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Genetic markers for PTSD risk and resilience among survivors of the World Trade Center attacks

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Abstract. We have previously reported the differential expression of 17 probe sets in survivors of the 9/11 attacks with current posttraumatic stress disorder (PTSD) compared to similarly exposed survivors with no lifetime PTSD. The current study presents an expanded analysis of these subjects, including genotype at FKBP5, a modulator of glucocorticoid receptor (GR) sensitivity. It includes data from additional subjects who developed PTSD following 9/11 but then recovered, distinguishing expression profiles associated with risk for developing PTSD, resilience, and symptom recovery. 40 Caucasians (20 with and 20 without PTSD, matched for exposure, age, and gender) were selected from a population-representative sample of persons exposed to the 9/11 attacks from which longitudinal data had been collected in four previous waves. Whole blood gene expression and cortisol levels were obtained and genome-wide gene expression was analyzed. 25 probe sets were differentially expressed in PTSD. Identified genes were generally involved in hypothalamic-pituitary-adrenal axis, signal transduction, or in brain and immune cell function. STAT5B, a direct inhibitor of GR, and nuclear factor I/A, both showed reduced expression in PTSD. Comparison of lifetime versus current PTSD identified overlapping genes with altered expression suggesting enduring markers, while some markers present only in current PTSD may reflect state measures. As a follow-up, direct comparisons of expression in current PTSD, lifetime-only PTSD, and control groups identified FKBP5 and MHC Class II as state markers, and also identified several trait markers. An analysis of indirect effects revealed that homozygosity for any of 4 PTSD risk-related polymorphisms at FKBP5 predicted FKBP5 expression, which mediated indirect effects of genotype on plasma cortisol and PTSD severity.

Keywords: Stress disorders, post-traumatic, gene expression, genotype, FKBP5 protein, human, cortisol, September 11 terrorist attacks, childhood trauma

1. Introduction

Although genetic factors contribute to posttraumatic stress disorder (PTSD) risk [1], susceptibility genes have not yet been unambiguously confirmed [2]. Moreover, while research has begun to uncover alterations in gene expression associated with PTSD [3,4], the degree to which these alterations represent trait risk markers, as opposed to state markers of disease status, remains unclear.

We recently noted 16 genes with differential expression in association with current posttraumatic stress disorder among subjects with a high degree of expo-

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sure to the World Trade Center attacks on 9/11 compared to similarly exposed controls with no lifetime PTSD [3]. Although this expression study provided information about markers and mechanisms of PTSD at the molecular level, it did not distinguish transient state markers of disease status from enduring trait markers of risk or resilience. The current paper aims to address this and extend our previous findings in several ways. First, we now include analyses of an additional 5 persons who had developed PTSD following 9/11 but then recovered, to distinguish state from trait gene expression markers. Second, we present data on genotype at FK506 binding protein 5 (FKBP5), an emerging PTSD risk marker which has been reported in association with PTSD symptoms [5], diagnosis [6], and risk factors [7], directly or in interaction with childhood adversity. Third, we include mediator analyses exploring the relationship between trait and state markers for PTSD (i.e., genotype, gene expression, neuroendocrinology, and symptom severity).

Several of the genes identified in our previous report are involved in hypothalamic-pituitary-adrenal (HPA) axis functioning [3]. The HPA axis is the major constituent of the neuroendocrine response to acute and chronic stress [8]. In PTSD, the fine-tuned regulation of the HPA axis is disturbed, which is indicated by reduced levels and an exaggerated responsiveness of ACTH and cortisol in these patients. Enhanced responsiveness of the glucocorticoid receptor (GR) appears to underlie these disturbances [9]. However, neuroendocrine investigations of PTSD have been complicated by findings of both trait [10–13] and state-related [14, 15] HPA axis alterations. It is therefore important to disentangle mechanisms underlying stable and state-dependent HPA axis alterations, a task for which samples including both current and remitted PTSD cases are well suited.

Genotype may also underlie trait vulnerability for PTSD. Indeed, adults abused in childhood who have genetic variations in the FKBP5 gene (involved in regulation of GR) appear to be at greater risk of developing PTSD to subsequent traumata as adults [5,6]. Lower expression of FKBP5 was associated with current PTSD in our recent report [3], and FKBP5 was identified as a gene of interest in a previous genome-wide microarray study of predictors of PTSD [4].

Among the obstacles in acquiring information about risk factors for PTSD have been difficulties in delineating groups of interest within the context of appropriate study designs. In studies of risk for PTSD using samples based on type of trauma exposure (e.g., rape victims, veterans), findings may reflect exposure characteristics or other commonalities among those who experienced the same type of event. Twin studies suggest that risks associated with PTSD are similar to those associated with exposure to the interpersonal violence that gives rise to this condition [16]. In samples of PTSD due to different types of events (e.g., accidents, combat trauma, rape), the effect of unmeasured risk factors on those being studied cannot be determined. This problem is compounded, even in prospective studies, by the recruitment of convenience samples rather than of subjects that represent the general population, or by failure to include similarly exposed persons without PTSD as comparisons. The opportunity of examining risk factors in a sample randomly recruited from a population exposed to varying levels of a single event has been rare.

The current study capitalizes on an opportunity to examine risk factors in a well-characterized population-representative sample of men and women from the New York City Metropolitan Area who were exposed to the World Trade Center attacks on 9/11 and recruited for the purpose of evaluating the effects of trauma exposure on the community. Because the risk factor for exposure to 9/11 was based on geography (i.e., proximity to the site), the risk factors for the development of PTSD are likely to be unrelated to those associated with the event that precipitated symptoms. Furthermore, because longitudinal data were obtained at yearly intervals beginning one month after 9/11 until blood drawing for genetic analysis, data regarding stability of PTSD were available, and not subject to recall bias.

Twenty participants meeting criteria for lifetime PTSD, assessed five years after 9/11, and 20 participants matched with respect to severity of exposure to 9/11, age, gender, and race were recruited for the purpose of performing whole blood genome-wide expression analysis to identify altered gene activity patterns as risk factors for PTSD. We predicted that some genes identified as differentially expressed in current PTSD [3] would also show altered expression in this lifetime sample, reflecting trait vulnerability factors; some genes altered in current PTSD would not be significantly different in lifetime PTSD, reflecting state markers or current disease status; and finally that some genes not identified in the current-only sample would be differentially expressed in lifetime PTSD, reflecting markers of recovery.

Since this study was part of a larger examination of psychological and biological risk factors for PTSD, we could relate gene expression to genotype, HPA axis pa-

rameters, and clinical outcome. We hypothesized that genotype at FKBP5 would predict FKBP5 expression. We further hypothesized FKBP5 genotype would predict HPA axis measures and PTSD severity, and that these relationships would be mediated by FKBP5 expression.

2. Methods and materials

2.1. Participants

Participants for this study were drawn from a larger epidemiological sample of individuals exposed to the attacks on the World Trade Center on September 11, 2001 [17]. A random sample of Caucasians who met criteria for PTSD in at least two out of four waves following 9/11 was invited by mail to participate in this study. After 20 participants were successfully recruited, a random sample of participants who had not met criteria for PTSD at any time were invited to participate, selected to match the PTSD participants in severity of trauma exposure (i.e., high direct vs. low and/or indirect exposure), age, and gender. All participants were Caucasians according to their responses in a self-report questionnaire asking for nationality, first language and ethnicity of the participant and all 4 grandparents. The study was approved by institutional review boards at the Mount Sinai School of Medicine and the New York Academy of Medicine. All participants provided written, informed consent and were then further screened to determine eligibility. Participants were not invited to participate further if they had psychotic or bipolar illness, alcohol or substance dependence, or major medical, endocrine, or neurological illness, confirmed by medical examination. No participant was in active treatment at the time of the study, and none were taking antidepressants.

2.2. Clinical evaluation

Diagnostic evaluation at the time of the blood draw (wave 5) was performed by trained psychologists with established interrater reliability on the Clinician Administered PTSD Scale [18] and the Structured Clinical Interview for the DSM-IV [19]. These scales, respectively, determined the presence of PTSD and confirmed the absence of other psychiatric disorders. To obtain a PTSD symptom severity score, participants completed the Posttraumatic Diagnostic Scale [20].

2.3. Blood drawing and processing

Fasting blood samples were obtained by routine venipuncture between 08:00–09:00 h. Plasma samples were frozen for the subsequent determination of cortisol by radioimmunoassay. The intra-assay and inter-assay coefficients of variation for this method were 4.0% and 6.8%.

For RNA, blood was processed using the PAXgene blood RNA stabilization system, which prevents degradation of many short-lived RNA transcripts and prevents further transcription and metabolic activity from altering the composition of the sample [21]. In addition, the samples were subjected to the globin mRNA reduction method, as this greatly improves the data quality of stabilized RNA samples hybridized to microarrays [22] (Liu et al., 2006). Gene expression studies were carried out using an Affymetrix GeneChip® Human Genome U133 Plus 2.0 Array containing over 47,000 transcripts for 38,500 well-characterized human genes, using standard methods.

To test whether the observed FKBP5 expression differences might be partially determined by genotypic variation, four single nucleotide polymorphisms (SNPs) were genotyped in the FKBP5 gene region (rs3800373, rs9296158, rs1360780, rs9470080), which were previously reported as associated with PTSD risk, symptoms, and diagnosis [5–7]. rs9296158, rs1360780, and rs9470080 are intronic SNPs, while rs3800373 is located in the 3' untranslated region. Genotyping was performed with a Roche LightCycler 480 System using allele-specific hybridization probes obtained from Metabion International AG (Martinsried, Germany); sequences are available upon request. None of the SNPs showed significant deviation from Hardy-Weinberg Equilibrium ($p > 0.09$); genotypes could be determined with a call rate of greater than 97%. Linkage disequilibrium (LD) structure was evaluated with HAPLOVIEW, version 4.0, (http://www.broad.mit.edu/mpg/haploview/) revealing r^2 between 0.77 and 0.95, which agrees with previous reports about the LD structure of this gene [5,6].

2.4. Statistical analysis

Groups were compared on demographic, clinical and biological measures using t-tests or chi-square tests as appropriate.

To analyze the microarray data, RNA expression was compared between cases and controls using dChip 2007 (build date Sept 5, 2007). Invariant Set Normalization

Table 1
Demographics, trauma exposure, and clinical characteristics in individuals with and without lifetime PTSD

	No PTSD ($n = 20$)	PTSD ($n = 20$)	Group comparisons
	Mean (SD) or % (n)	Mean (SD) or % (n)	
Demographics			
Age (yrs)	57.30 (13.19)	51.20 (15.88)	t (38) = 1.32, *ns*
Sex			χ^2 (1) < 0.0005, *ns*
Male	45.0% (9)	45.0% (9)	
Female	55.0% (11)	55.0% (11)	
Trauma Exposure			
CTQ[a] total score	6.42 (1.73)	11.97 (11.05)	$t(38) = -2.16$, $p < 0.05$
Total number of traumas	4.20 (2.35)	5.85 (3.54)	$t(38) = -1.74$, $p < 0.10$
Degree of 9/11 exposure	0.45 (0.61)	0.45 (0.69)	$t(38) < 0.005$, *ns*
Clinical Characteristics			
Current CAPS[b] scores			
Intrusive symptoms	2.11 (2.56)	7.89 (8.93)	$t(37) = -2.64$, $p < 0.05$
Avoidance	0.89 (1.97)	16.21 (13.34)	t (37) = −4.82, $p < 0.0005$
Hyperarousal	2.72 (4.71)	12.26 (8.86)	t (37) = −4.06, $p < 0.0005$
PDS[c] total score	3.45 (3.19)	18.95 (11.50)	$t(38) = -5.81$, $p < 0.0005$

[a]CTQ: Childhood Trauma Questionnaire [41];
[b]CAPS: Clinician-Administered PTSD Scale;
[c]PDS: Posttraumatic Stress Diagnostic Scale.

was carried out with all 40 arrays and model-based expression was evaluated using PM-MM probe data. Parameters were chosen using empirically derived false discovery rates (FDR). The use of t-test P-values for identifying differentially expressed genes showed a U-shaped curve in these analyses, with a minimum FDR at $P = 0.01$. Other parameters showed increased FDR with more restrictive filtering. Differentially expressed genes were therefore first identified using P-value of 0.01 or lower as the criterion. As an example, using these parameters and comparing 20 controls and 20 cases with lifetime PTSD led to the identification of genes with an empirical median FDR of 16% (from 200 permutations). Subsequently, the large proportion of these genes where the absolute expression differences were $\leqslant 50$ were flagged as low-expressing genes and removed from the current analyses.

To distinguish trait, state, and recovery markers, findings from these analyses were compared to the findings from our previous report [3]. Given the small sample size, this approach is exploratory, but is illustrative of a useful method for identifying transient versus persistent alterations in gene expression. As a follow-up to these exploratory analyses, analyses of variance (ANOVAs) were used to compare expression level between the current, lifetime only, and never PTSD groups for genes found to be differentially expressed by microarray analysis.

Finally, mediator analyses were conducted to test (1) the direct effect of genotype on FKBP5 gene expression, plasma cortisol and corticotrophin (ACTH), and PDS PTSD severity, and (2) whether effects of genotype on the latter three measures were mediated by FKBP5 expression. We tested the direct and indirect effects between and among these variables using Preacher and Hayes' bootstrapping method [23], based on 5,000 bootstrap samples. Due to the small sample size, FKBP5 genotype information was used to create a dichotomous variable indicating homozygosity for any of the four alleles previously reported in association with PTSD [5,6].

3. Results

3.1. Demographic and clinical characteristics

Table 1 shows clinical characteristics of the sample. Gender distribution between the PTSD and the traumatized control group was identical (9 men and 11 women in both groups, *ns*) and the two groups did not differ in age (57 ± 13 vs. 51 ±16; *ns*). Differences were observed in childhood traumatization and in severity of PTSD symptoms. Fifteen of 20 persons who had previously been diagnosed with PTSD following 9/11 still had PTSD at the time of the assessment.

3.2. Gene expression by microarray analysis

Our previous report on this sample restricted PTSD participants to those with current PTSD ($n = 15$) at the time of blood draw, comparing them to the controls

($n = 20$) [8]. Five additional participants found to have developed PTSD after 9/11, but no longer meeting criteria for this disorder five years later, were also included in the current analysis in order to distinguish state markers of PTSD status from trait risk markers of risk.

Analysis of expression profiles revealed 25 probe sets, corresponding to 25 genes, differentially expressed between the entire PTSD and traumatized control group (Table 2). All of these genes showed percent call rates of $>$ 95% in cases and/or controls, with the exception of probe set 40016_g_at, which showed a percent call rate of 65% in cases and 35% in controls (mostly due to M/marginal calls), and 233004_x_at, which had a percent call rate of 25% in cases and 5% in controls and was not further analyzed. Results in Table 2 show mean expression and "fold change" reflecting the difference in expression. Eight of these 25 genes were also identified in current PTSD (data presented in Yehuda et al., 2009 [3]); these are shaded in Table 2.

The eight genes identified in both our current and lifetime PTSD analyses may reflect stable risk factors for or "scars" of PTSD. The 17 genes identified only in this report might represent markers of recovery, whereas the nine identified only in current PTSD may be state markers of PTSD severity. To further understand these patterns, we directly compared the three diagnostic groups using ANOVA. The results of these analyses are presented in the last column in Table 2. All omnibus tests were significant (all $p < 0.05$). Planned comparisons revealed that thirteen genes were differentially expressed compared to controls in both current and past PTSD, suggesting trait vulnerability markers or scars. In contrast, two genes – FKBP5 and major histocompatibility complex (MHC), class II – were differently expressed in current PTSD compared to controls and to past PTSD, suggesting state markers of current PTSD status.

3.3. Genetic analysis of the FKBP5 locus

To determine whether genetic variation at the FKBP5 locus might be partially responsible for the alterations in FKBP5 expression and HPA axis measures, four SNPs in FKBP5 that had previously been associated with PTSD were genotyped. The SNPs were in high linkage disequilibrium, and hence results were very similar for each SNP. Due to this and the small sample size, we created a dichotomous variable indicating whether a case was homozygous for any of the four PTSD risk alleles (i.e., for rs1360780: TT; rs3800373: GG; rs9470080: TT; rs9296158: AA). In the context of complex diseases like PTSD, different polymorphisms in the same gene may have similar effects on phenotype (allelic heterogeneity) [24]. Direct and indirect effects of genotype on FKBP5 expression, plasma cortisol and ACTH, and PTSD severity were tested using 5,000 bootstrap samples.

Analyses indicated that mediational models were predictive of significant variance in plasma cortisol, $R^2 = 0.19$, $F = 4.47$, $p < 0.05$, and PTSD symptom severity, $R^2 = 0.16$, $F = 3.40$, $p < 0.05$, but not plasma ACTH, $R^2 = 0.03$, $F = 0.66$, *ns*. Risk genotype was significantly and negatively predictive of FKBP5 expression (probeset 224840_at), $B = -177.91$, t (38) $= -2.06$, $p < 0.05$ (Fig. 1A). FKBP5 expression, in turn, was predictive of both plasma cortisol, B = 0.01, t(38) = 2.72, $p < 0.05$ (Fig. 1B), and PTSD severity, $B = -0.03$, t(38) $= -2.44$, $p < 0.05$ (Fig. 1C). Genotype did not directly predict cortisol or symptom severity (both *ns*). However, analyses indicated that there were significant indirect effects of genotype, mediated through FKBP5 expression, on both cortisol, $B = -2.00$ (95% confidence interval [CI]: -3.35 to -0.98), and severity, $B = 4.59$ (95% CI: 1.29 to 8.54). The pattern of results was similar when using an alternate probeset for FKBP5 expression, 224856_at (data not shown). Homozygosity for any risk allele predicted lower expression of FKBP5, which was in turn associated with both lower cortisol and higher PTSD symptom severity.

4. Discussion

In this sample, we found gene expression changes between similarly-exposed persons with and without lifetime PTSD, some of which depended on current state, and some of which suggest enduring trait markers. Genotype of a promising candidate gene for PTSD risk, FKBP5, predicted FKBP5 expression; expression in turn mediated indirect effects of genotype on both cortisol levels and PTSD severity.

The current study is noteworthy in that it took advantage of a unique cohort ascertained following the 9/11 attacks in New York City to perform whole blood gene expression profiling in a subsample of persons who developed PTSD compared to similarly exposed controls who did not. This sample differs from other PTSD samples in that the primary risk factor for exposure to the precipitating traumatic event was geographic. Accordingly, the sample provides the opportunity to

Table 2

Microarray results for lifetime PTSD versus controls. These genes differentiated persons with ($n = 20$) and without ($n = 20$) lifetime PTSD. Order of probes is determined by the extent of difference (fold change). A (-) t value denotes gene expression in PTSD was larger than controls. Genes in Section I also differentiated persons with current PTSD ($n = 15$), compared to the same control group; genes in Section II only differentiated those with lifetime PTSD; and those in Section III differentiated those with current PTSD, but were not significant in the analysis of lifetime PTSD versus controls. The "Current v. Past v. Control" column indicates differences when expression levels in current PTSD (cur), remitted PTSD (pst), and control (ctl) groups were compared using ANOVA. Data for 202783_at, 218498_s_at, and 1562028_at were not available for these comparisons. Genes identified in current PTSD vs. controls are more fully described in Yehuda et al., 2009 [1]

AFFY_ID	Gene name	Control mean	SE	PTSD mean	SE	Fold change	t statistic	p value	Current v. past v. control
I. Identified in Both Lifetime and Current PTSD									
203668_at	MANNOSIDASE, ALPHA, CLASS 2C, MEMBER 1	232.80	11.98	314.02	14.21	1.35	−4.37	0.0001	cur = pst > ctl
1569601_at	CHROMOSOME 2 OPEN READING FRAME 34	379.30	18.03	306.64	15.85	0.81	3.03	0.0045	cur < ctl
1555088_x_at	SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 5B	702.10	35.49	551.03	36.95	0.78	2.95	0.0054	cur < ctl
201446_s_at	TIA1 CYTOTOXIC GRANULE-ASSOC RNA BINDING PROTEIN	324.57	19.99	250.41	16.87	0.77	2.84	0.0074	cur = pst < ctl
224600_at	CGG TRIPLET REPEAT BINDING PROTEIN 1	515.12	27.56	398.34	32.61	0.77	2.74	0.0095	cur < ctl
210201_x_at	BRIDGING INTEGRATOR 1	1889.97	104.6	1413.43	93.46	0.75	3.40	0.0016	cur = pst < ctl
213902_at	N-ACYLSPHINGOSINE AMIDOHYDROLASE (ACID CERAMIDASE) 1	865.97	44.54	653.26	32.01	0.75	3.88	0.0005	cur = pst < ctl
204633_s_at	RIBOSOMAL PROTEIN S6 KINASE, 90KDA, POLYPEPTIDE 5	767.93	48.56	564.03	40.59	0.73	3.22	0.0027	cur < ctl
II. Identified in Lifetime PTSD Only									
209041_s_at	UBIQUITIN-CONJUGATING ENZYME E2G 2 UBC7 HOMOLOG	102.37	15.48	180.17	16.31	1.76	−3.46	0.0014	cur = pst > ctl
232009_at	EGF-LIKE MODULE CONTAINING, MUCIN-LIKE, HORMONE RECEPTOR-LIKE 2	168.45	12.14	225.93	14.64	1.34	−3.02	0.0045	cur = pst > ctl
215909_x_at	MISSHAPEN-LIKE KINASE 1 (ZEBRAFISH)	187.96	10.58	239.91	15.27	1.28	−2.80	0.0085	cur = pst > ctl
214049_x_at	CD7 ANTIGEN (P41)	336.41	19.56	425.11	23.85	1.26	−2.88	0.0067	cur > ctl
236583_at	CONSENSUS INCLUDES GB:AA286867	242.60	14.13	296.21	12.06	1.22	−2.89	0.0065	cur = pst > ctl
225562_at	RAS P21 PROTEIN ACTIVATOR 3	356.77	15.50	424.46	17.93	1.19	−2.86	0.0070	cur = pst > ctl
200920_s_at	B-CELL TRANSLOCATION GENE 1, ANTI-PROLIFERATIVE	2784.53	63.07	3049.72	64.56	1.10	−2.94	0.0056	cur = pst > ctl
213729_at	HUNTINGTIN-INTERACTING PROTEIN A	531.96	10.83	583.08	13.33	1.10	−2.98	0.0051	cur = pst > ctl
233004_x_at	NUCLEAR FACTOR I/A	2879.86	76.16	2519.39	96.38	0.87	2.94	0.0058	cur < ctl
41387_r_at	JUMONJI DOMAIN CONTAINING 3	812.74	29.39	696.97	26.88	0.86	2.91	0.0061	cur = pst < ctl
223064_at	LOC51255 = RING FINGER PROTEIN 181	709.34	32.94	596.65	20.95	0.84	2.89	0.0069	cur = pst < ctl
232644_x_at	OCIA DOMAIN CONTAINING 1	374.06	15.34	309.09	18.14	0.83	2.74	0.0095	cur < ctl
213448_at	GLUCOSIDASE, BETA; ACID (INCLUDES GLUCOSYLCERAMIDASE)	301.66	12.52	245.05	11.83	0.81	3.29	0.0022	cur = pst < ctl
227961_at	CATHEPSIN B	512.42	27.60	408.38	26.47	0.80	2.72	0.0098	cur < ctl
40016_g_at	MICROTUBULE ASSOCIATED SERINE/THREONINE KINASE FAMILY MEMBER 4	442.09	21.77	349.82	19.72	0.79	3.14	0.0033	cur < ctl
206966_s_at	KRUPPEL-LIKE FACTOR 12	365.92	18.60	286.44	22.01	0.78	2.76	0.0090	cur < ctl
212837_at	KIAA0157 = FAMILY WITH SEQUENCE SIMILARITY 175, MEMBER B	294.28	15.28	225.69	14.25	0.77	3.28	0.0022	cur = pst < ctl
III. Identified in Current PTSD Only									
202783_at	NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE								
209703_x_at	METHYLTRANSFERASE LIKE 7A	Identified only in current PTSD; statistics in Yehuda et al., 2009							cur < ctl
213998_s_at	DEAD (ASP-GLY-ALA-ASP) BOX POLYPEPTIDE 17								cur > ctl
218498_s_at	ENDOPLASMIC OXIDOREDUCTIN-1-LIKE PROTEIN								
224702_at	MGC23909 = TRANSMEMBRANE PROTEIN 167A								cur < ctl
224840_at	FK506 BINDING PROTEIN 5								cur < pst = ctl
224856_at	FK506 BINDING PROTEIN 5								cur < pst = ctl
238900_at	MAJOR HISTOCOMPATIBILITY COMPLEX, CLASS II								cur < pst = ctl
1562028_at	CYCLIN D3								

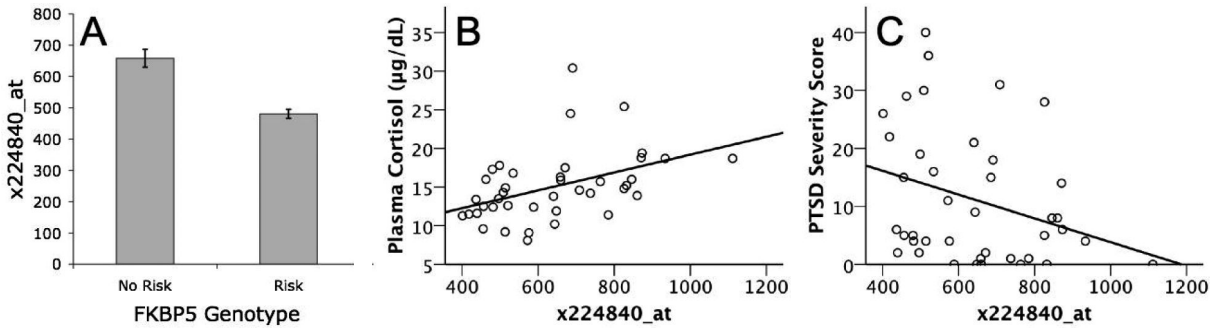

Fig. 1. Direct and indirect effects among genotype, FKBP5 expression, cortisol, and PTSD symptoms. Homozygosity for any of four PTSD risk-related SNPS in FKBP5 was associated with lower FKBP5 expression (panel A). Lower FKBP5 expression was associated with lower plasma cortisol (panel B) and with greater PTSD symptom severity (panel C). Analysis of 5,000 bootstrap samples revealed significant indirect effects of FKBP5 genotype on both cortisol and symptom severity, mediated by gene expression.

detect both risk factors and biomarkers contributing to PTSD in the absence of risk-seeking behavior, which is often confounded with the examination of PTSD. The current paper differs from our previous report on this cohort in its more comprehensive analyses, the inclusion of genotyping data, and in the inclusion of cases with remitted PTSD.

Several of the altered genes are known to be involved in signal transduction as well as in brain and immune cell function. In particular, a direct inhibitor of the nuclear translocation of activated GRs, STAT5B, was identified [25,26]. Since STAT5B is down-regulated in PTSD individuals, this could contribute to the consistently observed higher activity of GR in PTSD. Nuclear factor I/A (NFIA) was also found to be altered in gene expression. NFIA is a transcription factor which acts in concert with GR on a number of gene promoters, including the promoter of 11β-hydroxysteroid dehydrogenase type 2, whose activation regulates access of cortisol to the GR by converting cortisol to the glucocorticoid-inactive cortisone [27].

Therefore, two genes were identified that are involved in the actions of GR. STAT5B has also been identified in association with current PTSD [3]. However, two other GR-relevant genes previously associated with current PTSD (FKBP5 and MHC, class II) were not identified by microarray analysis in the present sample. While this implicates glucocorticoid dysfunction in the PTSD disease process, it also suggests that the mechanisms underpinning PTSD risk, resilience and recovery may differ from markers of diagnostic status. Specifically, genes showing alternate expression only in those with current PTSD may be state measures, while those altered in both current and lifetime PTSD may be consistent with risk or persistent metabolic change associated with PTSD. The eight genes in Section I of Table 2 represent this overlapping group of possible trait alterations. Finally, nine additional genes were identified in those with lifetime but not current PTSD, suggesting markers for recovery. ANOVAs conducted among the three diagnostic groups provided converging evidence that certain of these genes might represent state or trait markers. Interestingly, both FKBP5 and MHC class II were identified as state markers in these analyses, in which expression was lower in both current and remitted PTSD than in controls. This is consistent with the fact that these genes were both found to be differentially expressed in current PTSD [3] but not in lifetime PTSD compared to controls.

There have been two other studies examining gene expression following trauma exposure. In a study of persons exposed to severe trauma encountered in the emergency room who either did ($n = 8$), or did not ($n = 6$) meet criteria for PTSD at both a 1 month and 4 month follow-up, gene expression changes associated with several interesting molecular categories related to the stress response [4]. Given the proximity to trauma exposure, however, the findings may have reflected biological changes associated with recovering from the effects of trauma exposure, rather than with the development or persistence of chronic PTSD. Indeed, in the current sample, there was a dramatic recovery in PTSD prevalence between the first and second wave of data collection [28]. A subsequent whole blood gene expression profiling study of 16 subjects ($n = 8$ with PTSD) exposed to the Ramstein Airshow tragedy (20 years ago) attempted to examine processes associated with very chronic PTSD [29]. This study used a special microarray chip modified to specifically detect genes associated with the immune and stress responses. Interestingly, little overlap in gene expression was reported in the two studies. Both studies reported on very few

subjects, and neither study examined risk factors other than exposure to the focal trauma. The results of the current study underscore the relevance of risk factors in association with gene expression in predicting PTSD severity, which may lead to new effective personalized treatment approaches, considering both genotype and biomarkers [30].

Homozygosity for any of four alleles in the FKBP5 locus associated with PTSD risk was associated with lowered FKBP5 expression. This effect contrasts with observations in major depression that TT homozygosity at rs1360780 was not related to FKBP5 mRNA expression, and was associated with higher FKBP5 protein levels in lymphocytes [31]. These contrasting findings may be consistent with enhanced negative feedback inhibition of cortisol in PTSD, compared with blunted cortisol inhibition in depression [32]. There is also evidence that FKBP5 genotype may moderate the relationship between FKBP5 expression and cortisol/GR, [31] suggesting an additional mechanism which may underlie these different results. Finally, we cannot exclude the possibility that the previous study obtained different results because RNA was analyzed from a subset of lymphocytes [31], while here RNA from whole blood was investigated.

Furthermore, FKBP5 expression mediated indirect effects of genotype on both PTSD symptom severity and the PTSD risk factor of low cortisol. This pathway is consistent with FKBP5's role as an inhibitor of GR. As a cochaperone of the central heat shock protein hsp90 [33,34], FKBP5 participates in an intracellular negative feedback cycle resulting in lowered GR expression [35]. Furthermore, the mediational pathways identified here suggest a possible mechanism for previous findings that genotype at FKBP5 interacts with childhood trauma to predict PTSD severity [5] and diagnosis [6]. That is, individuals carrying one or more risk allele at this locus may be vulnerable to underexpression of FKBP5 in response to childhood trauma, which may, with other factors, lead to chronically low cortisol and a predisposition to PTSD. Future studies should test whether the mediational models reported here are moderated by childhood trauma severity. This gene has also been associated with recurrence of depressive episodes [31,36], suicide attempts [36], response to treatment [31,37,38], and impaired recovery of the stress response [39]; the present findings may therefore also be relevant in these areas.

There are some limitations which should be considered when interpreting these findings. First, the sample size limited the power of the study for the applied unbiased approach, and was particularly small for genotyping analyses. Further, the subsample studied was ethnically homogeneous to increase power to detect small changes in gene expression. Nevertheless, there were a number of significant findings, several of them in agreement with the model of enhanced GR responsiveness as one key feature of PTSD. Second, the small number of individuals with remitted PTSD led us to include both recovered and non-recovered participants in the lifetime PTSD group used in the initial microarray analysis; had more remitted participants been available, it would have been preferable to include them as a separate group in this analysis. Again, however, this approach identified several genes that did not emerge from analyses of current PTSD alone, and identified FKBP5 and MHC class II expression as potential state markers of PTSD status. Finally, the direct effect of genotype on cortisol and symptom severity did not reach significance. The sample may have been underpowered to detect a significant direct effect of genotype. However, significant mediational effects may exist even in the absence of a significant direct effect, due to a competing, unmeasured mediator exercising effects opposite to those of the observed mediator, in effect "canceling out" the direct effect [40].

It will be critical to carry out similar studies in larger cohorts to address these issues. Replication studies in additional Caucasian cohorts and generalization to other cohorts will also be important. Should these findings be replicated, the pattern of gene expression identified here can be evaluated as a diagnostic marker for PTSD, using techniques such as linear discriminant analysis. It would be especially interesting to carry out prospective studies where expression of the genes identified here are studied as pre-traumatic risk factors for subsequent PTSD. Such studies can be performed in large epidemiological cohorts and in unique populations such as military personnel. Similarly, animal models can be carried out that relate expression of the genes identified here to emergence of PTSD-like symptoms.

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