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Risk and Resilience in the Intergenerational Impacts of Childhood Trauma Among Black Americans: Moderating Roles of Epigenetic Aging and the Gut Microbiome

By

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An abstract of
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Abstract

Risk and Resilience in the Intergenerational Impacts of Childhood Trauma Among Black Americans: Moderating Roles of Epigenetic Aging and the Gut Microbiome

By Brooke G. McKenna

This dissertation examined the intergenerational associations between maternal childhood adversity and offspring symptoms of psychopathology among Black American mothers and children. Moreover, we explored whether two biological factors related to stress physiology – gut microbiome composition and epigenetic aging – may explain individual differences in offspring susceptibility to maternal childhood adversity. Specifically, we examined how maternal childhood trauma (measured via the Childhood Trauma Questionnaire) and maternal adverse childhood experiences (measured via the ACEs survey) related to offspring internalizing, externalizing, and posttraumatic stress symptomatology. We additionally explored whether maternal childhood maltreatment subtypes (i.e., emotional, physical, and sexual abuse) were differentially related to offspring outcomes. Our findings suggested that both maternal ACEs and maternal childhood trauma, particularly maternal emotional and sexual abuse, are predictive of offspring symptoms of psychopathology, with potentially varying impacts of different types of adversity. We also examined whether variation in the composition of the infant gut microbiome may moderate these intergenerational associations. Although limited evidence supported this hypothesis, we found that the significant association between maternal ACEs and offspring internalizing symptoms was marginally attenuated in offspring with a greater relative abundance of the protective bacteria *Lactobacillus.* In a separate study, we examined whether offspring epigenetic aging – a measure of DNA methylation differences that are associated with infant health outcomes – may moderate the association between maternal childhood trauma and offspring symptoms. In line with our hypothesis, we found that the significant associations between maternal childhood sexual abuse and offspring internalizing, externalizing, and posttraumatic stress symptoms were attenuated in offspring with accelerated epigenetic aging at birth. Taken together, these results suggest that individual differences in biological factors that relate to the development and regulation of the stress response may influence a child's susceptibility to stress, including the intergenerational impact of maternal stress. Findings from the current dissertation highlight the utility of better understanding the factors that contribute to these biological differences and whether targeting these modifiable processes could help interrupt the intergenerational transmission of trauma, particularly for those who are most profoundly impacted.

Risk and Resilience in the Intergenerational Impacts of Childhood Trauma Among Black Americans: Moderating Roles of Epigenetic Aging and the Gut Microbiome

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Tables and Figures

General Introduction

Chronically stressful or traumatic experiences can have a prolonged impact on psychosocial functioning and quality of life, and may result in the development of psychopathology including depression and posttraumatic stress disorder (PTSD). Moreover, increasing evidence suggests that the negative impacts of trauma may even extend to subsequent generations, as individuals with a maternal history of trauma demonstrate elevated symptoms of depression, PTSD, and other psychopathology (Plant et al., 2018). However, not all offspring of trauma-exposed mothers demonstrate these effects, and a clearer understanding of the factors that protect against the intergenerational effects of trauma is needed.

Epidemiological studies indicate that over 30% of Americans will experience depression, PTSD, or other stress- and trauma-related psychopathology in their lifetime (Kessler et al., 2005). Although the onset of clinically-significant disorders typically emerges in adolescence or early adulthood (Kessler et al., 2005), evidence suggests that symptomatology is often present far earlier in life. Elevated internalizing and externalizing symptoms during early childhood – commonly measured via the Child Behavior Checklist (CBCL/1.5-5) – have been shown to reliably predict the development of depression, PTSD, and psychopathology in later life (Hofstra et al., 2002; Petty et al., 2009). Moreover, recent evidence points to the utility of the CBCL/1.5-5 to measure posttraumatic stress symptoms in early childhood (Dehon & Scheeringa, 2006), which can capture early signs of emotion dysregulation, hyperarousal, and negative alterations to cognitions and mood. While no studies to date have examined the intergenerational impact of trauma on early childhood internalizing, externalizing, or posttraumatic stress symptoms, there is evidence that children with a maternal history of trauma demonstrate elevated rates of emotion dysregulation (Enlow et al., 2017; McDonnell & Valentino, 2016), heightened physiological

arousal (Jovanovic et al., 2011), and general symptoms of psychopathology (Bush et al., 2023). Building off these early findings, the current dissertation examines how maternal childhood trauma may influence offspring symptoms of psychopathology in early life to better elucidate the developmental factors that underlie the intergenerational effects of trauma.

It is particularly important to examine the intergenerational effects of trauma for Black Americans. Research indicates that Black Americans not only develop trauma-related psychopathology at higher rates but also experience more severe symptoms compared to White Americans (Roberts et al., 2011). These differences can be attributed to a variety of factors that reflect structural inequality in the United States, including socioeconomic disadvantage, racial discrimination, reduced access to mental healthcare services, and greater exposure to traumatic stress (Ellis et al., 2008; Loo, Fairbank, & Chemtob, 2005; Sibrava et al., 2019). In particular, Black Americans experience elevated rates of childhood adversity (Mersky & Janczewski, 2018; Roberts et al., 2011), which is a well-established risk factor for the later development of depression, PTSD, and other forms of psychopathology (Cloitre et al., 2005; Widom, 1999).

Together, this evidence highlights the importance of better understanding the factors that contribute to – and, more importantly, attenuate – the negative effects of childhood trauma and adversity for Black Americans. However, while studies of psychosocial factors have increasingly utilized more diverse samples, studies examining the biological factors that contribute to the development of psychopathology remain largely Eurocentric. The current dissertation provides a step towards reducing this disparity by examining whether and how two biological factors – epigenetic aging and gut microbiome composition – moderate the intergenerational impact of trauma in Black American mothers and children.

Research over several decades has sought to identify mitigating factors that are both protective against the negative effects of chronic or traumatic stressors and malleable through intervention. While much of this work has focused on modifiable psychosocial factors (e.g., social support, coping skills; Ozbay et al., 2007; Sinclair, Wallston, & Strachan, 2016), recent evidence suggests that biological systems such as the gut microbiome may be similarly protective and modifiable. The gut microbiome is intricately tied to brain functioning through a complex, multisystemic *gut-brain axis* (Osadchiy, Martin, & Mayer, 2019), and variation in gut microbiome composition has been associated with alterations to the stress response (Sudo et al., 2004) as well as depression, PTSD, and other psychological disorders (Dinan & Cryan, 2013; Leclercq, Forsythe, & Bienenstock, 2016). Importantly, experimental rodent studies have demonstrated that manipulation of the gut microbiome (via probiotics or gut microbiome transplants from one animal to another) can attenuate posttraumatic stress symptoms (e.g., anxiety) following early life trauma exposure (Liang et al., 2015). Further, developmental studies suggests that early childhood may be a particularly important period of intervention, as alterations to the gut microbiome in early life have been shown to reduce posttraumatic stress symptoms, while alterations in later life do not (Sudo et al., 2004). The current study combines and extends these findings by examining whether and how early life gut microbiome composition may protect against the intergenerational effects of childhood trauma. In turn, our findings may contribute to growing evidence supporting the gut microbiome as a potential target for clinical intervention, particularly for those at an increased risked for developing trauma-related symptomatology.

In addition to the gut microbiome, epigenetic processes have also been theorized to modulate the impact of chronic or traumatic stressors on the development of psychopathology.

Given that epigenetic processes influence the physiological systems that contribute to the link between stress and psychopathology (e.g., HPA-axis, inflammation), it has been proposed that differences in the epigenome may influence individual differences in susceptibility to stress (Al Jowf et al., 2021; Conradt et al., 2017; Smeeth et al., 2021). Although the interaction between epigenetic susceptibility and trauma exposure has not been examined in the context of intergenerational risk, evidence from intra-generational studies shows that epigenetic differences in genes associated with neuroendocrine functioning (Conradt et al., 2013) and depression risk (Smearman et al., 2016) moderate the relationship between early life stress and the development of psychopathology-related outcomes. As such, it is possible that epigenetic differences may explain why some offspring develop trauma-related symptomatology following maternal trauma exposure, while others do not. A clearer understanding of how epigenetic differences may exacerbate or buffer against the intergenerational effects of trauma would not only provide important insight for future research, but also hold important clinical implications. Increasing evidence suggests that early measurement of genetic – and, by extension, epigenetic – susceptibility may have clinical utility for identifying and intervening with children that are at higher risk for developing psychopathology, including trauma-related disorders (Torkamani, Wineinger, & Topol, 2018). However, there currently exist racial disparities surrounding the use of genetic and epigenetic measures for precision medicine (Martin et al., 2019). Given that the majority of large-scale genetic studies comprise individuals from predominantly European ancestries, far less is known about the factors that may contribute to increased risk for psychopathology in individuals of African ancestry (Duncan et al., 2019). The proposed study intends to examine how the offspring epigenome moderates the intergenerational impact of

trauma for Black American women and children in particular, as a step towards reducing inequities in clinical care and disparities in mental health outcomes.

Overall, there are several significant and innovative aspects of the current dissertation that would meaningfully contribute to the field. Together, these studies are the first to simultaneously examine the role of genetic factors for both the individual (i.e., DNA methylation) and their microbial community (i.e., the gut microbiome) on the relationship between maternal trauma and offspring symptoms of psychopathology. Further, they are among the first human studies to investigate how these factors influence symptomatology in early childhood, an important developmental period that can influence lifetime risk for psychopathology. Finally, no extant human study has examined these associations in an exclusively Black American sample. Understanding the interplay of these factors for Black American women and children is an important first step to understanding the broader racial disparities that exist for intergenerational impacts of childhood trauma on psychopathology.

Intergenerational and Early Life Associations of the Gut Microbiome and Stress-Related Symptomatology Among Black American Mothers and Children

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Abstract

Recent evidence suggests that maternal childhood adversity may have an intergenerational impact, with children of adversity-exposed mothers exhibiting elevated symptoms of psychopathology. At the same time, many children demonstrate resilience to these intergenerational impacts. Among the variety of factors that likely contribute to resilience, the composition of the gut microbiome may play a role in buffering the negative impacts of trauma and stress. The current study used a prospective, longitudinal design to test the novel hypothesis that offspring gut microbiome composition is a potential moderator in the relationship between maternal exposure to childhood adversity and offspring symptomatology (i.e., internalizing, externalizing, and posttraumatic stress symptoms). Maternal childhood adversity was self-reported during pregnancy via the Childhood Trauma Questionnaire and Adverse Childhood Experiences (ACEs) survey, and offspring symptomatology was assessed with the Child Behavior Checklist/1.5-5 when offspring were 2-4 years old. Offspring fecal samples were collected between these timepoints (i.e., during 6- to 24-month follow-up visits) for microbiome sequencing. Results indicated that maternal ACEs and the relative abundances of *Bifidobacterium*, *Lactobacillus*, and *Prevotella* were associated with offspring symptomatology. However, there was little evidence that microbial abundance moderated the association between maternal adversity and offspring symptoms. Overall, these findings further our understanding of how the gut microbiome associates with psychopathology, and informs future studies aimed at targeting modifiable factors that may buffer the intergenerational effects of childhood adversity.

Intergenerational and Early Life Associations of the Gut Microbiome and Stress-Related Symptomatology Among Black American Mothers and Children

The link between stress and psychopathology is well established, with particularly pronounced effects stemming from early life stress exposure (Heim & Binder, 2012). Childhood adversity is one of the most robust and pervasive risk factors for later psychopathology, with studies demonstrating increased risk for posttraumatic stress disorder (PTSD), depression, anxiety, and a variety of other adverse outcomes (Cicchetti & Toth, 2005). Moreover, emerging evidence indicates that, among women in particular, the prolonged impact of childhood adversity may not be limited to one's own lifetime but extend to future generations as well. Maternal childhood trauma has been associated with increased offspring risk for psychopathology across multiple levels of analysis, ranging from emotional and behavioral symptomatology (Plant et al., 2018) to physiological alterations that characterize PTSD and stress-related disorders (e.g., Buss et al., 2017, Daskalakis et al., 2021). Similarly, maternal adverse childhood experiences (ACEs) have been significantly associated with both symptoms of psychopathology (e.g., negative emotionality, behavioral dysregulation, internalizing and externalizing symptoms) and physiological correlates of psychopathology (e.g., cortisol, inflammatory cytokines, HPA-axis functioning, and epigenetic aging) in offspring (Cooke et al., 2021; Zhang et al., 2022). At the same time, many offspring demonstrate resilience to these intergenerational effects, spurring efforts to identify modifiable factors that can be protective in the context of intergenerational risk.

While a range of biological and environmental factors likely contribute to resilience to adversity, emerging evidence points to one important factor that warrants further investigation:

the gut microbiome (Leclercq et al., 2016). There is increasing support for a complex, bidirectional communication between the gut and the brain (commonly referred to as the *gut-brain axis*) that has been shown to play a role in the development of psychopathology (Foster, Rinaman, & Cryan, 2017). Moreover, early findings suggest that certain characteristics of the gut microbiome may be protective in the context of early childhood stress (Liang et al., 2015). The current study aims to examine whether and how variation in the gut microbiome may be protective against the impact of maternal childhood adversity, as a potential first step towards interrupting intergenerational risk for psychopathology.

1.1 The Gut Microbiome & Stress-Related Symptomatology

The gut microbiome contains thousands of microbe species, primarily represented by bacteria but also featuring viruses, fungi, and other microorganisms (Turnbaugh et al., 2007). Empirical evidence suggests that the composition of the gut microbiome – for example, microbial diversity and/or the abundance of certain bacteria relative to others – directly and indirectly modulates brain functioning, which in turn influences risk for psychopathology (Cryan et al., 2019). This modulation has been shown to occur through a bidirectional, multisystemic *gut-brain axis* involving the afferent nervous system (e.g., vagus nerve), immune system (e.g., regulation of inflammatory cytokines), limbic system (e.g., secretion and regulation of neurotransmitters, such as GABA and serotonin), and hypothalamic-pituitary-adrenal (HPA) axis (Osadchiy et al., 2019). Indeed, both rodent and human studies have demonstrated that changes to gut microbiome composition can lead to brain alterations that have been associated with symptoms of depression, anxiety, and PTSD (Bravo et al., 2011; Carbia et al., 2020; Tillisch et al., 2013).

Although there is a dearth of studies examining the role of the gut microbiome in the context of intergenerational stress, early *intra*-generational studies have provided evidence that the gut microbiome could play a moderating role in the association between adversity and psychopathology. For example, correlational human studies have found that, among adults exposed to trauma, the relative abundance of certain bacteria can distinguish individuals who develop PTSD from those who do not (Hemmings et al., 2017). Moreover, experimental studies have demonstrated that modification of the gut microbiome can attenuate the relationship between adversity and symptomatology. For example, rodent studies found that the association between early life stress and the development of stress-related symptoms (e.g., memory impairment, anxiety- and depressive-like behaviors) was reduced in rats that ingested a probiotic containing *Lactobacillus*, compared to rats that did not (Liang et al., 2015; Karen et al., 2021). Similar findings have been demonstrated for both rodents and humans among stress-exposed adults (Lew et al., 2019; Takada et al., 2016). Together these results suggest that certain bacteria or microbial compositions may be protective against the impact of early adversity. However, no human study to date has examined these relationships longitudinally, either intra- or intergenerationally.

1.2 Developmental & Cultural Factors

The human gut microbiome is established across the first few years of life (Matamoros et al., 2013; Palmer et al., 2007), and preliminary studies suggest that the brain may be particularly sensitive to variability in the microbiome during this developmental period. For example, an experimental study with germ-free mice (i.e., mice that lack any gut microbes) tested whether recolonizing the gut with protective bacteria such as *Bifidobacterium* could attenuate the impact of early life stress on the development of anxiety-like symptoms. Interestingly, results indicated

that introducing these bacteria in *early* life successfully attenuated the development of symptoms, while introducing the bacteria in *later* life did not (Sudo et al., 2004). Combined with evidence that brain development is especially sensitive to environmental and physiological exposures during the first years of life (Heijtz et al., 2016), these findings suggest that the gut microbiome may be a particularly important moderator of the link between childhood adversity and increased symptomatology during early development.

In addition to developmental timing, studies also suggest that the influence of the gut microbiome may vary according to racial and ethnic factors. The Human Microbiome Project found that racial/ethnic background was one of the strongest predictors of gut microbiome composition (Methé et al., 2012), and evidence suggests that a typical "healthy" gut microbiome composition is influenced by genetic, environmental, and dietary factors that vary across racial and cultural groups (Goodrich et al., 2014; Singh et al., 2017). Together, these findings highlight the importance of examining how gut microbiome composition may influence psychopathology within individuals of the same racial/ethnic background. Given consistent evidence that Black Americans are disproportionately exposed to childhood adversities and demonstrate elevated rates of posttraumatic stress symptomatology (Merskey et al., 2018; Roberts et al., 2011), the proposed study will specifically focus on this population to examine whether certain gut microbiome characteristics can ameliorate the intergenerational impact of childhood adversity.

1.3 The Present Study

The present study used a prospective, longitudinal design leveraging data from three related projects that comprise a sample of Black American mother-child dyads followed from pregnancy through three years postpartum: 1) The Pregnancy Study (Corwin et al., 2017), which examines the impact of social and environmental exposures on maternal and infant birth

outcomes, 2) The Infant Microbiome Study (Brennan et al., 2019), which focuses on maternal stress and the infant gut-brain axis in the perinatal period; and 3) The Environmental Influences on Child Health Outcomes Study (ECHO; Gillman & Blaisdell, 2018), which examines how biological, behavioral, and social factors relate to developmental outcomes in early childhood. We hypothesized that:

Hypothesis 1: Maternal childhood adversity (i.e., childhood trauma and ACEs) would be positively associated with offspring symptomatology (i.e., internalizing, externalizing, and posttraumatic stress symptoms).

Hypothesis 2: Offspring gut microbiome composition, as defined by microbial diversity and relative abundance of particular taxa, would be associated with offspring symptomatology. Specifically, alpha diversity, beta diversity, and the relative abundances of *Bifidobacterium* and *Lactobacillus* will each be negatively associated with offspring symptoms.

Hypothesis 3: The association between maternal childhood adversity and offspring symptomatology would be moderated by offspring gut microbiome composition. Specifically, microbial diversity and higher relative abundances of *Bifidobacterium* and *Lactobacillus* would attenuate the association between maternal childhood adversity and offspring symptoms.

Methods

2.1 Participants

Pregnant Black American women (n=106) were initially recruited from prenatal clinics at a public and private hospital within a large metropolitan city in the southeastern United States. Mothers first enrolled in the Pregnancy Study, where data was collected at two prenatal visits (typically during the second and third trimesters). After delivery, mothers and offspring were enrolled in the Infant Microbiome Study, which collected data at five time points across infants'

first 18 months of life (ages 1-week and 3-, 6-, 12- and 18-months). When the child reached two years of age, participants were invited to enroll in the ECHO Study, which conducted annual follow-up visits from ages two to five years. Inclusion criteria for the three studies included: 1) Black/African American race (via self-report); 2) Maternal age of 18-40 years; 3) Singleton pregnancy (verified by clinical record); 4) Maternal comprehension of written and spoken English; and 5) Absence of infant congenital disorders. Additional inclusion criteria for the current study included: 6) completion of at least one Pregnancy Study visit, 7) availability of microbiome data from at least one collection timepoint between 6- to 24- months, and 8) completion of an ECHO Study visit at 2- or 3-years. Sample characteristics are shown in Table 1.

2.2 Procedure

Study procedures were approved by Emory University's Institutional Review Board and informed consent was obtained for each participant at enrollment in the Pregnancy Study, Infant Microbiome Study, and ECHO Study. Data collection was conducted by trained laboratory staff in participants' homes or a laboratory setting. At the pregnancy visit, mothers self-reported on childhood trauma and ACEs. At the infancy and toddlerhood visits, fecal samples were collected from offspring for gut microbiome sequencing. At the toddlerhood visits, mothers reported on offspring symptomatology. Covariates relevant to gut microbiome composition (e.g., delivery mode, recent antibiotic use) and adversity (e.g., socioeconomic status) were collected at all time points.

2.3 Measures

2.3.1 Maternal Exposure to Trauma & Stress.

Childhood Trauma. Maternal childhood trauma was measured using the short form of the Childhood Trauma Questionnaire (CTQ; Berstein et al., 2003). The CTQ has 28 questions

Table 1. *Sample Characteristics and Descriptives*

	N = 106
Sociodemographic Characteristics	
Maternal Age	
Mean	25.66
Median [Min, Max]	25.00 [18, 35]
Maternal Education	
Some high school	6 (6%)
Graduated high school or GED	44 (42%)
Some college or technical school	36 (34%)
Graduated college	17 (16%)
Some graduate work or degree	3 (3%)
Maternal Marital Status	
Married/Cohabitating	17 (16%)
Single	89 (84%)
Delivery Mode	
Vaginal	74 (70%)
Cesarean	32 (30%)
Child Gestational Age at Birth	
Mean	38.74
Median [Min, Max]	39.10 [29.6, 41.4]
Child Sex	
Male	55 (52%)
Female	51 (48%)
Descriptives	
Maternal ACE total	
Mean	2.29
Median [Min, Max]	2.00 [0, 9]
Maternal CTQ total	
Mean	48.58
Median [Min, Max]	42 [28, 130]
CBCL Internalizing Score	
Mean	7.64
Median [Min, Max]	6 [0, 24.5]
CBCL Externalizing Score	
Mean	12.19
Median [Min, Max]	10.75 [0, 45.5]
CBCL Posttraumatic Stress Score	
Mean	3.74
Median [Min, Max]	3.00 [0, 10]
Ages at Data Collection	
Fecal Sample Collection Visit	
6 month	14 (13%)
12 month	6 (6%)
18 month	5 (5%)
24 month	81 (76%)
Age at Fecal Sample Collection (days)	
Mean	674.05
Median [Min, Max]	732 [179, 1016]

regarding experiences of emotional abuse, physical abuse, sexual abuse, emotional neglect, and physical neglect. Responses are rated on a 5-point Likert scale ranging from "1—Never True" to "5—Very Often True." Higher scores are associated with more severe neglect and abuse. The CTQ has been well validated in non-clinical and Black American samples (Liebschutz et al., 2018). Internal consistency for the CTQ total score in the current sample was high (Cronbach's $\alpha = 0.86$).

Adverse Childhood Experiences. Maternal experiences of childhood adversity were measured using a shortened form of the Adverse Childhood Experiences questionnaire (ACEs; Felitti et al., 1998), which eliminates items that overlap with the CTQ. The shortened form consists of ten items assessing adversities related to family dysfunction (e.g., mental illness, substance abuse, or suicidality within the household), parental loss (e.g., through divorce, imprisonment, death, or abandonment), and other childhood adversities (e.g., experiences of homelessness or foster care). Responses are rated in a yes/no format and items are coded as "0—Absent" or "1—Present." The total score is calculated by summing the items, with higher scores indicating more adverse experiences. Internal consistency in the current sample was adequate (Cronbach's $\alpha = 0.66$).

2.3.2 Offspring Symptomatology. Internalizing, externalizing, and posttraumatic stress symptoms were measured using the Child Behavior Checklist for Ages *1.5-5* (CBCL/1.5-5), a standardized form in which mothers report their children's behavioral and emotional symptoms (Achenbach & Ruffle, 2000). The CBCL/1.5-5 contains 100 items in which the mother indicates the option that best describes her child *now or within the past 2 months* with one of the following: 0 = *not true (as far as you know)*; 1 = *somewhat or sometimes true*; 2 = *very true or often true*. The internalizing symptoms score reflects the sum of 36 of these items, with possible

scores ranging from 0 to 72, and the externalizing symptoms score reflects the sum of 24 items, with possible scores ranging from 0 to 48. The posttraumatic stress symptoms scale is based on a sum of 15 items (Supplemental Table 1; Dehon & Scheeringa, 2006), with scores ranging from 0 to 30. The CBCL is a well-established measure of child emotional and behavioral concerns and demonstrates strong test-retest in ethnically- and socioeconomically-diverse samples (e.g., Ivanova et al., 2010). The 15-item posttraumatic stress symptoms subscale demonstrated adequate internal consistency within the current sample (Cronbach's $\alpha = 0.62$).

2.3.3 Gut Microbiome Composition. Mothers were instructed to collect offspring fecal samples according to protocols outlined by the Human Microbiome Project using a field-tested Stool Collection kit. Briefly, the kit contained three CatchAll swabs, MoBio tubes, and plastic biohazard bags. Mothers collected three CatchAll swabs of infant/toddler stool from a single diaper (plunging the swab into the stool in the diaper), placed each stool-coated swab in a plastic biohazard bag, and stored the samples in a home freezer. Mothers or lab staff transported the samples in an insulated bag to Emory, where lab staff transferred CatchAll swabs into pre-labelled MoBio tubes and placed all tubes in a -80 freezer for storage prior to assay. Laboratory assay and data processing procedures are described in detail in the Supplementary Materials. After processing, 54 offspring (50.9% of the total sample) had viable microbiome data from only the 24-month visit. Therefore, for offspring with viable microbiome data from multiple infancy visits, the oldest sample from age 6-24 months was selected to maximize developmental consistency across samples. We then removed samples with <300 read counts across all taxa, which resulted in a final sample of 106 offspring with data from 14 offspring at 6-months, six offspring at 12-months, five offspring at 18-months, and 81 offspring at 24-months (Table 1).

Finally, we removed taxa with 0 read counts, which resulted in a total of 114 taxa represented across the 106 samples (Figure 1).

2.4 Statistical Analysis.

Analyses were performed in R version 4.1.3. All analyses adjusted for covariates that have been previously associated with the infant microbiome: maternal SES, maternal age, maternal prenatal BMI, mode of delivery, gestational age at birth, breastfeeding status (at three months), offspring sex, visit time point and offspring age at stool collection, antibiotic use in the preceding two weeks, and illness in the preceding two weeks. Complete data was available for all covariates except breastfeeding status, which was missing for 25 infants. This data was imputed using predictive mean matching via the MICE package (Van Buuren & Groothuis-Oudshoorn, 2011).

Within the Phyloseq package (version 1.38.0), we used the *tax_glom* function to merge taxa classified at the level of species to calculate the corresponding genus level abundances (Figure 1). Alpha diversity – the diversity of genera represented in an individual's microbiome (i.e., *within*-subject diversity) – was analyzed using Shannon index. While other measures of alpha diversity exist, including the commonly used Simpson index, we selected Shannon index given its function in evaluating species richness, rather than evenness, as well as evidence that Shannon index has demonstrated more consistent and significant associations with psychopathology-related outcomes (Kuo & Chung, 2019). Beta diversity – the dissimilarity of an individuals' microbiome compared to others' (i.e., *between*-subject diversity) – was measured using two-dimensional principal coordinates (PC1 and PC2) obtained from the *ordinate* function with non-metric multidimensional scaling (NMDS) using the Bray-Curtis method. Robust linear

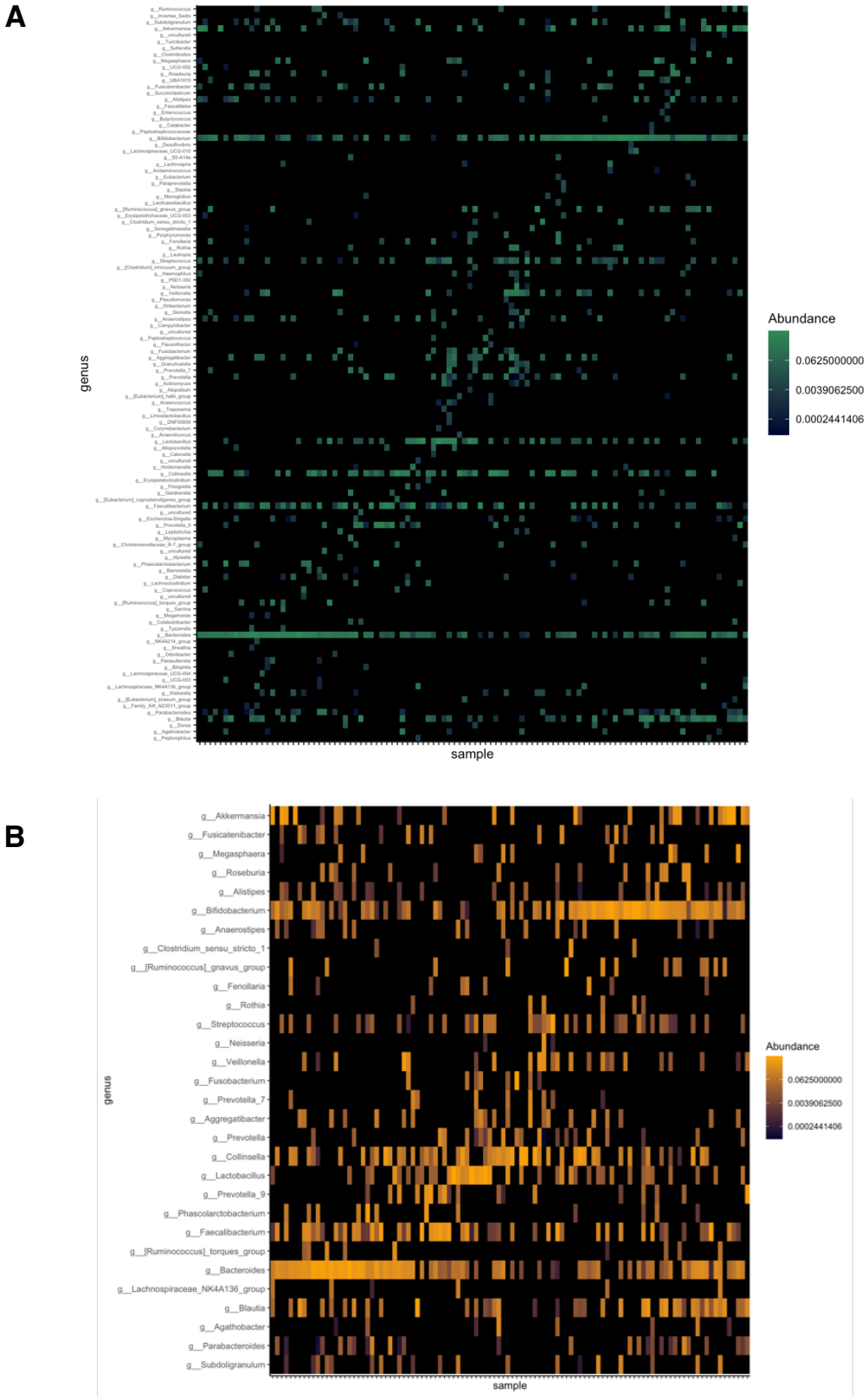

Figure 1. *Abundances of all (A) and the top 30 (B) genera represented in the sample (n=106).*

regressions were used to test for associations between diversity variables (i.e., Shannon index, PC1, PC2) and offspring symptomatology, controlling for covariates.

Robust linear regressions were also used to test our *a priori* hypotheses that maternal adversity would be positively associated with offspring symptoms (Hypothesis 1), and that *Bifidobacterium* and *Lactobacillus* would be negatively associated with offspring symptoms (Hypothesis 2) and would buffer the impact of maternal adversity on offspring symptoms (Hypothesis 3). Power analyses indicated that the main effect analyses were adequately powered ($f^2 > 0.059$, power = 0.8, $p = 0.05$) and the interaction analyses were adequately powered to detect medium and large effects but potentially underpowered to detect small effects ($f^2 > 0.08$, power = 0.8, $p = 0.05$). Given that the expected interaction effect sizes are not well established, we chose to report both significant and marginally significant interaction findings. Significant and marginally significant interactions were further probed to identify directionality of moderating effects.

Finally, we used the linear decomposition model (LDM; Hu and Satten, 2020) to explore whether the relative abundances of other genera (beyond *Bifidobacterium* and *Lactobacillus*) were associated with offspring symptomatology. LDM is a permutation-based analysis that can accommodate clustered data while maintaining validity for small sample sizes when it subjected to over-dispersion. In the LDM test, we attained FDR adjusted and unadjusted p-values < 0.05 for taxa that were individually associated with the outcome of interest before and after controlling for false discovery rate (FDR) at the genus level. Given the exploratory nature of this analysis, we chose to report both significant and marginally significant findings to inform more focused hypothesis-testing in future studies.

Results

3.1 Intergenerational Association of Maternal Adversity and Offspring Symptomatology

First, we examined whether maternal experiences of childhood adversity were intergenerationally associated with offspring symptomatology, after adjusting for maternal SES, maternal age, offspring age, and offspring sex. Results indicated that maternal ACEs were significantly associated with offspring internalizing symptoms (t=2.31, p=0.02; Figure 2a), externalizing symptoms (t=2.49, p=0.01; Figure 2b), and posttraumatic stress symptoms (t=2.20, p=0.03; Figure 2c). Maternal childhood trauma was not significantly associated with internalizing symptoms (t=1.86, p=0.065), externalizing symptoms (t=1.41, p=0.16), or posttraumatic stress symptoms (t=1.57, p=0.12).

3.2 Association of Offspring Gut Microbiome Composition and Offspring Symptomatology

Next, we examined the main effects of offspring gut microbiome composition on offspring symptomatology. Results indicated that offspring gut microbiome alpha diversity (i.e., Shannon index) was not significantly associated with internalizing symptoms, externalizing symptoms, or posttraumatic stress symptoms (Figure 3). Beta diversity PC1 was also not associated with internalizing (t=0.75, p=0.45), externalizing (t=1.56, p=0.12), or posttraumatic stress (t=1.34, p=0.18) symptoms, and beta diversity PC2 was not associated with internalizing symptoms (t=-1.39, p=0.17) or posttraumatic stress symptoms (t=-1.83, p=0.07). However, beta diversity PC2 was significantly associated with externalizing symptoms (t=-2.08, p=0.04) such that lower PC2 values were associated with greater symptomatology. To better understand how beta diversity PC2 represents microbial composition, we calculated the correlation coefficients between PC2 coordinates and the relative abundance of the genera hypothesized to contribute to offspring outcomes (i.e., *Bifidobacterium* and *Lactobacillus*). Beta diversity PC2 was not

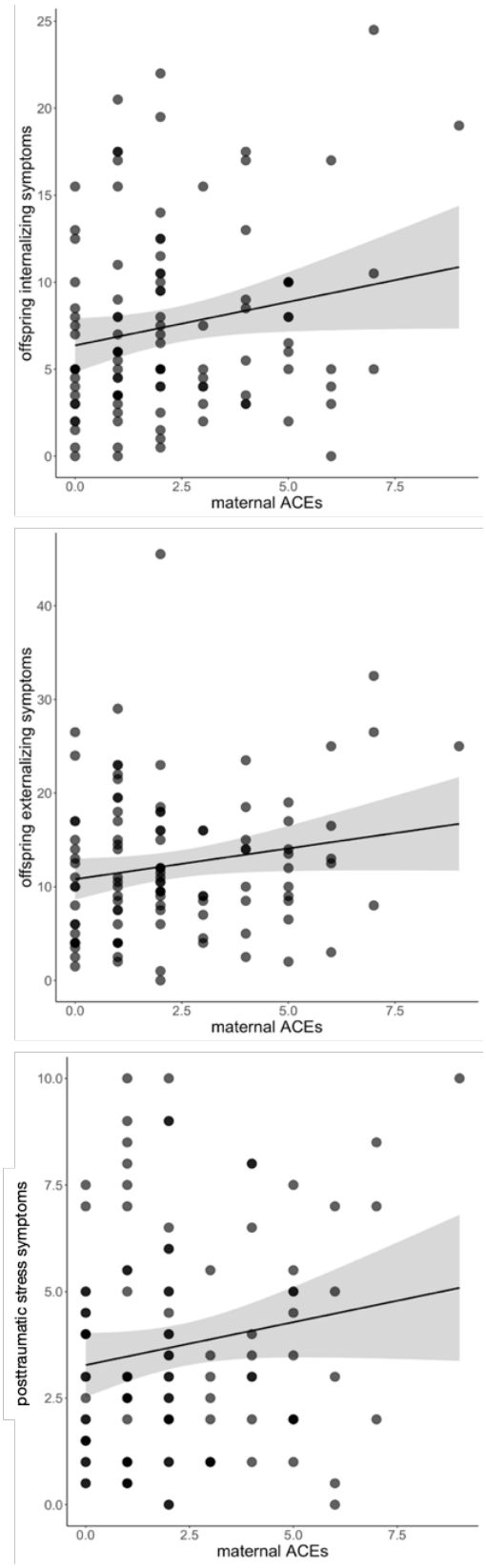

Figure 2. *Maternal ACEs were intergenerationally associated with offspring symptomatology.*

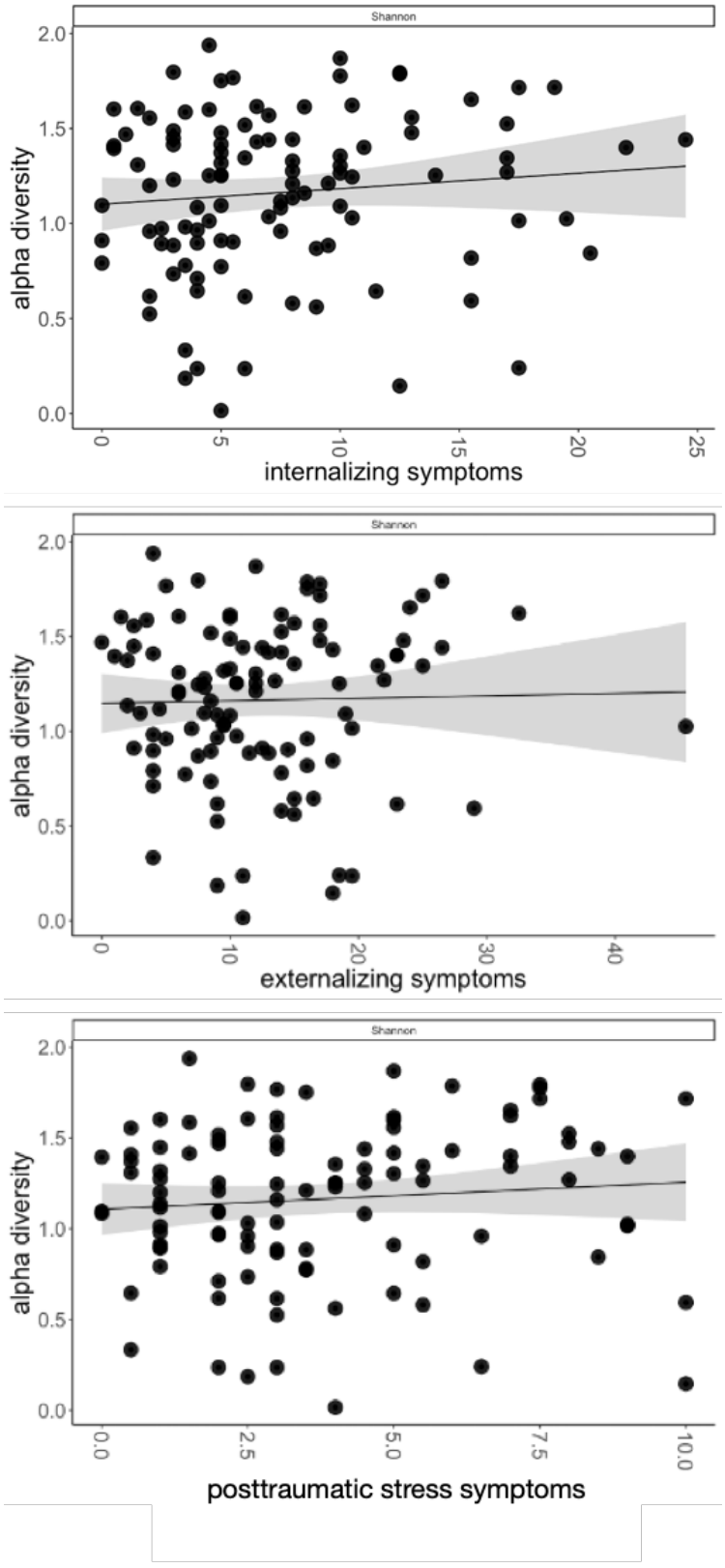

Figure 3. *Shannon's alpha diversity was not significantly associated with internalizing (t=1.2, p=0.22), externalizing (t=0.44, p=0.66), or posttraumatic stress (t=1.08, p= 0.28) symptoms.*

associated with *Lactobacillus* (r=-0.15, p=0.12) but demonstrated a strong positive association with *Bifidobacterium* (r=0.65, p<0.001) relative abundance. Together, these beta diversity results suggest that infant microbiomes characterized by low relative abundance of *Bifidobacterium* are associated with greater externalizing symptoms.

Next, we examined whether the relative abundance of *Bifidobacterium* and *Lactobacillus* influenced offspring symptomatology directly. Results indicated that a greater relative abundance of *Bifidobacterium* was associated with significantly lower externalizing symptoms (t=-2.14; p=0.02; Figure 4a) and posttraumatic stress symptoms (t=-1.98; p=0.03; Figure 4a). A greater relative abundance of *Lactobacillus* was significantly associated with greater externalizing symptoms (t=2.81; p=0.01; Figure 4a). All other associations were nonsignificant (including two marginally significant associations; Figure 4c).

Using LDM, we then conducted exploratory analyses to identify genera whose relative abundance was significantly associated with offspring symptomatology. Although no relationships were significant after FDR correction, results indicated that a greater relative abundance of *Prevotella* was significantly associated with higher internalizing symptoms (p=0.03; Figure 4b) and marginally associated with higher posttraumatic stress symptoms (p=0.08; Figure 4b), and a greater relative abundance of *Prevotella 7* was marginally associated with higher externalizing symptoms (p=0.06; Figure 4b). Results also corroborated the associations between *Bifidobacterium* and *Lactobacillus* and offspring posttraumatic stress symptoms reported above. LDM findings are illustrated in Figure 5.

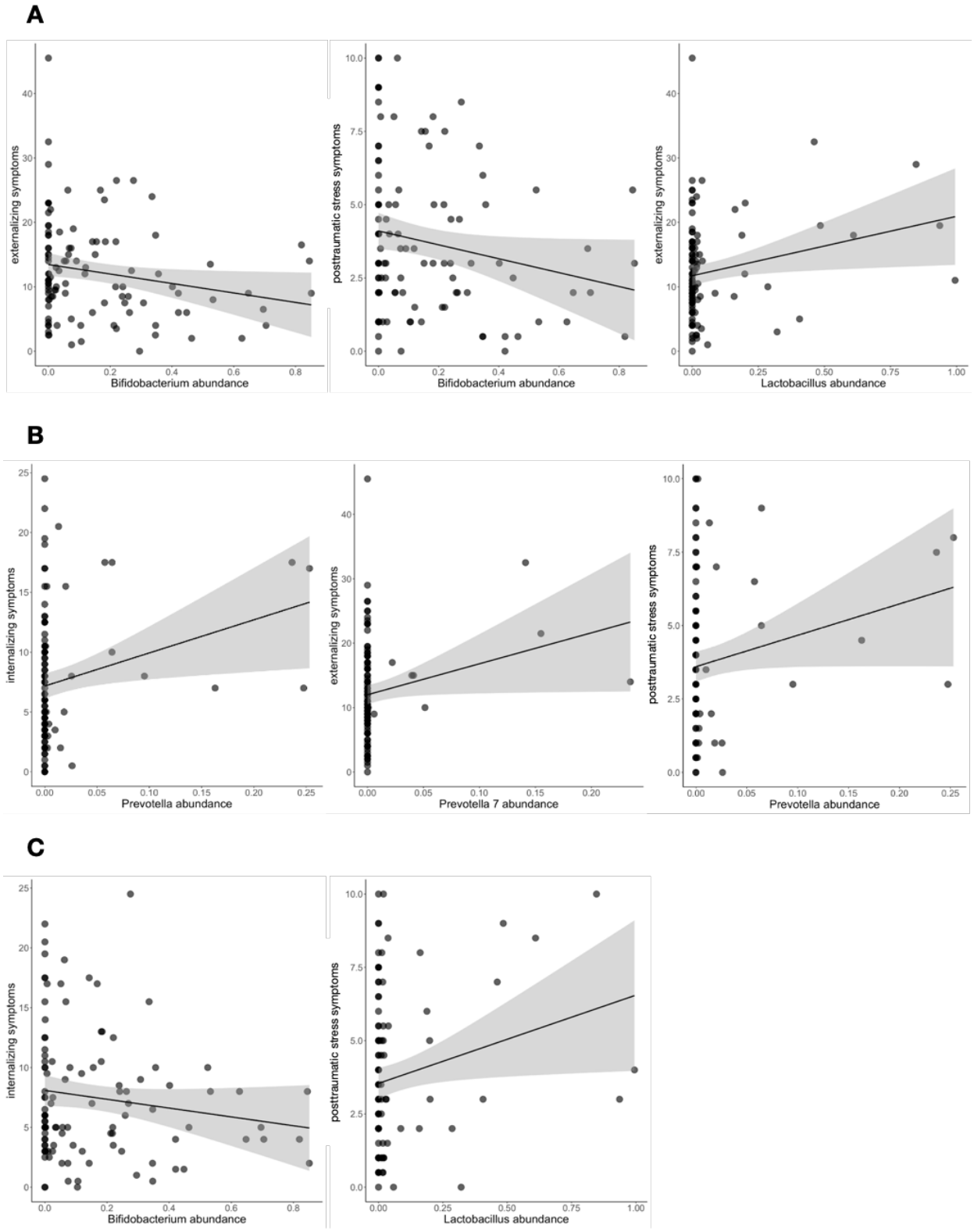

Figure 4. *Significant a priori hypothesis results are demonstrated in (A). Exploratory hypothesis results are demonstrated in (B). Marginally significant (p<0.10) findings from a priori hypotheses are demonstrated in (C).*

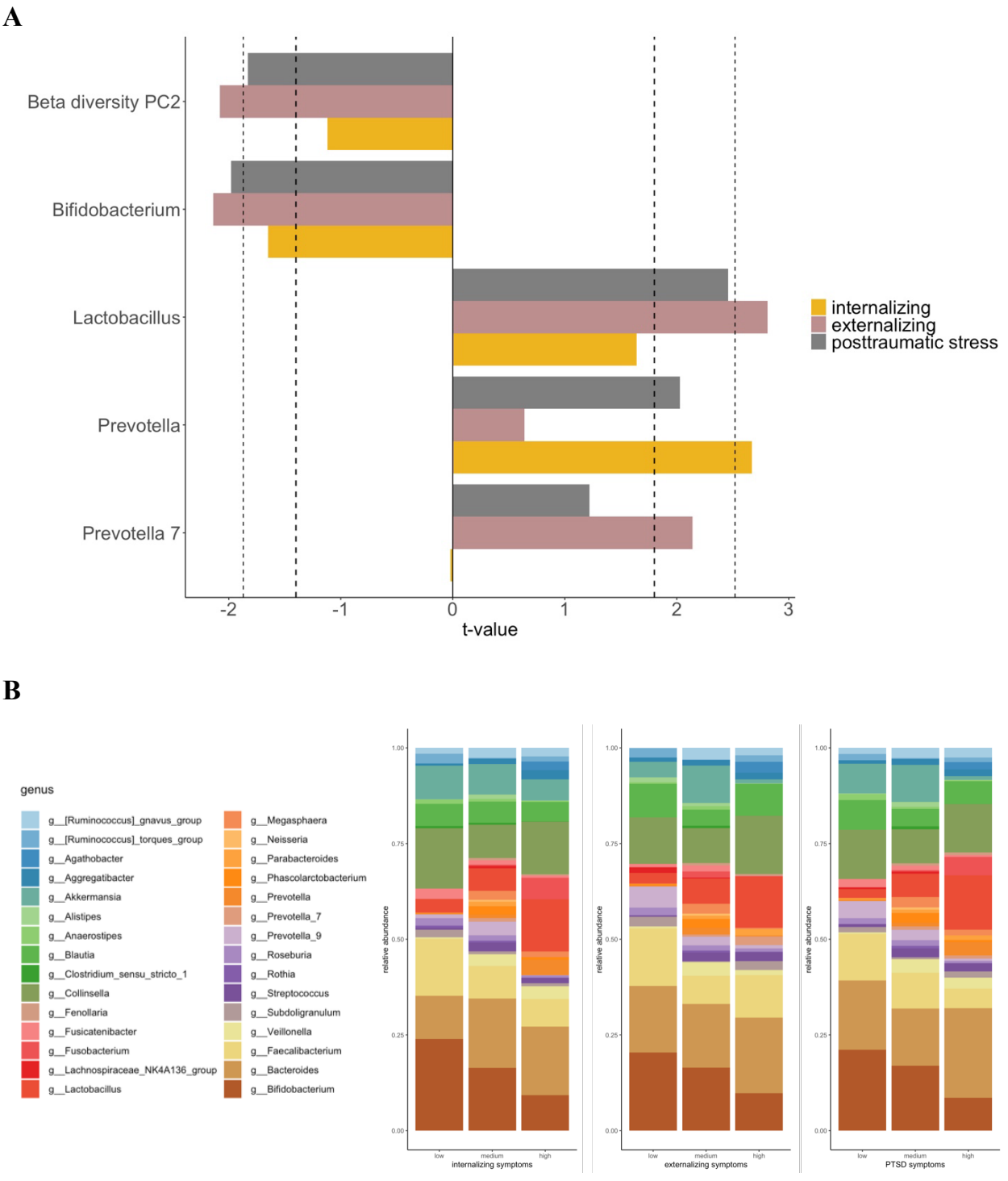

Figure 5. (A) *Associations between infant gut microbiome composition and symptomatology. Thick dotted lines indicate threshold for marginal significance (p<0.10), thin dotted lines indicate threshold for statistical significance (p<0.05).* (B) *Relative abundances of top 30 genera across low, medium, and high levels of symptomatology.*

3.3 Does Infant Gut Microbiome Composition Buffer Associations between Maternal Adversity and Offspring Symptomatology?

Finally, we examined whether the composition of the infant gut microbiome buffered the association between maternal childhood adversity and offspring symptomatology. Results indicated that a greater relative abundance of *Lactobacillus* may attenuate the impact of maternal ACEs on offspring internalizing symptoms, although the interaction was only marginally significant (t=-1.66, p=0.1). Upon plotting the interaction, it was apparent that the positive association between maternal ACEs and offspring internalizing symptoms was significant at low levels of *Lactobacillus* but was attenuated at higher levels of *Lactobacillus* (see Figure 6). All other interactions were nonsignificant.

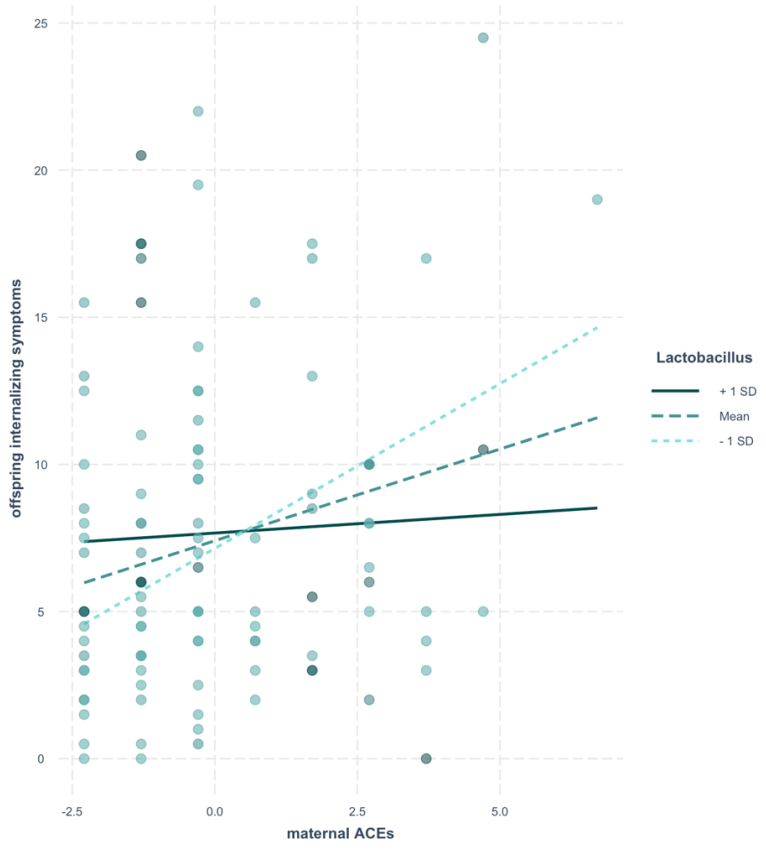

Figure 6. *The association between maternal ACEs and offspring internalizing symptoms was significant at low levels of* Lactobacillus *(light dotted line) but marginally attenuated at higher levels of* Lactobacillus *(dark solid line).*

Discussion

The current study is the first to show that variation in the gut microbiome during early life is associated with internalizing, externalizing, and posttraumatic stress symptoms in a community sample of Black American children. Moreover, we are the first to investigate whether greater microbial diversity or a higher relative abundance of protective bacteria may buffer the intergenerational effects of maternal trauma on offspring symptomatology. While we do not aim to establish causality in the present study, these findings offer an important first step towards identifying when and how the gut microbiome could serve as an early intervention target to reduce the impact of adversity on child psychopathology.

Our results indicated that alpha diversity (i.e., within-subject microbial richness) was not associated offspring symptomatology. While the early assumption was that microbial richness is indicative of gut health, our findings add to the growing evidence that assessing the health of the gut microbiome is more complex than the relatively reductive measure of microbial richness (Carlson et al., 2018; Nikolova et al., 2021; Sanada et al., 2020; Sordillo et al., 2019). Our findings did, however, indicate that beta diversity (i.e., between-subject diversity) may differentiate children with and without elevated symptomatology. Children with negative beta diversity PC2 values demonstrated greater externalizing symptoms, and secondary analyses indicated that negative PC2 values were characterized by low levels of *Bifidobacterium*. These associations aligned with our findings that externalizing symptoms were negatively associated with the relative abundance of *Bifidobacterium*. These results may be interpreted in several ways. First, it is possible that high levels of *Bifidobacterium* cause (or serve as a proxy for) greater microbial diversity, which in turn is protective. Alternatively, it may be the case that beta diversity PC2 values serve as a proxy for the relative abundance *Bifidobacterium,* which would

be consistent with prior studies that have found the relative abundance of *Bifidobacterium* to predict symptomatology even when beta diversity was not a significant predictor (e.g., Pulikkan et al., 2018). Bacteria within the *Bifidobacterium* genus are largely regarded as "protective" bacteria (despite some inconsistencies; see Nikolova et al., 2021), with experimental animal studies, correlational human studies, and human clinical trials demonstrating that *Bifidobacterium* is negatively associated with psychopathology (e.g., major depression, anxiety, ASD) as well as symptoms and correlates of psychopathology (Aizawa et al., 2016; Akkasheh et al., 2016; Groen et al., 2018, Pinto-Sanchez et al., 2017; Sarkar et al., 2016). Our findings extend this evidence by demonstrating that higher levels of *Bifidobacterium* predict lower symptoms of psychopathology during the sensitive period of early development.

Our results supported the a priori hypothesis that the relative abundance of *Lactobacillus* would predict offspring symptomatology. However, the direction of these associations was unexpected. Species within the *Lactobacillus* genus have generally been considered "protective" given evidence from intervention studies that probiotics containing strains of *Lactobacillus* can alleviate symptoms of depression, anxiety, and other forms of psychopathology (e.g., Foster & McVey Neufeld, 2013; Messaoudi et al., 2011). However, our study found that *greater* levels of *Lactobacillus* were associated with greater externalizing and (marginally) posttraumatic stress symptoms. While these results conflict with the general conception, they are not the first to suggest that greater levels of *Lactobacillus* may be detrimental in certain contexts. In fact, a recent review and meta-analysis found a positive association between *Lactobacillus* and adult psychopathology (i.e., major depressive disorder, schizophrenia; Nikolova et al., 2021) and a case-comparison study of children ages three to 16 found that children with autism spectrum disorder (ASD) demonstrate greater levels of *Lactobacillus* compared to healthy controls

(Pulikkan et al., 2018). Importantly, none of these studies examined relative abundance at the species level, and it is possible that different species within the Lactobacillacae family and *Lactobacillus* genus play differential roles such that some species are protective while others confer risk (Pulikkan et al., 2018). While we were not able to examine this in the present study, it is an important question for future studies that are able to differentiate bacteria at the species level. Alternatively, it may be the case that more abundant *Lactobacillus* is protective at certain developmental stages but not others. For example, it is developmentally typical for *Lactobacillus* to be in higher abundance during infancy when the diet is high in lactate (i.e., Pulikkan et al., 2018). Typically, as infants age and expand their diet, the relative abundance of *Lactobacillus* declines and the composition of the gut becomes more diverse. As such, it may be that high *Lactobacillus* that persists past infancy (as 80% of our sample was 18-24 months old) represents an immature or delayed gut microbiome, which could confer risk for externalizing and posttraumatic stress symptoms.

Our exploratory findings indicated that bacteria in the Prevotellacae family may also confer risk for psychopathology in early life. Specifically, higher levels of *Prevotella* were significantly associated with greater internalizing symptoms and marginally associated with greater posttraumatic stress symptoms, and higher levels of *Prevotella 7* were marginally associated with greater externalizing symptoms. While *Prevotella* are generally regarded as commensal bacteria (i.e., protective against invasive species and beneficial to overall health), there is increasing evidence that *Prevotella*-rich microbiomes are associated with increased inflammation (Iljazovic et al., 2021) and neurological markers of psychopathology (Tillisch et al., 2017). It has been suggested that *Prevotella* may be generally protective at low levels, but an overabundance past a certain threshold may influence physiological systems in a way that

confers risk for psychopathology (Iljazovic et al., 2021). It is also possible that, similar to with *Lactobacillus*, different species within the *Prevotella* and *Prevotella 7* genera may have differential functions such that certain bacteria are protective while others are detrimental.

Finally, we were interested in examining whether and how the infant gut microbiome may buffer the impact of early life stress on the development of psychopathology, with a particular focus on intergenerational stress. Our results indicated that maternal adverse childhood experiences (ACEs) were positively associated with offspring early life symptomatology across all domains (i.e., internalizing, externalizing, and posttraumatic stress symptoms). We also found a marginally significant buffering effect of *Lactobacillus* on the relationship between maternal ACEs and offspring internalizing symptoms. This potential buffering effect for internalizing symptoms was interesting, given that the relative abundance of *Lactobacillus* was associated with *increased* risk for externalizing and PTSD symptoms when examining main effects. It may be that *Lactobacillus* is not universally beneficial but may be useful as prevention or intervention for high-risk individuals. Indeed, an experimental rodent study found that the association between early life stress and posttraumatic stress symptoms was reduced in rats that ingested a probiotic containing *Lactobacillus*, compared to rats that did not (Liang et al., 2015). Similarly, a study examining both rodent and human models found that probiotics containing strains of *Lactobacillus* relieved stress-associated symptoms in subjects exposed to psychosocial stressors (Takada et al., 2016). However, we hesitate to overinterpret a marginally significant finding and these speculations require a more thorough examination from future studies that are better powered to detect small interaction effects.

4.1 Limitations and Future Directions

Our findings must be considered in the context of several limitations. First, due to insufficient data at each fecal sample timepoint (i.e., 6, 12, 18, and 24 months), we chose to combine data across timepoints to maximize sample size. While this strengthened the statistical power of our study, it may have also introduced noise given that the composition of the gut microbiome is relatively dynamic during this developmental period (Sordillo et al., 2019). It should also be noted that, while our sample size was large compared to most human microbiome studies, we lacked statistical power to detect small effects, particularly interaction effects. In addition, the use of 16S rRNA sequencing limited our ability to determine the specificity and functional relevance of our findings. Future studies utilizing metagenomic sequencing will yield better insight into how species-level (rather than genus-level) differences may contribute to differences in early risk for psychopathology and will allow for examination of the functional pathways impacted by these taxonomic differences. Finally, we relied solely on maternal report to assess offspring posttraumatic stress symptoms. Future research would benefit from including additional measures of offspring posttraumatic stress symptoms such as a standardized diagnostic assessment or physiological measures (e.g., startle responsivity) to corroborate our intergenerational findings.

4.2 Conclusion

Our findings add to the growing literature that gut microbiome composition during the sensitive period of early life is associated with symptoms of psychopathology later in development. However, despite early evidence and theory that a “healthy” gut microbiome may protect against the impacts of early life stress, there was limited evidence that variation in the infant gut microbiome buffers the intergenerational impacts of maternal childhood adversity. Overall, these findings further our understanding of how the gut microbiome associates with the

development of psychopathology, and informs future studies aimed at targeting modifiable factors that may buffer the intergenerational effects of childhood adversity.

Infant Epigenetic Aging Moderates the Link Between Maternal Childhood Trauma and Offspring Symptoms of Psychopathology

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Abstract

Increasing evidence suggests that maternal childhood trauma may have an intergenerational impact, with children of trauma-exposed mothers exhibiting elevated symptoms of psychopathology. At the same time, many children demonstrate resilience to these intergenerational impacts. Among the variety of factors that likely contribute to resilience, epigenetic processes have been suggested to play a role in attenuating the negative impacts of trauma and stress. The current study used a prospective, longitudinal design to test the novel hypothesis that offspring epigenetic aging – a measure of methylation differences that are associated with infant health outcomes – may moderate the relationship between maternal exposure to childhood adversity and offspring symptomatology (i.e., internalizing, externalizing, and posttraumatic stress symptoms). Maternal childhood adversity was self-reported during pregnancy via the Adverse Childhood Experiences (ACEs) survey and the Childhood Trauma Questionnaire, which assessed total childhood trauma as well as maltreatment subtypes (i.e., emotional, physical, and sexual abuse). Offspring blood samples were collected at or shortly after birth for methylation sequencing, and offspring symptomatology was assessed with the Child Behavior Checklist/1.5-5 when offspring were 2-4 years old. Results indicated that maternal childhood trauma, particularly emotional and sexual abuse, was predictive of offspring internalizing and posttraumatic stress symptoms (*p*s = 0.001 to 0.04). However, the associations between maternal sexual abuse and offspring symptomatology were significantly attenuated in offspring with accelerated epigenetic aging at birth. Overall, these findings further our understanding of how epigenetic processes may contribute to the link between stress and psychopathology, and informs future studies aimed at targeting modifiable factors that may buffer the intergenerational effects of childhood adversity.

Infant Epigenetic Aging Moderates the Link Between Maternal Childhood Trauma and Offspring Symptoms of Psychopathology

Exposure to childhood trauma is a prominent risk factor for the development of psychopathology (Cicchetti & Toth, 2005), and recent evidence suggests that the risk conferred by childhood trauma may even carry into future generations (Plant et al., 2018). At the same time, there are significant individual differences in people's susceptibility to childhood or intergenerational trauma. While roughly 40% of youth are exposed to childhood trauma or adverse childhood experiences (Crouch et al., 2019; Kessler et al., 2010) – and similar rates are estimated for maternal childhood trauma exposure (Plant et al., 2018) – only an estimated 5-20% go on to develop depression, posttraumatic stress disorder (PTSD), or other clinically significant disorders (Bromet et al., 2011; Kilpatrick et al., 2013). However, the biological factors that contribute to these differences in susceptibility remain unclear. To better understand potential protective mechanisms, the present study examines how epigenetic changes that reflect differences in biological aging may confer vulnerability or protection against the intergenerational impacts of maternal childhood trauma and adverse childhood experiences (ACEs).

Intergenerational Effects of Childhood Adversity

A broad body of literature has examined the detrimental impacts of childhood trauma – comprising experiences of maltreatment (i.e., emotional, physical, and sexual abuse) and neglect – as well as ACEs, which include non-traumatic stressors such as family dysfunction (e.g., parental substance abuse, parental incarceration). Trauma and adversity during childhood have prolonged consequences that not only increase risk for psychopathology in the individual but

also in their offspring (Su et al., 2022). Offspring of mothers who experienced childhood trauma demonstrate increased risk for psychopathology across multiple levels of analysis, ranging from emotional and behavioral symptomatology (Plant et al., 2018) to physiological alterations that characterize mood, trauma, and stress-related disorders (e.g., Buss et al., 2017, Daskalakis et al., 2021). Similarly, maternal ACEs have been significantly associated with both symptoms of psychopathology (e.g., negative emotionality, behavioral dysregulation, internalizing and externalizing symptoms) and physiological correlates of psychopathology (e.g., cortisol, inflammatory cytokines, HPA-axis functioning) in offspring (Cooke et al., 2021; Zhang et al., 2022). As such, it has been proposed that this intergenerational risk may be transmitted through alterations to the mother's physiological stress response, which influences the fetal development of her offspring's stress response to increase their sensitivity to environmental stressors and their risk for psychopathology (Babenko et al., 2015; Buss et al., 2017). Additionally, mothers with a history of childhood adversity have higher rates of perinatal psychopathology (e.g., depression, PTSD), which can influence mother-child attachment, parenting practices, and offspring exposure to childhood adversity, all of which confer risk for the development of psychopathology in offspring (Plant et al., 2018).

Interestingly, evidence from intra-generational studies suggests that the mechanisms underlying the links between childhood adversity and the development of psychopathology may differ according to the type of trauma exposure. For example, while childhood emotional, physical, and sexual abuse often cooccur and have generalized effects on the physiological mechanisms that confer risk for psychopathology (Cassier et al., 2018; Finkelhor et al., 2007; Noll et al., 2022), there is also increasing evidence that trauma subtypes differentially impact physiological and behavioral outcomes. Neuroendocrine studies, for instance, have found that

different forms of childhood maltreatment are associated with different patterns of HPA-axis activation. Findings from a recent study demonstrated that physical abuse was associated with greater HPA-axis activation following a stressor while emotional abuse was associated with lower activation but a longer recovery to baseline (Kuhlman et al., 2015). The authors posited that physical abuse may cause HPA-axis adaptations that make an individual more sensitive and reactive to threats in the environment, while emotional abuse may result in adaptations that lead to a more subtle but chronically activated stress response, which in turn may confer greater risk for depression and other psychopathology (Kuhlman et al., 2015). In line with this interpretation, some studies have demonstrated that childhood emotional abuse is more strongly associated with depressive symptoms compared to physical or sexual abuse (Infurna et al., 2016; Mandelli et al., 2015; Paul et al., 2015). However, the literature is mixed, as other studies have found that physically and sexually abused children are at a greater risk for depression (Cicchetti & Valentino, 2006). Moreover, no studies to date have examined the differential impact of trauma subtypes on *intergenerational* outcomes. This is an important next step given the shared social and physiological mechanisms underlying intra- and intergenerational transmission (Babenko et al., 2015; Buss et al., 2017).

Individual Differences in Intergenerational Transmission

Despite the increased risk conferred by maternal childhood trauma exposure, not all offspring of trauma-exposed mothers develop symptomatology. One factor that may contribute to these individual differences is offspring sex. Population studies show that many trauma-related outcomes – including depression, anxiety, and PTSD – are more prevalent in females than males (Kessler et al., 2005; Perkonigg et al., 2000), and evidence suggests that this is particularly the case in the context of childhood trauma (Breslau et al., 1997). However, a recent

review found that male offspring may be more sensitive to maternal stress and/or HPA-axis dysregulation during fetal development compared to female offspring (Sutherland & Brunwasser, 2018). Although a dearth of studies has examined offspring sex differences in the context of maternal trauma exposure prior to pregnancy, it has been recognized as an important area of future research (Buss et al., 2017).

There is increasing evidence that epigenetic factors may also influence susceptibility to childhood trauma exposure. Epigenetic factors refer to environmentally sensitive molecular changes that alter the expression of genes without changing the genetic sequence itself. These changes include histone modifications, changes to non-coding RNAs, and, most widely studied, DNA methylation at specific sites (referred to as CpG sites) that impact gene transcription. A variety of environmental factors have been shown to modify DNA methylation including nutrition, physical health, ecological exposures, and even psychological stressors such as trauma (Quach et al., 2017; Zannas et al., 2015). Indeed, epigenetic mechanisms have been proposed as a key molecular pathway linking childhood trauma exposure to the development of psychopathology (Nöthling et al., 2020). Epigenetics have also been suggested as a mechanism of intergenerational transmission (Daskalakis et al., 2021; Yehuda & Lehrner, 2018), although there remains limited evidence in humans that maternal exposure to stress has a direct impact on her offspring's epigenome (Smeeth et al., 2021). Nevertheless, recent studies suggest that differences in the offspring epigenome may influence differential susceptibility to maternal childhood trauma. In other words, in addition to mediating the link between trauma and psychopathology, epigenetic factors are also hypothesized to *moderate* the link between maternal childhood trauma and offspring psychopathology (Al Jowf et al., 2021; Conradt et al., 2017; Smeeth et al., 2021).

Although no studies to date have examined whether epigenetic factors moderate the intergenerational association between maternal *childhood* adversity and offspring psychopathology, findings from studies of maternal prenatal stress provide preliminary support for the moderating role of epigenetic factors. For example, one study found that methylation differences in *NR3C1*, a gene that influences neuroendocrine reactivity, moderated the relationship between maternal prenatal depression and infant self-regulation and health outcomes. Specifically, infants with high levels of methylation at a specific *NR3C1* CpG site were susceptible to maternal prenatal depression, while infants with low levels of methylation were protected (Conradt et al., 2013). Given the potential role of *NR3C1* in the development of psychopathology following maternal childhood trauma (Bowers & Yehuda, 2016), it is possible that similar effects may be demonstrated in the context of intergenerational trauma.

Epigenetic Age Acceleration

The above noted findings highlight the importance of considering epigenetic factors when evaluating susceptibility to maternal childhood trauma. However, based on evidence from genome-wide association studies that psychopathology stems from variation across hundreds (if not thousands) of genes (Duncan et al., 2018), the significance of epigenetic variation within a single candidate gene is disputed. As such, researchers are increasingly turning to epigenome-wide measures to more robustly assess the interplay between environmental and epigenetic factors. One such measure is epigenetic age, also referred to as DNA methylation age (DNAm age), which uses DNA methylation patterns across hundreds of CpG sites to estimate biological aging. Interestingly, the discrepancy between an individual's chronological age and their biological (i.e., epigenetic) age – often referred to as epigenetic age acceleration (EAA) – has been demonstrated as an informative biomarker of health and development (Horvath & Raj,

2018). While the majority of EAA studies have focused on predictors of adult health outcomes (e.g., GrimAge; Lu et al., 2019), recent studies have determined that DNA methylation patterns associated with length of gestation (i.e., DNAm gestational age) are a useful predictor of infant health and early development. Contrary to adult studies, where epigenetic age acceleration has been associated with a variety of negative health outcomes (Oblak et al., 2021), studies of infants indicate that epigenetic age acceleration is predictive of *better* outcomes such as higher birth weight (Knight et al., 2016) and fewer socioemotional problems (Simpkin et al., 2017). As such, it is possible that infant epigenetic age acceleration may be protective against exposures that are known to contribute to developmental delays and adverse psychosocial outcomes, such as maternal stress. Together, these findings highlight the potential utility of using DNAm gestational age to more robustly assess how epigenetic factors may influence early developmental outcomes in the context of maternal childhood trauma.

The Present Study

The present study used a prospective, longitudinal design leveraging data from three related projects that comprise a sample of Black American mother-child dyads followed from pregnancy through three years postpartum: 1) The Pregnancy Study (Corwin et al., 2017), which examines the impact of social and environmental exposures on maternal and infant birth outcomes, 2) the Maternal Stress and Infant Microbiome Study (Brennan et al., 2019), which focuses on maternal stress and the infant gut-brain axis in the perinatal period; and 3) our local cohort of the Environmental Influences on Child Health Outcomes Study (ECHO; Gillman & Blaisdell, 2018), which examines how biological, behavioral, and social factors relate to developmental outcomes in early childhood. We hypothesized that:

Hypothesis 1: Maternal childhood adversity – examined as childhood trauma and ACEs, separately – would be positively associated with offspring symptomatology (i.e., internalizing, externalizing, and posttraumatic stress symptoms).

Hypothesis 1.1 (exploratory): Subtypes of maternal childhood maltreatment (i.e., physical, sexual, and emotional abuse) would demonstrate differential impacts on offspring symptomatology.

Hypothesis 1.2 (exploratory): The association of maternal adversity and offspring symptomatology would be moderated by offspring sex.

Hypothesis 2: Offspring epigenetic aging would be negatively associated with offspring symptomatology. In other words, epigenetic age acceleration in infancy would predict lower symptomatology in early childhood.

Hypothesis 3: The association between maternal adversity and offspring symptomatology would be moderated by offspring epigenetic aging, such that the impact of maternal childhood adversity on offspring symptomatology would be attenuated in offspring with accelerated epigenetic aging.

Methods

Participants

Pregnant Black American women were initially recruited from prenatal clinics at public and private hospitals within a large metropolitan city in the southeastern United States. Mothers first enrolled in the Pregnancy Study, where data was collected at two prenatal visits (typically during the second and third trimesters). After delivery, participants were invited to enroll in the Maternal Stress and Infant Microbiome Study, where an infant blood sample was collected shortly after birth or at roughly 41-weeks gestational age. When the child reached two years of age, participants were invited to enroll in the ECHO Study, which conducted annual follow-up

visits from ages two to five years. Inclusion criteria for the three studies included: 1) Black/African American race (via self-report); 2) Maternal age of 18-40 years; 3) Singleton pregnancy (verified by clinical record); 4) Maternal comprehension of written and spoken English; and 5) Absence of infant congenital disorders. Additional inclusion criteria for the current study included: 6) completion of at least one Pregnancy Study visit, 7) completion of an ECHO Study visit at 2-, 3- or 4-years, and 8) availability of infant methylation data. This resulted in a final sample of 80 mother-child dyads. Sample characteristics are shown in Table 1.

Procedure

Study procedures were approved by Emory University's Institutional Review Board and informed consent was obtained for each participant at enrollment in the Pregnancy Study, Infant Microbiome Study, and ECHO Study. Data collection was conducted by trained laboratory staff in participants' homes or a clinical or laboratory setting. At the pregnancy visit, mothers self-reported on experiences of childhood adversity. At or shortly after birth, blood samples were collected from offspring, which were later used for methylation sequencing. At the toddlerhood visits, mothers reported on offspring socioemotional and behavioral symptomatology. Covariates relevant to intergenerational adversity and child development (e.g., maternal socioeconomic status, child age, child sex) were collected at all relevant time points.

Measures

Maternal Exposure to Adversity.

Childhood Trauma. Maternal childhood trauma was measured using the short form of the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 2003). The CTQ has 28-items that are used to compute a total score and maltreatment subscale scores including emotional abuse, physical abuse, and sexual abuse. Responses are rated on a 5-point Likert scale ranging from

"1—Never True" to "5—Very Often True." Higher scores are associated with more severe neglect and abuse. The CTQ has been well validated in non-clinical and Black American samples (Liebschutz et al., 2018). Internal consistency for the CTQ total score in the current sample was high (Cronbach's α = 0.89), as were those for the CTQ subscale scores (Emotional Abuse: Cronbach's α = 0.88; Physical Abuse: Cronbach's α = 0.78; Sexual Abuse: Cronbach's α = 0.91).

Adverse Childhood Experiences. Maternal experiences of childhood adversity were measured using a shortened form of the Adverse Childhood Experiences questionnaire (ACEs; Felitti et al., 1998), which eliminates items that overlap with the CTQ. The shortened form consists of ten items assessing adversities related to family dysfunction (e.g., mental illness, substance abuse, or suicidality within the household), parental loss (e.g., through divorce, imprisonment, death, or abandonment), and other childhood adversities (e.g., experiences of homelessness or foster care). Responses are rated in a yes/no format and items are coded as "0—Absent" or "1—Present." The total score is calculated by summing the items, with higher scores indicating more adverse experiences. Internal consistency in the current sample was adequate (Cronbach's α = 0.71).

Offspring Symptomatology. Internalizing, externalizing, and posttraumatic stress symptoms were measured using the Child Behavior Checklist for Ages *1.5-5* (CBCL/1.5-5), a standardized form in which mothers report their children's behavioral and emotional symptoms (Achenbach & Ruffle, 2000). The CBCL/1.5-5 contains 100 items in which the mother indicates the option that best describes her child *now or within the past 2 months* with one of the following: 0 = *not true (as far as you know)*; 1 = *somewhat or sometimes true*; 2 = *very true or often true*. The internalizing symptoms score reflects the sum of 36 of these items, with possible

scores ranging from 0 to 72, and the externalizing symptoms score reflects the sum of 24 items, with possible scores ranging from 0 to 48. The posttraumatic stress symptoms scale is based on a sum of 15 items (Dehon & Scheeringa, 2006), with scores ranging from 0 to 30. The CBCL is a well-established measure of child emotional and behavioral concerns and demonstrates strong test-retest in ethnically- and socioeconomically-diverse samples (e.g., Ivanova et al., 2010).

Infant DNA Methylation and Epigenetic Age Acceleration (EAA). Infant blood samples were collected in one of two ways. For 53 offspring, blood spots were collected at birth according to standard procedures, described in detail by Schroder et al. (2011). For 27 offspring, blood clots were collected from infants via heel stick at roughly 41 weeks-equivalent gestational age. DNA was extracted, processed, and sequenced using the MethylationEPIC BeadChip (Illumina) at the Emory Integrated Genomics Core. Initial quality control was performed using R package *minfi* (Ayree et al., 2014), which removed probes and samples with low signal and missing data. Cross reactive probes identified by McCartney and colleagues were removed (McCartney et al., 2016). Following quality control, the Noob normalization method implemented in *minfi* was used for dye bias equalization.

Epigenetic gestational age was calculated based on a weighted average of 148 CpG sites, according to procedures developed by Knight et al. (2016). In line with expectations, epigenetic gestational age was positively correlated with clinically estimated gestational age (Pearson correlation $r = 0.45$, $p < 0.001$). The difference between epigenetic gestational age and clinically estimated gestational age (i.e., epigenetic age acceleration) was defined by taking the residual of a linear regression of epigenetic gestational age on clinically estimated gestational age.

To account for the potential effects of cell type heterogeneity in infant blood, cell type proportions were estimated for CD4+ T cells, natural killer cells, B cells, neutrophils, and monocytes.

Statistical Analysis

Analyses were performed in R version 4.1.3. Complete data was available for all measures apart from the CTQ, which had missing data for five participants. This data was imputed using predictive mean matching via the MICE package (Van Buuren & Groothuis-Oudshoorn, 2011). All analyses adjusted for covariates that have been previously associated with intergenerational trauma and socioemotional development: maternal socioeconomic status (SES), offspring age at the time of the CBCL, and offspring sex. Analyses involving epigenetic age acceleration additionally adjusted for cell type proportions, biosample type (i.e., blood spot vs. blood clot), and maternal prenatal tobacco use. Multiple regressions were used to test our hypotheses that maternal childhood adversity would be positively associated with offspring symptoms (Hypothesis 1 and exploratory Hypothesis 1.1), that offspring epigenetic aging would be negatively associated with offspring symptoms (Hypothesis 2), and that offspring epigenetic aging (Hypothesis 3) and offspring sex (exploratory Hypothesis 1.2) would moderate the impact of maternal childhood adversity on offspring symptoms. Power analyses indicated that the analyses were adequately powered to detect medium and large effects but potentially underpowered to detect small effects ($f^2 > 0.078$, power = 0.8, $p = 0.05$).

Results

Descriptive statistics and bivariate correlations for the primary variables are displayed in Tables 1 and 2, respectively. Bivariate correlations indicated that maternal childhood trauma was

moderately correlated with maternal ACEs (r=0.43), and childhood maltreatment subtypes were highly correlated with each other (rs = 0.67 to 0.83). Offspring internalizing, externalizing, and posttraumatic stress symptoms were also highly intercorrelated (rs = 0.79 to 0.83). Maternal childhood adversity measures were not associated with moderator variables (i.e., offspring sex and epigenetic aging), other than a small correlation between maternal childhood emotional abuse and offspring sex (r=-0.22) such that male offspring had mothers with higher scores.

With respect to main effects, maternal childhood trauma was significantly correlated with offspring internalizing, externalizing, and posttraumatic stress symptoms (rs = 0.22 to 0.39), as were maternal sexual and emotional abuse (rs = 0.27 to 0.39). Maternal physical abuse was only significantly correlated with offspring internalizing symptoms (r=0.27). Offspring sex was significantly correlated with offspring externalizing symptoms, such that males demonstrated higher symptoms (r=-0.22); offspring sex was not correlated with internalizing or posttraumatic stress symptoms. Offspring epigenetic aging was significantly correlated with offspring externalizing (r=-0.26) and posttraumatic stress (r=-0.24) symptoms, but only marginally with internalizing symptoms (r=0.21).

Finally, with respect to potential confounders, maternal SES was significantly and negatively correlated with maternal childhood trauma, maternal childhood emotional abuse, and offspring internalizing symptoms. Additionally, offspring EAA differed for biosamples that were collected via blood spot at birth versus blood clot at 41 weeks-equivalent gestational age (Supplemental Table 1). As such, these variables were included as covariates in all appropriate regression analyses.

Table 1. *Sample characteristics and descriptive statistics (n=80)*

Sociodemographic Characteristics	
Maternal Age	
Mean	24.5
Median [Min, Max]	24.50 [18, 39]
Maternal Education	
Some high school	20 (25%)
Graduated high school or GED	33 (41%)
Some college or technical school	20 (25%)
Graduated college	6 (8%)
Some graduate work or degree	1 (1%)
Child Gestational Age at Birth (weeks)	
Mean	38.17
Median [Min, Max]	39 [34, 41]
Child Sex	
Male	35 (44%)
Female	45 (56%)
Child Age at CBCL (years)	
Mean	3.04
Median [Min, Max]	3 [1.96, 4]
Descriptives	
Maternal CTQ Total	
Mean	46.01
Median [Min, Max]	39 [25, 116]
Maternal Emotional Abuse	
Mean	8.25
Median [Min, Max]	6.00 [5, 25]
Maternal Physical Abuse	
Mean	7.88
Median [Min, Max]	6 [5, 25]
Maternal Sexual Abuse	
Mean	8.44
Median [Min, Max]	5 [5, 25]
Maternal ACE Total	
Mean	2.13
Median [Min, Max]	2.00 [0, 9]
CBCL Internalizing Score	
Mean	6.3
Median [Min, Max]	4 [0, 23]
CBCL Externalizing Score	
Mean	9.68
Median [Min, Max]	7.5 [0, 40]
CBCL Posttraumatic Stress Score	
Mean	3.06
Median [Min, Max]	2.00 [0, 13]

Table 2. *Bivariate correlations*

	Maternal CTQ Total	Maternal CTQ EA	Maternal CTQ PA	Maternal CTQ SA	Maternal ACES	Offspring Sex	Offspring EAA	Offspring Internalizing	Offspring Externalizing	Offspring Posttraumatic Stress
Maternal CTQ EA	**0.76 p<0.001**									
Maternal CTQ PA	**0.83 p<0.001**	**0.76 p<0.001**								
Maternal CTQ SA	**0.8 p<0.001**	**0.67 p<0.001**	**0.76 p<0.001**							
Maternal ACEs	**0.43 p<0.001**	**0.42 p<0.001**	**0.35 p=0.001**	**0.3 p=0.01**						
Offspring Sex	-0.14 p=0.2	**-0.22 p=0.047**	-0.09 p=0.41	-0.15 p=0.18	-0.04 p=0.71					
Offspring EAA	-0.07 p=0.51	0.002 p=0.99	0.07 p=0.54	-0.06 p=0.62	-0.07 p=0.54	0.18 p=0.11				
Offspring Internalizing Symptoms	**0.39 p<0.001**	**0.32 p=0.003**	**0.27 p=0.01**	**0.39 p<0.001**	0.06 p=0.61	-0.18 p=0.12	-0.21 p=0.06			
Offspring Externalizing Symptoms	**0.22 p=0.049**	**0.29 p=0.01**	0.12 p=0.31	**0.3 p=0.006**	0.02 p=0.88	**-0.22 0.048**	**-0.26 p=0.02**	**0.79 p<0.001**		
Offspring Posttraumatic Stress Symptoms	**0.27 p=0.02**	**0.3 p=0.01**	0.22 p=0.052	**0.27 p=0.01**	0.1 p=0.38	-0.07 p=0.51	**-0.24 p=0.03**	**0.82 p<0.001**	**0.83 p<0.001**	
Maternal SES	**-0.28 p=0.01**	**-0.28 p=0.01**	-0.14 p=0.21	-0.14 p=0.21	-0.001 p=0.99	0.15 p=0.18	0.17 p=0.13	**-0.26 p=0.02**	-0.21 p=0.06	-0.21 p=0.06
Offspring Age	-0.13 p=0.26	0.01 p=0.93	-0.11 p=0.32	0.02 p=0.87	-0.08 p=0.49	-0.08 p=0.5	0.13 p=0.27	-0.16 p=0.17	-0.12 p=0.28	-0.08 p=0.46

Note. CTQ = Childhood Trauma Questionnaire; EA = Emotional Abuse; PA = Physical Abuse; SA = Sexual Abuse; EAA = Epigenetic Age Acceleration

Intergenerational Association of Maternal Adversity and Offspring Symptomatology

First, we examined whether maternal experiences of childhood adversity were intergenerationally associated with offspring symptomatology, after adjusting for maternal SES, offspring age, and offspring sex. Results indicated that maternal ACEs were not significantly associated with any offspring outcomes (Table 3), and maternal childhood trauma was not significantly associated with offspring externalizing (t=1.19, p=0.24) or posttraumatic stress (t=1.75, p=0.09) symptoms. However, maternal childhood trauma was significantly positively associated with offspring internalizing symptoms (t=2.61, p=0.01). To further examine the impact of maternal childhood trauma, we then examined the individual contributions of maltreatment subtypes (i.e., emotional, physical, and sexual abuse). Results indicated that maternal experiences of childhood sexual abuse were associated with offspring internalizing (t=3.30, p=0.001), externalizing (t=2.43, p=0.02) and posttraumatic stress (t=2.20, p=0.03) symptoms. Maternal childhood experiences of emotional abuse were also associated with offspring internalizing (t=2.08, p=0.04) and posttraumatic stress (t=2.18, p=0.03) symptoms. Maternal childhood experiences of physical abuse were not associated with any offspring outcomes. All regression results are presented in Table 3.

Association of Offspring Sex and Early Childhood Symptomatology

Next, we examined the main effects of offspring sex on symptomatology. Results indicated no sex differences in internalizing, externalizing, nor posttraumatic stress symptoms after controlling for relevant covariates (Table 4).

Table 3. *Main effect of maternal childhood adversity on offspring symptomatology*

	Internalizing Symptoms		Externalizing Symptoms		Posttraumatic Stress Symptoms	
	t	*p*-value	t	*p*-value	t	*p*-value
Block 1 (Covariates)						
Maternal SES	**-2.58**	**0.01**	-1.83	0.07	-1.94	0.06
Offspring Sex	-1.3	0.2	-1.93	0.06	-0.43	0.67
Offspring Age	**-1.99**	**0.049**	-1.59	0.12	-1.13	0.26
Block 2 (Maternal Adversity)						
Childhood Trauma	**2.61**	**0.01**	1.19	0.24	1.75	0.08
Emotional Abuse	**2.08**	**0.04**	1.95	0.055	**2.18**	**0.03**
Physical Abuse	1.9	0.06	0.46	0.65	1.54	0.13
Sexual Abuse	**3.3**	**0.001**	**2.43**	**0.02**	**2.2**	**0.03**
Adverse Childhood Experiences (ACEs)	0.32	0.75	-0.05	0.96	0.79	0.43

Note. Separate regressions were conducted for each form of maternal adversity. Results are presented together for consolidation purposes.

Table 4. *Interaction of maternal childhood adversity and offspring sex to predict offspring symptomatology*

	Internalizing Symptoms		Externalizing Symptoms		Posttraumatic Stress Symptoms	
	t	*p*-value	t	*p*-value	t	*p*-value
Block 1 (Covariates)						
Maternal SES	**-2.79**	**0.01**	**-1.47**	**0.04**	**-2.04**	**0.04**
Offspring Age	-1.92	0.06	-1.47	0.15	-1.11	0.27
Block 2 (Main Effects)						
Maternal Adversity	(see Table 3)					
Offspring Sex	-1.3	0.2	-1.93	0.06	-0.43	0.67
Block 3 (Maternal Adversity x Offspring Sex)						
Childhood Trauma x Sex	-0.78	0.44	-1.66	0.1	-1.02	0.31
Emotional Abuse x Sex	-0.44	0.66	-1.63	0.11	-0.51	0.61
Physical Abuse x Sex	-0.36	0.72	-1.22	0.23	-0.32	0.75
Sexual Abuse x Sex	-0.63	0.53	**-2.3**	**0.02**	-0.73	0.47
Adverse Childhood Experiences (ACEs) x Sex	-1.11	0.27	-0.62	0.53	-1.28	0.2

Note. Separate interaction models were conducted for each form of maternal adversity. Results are presented together for consolidation purposes.

Does Offspring Sex Moderate Associations between Maternal Adversity and Offspring Symptomatology?

We then examined whether the intergenerational associations between maternal adversity and offspring symptoms differed across male and female offspring. Results demonstrated a significant interaction between maternal childhood sexual abuse and offspring sex to predict offspring externalizing symptoms (t=-2.30, p=0.02; Table 3). Upon further probing, results indicated that the significant association between maternal childhood sexual abuse and offspring externalizing symptoms was driven by male offspring, and there was no association for female offspring (Figure 1). No other sex differences were found (Table 4).

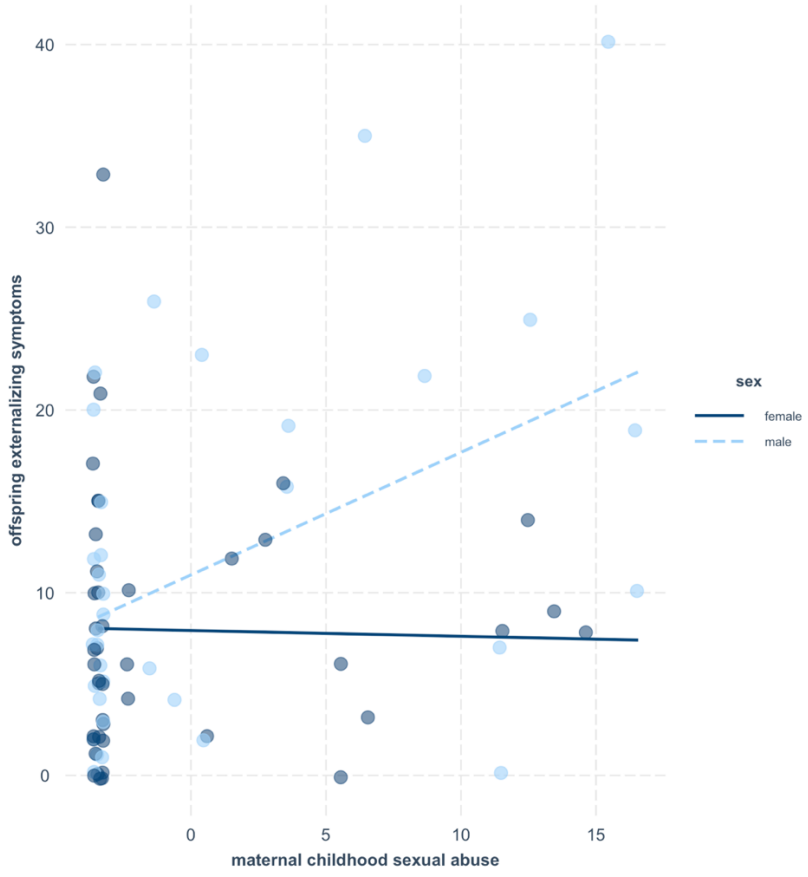

Figure 1. *Male offspring demonstrated elevated externalizing symptoms in the context of maternal childhood sexual abuse, while female offspring did not.*

Association of Infant Epigenetic Aging and Early Childhood Symptomatology

Next, we examined the main effects of offspring epigenetic aging on symptomatology. Although offspring epigenetic aging was initially correlated with offspring symptoms, regression results indicated that epigenetic aging was no longer significantly associated with internalizing, externalizing, or posttraumatic stress symptoms after adjusting for cell type proportions, maternal prenatal tobacco use, and other relevant covariates (Table 5).

Does Infant Epigenetic Aging Moderate Associations between Maternal Adversity and Offspring Symptomatology?

Finally, we examined whether infant epigenetic aging moderated the association between maternal adversity and offspring symptomatology (Table 6). Results indicated that epigenetic age acceleration attenuated the impact of maternal childhood sexual abuse on offspring internalizing ($t=-2.28$, $p=0.03$; Figure 2a), externalizing ($t=-2.48$, $p=0.02$; Figure 2b), and posttraumatic stress ($t=-2.56$, $p=0.01$; Figure 2c) symptoms. In addition, results demonstrated a cross-over interaction between maternal adverse childhood experiences (ACEs) and offspring epigenetic aging to predict offspring internalizing symptoms ($t=-2.53$, $p=0.01$; Figure 3). For offspring with lower epigenetic aging, maternal ACEs were associated with greater internalizing symptoms. Conversely, for offspring with accelerated epigenetic aging, maternal ACEs were associated with lower internalizing symptoms.

Discussion

In the present study, we examined the intergenerational impacts of different types of maternal childhood adversity on offspring behavioral outcomes and whether infant epigenetic aging might buffer these associations. We found that maternal experiences of childhood trauma were significantly associated with symptoms of psychopathology in her offspring, with

Table 5. *Main effect of offspring epigenetic aging on offspring symptomatology*

	Internalizing Symptoms		Externalizing Symptoms		Posttraumatic Stress Symptoms	
	t	*p*-value	t	*p*-value	t	*p*-value
Block 1 (Covariates)						
Maternal SES	**-2.21**	**0.03**	**-2.22**	**0.03**	**-2.08**	**0.04**
Offspring Sex	-1.42	0.16	**-2.5**	**0.01**	-0.8	0.43
Offspring Age	**-2.28**	**0.03**	**-2.13**	**0.04**	-1.49	0.14
Maternal Prenatal Tobacco Use	0.06	0.95	1.69	0.1	1.64	0.11
CD4+ T Cells	-0.31	0.76	-0.67	0.5	-0.49	0.63
Natural Killer Cells	1.99	0.05	2.18	0.03	1.76	0.08
B Cells	-1.35	0.18	-2.19	0.03	-1.3	0.2
Monocytes	-0.57	0.57	-1.18	0.24	-0.76	0.45
Neutrophils	-1.01	0.31	-1.63	0.11	-1.33	0.19
Biosample Source	-0.63	0.53	-1.18	0.24	-1.17	0.25
Block 2 (Offspring EAA)						
Epigenetic Aging	0.12	0.9	-0.43	0.67	-0.14	0.89

Table 6. *Interaction of maternal childhood adversity and offspring epigenetic aging predicting offspring symptomatology*

	Internalizing Symptoms		Externalizing Symptoms		Posttraumatic Stress Symptoms	
	t	*p*-	t	*p*-	t	*p*-value
Block 1 (Covariates)						
Maternal SES	**-2.58**	**0.01**	-1.83	0.07	-1.94	0.06
Offspring Sex	-1.3	0.2	-1.93	0.06	-0.43	0.67
Offspring Age	**-1.99**	**0.049**	-1.59	0.12	-1.13	0.26
Block 2 (Main Effects)						
Maternal Adversity	(see Table 3)					
Offspring EAA	(see Table 5)					
Block 3 (Maternal Adversity x Offspring EAA)						
Childhood Trauma x EAA	-1.18	0.24	-0.9	0.37	-1.07	0.3
Emotional Abuse x EAA	-0.94	0.35	-1.64	0.1	-1.69	0.1
Physical Abuse x EAA	-1.67	0.1	-0.33	0.74	-0.76	0.45
Sexual Abuse x EAA	**-2.28**	**0.03**	**-2.48**	**0.02**	**-2.56**	**0.01**
Adverse Childhood Experiences (ACEs) x EAA	**-2.53**	**0.01**	-1.07	0.29	-1.79	0.07

Note. Separate interaction models were conducted for each form of maternal adversity. Results are presented together for consolidation purposes.

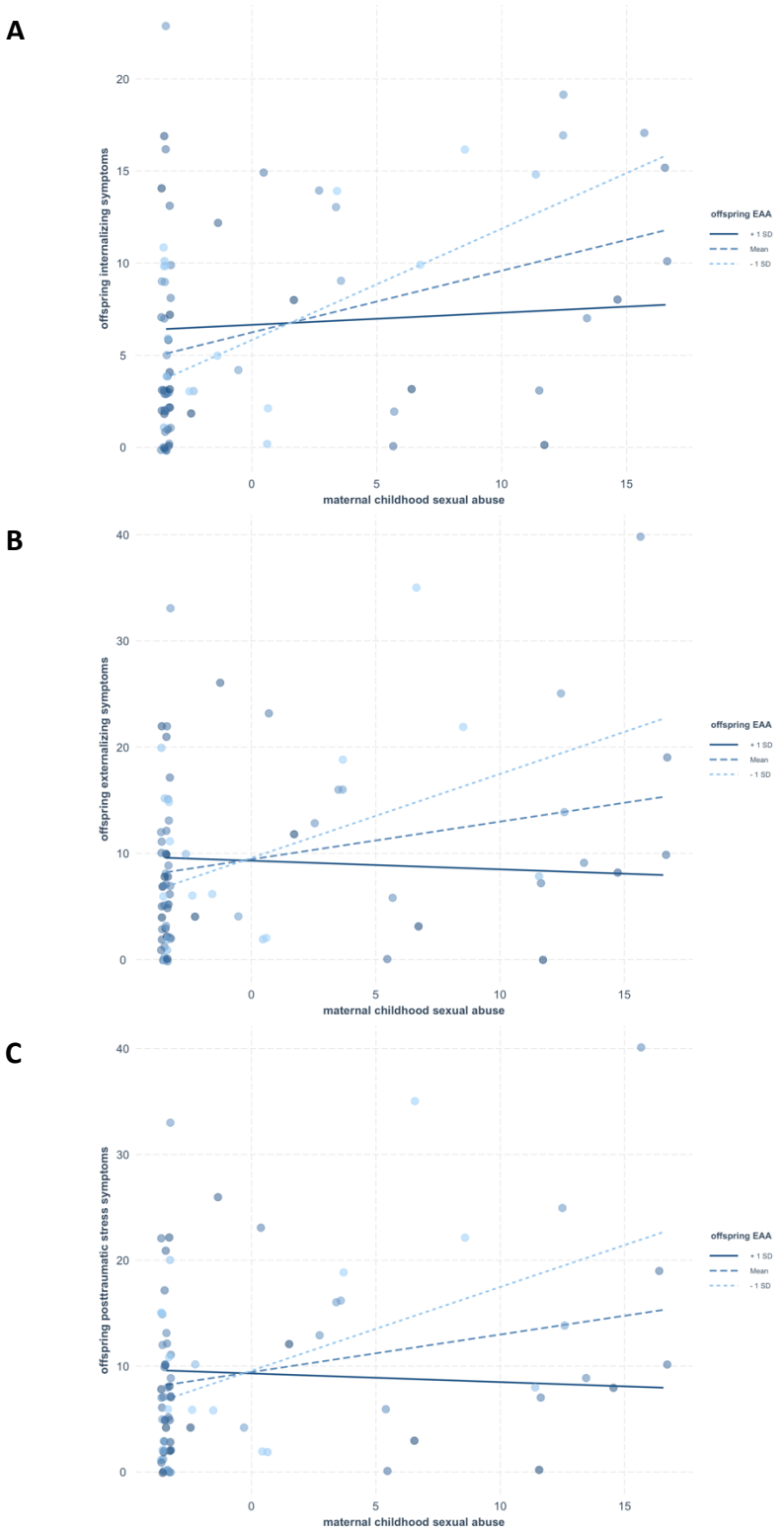

Figure 2. *The associations between maternal childhood sexual abuse and offspring internalizing (A), externalizing (B), and posttraumatic stress (C) symptoms were attenuated in offspring with accelerated epigenetic aging.*

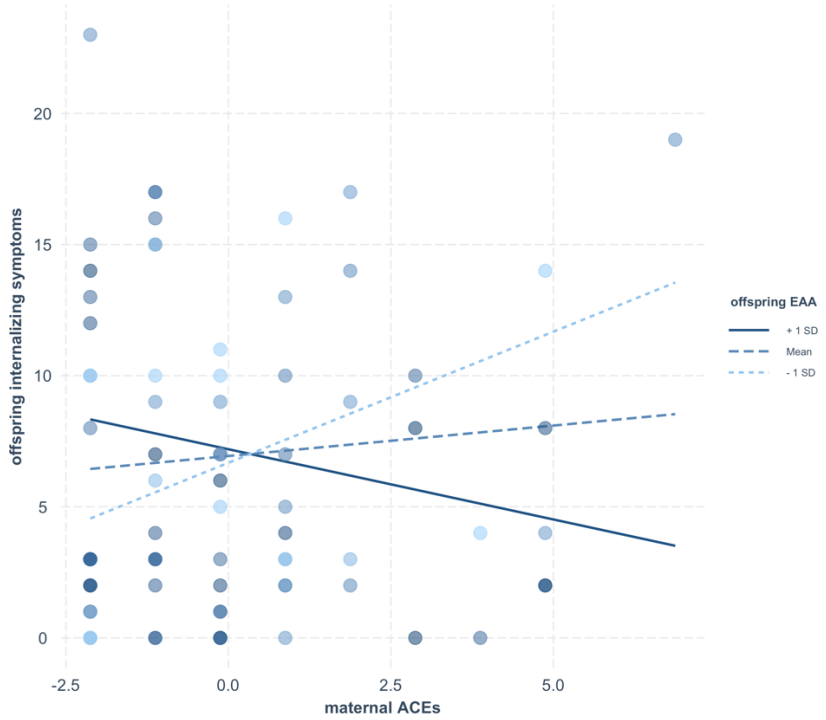

Figure 3. *Although there was not a significant main effect of maternal ACEs on offspring internalizing symptoms, there was a negative association for offspring with accelerated epigenetic aging and a positive association for offspring with lower epigenetic aging.*

particularly pronounced effects of emotional and sexual abuse on offspring symptoms. The intergenerational impact of sexual abuse, however, was attenuated in offspring with accelerated epigenetic aging at birth. These findings extend prior research demonstrating the differential impact of childhood maltreatment subtypes not only on an individual's risk for developing psychopathology, but also extending into future generations. Further, it provides novel evidence that epigenetic mechanisms may play an important role in moderating the influence of intergenerational stress on early developmental outcomes.

Across internalizing, externalizing, and posttraumatic stress symptoms, the associations between maternal sexual abuse and offspring symptomatology were significant for offspring with lower epigenetic aging at birth but were attenuated in offspring with accelerated epigenetic

aging. While this was the first study to examine the moderating effect of epigenetic aging in the context of intergenerational trauma, these findings are consistent with theoretical and empirical evidence from intra-generational studies that epigenetic differences associated with psychopathology-related mechanisms (e.g., neuroendocrine reactivity) moderate the relationship between early life stress and psychopathology (Al Jowf et al., 2021; Conradt et al., 2013; Conradt et al., 2017; Smearman et al., 2016; Smeeth et al., 2021). Prior studies suggest that epigenetic aging may reflect differences in neuroendocrine and inflammatory processes that in turn confer greater susceptibility to environmental stressors (Lu et al., 2019; Wolf et al., 2021). For example, epigenetic aging has been associated with methylation differences in the stress-regulatory gene *FKBP5* (Beach et al., 2022) as well as differential expression of inflammation genes (Wolf et al., 2021). Given that neuroendocrine and inflammation pathways have been shown to mediate the intra-generational relationship between childhood maltreatment (including childhood sexual abuse; Nunes et al., 2010) and the development of depression and other psychopathology (McCrory et al., 2012), it follows that differential expression of genes implicated in these pathways might influence an individual's susceptibility to maltreatment.

Importantly, similar mechanisms have been posited to mediate the *intergenerational* link between maternal maltreatment and offspring psychopathology. Evidence stemming from the fetal programming hypothesis has demonstrated that maternal experiences of adversity can cause alterations to her physiological stress response that then influence the development of her offspring's stress response during fetal development (Babenko et al., 2015; Buss et al., 2017). In turn, offspring of women who experienced maltreatment tend to demonstrate differences in fear and neuroendocrine systems that influence stress sensitivity and risk for psychopathology (Brand et al., 2010; Hendrix et al., 2020). Given these effects, it is possible that methylation differences

that are reflected by infant epigenetic age acceleration may also be acting on neuroendocrine and inflammation mechanisms to buffer the impact of maternal maltreatment on the development of offspring psychopathology. However, these mechanisms are still poorly understood and future research is needed to better understand the physiological processes that relate to infant epigenetic aging and how those processes play a role in the intergenerational transmission of maltreatment.

Our findings corroborate prior evidence that maternal childhood trauma is associated with greater offspring symptomatology (Bush et al., 2023) and additionally suggest that different maltreatment types confer differential risk for offspring psychopathology. The present study is the first to our knowledge that examines subtype differences in the association between maternal childhood maltreatment and offspring psychopathology, and it adds to the growing evidence that examining subtypes of childhood maltreatment is important for understanding its long-term impact. Specifically, we found that maternal experiences of childhood emotional and sexual abuse were associated with offspring internalizing, externalizing, and posttraumatic stress symptoms, while experiences of physical abuse were not. These results are consistent with evidence from intra-generational studies, which have found that both emotional and sexual abuse account for unique variation in adverse psychological and behavioral outcomes. For example, a systematic review of neuroimaging studies found that while some structural and connectivity differences are shared across all types of childhood maltreatment, sexual maltreatment confers unique risk for structural changes to reward circuitry and emotional abuse is uniquely associated with structural changes to fronto-limbic socioemotional networks (Cassier et al., 2018). These findings are interesting given the well-established role of reward circuits (Nestler & Carlezon, 2006) as well as fronto-limbic socioemotional networks (Du et al., 2017) in the development of depression and other trauma-related psychopathology. As such, it is possible that similar

mechanisms could underlie the intergenerational association between maternal abuse and offspring symptoms, particularly considering recent evidence that maternal childhood emotional (but not physical) neglect is associated with fronto-limbic connectivity in newborn offspring (Hendrix et al., 2020). Together, these findings highlight the importance of examining the effects of maternal maltreatment subtypes on offspring neural circuitry in future research.

It is also possible that maternal emotional and sexual abuse influence offspring risk for psychopathology through the same mechanisms as other forms of maternal stress, but they demonstrate a greater impact because they elicit a more chronic or severe stress response. For example, studies have shown that emotional abuse is associated with more chronic activation of the stress response compared to other forms of maltreatment (Kuhlman et al., 2015). In turn, it is possible that mothers who experience emotional abuse during childhood demonstrate more sustained changes to their HPA-axis that are then transferred to their offspring during pregnancy (Babenko et al., 2015; Buss et al., 2017). Additionally, based on evidence from a recent review that childhood sexual abuse confers the most unique risk (i.e., over and above the influence of other childhood adversities) for disorders characterized by HPA-axis dysregulation (Noll et al., 2022), it is possible that mothers who experience sexual abuse during childhood develop more significant alterations to their HPA-axis that then disproportionately influence the fetal programming of their offspring's stress response. While these interpretations are speculative, our findings highlight the importance of better understanding how different forms of childhood maltreatment may also differentially influence the physiological (e.g., neuroendocrine) systems that contribute to intergenerational risk.

Finally, we found a significant cross-over interaction between maternal ACEs and offspring epigenetic aging such that, for mothers with higher ACEs, offspring with lower

epigenetic aging demonstrated greater internalizing symptoms while offspring with accelerated aging demonstrated lower symptoms; however, for mothers with low ACEs, offspring with accelerated aging demonstrated slightly higher symptoms than those with lower epigenetic aging. These results suggest that accelerated epigenetic aging may be protective in the context of maternal adversity but detrimental in the absence of maternal adversity. The latter is inconsistent with the extant literature, given that epigenetic age acceleration has been associated with better infant health outcomes in samples that were not enriched for maternal ACEs (Knight et al., 2016; Simpkin et al., 2017). As such, we hesitate to overinterpret this finding without further investigation and replication. Similarly, we found that male – but not female – offspring demonstrated elevated externalizing symptoms following maternal experiences of sexual abuse. It is possible that these sex differences are related to the higher prevalence of externalizing disorders among males (Leadbeater et al., 1999), as we found a significant correlation between offspring sex and offspring externalizing symptoms such that males demonstrated greater symptoms. However, given that no other sex moderation effects were identified and a recent study in a larger sample (n=1,948) found no sex differences in the intergenerational relationship between maternal childhood trauma and offspring behavioral outcomes (Bush et al., 2023), this finding may have been a statistical artifact and requires replication as well.

Limitations

Our findings must be considered in light of several limitations. Although power analyses indicated a sufficient sample size to detect medium effects, our sample was relatively small; it is possible that we did not capture significant relationships of smaller magnitudes, and our findings require replication in a larger sample. In addition, while a strength of our study was its longitudinal design which provides some directionality for the relationship between maternal

childhood trauma, infant epigenetic aging, and offspring outcomes, we cannot establish causality from the present study. Our study was also limited to using peripheral tissue (i.e., blood) to assess DNA methylation. Although there is evidence that methylation in peripheral tissue is reflective of systemic changes to inflammation and may be comparable to methylation of brain tissue (Braun et al., 2019; Smeeth et al., 2021; Smith et al., 2014), it does not perfectly reflect DNA methylation within the central nervous system. Finally, we relied solely on maternal report to assess offspring symptoms of psychopathology. The CBCL is a well-validated measure of offspring socioemotional and behavioral symptoms and previous studies have validated maternal reports of child symptoms using standardized behavioral observations (Hinshaw et al., 1992). Nevertheless, studies have suggested that mothers' mental health can bias the report of her child's socioemotional health (Kohen et al., 1997), albeit to a minimal degree (Olino et al., 2021). Given prior evidence that maternal psychopathology is one mechanism by which maternal childhood maltreatment confers risk for offspring outcomes, controlling for maternal mental health posed the risk of masking the effects we aimed to examine. As such, future research would benefit from including additional measures of offspring outcomes such secondary reports, behavioral observations, or physiological measures (e.g., cortisol reactivity) to corroborate these findings.

Conclusion

Experiences of childhood trauma can have an intergenerational impact on mental health, and it is important to consider the type of maltreatment when assessing level of risk. Moreover, biological factors such as advanced epigenetic aging may reduce a child's susceptibility to intergenerational trauma in early life. Better understanding the physiological processes that contribute to "epigenetic resilience" against the prolonged impacts of childhood trauma has the

potential to deepen our understanding of developmental psychopathology and design biologically informed intervention and prevention efforts.

General Discussion

The overarching goal of this dissertation was to interrogate the intergenerational associations between maternal childhood adversity and offspring symptoms of psychopathology, and to examine whether microbiome composition or epigenetic aging might moderate these intergenerational associations. We hypothesized that greater maternal exposure to adverse childhood experiences (ACEs) and childhood trauma – both generally and with respect to specific maltreatment subtypes (i.e., emotional, physical, and sexual abuse) – would predict greater internalizing, externalizing, and posttraumatic stress symptomatology in offspring. Our results largely supported these hypotheses. In Study 1, maternal ACEs were predictive of offspring symptomatology, with the strongest effects shown for offspring externalizing symptoms. In Study 2, maternal childhood trauma was predictive of offspring symptomatology, with maternal sexual abuse and emotional abuse demonstrating the strongest effects for offspring internalizing and posttraumatic stress symptoms. We also hypothesized that these intergenerational associations would be attenuated in offspring with a protective gut microbiome composition and, separately, for offspring with accelerated epigenetic aging. We found partial support for these hypotheses. In Study 1, the impact of maternal ACEs on offspring symptomatology was not dependent on offspring gut microbiome composition, apart from a marginally significant attenuation of internalizing symptoms in offspring with greater abundances of *Lactobacillus.* In Study 2, however, the impact of maternal childhood trauma – in particular, childhood sexual abuse – on offspring internalizing, externalizing, and posttraumatic stress symptoms was significantly attenuated in infants with accelerated epigenetic aging. Taken together, these results suggest that alterations to biological factors such as epigenetics and the gut

microbiome may influence the transmission of stress not only within an individual but also across generations.

Differential Impacts of Maternal Childhood Adversities

Childhood trauma often co-occurs with ACEs (Maniglio et al., 2009), and intergenerational studies have demonstrated that both trauma and adversity during a mother's childhood are associated with offspring symptoms of psychopathology (Bush et al., 2023; Cooke et al., 2021; Zhang et al., 2022). However, few studies have separately examined ACEs and childhood trauma within the same sample, and no studies to date have explored the potentially varying impacts of specific childhood maltreatment types. Our results suggested that maternal ACEs and childhood trauma may differentially influence offspring outcomes, as maternal ACEs conferred the greatest risk for offspring externalizing symptoms while maternal childhood trauma conferred the greatest risk for offspring internalizing symptoms. Moreover, specific childhood maltreatment subtypes conferred differential risk, such that maternal physical abuse was not associated with offspring outcomes, but maternal emotional and sexual abuse were both associated with offspring internalizing and posttraumatic stress symptoms. While these differential relationships require replication, our findings are somewhat consistent with the extant literature. For example, a recent systematic review found that maternal ACEs were more consistently associated with offspring externalizing symptoms relative to internalizing symptoms (Cooke et al., 2021). Evidence from intra-generational research also suggests that ACEs (e.g., a negative home environment) are more strongly associated with the development and maintenance of externalizing symptoms relative to internalizing symptoms (Fanti & Henrich, 2010). In contrast, and in line with our findings, evidence from intra-generational studies indicates that childhood trauma – particularly sexual and emotional abuse – is most strongly

associated with internalizing symptoms and disorders such as depression and posttraumatic stress disorder (Noll et al., 2022). However, a recent review examining *intergenerational* associations found that maternal childhood trauma was more consistently associated with externalizing symptoms than internalizing symptoms, and the intergenerational associations with offspring mood disorders were null (Plant et al., 2018). Taken together, the literature remains mixed on whether and how different forms of maternal childhood stress may differentially influence offspring symptoms, but our findings highlight the importance of taking a nuanced measurement of childhood adversity when examining how it may influence risk for psychopathology into future generations.

Importance of Parsing Internalizing, Externalizing, and Posttraumatic Stress Symptoms

The present dissertation examined offspring outcomes according to internalizing, externalizing, and posttraumatic stress symptoms. Internalizing and externalizing problems are standard measures of early childhood risk for psychopathology, with internalizing problems characterized by symptoms such as fearfulness, anxiety, withdrawal, and low mood and externalizing problems characterized by hyperactivity, defiance, and aggressive or destructive behavior. Posttraumatic stress symptoms, while much less often studied in the context of early symptomatology, are also of relevance given the elevated rates of posttraumatic stress disorder in children of mothers who experience childhood maltreatment (Yehuda et al., 1998). Posttraumatic stress symptoms are defined by emotion dysregulation, somatic complaints, sleep difficulties, and anxiety or hypervigilance. The importance of parsing offspring internalizing, externalizing, and posttraumatic stress outcomes in this dissertation was twofold. First, evidence suggests that child symptomatology as early as age two is predictive of mental health trajectories into adolescence and even adulthood, and these trajectories vary for internalizing and externalizing

symptoms (Fanti et al., 2010). In general, internalizing symptoms tend to increase steadily across child development, particularly for females (Gilliom & Shaw, 2004; Bongers et al., 2003). Externalizing symptoms, on the other hand, tend to decrease or, for a minority of children, remain stable into adolescence (Fanti et al., 2010). Moreover, the rate and degree of change for internalizing and externalizing symptoms differs according to both shared and unique factors (Gilliom & Shaw, 2004). For example, while risk factors such as low socioeconomic status and maternal depression are predictive of both internalizing and externalizing symptoms, protective factors such as a positive and enriched home environment have been shown to reduce the trajectory of externalizing symptoms, but not the development of internalizing symptoms (Fanti & Henrich, 2010). Further, even when the risk factors are shared, they may function through different processes that would require unique interventions when addressing internalizing versus externalizing symptoms. For example, children with internalizing and externalizing problems both tend to experience social difficulties; for children with greater internalizing problems this may be due to social withdrawal, while for children with externalizing problems this may be due to social rejection (Fanti & Henrich, 2010). Although the developmental trajectories of childhood posttraumatic stress symptoms are less studied, preliminary research suggests that similar factors differentially influence posttraumatic stress trajectories as well (Le Brocque et al., 2009; Nugent et al., 2009; Self-Brown et al., 2013). As such, while most intergenerational studies to date have examined transdiagnostic outcomes or total problems (e.g., Bush et al., 2023), findings from the present dissertation highlight the importance of parsing offspring socioemotional and behavioral outcomes in early life to inform effective clinical interventions moving forward.

Roles of the Gut Microbiome and Epigenetic Age Acceleration

The results of the current dissertation suggest that epigenetic differences and, potentially, differences in the gut microbiome may alter a child's susceptibility to maternal childhood adversity. In Study 1, we found marginally significant evidence that children with a greater relative abundance of the protective bacteria *Lactobacillus* may be less susceptible to developing internalizing symptoms following exposure to maternal childhood adversity. In Study 2, we found consistent evidence that children with epigenetic age acceleration at birth were less susceptible to developing internalizing, externalizing, and posttraumatic stress symptoms following exposure to maternal childhood sexual abuse. Together, these findings demonstrate how differences in physiological systems that contribute to the stress response can influence a child's susceptibility to intergenerational adversity.

A variety of mechanisms underlie the link between maternal childhood adversities and offspring symptoms, including socioeconomic factors, parenting differences, and physiological alterations that influence the stress response of both mothers and offspring (Lehrner & Yehuda, 2018). It is through the last mechanism that the biological factors examined in this dissertation – the gut microbiome and epigenetic aging – might be hypothesized to influence the link between maternal adversity and offspring psychopathology. Maternal ACEs and childhood trauma have each been associated with alterations to offspring's stress response, including differences in HPA-axis reactivity and the inflammatory response, which have in turn been associated with increased risk for psychopathology (Babenko et al., 2015; Buss et al., 2017). Importantly, the gut microbiome has demonstrated a bidirectional relationship with both the HPA-axis and the inflammatory response (Osadchiy et al., 2019). This suggests that differences in the gut microbiome may help regulate these physiological processes and, in turn, influence a child's physiological susceptibility to maternal stress. Similarly, epigenetic aging has been associated

with markers of inflammation (Lu et al., 2019) as well as with differential methylation in HPA-axis and inflammation-related genes (Beach et al., 2022; Wolf et al., 2021). This suggests that differences in epigenetic aging may reflect differences in stress response systems that in turn alter a child's sensitivity to maternal stress. Taken together, it may be that maternal childhood adversity confers risk for offspring symptomatology via changes to offspring neuroendocrine and/or inflammatory processes, and these changes depend on (i.e., are moderated by) epigenetic or gut microbiome differences. Unfortunately, examining mechanisms of transmission was beyond the scope of the current dissertation so we were not able to examine this moderated mediation model. However, our findings provide a foundation and justification for future research to examine this hypothesis.

Clinical Implications

Findings from the present dissertation have several implications for future clinical research and intervention. First, our findings emphasize the significance of early life interventions that reduce child exposure to adversity and traumatic experiences, as this is not only beneficial to the child but also has the potential to decrease rates of psychopathology intergenerationally. Our results also highlight the importance of effective interventions that "break the cycle" of intergenerational transmission for mothers who have experienced childhood trauma or adversity. Indeed, there already exist several clinical studies and interventions for pregnant and postpartum women that have been shown to reduce stress and psychopathology for trauma-exposed mothers and, in turn, reduce risk for psychopathology in offspring (Bush et al., 2023; McKenna et al., 2022). These solutions primarily involve maternal mental healthcare and parenting interventions, delivered in a clinical setting. Importantly, more research is also needed to better understand the protective factors that occur through community and family practices

and have the potential to reduce risk for trauma-related psychopathology, both intra- and intergenerationally, on a broader scale. This is particularly important for the Black American families we focused on in the current dissertation, who are both disproportionately exposed to adversity and experience reduced access to or engagement with formal interventions and mental healthcare (Cooper et al., 2002). Prevention and intervention efforts at the individual, community, and societal levels are necessary to reduce the undue impact of childhood trauma and ACEs for Black Americans, particularly interventions that are culturally informed, leverage factors that already exist in the community (e.g., religious support systems), and focus on resilience, strength, and empowerment (Hampton-Anderson et al., 2021).

Our findings also provide preliminary evidence that interventions targeting epigenetic aging and, potentially, the gut microbiome could indirectly reduce the intergenerational impact of maternal childhood adversity on offspring risk for psychopathology. Interestingly, many of these interventions could play a dual role in reducing maternal stress while also positively influencing these biological factors. For example, maternal prenatal stress has been associated with both epigenetic aging and gut microbiome composition in infants (Suarez et al., 2018; Szyf, 2021; Zjilmans et al., 2015). As such, interventions that reduce maternal prenatal stress have the potential to improve offspring outcomes through multiple mechanisms. Enhancements to maternal prenatal care are also necessary, particularly for Black American women, to reduce the prevalence of preterm birth and other adverse birth outcomes that are suggested to negatively impact infant epigenetic aging and gut microbiome composition (Brennan et al., 2019; Knight & Smith, 2016). Broader solutions to address the structural and societal inequities – stemming from systemic racism – that contribute to higher rates of adverse birth outcomes among Black

American women (Dominguez et al., 2008; Giscombé & Lobel, 2005) are also of the utmost importance for breaking the cycle of mental health disparities that exist in the United States.

Finally, while no studies have examined this directly, it is possible that factors that positively influence epigenetic aging and gut microbiome composition may increase resilience to intergenerational stress exposure. For example, diet, exercise, and even protective factors such as social support may influence epigenetic aging and the gut microbiome in ways that reduce a child's physiological susceptibility to stress (Dill-McFarland, 2019; Hasan & Yang, 2019; Mehta et al., 2022; Monda et al., 2017; Quach et al., 2017). For the gut microbiome, there is also increasing evidence that pharmaceutical interventions (e.g., the delivery of probiotics) can reduce the development of psychopathology following stress exposure (Liang et al., 2015; Takada et al., 2016). Further research is needed to better understand how these protective factors may directly or indirectly improve a child's mental health outcomes through these biological processes, particularly for children at an elevated risk for psychopathology due to maternal histories of trauma and adversity.

Importance of Representation of Black Americans in Biopsychosocial Research

The present dissertation's focus on Black American mothers and children is important for several reasons. First, given that Black Americans are most profoundly exposed to childhood trauma and adversity (Merskey et al., 2018), concentrating on Black women's and children's experiences increases the utility by making a relatively larger impact when examining the consequences of childhood exposures. Second, relative to White Americans, Black Americans are more likely to develop posttraumatic stress symptoms following trauma exposure (Roberts et al., 2011). This higher susceptibility can be attributed to a conglomeration of factors, including the compounding impact of racism-related stressors as well as lower access to mental healthcare

resources (Cooper et al., 2002; Williams, 2018). Further, due to socioeconomic inequities, many Black Americans have reduced access to protective factors potentially relevant to epigenetic and gut microbiome health, such as access to unpolluted outdoor spaces and diets low in processed foods (Beyer et al., 2014; Leung et al., 2014). As such, while we would not expect the mechanisms underlying the intergenerational impacts of maternal childhood adversity to differ for individuals of different racial/ethnic identities, we might expect these associations to differ according to moderators that vary for individuals of different backgrounds. Indeed, a recent multi-cohort study examining the impact of maternal childhood adversity on offspring symptoms of psychopathology found that intergenerational associations generalized across individuals of different racial, ethnic, socioeconomic, and regional backgrounds (Bush et al., 2023). Yet, they also found that effect sizes were smaller for the cohort that was primarily comprised of Black women and children from the southeastern United States. The authors posited that these differences may be due to the presence of other adversities (e.g., racial stressors, neighborhood crime) that outweigh the impact of the measured stressors or may be due to unmeasured protective factors such as family and community support (Bush et al., 2023). Similarly, research suggests that a typical "healthy" gut microbiome composition varies for individuals of different racial/ethnic backgrounds, as it is influenced by genetic, environmental, and dietary factors that differ across racial and cultural groups (Goodrich et al., 2014; Singh et al., 2017). However, most human studies examining the gut microbiome and how it relates to the development of psychopathology focus on European or White American samples. Epigenetic aging studies, though slightly more representative, are also largely Eurocentric. Together, this evidence emphasizes the importance of continuing to increase representation of Black Americans in the

realms of developmental, biological, and psychological research to better understand individual differences in risk and resilience.

Limitations and Future Directions

The findings from the current dissertation must be interpreted in the context of several limitations, beyond what was described in each study. It is worth nothing that, in Study 1, we found that maternal ACEs but not childhood trauma was predictive of offspring psychopathology, and in Study 2 we found the reverse. Although the samples from both studies pulled from the same longitudinal cohort, a minority of participants (n=27) were represented in both samples due to the varying inclusion criteria of each study. Given that both study samples were relatively small and *a priori* power analyses indicated that we were underpowered to detect small effects, these discrepancies are likely an issue of statistical power. Indeed, similar analyses from larger samples have demonstrated significant effects of both childhood trauma and ACEs (Bush et al., 2023; Cooke et al., 2021; Zhang et al., 2022). It is also possible that the two samples systemically differed according to an unmeasured variable that may have influenced these differential findings.

Future studies would benefit from considering the impact of additional forms of individual, community, and systemic stressors (e.g., racial discrimination, neighborhood violence) to better parse the shared and differential impacts of childhood adversities and stressors. Indeed, a recent intra-generational study found that childhood experiences of racial discrimination had comparable associations with the development of internalizing symptoms as did ACEs (Bernard et al., 2022). While this has not been examined intergenerationally, based on our findings that maternal ACEs were predictive of offspring externalizing symptoms we may hypothesize that maternal childhood experiences of racial discrimination would demonstrate

similar associations. Additionally, based on intra-generational evidence that the impact of chronic stressors such as racial discrimination may compound the impact of trauma on the development of psychopathology (e.g., Mekawi et al., 2021), future research would benefit from exploring how chronic and lifetime stressors may contribute to the intergenerational risk posed by maternal childhood trauma and adversity.

Conclusion

Findings from the current dissertation add to the growing literature that maternal experiences of trauma and adversity in her own childhood can influence the development of psychopathology in her offspring's childhood. Moreover, it provides novel evidence that individual differences in biological systems that influence the physiological stress response may alter a child's susceptibility to the intergenerational impacts of maternal adversity. This work sets a foundation for future research to examine ways in which targeting biological processes such as epigenetic aging or the gut microbiome may help mitigate the harmful impacts of maternal childhood adversity and reduce the burden of trauma-related psychopathology for Black American women and children.

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