Published in final edited form as:
Psychosom Med. 2011 January ; 73(1): 83–87. doi:10.1097/PSY.0b013e3181fdd074.

Methylation at *5HTT* Mediates the Impact of Child Sex Abuse on Women's Antisocial Behavior: An Examination of the Iowa Adoptee Sample

Steven R. H. Beach, Ph.D.[1,*], **Gene H. Brody, Ph.D.**[1], **Alexandre A. Todorov, Ph.D.**[2], **Tracy D. Gunter, MD**[3], and **Robert A. Philibert, MD, Ph.D.**[4]

[1]University of Georgia

[2]Washington University, St. Louis

[3]Department of Neurology & Psychiatry, St Louis University School of Medicine

[4]Department of Psychiatry, Neuroscience and Genetics Programs, The University of Iowa

Abstract

Objective—To examine epigenetic processes linking childhood sex abuse to symptoms of *Antisocial Personality Disoder* (ASPD) in adulthood, the authors investigated the possibility that the link between childhood sex abuse and DNA methylation at the *5HTT* promoter might represent a pathway of long-term impact on symptoms of ASPD.

Method—DNA was prepared from lymphoblast cell lines derived from 155 female participants in the latest wave of the Iowa Adoptee Study. Methylation at 71 CpG residues was determined by quantitative mass spectroscopy and the resulting values were averaged to produce an average CpG ratio for each participant. Simple associations and path analyses within an Mplus framework were examined to characterize the relationships among childhood sex abuse, overall level of methylation among women, and subsequent antisocial behavior in adulthood. Direct effects of biological parent psychopathology and *5HTT* genotype were controlled.

Results—Replicating prior work, a significant effect of childhood sex abuse on methylation of the *5HTT* promoter region emerged for women. In addition, a significant effect of methylation at *5HTT* on symptoms of ASPD emerged.

Conclusions—Child sex abuse may create long-lasting changes in methylation of the promoter region of *5HTT* in women. Further, hypermethylation may be one mechanism linking childhood sex abuse to changes in risk for adult antisocial behavior in women. Better understanding of the methylome may prove critical in understanding the role of childhood environments on long-term psychiatric sequelae.

Keywords

ASPD; child sex abuse; epigenetic; methylation

[*]To whom correspondence should be addressed. Steven R. H. Beach, Ph.D., 510 Boyd GSRC, University of Georgia, Athens, GA. 30602 TEL 706-542-1806, FAX 706-542-6064, srhbeach@uga.edu.

Introduction

Complex behavioral patterns such as Antisocial Personality Disorder (*ASPD*) result from a combination of genetic and environmental factors, with a probable role for environmental experiences such as child maltreatment (1). However, the biological processes that mediate the long-term impact of child maltreatment on adult psychopathology are poorly understood. Epigenetic modifications in the form of DNA methylation status provide one potential pathway of interest. Alterations in methylation, in the form of changes in CpG motifs, may remain stable over a relatively long time in humans (2), making individual differences in methylation a potential biological marker of environmental contributions to the phenotypic divergence of those with similar genetic endowments (3). Because CpG motifs are potentially modifiable by environmental factors, they provide a plausible physical substrate by which environmental events may have lasting effects on behavior.

Supporting the potential impact of childhood experiences on epigenetic change, methylation differences have been demonstrated in the postmortem hippocampi obtained from suicide victims with a history of childhood abuse relative to those from either suicide victims with no childhood abuse or non-suicide controls (4,5). Likewise, in rodent models, early experience vis-à-vis the mother has been linked to lasting epigenetic change via CpG methylation (6,7).

In earlier work, we found that child abuse, including physical abuse, harsh parenting, and sex abuse, was associated with overall hypermethylation of the *5HTT* promoter region (8). A significant association also emerged in that sample between sex abuse alone and overall methylation of the CpG island at *5HTT* among females, $r(82) = .360$, $p < .001$. Other facets of child maltreatment did not account for additional variance beyond the effect of sex abuse on methylation at this locus for women. Reports of sex abuse among males were too few to allow for separate analyses.

In the current investigation we focus only on women in a non-overlapping sample of 155 female participants in the latest wave of the Iowa Adoption Studies (IAS). Extending the prior report, we also examine symptomatic outcomes for adult ASPD and the potential role of methylation in modifying the impact of genetic risk alleles. We simultaneously control for the potential impact of parental diagnostic status on both symptoms and level of methylation to rule out a range of potential third-variable explanations for observed associations.

The biological model guiding the analyses is based on experimental and naturalistic observations with primates, which show that a stressful environment during development can alter CNS serotonin system functioning to produce long-term effects on behavior (9). These effects include heightened aggressiveness, impaired impulse control (10), and problematic social behavior (11). Similar effects involving decreased responsiveness of the serotonin system have been observed in humans (12,13). We propose that methylation of the promoter region of 5HTT may contribute to diminished serotonergic system responsiveness in response to child sex abuse by decreasing expression of the serotonin transporter, thus creating potential for impulsive or aggressive behavior that ultimately may be labeled antisocial. Because carrying one or more copies of the s allele is associated with diminished expression of the transporter, we hypothesized that carriers of this allele might be particularly likely to show behavioral effects in response to down-regulation resulting from promoter methylation.

Methods

The overall methodologies for the IAS studies have been extensively described elsewhere (14,15), with all study procedures and protocols having been approved by the University of Iowa Institutional Review Board. The current study includes clinical data and biomaterial from 155 women randomly selected from those participating in the last round of the study (2004-current). In the wave of data collected in 1999–2004, when subjects were adults, they were asked about a range of symptoms as well as about childhood events. To assess childhood sex abuse, participants were asked, “Before you were age 16 years old, were there any sexual contacts between you and any family members, like a parent or step-parent, grandparent, uncle, aunt, brother, sister, or cousin? By sexual contact I mean their touching your sexual parts, your touching their sexual parts, or sexual intercourse.” They were also asked specifically “Was there sexual contact with a parent or grandparent?” These questions were combined to form the sex abuse scale, which yielded possible scores of 0, 1, and 2, with the highest score indicating involvement of a parent or grandparent in the abuse.

Methylation ratios for each of the 71 CpG residues in the *5HTT* promoter island were determined as previously described (16). Briefly, DNA was obtained from growth phase entrained EBV transformed lymphoblast cell lines, and the resulting DNA was bisulfite treated to convert unmethylated cytosine residues into thymidine (17). Then, methylation ratios for each of the CpG residues were determined using quantitative mass spectroscopy by Sequenom, Inc. (San Diego, CA).

Prior work indicates that a single factor captures the reliable variation in CpG methylation at *5HTT* (8). Accordingly, we used the average of all residue values as our index of overall methylation in the promoter region. Symptoms of ASPD were assessed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA-II). The SSAGA is a polydiagnostic instrument that assesses symptoms of antisocial personality disorder, among others, in a manner consistent with DSM-III-R, DSM-IV, and Feighner RDC (Research Diagnostic Criteria).

Data Analysis

Simple bivariate relationships were examined using Pearson's *r* as well as Spearman's rho. Because no differences in patterns of significant association were observed, only Pearson's *r is reported*. Model fitting was conducted using Mplus version 6 (18) with manifest indicators, simultaneous estimation of all paths, and maximum likelihood fit function. Mplus was chosen because it can incorporate variables with different levels of measurement and the impact of measurement assumptions can be examined by the use of alternative estimation strategies. In the baseline model, the direct effect of number of s alleles on outcomes was constrained to be 0, in keeping with observed simple correlations. We freely estimated all other paths in the hypothesized model (Figure 1). We then examined the fit of the hypothesized model using the overall chi-square test in each case and used change in chi-square ($\Delta\chi^2$) between nested models to guide the removal of non-significant paths that could be constrained to be zero without producing significant deterioration in model fit. This process yielded a final main-effects model with a non-significant χ^2 (i.e., a good fit; see Figure 2).

To test directly the hypothesis that methylation mediates the effect of child sex abuse on symptoms of ASPD for women, we constrained the direct path from sex abuse to symptoms of ASPD to be 0, leaving only the indirect pathway through methylation. We examined deterioration of overall model-data fit in terms of $\Delta\chi^2$, resulting in a fully mediated model (Figure 3). Finally, to provide a preliminary test of the expectation that methylation would potentiate the impact of the *s* allele, we added the interaction of genotype and methylation to

the final main effects model (Figure 4). Specifically, we centered the variables and then created a product term. This model is not nested because it introduces a new variable and directly tests the hypothesis that, by further down-regulating *5HTT*, increased methylation has greater impact on those with low activity alleles. To explicate the direction of the interaction, we examined simple correlations between methylation and ASPD symptoms within each level of genotype.

Results

A total of 155 female participants provided biomaterial for the study. Means, standard deviations and intercorrelations of key study variables are presented in Table 1. Of the women in the current sample, 13% (*n* = 15) reported one or more indications of child sex abuse. Average symptoms of ASPD ranged from 0 to 5, with a mean of .92 and a median score of .5. Z-transformed methylation ratios ranged from −1.66 to 4.07 with a median score of −0.127. Of the sample, 16% (n = 25) were homozygous for the *s* allele, 46% (*n* = 71) were heterozygous, and 38% (*n* = 59) were homozygous for the *l* allele. No significant differences in bivariate correlations emerged between participants homozygous for the long allele and the rest of the sample. Hardy-Weinberg equilibrium was computed as χ^2 (1) = .22, ns. Due to the inability of the mass spectroscopy technique to differentiate peaks, some methylation scores were reported as averaged aggregates. In computing mean methylation scores, these aggregates were weighted to reflect the number of peaks included in each score.

Parameter estimates

The baseline model had three degrees of freedom (Figure 2). In keeping with the directional hypotheses for all parameters, exact, one-tailed *p*-values are reported. As predicted, with all other relationships in the model controlled, a *small* positive association emerged between child sex abuse and symptoms of ASPD (*p* = .034). Significant positive relationships also emerged between reported child sex abuse and level of methylation at *5HTT* (*p* = .0001) and between methylation at *5HTT* and symptoms of ASPD (*p* = .001). Finally, a *small* significant positive relationship emerged between parental diagnosis and ASPD symptoms (*p* = .022). Thus, all hypothesized main effect relationships were in the direction predicted. No other relationships in the model were significant. Fit of the hypothesized baseline model was good, $\chi^2(3) = 1.104$, ns.

Test of mediation

To examine directly the possibility that the effect of child sex abuse on methylation mediated its relationship with symptoms of ASPD (*i.e., partially or fully accounted for the relationship*), we constrained the direct path from child sex abuse to symptoms of ASPD to be 0 (Figure 3). Using nested model comparisons based on chi-square difference tests (19), we determined change in chi-square. Because $\Delta\chi^2$ for nested chi-square models is itself distributed as chi-square, the resulting value was a χ^2 with 1 degree of freedom. The imposition of the constraint on the direct pathway yielded a non-significant result, $\Delta\chi^2(1) = 3.27$, ns; this indicates that the direct pathway is not needed to obtain a good fit to the data and supports the hypothesis that the direct effect of child sex abuse on symptoms of ASPD is mediated through methylation of *5HTT*. Standardized structural path coefficients for the final model are presented in Figure 3. Constraining the direct path in the model from child abuse to *ASPD* symptoms to be 0 had little effect on other structural relationships. The final model accounted for 9.0% of the variance in symptoms of ASPD.

Exploratory test of moderation

If both genotype and methylation exert regulatory control over gene expression, methylation can be expected to interact with 5HTT genotype to potentiate the effect of number of risk alleles. Variables that affect the strength of the relationship between a predictor and a dependent variable are called "moderators." So, we considered the possibility that genotype might "moderate" the impact of methylation on behavior. We examined this possibility in the current data set by centering the variables and then creating a product term that captured the combination of genotype and level of methylation. The interaction term was added to the final main effects model in Figure 3, creating a new, non-nested model that could not be compared directly to the main effects model. The resulting model, with standardized path coefficients, is presented in Figure 4. Introduction of the interaction term yielded a *small* significant pathway from the interaction term to ASPD symptoms ($p = .015$), but had little impact on other structural coefficients in the model. The resulting model increased total variance accounted for in symptoms of ASPD to 11.6%. Constraining the path from the interaction term to symptoms of ASPD to be 0 yielded a significant result, $\Delta\chi^2(1) = 4.55$, $p < .05$, indicating that the pathway from the interaction term to ASPD cannot be excluded from the model, supporting the hypothesis that methylation of the promoter region at *5HTT* changes the impact of the risk allele on symptomatic outcomes. The direction and magnitude of the association of methylation with symptoms of ASPD was examined within genotype. Correlations between methylation and symptoms of ASPD were $r(25) = .573$, $p = .001$, for those homozygous for the *s* allele; $r(71) = .269$, $p = .012$, for the heterozygous; and $r(59) = .143$, ns, for those homozygous for the *l* allele. In all cases, greater methylation was positively associated with symptoms of ASPD; this association, however, was not significant for those homozygous for the long allele.

Discussion

Replicating prior research (8), we found that the degree of methylation of the CpG island upstream from *SLC6A4* was associated with reported sex abuse during childhood among women. In addition, degree of methylation mediated the impact of childhood sex abuse on symptoms of ASPD reported in adulthood, even when the contribution of parental diagnosis was included in the model. In addition to its direct effect, methylation also potentiated the effect of *5HTT* risk alleles. Methylation was more strongly associated with symptoms of ASPD for those with a greater number of *s* alleles. Due to the small sample size, however, the finding that methylation interacted with genotype to predict symptoms of ASPD must be considered preliminary pending replication. Several other limitations of the study should be acknowledged. First, biomaterial was taken from lymphoblasts and not from the central nervous system. Consistency of DNA methylation, however, is surprisingly strong across many somatic tissues, including brain and lymphocytes. For example, in an examination that included lymphocytes, Fan and Zhang (20) demonstrated an inter-tissue correlation of 0.95, suggesting substantial validity for peripheral measurement of DNA methylation. Second, sex abuse was measured via retrospective reports. Third, we examined only one promoter within the serotonergic system and only one of several potential pathways contributing to ASPD symptoms. Fourth, we did not examine the "tri-allelic" characterization of 5HTT, potentially underestimating the gene's direct or interactive effects. Fifth, the sample is relatively small, highlighting the importance of future replication.

Overall, our results complement and expand prior work on the impact of childhood sex abuse on methylation (4,7). In particular, because women in the Iowa Adoptee sample were not biologically related to their abusers even though the abusers were family members, the patterns observed in the current study are not subject to the critique that observed associations could be secondary to passive gene-environment correlations or passive epigenetic-environment correlations. Further, the inclusion of diagnostic status of the

biological parent in the model controls any potential contribution to methylation conferred "in utero" through prenatal exposures secondary to parental ASPD as well as any other third variables associated with diagnosis of biological parent. It is noteworthy, however, that no relationship emerged between diagnosis of the biological parent and average methylation, suggesting that parent diagnosis did not exert an effect on symptoms through either direct or indirect effects on methylation at *5HTT*.

Sex abuse in the family commonly co-occurs with other forms of child maltreatment and can extend over many years, creating the potential for sustained high levels of stress while depriving the child of a sense of security in the home environment. The observed effect of sex abuse on methylation at 5HTT may reflect this combination of sustained stress and loss of security, as well as the impact of sustained stress arising from a variety of concurrent stressors. Unfortunately, the current sample is not large enough and our assessments not specific enough to examine the impact of severity of abuse in greater detail, and so we cannot draw conclusions about the relative impacts of sustained or repeated abuse compared to less chronic abuse, nor can we contrast the impact of early sexual contacts relative to those that occur later in childhood or adolescence.

The observed direct effects of sex abuse on methylation and of methylation on ASPD symptoms, as well as the significant interaction of methylation with genotype, suggest that epigenetic change in the form of altered methylation of key regulatory motifs may be one biological mechanism through which early adverse environments confer increased risk for psychopathology. The current results, although preliminary, suggest that stressors in the rearing environment interact with genetic vulnerability to exert long-term symptomatic consequences (21) to the extent that they produce changes in methylation at key promoter regions, thereby disrupting or modifying biological functions that might otherwise have developed normally. In this way, child sex abuse could have a biological impact that confers long-term risk for symptoms of ASPD; which, in turn, could potentially increase risk for other forms of psychopathology.

Acknowledgments

This study was supported by a grant to Dr. Philibert (DA015789) as well as support from the Center for Contextual Genetics and Prevention Studies (1P30DA027827) funded by the National Institute on Drug Abuse. On behalf of Dr. Philibert, the University of Iowa has filed intellectual property claims for certain uses of the methylation status of serotonin transporter promoter. We acknowledge the continuing intellectual contribution of Dr. Remi Cadoret. The content of this report is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute on Drug Abuse or the National Institutes of Health.

List of abbreviations

5HTT	serotonin transporter gene
CpG	a site at which cytosine (C) lies next to guanine (G) in the DNA sequence
ASPD	Antisocial Personality Disorder

References

1. Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Archives of General Psychiatry 2003;60:929–37. [PubMed: 12963675]
2. Eckhardt F, Lewin J, Cortese R, Rakyan VK, Attwood J, Burger M, Burton J, Cox TV, Davies R, Down TA, Haefliger C, Horton R, Howe K, Jackson DK, Kunde J, Koenig C, Liddle J, Niblett D, Otto T, Pettett R, Seemann S, Thompson C, West T, Rogers J, Olek A, Berlin K, Beck S. DNA

methylation profiling of human chromosomes 6, 20 and 22. Nature Genetics 2006;38:1378–85. [PubMed: 17072317]

3. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suñer D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu Y-Z, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. Proceedings of the National Academy of Sciences of the USA 2005;102:10604–9. [PubMed: 16009939]
4. McGowan PO, Sasaki A, Huang TCT, Unterberger A, Suderman M, Ernst C, Meaney MJ, Turecki G, Szyf M. Promoter-wide hypermethylation of ribosomal RNA gene promoter in the suicide brain [electronic article]. PLoS One 2008;3:e2085. [PubMed: 18461137]
5. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, Turecki G, Meaney MJ. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nature Neuroscience 2009;12:342–8.
6. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, Sharma S, Pearson D, Plotsky PM, Meaney MJ. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 1991;277:1659–62. [PubMed: 9287218]
7. Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. Journal of Child Psychology and Psychiatry 2007;48:224–44. [PubMed: 17355397]
8. Beach SRH, Brody GH, Todorov A, Gunter TD, Philibert RA. Methylation at SLC6A4 is linked to family history of child abuse: An examination of the Iowa Adoptee sample. American Journal of Medical Genetics: Part B Neuropsychiatric Genetics 2009;153B:710–713.
9. Shannon C, Schwandt ML, Champoux M, Shoaf SE, Suomi SJ, Linnoila M, Higley JD. Maternal absence and stability of individual differences in CSF 5-HIAA concentrations in rhesus monkey infants. American Journal of Psychiatry 2005;162:1658–64. [PubMed: 16135625]
10. Ichise M, Vines DC, Gura T, Anderson GM, Suomi SJ, Higley JD, Innis RB. Effects of early life stress on [11C]DASB positron emission tomography imaging of serotonin transporters in adolescent peer- and mother-reared rhesus monkeys. Journal of Neuroscience 2006;26:4638–43. [PubMed: 16641244]
11. Mehlman PT, Higley JD, Faucher I, Lilly AA, Taub DM, Vickers JM, Suomi SJ, Linnoila M. Correlation of CSF 5-HIAA concentration with sociality and the timing of emigration in free-ranging primates. American Journal of Psychiatry 1995;152:907–13. URL. [PubMed: 7538731]
12. Carver CS, Johnson SL, Joormann J. Serotonergic function, two-mode models of self-regulation, and vulnerability to depression: What depression has in common with impulsive aggression. Psychological Bulletin 2008;134:912–43. [PubMed: 18954161]
13. Virkkunen M, Rawlings R, Tokola R, Poland RE, Guidotti A, Nemeroff CB, Bissette G, Kalogeras KT, Karonen S-L, Linnoila M. CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic, violent offenders, fire setters, and healthy volunteers. Archives of General Psychiatry 1994;51:20–7. URL. [PubMed: 7506515]
14. Philibert RA. Merging genetic and environmental effects in the Iowa Adoption Studies: Focus on depression. Annals of Clinical Psychiatry 2006;18:219–22. [PubMed: 17162620]
15. Yates, W.; Cadoret, R.; Troughton, E. The Iowa adoption studies methods and results. In: LaBuda, M.; Grigorenko, E., editors. On the way to individuality: Methodological issues in behavioral genetics. Nova Science; Hauppauge, NY: 1998. p. 95-125.
16. Philibert RA, Madan A, Andersen A, Cadoret R, Packer H, Sandhu H. Serotonin transporter mRNA levels are associated with the methylation of an upstream CpG island. American Journal of Medical Genetics: Part B Neuropsychiatric Genetics 2007;144B:101–5.
17. Thomassin H, Oakeley EJ, Grange T. Identification of 5-methylcytosine in complex genomes. Methods 1999;19:465–75. [PubMed: 10579942]
18. Muthén, LK.; Muthén, BO. Mplus user's guide. 6th ed.. Authors; Los Angeles: 1998–2009.
19. Bollen, KA. Structural equations with latent variables. Wiley; New York, NY: 1989.
20. Fan S, Zhang X. CpG island methylation pattern in different human tissues and its correlation with gene expression. Biochemical and Biophysical Research Communications 2009;383:421–5. [PubMed: 19364493]

21. Suomi SJ. Risk, resilience, and gene × environment interactions in rhesus monkeys. Annals of the New York Academy of Sciences 2006;1094:52–62. [PubMed: 17347341]

Figure 1. Theoretical Model Showing Possible Direct and Indirect Pathways from Childhood Sex Abuse and Parent Diagnosis to Symptoms of ASPD

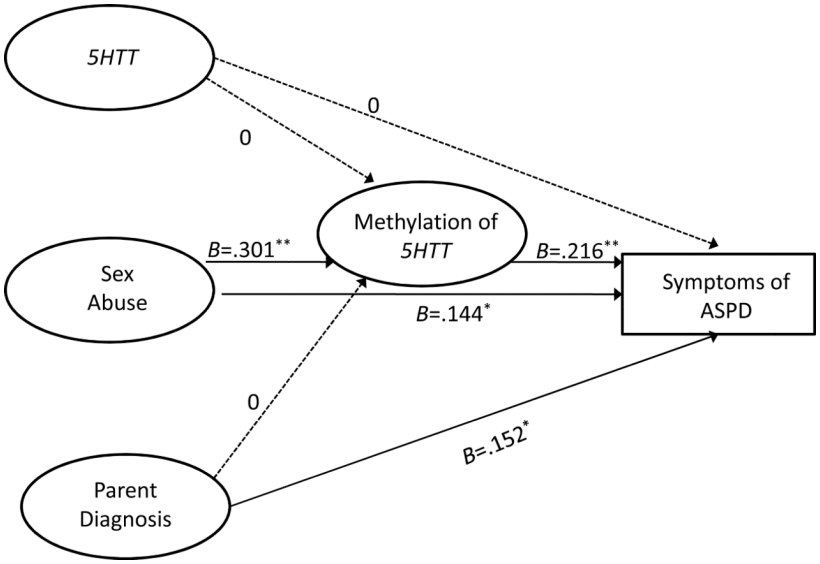

Figure 2. Final Main Effects Model showing Significant Paths from Sex Abuse to Methylation and ASPD, and a Significant Path from Parent Diagnosis to ASPD

Fit: $\chi^2(3) = 1.104$, ns

**$p < .01$

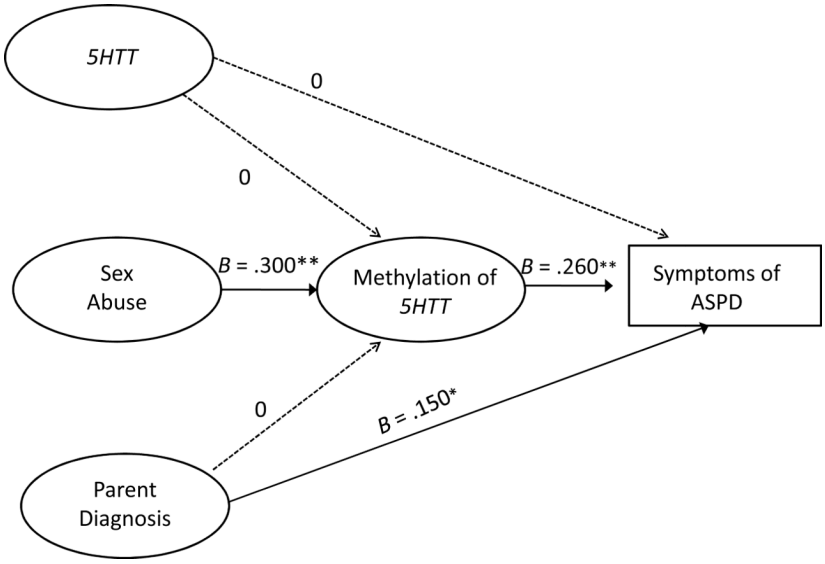

Figure 3. The Main Effect Model in which the Direct Path from Sex Abuse to ASPD is constrained to be Zero, indicating full mediation through methylation at *5HTT*

Fit: $\chi^2(4) = 4.373$, ns

**$p < .01$

Figure 4. Final Model with Significant Interaction of Methylation and *5HTT* Included
Fit: $\chi^2(5) = 5.12$, ns
**$p < .01$

Table 1

Correlations for full sample (n = 155) above the diagonal and correlations for homozygous *l* (n = 59) below the diagonal. Means and standard deviations for major study variables and demographics for the full sample and sub-sample.

Correlations						
Measures		**1**	**2**	**3**	**4**	**5**	**6**
1. Sex Abuse		--	.210	.301	.011	.067	−.040
2. ASPD Symptoms		**.309**	--	.271	.171	−.136	−.111
3. Methylation		**.329**	**.143**	--	.079	.104	−.007
4. Parent Diagnosis		**.098**	**.290**	**.094**	--	−.062	−.160
5. Age		**.056**	**−.235**	**.145**	**−.128**	--	−.144
6. Education		**−.086**	**−.186**	**−.002**	**−.188**	**−.127**	--
Means							
Full sample	*M*	.13	.92	.02	.43	41.10	14.26
	SD	.42	1.14	1.00	.50	7.74	1.99
ll sample	*M*	.20	.93	.01	.37	41.76	14.15
	SD	.55	1.10	.95	.49	8.22	1.76