BRIEF REPORT

Gene Expression Patterns Associated with Posttraumatic Stress Disorder Following Exposure to the World Trade Center Attacks

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Background: Although genetic risk factors for posttraumatic stress disorder (PTSD) in similarly traumatized cohorts can be confounded with risk for type of exposure, the primary risk for exposure to the 9/11 attack on New York City was proximity, allowing study of PTSD risk in a sample that is not confounded by exposure-related risk.

Methods: Thirty-five Caucasians (15 with PTSD, stratified for exposure, age, and gender) were selected from a population-representative sample of persons exposed to the attack from which longitudinal data had been collected in four previous waves. Whole blood gene expression and cortisol levels were obtained.

Results: Seventeen probe sets were differentially expressed in PTSD. Identified genes were generally involved in hypothalamic-pituitary-adrenal (HPA) axis, signal transduction, or brain and immune cell function. FKBP5, a modulator of glucocorticoid receptor (GR) sensitivity, showed reduced expression in PTSD, consistent with enhanced GR responsiveness. FKBP5 expression was predicted by cortisol when entered with PTSD severity in regression analysis. Quantitative polymerase chain reaction confirmed significant reductions in FKBP5. Also less expressed in PTSD were STAT5B, a direct inhibitor of GR, and major histocompatibility complex (MHC) Class II.

Conclusions: Consistent with observations of HPA axis dysfunction in PTSD, several genes involved in glucocorticoid signaling are differentially expressed among those with current PTSD.

Key Words: Childhood trauma, cortisol, gene expression, human FKBP5 protein, posttraumatic stress disorders, September 11 terrorist attacks

Genetic factors contribute to posttraumatic stress disorder (PTSD) risk (1). However, susceptibility genes have not yet been unambiguously confirmed (2). As PTSD is associated with enhanced responsiveness of the glucocorticoid receptor (GR), genes involved in the regulation of the GR may associate with PTSD risk (3). Adults abused in childhood who have genetic variations in the GR-regulatory gene FKBP5 are at greater risk for developing PTSD to subsequent exposures (4). Polymorphisms in FKBP5 have also been linked to the PTSD risk factor of peritraumatic dissociation (5). Moreover, using an unbiased genome-wide approach to identify PTSD predictors, FKBP5 gene expression was altered in acutely traumatized civilians with PTSD compared with those without PTSD (6).

This study examined risk factors in persons exposed to the World Trade Center (WTC) attacks on 9/11 drawn from a population-representative sample. Because proximity to the WTC was the primary risk factor for trauma exposure, development of PTSD was unlikely to be confounded by factors associated with risk for violence. Whole blood genome-wide expression analysis was used to identify altered gene activity patterns in highly exposed persons with, compared with those without, PTSD.

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Methods and Materials

Participants

A random sample of 20 Caucasians with high-magnitude exposure to 9/11 who met criteria for PTSD in at least two of four waves in a previously described longitudinal study (7) were recruited. Subjects were then stratified by age and gender to 20 similarly exposed Caucasians without PTSD. The study was approved by the Institutional Review Board (IRB) at the Mount Sinai School of Medicine. All participants provided written, informed consent. Participants were excluded if they had psychosis, bipolar disorder, substance dependence, or major medical, endocrine, or neurological illness. None were in active psychiatric treatment or taking antidepressants at the time of the study.

Clinical Evaluation

Psychologists with established interrater reliability administered the Clinician Administered PTSD Scale (CAPS) (8) and the Structured Clinical Interview for the DSM-IV (9) to determine the presence of PTSD and other psychiatric disorders. Five individuals with lifetime (assessed previously) but not current PTSD were excluded from analysis, yielding 15 cases with current PTSD and 20 cases with no lifetime PTSD. Participants also completed the Trauma History Questionnaire and the Childhood Trauma Questionnaire (10,11).

Blood Drawing and Processing

Fasting blood samples were obtained between 08:00 and 09:00 hours. Blood was processed using the PAXgene RNA stabilization system (Qiagen, Valencia, California) (12). Samples were subjected to the globin messenger RNA (mRNA) reduction

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Table 1. Demographics, Trauma Exposure, and Clinical Characteristics in Individuals With and Without Current PTSD

	No PTSD ($n = 20$) Mean (SD) or % (n)	PTSD ($n = 15$) Mean (SD) or % (n)	Group Comparisons
Demographics			
Age (years)	57.30 (13.19)	51.07 (15.35)	$t(33) = 1.29$, ns
Sex			$\chi^2(1) = .088$, ns
Male	45.0% (9)	40.0% (6)	
Female	55.0% (11)	56.0% (9)	
Trauma Exposure			
CTQ total score	6.42 (1.73)	13.93 (12.20)	$t(32) = -2.66$, $p = .012$
Total number of traumas	4.20 (2.35)	6.40 (3.52)	$t(33) = -2.22$, $p = .034$
Degree of 9/11 exposure	.45 (.61)	.40 (.63)	$t(33) = .24$, ns
Clinical Characteristics			
Current CAPS scores			
Intrusive symptoms	2.11 (2.56)	10.00 (9.28)	$t(30) = -3.46$, $p = .002$
Avoidance	.89 (1.97)	21.36 (11.53)	$t(30) = -7.43$, $p < .0005$
Hyperarousal	2.72 (4.71)	12.64 (9.06)	$t(30) = -4.01$, $p < .0005$
PDS total score	3.45 (3.19)	22.80 (10.04)	$t(33) = -8.12$, $p < .0005$

CAPS, Clinician-Administered PTSD Scale; CTQ, Childhood Trauma Questionnaire; ns, nonsignificant; PDS, Posttraumatic Stress Diagnostic Scale; PTSD, posttraumatic stress disorder.

method to improve the data quality of stabilized RNA samples hybridized to microarrays. Gene expression studies were carried out using Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, California) containing 47,000 transcripts. Plasma cortisol was measured using radioimmunoassay.

Quantitative polymerase chain reaction (qPCR) was performed to validate FKBP5 gene expression. For this, total RNA was used to generate complementary DNA (cDNA) using SuperScript II Reverse Tanscriptase (Invitrogen, Carlsbad, California). Gene expression probes and primers for FKBP5 were designed based on the National Center for Biotechnology Information (NCBI) sequence (NM_004117.2) using the ProbeFinder v2.41 software (Roche, Madison, Wisconsin, http://www.universalprobelibrary.com). Four different exon junctions, including exon 4 to 5 (4,5), exon 7 to 8 (7,8), exon 8 to 9 (8,9), and exon 11 to 12 (11,12), were quantitatively amplified. Quantitative polymerase chain reaction was performed on an ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, California). Samples were analyzed in quadruplicate and evaluated using the Sequence Detection Software v2.2.1 (Applied Biosystems).

Statistical Analysis

RNA expression in the two groups was compared using dChip (build date January 2009, Cheng Li *et al.*, Cambridge, Massachusetts). Invariant set normalization was carried out with all 35 arrays and model-based expression was evaluated using perfect match (PM)-mismatch (MM) probe data. To limit subsequent analyses to probes showing significant variation and adequate representation, the probes were then filtered such that the coefficient of variation was greater than .25 and less than 1000 and present calls in a minimum of 14 samples, resulting in 8878 probe sets for further analyses. Parameters for identifying differentially expressed genes were then identified using a t test p values of .01 and an absolute expression difference >50. This resulted in a median (empirical) false discovery rate of ~17% (a median of three genes found when samples were randomly assigned to control or to experimental groups in 200 replications).

Data analysis for qPCR was performed using qBase (Dayton, Ohio). Reference genes chosen from glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin, beta (ACTB), beta-2-microglobulin (B2M), and ribosomal protein, large, P0 (RPLP0) were included based on the minimal coefficient of variation. A normal control sample was run in each plate as a normalization control set to a value of 1. Transcript sequence and Universal Probe Library (UPL) probe information are included in Supplementary Tables 1 and 2. Analysis of variance (ANOVA) compared the expression levels between PTSD and control subjects.

Stepwise regression analyses were performed to predict the contribution of trauma-related variables and plasma cortisol levels to expression of two genes associated with GR activity. The Holm-Bonferroni (13) procedure was applied to correct for multiple comparisons.

Results

Table 1 shows clinical characteristics of the sample. Differences were observed in childhood traumatization, number of lifetime traumas, and severity of PTSD symptoms.

Analysis of expression profiles revealed 17 probe sets, corresponding to 16 genes, differentially expressed between those with and without PTSD (Table 2). All genes showed present call rates of >90% in cases and control subjects, with the exception of probe set 238900_at, which showed a present call rate of 60% in control subjects and 26% in cases (mostly due to marginal [M] calls). FKBP5 was altered in a direction consistent with enhanced GR responsiveness, using two different probe sets (224840_at and 224856_at).

We next undertook a validation study of FKBP5 because of prior findings and its direct relevance to GR responsiveness (14). Furthermore, stepwise regression identified FKBP5 to be associated with gene × childhood trauma interaction (R.Y., unpublished observations, 2008), consistent with a recent study (4). Four pairs of primers flanking four introns were designed and used for qPCR. There were significant decreases in FKBP5 in PTSD, confirming the results of the microarray analysis (Figure 1).

Additional exploratory analyses were performed to determine predictors of gene expression for FKBP5 and STAT5B. These proceeded in three steps: 1) age and gender; 2) cortisol, lifetime

Table 2. Control Versus Current PTSD

Affy ID	Gene Name	Control Mean	SE	PTSD Mean	SE	Fold Change	*t* Statistic	*p* Value
203668_at	MANNOSIDASE, ALPHA, CLASS 2C, MEMBER 1	238.08	13.46	317.06	19.63	1.33	3.32	.0027
213998_s_at	DEAD (ASP-GLU-ALA-ASP) BOX POLYPEPTIDE 17	211.93	12.32	281.06	19.05	1.33	3.05	.0054
213902_at	N-ACYLSPHINGOSINE AMIDOHYDROLASE (ACID CERAMIDASE) 1	857.36	43.21	687.73	41.15	.80	−2.84	.0076
224840_at	FK506 BINDING PROTEIN 5	700.63	41.62	548.95	34.94	.78	−2.79	.0087
1562028_at	CYCLIN D3	350.84	20.74	272.22	19.16	.78	−2.78	.0088
202783_at	NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE	257.77	13.11	199.72	14.95	.77	−2.92	.0065
224856_at	FK506 BINDING PROTEIN 5	306.10	19.52	236.83	13.96	.77	−2.89	.0069
1569601_at	CHROMOSOME 2 OPEN READING FRAME 34	403.53	21.77	311.98	18.49	.77	−3.21	.0030
210201_x_at	BRIDGING INTEGRATOR 1	1950.79	112.00	1493.97	101.69	.77	−3.02	.0049
224702_at	MGC23909 = TRANSMEMBRANE PROTEIN 167A	649.87	41.26	496.58	32.22	.76	−2.93	.0062
1555088_x_at	SIGNAL TRANSDUCER ACTIVATOR OF TRANSCRIPTION 5B	753.96	42.75	571.54	47.05	.76	−2.87	.0073
201446_s_at	TIA1 CYTOTOXIC GRANULE-ASSOC RNA BINDING PROTEIN	346.43	21.42	256.02	20.10	.74	−3.08	.0042
224600_at	CGG TRIPLET REPEAT BINDING PROTEIN 1	536.71	30.65	395.24	34.13	.74	−3.08	.0043
218498_s_at	ENDOPLASMIC OXIDOREDUCTIN-1-LIKE PROTEIN	193.09	13.64	141.29	10.04	.73	−3.06	.0045
209703_x_at	METHYLTRANSFERASE LIKE 7A	220.78	11.71	160.84	16.72	.73	−2.94	.0068
204633_s_at	RIBOSOMAL PROTEIN S6 KINASE, 90KDA, POLYPEPTIDE 5	831.25	57.19	561.34	43.92	.68	−3.74	.0007
238900_at	MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS II, DR BETA	98.71	24.93	22.74	9.23	.23	−2.86	.0087

The table lists all genes that differentiated persons with current (n = 15) PTSD compared with a control group (n = 20).
PTSD, posttraumatic stress disorder.

PTSD severity, total lifetime traumatic events, childhood trauma; and 3) the interactions of cortisol with the three variables in the second step. Cortisol levels did not significantly predict gene expression of any gene except FKBP5 (for probe 224840_at: r = .473, p = .010; for probe 224856_at: r = .411, p = .027; controlling for age, gender, and PTSD severity).

The interaction of cortisol levels and childhood trauma predicted STAT5B (probe 1555088_x_at: r = −.460, p = .022; controlling for variables in the previous steps). These three findings remained significant after performing the Holm-Bonferroni procedure to correct for multiple comparisons. The groups did not differ in cortisol levels (M ± SD = 15.28 ± 5.74 μg/dL, 14.21 ± 3.28 μg/dL for PTSD, control subjects; $F(1,31)$ = .61, ns, controlling for age and gender).

Discussion

Several of the identified regulated genes are involved in GR regulation, signal transduction, and brain and immune cell function. FKBP5 acts as an inhibitor of GR (via its function as a co-chaperone of the central heat-shock protein 90 [Hsp90]) (14) and has been associated with recurrence of depression and impaired recovery of the stress response (15–17). Here, FKBP5 expression was significantly lower in PTSD than in control subjects. Similarly, STAT5B is a direct inhibitor of the nuclear translocation of activated GR (18) and was also downregulated in PTSD+ individuals. The decreased expression observed for both genes is consistent with observations of higher activity of the GR in PTSD. The reduced expression of the major histocompatibility complex (MHC) Class II gene is also compatible with observations of abnormally reduced cortisol levels in these patients, as expression of this gene family is stimulated by glucocorticoids (19).

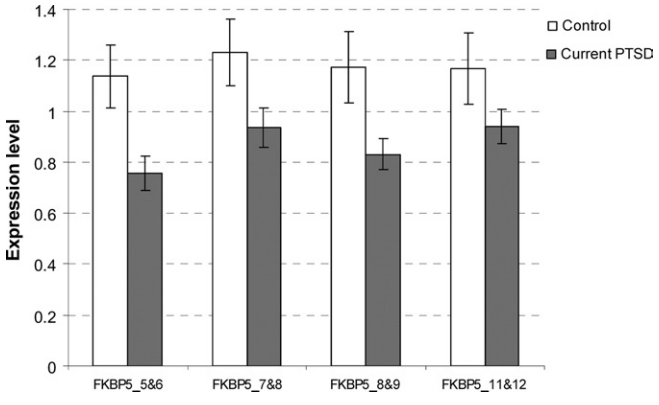

Figure 1. qPCR of FKBP5. qPCR for FKBP5 was carried out in all subjects using probes directed across four intron boundaries (identified for each probe set). Comparisons were made between control subjects and individuals with current PTSD. qPCR, quantitative polymerase chain reaction.

This study conducted genome-wide expression analyses in the context of a neuroendocrine measure. Cortisol levels were associated with FKBP5, though no group differences in cortisol were observed using a single morning estimate. The association suggests that changes in FKBP5 may facilitate enhanced GR responsiveness, leading to low cortisol levels. Alternatively, low cortisol levels may regulate the expression of FKBP5 via alterations in GR responsiveness, as has been previously reported (20). That expression levels of FKBP5 were in the opposite direction of those observed in recurrent depression (15) suggests that gene expression in PTSD may be regulated by different mechanisms than in depression, possibly including epigenetic mechanisms. The interaction of cortisol and childhood trauma severity in the prediction of STAT5B implicates early developmental influences on GR regulation and responsiveness.

Several limitations should be considered when interpreting these findings. First, only subjects with current PTSD are included, thus the study cannot distinguish between genes associated with risk, recovery, or resilience. Analyses including cases of remitted PTSD may highlight other relevant genes. For instance, an analysis including the five additional cases with remitted PTSD showed altered expression of nuclear factor I/A, a transcription factor that acts in concert with GR on gene promoters including that for 11β-hydroxysteroid dehydrogenase type 2 (R.Y., unpublished data, 2008). Second, only Caucasians were studied, thus generalizability of results could not be established.

However, an FKPB5 by childhood trauma interaction has been observed in an African American cohort (4). Third, the power of the study was limited for the applied unbiased approach. These factors point to the need for replication. Nonetheless, many of the identified genes are consistent with enhanced GR responsiveness, a salient component of PTSD.

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Supplementary material cited in this article is available online.

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