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Methylation at *SLC6A4* is linked to Family History of Child Abuse: An Examination of the Iowa Adoptee Sample

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To the Editor:

In this letter we describe novel, preliminary work, examining a possible mechanism of the Gene-environment interactions thought to moderate the response of individuals to stressful life events. The molecular mechanisms through which this moderation may be accomplished are currently unknown but some have suggested DNA methylation (Lui and others, 1997; McGowan and others 2009). In order to test this hypothesis, we analyzed the relationship of child abuse to methylation of cytosine residues in the promoter region of the serotonin transporter gene in DNA from 96 male and 96 female subjects from the Iowa Adoptee Studies using a principal components analysis. The results from this preliminary work suggest a lasting effect of child abuse on overall methylation levels in both males and females.

It is commonly appreciated that complex behavioral illnesses result from the interplay of genetic and environmental factors. Over the past several years, large, systematic case and control studies have made significant inroads into the role of DNA sequence variation into psychiatric syndromes such as Major Depression and Schizophrenia (Boomsma and others 2008; O'Donovan and others 2008a; O'Donovan and others 2008b). These cross sectional studies indicate that the contribution of any one genetic variant to behavioral illness is limited and suggest that other factors such as epigenetic effects may play a more prominent role in disease pathology than previously thought (Neale and Purcell 2008).

In humans, methylation of the 5' carbon of cytosine-guanosine dinucleotide pairs (CpG) is the predominant, almost exclusive, form of DNA methylation. Although its exact effect is contextually dependent, methylation of CpG residues in CpG "islands" (areas of increased CpG residue frequency) in the promoter regions of genes is strongly associated with attenuation of mRNA expression from that locus. Hence, CpG methylation could serve as a mechanism for the cellular fine-tuning of gene expression in response to extracellular events such as changes in diet, exposure to hormones or chemical stress. In contrast, less attention has been paid to how adverse family environments may affect epigenetic processes, and ultimately differential gene-expression. If psychosocial stressors do affect the intracellular millieu, measuring the extent of DNA methylation would allow a more precise definition of how environmental risk mechanisms contribute to key outcomes of psychiatric interest.

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Child abuse is an environmental stressor with the potential to have an effect on phenotypic outcomes. Child abuse has been associated with later risk for posttraumatic stress disorder (PTSD) and depression, (MacMillan and others 2001; Mullen and others 1996) as well as increased physiological reactivity in response to stress (Heim and Nemeroff 2001; Weiss and others 1999) and possible changes in CNS functioning (Liu and others 1997; Weiss and others 1999). In addition, exposure to child abuse has been associated with lasting dysregulation of the hypothalamic-pituitary-adrenal axis and hypersecretion of corticotrophin-releasing factor in response to interpersonal stress in adulthood (Heim and others 2000). More recently, epigenetic differences have been demonstrated in the postmortem hippocampus 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 (McGowan and others 2009), further suggesting the potential importance of child abuse as a risk factor for epigenetic change. Because early maltreatment within a family environment may be particularly important in producing long-term epigenetic change (Gunner and Quevedo 2007) and early experience has been linked to epigenetic change via CpG methylation in animal models (Kaffman and Meaney 2007), we focused our assessment on severe stress occurring in the family context before age 16.

The serotonin transporter gene (*SLC6A4* or *5HTT*) is of particular interest in the context of understanding the effects of child abuse. The transporter is a key regulator of serotonergic neurotransmission. Changes in serotonergic neurotransmission are implicated in a range of disorders including migraine headaches, major depression (MD), autism and alcoholism (Conroy and others 2004; Feinn and others 2005; Glover and others 1993). Although it is commonly appreciated that a substantial portion of gene activity is contributed by heritable factors, over the past several years it has also become apparent that gene-environment interactions (GxE), which are defined as environmental effects whose impact is dependent on gene status, also make substantial contributions to neuropsychiatric illness (Moffitt and others 2005). Some (Caspi and others 2003; Kendler 2005), but not all studies (Surtees and others 2006), have suggested that GxE effects at the *SLC6A4* are particularly salient in the development of MD. A variable nucleotide repeat (referred to as 5HTTLPR), which is approximately 1,400 bp upstream of the transcription start site has been a primary focus of attention. The properties of the short or "*S*" allele in this polymorphism have been associated with decreased mRNA transcription (Bradley and others 2005; Lesch and others 1996; Philibert and others 2007), decreased protein production (Stoltenberg and others 2002), and increased vulnerability to alcohol dependence (AD) and MD in the presence of stressors (Feinn and others 2005; Lotrich and Pollock 2004). However, the amount of variance in mRNA production accounted for by 5HTTLPR variants is relatively modest (i.e., <10%) (Bradley and others 2005). It is reasonable to assume that the regulatory mechanism(s) controlling *SLC6A4* activity involves other *cis* and *trans* acting elements, suggesting the potential importance of examination of methylation of the *SLC6A4* promoter island.

Clinical data and biomaterial came from 96 males and 96 female subjects who were randomly selected from the participants in the last wave of the Iowa Adoption Study (2004-current). Male participants ranged in age from 35 to 69 with a mean of 49 at the time of the blood draw. Female participants ranged in age from 35 to 65 with a mean of 47 at the time of the blood draw. DNA was obtained from growth phase entrained EBV transformed lymphoblast cell lines. The resulting DNA underwent bisulfite conversion of unmethylated cytosine residues to thymidine (Thomassin and others 1999). Methylation ratios for each of the CpG residues were determined using quantitative mass spectroscopy by Sequenom, Inc. (San Diego, CA). After deleting those residues for which fewer than 90% of males or females had values (N=9), as well as the redundant peaks which could not be deconvoluted or were perfectly correlated with other peaks (N=36), 26 of the 71 CpG site values were included in the analyses. To examine methylation density, we extracted the first factor from

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a principal component analysis of the methylation levels at the 26 CpG sites, corrected for gender, thereby describing overall methylation density across much of the promoter region for males and females. As described below, the first factor weighted residues early in the promoter region more heavily than those later in the promoter region.

To assess childhood physical abuse, participants were asked “Did your Mother/Father ever physically punish you so hard that you hurt the next day or had to see a doctor?” Parental physical abuse was scored as “present” if either question was answered in the affirmative and “not present” otherwise. To assess childhood sexual 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?” Sexual abuse was scored as “present” if either question was answered in the affirmative and “not present” otherwise. The “child abuse” scale was scored “present” if either physical or sexual abuse was present. Accordingly, child abuse was scored as “0” if all answers to the questions outlined above were negative but “1” if any of the answers were positive.

The relationship of child abuse to overall methylation is presented in Figure 1 using untransformed factor scores so that scores indicate a weighted mean, with larger scores indicating greater overall methylation early in the promoter region (CpG1-17). Specifically, the factor score was strongly correlated with specific CpG residue values in the region (CpG1-17), with correlations ranging from .37 to .69, and with a median correlation of .59. Conversely, in the remainder of the promoter region (CpG20–71) correlations ranged from −.01 to .39, with a median correlation of .25. The relationship between child abuse and normalized principal component methylation scores were examined in a regression with gender, child abuse and the interaction of gender and child abuse as predictors. There was a significant effect of Child Abuse on overall methylation levels (b = .856, $F(1,167) = 13.11$, $p = .0004$); but no other significant effects were identified.

We also examined specific CpG loci. No specific site emerged as significantly differently methylated as a function of child abuse for males. However, methylation levels were elevated across the entire promoter region for abused males relative to non-abused males. In females, two loci were significantly hypermethylated for those who experienced child abuse: CpG1 (25586514) ($U = 4465$, $p = .0001$, empirical $p = .0037$); CpG3 (25586527)($U = 4371$, $p = .0064$, empirical $p = .003$). In addition, methylation was elevated for females, albeit non-significantly, across much of the remainder of the promoter region. Figures are available upon request.

Several limitations should be noted. First, we lacked the power to examine the role of gene methylation in addition to genetic factors in accounting for specific outcomes such as major depression, nor could we examine the role of methylation in mediating the impact of child abuse on depression. This should be a focus of attention in future research with a larger sample. Second, biomaterial was taken from lymphoblasts and so may not provide a direct index of methylation in the central nervous system or tissue specific patterns of methylation of potential interest. Third, we examined only the first factor that could be extracted from the methylation residue data, reducing the number of loci contributing to the analyses. It is possible that there are additional meaningful factors that capture variance and have consequences for gene transcription. In particular, the principal component score we extracted was dominated by variance in loci early in the promoter region (CpG1-17). To the extent that variation later in the promoter region is important in regulating transcription, this may be better captured by a second component.

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In summary, we find that methylation levels of the CpG island upstream from *SLC6A4* are associated with report of abuse during childhood in both males and females. This suggests that child abuse may have lasting effects on gene regulation in adults and highlights the importance of examination of the psychological mechanisms involved in such effects. These findings also provide further motivation for continued exploration of the role of the methylome in accounting for gene-environment interactions and they way they may modulate the risk for psychiatric disorders. The finding of an effect of adverse childhood experiences on level of methylation at *SLC6A4* was anticipated both because of the strong evidence indicating effects of child abuse on clinical outcomes, and because methylation is a likely physical basis for the impact of adverse events on clinical outcomes.

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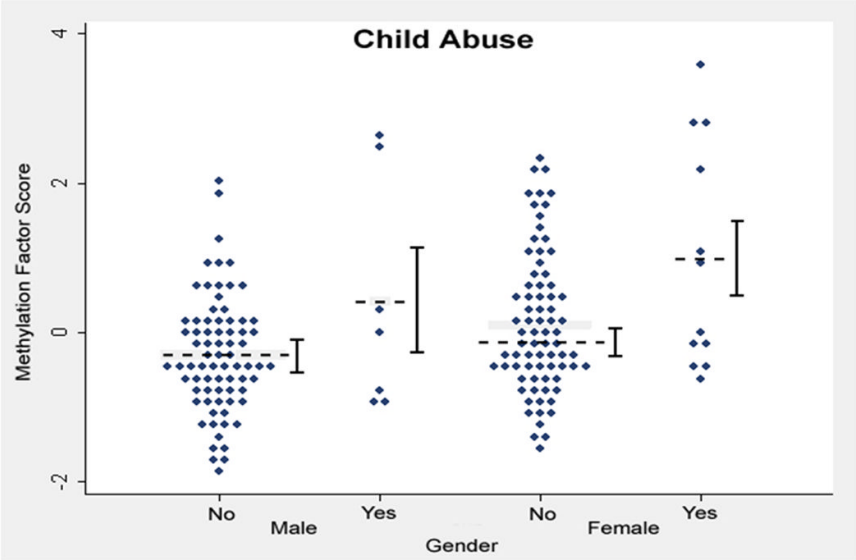

Figure 1.

Factor scores for males and females reporting child abuse or not reporting child abuse. The first factor is plotted and reflects primarily CpG ratio values (methyl CpG/Total CpG) early in the promoter region (i.e. CpG1 to CpG17). Values for females are on the right and values for males are on the left. Within gender, values for abused are on the right and values for non-abused are on the left.