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Impact of Child Sex Abuse on Adult Psychopathology: A Genetically and Epigenetically Informed Investigation

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Abstract

Genetic, environmental, and epigenetic influences and their transactions were examined in a sample of 155 women from the Iowa Adoptee sample (IAS) who had been removed from their biological parents shortly after birth, and assessed when participants were an average of 41.10 years old. We observed an interactive effect of child sex abuse (CSA) and biological parent psychopathology (i.e., genetic load) on substance abuse as well as a main effect of CSA on substance abuse in adulthood. We also observed main effects of CSA and genetic load on depression and on antisocial characteristics. As predicted, CSA, but not genetic load or later substance abuse, was associated with epigenetic change. In addition, the interaction between genetic load and CSA predicted epigenetic change, indicating a potential genetic basis for differential impact of CSA on epigenetic change. Finally, epigenetic change partially mediated the effect of CSA on antisocial characteristics. The results suggest the relevance of genetic and epigenetic processes for future theorizing regarding marital and family precursors of several forms of adult psychopathology. Implications for preventive intervention are discussed.

Keywords

ASPD; child sex abuse; epigenetic; methylation; substance use

Child sex abuse (CSA) is an unfortunate, but frequent occurrence, with estimates of lifetime prevalence converging on 16.8% for women and 7.9% for men (Gorey & Leslie; 1997). On

average, a closer relationship to the abuser results in worse outcomes (Trickett, Noll, Reiffman, & Puttnam, 2001), leading to particularly negative outcomes for father-daughter abuse, which is often characterized by earlier onset and longer duration of abuse (Mennen & Meadow, 1995; Trickett, Reiffman, Horowitz, & Puttnam, 1997). Adverse outcomes for women exposed to CSA range across several domains including depression, personality disturbance, and substance abuse (e.g. Fergusson, Horwood, & Lynskey, 1996); and, even when other aspects of family disruption are controlled, sex abuse often continues to account for a substantial amount of variance in outcomes (e.g., Kendler, Bulik, Silberg, Hettema, Myers, & Prescott, 2000).

Kendler et al. (2000) found that the strongest effects of CSA were on later drug dependence and alcohol dependence, with substantial effects of CSA across a range of additional forms of adult psychopathology. Similarly, Dinwiddie et al. (2000) found a significant increase in odds of major depression and alcohol dependence among those who had been sexually abused. Nelson et al. (2002) found that among twins discordant for the experience of CSA, the twin with the sex abuse history was significantly more likely to have depression, conduct disorder, and alcohol and nicotine dependence, again suggesting that some effects are attributable specifically to CSA rather than to more general negative aspects of family environment.

Despite accumulating evidence linking CSA to increased liability for adult psychopathology, establishing a causal role for CSA in the development of adult psychopathology is challenging and biological mechanisms are not entirely clear. In particular, because retrospective reports are typically used to assess CSA, and because CSA typically co-occurs with other challenging family conditions and adverse experiences, conclusions in the area must typically be offered with methodological caveats. However, available data suggest that false positive reports of CSA are relatively uncommon among women (Hardt & Rutter, 2004), and that retrospective reports of CSA among women have good validity and predictive efficiency (Widom & Morris, 1997). In addition, when multiple potential reporters are available, retrospective reports of child sex abuse show strong convergence with external reporters (Nelson, Lynskey, Heath, Madden, & Martin, 2010).

Because of a combination of factors including relatively high frequency, good validity, and well established associations with biological changes such as long-term elevations in hypothalamic-pituitary-adrenal axis activity (Heim et al., 2000), retrospective reports of child sex abuse provide a useful starting point for the examination of potential biological pathways of influence linking adverse childhood experiences to negative outcomes in adulthood. In the current investigation we examine the initiation of "epigenetic change" in the form of DNA methylation as one such potential biological pathway.

Epigenetic Change Processes

Epigenesis is defined as the production of stable changes in DNA expression that do not result from change in DNA sequence (Jiang, Bressler, & Beaudet 2004). In humans, DNA methylation is the epigenetic mechanism that has received the greatest attention. DNA Methylation has the effect of reducing the expression of gene products associated with the methylated region of DNA, either by creating a physical barrier to transcription factor binding proteins, thus partially inhibiting gene transcription and reducing the production of mRNA, or by recruiting proteins that initiate "chromatin remodeling", ultimately generating a different shape for the methylated region of DNA that in some cases completely inhibits gene transcription. The relationship between promoter DNA methylation and changes in gene activity are well established (Murrell, Rakyar, & Beck, 2005); hyper-methylation in promoter associated CpG islands typically results in decreased transcription of downstream

genes whereas experimentally induced hypomethylation typically leads to an increase in gene transcription (e.g., Hansen & Gartler, 1990). It also has been shown that individual differences in DNA methylation are predictive of individual differences in gene expression (e.g., Bell et al., 2011; Plume et al., 2012), highlighting the value of DNA methylation as an individual difference variable.

Methylation of Gene Promoters

CpG pairs, which are the targets of DNA methylation, are not randomly distributed across the human genome. Concentrated regions of CpG pairs, known as "CpG islands," and are often found in close proximity to gene promoters, i.e., areas of the gene known to be particularly important in regulating level of gene expression. One promoter region that has been well examined is the *5HTT* promoter region. *5HTT* is a key regulator of serotonergic neurotransmission, localized to 17p13 and consisting of 14 exons and a single promoter. The promoter associated CpG island for *5HTT* is comprised of 799 base pairs and surrounds exon 1. In keeping with usual mechanisms of regulation of gene activity, methylation of the CpG island associated with the promoter region for *5HTT* is known to be associated with reduced transcription (e.g. Vijayendran, et al., 2012), making it an attractive target for epigenetic assessment.

Methylation Ratios

Measurement of gene methylation has progressed rapidly in the past decade. Currently, level of methylation in a given sample is determined by using a chemical process involving sodium bisulfate to change all unmethylated "cytosines" (i.e., the "C" in the CpG island) to uracil. This makes it straightforward to count the number of remaining "cytosines" and to compare that count to the total number of "cytosines plus uracils." After counting the number of uracils and cytosines, overall methylation is typically reported as the fraction of molecules in a sample that are methylated at a specific location. Thus, methylation values have a range from "0," indicating that none of the cells in the sample are methylated, (i.e. all cytosines were converted to uracil), to "100," indicating that all of the cells in the sample were methylated at the location of interest (i.e. no cytosines were converted to uracil).

Hypothesized Effect of Sex Abuse on Epigenetic Change

We hypothesized that CSA would have a measureable biological impact due to its effect on increased physiological reactivity (Heim & Nemeroff, 2001; Weiss, Longhurst, & Mazure, 1999). Specifically, we expected that CSA would lead to epigenetic change by inducing a substantial and sustained stress response that would be associated with epigenetic reprogramming. Changes in serotonergic activity due to methylation of the promoter region of *5HTT* would, in turn, be associated with increased vulnerability for psychopathology. Accordingly, we hypothesized 1) that CSA would predict adult psychopathology for women, replicating prior research (e.g., Nelson et al., 2002), 2) that CSA would predict methylation, replicating prior work in an independent sample (Beach et al., 2010a), and 3) that some of the impact of CSA on psychopathology would be correlated with, and perhaps mediated by, degree of epigenetic change.

Hypothesized Effect of Genetic Load

Prior research creates an expectation that not all individuals will be affected to the same degree by adverse childhood events, including CSA. Research with adoptees suggests that "genetic load," i.e., having a parent with major psychopathology, , will moderate environmental effects. For example, Cadoret, Yates, Troughton, Woodworth, and Stewart (1995) found a main effect association between greater genetic load and increased likelihood

of negative behavioral outcomes in adolescence and adulthood, as well as an interaction between genetic load and an adverse adoptive home environment. The interaction indicated that there was a negative effect of an adverse home environment for those youth who had greater genetic load, but not for those without genetic load (See also Cutrona et al., 1994). In keeping with this pattern we anticipated that genetic load, in the form of having a biological parent with major psychopathology, would increase the deleterious impact of CSA on adult outcomes. In addition, we examined the possibility that genetic load would increase the impact of CSA on methylation. Although we did not examine candidate genes in the current study, the predictions for genetic load were conceptually similar to those based on the genetic susceptibility and genetic sensitivity models, i.e. perspectives that highlight the potential moderating impact of specific susceptibility genes (Belsky & Pluess, 2009; Boyce & Ellis, 2005). These perspectives also lead to the expectation that, for genetic reasons, some individuals will be more strongly affected by adverse events occurring during development.

Model to be Tested

Previously, we reported that child sex abuse occurring in the family was associated with symptoms of Antisocial Personality Disorder (ASPD) among women (Beach et al., 2011). In the current investigation we elaborated this model by expanding our definition of sex abuse to include all incidents of sex abuse, including those that did not involve family members. We also expanded our examination to include measures of commonly studied adult outcomes of child sex abuse including depression (MD) and substance abuse. In addition, in the current study we examined genetic moderation of CSA effects using the aggregate genetic variable of "genetic load." The expanded model (see Figure 1) allowed us to contrast different sources of effects in the context of a genetically and epigenetically informed model.

We used an adoption design to maximize our power to detect aggregate genetic influences. The adoption design used in the current investigation also minimized passive gene-environment correlations (passive rGE), i.e., correlations of genetic load with family environment and adverse family events, providing additional support for the impact of sex abuse and associated adverse childhood events by ruling out potential genetic confounds and disentangling the impact of adverse childhood events from biological parent genetics.

Potentially Confounding Effect of Substance Abuse

A potential concern regarding the association of epigenetic change and outcomes is that the relationships between methylation and symptoms in women could be secondary to adult substance use patterns. That is, if methylation is influenced by later substance use disorders, then observed associations of CSA with methylation and other outcomes may be caused by later substance abuse rather than being a direct effect of CSA. Prior research has noted associations between epigenetic change at several loci and substance use (Philibert, Beach, Gunter, Brody, Madan, & Gerrard, 2010; Philibert, Gunter, Beach, Brody, & Madan, 2008), making this an important concern. Accordingly, examination of a model that includes substance use as a predictor of methylation provides an important additional, stringent test of the relationships hypothesized in Figure 1.

Specific Hypotheses

Hypothesized relationships are presented in Figure 1. We did not hypothesize a significant effect of genetic load on the occurrence of sex abuse because CSA tends to be driven by perpetrator access rather than victim characteristics.¹ In keeping with prior research on the stressful nature of CSA, we hypothesized an effect of sex abuse on methylation. However, because most methylation effects are erased during gametogenesis (Cedar & Bergman,

2012), we did not anticipate an effect of genetic load on methylation even though this possibility was examined. Accordingly, the relationship is indicated by a dotted line. As noted above, we anticipated that biological parent psychopathology would intensify the impact of CSA on methylation and intensify the effect of CSA on adult psychopathology outcomes. In keeping with our expectation that epigenetic change might be one pathway from sex abuse to psychopathology, we hypothesized a significant association of methylation with adult psychopathology outcomes in addition to significant effects of genetic load on adult outcomes. Finally, we hypothesized that links between sex abuse and methylation would remain significant after controlling for later substance abuse (these associations are not shown in model and were tested separately).²

Methods

Participants

The Iowa adoptees and their adoptive parents were recruited from Iowa adoption agencies when the adoptees were in young adulthood starting in 1975. Adoptees were determined to be at genetic risk after review of records for both biological parents to identify biological parent psychopathology (either alcoholism, depression, or antisocial behavior). Genetic load was considered positive if either biological parent had a history of substance use, antisocial behavior, or depression based on agency, prison, hospital, or institutional records. A record search was conducted for all biological parents, which involved matching biological parents' names to those of patients seen at major public psychiatric treatment centers in the state of Iowa, and to names of inmates in the State of Iowa Correctional system. Available hospital records were reviewed by independent psychiatrists who provided diagnoses, resulting in kappas of .7 to .9 for diagnoses of alcohol abuse/dependence and ASPD. By design, approximately half of biological parents were considered positive for genetic load. Control adoptees without genetic load were matched to those positive for genetic load on the basis of age, sex, and age of mother at birth. Participants were largely White (94%) and had a mean age of 41.10 (SD = 7.74) at the time of their primary diagnostic interview (1999–2004), and had a mean of 14.26 years (SD = 1.99) of education. Initial sample recruitment targeted a similar number of males and females. However, CSA was reported 10 time more frequently by female than male participants in the broader sample (Beach et al., 2010b), making it impractical to study effects of CSA among males or to compare effects across gender. It is not known whether this reflects a true difference in prevalence in this sample, under reporting by males in the sample, or attrition from the male portion of the sample. Accordingly, only the female portion of the sample (N = 155) was used in the current investigation. The recruitment and assessment procedures used in this study have been described in detail elsewhere (Philibert et al., 2007; Yates et al., 1998), and all procedures were approved by the University of Iowa Institutional Review Board.

Measures

All clinical data in the current report, including CSA experiences (0 to maximum of 3), and symptom counts for MD (0 to maximum score of 9), alcohol dependence (AD; 0 to maximum score of 7), nicotine dependence (ND; 0 to maximum score of 7), cannabis dependence (CD; 0 to maximum score of 7) and ASPD (0 to maximum score of 7), were derived from structured interviews using the Structured Assessment for Genetic Studies of Alcoholism (SSAGA; Bucholz et al., 1994). The SSAGA is a polydiagnostic instrument that

¹There is, however, evidence of child effects on occurrence of physical abuse suggesting that children with characteristics that make them harder to care for have increased risk of physical abuse, emotional maltreatment, or neglect (e.g., Ammerman & Patz, 1996).

²We also examined the impact of adoptive parent problems, including psychopathology in the adoptive mother or father. Although there were significant main effects on outcomes, this did not affect the pattern of significant effects for any of the relationships examined in Figure 1

has been shown to have acceptable kappas for life-time diagnoses, with kappas of .65 for major depression, .70 for ASPD, and > .70 for all substance dependence diagnoses (See Bucholz et al., 1994 for additional detail).

Symptom count scores for "substance dependence" were created by taking the harmonic mean of lifetime maximum symptoms of alcohol, marijuana, and nicotine dependence scores obtained at the primary interview (1999–2004). Because the complete depression interview was given to all participants only at their primary interview, depression symptom counts from the first interview were used to calculate depression scores.³ For ASPD, the complete set of questions was asked at a second interview (2004–2008), so, the average of the two interviews was used to increase reliability of assessment.⁴ To assess child sex abuse, participants were asked about physical sexual contact (touching other/other touching them or attempted/completed intercourse) that occurred prior to age 16 and involved family or non-family members. If absent for family and non-family sources, respondents received a score of "0;" if present in the family they received a score of "1;" if the incident involved a parent or grandparent they received a score of "2." If abuse also was perpetrated by a non-family member more than five years older than them prior to their 16th birthday, their score was incremented by "1."⁵ The scoring strategy was designed to reflect the more negative effect of sex abuse when the relationship to the perpetrator is closer.

Biological Measures—Genetic and epigenetic measurements were conducted using blood contributed by each of the subjects during the last wave (2004–2008) of the study, lymphoblast cell lines were prepared from lymphocytes using standard EBV transformation techniques (Caputo, Thompson, McClintock, Reid, & Hay, 1991). The methylation status of 71 of the 81 CpG residues assayed in the 799 bp CpG island surrounding exon 1 of *5HTT*, a gene localized to 17p13, was determined by Sequenom (San Diego, USA; Philibert, Sandhu, Hollenbeck, Gunter, Adams, & Madan, 2008). Coded aliquots of DNA were bisulfite treated under basic conditions to convert unmethylated cytosines to uracils (Thomassin, Oakeley, & Grange, 1999). The region of DNA corresponding to the CpG island was then PCR amplified using two separate amplicons, using primers specific for bisulfite converted DNA and touchdown PCR procedures (Philibert et al., 2008). For each locus, methylation values potentially ranged across individuals from "0," indicating no methylation, to "1," indicating complete methylation. Methylation level was then averaged across all loci assessed within the promoter region.

Plan of Data Analysis

Simple bivariate relationships were examined using Pearson's *r*. Model fitting was conducted using Mplus V.6 (Muthén & Muthén, 1998–2010) with manifest indicators, simultaneous estimation of all paths, and maximum likelihood fit function. In the baseline model, all effects were estimated freely, providing estimates of pathways in a saturated model with no degrees of freedom. We then constrained non-significant interaction effects, yielding a model with 2 degrees of freedom, allowing significance testing for overall fit of the model as well as significance testing for the individual pathways. Subsequently we used a Monte Carlo simulation based on the observed covariance structure to examine power to detect a significant overall model and specific hypothesized pathways. Finally, we tested separately the possibility of spurious associations due to substance use by examining a model in which substance use was included as a predictor of methylation effects. To assess

³Analyses were also conducted using maximum symptom counts for depression. The overall pattern of effects was unchanged.

⁴Analyses were also conducted using time 1 ASPD symptoms only, yielding a similar pattern of effects. Likewise, recoding CSA to 0 vs. 1 yielded minimal change.

⁵Eleven women indicated sexual contact with a non-family member who was 5 or more years older prior to the age of 16, but no sex abuse in the family. When analyses were restricted to sex abuse by a family member, the pattern of significant results was unchanged.

robustness of the results we also examined the impact of varying the operationalization of depressive symptoms and symptoms of ASPD.

Results

Sample Descriptive Statistics and Correlations

Means, standard deviations and intercorrelations of key study variables are presented in Table 1. Of the women in the current sample, 16.8% ($n = 26$) reported child sex abuse. Mean symptoms were as follows: ASPD = .92, median = .5, $sd = 1.14$; MD = 3.42, median = 3, $sd = 3.38$; Substance Abuse = .21, $sd = .82$. For the Z-transformed methylation ratios, the interquartile (IQR) range was $-.73$ to $.51$, with a median score of -0.145 . Skewness was minimal, skewness = 1.086. There was a positive association between child sex abuse and symptoms of ASPD ($r = .315$; $p < .001$). Child sex abuse was also associated with depression ($r = .299$, $p < .001$), and substance use ($r = .340$, $p = .001$). Significant positive relationships emerged between reported child sex abuse and level of methylation ($r = .318$; $p < .001$), between methylation and symptoms of ASPD ($r = .271$; $p = .001$), and between methylation and MD ($r = .165$; $p = .04$). A significant positive relationship emerged between genetic load (i.e., presence of a diagnosis in the biological parent) and ASPD symptoms ($r = .171$; $p = .034$), as well as genetic load and MD ($r = .204$; $p = .011$), but there was no significant association of genetic load with substance use or methylation. As predicted, there was no significant association of genetic load with CSA. There were significant zero-order associations between ASPD and all other outcomes and predictors in the model. No additional zero-order relationships were significant. All zero-order correlations can be found in Table 1.

Model Testing

Following the plan of analysis outlined above, we examined hypothesized main effects and interaction effects relationships using MPLUS in a fully saturated model with no degrees of freedom. We then reran the model constraining the two non-significant interaction effects, yielding a model with 2 df which is described below.⁶ Standardized pathways are indicated in Figure 2. The constrained model provided adequate fit to the data $\chi^2(2) = 1.163$, $df = 2$, $p = .559$. RMSEA = .000 and CFI = 1.000. Exact, two-tailed, p -values for significant effects are reported unless the p -value is $< .001$. With all other relationships in the model controlled, there was a strong association of CSA with methylation ($\beta = .321$; $p < .001$), as well as, a significant interaction of biological parent diagnosis and CSA ($\beta = .163$, $p = .027$) in the prediction of methylation. The effect of genetic load on methylation was non-significant.

For symptoms of ASPD, there was an effect of biological parent's diagnosis ($\beta = .161$; $p = .028$) as well as an effect of methylation on ASPD ($\beta = .175$; $p = .023$). There was also a significant effect of CSA on ASPD ($\beta = .262$; $p = .001$). The interaction of biological parent psychopathology with CSA was non-significant. For symptoms of MD, there was an effect of CSA ($\beta = .284$; $p < .001$) as well as an effect of biological parent's diagnosis ($\beta = .205$; $p = .005$). All other effects were non-significant (all p 's $> .2$). For symptoms of substance use there was a significant effect of CSA ($\beta = .333$; $p < .001$), and a significant interaction of biological parent psychopathology with CSA ($\beta = .144$; $p = .041$).

Total variance accounted for by the model for each outcome were: for MD, $R^2 = .157$; for substance abuse $R^2 = .136$; for ASPD, $R^2 = .146$. The indirect pathway from CSA to ASPD

⁶Because of the relatively small number of women reporting sex abuse, we conducted power analyses to examine our ability to detect relationships between sex abuse and other variables in our model within a multivariate framework. Power to detect significance of the overall model was $> .90$. Power to detect individual significant pathways ranged from .94 to .99.

through methylation was significant $p = .047$, indicating that methylation is a significant partial mediator of the main effect of CSA on ASPD.

Interaction of CSA with Genetic Load

The two significant interaction effects portrayed in Figure 2 were plotted to examine the pattern of effects. In both cases the effect was in the hypothesized direction, i.e., there was greater impact of CSA for those with greater genetic load. The simple main effect of CSA on methylation for those with greater genetic load was strong ($\beta = .944, p < .001$), whereas for those with no genetic load the effect was marginal ($\beta = .331, p = .077$). Similarly, the simple main effect of CSA on symptoms of substance dependence for those with greater genetic load was strong ($\beta = .789, p < .001$) whereas for those with no genetic load the effect was marginally significant in the same direction ($\beta = .297, p = .055$).

Tests of Spuriousness and Generalizability

To examine potential spuriousness in the associations of CSA with methylation that might be introduced by the association of CSA with both methylation and with later substance use, we examined an alternative model in which substance use was a predictor rather than an outcome. This model resulted in similar estimates of standardized effects on methylation, with highly significant associations between CSA and methylation ($\beta = .311, p < .001$), and a significant interaction of biological parent psychopathology and CSA ($\beta = .159, p = .034$), but no significant main effect of substance use on methylation ($\beta = .027, p = .736$). Accordingly, the conceptual model does not appear to be vulnerable to the potential criticism that later substance use inflates the association of CSA with methylation.

Discussion

The current investigation supports and extends prior research on the effect of sex abuse on a range of outcomes by indicating that the impact of CSA on adult psychopathology of women persists even after controlling for genetic risk in the form of a biological parent with major psychopathology (cf. Kendler et al., 2000; Dinwiddie et al., 2000; and Nelson et al., 2002). CSA predicted symptoms of antisocial personality, depression, and substance use. In addition, prior work suggesting the enhanced susceptibility of those with greater genetic load was also replicated, with greater effects of CSA on methylation and on substance abuse for those who had a genetic vulnerability. Conversely, there was no evidence that genetic load correlated with the occurrence of sex abuse, providing no evidence that the observed associations of CSA with adult psychopathology are attributable to gene-environment correlations (rGE). Likewise, separate analyses controlling for later symptoms of substance dependence did not reduce the effect of CSA on methylation, further reducing concerns about spuriousness of the association. Finally, the pattern of results suggest that there is more than one pathway by which CSA exerts its effects across adult outcomes, with some significant effects on outcomes being mediated through epigenetic changes (i.e. symptoms of ASPD), but other effects occurring independently of change in methylation at 5HTT (i.e., symptoms of depression and symptoms of substance abuse).

The link between methylation and CSA provides some corroboration for the view that reports of CSA often reflect a highly stressful experience that may set the stage for biological changes, and ultimately for later psychopathology. Because measurement of methylation is independent of self-report and is not subject to potential biases introduced by retrospective reports, the presence of a significant indirect pathway from CSA to methylation to symptoms of antisocial personality, even after controlling for the effect of genetic load, suggests a relationship between CSA and later outcomes that goes beyond self-report bias. Further, in supplementary analyses, this relationship also survived controls for

the influence of other aspects of the adoptive family (see footnote 2) and the introduction of substance use as a control variable.

However, limitations of the current design preclude viewing methylation of the CpG island of *5HTT* as uniquely indicative of CSA. Because CSA is often associated with other adverse experiences during development and young adulthood, it is difficult to untangle effects of CSA from all other possible co-occurring adverse childhood experiences, and we cannot claim to have done so in the current investigation. In particular, because CSA occurs more frequently in children from socially deprived and disorganized family backgrounds (e.g., Mullen et al. 1993) it is likely that girls experiencing CSA also experienced other markers of greater family disruption and deprivation. In addition, because of overlap between physical, emotional and sexual abuse, it is likely that girls who experienced CSA were more likely to have experienced other forms of abuse as well (e.g., Fergusson, Horwood, & Lynskey, 1996). Indeed, Mullen et al. (1996) reported that women with CSA reported five times the rate of physical abuse and three times the rate of emotional deprivation as others without CSA. The current analyses also did not examine variation in experience of CSA as a predictor of outcomes because the sample size was too small to allow reliable estimation of parameters related to frequency, intensity, and response. Similarly, the design of the study does not allow for standardized characterization of life stress across adult development or adult victimization experiences. Accordingly, we do not know if adult sexual trauma or other highly stressful circumstances in adulthood would produce a similar or different pattern of effects, or if they would interact with epigenetic change in predicting adult psychopathology.

Finally, it is likely that epigenetic change in one area is correlated with changes in other areas of the genome as well. This suggests caution in attributing the effect of sex abuse on outcomes to methylation in any one region of the genome. Instead, it will be important to examine the broader patterning of methylation effects using larger samples and genomewide methodologies.

Because no rGE was observed for sex abuse, helping to rule out active or evocative rGEs as an explanation for the association of sex abuse with adult outcomes, the current results strengthen the argument that the effect of sex abuse on psychopathology outcomes is not spurious or entirely attributable to a shared genetic diatheses with adult psychopathology. In addition to strengthening the premise that CSA may be important in understanding the causal chain leading to adult outcomes, this pattern also sets the stage for examination of interactions between CSA and genetic load. Accordingly, we were able to characterize the role of genetic load in moderating the impact of CSA on adult outcomes. Because this was not an investigation of candidate genes, however, the current results do not indicate which genes account for the increased sensitivity of the "at risk" group. Nonetheless, the pattern of results supports continued examination of gene by family environment interaction effects at the molecular level (cf. van IJzendoorn et al., 2011).

Likewise, the significant impact of genetic load on outcomes suggests that aggregate genetic influence is an important part of any conceptual model for developmental impacts on symptoms of MD and ASPD. Interestingly, for substance use outcomes, genetic load exercised its influence primarily by increasing the negative impact of CSA rather than exerting a main effect on substance use outcomes, suggesting that the processes linking genetic vulnerability to substance use outcomes are somewhat different than those linking it to depression and symptoms of ASPD.

Other Limitations

The current investigation is limited in several additional ways. First, the current study is limited by its reliance on retrospective reports of sex abuse, a common concern in this area (Hardt & Rutter, 2004), as well as reliance on a single reporter for all major variables other than methylation and genetic load. In particular, it is possible that sex abuse was under reported, potentially weakening observed relationships in the data. At a minimum, under reporting may provide a partial explanation for our inability to examine the impact of CSA among male participants. Second, the assessment of genetic load was based on a review of records rather than direct assessment of biological parents and so there may have been undetected psychopathology in the "low genetic load" group which could have led to underestimates of the main and interaction effects of genetic load on outcomes, and perhaps underestimates of rGE effects as well. Another limitation of the current methodology is that, like most work with humans, our assessment of methylation relied on peripheral measures (lymphocytes) rather than central measures of methylation based on methylation of genes in neuronal tissue. However, the use of peripheral measures is rendered more plausible in the current investigation because *5HTT* is expressed in both lymphocytes and in key systems within the central nervous system, and work by Rollins, Martin, Morgan, and Vawter (2010) has shown that peripheral measures of gene expression predicts central expression for genes expressed similarly in each cell type. Finally, the current study reports associations only for females, a circumstance forced on us by the small number of males in the IAS who reported experiencing child sex abuse. Consequently, the current sample was small, and so the results require replication in larger samples or samples with larger numbers of abused individuals. In the future, measures of genome-wide methylation may help elaborate the effect of child sex abuse and better characterize gender differences.

Despite the limitations of the data, the current results provide conceptual support for family targeted interventions to prevent CSA as a way of reducing propensity for adult psychopathology. In particular, the absence of observed rGE effects in combination with the presence of direct effects of CSA on outcomes as well as indirect effects mediated through methylation provides support for the potential value of family interventions to prevent adult psychopathology by preventing CSA. Further, interaction effects with genetic load suggest that such prevention effects are particularly important in the context of genetic vulnerability. It is possible that some existing family based programs may prevent sex abuse, but this remains an area in need of further research (Putnum, 2003).

Broadly, the current investigation suggests the need for greater exploration of biological, epigenetic, and genetic pathways to complement and expand current models of effects of CSA as well as other adverse childhood events and experiences, potentially leading to new points of preventive intervention. The current results also illustrate the potential for a particular adverse event, and the various stressors correlated with that experience, to influence a range of negative outcomes, suggesting that some childhood stressors, such as sex abuse, may have effects across a range of outcomes, with the ultimate impact for a given individual depending in part on that person's vulnerabilities and strengths. Accordingly, the current investigation suggests the value of broadening the range of symptomatic outcomes examined in the context of adverse childhood experiences, giving increased attention to the broad range of negative outcomes that may be prevented by successful family based intervention and highlighting the importance of preventive efforts for those who are most susceptible.

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Figure 1. Conceptual Model Showing Pathways from Sex Abuse (CSA) and Biological Parent Psychopathology (Genetic Load) to Adult Outcomes (ASPD, Depression, Substance Use), including a Potential Epigenetic Pathway and the Potential moderating Role of Genetic Load. Hypothesized Significant Effects are Indicated by Solid Lines. Hypothesized Non-significant Effects are Indicated by Dotted Lines.

Figure 2.

Final Model Showing Pathways from Childhood Sex Abuse and Parent Diagnosis to Symptoms of Depression, ASPD, and Substance Dependence. Standardized Path Coefficients are shown in the model. $\chi^2=1.163$, $df=2$, $p=.559$. RMSEA=.000 and CFI=1.000.

$^{**}p < .01$; $^*p < .05$, $^{\dagger}p < .10$ (two-tailed tests), $n=155$

Table 1

Correlations Between Major Study Variables with Means and SDs

	1	2	3	4	5	6	7
<u>Measures</u>							
1. Sex Abuse	--	.314	.318	-.017	.340	.299	.061
2. ASPD Symptoms	--	--	.271	.171	.433	.274	-.147
3. Methylation	--	--	--	.079	.165	.165	.104
4. Bio Parent Diagnosis	--	--	--	--	.081	.204	-.056
5. Substance Abuse	--	--	--	--	--	.142	.010
6. Depression	--	--	--	--	--	--	-.013
7. Age	--	--	--	--	--	--	--
Mean	.22	.92	.02	.43	.21	3.42	41.10
SD	.54	1.14	1.00	.50	.82	3.38	7.74

Note. Pearson correlations for total sample (N= 155). Correlations in the table below *r*= .159 are not significant (and are in italics).