Intergenerational Effects of Maternal Holocaust Exposure on *FKBP5* Methylation

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Objective: There is growing evidence that exposure to trauma prior to conception can affect offspring. The authors have reported that adult offspring of Holocaust survivors showed lower methylation of FK506 binding protein 5 (*FKBP5*) intron 7, site 6 compared with Jewish comparison volunteers. The present study sought to replicate this finding in a larger sample and to examine parental and offspring correlates of observed effects.

Methods: Cytosine methylation was measured in blood using pyrosequencing. The independent replication sample consisted of 125 Holocaust offspring and 31 control subjects. Additional analyses, performed in a larger sample of 147 offspring and 40 control subjects that included the 31 previously studied participants, examined associations of parental trauma–related variables (i.e., sex of the exposed parent, parental posttraumatic stress disorder, age at Holocaust exposure) and offspring characteristics (i.e., childhood trauma exposure, lifetime psychiatric diagnoses, psychotropic medication use, *FKBP5* rs1360780 genotype, *FKBP5* gene expression, and neuroendocrine measures) with offspring *FKBP5* methylation.

Results: *FKBP5* site 6 methylation was significantly lower in Holocaust offspring than in control subjects, an effect associated with maternal Holocaust exposure in childhood and with lower offspring self-reported anxiety symptoms. *FKBP5* gene expression was elevated in Holocaust offspring. *FKBP5* methylation was associated with indices of glucocorticoid sensitivity but not with basal *FKBP5* gene expression.

Conclusions: This study replicates and extends the previously observed decrement in *FKBP5* intron 7, site 6 methylation in Holocaust offspring. The predominance of this effect in offspring of mothers exposed during childhood implicates maternal developmental programming as a putative mechanism.

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That offspring may be affected by parental trauma exposures occurring prior to conception was initially supported by an increased prevalence of posttraumatic stress disorder (PTSD) and of mood and anxiety disorders in offspring of Holocaust survivors (1). Similar consequences of parental exposure to combat, displacement, and genocides have also been reported (2, 3). Many putative mechanisms have been proposed to explain the effects of parental trauma, including impaired attachment and parenting, in utero perturbations, and alterations to the germline of exposed parents (3).

Initial examination of the biological correlates of parental Holocaust trauma focused on the hypothalamic-pituitary-adrenal (HPA) axis and on two genes related to glucocorticoid signaling: *NR3C1*, encoding the glucocorticoid receptor, and FK506 binding protein 5 (*FKBP5*), encoding a glucocorticoid receptor co-chaperone that has been shown to be altered in PTSD (4, 5). The HPA axis is subject to early developmental programming, including via epigenetic alterations to *FKBP5* and *NR3C1* (6, 7). Changes in DNA methylation of the *NR3C1* promoter, glucocorticoid receptor responsiveness, and ambient cortisol levels have been demonstrated in offspring of Holocaust survivors in relation to parental sex and PTSD (8), prompting a preliminary study examining DNA methylation on a region of the *FKBP5* gene containing functional glucocorticoid response elements (5). The FKBP5 protein moderates translocation of the bound glucocorticoid receptor and is a regulator of glucocorticoid receptor responsivity (7, 9). *FKBP5* was identified in the first genome-wide transcriptomics study of PTSD as one of several mRNAs that distinguished trauma-exposed persons with and without PTSD, independent of *FKBP5* genotype (10). Functional epigenetic alterations to *FKBP5* have been observed in association with psychiatric vulnerability, resilience, and symptom improvement in PTSD (7, 9, 11). Increased methylation at *FKBP5* intron 7,

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specifically at CpG site 6, was observed in Holocaust survivors, but lower methylation at this site was seen in their offspring compared with respective control subjects (5).

Because the sample size in our original pilot study (5) was small (constrained by availability of DNA from both parents and their offspring), potential contributors to *FKBP5* methylation, such as parental sex, age at exposure, and PTSD status or offspring trauma history or psychopathology, could not be examined. The present study was conducted to replicate and extend the previous findings in a larger sample and to determine salient correlates of the effect.

METHODS

Participants

Participants were recruited for two studies (study 1, 2001–2006; study 2, 2009–2011) designed to examine endocrine and molecular correlates of parental PTSD in five groups (Holocaust offspring with and without maternal and/or paternal PTSD, and comparison subjects). Data for 22 Holocaust offspring and nine control subjects from study 1 were previously published (5) and are designated as sample 1a. The remaining 48 offspring and 17 control subjects constitute sample 1b. Study 2 contributed 77 offspring and 14 control subjects. Thus, 156 participants (125 offspring and 31 control subjects) made up an independent replication sample to test methylation differences between Holocaust offspring and control subjects. Data from all 187 participants were used to test associations of parental and offspring characteristics with *FKBP5* methylation. Study procedures were approved by the institutional review boards at the Icahn School of Medicine at Mount Sinai and the Bronx Veterans Affairs Medical Center, and all participants provided written informed consent.

Holocaust offspring were conceived after parental Holocaust-related exposure(s), and parents of Jewish control subjects were generally from North America, as previously described (5). Exclusion criteria included lifetime psychotic, obsessive-compulsive, or bipolar disorders, current substance misuse, major medical illness, and treatment with steroids. Current PTSD status was exclusionary for Holocaust offspring; lifetime PTSD status was exclusionary for control subjects.

Procedures

Clinical evaluation. Psychiatric diagnoses were determined using the Structured Clinical Interview for DSM-IV (12), and PTSD was assessed using the Clinician-Administered PTSD Scale for DSM-IV (13). Participants completed the Beck Depression Inventory (14), the Spielberger State-Trait Anxiety Inventory (15), the Parental Bonding Instrument (16), and the Childhood Trauma Questionnaire (17). Parental psychopathology was assessed using the Family Informant Schedule and Criteria for DSM-IV (18) and the Parental PTSD Questionnaire (19).

Biological measures. Methods for *FKBP5* methylation, rs1360780 genotype, *FKBP5* gene expression, immune cell type differentiation and proportion, and details justifying covariates associated with biological assays are described in the online supplement and have been published elsewhere (5, 7, 20, 21).

Statistical Methods

Group comparisons. Group comparisons were made using chi-square analyses or analyses of variance and covariance (ANCOVAs), and partial correlation was used to explore relationships of symptom severity with molecular markers. Ages at maternal and paternal Holocaust exposure were categorically defined as occurring in childhood (≤11 years of age) or later (≥12 years). *FKBP5* expression data were log-transformed to minimize potential outlier effects; raw means are presented.

Covariate selection. Offspring age, sex, and study (i.e., study 1 or 2) were entered as covariates in all analyses; body mass index (BMI) and batch (batch 1–3) were additionally entered in analyses of methylation data. These covariates are justified as follows. Age differed across the two studies (study 1: mean age, 47.6 years [SD=7.5]; study 2: mean age, 56.4 years [SD=8.6]; $F=57.57$, df=1, 186, $p<0.0005$). Offspring sex was not evenly distributed, and *FKBP5* site 6 methylation was lower among men (mean=62.01, SD=0.75) than women (mean=67.17, SD=0.53) ($F=30.37$, df=1, 180, $p<0.0005$, controlling for age, BMI, study, batch, and control or offspring group). BMI was associated with site 6 methylation ($r=-0.156$, df=181, $p=0.035$). The designation of "limited model" was assigned to analyses including only these essential covariates.

An expanded set of covariates additionally accounted for the potential contribution(s) of maternal and paternal PTSD, offspring childhood adversity, offspring diagnoses of PTSD, depression and anxiety disorders, current psychotropic medication use, and rs1360780 genotype. Results based on the expanded model show reduced degrees of freedom due to variably missing covariate data. The analyses yielded similar results; results of the limited model are presented only when they differ from the expanded model.

RESULTS

Descriptive Demographic and Clinical Findings

Demographic and clinical characteristics are detailed in Table 1. Offspring sought psychiatric care and were taking psychotropic medication(s) more often, demonstrated more anxiety disorders and PTSD, self-reported higher symptom severity, and showed lower parental bonding scores than control subjects.

Characteristics of parental Holocaust exposure and PTSD. Holocaust exposure was reported for both parents by 75.5% (N=111) of offspring; 15.6% (N=23) had paternal exposure only, and 8.8% (N=13) had maternal exposure only. Maternal and paternal PTSD were reported by 54.5% (N=79) and 41.4% (N=60) of Holocaust offspring, respectively, with 37.7%

TABLE 1. Clinical characteristics of participants in a study of the intergenerational effects of Holocaust exposure on *FKBP5* methylation

Variable	Control Subjects (N=40)		Offspring of Holocaust Survivors (N=147)		Analysis		
					ANCOVA[a]		
	Mean	SE	Mean	SE	F	df	p
Age (years)	51.09	1.29	51.99	0.66	0.38	1, 183	n.s.
Interval from 1945 to birth year (years)	10.86	1.29	9.97	0.67	0.57	1, 183	n.s.
Body mass index	26.72	0.78	25.93	0.4	0.37	1, 182	n.s.
Education (years)	17.26	0.42	17.04	0.22	0.21	1, 178	n.s.
					Chi-square[b]		
	N	%	N	%	χ^2	df	p
Male	22	55.0	102	69.4	2.83	1	n.s.
Under care of psychiatrist (lifetime)	7	17.9	54	37.5	5.73	1	0.017
Psychotropic medication (current)	4	10.0	46	31.5	8.58	1	0.003
Maternal PTSD[c]	0	0.0	79	54.5	52.66	1	<0.0005
Paternal PTSD[c]	0	0.0	60	41.4	36.45	1	<0.0005
Diagnosis[d]							
Major depressive disorder (current)	1	2.7	12	8.3	1.69	1	n.s.
Major depressive disorder (lifetime)	14	37.8	81	55.9	3.86	1	n.s.
Anxiety disorder (current)	7	18.9	60	41.4	6.92	1	0.009
Anxiety disorder (lifetime)	7	18.9	75	51.7	13.79	1	<0.0005
PTSD (lifetime)	0	0.0	11	7.5	5.51	1	0.019
					ANCOVA[a]		
	Mean	SE	Mean	SE	F	df	p
Participant-rated scales							
Childhood trauma severity[e]	7.30	0.43	8.00	0.22	2.15	1, 174	n.s.
Maternal care and overprotection[f]	5.63	1.40	2.11	0.72	4.96	1, 167	0.027
Paternal care and overprotection[f]	6.08	1.30	2.17	0.68	7.01	1, 163	0.009
Depressive symptom severity[g]	5.95	1.24	9.83	0.63	7.73	1, 176	0.006
Suicidality[h]	0.01	0.06	0.14	0.03	4.26	1, 175	0.040
Anxiety symptom severity[i]	28.26	3.71	38.78	1.87	6.35	1, 170	0.013
PTSD severity (current)[j]	0.89	1.93	7.29	0.98	8.63	1, 172	0.004
PTSD severity (lifetime)[j]	3.42	2.91	17.61	1.48	18.66	1, 172	<0.0005
					Chi-square[b]		
	N	%	N	%	χ^2	df	p
FKBP5 rs1360780 "risk" allele[k]	18	46.2	67	46.5	0.002	1	n.s.

[a] Analyses of covariance (ANCOVAs) were covaried for age, sex, and study (offspring age and interval were covaried for sex and study).
[b] The likelihood ratio (chi-square statistic) is provided for all chi-square analyses.
[c] The assigned parental posttraumatic stress disorder (PTSD) status was based on the Parental PTSD Questionnaire.
[d] Depression and anxiety disorder diagnoses were assigned using the Structured Clinical Interview for DSM-IV; PTSD diagnoses were assigned based on the Clinician-Administered PTSD Scale for DSM-IV (CAPS).
[e] Measured with the total score on the Childhood Trauma Questionnaire.
[f] Measured with the Parental Bonding Instrument.
[g] Measured with the Beck Depression Inventory (BDI).
[h] Measured with the BDI suicidality item.
[i] Measured with the total score on the State-Trait Anxiety Inventory.
[j] Measured with the CAPS total scores for current and lifetime symptom severity.
[k] rs1360780 homozygous or heterozygous for the minor ("risk") allele (see text).

(N=55) reporting one parent with PTSD and 29% (N=42) reporting PTSD for both parents.

Mean age at the beginning of the Holocaust was 15.0 years (SD=7.8) for mothers and 19.9 years (SD=8.6) for fathers. A total of 19.7% (N=29) of Holocaust offspring were born to mothers who were ≤11 years old at the start of the Holocaust, and 16.3% (N=24) had fathers who were ≤11 years old at the start of the Holocaust. Comparing

childhood with later parental exposure yielded no significant difference in the frequency of maternal (58.6% and 66.7%, respectively) or paternal (45.8% and 44.4%, respectively) PTSD (χ^2=0.619, df=1, n.s., and χ^2=0.015, df=1, n.s., respectively).

FKBP5 Intron 7, Site 6 Methylation in Holocaust Offspring and Control Subjects

The percentage of *FKBP5* site 6 methylation was lower in Holocaust offspring (mean=67.12%, SE=0.53) than in control subjects (mean=69.64%, SE=1.09) (F=4.26, df=1, 150, p=0.041, pη^2=0.028) for the replication sample, and for the entire sample (offspring: mean=64.87%, SE=0.48; control subjects: mean=67.49%, SE=0.93; F=6.26, df=1, 180, p=0.013, pη^2=0.034). Including covariates in the expanded model strengthened this difference (F=7.70, df=1, 156, p=0.006, pη^2=0.048) (Figure 1).

Because offspring sex was a significant covariate in the above analysis, a two-way ANCOVA was performed. There were significant effects of sex (male: mean=63.06%, SE=0.84; female: mean=67.62%, SE=0.69; F=16.80, df=1, 179, p<0.0005) and group (control subjects: mean=66.7%, SE=0.93; offspring: mean=63.94%, SE=0.52; F=6.92, df=1, 179, p=0.009), but the absence of a significant interaction between the two (F=0.96, df=1, 179, n.s.) indicates that sex did not account for the reduction in *FKBP5* methylation observed in Holocaust offspring.

Table S1 in the online supplement details group comparisons for additional *FKBP5* sites on intron 7 to highlight the specificity of the effect of parental Holocaust exposure at site 6 and to demonstrate that mean intron methylation was also reduced. The intercorrelations among sites presented in Table S2 in the online supplement indicate that site 6 is part of a functional unit with other sites on intron 7. There was no difference in site 6 methylation by genotype (protective allele: mean=65.01%, SE=0.61; risk allele: mean=65.74%, SE=0.65; F=0.65, df=1, 176, n.s.).

FKBP5 Gene Methylation and Offspring Symptom Profiles

Lower site 6 methylation was significantly associated with reduced anxiety (r=0.169, df=167, p=0.028) but not with childhood trauma (r=0.130, df=171, n.s.), parental bonding (maternal: r=0.027, df=166, n.s.; paternal: r=0.115, df=162, n.s.), depression severity (r=0.000, df=173, n.s.), or PTSD severity (current: r=0.090, df=169, n.s.; lifetime: r=0.083, df=169, n.s.).

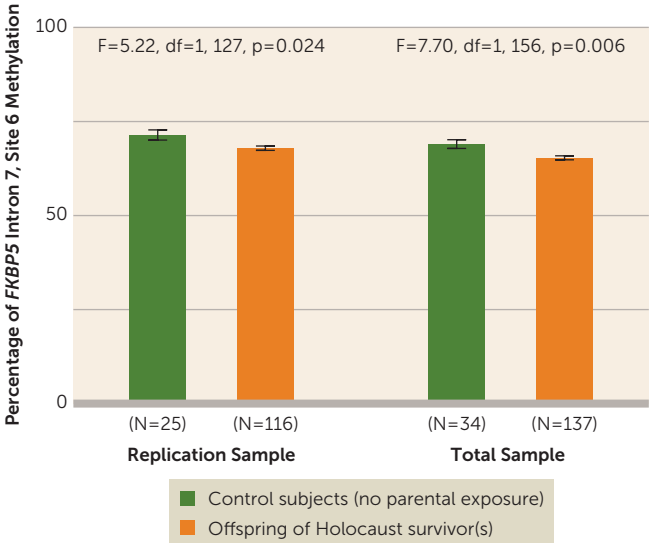
Effect of Sex of Holocaust-Exposed Parent

Maternal exposure was associated with lower site 6 methylation (F=2.78, df=3, 154, p=0.043), but methylation for paternal exposure did not differ significantly from that of control subjects (Figure 2A).

Effects of Maternal or Paternal PTSD

There were no significant site 6 methylation differences in association with maternal or paternal PTSD (maternal PTSD: F=0.26, df=1, 156, n.s.; paternal PTSD: F=0.35, df=1, 156, n.s.; or with the interaction between maternal PTSD and paternal PTSD: F=0.43, df=1, 156, n.s.).

FIGURE 1. Comparison of *FKBP5* intron 7, site 6 gene methylation between offspring of Holocaust survivors and Jewish control subjects[a]



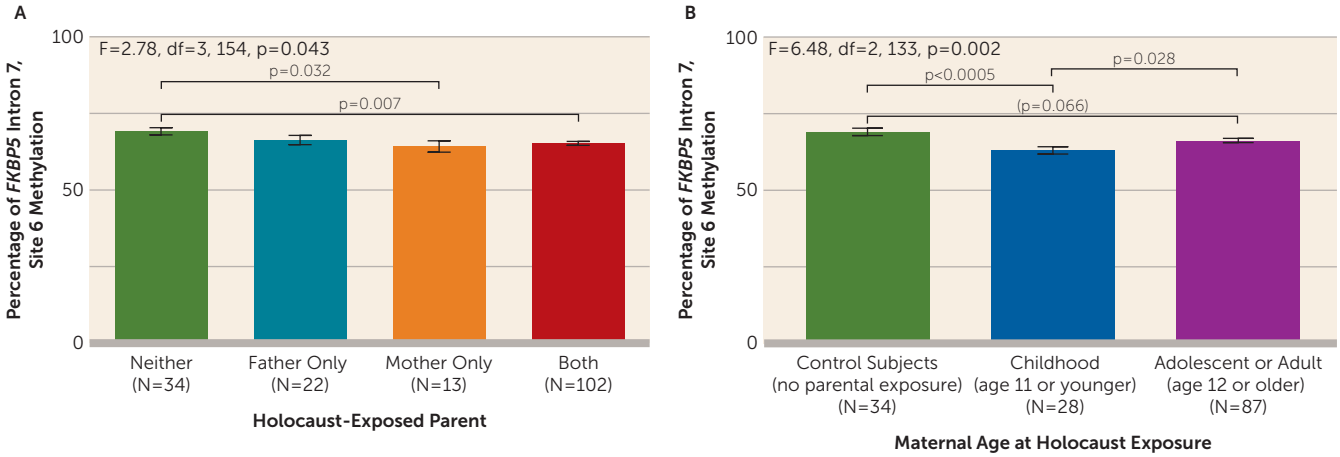
[a] Data on the left represent results for the independent replication sample, and data on the right represent findings for the total sample, including previously published data. While the means in the text represent corrected means based on the limited model (covariates: age, sex, body mass index, batch, and study), the data in this figure and in Figure 2 show corrected means for analyses of covariance based on an expanded model (additional covariates: maternal and paternal posttraumatic stress disorder [PTSD]; severity of childhood trauma [Childhood Trauma Questionnaire total score]; lifetime anxiety disorder, major depression, and PTSD diagnoses; psychotropic medication use; and *FKBP5* rs1360780 genotype). Error bars indicate standard errors of the mean.

Effects of Maternal Childhood Holocaust Exposure on *FKBP5* Methylation

As shown in Figure 2B, significantly lower site 6 methylation was observed in offspring whose mothers were exposed in childhood compared with control subjects (p<0.0005) and compared with offspring of mothers exposed later in life (p=0.028), although the latter group did not differ significantly from control subjects (p=0.066) (F=6.48, df=2, 133, p=0.002; pη^2=0.089). When accounting additionally for paternal age at Holocaust exposure, the finding remained significant (F=6.78, df=1, 114, p=0.010). However, an apparent effect of paternal age at exposure on site 6 methylation (F=4.01, df=2, 142, p=0.020) was no longer significant when also accounting for maternal age at exposure (F=0.55, df=1, 107, n.s.).

As shown in Table 2, clinical measures that distinguished Holocaust offspring from control subjects were largely associated with older maternal age at exposure. Generally, offspring with childhood maternal Holocaust exposure did not differ significantly from control subjects or from offspring born to mothers with later exposure.

FIGURE 2. Intergenerational effects of parental Holocaust exposure on offspring *FKBP5* intron 7, site 6 methylation by sex of the exposed parent and age at maternal exposure[a]



[a] Panel A illustrates offspring *FKBP5* intron7, site 6 methylation according to the sex of the Holocaust-exposed parent(s). Corrected means are based on analyses of covariance (ANCOVAs) (the expanded model) comparing control subjects, offspring with maternal exposure only, paternal exposure only, and maternal and paternal Holocaust exposure. Using the limited model, the overall effect fell short of significance (F=2.401, df=3, 178, p=0.069), but there were significant post hoc comparisons with control subjects for maternal exposure only (p=0.028) and for both maternal and paternal exposure (p=0.026), but not for paternal-only exposure. Panel B illustrates offspring *FKBP5* intron 7, site 6 methylation based on ANCOVA (the expanded model) comparing control subjects, offspring with maternal exposure in childhood, and offspring with later maternal exposure (in adolescence or adulthood). Results indicate that *FKBP5* site 6 methylation for offspring of mothers with childhood Holocaust exposure is significantly lower than that for control subjects ($p<0.0005$) and for offspring of mothers exposed later in life (p=0.028). The latter group shows reduced methylation compared with control subjects, but not to a significant level (p=0.066). Results were similar using the limited model (F=6.62, df=2, 155, p=0.002; $p\eta^2=0.079$), with significant post hoc comparisons of offspring with maternal childhood exposure relative to control subjects ($p<0.0005$) and to offspring with later maternal exposure (p=0.009); site 6 methylation did not differ significantly between the latter group and control subjects (p=0.129). Error bars indicate standard errors of the mean.

FKBP5 mRNA Expression

FKBP5 gene expression, available only for participants in study 2, was higher in Holocaust offspring than in control subjects (control subjects: mean=0.78, SE=0.15; Holocaust offspring: mean=1.13, SE=0.06; F=6.82, df=1, 84, p=0.011). The effect was maintained when controlling additionally for genotype (F=6.41, df=1, 80, p=0.013) but was no longer significant using the expanded model (F=2.89, df=1, 73, p=0.093), a difference likely reflecting an effect of lifetime anxiety disorder, the only significant covariate in the latter analysis (F=14.18, df=1, 73, $p<0.0005$). Indeed, *FKBP5* expression was positively correlated with self-reported anxiety ratings (r=0.246, df=81, p=0.025) and was higher among those with (mean=1.27, SE=0.08) relative to those without (mean=0.84, SE=0.09) lifetime (F=16.12, df=1, 83, $p<0.0005$) or current (F=12.77, df=1, 83, p=0.001) anxiety disorders. There was no significant association between basal (i.e., unstimulated) *FKBP5* gene expression and site 6 methylation (r=−0.003, df=83, n.s.).

Relationship of *FKBP5* Methylation to Neuroendocrine Measures

As shown in Table 3, site 6 and mean intron 7 methylation (sites 3–6) were negatively correlated with basal cortisol levels (r=−0.308, df=100, p=0.002, and r=−0.364, df=100, $p<0.0005$, respectively), with cortisol decline following 0.50 mg of oral dexamethasone (r=−0.287, df=93, p=0.005, and r=−0.344, df=93, p=0.001), and with weaker glucocorticoid sensitivity as determined by the lymphocyte lysozyme $IC_{50\text{-}DEX}$ (r=0.220, df=98, p=0.028, and r=0.332, df=98, p=0.001). Table 3 also details associations between *FKBP5* methylation, expression, endocrine markers, and previously reported glucocorticoid receptor (*NR3C1*) 1F promoter methylation and gene expression (4). *FKBP5* intron 7 and glucocorticoid receptor 1F promoter methylation were not significantly correlated; however, each was associated with distinct, but also overlapping, neuroendocrine measures.

DISCUSSION

The results reported here replicate the previous observation of reduced methylation at a CpG site in an intronic enhancer of the *FKBP5* gene in adult offspring of Holocaust survivors (5). Replication of this finding in offspring studied years apart, using DNA extracted from whole blood and peripheral blood mononuclear cells, with the use of similar pyrosequencing methods across three laboratories, increases confidence in the finding. Offspring age, childhood trauma severity, psychiatric diagnoses, suicidality, parental PTSD, psychotropic medication, *FKBP5* genotype, or differential immune cell count did not account for the decrement in site 6 methylation. Rather, reduced *FKBP5* methylation was associated with maternal Holocaust exposure and was particularly evident in offspring of mothers exposed as children. While the effect for the comparison between Holocaust offspring and control subjects was modest, the effect comparing

offspring with maternal childhood exposure and control subjects was large.

An effect in a single area on a functionally relevant gene in peripheral blood may serve as a sentinel for significant functional effects, particularly if the observed change is reliable and correlated with changes in methylation of that gene in other important target tissues. The present finding builds on translational work demonstrating correlations of glucocorticoid-induced changes in *FKBP5* enhancer methylation between rodent peripheral blood and hippocampus (22). In human tissue, DNA methylation of intron 7, site 6 was shown to be affected by glucocorticoids in peripheral blood cells and in a multipotent hippocampal progenitor cell line (7). Stable DNA demethylation of *FKBP5* intron 7 in response to glucocorticoids in human hippocampal cells was present during early but not later phases of cellular proliferation and differentiation (7). In these models, *FKBP5* intron 7 methylation was correlated with glucocorticoid-induced *FKBP5* expression and glucocorticoid receptor sensitivity as determined in an ex vivo lymphocyte model (similar to results shown in Table 3), demonstrating the functional relevance of a methylation change in this intronic region; *FKBP5* methylation was not, however, correlated with basal mRNA expression, as shown in this study and described elsewhere (23).

When bound to the glucocorticoid receptor protein complex, *FKBP5* reduces ligand binding, nuclear translocation, and functional sensitivity of the glucocorticoid receptor (7, 9). The significant correlations of *FKBP5* methylation in peripheral blood with endocrine measures imply that small effects observed in blood cells may reflect functionally significant alterations in other stress responsive systems. Moreover, small differences in intron 7 DNA methylation in peripheral blood have been associated with structural and functional brain alterations (7, 24). Methylation at CpG sites within the glucocorticoid response elements of intron 7 was intercorrelated and likely functions as a unit—with site 6 best representing the unit—a finding previously demonstrated in a reporter gene assay (7). Indeed, correlations between endocrine measures and mean methylation of the intron were more robust than those with site 6 methylation.

In the present study, as in the pilot (5), the effect of maternal Holocaust exposure on site 6 methylation was independent of genotype and was not associated with trauma-related psychopathology. Rather, lower site 6 methylation was associated with diminished self-reported anxiety symptoms, suggesting the possibility of a protective effect. Offspring of mothers exposed in childhood showed the greatest reductions in methylation but also the least psychopathology. Reduced intronic and site 6 methylation in offspring contrasts with increased site 6 methylation observed in Holocaust survivors (although, interestingly, parental and offspring methylation at site 6 were positively correlated) (5).

FKBP5 intron 7 methylation was not correlated with methylation of the *NR3C1* gene, although both were associated with slightly different HPA axis measures, suggesting that methylation in genes that are known to interact may be independently regulated and associated with distinct aspects of trauma exposure or its sequelae (25). For instance, past work has shown that glucocorticoid receptor 1F promoter methylation predicted response to psychotherapy for PTSD, while posttreatment *FKBP5* promoter methylation was associated with treatment-induced symptom change (11). Moreover, glucocorticoid receptor methylation has been shown to be differentially associated with maternal and paternal PTSD, a finding linked with increased trait anxiety, depression, and risk for PTSD (4).

Within *FKBP5* intron 7, different CpG sites appear to be subject to distinct influences, as indicated by the lack of association between site 6 methylation and childhood trauma, whereas methylation of other intron 7 sites (particularly site 3) has been shown in this and other studies (5, 7, 24, 26) to interact with the rs1360780 genotype to increase risk for the development of PTSD in the presence of childhood trauma. Only one single-nucleotide polymorphism was measured in the present investigation; however, other genetic influences on site 6 methylation may be detected in future studies using genome-wide approaches. That *NR3C1* and *FKBP5* methylation relate to distinct clinical correlates suggests a complex interaction between the two systems that could be further explored to better understand sources of variation in molecular and endocrine phenotypes.

The finding of decreased *FKBP5* methylation with earlier maternal age at Holocaust exposure is consistent with a previous observation that maternal age at exposure and PTSD were independently associated with reduced urinary cortisol levels in adult offspring (27). Offspring born to mothers with childhood exposure showed elevated 11-β-hydroxysteroid dehydrogenase (11-β-HSD-2) activity (28), a finding directionally opposite to that in Holocaust survivors (29). Survivors who were younger at age at exposure demonstrated the lowest 11-β-HSD-2 enzyme activity (29), but their adult children had the highest levels of urinary 11-β-HSD-2 activity (28). Interestingly, methylation of both *FKBP5* and *HSD11B2* is regulated in the placenta to moderate embryonic exposure to maternal glucocorticoids (6).

A maternal effect may be attributable to in utero glucocorticoid exposure (2, 30–32) and reflect preconception molecular effects on gametes or altered placental neuroendocrine stress physiology, with effects on fetal brain development in association with maternal childhood adversity (30, 33). Theoretically, trauma occurring prior to puberty may precipitate changes to the oocyte that are maintained throughout embryogenesis and/or are reestablished after conception, potentially influencing the intrauterine environment (2, 34). Before puberty, oocytes are still in a haploid demethylated state and are vulnerable to environmental perturbations (35). However, we know of no studies that have examined the possibility of epigenetic transmission through oocytes in humans or animals. That early maternal age at trauma exposure is a significant

TABLE 2. Clinical characteristics of control subjects and of Holocaust offspring by maternal age at exposure in a study of *FKBP5* methylation

Variable	[1] Control Subjects (N=40)		[2] Exposure Age ≤11 Years (N=29)		[3] Exposure Age ≥12 Years (N=95)		ANCOVA Post Hoc Tests or Chi-Square Pairwise Comparisons					
										[1] Compared With [2]	[1] Compared With [3]	[2] Compared With [3]
							ANCOVA[a]					
	Mean	SE	Mean	SE	Mean	SE	F	df	p			
Age (years)	51.2	1.13	47.55	1.31	54.87	0.73	12.96	2, 157	<0.0005	0.038	0.007	<0.0005
Interval from 1945 to birth year (years)	10.8	1.13	14.50	1.30	7.04	0.73	13.50	2, 157	<0.0005	0.033	0.007	<0.0005
Body mass index	26.70	0.81	24.40	0.097	26.54	0.97	2.09	2, 156	n.s.	n.s.	n.s.	n.s.
Education (years)	18.10	0.70	18.45	0.82	26.54	0.54	1.28	2, 74	n.s.	n.s.	n.s.	n.s.
							Chi-square[b]					
	N	%	N	%	N	%	χ^2	df	p			
Male	20	45.5	8	27.6	26	27.4	4.69	2	0.096	n.s.	0.043	n.s.
Under care of psychiatrist (lifetime)	7	17.9	8	24.1	40	34.7	10.22	2	0.006	n.s.	0.004	n.s.
Psychotropic medication (current)	4	10.0	7	28.6	33	35.1	8.6	2	0.014	n.s.	0.002	n.s.
Maternal PTSD[c]	0	0.0	17	58.6	62	66.0	71.12	2	<0.0005	<0.0005	<0.0005	n.s.
Paternal PTSD[c]	0	0.0	8	27.6	40	43.0	37.96	2	<0.0005	<0.0005	<0.0005	n.s.
Diagnoses[d]												
Major depressive disorder (current)	1	2.7	3	10.3	8	8.6	2.06	2	n.s.	n.s.	n.s.	n.s.
Major depressive disorder (lifetime)	14	37.8	14	48.3	57	61.3	6.27	2	0.044	n.s.	0.015	n.s.
Anxiety disorder (current)	7	18.9	11	37.9	39	41.9	6.62	2	0.036	n.s.	0.01	n.s.
Anxiety disorder (lifetime)	7	18.9	12	41.4	52	55.9	15.75	2	<0.005	0.045	<0.0005	n.s.
PTSD (lifetime)	0	0.0	2	6.9	7	7.4	5.25	2	0.073	n.s.	0.024	n.s.
							ANCOVA[a]					
	Mean	SE	Mean	SE	Mean	SE	F	df	p			
Participant-rated scales												
Childhood trauma severity[e]	7.27	0.43	7.51	0.51	8.14	0.28	1.58	1, 150	n.s.	n.s.	n.s.	n.s.
Maternal care and overprotection[f]	5.73	1.41	3.37	1.67	1.11	0.94	3.59	2, 145	0.030	n.s.	0.009	n.s.
Paternal care and overprotection[f]	6.2	1.28	4.62	1.55	1.15	0.87	5.53	2, 141	0.005	n.s.	0.002	n.s.
Depressive symptom severity[g]	5.84	1.25	9.13	1.49	10.79	0.80	5.37	2, 151	0.006	n.s.	0.001	n.s.
Suicidality[h]	0.003	0.05	0.11	0.9	0.15	0.04	2.49	2, 152	n.s.	n.s.	n.s.	n.s.
Anxiety symptom severity[i]	21.2	3.63	34.21	4.40	42.08	2.32	5.85	2, 146	0.004	n.s.	0.001	n.s.
PTSD symptom severity (current)[j]	1.01	1.89	6.23	2.25	7.74	1.24	4.36	2, 147	0.014	n.s.	0.004	n.s.
PTSD symptom severity (lifetime)[j]	3.21	2.84	15.39	3.39	18.73	1.87	10.26	2, 147	<0.0005	0.006	<0.0005	n.s.

continued

TABLE 2, *continued*

Variable	[1] Control Subjects (N=40)		[2] Exposure Age ≤11 Years (N=29)		[3] Exposure Age ≥12 Years (N=95)		ANCOVA Post Hoc Tests or Chi-Square Pairwise Comparisons			[1] Compared With [2]	[1] Compared With [3]	[2] Compared With [3]
							Chi-square[b]					
	N	%	N	%	N	%	χ^2	df	p			
FKBP5 rs1360780 "risk" allele[k]	20	47.6	12	41.4	38	41.3	0.50	2	n.s.	n.s.	n.s.	n.s.

[a] Analyses of covariance (ANCOVAs) were covaried for age, sex, and study (offspring age and interval were covaried for sex and study).
[b] The likelihood ratio (chi-square statistic) is provided for all chi-square analyses.
[c] The assigned parental posttraumatic stress disorder (PTSD) status was based on the Parental PTSD Questionnaire.
[d] Depression and anxiety disorder diagnoses were assigned using the Structured Clinical Interview for DSM-IV; the assigned PTSD diagnosis was based on the Clinician-Administered PTSD Scale for DSM-IV (CAPS).
[e] Measured with the total score on the Childhood Trauma Questionnaire.
[f] Measured with the Parental Bonding Instrument with combined scores for care and overprotection.
[g] Measured with the Beck Depression Inventory (BDI).
[h] Measured with the BDI suicidality item.
[i] Measured with the total score on the State-Trait Anxiety Inventory.
[j] Measured with the CAPS total scores for current and lifetime symptom severity.
[k] rs1360780 homozygous or heterozygous for the minor ("risk") allele (see text).

TABLE 3. Associations of *FKBP5* and *NR3C1* methylation and expression with glucocorticoid neuroendocrine indices in a study of *FKBP5* methylation

Glucocorticoid Measure	Methylation and Expression of *FKBP5* Intron 7 and *NR3C1* 1F														
	FKBP5 Site 6[a]			*FKBP5* Sites 3–6[a]			*FKBP5* Expression[b]			*NR3C1* 1F Sum Percentage[c]			*NR3C1* Expression[c]		
	r	df	p	r	df	p	r	df	p	r	df	p	r	df	p
Glucocorticoid receptor sensitivity[d]	0.220	98	0.028	0.332	98	0.001	0.018	82	n.s.	0.052	84	n.s.	0.006	86	n.s.
Baseline plasma cortisol	−0.308	100	0.002	−0.364	100	<0.0005	−0.059	83	n.s.	−0.022	85	n.s.	0.202	87	n.s.[e]
Postdexamethasone plasma cortisol[f]	0.060	93	n.s.	0.057	93	n.s.	−0.040	76	n.s.	0.212	77	n.s.	0.175	76	n.s.
Cortisol decline following dexamethasone[f, g]	−0.287	93	0.005	−0.344	93	0.001	−0.071	76	n.s.	−0.133	77	n.s.	−0.282	76	0.012
Percentage of cortisol suppression to dexamethasone[f, h]	−0.042	93	n.s.	−0.061	93	n.s.	0.053	76	n.s.	−0.197	77	n.s.	−0.223	76	0.050

[a] Partial correlation controlling for age, sex, body mass index (BMI), cohort, study, and control or offspring group.
[b] Partial correlation controlling for age, sex, BMI, expression batch, and control or offspring group.
[c] Partial correlation controlling for age, sex, BMI, and control or offspring group.
[d] Glucocorticoid receptor sensitivity is estimated in live cultured lymphocytes incubated with increasing concentrations of dexamethasone and defined as the lysozyme $IC_{50\text{-}DEX}$, or the concentration of dexamethasone at which lymphocyte lysozyme production is inhibited by 50%.
[e] Partial correlation of baseline 8:00 a.m. plasma cortisol and *NR3C1* expression does not reach significance (r=−0.202, df=87, p=0.058). All other correlations, p>0.10.
[f] Controlling for dexamethasone levels.
[g] Cortisol decline (baseline minus postdexamethasone cortisol).
[h] The percentage of cortisol suppression was calculated as follows: 100×([baseline − postdexamethasone cortisol]/baseline cortisol).

determinant of an intergenerational effect on site 6 (and intron 7) methylation may also imply a differential effect on subsequent interpersonal interactions between mother and child, including parental bonding and attachment (36). It is reasonable to infer that maternal age at exposure may have influenced child-rearing practices, a possibility suggested by the finding of reduced psychopathology among offspring raised by mothers exposed in childhood. Moreover, parental care and overprotection ratings differed from control subjects only for offspring whose mothers were exposed later in life. These data underscore the complexity of assigning specific mechanisms for the observed effects, because even if an epigenetic alteration involves a post-conception change to germ cells or in utero perturbations, the impact of child rearing, attachment, and other postnatal environmental characteristics may be the more important drivers of change.

The lack of a finding specific to paternal exposure should not be interpreted to reflect a lack of epigenetic effects of paternal trauma. Indeed, several animal and human studies

suggest that there are potent effects on offspring transmitted through sperm (26, 31, 34). It is also not possible to know, when both parents have been exposed to severe trauma at slightly different ages, what the synergistic effects of that exposure might be on their offspring.

Data from the present cross-sectional study in adult offspring cannot speak to the mechanisms through which epigenetic alterations may have been acquired. It is nonetheless interesting to speculate whether the findings help explain increased offspring vulnerability to psychopathology or reflect an adaptation to optimize offspring preparedness or response to adversity in their own lives (37). Disinhibited *FKBP5* expression might promote caution or vigilance in an adverse environment, behaviors elicited by increased *FKBP5* expression in animal experiments (38). Consistent with these behaviors, reduced methylation of *FKBP5* intron 7 was associated with diminished anxiety following exposure-based psychotherapy (39). The significant associations of increased anxiety with higher *FKBP5* mRNA levels, and of decreased anxiety with lowered *FKBP5* methylation, would be consistent with such adaptive effects. The observation that lower site 6 and mean intron 7 *FKBP5* methylation was associated with reduced glucocorticoid sensitivity and increased basal cortisol levels suggests that individuals with lower *FKBP5* methylation display fewer PTSD-like endocrine alterations, which have been associated with elevated risk for psychopathology (8, 27). Certainly, genome-wide studies may identify broader functional epigenetic and transcriptomic profiles associated with intergenerational trauma and risk or resilience in offspring, and they would provide an important context for the present findings.

The potential functional role of any single observed epigenetic alteration requires further study, and the regulation of any specific gene transcript requires knowledge of other genes, epigenetic marks, microRNAs, and additional regulators. This study is limited by the lack of genome-wide data, the single cross-sectional observation, and the uneven sample sizes for the Holocaust offspring and comparison groups. A further limitation is that although a strong signal was detected for maternal childhood exposure, the number of participants with only fathers exposed may have been too small to test the contribution of the paternal germline to the present findings.

Nonetheless, the previous observation of an association of parental Holocaust exposure with *FKBP5* DNA methylation in adult offspring has been replicated in a larger cohort. Previous work is extended by the new observation that effects on offspring *FKBP5* intron 7 methylation and *FKBP5* gene expression are associated with maternal exposure and, for the former, with maternal childhood trauma exposure. Our study cannot distinguish determinants of the observed effects, including a preconception effect on germ cells, differences in the in utero milieu, alterations in postnatal care, or other influences (3, 32). However, the relative stability of the observation across cohorts, in adults born years or sometimes decades after parental exposure, provides an impetus for basic science studies in this field. Prospective intergenerational studies are needed to elucidate the mechanisms for such trauma effects, as well as their functional consequences for vulnerability, adaptation, and resilience.

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REFERENCES

1. Yehuda R, Schmeidler J, Wainberg M, et al: Vulnerability to posttraumatic stress disorder in adult offspring of Holocaust survivors. Am J Psychiatry 1998; 155:1163–1171
2. Yehuda R, Lehrner A: Intergenerational transmission of trauma effects: putative role of epigenetic mechanisms. World Psychiatry 2018; 17:243–257
3. Leen-Feldner EW, Feldner MT, Knapp A, et al: Offspring psychological and biological correlates of parental posttraumatic stress: review of the literature and research agenda. Clin Psychol Rev 2013; 33:1106–1133
4. Yehuda R, Daskalakis NP, Lehrner A, et al: Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. Am J Psychiatry 2014; 171:872–880
5. Yehuda R, Daskalakis NP, Bierer LM, et al: Holocaust exposure induced intergenerational effects on FKBP5 methylation. Biol Psychiatry 2016; 80:372–380
6. Monk C, Feng T, Lee S, et al: Distress during pregnancy: epigenetic regulation of placenta glucocorticoid-related genes and fetal neurobehavior. Am J Psychiatry 2016; 173:705–713
7. Klengel T, Mehta D, Anacker C, et al: Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. Nat Neurosci 2013; 16:33–41

8. Lehrner A, Bierer LM, Passarelli V, et al: Maternal PTSD associates with greater glucocorticoid sensitivity in offspring of Holocaust survivors. Psychoneuroendocrinology 2014; 40:213–220
9. Zannas AS, Wiechmann T, Gassen NC, et al: Gene-stress-epigenetic regulation of FKBP5: clinical and translational implications. Neuropsychopharmacology 2016; 41:261–274
10. Yehuda R, Cai G, Golier JA, et al: Gene expression patterns associated with posttraumatic stress disorder following exposure to the World Trade Center attacks. Biol Psychiatry 2009; 66:708–711
11. Yehuda R, Daskalakis NP, Desarnaud F, et al: Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in combat veterans with PTSD. Front Psychiatry 2013; 4:118
12. Spitzer RL, Williams JBW, Gibbon M, et al: Structured Clinical Interview for DSM-IV (SCID). New York, New York State Psychiatric Institute, Biometrics Research, 1995
13. Blake DD, Weathers FW, Nagy LM, et al: The development of a Clinician-Administered PTSD Scale. J Trauma Stress 1995; 8:75–90
14. Beck AT, Ward CH, Mendelson M, et al: An inventory for measuring depression. Arch Gen Psychiatry 1961; 4:561–571
15. Spielberger CD, Gorsuch RL, Lushene R, et al: Manual for the State-Trait Anxiety Inventory. Palo Alto, Calif, Consulting Psychologists Press, 1983
16. Parker G, Tupling H, Brown LB: A parental bonding instrument. Br J Med Psychol 1979; 52:1–10
17. Bernstein DP, Stein JA, Newcomb MD, et al: Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl 2003; 27:169–190
18. Mannuzza S, Fyer AJ, Endicott J, et al: An extension of the acquaintanceship procedure in family studies of mental disorder. J Psychiatr Res 1992; 26:45–57
19. Yehuda R, Labinsky E, Tischler L, et al: Are adult offspring reliable informants about parental PTSD? A validation study. Ann N Y Acad Sci 2006; 1071:484–487
20. Newman AM, Liu CL, Green MR, et al: Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 2015; 12:453–457
21. Arloth J, Bogdan R, Weber P, et al: Genetic differences in the immediate transcriptome response to stress predict risk-related brain function and psychiatric disorders. Neuron 2015; 86:1189–1202
22. Ewald ER, Wand GS, Seifuddin F, et al: Alterations in DNA methylation of Fkbp5 as a determinant of blood-brain correlation of glucocorticoid exposure. Psychoneuroendocrinology 2014; 44:112–122
23. Klinger-König J, Hertel J, Van der Auwera S, et al: Methylation of the FKBP5 gene in association with FKBP5 genotypes, childhood maltreatment and depression. Neuropsychopharmacology 2019; 44:930–938
24. Tozzi L, Farrell C, Booij L, et al: Epigenetic changes of FKBP5 as a link connecting genetic and environmental risk factors with structural and functional brain changes in major depression. Neuropsychopharmacology 2018; 43:1138–1145
25. Tyrka AR, Ridout KK, Parade SH: Childhood adversity and epigenetic regulation of glucocorticoid signaling genes: associations in children and adults. Dev Psychopathol 2016; 28:1319–1331
26. Binder EB, Bradley RG, Liu W, et al: Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. JAMA 2008; 299:1291–1305
27. Bader HN, Bierer LM, Lehrner A, et al: Maternal age at Holocaust exposure and maternal PTSD independently influence urinary cortisol levels in adult offspring. Front Endocrinol (Lausanne) 2014; 5:103
28. Bierer LM, Bader HN, Daskalakis NP, et al: Elevation of 11β-hydroxysteroid dehydrogenase type 2 activity in Holocaust survivor offspring: evidence for an intergenerational effect of maternal trauma exposure. Psychoneuroendocrinology 2014; 48:1–10
29. Yehuda R, Bierer LM, Andrew R, et al: Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors. J Psychiatr Res 2009; 43:877–883
30. Moog NK, Entringer S, Rasmussen JM, et al: Intergenerational effect of maternal exposure to childhood maltreatment on newborn brain anatomy. Biol Psychiatry 2018; 83:120–127
31. Klengel T, Dias BG, Ressler KJ: Models of intergenerational and transgenerational transmission of risk for psychopathology in mice. Neuropsychopharmacology 2016; 41:219–231
32. Monk C, Spicer J, Champagne FA: Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways. Dev Psychopathol 2012; 24:1361–1376
33. Moog NK, Buss C, Entringer S, et al: Maternal exposure to childhood trauma is associated during pregnancy with placental-fetal stress physiology. Biol Psychiatry 2016; 79:831–839
34. Chan JC, Nugent BM, Bale TL: Parental advisory: maternal and paternal stress can impact offspring neurodevelopment. Biol Psychiatry 2018; 83:886–894
35. Cortessis VK, Thomas DC, Levine AJ, et al: Environmental epigenetics: prospects for studying epigenetic mediation of exposure-response relationships. Hum Genet 2012; 131:1565–1589
36. Yehuda R, Bierer LM: Transgenerational transmission of cortisol and PTSD risk. Prog Brain Res 2008; 167:121–135
37. Burton T, Metcalfe NB: Can environmental conditions experienced in early life influence future generations? Proc Biol Sci 2014; 281: 20140311
38. Sawamura T, Klengel T, Armario A, et al: Dexamethasone treatment leads to enhanced fear extinction and dynamic Fkbp5 regulation in amygdala. Neuropsychopharmacology 2016; 41:832–846
39. Roberts S, Keers R, Breen G, et al: DNA methylation of FKBP5 and response to exposure-based psychological therapy. Am J Med Genet B Neuropsychiatr Genet 2019; 180:150–158