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Madison Malone December 7, 2015

Estrogen Receptor Alpha Gene (*ESR1*): Genetics, Epigenetics, Early Life Abuse and Depression in Women

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Abstract

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The current study investigates the association between the environment and underlying genetics on selective epigenetic methylation and adult depression outcomes. The specific aims of this study were to examine (1) whether early-life abuse associates with methylation of CpG sites across the Estrogen Receptor Alpha gene (*ESR1*), (2) the association between *ESR1* single nucleotide polymorphisms (SNPs) and *ESR1* CpG methylation, (3) whether CpG methylation may serve as a mediator between early-life abuse and adulthood depression, and (4) how early-life abuse may interact with CpG methylation to be associated with adult depression. The study focuses exclusively on a female sample, with 301 adult females drawn from the Grady Trauma Project, a large cross-sectional research study in Atlanta, focused on genetic and environmental predictors of Post Traumatic Stress Disorder. Linear regressions were used to test the study aims, and correction for multiple testing was accomplished with Bonferroni and False Discovery Rate calculations. We found that (a) exposure to early-life abuse associated with methylation of several *ESR1* CpG sites, with two surviving correction. While *ESR1* methylation (b) did not serve as a mediator between early-life abuse and adult depression ($p>0.05$), (c) methylation may moderate the association between early-life abuse and adult depression ($p<0.05$), though no moderator analyses survived correction for multiple tesing. We also found that (d) several *ESR1* SNPs may associate with nearby CpG site methylation, with one SNP-CpG pair surviving stringent correction, suggesting that genetic and epigenetic variability is not independent. This research further extends the scientific work exploring the interplay between our environment and

our biology in the development of adult outcomes. In context, understanding the biological implications of early-life adversity would give credence to intervention for those living in adverse enviroments and provide an outcome (methylation) that may be more proximally measured to assess the impact of adversity and/or intervention. This study is an initial step in extending animal literature assessing the role of *ESR1* regulation in the link between early adversity and adult outcomes.

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Introduction

There exists a well-established link between early-life stress and increased risk for poor behavioral and health outcomes, such as disease and depression (Heim, Shugart, Craighead, & Nemeroff, 2010; Miller, Chen, & Parker, 2011). As a result, there is a growing body of research dedicated to exploring potential mechanisms that could account for these associations. Epigenetics has surfaced as a potential mechanism by which the environment can "get under the skin" and change our biology (Meany, 2010). Epigenetics, literally "on top of the genome," refers to modifications of DNA structure that do not change the gene sequence, but rather regulate gene expression by inducing structural change (Bird, 2002). DNA methylation is one type of epigenetic modification in which a methyl group covalently binds to a cysteine on the DNA strand, predominantly at CpG sites in which a cysteine and guanine base pair occur next to one another in the gene sequence and are bound by a phosphate bond (Bird, 2002). When methylation occurs, particularly in promoter regions, it tends to block the binding of transcription factors, thus reducing gene expression (Klose & Bird, 2006).

Much of the excitement around epigenetics spurs from documented epigenetic changes that occur in response to specific environmental effects. For example, in rodent literature, nonhuman primate literature, and some initial human studies, differences in exposure to early maternal nurturance or exposure to stressful experiences, such as abuse, have been associated with differential methylation of a number of genes (e.g. Beach, Brody, Todorov, Gunter, & Philibert, 2010; Champagne, 2008; Essex et al., 2013; Kinnally et al., 2011; Klengel et al., 2013; Massart et al., 2014**;** McGowan et al., 2009; Nieratschker et al., 2014; Roth & Sweatt, 2011; Suomi, 2013; Szyf, 2013).

To date, there has been particular focus on how environmental stimuli may influence the epigenetics of the stress response system (McGowan et al., 2009; Oberlander, Winberg, Papsdorf, Grunau, Misri, & Devlin, 2008; Szyf & Bick, 2012; Szyf, Weaver, Champagne, Diorio, & Meaney, 2005). For example, low nurturance and maternal separation have been associated with higher methylation of the glucocorticoid receptor gene in rodents (Meaney & Szyf, 2005; Francis, Champagne, & Meaney, 2000; Nair & Young, 2006). In humans, exposure to childhood abuse is associated with higher methylation of the glucocorticoid receptor gene in post-mortem brain tissue (McGowan et al., 2009). The mechanism by which this environmentally-sensitive methylation may occur is still largely unknown, especially in humans, although some insights have recently emerged from experiemental model systems (Feil & Fraga, 2012). In addition to genes involved in the stress response system, the animal literature has also explored the role of the early environment in influencing the methylation of the Estrogen Receptor Alpha gene (*ESR1*). (Champagne, Weaver, Dorio, Dymov, Szyf, & Meaney, 2006; Matsuda, 2014).

These studies suggest that the early environment, particularly the level of maternal nurturance, may influence methylation of *ESR1* in areas of the brain related to parental and social behaviors (Champagne et al., 2006). No study to our knowledge has explored the role of the early environment on *ESR1* methylation in humans. This study question is derived from rodent literature. However, development of primates and rodents largely differs. Despite the lack of a directly translatable model, it is possible that some aspects of the rodent model may be applicable to humans (Suderman et al., 2012). Therefore, this exploratory study addresses an important unanswered question and is a starting point to investigate how rodent studies may

provide insight into the epigenetic and psychological effects of aversive human childhood experience.

The overall aim of this study is to examine the role of the early environment on the methylation of *ESR1* and its potential link with adult outcomes. We will examine this by exploring the association between the early environment, particularly childhood abuse, and methylation of *ESR1*. Further, to explore whether *ESR1* methylation could serve as a mechanism underlying later adult outcomes, we will explore whether *ESR1* methylation may either mediate or moderate the association between childhood abuse and adulthood depression.

Methylation is of particular interest given its role in the regulation of gene expression. However, regulation of gene expression is complex and influenced by a number of factors including single nucleotide polymorphisms (SNPs), in addition to CpG site methylation (Gibbs et al., 2010). While genetics and epigenetics have been primarily studied separately to date, recent studies have shown that they have some correlation, and that the level of CpG methylation can be associated with nearby SNPs (Bell, Tsai, Yang, Pidsley, Nisbet, & Glass, 2012; Smith et al., 2014). Therefore, exploring the association between SNPs and methylation, as well as the role of the early environment, can allow for a more comprehensive assessment of *ESR1* regulation. The current study will test for associations between *ESR1* SNPs and CpG site methylation.

Background

ESR1 is the gene that codes for Estrogen Receptor Alpha (ERα), a nuclear steroid receptor for estrogen, and is located on chromosome 6q25.1 (Kim et al., 2010). While there are two other known estrogen receptors, ERβ and G protein-coupled estrogen receptor 1 (GPER;

also G protein-coupled receptor 30, GPR30), most animal studies that examine the relationship between the early environment and estrogen focus on *ESR1*. ERα is highly expressed in the rodent medial preoptic area (MPOA), a region known to be involved in maternal behavior. Further, offspring MPOA ERα levels seem to reflect the level of maternal care received, and this differential expression persists from neonatal life into adulthood (Champagne, 2008).

A number of rodent studies have noted differential methylation, as well as differential expression of *ESR1,* in response to variable early environments, particularly the level of maternal nurturance during early life. In a 2006 study, Champagne et al. conducted an experiment to test the impact of mother-infant nurturance on female rat pup *ESR1* regulation and future maternal behavior. Maternal nurturance was measured as frequency of licking and grooming (LG) behavior towards the pups. LG behavior is normally distributed within a laboratory population, and therefore female mothers can be categorized as exhibiting "high LG" or "low LG" behavior, relative to the mean. Results indicated that female offspring reared by high LG mothers, compared to offspring raised by low LG mothers, exhibit higher LG behaviors toward their own pups. Further, these high LG offspring tend to have similar *ESR1* methylation and expression levels as their mothers, where higher LG was associated with lower *ESR1* methylation and greater expression (Champagne et al., 2006). To begin to differentiate environment from genetic predisposition, in subsequent studies pups from high LG and low LG environments were cross-fostered. Interestingly, *ESR1* methylation, and future maternal behavior, tended to match that of the foster mother (Champagne et al., 2006). Studies of maternal abuse in macaque monkeys have similarly found that infant abuse is transmitted intergenerationally, and that this transmission occurs as a result of early experience, rather than genetic inheritance (Maestripieri, 2005).

In addition to maternal behaviors, dynamic estrogen regulation may also be important for other social and bonding behaviors. For example, in female rodents, estradiol, the ligand of ERα, influences proceptive and receptive behavior towards male rodents and has been shown to increase aggression in response to mate competition and decrease kin interaction (Manuck et al., 2011). One mechanism by which this may happen is through estrogen's regulation of the oxytocin receptor. Oxytocin has been recognized as critical in a number of aspects of social behavior, perhaps by increasing the salience of, and thus an animal's sensitivity towards, social cues in the environment (Bartz, Zaki, Bolger, & Ochsner, 2011; Insel & Young, 2001; Ross & Young, 2009). From rodent research, it is evident that in certain areas of the brain, exposure to estrogen results in an increase in oxytocin receptor expression (Pfaff, Waters, Khan, Zhang, & Numan, 2011). In animals with high nurturance, who are reported to have less *ESR1* methylation and greater *ESR1* expression, exposure to estrogen results in increases in oxytocin receptor levels, possibly through the action of an estrogen response element in the promoter of the oxytocin receptor gene, *OXTR* (Champagne, 2011; Champagne et al., 2006; Zingg & Laporte, 2003). It is therefore possible that the regulation of the estrogen receptor may also influence regulation of the oxytocin receptor, and that this may serve as one mechanism by which the early environment could influence later social behaviors.

Estrogen may also play a role in emotion regulation and risk for psychopathology (i.e. Manuck et al., 2011; Westberg et al., 2003; Feng et al., 2001; Sundermann, Maki, & Bishop, 2010; Pinsonneault, Sullivan, Sadee, Soares, Hampson, & Steiner, 2013). Various studies have investigated the association of *ESR1* SNPs and mood in humans. Estrogen may have implications for mood modulation, as ERα mRNA is highly expressed in the amygdala and hypothalamus, areas of the brain involved in emotional regulation (Östlund, Keller, & Hurd, 2003). Tsai, Wang,

Hong, and Chiu (2003) found that two *ESR1* SNPs (PvuII, rs2234693 and XbaI, rs9340799) were associated with major depressive disorder in females. Additional studies have found that some *ESR1* polymorphisms are associated with depression in post-menopausal women, risk for Premenstrual Dysphoric Disorder, and anxiety (Huo et al., 2007; Kim et al., 2010; Shepard, Michopoulos, Toufexis, & Wilson, 2009). Additionally, estrogen has been found to influence the serotonin system and expression of the serotonin receptor (5HT), serotonergic proteins, and the serotonin reuptake transporter (Shepard et al., 2009; Amin, Canli, & Epperson, 2005), and serotonin is classically associated with depression. Further, while drugs that influence the serotonin system have been implicated in depression risk, estrogen supplementation therapies have also been suggested as possible treatments for depression in post-menopausal women (Soares, Almeida, Joffe, & Cohen, 2001). However, no study to our knowledge to date has explored associations between *ESR1* methylation and depression.

Sex differences in depression, as well as in the estrogen system, are well reported (Radloff, 1975; McEwen & Alves, 1999). For example, sexually dimorphic ER expression has been reported in the brain (Wilson, Westberry, & Trout, 2011). This dimorphic expression has been noted in areas of the limbic and hypothalamic systems, parts of the brain involved in processing emotion and mood (Weiland, Orikasa, Hayashi, & McEwen, 1997; Yokosuka, Okamura, & Hayashi, 1997). Therefore, an initial examination of the relationship of *ESR1* and mood should take sex into account. The current study cohort is thus completely female.

Stemming from the current literature, the aim of this study is to examine genetic, epigenetic and environmental factors that influence the regulation of *ESR1* and how these factors are associated with depressive symptoms in female adults. The study will assess whether: (1) early-life abuse is associated with methylation of CpG sites across *ESR1*, (2) *ESR1* SNPs are

associated with *ESR1* CpG methylation, (3) CpG methylation may serve as a mediator between early-life abuse and adult depression, and (4) whether CpG methylation might moderate the relationship between early-life abuse and adult depression.

Method

Participants

Participants in the current sample were a part of a larger study, the Grady Trauma Project, which aims to explore how environments and genetics are associated with the development of Post-Traumatic Stress Disorder. The majority of participants in the current sample are African American individuals of low socioeconomic status, living in an urban area (Gillespie et al., 2009). Researchers recruited individuals in the primary care clinic or obstetrical-gynecological clinic waiting rooms of Grady Hospital, a metropolitan hospital in Atlanta, GA. Those interested in participating provided written, informed consent, and completed a verbal interview as well as a blood draw for genetic and epigenetic data collection. Participants for the current study consist of all females in the Grady Trauma Project cohort with estrogen receptor methylation data (N=301). Our sample consisted of 279 African American and 22 non-African American women. Mean age was 40.73 (SD=13). All study procedures were approved by the Emory University School of Medicine Institutional Review Board and by the Grady Health Systems Research Oversight Committee.

Measures

Demographics of age, sex, and race were collected through self-report. Exposure to childhood abuse was obtained from The Childhood Trauma Questionnaire (CTQ), a retrospective self-report survey, which was used to evaluate each participant's childhood exposure to physical,

emotional, and sexual abuse as well as emotional and physical neglect. Abuse data was used in the current study, as relatively few individuals reported experiencing neglect alone. Using established cut-off scores, each individual was classified as experiencing (0) none to mild abuse, (1) moderate, or (2) severe abuse of any kind (physical, sexual, or emotional) (Bernstein & Fink, 1998; Bernstein et al., 2003; Fink, Bernstein, Handelsman, Foote, & Lovejoy, 1995). Depressive symptoms were determined through the widely used 21-item Beck Depression Inventory (BDI; Beck, Steer, & Brown, 1996). Total BDI scores were used as outcomes in the data analyses.

Participants provided whole blood samples, collected in EDTA tubes, to be used for DNA extraction. PicoGreen (Invitrogen) was used to quantify DNA and quality was assured on agarose gel. Omni-Quad 1M or the Omni Express BeadChip (Illumina) was used to genotype each sample. One microgram of DNA was treated with bisulfite to examine cytosine to thymine conversion with the EZ DNA Methylation-Gold kit (Zymo Research). Genome-wide DNA was amplified, fragmented, and hybridized to the HumanMethylation450 BeadChip (Illumina). A BeadStation 500GX scanned the BeadChips, and the beta value methylation level for each CpG locus was queried with the Methylation Module of BeadStudio software. Beta values were considered missing for p-values above 0.001. Samples with probe detection rates below 95% and those with average intensity of less than 50% of experimental sample mean or less than 2000 arbitrary unites (AU) were removed using CpGassoc (Barfield, Kilaru, Smith, & Conneely, 2012). If data for a particular CpG site was missing for more than 10% of samples, analysis of that CpG site was excluded.

Statistical Analysis

Study Aims

To test each study question, we performed a series of regressions, controlling for covariates. To test the association between abuse and CpG methylation, we performed linear regressions for abuse predicting methylation of each of the *ESR1* CpG sites. Next, to test for genetic and epigenetic associations, we performed linear regressions with each *ESR1* SNP predicting methylation of the CpG sites associated with abuse at $p < 0.05$. To test whether methylation served as a mediator to depression, for each CpG site that was associated with abuse, we performed linear regressions with the CpG site predicting depression. Lastly, to test whether methylation may moderate the association between abuse and depression, we created interaction terms for each CpG site and abuse term and performed linear regressions predicting depression.

Covariates

All models controlled for important covariates. Specifically, models controlled for age, genetic ancestry, and cellular heterogeneity (% of leukocyte subtypes), all of which may associate with methylation. The cellular heterogeneity was determined by evaluating the proportion of granulocytes and lymphocytes in the whole blood DNA sample using the method described by Houseman and colleagues (2012). Using sample methylation data, cell types were determined using data from (GEO GSE35069), which provides information on CpG sites that are tissue specific given that methylation differs across cell types (Houseman, Accomando, & Koestler, 2012; Koestler et al., 2013; Sun et al., 2013), and the cell type proportions were calculated for each sample.

Statistical Tests

SPSS Statistics Version 22 was used to perform linear regressions for abuse-CpG associations, SNP-CpG associations, CpG-depression associations, and CpG-abuse interaction

analyses. To provide a thorough statistical report, all results with a $p < 0.05$ are given. Survival of False Discover Rate (FDR) and Bonferroni correction for multiple testing is also provided. Bonferroni correction was calculated by dividing α signifance level (0.05) by the number of tests performed. FDR was calculated at a significance level of q=0.1. P-values for all tests were ranked in order of significance and the FDR comparison p-value was calculated by dividing the p-value rank by the number of tests, then multiplying by the significance level q. Tests survived correction for multiple testing if the p-value for the individual test was less than the cutoff corrected p-value for that test.

Results

Study Cohort

Our cohort contained a total of 301 women. Data on childhood exposure to abuse was available for 296 of those women and approximately half of these women were exposed to moderate (28%, N=82) or severe (26%, N=78) abuse (Table 1). Across these categories there were no significant differences in age or blood cellular composition. As expected, those that experienced more childhood abuse had significantly higher levels of adult depression symptoms ($p < 0.001$). A total of 68 CpG sites and 139 SNPs within *ESR1* were analyzed. Average methylation levels varied across CpG sites (Table 2). CpG site correlations are also provided (Table 6).

Early life abuse associates with methylation of 7 CpG sites

We examined the association between abuse and the methylation level of 68 CpG sites within *ESR1* (Table 3, $p < 0.05$, FDR and Bonferroni reported). We found that abuse was associated with lower methylation at 2 CpG sites and higher methylation for 5 CpG sites (See

Figure 1). Two of these sites (cg00920970, associated with higher methylation, and cg06877423, associated with lower methylation) survive FDR correction, and cg06877423 also survives more stringent Bonferroni correction ($p < 7.4 \times 10^{-4}$).

Overall, the majority of the 7 CpG sites fall within a binding site for one or more transcription factors, according to the UCSC Genome Browser (Kent et al., 2002) (Figure 1). Of the 5 sites where abuse was associated with higher methylation at $p < 0.05$ (cg23467008, cg00655307, cg15980539, cg00920970, cg20253551), 4 are located within an intron that is present in the majority of ERα splice variants, the various mRNA transcripts that can be produced from a single gene as a function of alternative splicing, and the 5th is located in an exon in the majority of splice variants. Four (cg00655307, cg15980539, cg00920970, cg20253551) are located in a single promoter, and 3 (cg15980539, cg00920970, cg20253551) are located within a single CpG island (Table 3; Figure 1). Though abuse was associated with higher methylation at these sites, overall these sites had relatively low methylation (see Table 2; mean methylation range from 0.012-0.039).

Of the 2 sites where abuse was associated with lower methylation at $p < 0.05$ (cg08415493 and cg06877423), both were located in introns in all ERα variants. Methylation of these sites is significantly correlated ($p < 0.01$). Though abuse was associated with lower methylation at these sites, overall these sites had relatively high methylation (See Table 2; mean methylation 0.855 and 0.889, respectively). Methylation values are given as proportions in which larger numbers indicate higher methylation.

Genetic and Epigenetic association

We examined the association between genotypes for each of the 139 *ESR1* SNPs and methylation of the 7 CpG sites found to associate with abuse in our sample. All but one CpG site

was associated with 3 or more SNPs (Table 4). Conversely, 29 SNPs (21%) were associated with at least one of these 7 CpG sites. One SNP-CpG association (cg08415493-rs543650) withstood FDR and Bonferroni correction for multiple testing (973 tests). This SNP is located within an intron and the CpG site is located within an intron in all splice variants (Kent et al., 2002). Abuse was associated with lower methylation at this CpG site, and both SNP and abuse remain associated with methylation when both are included in a model predicting methylation (B=0.24, $p < 0.001$; B= -0.16, p=0.01, respectively).

CpG methylation does not mediate the association between abuse and depression

Though abuse associated with 7 CpG across *ESR1*, none of these 7 CpG sites were significantly associated with depressive symptoms. Therefore, methylation of these sites did not serve as a mediator between abuse and depression ($p > 0.05$).

CpG methylation moderates abuse and depression

While methylation did not serve as a mediator, differences in methylation may moderate the association between abuse and depression. To test this, interaction variables were created for each CpG site and abuse term. Ten CpG sites interacted with abuse in association with depressive symptoms (Table 5, $p < 0.05$), however these did not survive correction for multiple testing. For the majority (90%), there was a stronger association between abuse and depression in the presence of lower methylation. Five of the CpG sites were directly associated with abuse in the above analyses. Four of the 10 CpG sites are located within a promoter and 2 within a CpG island. Eight CpG sites were found in introns that are present in the majority of *ESR1* splice variants. The other 2 CpG sites were found in exons in the majority of variants.

Discussion

In this study we investigated the interplay between genetics, epigenetics, early-life abuse, and depressive symptoms in a cohort of 301 female participants in the Grady Trauma Project. In the current study, we found that early-life abuse was associated with methylation of 7 CpG sites across *ESR1,* with a few surviving correction for multiple testing. SNPs and CpG methylation are not independent, as we found multiple SNP-CpG associations with one surviving correction for multiple testing. While methylation of these sites did not serve as a mediator between early-life abuse and adult depression symptoms, we found that 10 CpG sites across *ESR1* may moderate the association between early-life abuse and adult depressive symptoms ($p < 0.05$), though these results did not withstand correction. These findings are preliminary, as this is one of the first reports to explore this question in humans and extend the animal literature on early environments and *ESR1*. However, this report may serve as initial step for future studies examining the role of the environment on human epigenetics and the role of *ESR1* in gene-environment interactions.

Interpretation of Findings

Early life abuse and Methylation

Initial work suggests that certain environments, particularly adverse early environments, may associate with methylation patterns of stress-related genes (Champagne et al., 2006; McGowan et al., 2009). In our study, we found that early-life abuse was associated with methylation of 7 CpG sites across *ESR1*. Two of these sites (cg08415493 and cg06877423) showed lower relative methylation in association with abuse. Contrastingly, 5 sites (cg23467008, cg00655307, cg15980539, cg00920970, and cg20253551) showed higher relative methylation in association with abuse. In the animal literature, more adverse early environments, conceptualized as low maternal LG behavior, are primarily associated with higher *ESR1* methylation and lower *ESR1* expression (Champagne et al., 2006). The current study is cross-sectional in nature and

thus causality cannot be drawn. However, if findings replicate, longitudinal studies may be warranted. It is possible that experiencing abuse may result in reduced methylation of some CpG sites, and at the same time increased methylation of other sites. Further, investigating how methylation changes at these sites may influence *ESR1* expression and regulation will be an informative next step. Many of these *ESR1* CpG sites are located within promoter regions and within binding sites for transcription factors and therefore may be involved in regulating gene expression.

While it is possible that early-life environment may impact *ESR1* methylation in humans, with two sites surviving FDR correction and one surviving stringent Bonferroni correction, we did not find that *ESR1* methylation directly associated with adult depression symptoms. Therefore, it does not appear that methylation of the *ESR1* CpG sites investigated in this study serve as mediators between early-life abuse and adult depression. The current study was limited in the range of measures available, given the goals of the original study, The Grady Trauma Project. Therefore, future work that explores other potential mediators that may be tied to estrogen, such as age of menses, may yield positive results. Additionally, as estrogen affects many body systems, it is possible that estrogen may have relatively variable effects, lowering the ability to detect a direct correlation. Further, many factors are involved in the development of depression. While estrogen has been assocated with depression (Cohen, Soares, Poitras, Prouty, Alexander, & Shifren, 2003; Schmidt et al., 2000), and estrogen receptor methylation may play a role in depression risk (Mehta et al., 2014; Ryan & Ancelin, 2013), there are a number of other factors that influence depression outcomes.

The mean BDI score of our cohort was 16.6 (SD= 13.1), which is within the “mild” range of depressive symptoms. To provide context, a recent study of normative undergraduate college

students described a mean BDI score of 11.0 (SD= 8.2), which falls within the "minimal" depressive range. (Storch, Roberti, & Roth, 2004). The relatively high mean depression score of our cohort may have influenced our ability to statistically examine the relationships between *ESR1* methylation and depression. However, there is substantial variability in the depression scores of this cohort, suggesting that a high mean depression score does not explain our lack of statistical associations.

Genetic and Epigenetic association

Literature has consistently indicated that methylation at specific CpG sites may be associated with nearby SNPs (Bell et al., 2012; Smith et al., 2014). Therefore, incorporating SNPs in methylation studies may provide a more comprehensive assessment. Among the 7 CpG sites associated with abuse, we found that the majority associate with 3 or more SNPs, and 1 SNP-CpG association withstood stringent correction (cg08415493-rs543650). This CpG was associated with abuse, and remained associated when controlling for the SNP. The SNP is located within an intron in all splice variants of *ESR1*. Current *ESR1* SNP human literature has not looked at this SNP in depth. However, if this CpG site is involved in *ESR1* regulation, it may serve as a functional link to *ESR1* expression or regulation. SNPs were included in this study in an effort to create a comprehensive analysis, as SNPs and CpG methylation are not independent. Additional inclusion of SNPs and CpG methylation in future studies investigating early-life abuse and adult outcomes may continue to promote a comprehensive understanding of how genetic variation may influence these associations.

CpG methylation moderates the relationship between early life abuse and adult depression

Ten CpG sites were found to interact with early-life abuse to predict adult depression. For 9 of these CpG sites (90%) there existed a stronger association between abuse and depression in the presence of lower methylation levels. In other words, individuals with lower *ESR1* methylation at these sites tended to have higher levels of depression if exposed to abuse. This suggests that individuals with lower methylation at these sites may be more sensitive to abuse or to the development of adverse outcomes following abuse (Table 5, Figure 2). Eight of these sites are located within introns, with the majority falling within a transcription factor binding region, suggesting that they may play a role in regulating gene expression. However, none of these sites survived correction for multiple testing, suggesting that our findings may be due to Type 1 error and are in need of replication.

Five CpG sites were found to associate with abuse both directly and to interact with abuse history to predict depression symptoms at $p < 0.05$. For all of these sites, abuse was directly associated with higher methylation, though lower methylation was associated with a stronger relationship between abuse and depression. The level of methylation across each CpG site varies among individuals. While functional interpretation is limited, it is possible that exposure to abuse may result in some increase in methylation at these sites, but that overall, those with lower methylation at these sites may be more vulnerable to poor outcomes following abuse. Prior to replication, interpretation is quite speculative, results should be considered preliminary, and the number of analyses should be taken into account.

The interaction of early-life adversity and individual genetic predispositions may also alter an individual's vulnerability to psychological disorders. Human and animal neuroimaging studies indicate that sex steroids are involved in neural connectivity between the prefrontal cortical and subcortical limbic regions of the brain, and that these connections contribute to the

sensitivity of the brain's response to emotional stimuli (Ladoucer, 2012). There also exists an association between depression and puberty in girls. It has been shown that the risk of future affective disorders may be mediated by pubertal changes in brain regions involved in emotion regulation (Blakemore & Mills, 2014).

Other studies have found that genetic and epigenetic variants may affect the robustness or sensitivity of an individual to environmental stimuli. For example, both genetic and epigenetic (CpG methylation) variation in the human oxytocin receptor gene (*OXTR*) have been found to confer differential sensitivity to social environments. Correspondingly, exposure to early-life adversity has been associated with greater adverse outcomes later in life in both animals and humans (Barrett, Arambula, & Young, 2015). Smearman, Winiarski, Brennan, Najman, and Johnson (2015) recently investigated the association between early life and *OXTR* methylation, also drawing from the Grady Trauma Project sample, and found an interaction in which lower *OXTR* methylation was associated with more adult depression and anxiety symptoms among those who experienced childhood abuse.

A few initial reports have proposed the possibility that epigenetic modification may be passed down to future generations (Morgan, Sutherland, Martin, & Whitelaw, 1999; Skinner, 2011; Dias & Ressler, 2014). Investigating the trans-generational inheritance of *ESR1* methylation may provide insight into the observed underlying variation in methylation previously denoted. Such a study could also propose a possible mechanism by which dynamic changes in epigenetics in response to environmental stimuli could impact the fitness of an individual from an evolutionary standpoint.

Overall, our findings are suggestive of possible associations between early environment, epigenetic changes, and adult depression. While many tests were significant at $p < 0.05$, due to

our large number of statistical tests, very small p-values were required to withstand correction for multiple testing in many cases. If the effect sizes seen in this study are true and accurate, a larger sample size could result in higher power statistics and provide more significant findings past correction for multiple testing.

Translatability of rodent studies

ESR1 in humans, rats, and mice

The majority of studies addressing the question of early-life adversity and methylation of *ESR1* to date have been in rodents. The translational ability of this rodent work to humans may depend, to a degree, on the structural and functional similarity of this gene in rodents as compared to humans. In humans, rats, and mice, *ESR1* is preceded by multiple promoters that make different mRNA variants when spliced. All mRNA transcripts product the same protein, suggesting that different promoters may be utilized in regulating cell differentiation and development (Wilson, Westberry, & Prewitt, 2008). While these three species have similar *ESR1* gene structures, there are important differences. The human *ESR1* gene contains 7 upstream promoters that can initiate transcription (A, B, C, D, T1/T2, E1/E2, F); the rat gene contains at least 4 known promoters (O/B 87% homology to humans, 0N, 0S 73%, C); and the mouse gene contains 6 promoters that produce 5 variants (A 63%, B 65%, C 89%, F1 73%, F2, H, where F1 and F2 are spliced together). Promoters denoted by the same letter are comparable across species (Wilson et al., 2008). While there may be sufficient homology to make certain conservative comparisons across rodent and human forms of *ESR1,* we encourage caution when interpreting results across different species.

As was noted previously, the neural development of humans and primates compared to rodents is also different. Comparative data indicates that humans have undergone more neural

development by the time of birth as compared to rodent species. Therefore, the experience of abuse or maternal separation in early life for pups occurs in a similar developmental time period to the third trimester for humans; therefore, in humans, these experiences are occurring later along the timeline of brain differentiation (Clancy, Darlingon & Finlay, 2001). Estrogen also differentially impacts the development of primates and rodents. In both male and female rodents, early development is impacted by estrogen (Matsuda, 2014). In primates, the HPG axis is actively repressed until this inhibition is released for the onset of puberty (McCarthy, 2013), however pre-pubertal sex steroid production by the adrenal glands during adrenarche may play a role in the brain development of some species (Spear, 2000).

While our study focused on the effects of childhood environment, rather than neonatal environment as was used in the rodent literature, literature suggests that neural development and sensitivity may continue beyond infanthood and into adolescence. As discussed by Ladoucer (2012), some longitudinal studies suggest that adolescence may be a period of vulnerability, during which time, adverse events can drastically change developmental trajectories and risk for emotional and physical health outcomes.

Early-life trauma has been shown to be associated with long-term neural changes, including coping style, emotional regulation, and neurochemistry. In rats, maternal-infant interaction is involved in regulating the development of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis in pups. Early-life adversity also has a substantial impact on primate behavior, although the role of LHPA axis function has not been clearly determined relative to the consistent observations of LHPA alteration described in rodents. This may be due to the fact that the primate brain is more mature than the rat brain at birth (Sanchez, Ladd, & Plotsky, 2001). Given the endocrine changes before and during puberty, maturation may exert direct and indirect

influences on neural systems on both a molelcular and functional level (Blakemore & Mills, 2014).

Neglect vs Abuse

Much of the animal work looking at the early environment assesses the level of nurturance, specifically the amount of LG behavior by the mother. While low LG is conceptualized as relatively more adverse in these studies, how this may translate to a comparable human environment is not well known. It is possible that this model may be more representative of neglectful environments, and studies that distinctly assess exposure to early-life neglect, distinct from physical, sexual or emotional abuse, may better translate this question into human studies. In the current sample, abuse and neglect were highly correlated (data not shown) with only a few individuals experiencing only neglect unaccompanied by abuse. Therefore, given the variable nature of human environmental exposures, we decided to focus on abuse in this study to reduce some variability in beginning to address this question in human samples. While neglect and abuse are often correlated, they may affect our biology in different ways and through different mechanisms. We encourage future research that can separately distinguish and measure the impact of both neglect and abuse on epigenetic and behavioral outcomes.

Limitations

Using peripheral blood tissue as source of methylation data

A practical limitation of our study, and many studies assessing DNA methylation in humans, is restriction to peripheral tissue. In the context of our study questions concerning childhood abuse and adult depression, ideal tissue would include brain tissue samples, e.g. from the amygdala, bed nucleus of the stria terminalis, or hypothalamus (McEwen, 2002). Being able to utilize tissue from these sites of interest would allow us to draw more direct insights into

methylation patterns of *ESR1, ESR1* regulation and expression, and its association with behavioral outcomes. An important step in this research is to continue to explore and more deeply understand the degree to which peripheral blood tissue can be used as a surrogate to brain tissue samples. Initial studies have compared blood and brain inter-individual DNA methylation profiles, and have suggested that some variation in methylation brain patterns were reflected in the blood (Sommershof et al., 2009; Davies et al., 2012). A study in rhesus macaques found that DNA methylation changes resulting from early-life adversity lasted into adulthood and manifested similarly in both the prefrontal cortex and circulating T cells in several classes of genes, for example *FKBP5* which is shown to be associated with mood disorders (Erwald et al., 2014; Menke et al., 2013). Several studies have also concluded that blood may provide a promising surrogate for brain tissue in human methylation studies (Horvath et al., 2012; Provençal et al., 2012). In a study investigating Parkinson's Disease (PD), distinct and concordant methylation patterns within genes involved in PD were found in both brain and blood samples (Masilah, Dumaop, Galasko, & Desplats, 2013). These findings suggest that peripheral blood may be useful in studying the complex neural epigenome, though conclusive research should follow-up using brain tissue. In addition to blood, new research suggests that saliva may provide a more accurate insight into patterns of brain tissue methylation (Smith et al., 2015). Future studies should consider the utility of both saliva and blood samples in studying behavioral epigenetics.

Dynamic vs Stable changes in the Epigenome

Events in prenatal and early life have been shown to affect gene expression through epigenetic regulation past the time of exposure. Estrogens play a role in regulating neurotransmitter synthesis via transcription and are known to influence the release of

acetylcholine and dopamine, both of which are known to modulate social behavior. Estrogen, as well as ERα specifically, is implicated as a regulator of the oxytocin system, another "social hormone" (Young, Wang, Donaldson, & Rissman, 1998). Studies have also shown that estrogen influences serotonin metabolism, particularly in the raphe nuclei. ERs are expressed in this region of the brainstem and have been identified on serotonergic neurons in rats, mice, and macaques. Additionally, ER activation results in increased serotonin release (Borrow & Cameron, 2014).

Therefore, estrogens may be important priming neurons for social and behavioral processing and mood disorders. These findings may help to explain how estrogen may use different molecular pathways to induce both long-term and short-term effects (Ervin, Lymer, Matta, Clipperton-allen, Kavaliers, & Choleris, 2015). These rapid and dynamic changes can also maintain stable alterations in gene expression over time (Champagne, 2010). For example, in some studies of maternal-pup care, estradiol in rat offspring of low LG moms lacks the ability to regulate oxytocin activity, suggesting a lasting alteration of ER expression (Wilson, Westberry, & Prewitt, 2008). However, the stability and persistence of these changes do not necessarily imply permanence.

While this system of gene regulation may be heritable as is evident in Champagne's maternal LG studies, it is also dynamic. Experience, memory, and fear conditioning can all influence epigenetic changes in the adult nervous system (Sweatt, 2009). In addition, substantial changes in quality of life and pharmacological manipulation can reverse the epigenetic effects of early-life adversity. Some evidence suggests that environmental enrichment may induce histone acetylation, which produces the opposite effect of DNA methylation.

Studying other epigenetic mechanisms such as histone acetylation could provide a next step in investigating how the epigenome is responsive to early environment and may influence adult outcomes. Epigenetic plasticity is possible, however the pathways and mechanisms of gene regulation may vary across developmental stages (Champagne, 2010). Because of the dynamic nature of epigenetics, longitudinal studies could provide valuable insight into the long-term effects of childhood environment on epigenetics. Such studies could also address how these modifications are influenced by later-life environment and experiences. Furthermore, multi-generational studies could provide valuable information as to how epigenetic modifications are passed-down.

It is important to consider that the methylation observed in this cross-sectional study may be the result of recent environmental effects, or a combination of both early-life and current environment, if findings hold in humans. Seeking to determine the effect of early-life abuse on epigenetic methylation via adulthood methylation measures therefore poses several limitations. However, this study may provide some insight into the long-term affects of early-life environment and the longevity of epigenetic changes. To address the potentially confounding effects of current environment on *ESR1* methylation, future studies may consider including additional outcome measures to assess current environment and recent stress, and controlling for these variables in their analyses.

Future directions

Estrogen Receptor Types

Animal literature has consistently studied the association between early-life stress and *ESR1* regulation. While our study sought to investigate this association in humans in parallel with existing animal literature, there are other estrogen receptors that may be important to

consider. Estrogen receptors are historically described as nuclear receptors. Both ERα and ERβ mediate gene expression through genomic signaling, forming hetero- or homo-dimers (King & Green, 1984; Green et al., 1986; Kuiper, Enmark, Pelto-Huikko, Nilsson, & Gustafsson, 1996). These nuclear receptors have been found to interact with transcription factors to modify DNA expression and gene regulation (Schultz-Norton, Ziegler, & Nardulli, 2011). Recently, a membrane G protein-coupled estrogen receptor (GPER) has been found to mediate some rapid effects of estrogen that were known to be inconsistent with the classical genomic effects of estrogen binding to intracellular ERs (Prossnitz & Barton, 2014). However, some studies have described the effects of GPER on gene expression as well (Prossnitz & Maggiolini, 2009). Future studies that also investigate the relationship between GPER and ERβ and early-life experience may help us gain a more comprehensive understanding of estrogen as a mediator of environmental factors and genomic changes.

Critical Windows and Canalization

During early life, neural connections and circuits rapidly remodel and develop in response to the environment, however this plasticity changes over the course of development. The post-natal period is particularly sensitive to environmental factors, and therefore may provide a critical period or "window of opportunity" for epigenetics to mediate the effects of external stimuli on long-term biological changes (Guerro-Bosagna & Skinner, 2012). Such critical periods in neuronal development are not uncommon and are implicated in various developmental contexts (Fagiolini, Jensen, & Champagne, 2009; Makinodan, Rosen, Ito, & Corfas, 2012; Khan et al., 2015). Additionally, sustained environmentally-induced epigenetic changes have been demonstrated in the context of early-life experience and its impact on HPA axis activity, reproductive hormone systems, BDNF methylation, and glucocorticoid receptor

expression (Meaney, 2001; Champagne et al., 2006; McGowan et al., 2009; Roth & Sweatt, 2011).

"Canalization" could also play a role in robust responses to environmental factors, in which underlying genetic and epigenetic alterations established during the critical period would mediate the impact of stimuli on an individual's biology at a later time (Gursky, Surkova, & Samsonova, 2012). It is possible that early life may be particularly important in determining developmental trajectories through differential methylation of certain genes, however the degree to which this methylation may change over time or continue to be responsive to environmental stimuli or experiences is not yet well known. As our study is cross-sectional in nature, longitudinal work is needed to develop a more comprehensive understanding of how early life, epigenetics, and adult outcomes are related.

Implications and next steps

Estrogen affects the brain throughout development. The production of estrogen and the regulation of *ESR1* are complex processes that are likely influenced by a wide variety of factors and play vital roles in a variety of other body systems. Very little is known about how estrogen influences neural development and plasticity throughout human life. While there is a large body of research indicating that estrogen levels change across the lifespan, the mechanism by which these changes occur, whether epigenetic or otherwise, is vastly unexplored.

In this study, abuse was found to associate with a number of *ESR1* CpG sites, with two sites surviving correction. While none of the CpG sites analyzed were found to act as mediators between early-life abuse and adult depression, there are a number of other potential adult outcomes to consider. For example, Belsky, Steinberg, Houts, and Halpern-Felsher (2010) propose that adverse early-life experience, such as maternal harshness, may influence pubertal

timing in females. A study of the interplay among early-life abuse, *ESR1* methylation, and pubertal timing could provide interesting insight into how early life affects developmental timing. Additionally, studying adult anxiety or PTSD could yield additional data to supplement our findings on depression in the current study.

Recent studies have explored associations between early-life abuse and oxytocin (Smearman et al., 2015), and as noted previously, oxytocin and estrogen are known to influence the production of one another (Pfaff et al., 2011). Future studies investigating the predictive value of ERα on *OXTR* expression and how these systems may work together to create physiological change could provide valuable insight as to how the epigenome responds to early-life stress and may influence variable adult outcomes. It remains relatively unknown as to whether and to what degree estrogen may provide a mechanism for *OXTR* to impact social behavior, particularly in humans.

Conclusion

In this study we investigated how environmental and genetic factors influence *ESR1* methylation and depression in a large sample of women assessed through the Grady Trauma Project. In our study, we found that exposure to early-life abuse associated with methylation of several *ESR1* CpG sites, with two surviving correction, though it did not serve as a mediator between early-life abuse and adult depression. However, *ESR1* CpG site methylation may moderate the association between early-life abuse and adult depression. We also found that several *ESR1* SNPs may associate with nearby CpG site methylation, with one SNP-CpG pair surviving stringent correction, suggesting that genetic and epigenetic variability is not independent. This research further supports an interplay between our environment and our

biology in the development of adult outcomes. In a broader context, understanding the biological implications of early-life adversity would give credence to intervention for children living in adverse enviroments and provide an outcome in methylation that may be more proximally measured to assess the impact of adversity and/or intervention. This study is an initial step in extending animal literature assessing the role of *ESR1* regulation in the link between early adversity and adult outcomes.

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Tables and Figures

Table 1.

Study Variables Among those with None to Mild, Moderate, or Severe Abuse

Abuse: Physical, Emotional, or Sexual

	None to Mild	Moderate	Severe	p-value
	(N=136)	(N=82)	(N=78)	
Age (Mean, SD)	40.84 (13.558)	38.99 (13.363)	42.04 (11.355)	0.322
% Granulocytes	61.03 (9.744)	62.01 (12.335)	62.83 (11.251)	0.497
% Lymphocytes	14.93 (5.908)	15.06 (7.528)	13.88 (4.941)	0.404
BDI Score	12.34 (10.714)	16.53 (12.239)	24.25 (14.642)	p<0.001

Note: One way ANOVAs were used to compare means across depression categories. Percent granulocyte and lymphocyte reflects the percentage of that cell type in the blood sample that was used for methylation analyses. It representes cellular heterogeneity in the sample. BDI is the Beck Depression Inventory. Scale range 0-63 with higher scores representing higher depressive symptoms. The cut-off score for depression is 13. The amount of missing data varied between the variables; total N ranged from 288-301.

Table 2.
Descriptive Statistics for CpGs

CpG Site	CpG Position	N	Minimum	Maximum	Mean (SD)
cg19449067	152011103	299	0.572	0.962	0.825 (0.071)
cg25338972	152011178	300	0.801	0.999	0.970 (0.026)
cg06611115	152011200	300	0.935	0.997	0.981 (0.010)
cg18745416	152011399	300	0.556	0.917	0.738 (0.078)
cg08161546	152011415	300	0.363	0.869	0.616 (0.111)
cg04211581	152011656	300	0.026	0.281	0.112 (0.052)
cg18007957	152011666	300	0.009	0.399	0.113 (0.068)
cg22157087	152012887	300	0.343	0.850	0.614 (0.112)
cg25565730	152085565	300	0.111	0.418	0.244 (0.054)
cg17741339	152085619	298	0.080	0.497	0.243 (0.069)
cg18132851	152085641	299	0.060	0.552	0.240 (0.089)
cg08415493	152124815	300	0.771	0.911	0.855 (0.023)
cg09646983	152125861	299	0.932	1.000	0.993 (0.006)
cg08907436	152125965	300	0.916	0.996	0.985 (0.007)
cg07619683	152126080	296	0.811	1.000	0.990 (0.024)
cg07189962	152126092	300	0.443	1.000	0.994 (0.038)
cg07584093	152126180	300	0.855	1.000	0.992 (0.012)
cg10441070	152126250	300	0.807	0.979	0.929 (0.035)
cg17706972	152126337	300	0.950	0.996	0.982 (0.008)
cg01321962	152126441	300	0.952	1.000	0.998 (0.003)
cg20893956	152126736	300	0.569	0.902	0.774 (0.062)
cg24764793	152126745	300	0.807	0.991	0.937 (0.037)
cg07746998	152126785	300	0.538	0.800	0.686 (0.051)
cg21157690	152126895	300	0.596	0.891	0.785 (0.054)
cg17264271	152126938	300	0.522	0.807	0.699 (0.056)
cg15543523	152127812	300	0.905	1.000	0.995 (0.009)
cg26089753	152127821	300	0.958	1.000	0.993 (0.007)
cg08884395	152127887	299	0.889	0.987	0.956 (0.018)
cg01715172	152128024	300	0.795	0.999	0.973 (0.030)
cg21608605	152128258	300	0.004	0.101	0.035 (0.019)
cg20627916	152128328	300	0.005	0.409	0.112 (0.071)
cg07671949	152128338	300	0.040	0.310	0.140 (0.058)
cg23164938	152128366	298	0.000	0.242	0.036 (0.037)
cg23165623	152128411	300	0.013	0.094	0.037 (0.016)
cg21614759	152128426	300	0.000	0.020	0.005 (0.004)
cg19411146	152128471	300	0.020	0.181	0.056 (0.022)

cg21950534	152128483	300	0.030	0.142	0.062 (0.020)
cg11813455	152128515	300	0.006	0.187	0.044 (0.025)
cg24900983	152128528	300	0.045	0.287	0.126 (0.049)
cg05171584	152128535	300	0.056	0.284	0.132 (0.045)
cg23467008	152128537	300	0.002	0.083	0.018 (0.011)
cg22839866	152128584	299	0.010	0.070	0.027 (0.011)
cg23009221	152128588	300	0.052	0.171	0.090 (0.020)
cg13612689	152128634	300	0.011	0.090	0.034 (0.014)
cg27316393	152128675	300	0.008	0.097	0.030 (0.015)
cg00655307	152128743	300	0.010	0.133	0.039 (0.018)
cg01777019	152128805	300	0.001	0.047	0.009 (0.006)
cg15980539	152128865	299	0.003	0.146	0.035 (0.023)
cg11251858	152129036	300	0.017	0.193	0.030 (0.029)
cg00920970	152129388	300	0.007	0.069	0.032 (0.011)
cg20253551	152129400	300	0.001	0.050	0.012 (0.008)
cg02285263	152129749	300	0.004	0.051	0.018 (0.008)
cg02720618	152129791	300	0.001	0.038	0.007 (0.006)
cg04063345	152130058	300	0.476	0.962	0.812 (0.091)
cg15626350	152130207	300	0.419	0.930	0.725 (0.102)
cg00601836	152130332	300	0.536	0.948	0.834 (0.073)
cg06877423	152200760	298	0.588	0.999	0.889 (0.068)
cg03732055	152201038	300	0.710	1.000	0.993 (0.025)
cg21265702	152201605	300	0.898	0.983	0.956 (0.013)
cg10939667	152201611	300	0.961	0.995	0.985 (0.005)
cg09414638	152239860	300	0.898	1.000	0.989 (0.015)
cg19369424	152264237	300	0.951	1.000	0.993 (0.008)
cg07455133	152379044	300	0.730	0.955	0.885 (0.043)
cg12209876	152381560	300	0.972	0.999	0.994 (0.004)
cg25490334	152387590	300	0.946	0.994	0.979 (0.009)
cg02404255	152419175	300	0.950	1.000	0.993 (0.005)
cg03037684	152421333	300	0.541	0.774	0.644 (0.043)
cg07059469	152421432	300	0.288	0.777	0.492 (0.107)

Note: Descriptive statistics are presented each CpG site. "CpG position" describes the position of the CpG site on the chromosome.

Table 3. *Abuse Predicting CpG Site Methylation*

	Model			Abuse Variable			Methylation level with stronger association
CpG site	R-squared	F-statistic(1,3)	F p-value	(stnd) Beta	t-statistic	p-value	
cg08415493	0.071	2.951 (7, 278)	0.005	-0.167	-2.803	0.005	Lower
cg23467008	0.117	5.136 (7, 278)	1.70E-05	0.144	2.483	0.014	Higher
cg00655307	0.122	5.364 (7, 278)	9.00E-06	0.124	2.150	0.032	Higher
cg15980539	0.144	6.514 (7, 277)	4.13E-07	0.123	2.146	0.033	Higher
cg00920970	0.078	3.254 (7, 278)	0.002	0.193	3.254	0.001*	Higher
cg20253551	0.083	3.492 (7, 278)	0.001	0.16	2.709	0.007	Higher
cg06877423	0.063	2.567 (7, 276)	0.014	-0.232	-3.866	0.000139**	Lower

Note: The association between abuse and CpG site methylation of 68 CpG sites was tested in a linear regression controlling for age, cellular heterogeneity, and genetic ancestry. Standardized betas are presented. To enhance interpretation, the final column indicates whether abuse is more strongly associated with methylation among those one standard deviation above (Higher) or below (Lower) than the mean methylation for that specific CpG site. Abuse was associated with higher methylation of 5 sites and lower methylation of 2 sites. All models presented reach significance at $p<0.05$. * = withsthood FDR correction for multiple testing. ** = withstood FDR and Bonferroni correction.

Table 4.

Association between SNPs and methylation of specific CpG sites

		SNP Predictor Statistics		
CpG site	SNP	Beta	t-statistic	p-value
cg00655307	rs11964281	-0.172	-2.901	0.004
cg00655307	rs17082000	0.133	2.221	0.027
cg00655307	rs2881766	-0.130	-2.169	0.031
cg00655307	rs1643821	-0.123	-2.063	0.040
cg00655307	rs827423	0.120	1.996	0.047
cg00655307	rs9397459	-0.119	-1.993	0.047
cg00920970	rs1293936	-0.158	-2.574	0.011
cg00920970	rs1159327	0.152	2.457	0.015
cg00920970	rs1293942	0.146	2.389	0.018
cg00920970	rs11964488	-0.147	-2.389	0.018
cg00920970	rs11964281	-0.139	-2.261	0.025
cg00920970	rs6557164	0.132	2.136	0.034
cg00920970	rs3020334	-0.129	-2.080	0.039
cg00920970	rs9322336	-0.126	-2.032	0.043
cg06877423	*not associated with any tested SNPs*			
cg08415493	rs543650	0.254	4.163	4.3×10^{-5}**
cg08415493	rs2881766	0.210	3.403	0.001
cg08415493	rs11964281	0.190	3.077	0.002
cg08415493	rs7761133	-0.183	-2.950	0.003
cg08415493	rs9340776	0.177	2.834	0.005
cg08415493	rs1293942	-0.163	-2.642	0.009
cg08415493	rs1159327	-0.163	-2.603	0.010
cg08415493	rs827423	-0.159	-2.549	0.011
cg08415493	rs11967900	-0.152	-2.407	0.017
cg08415493	rs2077647	0.148	2.375	0.018
cg08415493	rs11964488	0.145	2.329	0.021
cg08415493	rs3020334	0.139	2.216	0.028
cg08415493	rs3020333	0.138	2.198	0.029
cg08415493	rs6902771	0.133	2.148	0.033
cg08415493	rs2941740	-0.133	-2.119	0.035
cg15980539	rs9383598	0.175	2.934	0.004
cg15980539	rs9397459	-0.165	-2.811	0.005
cg15980539	rs11155823	-0.146	-2.422	0.016
cg20253551	rs851995	0.191	3.163	0.002
cg20253551	rs2881766	-0.154	-2.525	0.012
cg20253551	rs1999805	0.152	2.482	0.014

cg20253551	rs9383939	-0.146	-2.370	0.019
cg20253551	rs543650	-0.144	-2.350	0.020
cg20253551	rs827423	0.142	2.319	0.021
cg20253551	rs2144025	-0.138	-2.270	0.024
cg20253551	rs1336981	-0.131	-2.138	0.033
cg20253551	rs1890010	-0.127	-2.049	0.041
cg20253551	rs7761133	0.124	2.016	0.045
cg23467008	rs1293942	0.137	2.293	0.023
cg23467008	rs9383598	0.137	2.243	0.026
cg23467008	rs17082000	0.130	2.170	0.031
cg23467008	rs11155823	-0.130	-2.108	0.036

Note: The association between SNPs and methylation of the 7 CpG sites associated with abuse were determined using linear regressions controlling for age, cellular heterogeneity, and genetic ancestry. Standardized betas are presented. ** = withstood FDR and Bonferroni correction.

Table 5.
Association between Childhood Abuse and Depresion Symptoms: Potential Moderating Role of ESR1 *CpG Site methylation*

AbusexCpG Interaction Model	Depression			Methylation level with Stronger Association
	Beta	t-statistic	p-value	
cg18132851	-0.123	-2.125	0.035	Lower
cg08415493	0.146	2.524	0.012	Higher
cg07619683	-0.158	-2.707	0.007	Lower
cg19411146	-0.115	-2.01	0.045	Lower
cg24900983	-0.135	-2.322	0.021	Lower
cg23467008	-0.147	-2.37	0.018	Lower
cg00655307	-0.124	-2.051	0.041	Lower
cg15980539	-0.145	-2.406	0.017	Lower
cg00920970	-0.122	-2.13	0.034	Lower
cg07059469	-0.158	-2.749	0.006	Lower

Note: The interaction between abuse and CpG site methylation was tested using linear regression predicting depression symptoms, controlling for age, cellular heterogeneity, and genetic ancestry. To enhance interpretation, the final column indicates whether abuse associates with greater depression among those with Higher (one standard deviation above the mean) or Lower (one standard deviation below the mean) methylation for that specific CpG. No associations survived FDR or Bonferroni correction.

Table 6.
CpG Correlations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
2 cg25338972	.620*																									
3 cg06611115	.502*	.599*																								
4 cg18745416	.814*	.613*	.551*																							
5 cg08161546	.785*	.608*	.520*	.882*																						
6 cg04211581	.568*	.402*	.332*	.686*	.755*																					
7 cg18007957	.606*	.449*	.383*	.725*	.767*	.860*																				
8 cg22157087	.333*	.244*	.195*	.442*	.500*	.441*	.454*																			
9 cg25565730	.209*	.116		.168*				-.268*																		
10 cg17741339	.158*	.151*	.134					-.151*	.546*																	
11 cg18132851	.173*			.129				-.324*	.834*	.641*																
12 cg08415493	-.385*	-.258*	-.227*	-.449*	-.418*	-.386*	-.407*		-.421*	-.150*	-.298*															
13 cg09646983				-.130	-.209*	-.235*	-.195*	-.211*			.129	.173*														
14 cg08907436	-.169*			-.208*	-.203*	-.234*	-.206*	-.140	-.121			.333*	.494*													
15 cg07619683																										
16 cg07189962																										
17 cg07584093					-.120		-.114						.219*	.252*												
18 cg10441070	-.373*	-.193*	-.137	-.370*	-.345*	-.283*	-.266*	.116	-.567*	-.222*	-.431*	.614*		.330*			.251*									
19 cg17706972						-.178*	-.183*					.241*	.298*	.264*			.202*	.253*								
20 cg01321962																			.168*							
21 cg20893956	-.428*	-.332*	-.285*	-.565*	-.660*	-.649*	-.701*	-.524*	.196*	.196*	.272*	.271*	.295*	.195*				.151*	.267*							
22 cg24764793	-.254*	-.183*	-.160*	-.348*	-.415*	-.390*	-.458*	-.369*	.245*	.206*	.285*	.153*	.277*	.216*					.282*		.691*					
23 cg07746998	-.443*	-.346*	-.292*	-.567*	-.635*	-.581*	-.649*	-.476*	.124	.127	.203*	.317*	.285*	.231*				.164*	.228*		.823*	.706*				
24 cg21157690	-.414*	-.279*	-.256*	-.526*	-.616*	-.588*	-.648*	-.416*	.174*	.264*	.251*	.362*	.300*	.277*			.133	.216*	.226*		.805*	.637*	.769*			
25 cg17264271	-.408*	-.355*	-.364*	-.558*	-.627*	-.601*	-.647*	-.490*	.251*	.169*	.273*	.319*	.289*	.215*					.174*		.821*	.651*	.800*	.792*		
26 cg15543523	-.170*	-.131		-.237*	-.257*	-.281*	-.323*	-.175*			.133	.194*	.294*	.265*			.131		.324*	.126	.328*	.330*	.351*	.338*	.300*	
27 cg26089753		.129				-.152*	-.160*	-.134				.192*	.236*	.229*				.142	.152*		.201*	.123	.150*	.175*	.149*	.236*
28 cg08884395	.159*	.189*	.204*	.176*									.200*	.253*					.159*							
29 cg01715172													.260*	.256*			.250*	.124						.174*	.113	
30 cg21608605	.146								.438*	.421*	.468*	-.235*		-.139				-.261*	-.132					.114		
31 cg20627916								-.146	.450*	.352*	.422*	-.198*						-.294*								
32 cg07671949								-.239*	.587*	.400*	.564*	-.272*					-.128	-.409*			.146	.168*			.119	
33 cg23164938								-.187*	.618*	.501*	.575*	-.273*						-.385*			.187*	.222*	.160*	.190*	.215*	
34 cg23165623								-.242*	.696*	.478*	.606*	-.312*		-.146			-.121	-.417*			.193*	.218*	.121	.158*	.208*	
35 cg21614759								-.190*	.537*	.360*	.480*	-.328*		-.153*				-.408*				.126				
36 cg19411146	.318*	.181*	.163*	.283*	.279*	.256*	.283*		.545*	.339*	.441*	-.414*		-.151*				-.527*	-.120		-.195*		-.219*	-.207*	-.135	
37 cg21950534	.267*			.219*	.179*	.196*	.216*		.575*	.327*	.480*	-.480*		-.253*				-.515*	-.140					-.123		-.163*
38 cg11813455	.270*	.116		.261*	.232*	.177*	.213*		.540*	.296*	.440*	-.425*	-.125	-.233*			-.141	-.551*	-.192*				-.141	-.117		
39 cg24900983	.254*			.188*				-.213*	.820*	.488*	.709*	-.540*		-.169*			-.170*	-.610*			.119	.238*			.127	
40 cg05171584	.361*	.237*	.186*	.328*	.293*	.263*	.269*		.700*	.387*	.576*	-.608*		-.208*			-.116	-.593*	-.136							-.136
41 cg23467008	.212*			.131				-.195*	.637*	.397*	.543*	-.466*		-.155*			-.176*	-.560*	-.117			.135				
42 cg22839866	.165*			.149				-.182*	.671*	.327*	.508*	-.454*		-.186*				-.488*				.121				
43 cg23009221	.167*			.124	.144	.127	.145	-.157*	.600*	.296*	.494*	-.437*		-.160*			-.143	-.485*	-.160*	-.122		.118				
44 cg13612689	.302*	.140		.268*	.223*	.182*	.171*	-.174*	.592*	.331*	.461*	-.446*		-.191*				-.541*	-.153*	-.121						-.139
45 cg27316393	.140			.129	.117		.121		.338*		.253*	-.244*					-.134	-.310*								
46 cg00655307	.322*	.160*		.290*	.265*	.257*	.240*		.646*	.327*	.541*	-.568*		-.233*			-.174*	-.611*	-.167*							
47 cg01777019	.136			.125	.126	.134	.197*		.160*			-.218*		-.269*				-.270*	-.164*	-.181*				-.134		
48 cg15980539	.230*			.224*	.202*	.216*	.183*	-.147	.570*	.279*	.472*	-.503*		-.198*				-.579*								
49 cg11251858	.186*			.177*	.204*	.243*	.218*		.274*	.146	.229*	-.308*		-.156*				-.351*			-.126			-.203*		
50 cg00920970					.149*	.156*		.186*		.137	-.361*	-.186*	-.208*				-.121	-.327*								-.116
51 cg20253551	.131			.157*	.153*	.199*	.188*		.198*		.140	-.363*		-.206*			-.172*	-.311*	-.205*	-.168*						
52 cg02285263		-.128	-.132		-.141	-.179*	-.129	-.264*	.437*	.222*	.407*	-.262*		-.119				-.240*			.297*	.255*	.314*	.271*	.349*	
53 cg02720618								-.260*	.415*	.230*	.384*	-.198*					-.233*	-.296*			.241*	.267*	.260*	.227*	.289*	
54 cg04063345	-.458*	-.343*	-.322*	-.568*	-.651*	-.623*	-.687*	-.530*	.269*	.276*	.364*	.253*	.219*	.133				.116	.189*		.806*	.578*	.763*	.769*	.788*	.278*
55 cg15626350	-.477*	-.375*	-.371*	-.595*	-.676*	-.667*	-.702*	-.521*	.266*	.276*	.335*	.253*	.266*	.208*					.180*		.812*	.595*	.784*	.791*	.803*	.341*
56 cg00601836	-.545*	-.413*	-.336*	-.645*	-.713*	-.691*	-.754*	-.539*	.189*	.173*	.257*	.315*	.338*	.249*			.124	.183*	.216*		.798*	.559*	.760*	.743*	.760*	.325*
57 cg06877423				-.146			-.117		-.150*			.255*		.132			.135	.258*	.172*							.126
58 cg03732055																										
59 cg21265702		.170*	.201*										.202*	.184*					.240*		.170*			.133		
60 cg10939667					-.116	-.203*	-.201*	-.219*				.207*	.244*	.229*					.164*		.218*	.129	.140	.184*	.244*	.131
61 cg09414638	-.206*			-.281*	-.237*	-.260*	-.174*		-.137	-.198*	-.161*	.276*		.134	.169*		.158*	.255*	.150*		.163*	.122	.159*	.180*	.144	.130
62 cg19369424					-.140	-.124				-.147		.118						.147	.212*	.150*						
63 cg07455133	-.220*	-.118		-.182*	-.138		-.118	.129	-.554*	-.310*	-.484*	.347*		.171*			.125	.441*	.131							
64 cg12209876												.155*	.227*	.185*			.120	.131	.256*		.120	.161*	.133	.135	.118	
65 cg25490334	-.126			-.206*	-.224*	-.254*	-.283*		-.224*		-.137	.414*	.234*	.334*			.269*	.408*	.193*		.185*		.159*	.223*	.167*	.132
66 cg02404255	.207*		.194*	.150*	.171*				.114										.130							
67 cg03037684	.179*	.146		.156*	.160*	.121	.129		.197*		.261*															
68 cg07059469	.170*	.154*		.168*	.113			-.192*	.412*	.220*	.443*	-.156*						-.296*			.178*	.241*	.178*	.186*	.185*	

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29	.179*	.294*																								
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31				.429*																						
32				.401*	.468*																					
33				.465*	.495*	.535*																				
34				.550*	.553*	.682*	.715*																			
35		-.154*		.361*	.381*	.476*	.604*	.653*																		
36				.413*	.389*	.492*	.525*	.579*	.478*																	
37	-.139			.489*	.436*	.540*	.567*	.632*	.548*	.614*																
38				.433*	.455*	.496*	.518*	.574*	.484*	.640*	.636*															
39				.492*	.494*	.675*	.689*	.787*	.633*	.632*	.728*	.662*														
40				.440*	.479*	.572*	.531*	.673*	.570*	.629*	.700*	.695*	.853*													
41				.436*	.456*	.547*	.574*	.688*	.553*	.573*	.621*	.556*	.789*	.745*												
42		-.127		.380*	.398*	.492*	.535*	.654*	.645*	.519*	.628*	.589*	.764*	.723*	.663*											
43	-.155*			.377*	.398*	.522*	.494*	.608*	.561*	.504*	.639*	.546*	.716*	.660*	.643*	.683*										
44				.362*	.364*	.497*	.491*	.562*	.477*	.541*	.665*	.594*	.684*	.715*	.610*	.631*	.610*									
45				.188*	.197*	.284*	.227*	.352*	.317*	.364*	.398*	.349*	.390*	.391*	.345*	.431*	.350*	.455*								
46				.431*	.474*	.544*	.543*	.674*	.539*	.619*	.696*	.634*	.782*	.777*	.729*	.685*	.666*	.704*	.416*							
47	-.190*			.148			.127	.148	.259*		.251*	.179*	.180*	.232*	.222*	.217*	.298*	.252*	.238*	.275*						
48				.275*	.371*	.443*	.444*	.542*	.526*	.510*	.545*	.492*	.693*	.683*	.657*	.598*	.568*	.570*	.333*	.700*	.251*					
49	-.175*			.209*	.124	.160*	.148	.246*	.261*	.289*	.340*	.225*	.315*	.410*	.346*	.319*	.375*	.453*	.356*	.415*	.295*	.404*				
50		-.145		.124	.131	.126	.260*	.249*	.244*	.266*	.342*	.260*	.303*	.276*	.352*	.331*	.297*	.287*	.225*	.361*	.203*	.321*	.217*			
51	-.161*		-.147		.197*	.188*	.143	.253*	.330*	.196*	.353*	.296*	.317*	.389*	.296*	.381*	.327*	.449*	.259*	.468*	.317*	.424*	.358*	.356*		
52	-.173*			.290*	.318*	.356*	.336*	.419*	.363*	.177*	.380*	.274*	.472*	.346*	.382*	.473*	.411*	.367*	.218*	.368*	.152*	.399*	.149*	.233*	.354*	
53				.225*	.342*	.405*	.298*	.430*	.355*	.238*	.319*	.270*	.467*	.369*	.387*	.440*	.360*	.299*	.216*	.402*	.171*	.428*	.138	.241*	.396*	.675*
54				.119	.177*	.184*	.218*	.257*		-.151*		-.127	.170*										-.128			.377*
55			.137		.145	.187*	.245*	.247*		-.144			.177*		.121											.410*
56	.127		.160*				.148	.148		-.238*		-.193*		-.132												.293*
57			.150*		-.150*	-.164*					-.251*	-.199*	-.126	-.165*	-.151*	-.209*	-.202*	-.148	-.207*	-.253*		-.179*		-.147	-.215*	-.187*
58																										
59	.186*	.209*	.133																-.155*					-.129	-.146	
60	.243*		.128								-.152*						-.117			-.130	-.147		-.144	-.175*	-.189*	
61	.290*		.173*		-.164*		-.115		-.196*	-.192*	-.191*	-.198*	-.194*	-.199*	-.187*	-.185*	-.236*	-.192*		-.182*		-.175*	-.136	-.143	-.170*	-.153*
62				-.277*			-.120	-.160*	-.199*	-.167*	-.135	-.158*	-.136	-.187*	-.259*	-.208*	-.163*	-.213*		-.206*	-.199*		-.261*	-.147	-.210*	
63				-.483*	-.383*	-.543*	-.376*	-.515*	-.349*	-.484*	-.433*	-.394*	-.524*	-.456*	-.392*	-.410*	-.383*	-.320*	-.215*	-.413*		-.313*	-.143			-.261*
64	.162*	.200*							-.140		-.148					-.179*	-.158*	-.146	-.158*	-.127	-.143		-.146	-.179*	-.158*	-.181*
65	.217*	.121	.239*	-.206*	-.183*	-.240*	-.193*	-.245*	-.259*	-.277*	-.367*	-.252*	-.308*	-.338*	-.253*	-.340*	-.347*	-.323*	-.219*	-.342*	-.231*	-.302*	-.252*	-.233*	-.351*	-.252*
66	.167*																							-.133		
67				.175*		.156*				.130	.142	.149*	.145	.145			.154*	.159*		.184*						.128
68				.188*	.212*	.290*	.241*	.223*	.177*	.222*	.219*	.278*	.355*	.312*	.238*	.257*	.269*	.299*		.321*		.301*				.293*

	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
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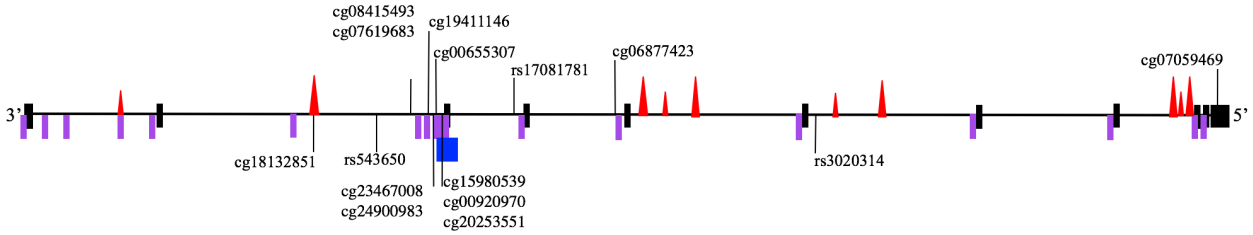
54	.333*														
55	.352*	.933*													
56	.222*	.818*	.851*												
57	-.123														
58															
59		.140		.137											
60		.198*	.148	.164*	.154*		.250*								
61			.114	.157*	.214*			.189*							
62								.183*	.163*						
63	-.243*				.139				.159*	.144					
64		.174*			.234*		.279*	.217*	.128						
65	-.266*	.166*	.163*	.205*	0.14		.158*	.283*	.196*	.303*	.256*	.145			
66							.123	.129	.189*	.179*		.166*			
67	.141										-.139				
68	.297*	.205*	.221*	.151*							-.304*				.790*

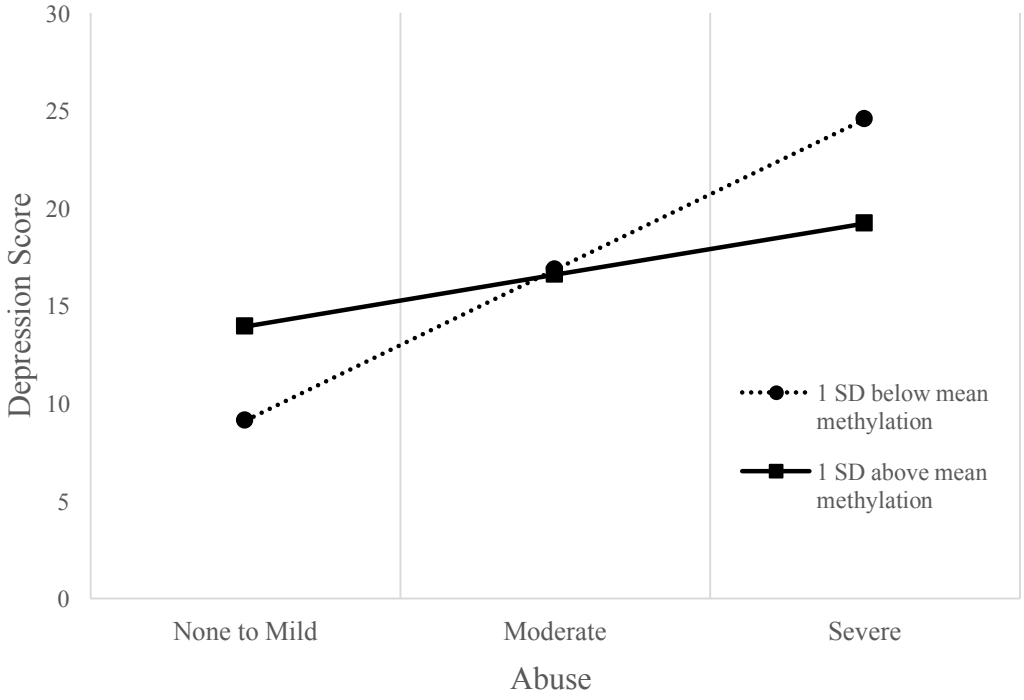
Note: CpG correlations were determined with 2-tailed Pearson correlation tests. Only values with $p<0.05$ were reported. * indicates $p<0.01$

Figure 1.
ESR1 *diagram with CpG sites and SNPs*



Note: Black bars indicate exons; thin black lines indicate exons; purple bars indicate alternative promoters; blue bars indicate CpG islands; red triangles indicate high regulatory factor binding activity (UCSC Genome Browser).

Figure 2.
cg07059469 Interaction with Abuse and Depression



Note: This graph is included as an illustration of the data presented in Table 5. From this data we were able to create a linear equation to reflect the interaction for each CpG site. Depression score was measured with the BDI (0-63, score of 13 or above is considered depressive) and was determined for none to mild, moderate, and severe abuse by calculating the y value for each x=0, 1, 2, respectively.