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Childhood Maltreatment and Methylation of *FKBP5*

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Abstract

A growing body of evidence suggests that alterations of the stress response system may be a mechanism by which childhood maltreatment alters risk for psychopathology. FK506 binding protein 51 (FKBP5) binds to the glucocorticoid receptor and alters its ability to respond to stress signaling. The aim of the present study was to examine methylation of the FKBP5 gene (*FKBP5)*, and the role of an *FKBP5* genetic variant, in relation to childhood maltreatment in a sample of impoverished preschool-aged children. One hundred seventy-four families, including n=69 with child welfare documentation of moderate-severe maltreatment in the past six months, participated in this study. Children ranged in age from 3 to 5 years, and were racially and ethnically diverse. Structured record review and interviews in the home were used to assess a history of maltreatment, other traumas, and contextual life stressors, and a composite variable assessed the number exposures to these adversities. Methylation of two CpG sites in intron 7 of *FKBP5* was measured via sodium bisulfite pyrosequencing. Maltreated children had significantly lower levels of methylation at both CpG sites ($p<.05$). Lifetime contextual stress exposure showed a trend for lower levels of methylation at one of the sites, and a trend for an interaction with the *FKBP5* polymorphism. A composite adversity variable was associated with lower levels of methylation at one of the sites as well ($p<.05$). FKBP5 alters glucocorticoid receptor responsiveness and *FKBP5* gene methylation may be a mechanism of the bio-behavioral effects of adverse exposures in young children.

Introduction

Childhood maltreatment significantly increases risk for behavior problems (Carr et al., 2013; Hickman et al., 2013; Kim-Spoon, Cicchetti & Rogosch, 2013; Mills et al., 2013; Norman et al., 2012) which can be identified as early as 12-48 months of age and are strongly correlated with the development of significant psychopathology (Briggs-Gowan et al., 2006;

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Mesman & Koot, 2001; Roza et al., 2003). Childhood maltreatment is also significantly associated with adult psychopathology (Brown & Anderson, 1991; Bryer et al., 1987; Kendler et al., 1993; Lewinsohn, Hoberman & Rosenbaum, 1988) and among those with disorders, a history of maltreatment predicts greater severity, worse prognosis, and multiple comorbidities (Nanni, Uher & Danese, 2012; Widom, DuMont & Czaja, 2007). A number of biological mechanisms have been proposed to mediate risk for psychopathology following exposure to maltreatment. Alterations of neural structure and function and other biological changes have been observed in children and adults with a history of maltreatment (Cicchetti, 2015; Cicchetti, Handley & Rogosch, 2015; Hart & Rubia, 2012; Philip et al., 2015; Ridout et al., 2014; Ridout et al., 2015; Toth et al., 2013; Tyrka et al., 2013; Tyrka et al., 2015b). Identifying these mediating factors is critical for understanding how early childhood maltreatment contributes to risk of psychopathology in order to prevent long-term sequelae.

Epigenetic modifications to the genome are of major interest in this effort because they can stably alter gene expression in response to environmental exposures. There is a growing body of literature to support the importance of epigenetic changes in mediating the effects of childhood maltreatment on psychopathology and on biological systems involved in the development of psychopathology (Guintivano & Kaminsky, 2014; Januar, Saffery & Ryan, 2015; Lutz et al., 2015). Epigenetic modifications alter gene expression but do not change the DNA sequence and thus allow for elaboration of the genome beyond what is determined by the DNA (Szyf, 2015). Accumulating evidence indicates that childhood maltreatment may exert long-lasting effects through epigenetic changes (Lutz & Turecki, 2014; Szyf, 2011; Tyrka et al., 2015a). DNA methylation, involving the addition of a methyl group at sites where a cytosine nucleotide occurs next to a guanine nucleotide (CpG dinucleotides), is thought to be the most stable form of epigenetic alteration (Reik, 2007). Methylation can block the binding of transcription factors or interfere with gene expression through other mechanisms (Klengel et al., 2014; Lee et al., 2011; Yang et al., 2014). Consistent with this, genes that are highly expressed typically have low levels of promoter methylation (Sasaki, de Vega & McGowan, 2013; Wagner et al., 2014; Zhang et al., 2013).

Maltreatment and the development of the physiologic stress response

Research examining the biologic underpinnings of the associations between childhood maltreatment and psychopathology highlights the importance of the physiologic stress response system, and in particular, the hypothalamic pituitary adrenal (HPA) axis. In response to stressful stimuli, glucocorticoids are released and exert cellular responses by binding at the intracellular glucocorticoid receptor (GR) (Tyrka et al., 2013). Glucocorticoid receptors are distributed throughout the body and brain where they regulate basal physiologic function and effect changes in various organ systems and tissues that promote adaptive responding to acute stressors (de Kloet, Joels & Holsboer, 2005; Kadmiel & Cidlowski, 2013). Activation of the GR through cortisol binding also involves a negative feedback mechanism that inhibits release of cortisol and prevents damaging effects of chronic activation (Herman et al., 2012; Laryea et al., 2015). Thus, changes in GR number or function can influence the activity of the stress response system and consequently, the biological adaptation to stressful or traumatic experiences.

Studies examining the development of the stress response system reveal that early environment is linked to abnormalities in HPA axis function, with exaggerated or attenuated basal cortisol and stress-induced cortisol concentrations (Carpenter et al., 2007; Carpenter et al., 2009; Dozier et al., 2006; Elzinga et al., 2008; Gonzalez, 2013; Gunnar & Vazquez, 2001; Heim et al., 2001; Heim et al., 2000b; Klaassens et al., 2009; McCrory, De Brito & Viding, 2010; Tyrka et al., 2009; Tyrka et al., 2010). Excessive activation of the HPA axis over time or in response to extreme stress may result in down-regulation of this system (Fries et al., 2005; Heim, Ehlert & Hellhammer, 2000a; McEwen, 2007; Pryce et al., 2005; Tyrka et al., 2008). For example, in comparison with children who were not maltreated, maltreated children have greater variance in cortisol levels at baseline, and those with high baseline cortisol levels are more likely than nonmaltreated children to have blunted cortisol levels over time (Doom, Cicchetti & Rogosch, 2014). Several studies of adults with a history of early life stress show attenuated cortisol response to stress (Carpenter et al., 2007; Carpenter et al., 2011; Carpenter et al., 2009; Elzinga et al., 2008; Klaassens et al., 2009), suggesting that the physiologic effects of maltreatment are long lasting.

The HPA axis and risk for psychopathology and other medical conditions

Converging lines of evidence indicate that excessive exposure to glucocorticoid activity may be involved in the pathogenesis of depressive and anxiety disorders. Stimulation of the HPA system through chronic glucocorticoid treatment produces anxiety- and depressive-like behaviors and causes dendritic atrophy, pyramidal cell loss, and suppression of hippocampal neurogenesis (Cameron & Gould, 1994; Dachir et al., 1993; Gould, Woolley & McEwen, 1991; Magarinos, Orchinik & McEwen, 1998; Watanabe, Gould & McEwen, 1992; Woolley, Gould & McEwen, 1990). Altered cortisol responses are associated with internalizing behaviors (Beauchaine, Crowell & Hsiao, 2015; Hartman et al., 2013), externalizing behaviors (Hartman et al., 2013; Marsman et al., 2008), suicidal ideation (Beauchaine et al., 2015), and post-traumatic stress disorder (PTSD) (de Kloet et al., 2008; de Kloet et al., 2006; Golier et al., 2014), and depression (Ciufolini et al., 2014; Colich et al., 2015) (de Kloet et al., 2008; Suzuki et al., 2013). The HPA axis directs the secretion of glucocorticoids, which potentiate systemic inflammation, oxidative stress, and advance cellular aging; factors that may underlie the association of stress, psychopathology, and somatic conditions (Bauer, Jeckel & Luz, 2009; Wolkowitz et al., 2010). Evidence suggests that children with certain genetic alleles that alter the function of the HPA axis may be at a higher risk of developing psychopathology after experiencing maltreatment and that childhood maltreatment exerts its effects on HPA axis function through epigenetic modifications of key regulatory genes (Klengel & Binder, 2015a; Nugent et al., 2011; Turecki & Meaney, 2014; Tyrka et al., 2012).

Genetic and epigenetic factors influencing the HPA axis

There are multiple levels of regulation and control that can help determine the function of the HPA axis in response to stress (Booij et al., 2013; Stankiewicz, Swiergiel & Lisowski, 2013). These include genetic variants such as single nucleotide polymorphisms (SNPs) that influence levels of gene expression as well as epigenetic regulation of HPA axis-involved genes (Booij et al., 2013; Gillespie et al., 2009; Lutz & Turecki, 2014; Stankiewicz et al., 2013). For example, the GR is regulated by a number of mechanisms allowing for tissue-

specific control of its expression and adaptation to stress exposure (Binder, 2009; Lutz et al., 2015; Spijker & van Rossum, 2012; Vandevyver, Dejager & Libert, 2014), altering the feedback on and function of the HPA axis. Early environmental experiences are associated with epigenetic changes to the promoter region of the GR gene, *NR3C1*, that influence GR expression and potentially alter HPA-axis set points and responses to stress (Turecki & Meaney, 2014; van der Knaap et al., 2015a). *NR3C1* methylation is associated with a greater risk of internalizing symptoms and disorders (Radtke et al., 2015; van der Knaap et al., 2015b). Recently, our group reported work with the present sample showing that higher *NR3C1* methylation is associated with childhood maltreatment and other adversities in preschool-aged children (Tyrka et al., 2015a).

The role of *FKBP5* in GR regulation and the HPA axis

An important regulator of the GR is the FK506 binding protein 51 (FKBP5). In response to stressors, cortisol is released, travels systemically through the blood and moves into the cytoplasm of cells (Kadmiel & Cidlowski, 2013). Cortisol binding to the GR in the cytosol induces translocation of the GR into the nucleus, where it binds to specific regions of the genome called glucocorticoid response elements. The bound GR serves as a transcription factor at cortisol-responsive genes, and thereby activates systems necessary to cope with stressors (Magee et al., 2006). The GR also provides negative feedback to the brain to shut down the HPA stress response. FKBP5 mediates an additional negative feedback loop on glucocorticoids. GR activation results in rapid induction of FKBP5 which binds to the GR and decreases its ability to bind cortisol and to translocate to the nucleus. Thus, FKBP5 decreases systemic sensitivity to cortisol and reduces GR-mediated negative feedback modulation of the HPA axis (Binder, 2009; Cioffi, Hubler & Scammell, 2011; Schmidt et al., 2015; Tatro et al., 2009).

Genetic variation in the *FKBP5* gene confers altered GR function and a poorly regulated neuroendocrine response to stress (Zannas & Binder, 2014). A single nucleotide polymorphism (SNP) in *FKBP5* (C to T SNP in intron 2, rs1360780) increases the ability of the GR to bind to the glucocorticoid response elements and induce *FKBP5* expression (Zannas & Binder, 2014). This “risk” T allele is associated with GR resistance (Hohne et al., 2015; Ising et al., 2008; Menke et al., 2013) and has been linked with PTSD, depressive and anxiety symptoms and disorders, and suicide (Leszczynska-Rodziewicz et al., 2014; Suzuki et al., 2014; Szczepankiewicz et al., 2014; VanZomeren-Dohm et al., 2015; Zannas & Binder, 2014).

Recent groundbreaking work by Klengel and colleagues (Klengel et al., 2013) examined the rs1360780 SNP and methylation of functional glucocorticoid response elements in *FKBP5* in blood in relation to childhood maltreatment. In comparison with controls, adults with a history of childhood trauma had lower levels of methylation in regulatory regions of intron 7 of *FKBP5* in those with the rs1360780 risk allele, and this was associated with decreased GR sensitivity. Moreover, glucocorticoid teratment of human hippocampal progenitor cells also induced long-lasting demethylation of this regulatory region (Klengel et al., 2013). One other study assessed *FKBP5* methylation and found effects of maltreatment in saliva DNA from children ages 5-14 (Weder et al., 2014), but this involved a 450K methylation array

which did include the region studied by Klengel and colleagues. No prior work has examined early childhood, and this may be a sensitive period for the development of these epigenetic effects (Klengel et al., 2013). The aim of the present study was to examine *FKBP5* methylation, and the role of the *FKBP5* genetic variant, in relation to childhood maltreatment in a sample of impoverished preschool-aged children.

Methods

One-hundred and seventy-four children were included in this study, including sixty-nine with child welfare documentation of moderate-severe maltreatment in the past six months. Children ranged in age from 3 to 5 years (M = 49.87 months; SD = 8.4 months), were racially and ethnically diverse (38 White non-Hispanic, 81 Hispanic, 29 Black, 26 other races), and 84 were male. Most caregivers (n=161) were biological mothers. Thirty-one caregivers had less than a high school degree, 65 completed high school, 61 had some post-secondary education, 16 had a bachelor's degree, and one did not provide education information. Ninety-eight caregivers were unemployed and 156 of the families qualified for public assistance. No more than one child per family was included.

Procedure

Families with a maltreated child were identified from the local child welfare agency and an emergency maltreatment assessment service via record review. Families of children with no indicated case of maltreatment within the past six months were recruited at a pediatric medical clinic during a well-child visit and at childcare centers. Based on review of available medical records and parent report, children with a chronic medical or neurologic condition, medication use, obesity, and failure-to-thrive were excluded. Those with acute illness or medication use were included no less than 2 weeks following resolution of illness and medication use.

Families completed two home visits and questionnaires between the visits. The first home visit, during which caregivers completed interviews on child stress exposure and a saliva sample for DNA isolation was collected from the children, is the focus of the current report.

Measures

Socioeconomic adversity—Indicators of socioeconomic adversity (parental education ≤ high school degree, parental unemployment, and single parenthood) were summed.

Child maltreatment status—All families consented to examination of child welfare records to determine maltreatment status. Trained research staff coded the records using the System for Coding Subtype and Severity of Maltreatment in Child Protective Records (Barnett, Manly & Cicchetti, 1993). Five maltreatment subtypes and severity scores ranging from 1 (least severe) to 5 (most severe) were derived. Children with a case of moderate to severe levels of maltreatment (score of 3-5) within the last six months were considered as part of the maltreated group (n=69). Eight children had substantiated cases of physical abuse, 13 sexual abuse, 8 physical neglect/failure to provide, 21 physical neglect/lack of supervision, and 47 emotional maltreatment. Three of the maltreated children were removed

from the home and were in the care of their maternal grandmother. The comparison group included children who had never had a substantiated case of maltreatment. In addition five children had an episode of moderate maltreatment that occurred at least 18 months prior to participation. Results were consistent whether these children were in the maltreatment or comparison group. Because n=5 is insufficient for a separate analysis, these children were included in the comparison group.

Contextual stress interview—Caregivers completed a semi-structured interview developed in our laboratory to assess the child's experience of contextual stressors in the past month and in the child's lifetime. Categories were: death of a caregiver, separation from a caregiver, frequent change of residence or homelessness, inadequate food or clothing, and other events including witnessing neighborhood violence or parental arrest. Each domain was scored positive if at least one episode occurred, and domains were summed for past month and lifetime. Possible scores ranged from 0 (no stressors) to 5 (stressors in all five domains) for each summary scale. Past month contextual stressors ranged from 0 to 3, with mean of 0.64 and SD of 0.84, and lifetime contextual stressors ranged from 0 to 4 with mean of 1.39 and SD of 1.24.

Traumatic life events—The Diagnostic Infant and Preschool Assessment (Scheeringa & Haslett, 2010) interview was conducted with caregivers to assess child experiences of traumatic life events. Interviews were conducted by trained clinical social workers and PhD level psychologists, reviewed in a group supervision format, and scored based upon group consensus. The number of types of traumas experienced in the child's lifetime was summed. Categories were: experiencing an accident, animal attack, man-made disaster, natural disaster, witnessing violence, accidental burning, medical emergency/hospitalization/ invasive medical procedure, kidnapping, and other events such as a near drowning. Physical and sexual abuse were not included in this variable because they were assessed as maltreatment (above). Possible scores ranged from 0 to 9, and in the present sample ranged from 0 to 4 with mean of 0.93 and SD of 1.03.

Adversity Composite—The number of types of maltreatment experienced, the number of lifetime contextual stressors, and the number of other traumatic life events were summed to create an adversity composite. Possible scores ranged from 0 to 18, and in the sample ranged from 0 to 9 with a mean of 2.93 and a SD of 2.47.

Genotyping—Saliva samples were obtained using the Oragene DISCOVER kits (OGR-575) for Assisted Collections (DNA Genotek, Kanata, Ontario, Canada), and DNA was isolated following the manufacturer's instructions. Samples were genotyped for the SNP rs1360780 through an allelic discrimination assay using predesigned Taqman primers (part #C_8852038_10, Life Technologies) and Taqman universal master mix (Life Technologies) via established protocols as directed by the manufacturer on a Bio-Rad CFX connect. Genotype data were available for 156 children for this report.

***FKBP5* and *NR3C1* Methylation—**Sodium bisulfite modification was performed with 500 ng of DNA using the EZ DNA methylation Kit (Zymo Research, Irvine, CA, USA). PyroMark Assay Design software version 2.0.1.15 (Qiagen) was used to design the

pyrosequencing assays. The PyroMark (Qiagen) PCR kit and forward and reverse primers were used to amplify *FKBP5* intron 7 and regions of the *NR3C1* promoter, and PCR products were sequenced using a PyroMark MD system (Qiagen) as previously described (Paquette et al., 2014; Tyrka et al., 2015a). Percent DNA methylation at each CpG locus was quantified with the PyroMark CpG software, version 1.0.11 (Qiagen). All procedures were performed following manufacturer's protocols. Each sequencing run contained no template and genomic DNA negative controls, as well as methylated and unmethylated controls (Epitect). A bisulfite conversion control within the assay sequence was used to assess conversion efficiency. PCR, sequencing primers (Integrated DNA Technologies, Inc, Coralville, IA) and pyrosequencing assay sequences are available from the corresponding author upon request.

Our main focus was on *FKBP5*, but because we previously found effects of maltreatment on *NR3C1* methylation, we also examined the association of *NR3C1* and *FKBP5* methylation. The percent of alleles that were methylated was used in statistical analyses. Consistent with other studies on *NR3C1* methylation in a variety of cell types, methylation levels were low for *NR3C1*. For CpG sites in *NR3C1* region 1_D, percent methylation ranged from 0 to 7.02 with mean of 1.10 and SD of 0.19 across the whole region. Region 1_F methylation varied from 0 to 6.42. The mean and SD of the whole region was 1.47 and 0.26. For 1_H, the range of methylation was 0 to 7.11, and the mean across the region was 1.24 and SD of 0.20. For *FKBP5,* two CpGs in intron 7 (Chr 6: 35558488, CpG 1 and 35558514, CpG2) were studied based on findings of Klengel and colleagues (Klengel et al., 2013); percent methylation ranged from 79.00 to 98.00 with mean of 88.02 and SD of 2.76 for CpG 1 and mean of 88.61 and SD of 3.73 for CpG 2.

Results

Preliminary Analyses

Child age, race, sex, and socioeconomic adversity were not associated with *FKBP5* methylation at CpG 1 or CpG 2. Child age, race, and socioeconomic adversity were not associated with *NR3C1* methylation, but males had greater mean methylation at 1_F than females (p =.006), so child sex was included in models testing associations with *NR3C1* methylation. Child age, sex, and race did not differ according to *FKBP5* genotype.

FKBP5 Methylation in Association with Childhood Maltreatment and Other Adversities

As illustrated in Figure 1, maltreated children had lower levels of *FKBP5* methylation at CpG 1 and CpG 2 (t = 3.16, p = .002 and t = 2.29, p = .023, respectively).

With respect to other forms of adversity, there was a trend level association of lifetime contextual stress and methylation of *FKBP5* at CpG 1 (r = −.14, p = .064) but not CpG 2 (p = .54). In contrast, past month contextual stress and the number of traumatic life events were not associated with *FKBP5* methylation at either CpG site.

The adversity composite was negatively associated with methylation of *FKBP5* at CpG 1 (r = −.17, p = .026) but not CpG 2 (p = .36).

FKBP5 Methylation and *NR3C1* Methylation

Methylation of *FKBP5* at CpG 1 was negatively associated with mean methylation of *NR3C1* at Exon 1_F ($r = -.17$, $p = .023$), but not exons 1_D or 1_H. This association remained after controlling for maltreatment ($p = .04$) or the adversity composite ($p = .051$). No significant associations were observed between methylation of *FKBP5* at CpG 2 and methylation of *NR3C1* at any of the alternate first exons. The associations of the adversity variables and *FKBP5* methylation described above were consistent when methylation of *NR3C1* at exons 1_D, 1_F, and 1_H were covaried in the models.

Associations of *FKBP5* Genotype, Adversity, and *FKBP5* Methylation

Seventy-seven children were homozygotes for the C allele, 68 were CT heterozygotes, and 11 were homozygotes for the T allele. The minor allele frequency (MAF) was .29 for the sample, and the distribution conformed to the Hardy-Weinberg equilibrium ($p = .44$). Our overall sample MAF was not statistically different from the National Center for Biotechnology Information (NCBI) SNP database (dbSNP)-reported MAF of .33 (p = .44). Sample age, sex, and race did not differ according to genotype. Calculated MAF for males and females were .34 and .27 respectively; the distribution conformed to the Hardy-Weinberg equilibrium ($p = .65$ and .35, respectively). Calculated MAFs for Whites, Blacks and Hispanics were .3, .29, and .28 respectively; the distribution conformed to the Hardy-Weinberg equilibrium ($p = .36$, .23, and .40 respectively). These did not significantly differ from the MAF reported in the NCBI dbSNP for these populations of .21, .40, and .23 respectively ($p = .36$, .23, and .40, respectively).

There was no main effect of *FKBP5* genotype on methylation of *FKBP5*. Child maltreatment status, past month contextual stress, and the number of traumatic life events did not moderate links between *FKBP5* genotype and methylation of *FKBP5*. There was a trend-level interaction of *FKBP5* genotype and lifetime contextual stress; Children with no lifetime contextual stress with the protective CC genotype had the highest levels of *FKBP5* CpG 1 methylation, but this did not reach significance ($F(1,152) = 3.07$, $p = .082$). The interaction of lifetime contextual stress and *FKBP5* genotype was not associated with methylation of CpG 2 ($p = .321$).

Discussion

The central finding of this study is that childhood maltreatment was associated with lower levels of methylation of *FKBP5* at two sites within a regulatory region in intron 7 of this gene. These sites were found by Klengel and colleagues (Klengel et al., 2013) to exhibit lower levels of methylation in adults with a history of childhood adversity, with a moderating effect of rs1360870 variation. FKBP5 regulates GR sensitivity and function, so *FKBP5* demethylation may be a mechanism of the effect of maltreatment on HPA axis dysfunction and associated risk for psychiatric and other stress-associated conditions.

FKBP5 plays a dynamic role in the regulation of the HPA axis. Prolonged glucocorticoid exposure increases FKBP5 levels, which in turn limit the activation and translocation of GRs, creating a negative feedback loop that ultimately downregulates FKBP5 activation

(Klengel & Binder, 2015b; Klengel et al., 2013; Lee et al., 2011). Methylation of *FKBP5* decreases the induction of FKBP5 that occurs following glucocorticoid binding to GR, and demethylation can be induced by prolonged glucocorticoid exposure (Klengel et al., 2013; Lee et al., 2011). Thus, demethylation at glucocorticoid response elements in *FKBP5* allows for another level of feedback-loop control in response to glucocorticoids. Our finding in the present study that methylation of *FKBP5* and *NR3C1* 1_F were negatively correlated with each other and were associated with maltreatment in different directions suggests that both of these negative regulators of HPA axis activation are at work. These epigenetic changes may serve to establish a steady-state level and counteract elevated levels of glucocorticoids (Lee et al., 2011).

Converging lines of evidence indicate that methylation of glucocorticoid response elements in *FKBP5* in DNA from the periphery may have functional effects in the body and may reflect changes to GR sensitivity in the brain. Treatment with glucocorticoids was associated with lower levels of methylation of a key regulatory locus in *Fkbp5* in blood in mice, which in turn was associated with important physiological changes in glucocorticoid target tissues, including atrophy of the thymus, spleen, and the adrenal glands (Lee et al., 2011). Although epigenetic modifications to DNA are often tissue-specific, glucocorticoid-induced demethyation of regulatory regions of *Fkbp5* in blood was associated with anxiety-like behavior (Lee et al., 2011) and levels of *Fkbp5* methylation in blood were significantly correlated with both methylation and gene expression in hippocampus (Ewald et al., 2014). Demethylation of the regulatory region examined in the present study was also shown in a human hippocampal progenitor cell line following glucocorticoid exposure (Klengel et al., 2013). Taken together, these findings suggest that studies using peripheral markers in humans may have direct implications for brain function. It is important to note that we utilized saliva DNA, which may originate from a combination of blood leukocytes (Endler et al., 1999) (Thiede et al., 2000) and buccal epithelial cells. Some evidence indicates that saliva DNA may actually more closely reflect methylation patterns in the brain than DNA from leukocytes (Smith et al., 2015).

This system is complex, and the literature regarding the functional and dynamic effects on systemic cortisol levels and psychopathology remains at an early stage. There is evidence that lower levels of *FKBP5* methylation are associated with higher *FKBP5* expression (Lee et al., 2011; Sarapas et al., 2011), and reduced GR sensitivity (Klengel et al., 2013). *FKBP5* expression is positively associated with cortisol levels (Lee et al., 2011; Sarapas et al., 2011). However, early stress and PTSD are often associated with low levels of cortisol (Morris, Compas & Garber, 2012; Tyrka et al., 2013) and increased sensitivity of the GR has been shown in some studies (Rohleder et al., 2004; Daskalakis, Lehrner & Yehuda, 2013). There is also evidence of lower FKBP5 expression in PTSD (Yehuda et al., 2009). One report indicated that the *FKBP5* risk allele that confers higher *FKBP5* expression is linked to blunted cortisol response to dexamethasone challenge in adults over age 50, consistent with elevated GR sensitivity (Fujii et al., 2014). Thus, it is not clear how findings of lower methylation and reduced GR sensitivity are related to some of these other findings, and more work is needed to clarify the role of *FKBP5* in regulating glucocorticoid signaling in the wake of childhood maltreatment.

Most of the families in our sample were impoverished and exposed to a variety of contextual stressors, though we did not observe significant effects with our measure of socioeconomic adversity, and we only saw trend-level effects of contextual stressors. There may be effects of early-life or in utero exposures that we did not measure. It is also possible that undocumented cases of maltreatment, such as neglect, may have occurred in the non-maltreated group and this would have diminished the magnitude of the effect. Moreover, maltreatment characteristics, such as type, timing of onset, and chronicity may influence DNA methylation patterns and this should be examined in future studies.

In summary, this study shows that maltreatment in preschool-aged children is associated with lower levels of methylation of a functional glucocorticoid response element of *FKBP5*. Methylation of this region decreases the induction of *FKBP5* that occurs when the GR is activated by glucocorticoids. Future work should examine associations of diurnal and provoked cortisol concentrations in relation to *FKBP5* and *NR3C1* methylation in maltreated children. Lower levels of methylation of *FKBP5* and associated increases in *FKBP5* induction by activated glucocorticoid receptor may explain some of the neuroendocrine dysfunction and associated psychiatric and medical morbidity that result from childhood maltreatment.

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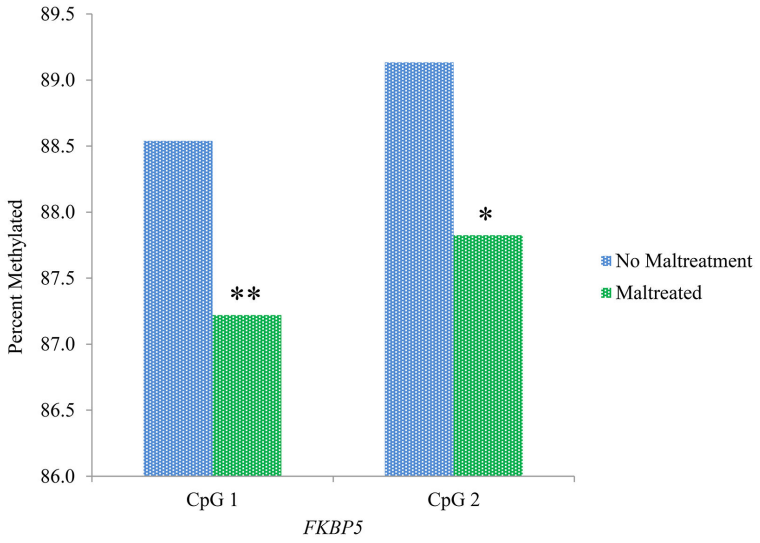

Figure 1.

Association of child maltreatment with methylation of *FKBP5*

Note. ** $p < .005$; * $p < .05$.