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Emily Ronkin 4/17/2012

DNA Methylation and Cognitive Functioning in Preschool Aged Children

by

Emily Ronkin

Patricia A. Brennan
Adviser

Psychology

Patricia A. Brennan
Adviser

Katrina Johnson
Committee Member

Heather R. Clarke
Committee Member

2012

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Emily Ronkin

Patricia A. Brennan
Adviser

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Abstract

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Novel research concerning a phenomenon termed epigenetics has shown that the environment can interact with, and alter, what were previously thought to be permanent, genetic predispositions toward behavior. This study examines one such epigenetic mechanism, DNA methylation, and its association with child cognitive outcomes during the preschool period. 74 women and their children were recruited from the Emory Women's Mental Health Program (WMHP) to take part in this study. Cord blood collected at delivery was used to assay methylation of the brain-derived neurotrophic factor (BDNF) gene. Obstetric and delivery complication data were obtained from hospital records. When the child was 2.5 to 5.5 years of age maternal mental illness history and cognitive outcomes were measured. These data were used to test the hypotheses that there would be an association between higher methylation levels and negative cognitive outcomes, exposure to early life stress and negative cognitive outcomes, and finally that higher levels of methylation would interact with exposure to early life stress to predict more negative cognitive outcomes than the other children. Previous literature demonstrated relationships between methylation of BDNF, early life stress, and cognitive functioning. No significant associations were found between higher levels of methylation and lower levels of cognitive functioning or between exposure to early life stressors and lower levels of cognitive functioning. A few significant interactions were found between methylation levels and early life stressors predicting cognitive functioning, however all but one pointed in the direction opposite of what was hypothesized. Since this study found conflicting results and this field is so new, further research is needed.

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According to the diathesis stress model, environmental factors and genetic factors interact across the lifespan to determine various behavioral, psychological, and cognitive outcomes. Early childhood has been found to be an especially critical time period for the interaction of genes and the environment in the prediction of cognitive outcomes because of the high level of neuroplasticity of the developing brain (Nelson et al., 2007). Candidate genes that are most relevant for cognitive outcomes include catechol-O-methyltransferase (COMT), the dopamine receptor genes D1, D2, and D4, as well as brain-derived neurotrophic factor (BDNF) (Savitz, Solms & Ramesar, 2005). Studies of gene-environment interactions commonly examine a polymorphism of a particular gene in interaction with an environmental stressor. More recently, however, scientists have begun to examine the relationship between gene expression and methylation—a phenomenon referred to as "epigenetic", meaning "on top of the genome"—as a potential source of genetic risk. Recent investigations have begun to highlight the potential role of epigenetics in predicting growth and development (Kiefer, 2007), but no previous studies have examined epigenetic factors and their association with specific cognitive outcomes in childhood. The goal of the present study is to examine how the methylation of the BDNF gene may be related to the cognitive functioning of preschoolers and if this relationship is moderated by exposure to postnatal life stressors.

Epigenetics

Traditionally, it has been debated whether different traits reflecting personality, capability, and physicality are due to nature or nurture. However, scientific research has recently been focusing on how nature and nurture can work together to result in these trait differences (Champagne & Mashoodh, 2009). Each individual has a genome made up of a sequence of

DNA nucleotide bases: Cytosine, guanine, adenine, and thymine. These bases combine in different ways to form genes, each of which is preceded by a region of bases called the promoter. Gene expression occurs when an enzyme, or a transcription factor, called RNA polymerase binds to the promoter region of the gene. Promoters for genes that are frequently expressed lie in certain regions of DNA called CpG islands. These regions are stretches of DNA that have a high concentration of cytosine bases and guanine bases separated by a single phosphate bond. Once the process of transcription is initiated, an mRNA transcript of the important DNA sequence is created that can be further expressed into a protein via translation. These proteins then work to regulate cell activity. However, in order for the protein to exist, the DNA's environment must be conducive to gene expression by providing the RNA polymerase and allowing it to bind to the promoter.

Gene expression is controlled by factors that promote or obstruct transcription. As noted above, factors that affect gene expression without altering the specific DNA sequence are referred to as epigenetic (Champagne & Mashoodh, 2009). One epigenetic mechanism that has the capability to cause enduring changes in gene activity is DNA methylation. DNA methylation occurs when a methyl group binds to the promoter region of a gene and blocks the RNA polymerase from binding to the promoter and transcribing the gene, thus reducing the likelihood of transcription and eventual protein production (Champagne & Mashoodh, 2009). CpG islands are particularly prone to DNA methylation because of the presence of gene promoters in these regions of the genome.

It is important to understand how the outside environment impacts the cellular environment in order to control gene and protein expression. This question has primarily been examined in studies using rodents. For example, one seminal study in this area found that

differences in maternal care in some rodent species cause individual variations in the expression of genes associated with the stress response (Weaver et al., 2004). A prolonged response to stress is associated with low levels of glucocorticoid receptors in the hippocampus, and this study found that decreased maternal care accompanied elevated levels of DNA methylation, silencing the gene, and leading to fewer glucocorticoid receptors in the brain. This situation is mirrored in humans: A similar study found that newborns prenatally exposed to increased maternal depression and anxiety in the third trimester had increased methylation at the human glucocorticoid receptor gene and altered HPA stress reactivity (Oberlander et al., 2008). These studies exemplify how DNA methylation, an epigenetic mechanism, changes gene expression in response to the environment—in this particular case, differences in parenting and maternal psychopathology.

BDNF

BDNF is a neurotrophin, or a growth factor. It is involved in synaptic plasticity and promotes neuronal differentiation, proliferation, and survival (Klein et al., 2011). BDNF is expressed not only in the brain, but in the peripheral regions of the body as well. Genes on human chromosome 11 code for BDNF and the current study examines the percentage of DNA methylation on two CpG islands, cg16257091 and cg27351358, within the BDNF gene. It has been demonstrated in previous literature that the amount of methylation of these CpG islands negatively correlates with the level of BDNF expressed in the brain and blood serum (Fuchikami et al., 2011).

Genetics and Cognition

Previous research linking the BDNF gene and cognitive functioning has focused on a common single nucleotide polymorphism in the BDNF gene where a methionine amino acid

replaces a valine at codon number 66 (annotated as val66met). For example, one representative study examining this polymorphism found that the met allele was associated with poorer episodic memory, lower hippocampal volume, and worse performance on the Wisconsin Card Sorting Test, a test of executive functioning (Savitz et al., 2005). Animal studies have examined how the level of expressed BDNF in the blood serum and brain influence cognitive functioning. One body of research examined the fundamental action of BDNF in mice by knocking out BDNF from their forebrains. Studies of this type have found that by selectively deleting BDNF, specific forms of spatial learning and memory were seriously impaired (Gorski, Balogh, Wehner, & Jones, 2003).

In humans, the research produces more conflicting results. One study found BDNF serum levels to be a biomarker of general cognitive functioning in older women, but not men, showing a positive correlation between the level of BDNF in the serum and scores on cognitive tests (Komulainen et al., 2008). It has also been demonstrated that physical activity in humans mediated by short-term increases of BDNF serum levels, increased learning in short-term tasks as well as prevented long-term cognitive decline. Cognitive functioning continued to operate at higher levels with continued serum level increases of BDNF, showing a positive relationship between BDNF and cognition (Winter et al., 2007). Despite this literature linking BDNF to cognition, there are some reports of levels of BDNF showing no significant associations to cognitive measures (Miyajima et al., 2007).

The Moderating Role of the Environment

The diathesis stress model suggests that inherited vulnerabilities are more likely to result in deleterious outcomes in particular "high-risk" environments. Although epigenetic factors in some sense represent a combination of genetic and environmental vulnerabilities, their impact on

outcome may still be dependent on more proximal environmental effects. The present study draws its data from a sample of women who were treated during pregnancy for mood and anxiety disorders. The average age at delivery is 34.1 years, which suggests the increased likelihood of exposure to perinatal complications due to higher maternal age (Jacobsson, Ladfors, & Milson, 2004). Therefore the children being studied may be at increased risk for negative cognitive outcomes due to additional exposures to maternal symptoms during early childhood (Brennan, Hammen, Andersen, Bor, & Najman, 2000), or due to higher levels of delivery complications (Rees & Inder, 2005). The use of such a "high-risk" sample enables us to examine the interaction between epigenetic vulnerability (the increased methylation of BDNF gene) and both maternal mental illness and delivery complications in the prediction of preschool cognitive outcomes.

Maternal Mental Illness and Cognitive Outcomes

Cognitive functioning refers to the mental processes that include, but are not limited to, memory, attention, perception, thinking, reasoning, problem solving, pattern recognition, decision making, and executive functioning. Cognitive functioning in early childhood is influenced by a variety of factors, including maternal mental illness (Brand & Brennan, 2009). For example, one recent study examined a collection of maternal risk factors, including mother's anxiety and mother's mental health, and found that these risks contributed to the variance of IQ scores in 4-year-olds (Sameroff, Seifer, Baldwin, & Baldwin, 2008). Another study demonstrated that maternal mood and stress measured when the child was one month old were associated with behavioral and emotional regulation several years later (Anhalt, Telzrow, & Brown, 2007).

A separate prospective longitudinal study by Laucht, Esser, & Schmidt (2004) followed children exposed to familial conflict and stress at and after birth and found significant influences of family adversity on child motor and cognitive functioning. A recent review of the literature in this area reported that maternal mental illness (manifested primarily in maternal anxiety and depression) experienced in the perinatal period has persistent negative consequences on the behavioral, cognitive, and psychological development throughout the child's lifetime. Areas of impact include motor and mental development, language development, and intelligence. It was also found that chronic exposure to maternal anxiety or depression in early childhood, rather than acute exposure during the postpartum period, more strongly predicted these negative outcomes (Brand & Brennan, 2009).

Delivery Complications and Cognitive Outcomes

Delivery complications include hypoxic or hypoxic-ischemic episodes, or any kind of severe oxygen deprivation, prematurity, low birth weight, small for gestational age, any type of growth restriction, and low APGAR scores. There is an extensive literature linking these types of complications to negative cognitive outcomes.

It has been demonstrated that high levels of prenatal oxygen deprivation can result in neurodevelopmental impairments in the infant and poor educational outcomes later in childhood (Van Handel, Swaab, de Vries, & Jongmans, 2010). For example, acute insults in late gestation, like umbilical cord occlusion, result in widespread cell death in the brain leading to lower levels of cognitive functioning later in life (Rees & Inder, 2005). Similar findings have been noted in animal research. For example, one study found that the mice exposed to severe oxygen deprivation (75 minutes of hypoxia) experienced negative motor, behavioral, and cognitive consequences (van der Kooij et al., 2010).

Current Study

The current study will examine gene-environment effects on cognitive functioning in a sample of preschool aged children whose mothers have undergone treatment for a major mental disorder. As noted above, exposure to the symptoms of maternal mental illness at a young age as well as delivery or obstetric complications can have adverse consequences on cognition in early childhood. There is also evidence for a relationship between BDNF and cognitive functioning suggesting that the amount of methylation in BDNF genes might be associated with children's cognitive outcomes. On the basis of the previous empirical literature we hypothesize that:

1. Higher levels of methylation of BDNF genes will be associated with lower levels of cognitive functioning;
2. Higher levels of obstetric complications and exposure to maternal mental disorder symptoms in the postnatal period will be associated with lower levels of cognitive functioning; and
3. A combination of genetic risk (increased methylation of BDNF genes) and environmental risks (obstetric complications and maternal mental illness exposure) will interact to predict cognitive functioning of the children in the sample.

Method

Participants

The sample consisted of 74 women and their children (32 male and 42 female), recruited from the Emory University Women's Mental Health Program. Ages of the children ranged from 2.46 to 5.56 years old, with a mean of 3.10 years and a standard deviation of 0.79 years. Mother's age at delivery ranged from 23.0 years to 45.5 years with an average value of 34.1

years and a standard deviation of 4.95 years. 87% of the children were Caucasian, 4% were African-American, 3% were Asian, and 7% were multi-racial.

Procedure

The participants were recruited for the current study through the Emory University Women's Mental Health Program (WMHP). The women had been treated at the WMHP during pregnancy for a major mental disorder, and many of them had been treated with psychotropic medications for their disorders. WMHP researchers collected umbilical cord blood at the time of birth and conducted a genome-wide methylation profile as well as multiple other measures before, during, and after pregnancy.

Once the child reached the ages of approximately 2.5-5.5 years, the mother and child were recruited to participate in the current study of preschool outcomes. The mother and child were scheduled and came in for a 3-4 hour session in the Biological Underpinnings Involved in learning and Development (BUILD) lab in the Psychology Department at Emory University. Once the mother completed the necessary consent forms, a trained examiner completed the Structural Clinical Interview for the DSM-IV-TR (SCID) with the mother. When the interview was finished, the mother filled out the Behavioral Rating Inventory of Executive Functioning-Preschool Edition (BRIEF). The child was administered the Differential Abilities Scale-Second Edition (DAS-II) as well as other tasks of cognition, frustration, and executive function.

Measures

BDNF CpG Island DNA methylation

The percent methylation of each of the CpG islands within the BDNF gene on chromosome 11 was determined using a HumanMethylation 27 BeadChip (Ilumina). 1 μg of DNA collected at birth from umbilical cord blood was bisulfite-treated, whole-genome amplified,

fragmented, and hybridized to the chip. A sample of pooled female DNA was included in each assay as a control and assessed for reproducibility. The methylation levels of two CpG islands were used in the study: cg16257091 (BDNF 091) and cg27351358 (BDNF 358). Refer to Table 1 for BDNF methylation statistics.

Differential Abilities Scale-Second Edition (DAS-II)

The DAS-II is a multi-subtest battery composed of 10 core subtests and 10 diagnostic subtests. The current research uses the lower level Early Years Battery for ages 2-6, which consists of Verbal Ability and Nonverbal Ability. The Verbal Ability section is made up of Verbal Comprehension, pointing to specific objects or following examiner instructions (e.g. "Show me the shoe", "Put the cat in the box"), and Naming Vocabulary, naming objects or pictures (e.g. the examinee might be shown a picture of a rocket and asked "What is this called?"). The Nonverbal Ability section is made up of Picture Similarities, placing a card with a picture below 4 possible pictures and asked with which it goes best (e.g. the examinee is given a card with a triangle and asked to put it next to the triangle it matches out of 4 shape options), and Pattern Construction, which requires reproducing designs using different shapes (e.g. construct a square using solid cubes).

The DAS-II has high internal reliability for the Verbal Ability subtest ($\alpha = .89$) and for the Nonverbal Ability subtest ($\alpha = .89$). The measure also has high test-retest reliability; the stability coefficient for the Nonverbal Ability subtest is .77.

The DAS-II has satisfactory validity with other measures of intelligence. There is also established validity with the Bayley Scales of Infant Development—Second Edition, the Wechsler Intelligence Scale for Children—Fourth Edition (WISC-IV), and the Wechsler Preschool and Primary Scale of Intelligence—Third Edition (WPPSI-III) (Elliott, 2007).

Behavioral Rating Inventory of Executive Function-Preschool Edition (BRIEF)

The BRIEF is a standardized rating scale of the executive and cognitive functioning of 2 to 5 year olds. The BRIEF consists of 63 items (e.g. "Cannot stay focused on a particular topic when speaking", "Over-reacts to small problems") in 5 non-overlapping scales completed by the mother on a standardized rating scale (e.g. Often, Sometimes, Never). The scales compose a Global Executive Composite (GEC) and three overlapping summary indexes. The current research used the three overlapping summary indexes for analyses. The Inhibitory Self-Control Index (ISCI) is comprised of the Inhibit scale and the Emotional Control scale and measures the ability to control emotional, behavioral and physical responses and actions via inhibitory control. The Flexibility Index (FI) is comprised of the Shift scale and the Emotional Control scale and measures the ability to emotionally, behaviorally, and physically respond to situations flexibly. The Emergent Metacognition Index (EMI) is comprised of the Working Memory scale and the Plan/Organize scale and measures the child's ability to hold ideas in working memory in order to plan and organize responses to problems.

There is high internal consistency (α = .80-.98) and the test-retest correlation coefficient is .82 for parents. The BRIEF has two validity scales—the Negativity scale and the Inconsistency scale. The Negativity scale measures the extent to which the mother answered BRIEF elements about the child in an overly negative way while the Inconsistency scale measures the extent to which similar BRIEF items were answered about the child in an inconsistent fashion.

There is established convergent validity with other measures of inattention, impulsivity, and learning skills and demonstrates divergent validity against measures of emotional and behavioral functioning (Gioia, Isquith, Guy & Kenworthy, 2012).

Obstetric records

Delivery complications were obtained from obstetric records collected by the WMHP. Two measures were chosen for the current study on the basis of sufficient variability and their conceptual match to delivery stress/hypoxia. The first measure is the Apgar, taken 1 minute after birth by the hospital doctors in order to assess newborn health. The Apgar score is comprised of 5 components each on a scale of 0-2, which are then summed so the resulting score ranges from 0-10. The five components are appearance (measuring skin color or complexion), pulse, grimace (measuring response to stimulation), muscle tone (measuring flexion of limbs), and breathing.

The second measure of delivery complications is whether the newborn experienced a nuchal cord complication. This complication occurs when the cord is wrapped all the way around the fetal neck during gestation or labor and delivery. This is a fairly common problem occurring in 21.7% to 0.3% of all births (Miser, 1992) and the probability increases as gestational age increases (Clapp, Stepanchak, Hashimoto, Ehrenberg & Lopez, 2002).

Structured Clinical Interview for DSM Disorders (SCID-I)

The SCID-I is a semi-structured clinical interview administered to the mothers to determine DSM-IV-TR Axis I diagnoses. Reliability for the separate DSM diagnoses assessed is reported in terms of kappa and is medium to high ($\kappa > .57$) for each category (Zanarini, Skodol, Bender, Dolan & Sanislow, 2000). The SCID-I has high validity (Fennig, Craig, Lavelle, Kovasznay & Bromet, 1994). The SCID-I was used in this study to assess the number of months the child was exposed to maternal mental illness. Typical maternal mental illnesses children were exposed to in early life include Major Depressive Disorder, Bipolar I, Generalized Anxiety Disorder (GAD), Post-traumatic Stress Disorder (PTSD) and Obsessive Compulsive Disorder (OCD).

Results

The ranges, means, and standard deviations of all the variables examined in the current study can be found in Table 1.

In order to test for potential confounds, correlations were conducted between the dependent measures used—DAS Verbal, DAS Nonverbal, BRIEF ISCI, BRIEF FI, and BRIEF EMI—and potential confounding variables. The potential confounds tested were the Clinical Global Impression (CGI) rating given during pregnancy, Beck Depression Inventory (BDI) scores during pregnancy, exposure to antidepressants, anti-epileptic drugs, lithium, antipsychotics, hypnotics, benzodiazepines, folate, or ethanol during pregnancy, child sex, child age at time of testing, estimated gestational age of the child at delivery, mother's age, number of children that the mother has, mother's highest level of education, and child race (dummy coded as either white versus non-white or white versus black).

Significant relationships were found between the DAS Verbal subtest and the BDI score during pregnancy, $r(63) = -.40$, $p<.01$, number of children that the mother has had, $r(67) = -.26$, $p<.05$, mother's highest level of education, $r(67) = .28$, $p<.05$, and white versus non-white, $r(72) = -.25$, $p<.05$. The DAS Nonverbal subtest was significantly correlated with mother's age, $r(64) = .34$, $p<.01$. The BRIEF ISCI was significantly correlated with anti-epileptic drug exposure during pregnancy, $r(69) = .24$, $p<.05$ as was the BRIEF EMI, $r(69) = .34$, $p<.01$. The BRIEF FI was not significantly correlated with any potential confounds. Significant confounds were controlled for in later analyses undertaken for hypothesis testing.

The first hypothesis was that there would be a negative correlation between percent methylation of the CpG islands within the BDNF genes and cognitive functioning in preschool aged children. This hypothesis was tested using partial correlation analyses controlling for potential confounds. No associations were found to be significant (see Table 2).

The second hypothesis was that there would be significant correlations between levels of obstetric complications and exposure to maternal mental illness and scores of cognitive functioning. Contrary to the hypothesis, partial correlations revealed no significant associations between these variables (see Table 3).

The third hypothesis was that children with increased methylation of the BDNF genes and exposure to a higher environmental risk (operationalized by higher percentage exposure to maternal mental illness and higher obstetric complications) would have lower cognitive functioning than the other children in the sample. Linear regressions were used to analyze these predicted interactions. Covariates were entered into the first block, main effect terms were entered into the second block, and interaction terms were entered into the final block of each regression. Statistics representing the final blocks (interaction results) of these analyses are presented in tables 4 to 8. The regressions revealed the following significant interactions: BDNF358 and nuchal cord interacted to predict DAS verbal scores, BDNF358 and Apgar 1 scores interacted to predict DAS nonverbal scores, BDNF358 and percentage of the child's life he or she has been exposed to maternal mental illness interacted to predict BRIEF ISCI scores, BDNF091 and Apgar 1 scores interacted to predict BRIEF EMI scores, BDNF091 and nuchal cord interacted to predict BRIEF EMI scores, and BDNF358 and percentage of the child's life he or she has been exposed to maternal mental illness interacted to predict BRIEF ISCI scores.

Median splits were undertaken to test for the direction of the interaction effects. Association between BDNF and cognitive outcomes were analyzed using linear regressions after dividing the sample at the median of the environmental risk factor into high and low.

Only one of the patterns of interactions fit the predictions of Hypothesis 3: For children above the median on Apgar 1 scores, the association between BDNF358 methylation and DAS

nonverbal scores was in the positive direction, $t(51) = .68$, $p>.05$. In contrast, for children below the median on Apgar 1 scores, the association between BDNF358 and DAS nonverbal scores was significant and in the expected negative direction, $t(13) = -2.77$, $p<.05$.

All other interaction patterns were in the opposite direction than was predicted. That is, in each case the expected relationship between BDNF methylation and cognitive outcomes was more apparent in the children at *lower* postnatal risk, rather than higher.

Specifically, for children above the median on nuchal cord, the association between BDNF358 methylation and DAS verbal scores was positive, $t(16) = 2.23$, $p>.05$. In contrast, for children below the median on nuchal cord, the association between BDNF358 methylation and DAS verbal scores was in the expected, negative direction, $t(42) = -1.24$, $p>.05$. In addition, for children above the median on nuchal cord, the association between BDNF091 methylation and BRIEF EMI scores was significant and negative, $t(19) = -2.79$, $p<.05$. In contrast, for children below the median on nuchal cord the association between BDNF091 and BRIEF EMI scores was nonsignificant, but in the expected, positive direction, $t(49) = 1.13$, $p<.05$.

A similar pattern of results was seen with the postnatal risk factor of exposure to maternal mental illness. Again, contrary to the third hypothesis, for children above the median on percentage of the child's life he or she has been exposed to maternal mental illness, the association between BDNF358 methylation and BRIEF ISCI scores was in the negative direction, $t(37) = -.81$, $p>.05$. In contrast, for children below the median on percentage of child's life he or she has been exposed to maternal mental illness, the association between BDNF358 and BRIEF ISCI scores was in the expected, positive direction, $t(31) = 1.02$, $p>.05$. And for children above the median on percentage of the child's life he or she has been exposed to maternal mental illness, the association between BDNF358 methylation and BRIEF EMI scores was also in the

negative direction, $t(37) = -1.33$, $p>.05$. In contrast, for children below the median on percentage of child's life he or she has been exposed to maternal mental illness, the association between BDNF358 and BRIEF EMI scores was significant and in the expected, positive direction, $t(31) = 2.41$, $p<.05$.

And finally, for children above the median on Apgar 1 scores (i.e., those with lower postnatal risk), the association between BDNF091 methylation and BRIEF EMI scores was in the expected, positive direction, $t(53) = 1.16$, $p>.05$. In contrast, for children below the median on Apgar 1 scores, the association between BDNF091 and BRIEF EMI scores was in the negative direction, $t(16) = -1.37$, $p>.05$.

Discussion

The first hypothesis was that higher levels of methylation in BDNF genes (i.e., BDNF091 and BDNF358) would predict lower scores on the two DAS subtests and higher scores (worse functioning) on the three BRIEF summary indexes. Our results did not support this hypothesis and are inconsistent with previous literature (Komulainen et al., 2008; Miyajima et al., 2007). Specifically, previous studies have found a significant relationship between cognitive functioning and serum levels of BDNF (Gorski et al., 2003; Komulainen et al., 2008; Winter et al., 2007). This lack of corroboration could be due to a number of reasons. One issue may be the particular cognitive measures used in the present study. Previous animal research has linked BDNF levels to deficits in spatial learning and memory (Gorski et al, 2003). Our measures assess verbal abilities, nonverbal abilities (including picture similarities and pattern construction), and executive functioning (comprised of emotional control, behavioral inhibition, working memory, and planning and organization). It could be that the measures of cognition used here are not the

measures of cognition primarily influenced by BDNF, so they were not affected when the BDNF genes were methylated.

Another potential reason for our lack of significant results might be that most studies look at BDNF serum levels with regards to aging, especially memory problems associated with aging (Komulainen et al., 2008). It could be that low levels of BDNF in the serum only lead to cognitive problems later in life and that levels of BDNF at birth are not implicated in cognitive problems experienced in early childhood.

Another conceivable reason that our results were inconsistent with previous findings is that BDNF is activity-dependent and appears to act on a much shorter temporal scale than other proteins. Studies have found that BDNF causes rapid changes at the synaptic levels when it is expressed as a result of neuronal activity (Savitz et al., 2005). Perhaps the single measure of BDNF methylation taken at birth was not an accurate reflection of the levels of BDNF expressed in the brain in later childhood.

It has been demonstrated in previous literature that methylation levels in adulthood correlate with peripheral and brain serum level BDNF (Fuchikami et al., 2011). However, it is possible that umbilical cord blood methylation levels are not indicative of peripheral or brain levels of BDNF later in childhood, so perhaps our measure of BDNF did not correctly mirror BDNF brain or serum levels at the time of testing.

Many candidate gene studies suggest that the Val66Met polymorphism may be more predictive of cognitive functioning than the serum level of BDNF (Savitz et al., 2005; Miyajima et al., 2007). It is possible that cognitive functioning is more related to the polymorphism of the BDNF gene rather than its methylation level. Future research might compare and contrast the

impact of the polymorphism versus the methylation levels of BDNF in the brain, as well as associated cognitive function outcomes.

Our second hypothesis was that stress early in life, measured by obstetric complications during labor and delivery as well as exposure to maternal mental illness after birth, would be associated with impaired cognitive functioning. The results of the partial correlations conducted did not support this hypothesis and were inconsistent with previous literature (Brennan et al., 2000; Van Handel et al., 2010). Numerous studies have found that early life exposure to maternal mental illness influences cognitive functioning (Brand & Brennan, 2009; Anhalt et al., 2007). The current results show no significant associations between percentage of the child's life he or she has been exposed to maternal mental illness and any of the cognitive outcomes measured. This could be because the sample, despite being termed "high-risk" was primarily composed of high-functioning women who have raised their children in an enriched environment. The positive environment could have an opposing response to the adverse impact of maternal symptoms, resulting in no significant cognitive impairments.

Previous literature has established a relationship between delivery complications, especially those related to oxygen deprivation, and lower levels of cognitive functioning later in life (Van Handel et al., 2010; Rees et al., 2005). Nevertheless, the current research found no significant direct association between nuchal cord or Apgar scores and negative cognitive outcomes. These findings may be due to the fact that these particular obstetric complications may or may not be reflective of hypoxia. More direct measures of perinatal oxygen deprivation may yield stronger associations with cognitive outcomes.

Our third hypothesis was that children with an increased genetic risk, measured by BDNF methylation *and* increased environmental risk would have lower cognitive functioning than any

of the other children in the sample. Although one finding supported this hypothesis, the majority of our results suggested the opposite; that is, we found that higher BDNF methylation levels were associated with *lower* levels of cognitive functioning in children who experienced more negative postnatal environments. Barker's hypothesis may provide an explanation for these conflicting results.

Barker's hypothesis was originally developed in order to explain how reduced fetal growth was associated with health problems later in life. Barker asserted that a pregnant woman, when faced with adverse nutritional conditions, can adjust the developmental and growth trajectories of her unborn child in a way so that he or she is better prepared for survival in an environment with limited resources, highlighting the importance of matching the prenatal environment with the postnatal environment in order to be best primed to survive (Barker, 1995). However, this theory can be altered to account for psychological outcomes as well. Perhaps the original genetic or environmental risk the fetus is faced with during gestation prepares the infant to function optimally in an *adverse* environment after birth.

In terms of the current research, the children who encountered more negative prenatal conditions (and had higher methylation of the BDNF gene as a result) were potentially primed to perform better in a more negative environment (e.g., one with greater stressors). This could explain our finding that higher BDNF methylation was associated with improved cognitive functioning in postnatal environments characterized by higher risk. Because these results were unexpected, replication of the findings will be necessary to assess the validity of this interpretation.

It should also be noted that while we found that lower Apgar scores (i.e., those that are unhealthier) interacted with more BDNF358 methylation to predict lower scores (better

performance) on the BRIEF EMI, this is the only interaction we found that was consistent with our a priori hypothesis, suggesting the possibility of a Type 1 error, or the likelihood that this result occurred by chance.

Limitations

One limitation of this research is the fact that cognitive functioning is a complex trait. This means that cognition is influenced by the action of many genes potentially interacting with the environment. Other genes implicated in cognitive functioning include COMT, involved in attention, and the NMDA receptor gene, involved in learning and memory. Since cognitive functioning has so many varied influences and causes, it is difficult to pinpoint BDNF as one specific gene that can significantly alter the trait.

Another major limitation of the study was that there was very little variability in the methylation of the BDNF gene in the cord blood. Other studies found that at birth umbilical levels of BDNF methylation are generally low and have low variability (Non, Binder, Barault, Rancourt, & Kubzansky, 2011), so maybe the variability of methylation, or potentially just the serum levels, increases during growth and development. Perhaps a stronger relationship between BDNF methylation and cognition would be detected later on in development. This point is evidenced further by previous literature that demonstrated a relationship between BDNF levels and cognition and memory problems in aging adults (Komulainen et al., 2008).

Related to this, a further limitation of the current study is that only a single measure of BDNF methylation was taken of the children and it was from umbilical cord blood at birth. Since, as mentioned above, BDNF is activity-dependent (Savitz et al., 2005) this single measure of BDNF methylation may not be indicative of the serum levels 2.5 to 5.5 years later. It would be beneficial in future research to look at measures of BDNF methylation across a number of

different intervals and with a number of different activities to get a sense of how methylation patterns and expression of BDNF changes over development and what particular activities change methylation levels and expressed levels of BDNF.

Another major limitation of the current study was that the sample was fairly homogenous. Most of the women studied were high-functioning despite their mental illness and have provided an enriched environment for their children to grow in, despite their "high-risk" label. Future research can look to see if certain variables encompassing environmental differences, such as a greater variability in socioeconomic circumstances, or exposure to a structured learning environment, change the findings at all.

In terms of future directions, a sample that included healthy controls exposed to no maternal mental illness should be included in order to increase the variability of the sample. Also, it would be beneficial to conduct a longitudinal study with the current sample, assessing cognitive functioning and methylation levels across multiple years. In addition, conducting research examining more candidate genes related to cognitive functioning would give a better idea of what genetic factors influence this complex trait.

Overall, our results have primarily yielded consistent outcomes conflicting with the previous literature, indicating that DNA methylation influences on cognitive functioning is a complex topic needing more research in order to be fully understood. Since epigenetics as related to gene-environment interactions is such a new field and so little is known about the topic, it is important that we continue to study how different genetic risks can interact with environmental factors to change gene expression and thus, behavior and functioning.

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Table 1

Descriptives of BDNF, moderators, and DVs

	N	Range	Min.	Max.	Mean	SD
BDNF 091	74	.07	.02	.10	.06	.02
BDNF 358	74	.13	.05	.19	.10	.03
DAS Verbal	72	61	78	139	107.64	10.47
DAS Nonverbal	69	52	75	127	102.41	11.07
BRIEF ISCI	71	61	33	94	49.49	12.72
BRIEF FI	71	59	35	94	49.58	11.56
BRIEF EMI	71	49	33	82	48.10	11.31
% of Child's Life Exposed to Mat. Mental Illness	73	100	0	100	32	39
Apgar 1	74	7	2	9	7.58	1.37
Nuchal cord	73	1	0	1	.29	.46

Table 2

Correlations between BDNF Methylation and Cognitive Measures

Cognitive Measure	BDNF091Correlation	BDNF358 Correlation
DAS Verbal	-.03	-.04
DAS Nonverbal	-.05	-.09
BRIEF ISCI	.13	-.00
BRIEF FI	.01	-.00
BRIEF EMI	.06	.02

Note *p<.05

Table 3

Partial Correlations between Moderators and DAS Verbal

	% of Child's Life Exposed to Mat. Mental Illness	Apgar 1	Nuchal cord
DAS Verbal	-.05	.05	-.03
DAS Nonverbal	-.05	.03	.20
BRIEF ISCI	.19	.05	-.03
BRIEF FI	.20	.10	-.01
BRIEF EMI	.16	-.10	-.08

Note *p<.05

Table 4

BDNF Methylation by Environment Interactions Predicting DAS Verbal Scores

BDNF091

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	.562	.009	.457
Apgar 1	.505	.008	.480
Nuchal cord	.190	.003	.665

BDNF358

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	.014	.000	.905
Apgar 1	.855	.013	.360
Nuchal cord	5.581	.080	.022*

Note *p<.05

Table 5

BDNF Methylation by Environment Interactions Predicting DAS Nonverbal Scores

BDNF091

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	.493	.007	.485
Apgar 1	1.711	.025	.196
Nuchal cord	.047	.001	.829

BDNF358

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	.120	.002	.730
Apgar 1	4.475	.061	.039*
Nuchal cord	2.074	.029	.155

Note *p<.05

Table 6

BDNF Methylation by Environment Interactions Predicting BRIEF ISCI Scores

BDNF091

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	1.737	.024	.192
Apgar 1	2.657	.037	.108
Nuchal cord	.100	.001	.752

BDNF358

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	4.377	.059	.040*
Apgar 1	.101	.001	.752
Nuchal cord	.067	.001	.796

Note *p<.05

Table 7

BDNF Methylation by Environment Interactions Predicting BRIEF EMI Scores

BDNF091

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	1.508	.020	.224
Apgar 1	6.291	.079	.015*
Nuchal cord	4.504	.058	.038*

BDNF358

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	9.994	.119	.002*
Apgar 1	.626	.009	.432
Nuchal cord	.267	.004	.607

Note *p<.05

Table 8

BDNF Methylation by Environment Interactions Predicting BRIEF FI Scores

BDNF091

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	.720	.010	.399
Apgar 1	.157	.002	.693
Nuchal cord	.908	.014	.344

BDNF358

Moderator	F Change	R Square Change	Sig. F Change
% of Child's Life Exposed to Mat. Mental Illness	2.714	.038	.104
Apgar 1	.056	.001	.814
Nuchal cord	.269	.004	.606

Note *p<.05