remains to be established if some of the robust differences among PBMCs in gene transcripts related to transcriptional activation, intracellular signaling pathways, cell cycle and apoptosis, might be indicative of parallel changes occurring among cell populations more relevant to central stress reactivity. Genomic variation may drive related transcriptional reactivity among glial cells that share closer embryonal derivation to leukocytes or even among neuronal cells. Reduced hippocampal volumes have been described among PTSD patients.[40] Altered neuroendocrine reactivity, signal transduction, and cellular proliferation and demise among neural and glial cells have been implicated in the hippocampal volume depletion,[39–41] as well as in fear avoidance formation[42] and memory consolidation[43] processes, and in some of the protective effects induced by antidepressant drugs.[44,45] It is thus tempting to suggest that our results may denote reduced potential for neural plasticity in response to stress among affected trauma survivors.

Despite the small sample size, gene expression patterns observed were reproducible and robust across statistical tests and alternative phenotype measures. Naturally, to generalize the diagnostic utility of our results, larger sample size will be required, as well as studies focusing on specific markers within the signatures that we discovered. Following up on specific genes or pathways was beyond the scope of this study. Pursuing the relevance of our findings to CNS processes will require further specific investigation of implicated genes, employing post-mortem brain studies, *in vivo* brain imaging or animal stress paradigms. Altered expression may result from genomic sequence variation, and implicated transcripts may be further pursued through informing candidate gene mutation screen

and association studies among affected trauma survivors (eg Kakiuchi *et al*[39]).

Our results suggest that PBMC gene expression signatures are informative and predictive of the PTSD outcome among survivors of trauma, and correlate with the essential neuropsychiatric dimensions that compose the disorder. This is the first evidence that peripheral gene expression signatures may harbor information relevant for the identification and course of mental disorders. This suggests the more general heuristic prospect that other organ-related disorders may be approached through the study of accessible blood cells. Results should encourage research into the diagnostic value of gene expression signatures in PBMCs as well as illuminating the mechanistic factors that determine these changes.

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Supplementary Information accompanies the paper on Molecular Psychiatry website (http://www.nature.com/mp)