Prenatal Cannabis Use and Offspring Autism-Related Behaviors:
Examining Maternal Stress as a Moderator in a Black American Cohort

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By

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Abstract

The prevalence of both autism spectrum disorder (ASD) diagnoses and cannabis use during pregnancy are increasing. While researchers are examining these phenomena independently, few have examined their possible association. Some studies have suggested that prenatal cannabis use might be associated with increased risk of ASD, but further research is needed to substantiate these findings. Additionally, prenatal stress has been identified as a risk factor for ASD. Black mothers and mothers of lower socioeconomic status (SES) may be especially likely to experience high levels of prenatal stress due to additional exposure to race- and poverty-related stressors. This project aimed to examine the impact of prenatal cannabis use and prenatal distress (i.e., racial discrimination, perceived stress and lower SES) on child autism-related behaviors in a sample of 172 Black mother-child pairs, with special attention to how maternal experiences of discrimination and socioeconomic status affect this association. We hypothesized that both prenatal cannabis use and prenatal stress would predict increased levels of child ASD-related behaviors, and that the association between prenatal cannabis use and child ASD-related behaviors would be stronger in cases of higher prenatal distress. We found no significant associations between prenatal cannabis use and autism behaviors and no moderation effects. We did, however, find that prenatal stress predicted autism behaviors reported on the Achenbach Childhood Behavior Checklist (CBCL) in our sample. These findings replicate previous work on prenatal-ASD associations and add to the limited literature on cannabis-ASD associations. In light of the current trends in the perception and use of cannabis during pregnancy, we emphasize the need for more rigorous investigation of the long-term effects of prenatal cannabis exposure.

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Introduction

Autism Spectrum Disorder (ASD) represents a wide continuum of associated cognitive and neurobehavioral deficits, including deficits in socialization and communication, with restricted and repetitive patterns of behavior (American Psychiatric Association, 2013). The reported prevalence of ASD has increased in the U.S. in the last several decades, with recent estimates as high as 1 in 54 children (Maenner et al., 2020). Of note, ASD prevalence rates have historically been lower for Black children and children of low socioeconomic status (SES). Population-based surveillance data from 11 sites across the U.S. in 2012 concluded that the estimated prevalence of ASD in non-Hispanic white children was 20% higher than non-Hispanic Black children (Imm,White, & Durkin, 2019). Additionally, ASD prevalence was lowest in the low educational attainment tertile and highest in the high educational attainment tertile, consistent with a dose–response association between census tract educational attainment and population-based ASD prevalence in 2010 (Durkin et al., 2017). Importantly, these associations are specific to *diagnoses* of ASD.

Published in 2013, the 5th Edition Diagnostic Statistical Manual (DSM-5) was the first version of this diagnostic manual to characterize autism on a continuous spectrum (American Psychiatric Association, 2013). This new definition follows the trend of the field of psychopathology, which is largely moving towards a more continuous variable conceptualization rather than categorical yes/no disorder diagnoses (Insel et al., 2010). With the shift to this new conceptualization, researchers have found that minority parents and parents with lower education report increased observations of ASD behaviors in their children compared to their white and more educated counterparts, despite the lower prevalence of ASD diagnoses noted above. This contrast may reflect increases in medical care access and service delivery in non-Hispanic white and higher SES communities (Bishop-Fitzpatrick & Kind, 2017; Chiang, et al., 2018; Khowaja,

Hazzard, & Robins, 2015). As the field has moved toward examining psychopathology on a continuum, I will be examining ASD-related *behaviors* rather than *diagnoses* in the current study.

The DSM-5 also specifies that ASD symptoms must be present in the "early developmental period" (American Psychiatric Association, 2013). Although twin and family studies suggest a strong genetic component in the etiology of ASD, recent evidence indicates that up to 40–50% of variance in autism spectrum disorder liability may be determined by environmental factors (Modabbernia, Velthorst, & Reichenberg, 2017). A significant role for environmental factors in the etiology of ASD is also consistent with the quickly increasing rates of this disorder over the past few decades (Hertz-Picciotto, Schmidt, & Krakowiak, 2018). The "developmental origin of health and disease" hypothesis suggests that environmental influences on psychopathology may originate as early as the prenatal period (Barker, 2007). This hypothesis therefore underlines the importance of investigating the mechanisms of fetal exposure and other perinatal factors in early neurodevelopment. In line with this theory, increasing attention has been focused on the role that prenatal exposures, including environmental toxicants, may play in increasing the risk of ASD. The current study will focus on two of these potential prenatal environmental influences: maternal cannabis use and maternal stress during pregnancy.

Cannabis Use

With the legalization of cannabis in various states in the U.S., there has been a decrease in the perception of risk and a concomitant increase in reports of cannabis use by pregnant women (Jarlenski et al. 2017; Westfall et al., 2006). For example, one study found that prevalence of cannabis use among pregnant women increased 62% from 2002 to 2014 (Brown et al., 2017). Importantly, cannabis potency has also increased over the past few years with some

confiscated samples containing more than 80% Tetrahydrocannabinol (THC), the psychoactive component of cannabis (Mehmedic et al., 2010). Animal studies have found that THC can pass the placental barrier and reach the fetus, and fetal exposure in humans has been linked to poorer neonatal and cognitive outcomes (Frau et al., 2019; Nashed, Hardy & Laviolette, 2021). In light of these findings and in conjunction with the reported increased potency and increased prenatal use, it is imperative for us to better understand how prenatal cannabis exposure affects child neurodevelopment.

The endocannabinoid system, a molecular signaling system of cannabinoid receptors, also plays a role in social behavior and emotionality which are two aspects of human behavior that are altered in individuals with ASD (Wall & Crawford, 2019). Recent population level, epidemiological research has found that rising cannabis use is linked with increasing rates of ASD in 50 of 51 states in the U.S. (including Washington D.C.) and that when the nine highest cannabis use states are grouped together, ASD is rising significantly faster there than elsewhere (Reece & Hulse, 2019). Only a few empirical studies have tested the association between prenatal cannabis use and ASD at an individual level. In 2015, Wood and colleagues found that first trimester cannabis use predicted higher offspring ASD scores using a behavioral scale. More recently, Corsi and colleagues (2020) found an association such that children with prenatal cannabis exposure had an increase of 50% in the risk of ASD diagnosis. Authors called for further research to replicate these findings in a prospective sample with controls for potential confounds – a gap this project can fill.

One of the best known risk factors for ASD is biological sex. ASD is 4.3 times as prevelant among boys than girls. Prenatal cannabis use also appears to affect males differently than females (Maenner et al., 2020). Using an animal model, Frau and colleagues (2019) found

that male rats that were exposed to THC *in utero* exhibited a hyperdopaminergic state that lead to increased behavioral sensitivity in preadolescence that could manifest in aberrant associative learning and abnormal reward processing. In contrast, prenatal THC does not affect the mesolimbic dopaminergic system, or socioemotional behavior, in preadolescent female rats (Traccis et al., 2021).

Maternal Stress

In their review of the association between maternal prenatal stress and offspring ASD, Kinney and colleagues (2008) summarized numerous studies involving different species, research designs, types of prenatal stressors, and measures of postnatal sensitivity to stress. They concluded that prenatal stress is significantly associated with a number of ASD related behaviors. For example, prenatal stress can make individuals hyper-aroused when faced with novel or challenging postnatal stimuli. Individuals that are hyper-aroused are, in turn, more likely to engage in stereotyped motor behaviors (Kinney et al., 2008). Class and colleagues (2014) evaluated mothers' bereavement stress preconception, during pregnancy, and postnatally. They found no associations between preconception stress and ASD, but replicated findings of associations of maternal stress during pregnancy and increased rates of offspring ASD.

It is important to acknowledge how race is associated with the amount of stress experienced by pregnant women. A recent study that directly compared stressors experienced by non-Hispanic White and Black women in pregnancy found that non-Hispanic Black women experienced higher levels of biological indicators of stress, relative to non-Hispanic White women in the sample at the same SES (Borders, 2015). According to Robert Clark's biopsychosocial model of racism as a stressor (Clark et al., 1999), these findings may be partially due to additional race-related stressors such as racial discrimination. In fact, Nancy Krieger

(1990) examined the impact of racial and gender discrimination on hypertension, a condition understood to be caused in part by stress and internalized anger, and found that Black women who internalized unfair treatment were 4.4 times more likely to report hypertension. Additionally, Murry and colleagues (2001) examined the impact of racial discrimination on Black American women's ability to manage stress, function psychologically, and maintain family relationships. Results suggested that Black American mothers who encountered high levels of racial discrimination were more likely to experience psychological distress.

Lower SES has also been associated with increased stress. Individuals from lower SES report greater exposure to stressful life events and greater impact of these events on their lives than individuals from higher SES (Leffman & Combs-Orme, 2014). Morever, Santiago, Wadsworth, and Stump (2011) examined SES, income, neighborhood disadvantage and found that poverty-related stress was directly related to anxious/depressed symptoms. Because children are already at a disadvantage in under-resourced conditions, it is reasonable to hypothesize that the negative impacts of prenatal cannabis exposures would be more prominent in children from low SES backgrounds. For instance, Khoury, Jamieson & Milligan (2018), found prenatal SES moderated the relationship between prenatal alcohol exposure and child behavior problems. Children from low SES families suffered from greater behavioral issues as a result of prenatal alcohol exposure compared to those from high SES families. Therefore, evidence supports that cannabis-exposed infants may develop differently depending on their family’s level of SES.

In his biopsychosocial model of stress and disease, Hector Myers (2009) proposed that the persistent racial/ethnic health disparities can best be understood as the byproducts of the complex interactions between race/ethnicity and SES factors. The model also acknowledged that the hypothesized stress-biological processes-disease pathway is likely moderated by a number of

psychosocial and behavioral factors, including substance use. Under this model, I would expect the reported psychological distress, racial discrimination, and socioeconomic status of mothers to each interact with prenatal cannabis exposure to increase the likelihood of ASD behaviors in their children.

The Present Study

As suggested by the empirical literature to date, both prenatal cannabis use and prenatal stress are associated with an increase in the risk of ASD in children. However, there is no overlap in the toxicant and stress literatures despite studies showing that stress-related factors such as socioeconomic status, maternal anxiety, depression, and trauma are associated with higher odds of cannabis use among pregnant women (Oh et al, 2017; Young-Wolff et al, 2020). *This project aims to fill the gap between prenatal toxicant and stress literatures as they relate to autism outcomes.*

Additionally, the existing literatures linking stress and toxicants to ASD independently are not without methodological weaknesses. Most of the research evaluating prenatal cannabis use utilizes retrospective maternal self-report which is subject to recall-bias (Reece & Hulse, 2020). Alternatively, the current project is prospective: mothers were asked about their substance use during their pregnancy by clinical providers and research staff. Self-reported substance use is also vulnerable to underreporting due to social desirability - some mothers might feel shame and/or be fearful of being judged. To help diminish this effect, we will also evaluate a portion of our sample's self-reported substance use with biological assays obtained from urine samples.

The majority of the studies in the stress and ASD literature evaluate only a single component of stress. Turner and Avison (2003) compared a life events checklist alone to a wider range of measures of stress--recent life events, chronic stressors, lifetime major events, and

discrimination and found that life events alone systematically underestimated stress exposure among Black Americans relative to their White counterparts and among persons of lower socioecnomic status relative to their more advantaged counterparts. Therefore, by employing similarly comprehensive methods of evaluating stress, this project provides a more holistic approach that might better address the relevant stressors in Black American communities.

Lastly, most studies examining ASD use diagnostic labels as opposed to continuous variables that can capture a broader scope of ASD-related behaviors. By utilizing continuous measures of ASD-related behaviors, this project aims to capture the extent to which prenatal cannabis use and stress impact the full spectrum of these behaviors. Furthermore, to our knowledge, this will be one of the few studies to examine ASD behaviors in an all Black sample (Exception: Constantino et al., 2020). This within-group evaluation is especially important given the aforementioned racial disparities in ASD prevalence. This work will contribute to a better understanding of within-race risk for ASD, providing insight into risk and protective factors relevant to Black American families.

Objective

The overarching goal of the current project is to examine the association between maternal cannabis use in pregnancy, maternal stress, and child autism-related behaviors with special attention to how maternal experiences of discrimination and socioeconomic status affect these associations.

Specific Aims

Aim 1: To examine the association between prenatal cannabis use and child ASD-related behaviors. We hypothesize that prenatal cannabis use will predict increased levels of child ASD-related behaviors, and that this association will remain significant when covariates such as child

biological sex, socioeconomic status, and maternal tobacco and alcohol use in pregnancy are controlled.

Aim 2: To examine the association between prenatal stress and child ASD-related behaviors. We hypothesize that prenatal stress will predict increased levels of child ASD-related behaviors and that this association will remain significant when covariates such as child biological sex, socioeconomic status, and maternal tobacco and alcohol use in pregnancy are controlled.

Aim 3: To examine prenatal stress and racial discrimination as potential moderators of the association between prenatal cannabis use and child ASD-related behaviors. We hypothesize that positive associations between prenatal cannabis use and child ASD-related behaviors will be stronger in cases of (a) higher prenatal distress, (b) higher racial discrimination, and (c) lower SES.

Methods

Participants

The participants in this study were 172 Black American children and their mothers recruited from an ongoing longitudinal study that follows Black American women through pregnancy (Corwin et al., 2017) and again at child ages two through six years, as part of the ECHO consortium (Padula et al., 2020). Women were recruited for the larger ongoing prenatal study during the first trimester of pregnancy from Grady Memorial Hospital, a public hospital, and Emory Midtown Hospital, a private hospital in Atlanta, resulting in a socioeconomically diverse sample. Exclusion criteria for follow-up were: 1) non-singleton birth, 2) fetal death prior to labor and 3) major congenital anomalies.

Procedures

Pregnant Black women (ages 18-40 years) were recruited from the Emory and Grady prenatal clinics at 8-14 weeks' gestation (as determined by standard criteria based upon last menstrual period and/or first trimester ultrasound). In the context of the prenatal study, mothers reported on their cannabis use and stress in their second (between 8 and14 weeks) and third (between 24 and 30 weeks) trimesters. Participants were then invited to participate in a separate study on child health outcomes when their children reached the age of two years. Child ASD behaviors were assessed in the child follow up study as described in detail below.

Measures

Maternal Prenatal Substance Exposure:

The *Timeline Follow-back Interview (*TLFB) was administered by research interviewers to the mothers twice during pregnancy to assess prenatal substance use. This survey assesses the consumption of a variety of drugs (i.e., tobacco, cannabis, alcohol, cocaine, etc.) during the last month and how many total times mothers used these substances (Sobell & Sobell, 1992).

Medical Record Review: Maternal reports of cannabis use were also obtained from clinical provider notes within the medical record. Providers typically conducted a 'clinical interview' during the initial prenatal appointment where they take an in-depth history. The provider documentation usually says something like "positive for marijuana use" or "uses marijuana" or "no substance use" and this reflects what the provider ascertained during the clinical interview. Providers had the ability to update the history and document substance use that occured after the initial encounter.

Nicotine and Cannabis Metabolites were assessed from maternal urine collected twice during pregnancy, and used for biological assay. Specifically, the conjugated and non-conjugated species of cotinine (COT), 3-OHCOT, and tetrahydrocannabinolic acid (THCA) were measured by liquid chromatography (LC) with an electrospray ionization (ESI) interface-tandem mass spectrometry (MS/MS) with isotope dilution quantification. In each analytical run, 78 unknown samples are analyzed concurrently with a 10-point calibration curve, one matrix blank sample, six quality control samples (all prepared using pooled non-smokers' urine), and one solvent blank sample. The method has relative standard deviations <10% across the calibration range. The limits of detection are 1.25 ng/mL for COT and 3-OH-COT and 5 ng/mL for THCA. For all analyses, strict quality assurance and quality control (QA/QC) procedures are followed. In addition, method accuracies are validated by analyses of certified standard reference material (SRM) 3672, 3673, and 1507b obtained from the U.S. National Institute of Standards and Technology (NIST). Urine samples were not initially collected in the prenatal study, so these data are only available on a subsample of 77 particpants.

In the current study, we used categorical measures of cannabis, tobacco, alcohol , and other drug use (yes/no) across pregnancy. We collapsed substance use reported via TLFB and medical record review such that mothers who endorsed substance use in either was coded as yes. In our sample, 35% of mothers reported cannabis use, 18% tobacco use, 10% alcohol use, and 1% other illicit substances.

Autism Spectrum Disorder (ASD) Behaviors: At the age 2 follow-up, children were assessed for ASD Behaviors using three separate measures as described below.

Autism Diagnostic Observation Schedule (ADOS) is a semi-structured, clinician-administered assessment of communication, social interaction, and play (Lord et al., 2000). Developmentally appropriate modules were chosen for each follow up visit. In these modules, children were asked to do a number of tasks, such as tell a story or play a make-believe game. Each task loads onto one of three domains: Communication (e.g., conversation, reporting of events), Reciprocal Social Interaction (e.g., quality of social response, overall quality of rapport), and Stereotyped Behaviors and Restricted Interests (e.g., Unusual sensory interest in play material/person). A total ADOS score provides a continuous measure of ASD behaviors and will be used for this study.

Modified Checklist for Autism in Toddlers (M-CHAT) was also administered to the mothers at the ECHO 2 year visit to assess for developmental delays. The M-CHAT is a 20-item autism screener that assesses developmental delays. Although the MCHAT is most typically used in high-risk populations of toddlers at risk for autism, it has recently demonstrated concurrent and predictive validity for cognitive development in community samples (Robins et al., 2001). Total scores on the M-CHAT were used as a continuous measure of ASD behaviors in this study.

The *Achenbach Child Behavior Checklist* (CBCL) 1.5-5 was completed by the mothers at the ECHO age 2 follow up, focusing on her observations of her child's behavior. The CBCL is a widely used measure for assessing child behavior and has been shown to have excellent reliability and validity (Achenbach & Rescorla, 2000). For this study I used the Pervasive Developmental Problems (PDP) scale which was constructed based on DSM-IV criteria and consists of 13 items (Cronbach's alpha = 0.75; Achenbach, Dumenci, & Rescorla, 2003). Studies show that children with ASD score significantly

higher on the PDP scale compared to both low- and high-risk counterparts (Rescorla et al., 2019).

I ran correlations between ASD outcome measures to assess whether a latent variable approach might be appropriate. We found that ASD measures were significantly correlated with each other (see **Table 1**). However, because the correlations were lower than expected and different measures of ASD were correlated with different predictors, we opted to evaluate each measure separately.

Maternal Prenatal Psychological Distress: Pregnant women provide self-report measures of perceived stress, anxiety, and depressive symptoms at two prenatal visits (in mid and late pregnancy) using the following measures:

Perceived Stress Scale (PSS) is a 14-item questionnaire that measures experiences of stress in the last month. PSS scores significantly correlate with HPA axis function during pregnancy and postpartum (Cohen, Kamarck, & Mermelstein, 1983).

Spielberger State-Trait Anxiety Inventory (STAI) measures current stress and anxiety as well as anger traits using a 20-item inventory (scored 0-1). It has been widely used in perinatal studies and is well-validated in minority and low-literacy populations stress (Gaudry et al., 1975; Spielberger et al., 1970).

Edinburgh Depression Scale (EDS) is a 10-item scale that assesses depressive symptoms. This measure has been shown to have acceptable sensitivity and specificity in community samples and good construct validity when compared with structured clinical interviews (Cox et al., 1987).

Principal components analysis (PCA) revealed that the PSS, STAI and EDS loaded together onto a single factor. Therefore, we standardized scores on each of these

measures, combined them for each pregnancy timepoint, and then averaged across timepoints to create a single maternal prenatal stress measure for the purpose of data analysis.

Socioeconomic Status: SES is assessed via maternal self-reports of education, income, marital status, and insurance type at the prenatal visits as derived from a second component of the PCA with maternal prenatal psychological distress.

Racial Discrimination: Pregnant women self-reported their experiences of discrimination at the first prenatal visit.

Jackson, Hogue, Phillips Contextualized Stress Measure (JHP) is a measure designed to assess chronic intersectional racial and gendered stress (Jackson, 2005). This tool is a multidimensional measure created from focus groups and interviews as part of community-based participatory research where Black American women were asked to elaborate on their particular racial and gendered stressors and stress mediators. The JHP consists of subscales for assessing racism, gendered roles and burden, abuse and neglect, workplace stress, coping, social support, and affective stress responses (distress). The racism subscale, which is what will be used for this project, reflects ongoing concerns over their children's exposure to racism, inequitable neighborhood resources, White privilege, and racial stereotypes (e.g., “I have to go outside of Black American communities to provide the educational and other resources I desire for my children”).

The *Krieger Experiences of Discrimination Scale* assesses self-reported experiences of race-based discrimination across the lifespan (Krieger et al., 2005). On this measure, individuals are asked whether, and how many times, they have been discriminated against in 9 different situations (e.g., “getting hired or getting a job” and

"getting services in a store or restaurant"). Consistent with epidemiological research showing that experiencing discrimination across a greater number of different situations predicts psychological symptoms among AA women (Ertel et al., 2012), we measured the number of different situations in which women experienced discrimination. Our discrimination summary score therefore ranged from 0 to 9, with higher scores representing discrimination in more situations.

In our sample, discrimination scores were correlated with each other (r=. 33). Therefore, we computed a combined racism score by summing the standardized scores from the JHP and the Krieger.

Results

Descriptive Statistics & Exploratory Correlations

Table 2 displays descriptive statistics for the study variables in the current sample. In this table, we compared cannabis users to non-cannabis users. Of note, there was a statistically significant difference in education level, marital status, and prenatal tobacco use between mothers who used cannabis and those who did not. As described previously, these differences were expected based on the literature. There were no other significant differences between mothers who did and did not use cannabis during pregnancy.

Preliminary Analyses

Table 3 displays bivariate correlations between our ASD measures and the covariates and proposed moderators. The covariates included in all analyses were child biological sex, prenatal tobacco use, prenatal alcohol use, and other illicit drug use during pregnancy. The sex and substance exposure covariates were determined a priori based on previous literature. Socioeconomic status was added as a covariate for Aims 1 and 2 because we saw that the SES-

related factors maternal education and marital status were significantly correlated with cannabis use.

The ADOS was significantly correlated with child sex and tobacco use during pregnancy. Surprisingly, we did not see these expected associations with the other, maternally reported measures of autism behavior.

Testing the Hypotheses

To test Aim 1, we conducted an analysis of covariance (ANCOVA) to test if prenatal cannabis use predicts ASD-related behaviors. We found no evidence that self-reported prenatal cannabis exposure impacts child ASD behaviors measured by the ADOS ($F_{(1,170)}$ =.01, p=.98), M-CHAT ($F_{(1,170)}$ =.71, p=.40), or CBCL ($F_{(1,170)}$ =3.62, p=.06; see **Table 4**). We then controlled for sex, socioeconomic status, tobacco, alcohol , and other drug exposures. With these covariates included, prenatal cannabis still did not predict the autism behaviors of the children in our sample (**Table 5)**.

For the subsample we collected urine from, we also examined the association between THC levels and autism behaviors using a linear regression. We again found no support for an association between biologically measured THC and the ADOS (Adjusted R^2= -.01, $F_{(1,75)}$= .09, p=.77), M-CHAT (Adjusted R^2=.03 , $F_{(1,75)}$= 3.28, p=.07), or CBCL (Adjusted R^2= -.01, $F_{(1,75)}$= .03, p= .86). With covariates included, THC still did not predict the autism behaviors of the children in our sample.

To test Aim 2, we conducted linear regressions to test if prenatal distress predicts ASD-related behaviors. We found that prenatal distress predicted autism behaviors reported on the CBCL (ΔR^2= .07, t=3.65, p<.001), but not the ADOS (ΔR^2= 0, t=-.17, p=.87), or M-CHAT (ΔR^2= .01, t=.98, p=.33; **Table 6)**. Prenatal distress significantly predicted CBCL scores when

the previously described covariates were included in the model (ΔR^2=.07 , t=3.64, p<.001; **Table 7).**

For Aim 3, we created interaction terms of (a) prenatal cannabis use and prenatal maternal psychological distress, (b) prenatal cannabis use and racial discrimination, and (c) prenatal cannabis use and SES to test whether these significantly predict ASD-related behaviors above and beyond main effects. Each stressor variable was examined in a separate regression model. Using SPSS PROCESS, we found that SES, experiences of discrimination, and maternal distress did not interact with cannabis to predict any of the ASD measures (see **Table 8**). Results were similar when covariates were included in the models.

Discussion

This study examined the association between cannabis use in pregnancy and child autism-related behaviors and evaluated prenatal stressors as moderators of this relationship. We found no significant associations between prenatal cannabis use and autism behaviors. These findings add to the mixed literature on cannabis-ASD associations. Although some recent reports have indicated an association between prenatal cannabis exposure and ASD (Corsi et al., 2020; Wood et al., 2015), others have not (DiGuiseppi et al., 2021). Importantly the current study focused on continuous measures of ASD behaviors, finding that maternal prenatal cannabis use did not increase risk for these behaviors; we did not examine risk for diagnosis per se. Our sample consisted of Black mother child dyads, who have not been well represented in previous literatures and for whom disparities in ASD care have been noted. As such it adds unique data to the literature.

Our findings suggest that other prenatal risks might be more pertinent in the prediction of child ASD related behaviors, namely prenatal stress and prenatal tobacco use. These results

replicate previous findings in the literature (Hertz-Picciotto et al., 2022; Kinney et al., 2008), and extend them to a Black sample.

Contrary to our expectations, children were not at greater risk for ASD-related outcomes if their mothers used cannabis and were exposed to high levels of stress in pregnancy. It might be that other combinations of prenatal exposures are more relevant to ASD risk, such as prenatal toxicant exposures and prenatal stress. Future studies should explore this possibility, particularly in Black samples where structural racism might lead to greater exposures.

Much is still unknown about the mechanisms through which prenatal cannabis exposure might impact child development. Proposed mechanisms include neonatal outcomes such as lower gestational age at birth and lower birth weight. In our sample, we did not see significant differences in these neonatal outcomes between exposed and non-exposed infants, despite previous findings. Another proposed mechanism by which parental cannabis use might impact ASD is through genetic modification. Research supports the notion that prenatal cannabis exposure alters genes and molecular pathways that are associated with psychopathology such as ASD and anxiety (Rompala, Nomura, & Hurd, 2021; Smith et al., 2020). Schrott and colleagues (2020) found that cannabis use is associated with widespread DNA methylation changes in human and rat sperm. They specifically identified these changes in Discs-Large Associated Protein 2 (DLGAP2), a gene involved in synapse organization, neuronal signaling, and strongly implicated in autism. This suggests that fathers' preconceptual cannabis use could also affect the development of child autism symptoms. The current study did not gather paternal reports of cannabis use, leaving this question untested in our sample.

Most of the research examining cannabis biomarkers solely focuses on THC, and not the other components of cannabis including cannabinoidol (CBD), It is also possible that the method

of consumption (i.e. smoking versus vaping versus eating) could alter how it's metabolized and therefore how it affects the fetus. Moreover, little research has examined the impact of postnatal cannabis exposure including transfer through breastfeeding. Future work is needed to better assess these unaddressed issues.

Strengths and Limitations of the Current Study

Strengths of the current study included the use of biological and self report measures of prenatal cannabis use, continuous measures of autism behaviors, and the inclusion of multiple measures of each construct. It is possible that our sample was too small for moderation analyses. Because of our limited sample size, we were unable to take a closer look at the role of other factors which might also shed light on the proposed associations such as child biological sex and gestational age.

A strength of the study is the high rate of cannabis users in our sample. Clinical reports of cannabis use prevalence during pregnancy vary widely from 3% to upwards of 35% in North America (Nashed, Hardy, & Laviolette, 2021). Our sample reported cannabis use during pregnancy at the upper end of this range with 35% of mothers self-reporting cannabis use. This high rate of use would allow us to detect most effects, save a really small effect size, which increases our confidence that such an association truly does not exist. Of note, the prevalence rate of two studies that did find significant associations between prenatal cannabis exposure and autism behavios were .6% and 18% respectively (Corsi et al., 2020; Wood et al., 2015). Relatedly, neither of these studies reported on the race/ethnicity of their samples.

Another strength of this study is that it evaluated associations in an all Black sample. When racial disparities are reported, it is often assumed that this is due to experiences of discrimination

and/or SES. By specifically examining these constructs in our sample, we were able to control for these potential confounds.

This study also utilized a community sample with relatively low rates of autism symptoms. It is possible that the children in our sample had lower rates of autism behaviors due to attrition if parents of children with more severe behavioral presentations were less likely to attend the follow-up visit. Although autism is reliably diagnosed at age 2, research shows that Black children tend to be diagnosed later than White children (Mandell et al., 2002; Maenner et al., 2020). A longitudinal study that extends to later child ages to assess ASD-related behaviors may yield different results.

Conclusion

In conclusion, we replicated previous findings that prenatal stress predict parent reported autism behaviors. We found no evidence that prenatal cannabis use increases risk for autism-related behaviors in a sample of Black children. Given the mixed findings in the literature, the clinical significance of our current work is unclear. Upon replication, these findings may inform health policy recommendations regarding cannabis use and its legalization.

References

Achenbach, T. M., Dumenci, L., & Rescorla, L. A. (2003). DSM-oriented and empirically based approaches to constructing scales from the same item pools. *Journal of clinical child and adolescent psychology*, *32*(3), 328-340.

Achenbach, T. M., & Rescorla, L. A. (2000). ASEBA preschool forms & profiles. *Burlington, VT: Research Center for Children, Youth and Families, University of Vermont*.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: Publisher.

Barker D. J. (2007). The origins of the developmental origins theory. J. Intern. Med. 261 412–417. 10.1111/j.1365-2796.2007.01809.x

Bishop-Fitzpatrick, L., & Kind, A. J. (2017). A scoping review of health disparities in autism spectrum disorder. Journal of Autism and Developmental Disorders, 47(11), 3380-3391.

Borders, A. E., Wolfe, K., Qadir, S., Kim, K. Y., Holl, J., & Grobman, W. (2015). Racial/ethnic differences in self-reported and biologic measures of chronic stress in pregnancy. *Journal of Perinatology*, *35*(8), 580-584.

Brown Q. L., Sarvet A. L., Shmulewitz D., Martins S. S., Wall M. M., Hasin D. S. (2017). Trends in marijuana use among pregnant and nonpregnant reproductive-aged women, 2002-2014. *JAMA* 317 207–209. 10.1001/jama.2016.17383

Chiang, T. L., Lin, S. J., Lee, M. C., & Shu, B. C. (2018). Advanced maternal age and maternal education disparity in children with autism spectrum disorder. *Maternal and Child Health Journal*, *22*(7), 941-949.

Clark, Rodney, et al. “Racism as a Stressor for African Americans.” American Psychologist, 1999, p. 12.

Class, Q. A., Abel, K. M., Khashan, A. S., Rickert, M. E., Dalman, C., Larsson, H., Hultman, C., Langstrom, N., Lichtenstein, P., & D'Onofrio, B. M. (2014). Offspring psychopathology following preconception, prenatal and postnatal maternal bereavement stress. *Psychological Medicine*, *44*(1), 71-84.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of health and social behavior*, 385-396.

Constantino, J. N., Abbacchi, A. M., Saulnier, C., Klaiman, C., Mandell, D. S., Zhang, Y., Hawks, Z., Bates, J., Klin, A., Shattuck, P., Molholm, S., Fitzgerald, R., Roux, A., Lowe, J.K. , & Geschwind, D. H. (2020). Timing of the diagnosis of autism in African American children. *Pediatrics*, *146*(3).

Corsi, D. J., Donelle, J., Sucha, E., Hawken, S., Hsu, H., El-Chaâr, D., Bisnaire, L., Fell, D., Wen, S.W., & Walker, M. (2020). Maternal cannabis use in pregnancy and child neurodevelopmental outcomes. *Nature Medicine*, *26*(10), 1536-1540.

Corwin, E. J., Hogue, C. J., Pearce, B., Hill, C. C., Read, T. D., Mulle, J., & Dunlop, A. L. (2017). Protocol for the Emory University African American Vaginal, Oral, and Gut Microbiome in Pregnancy Cohort Study. *BMC Pregnancy and Childbirth*, *17*(1), 161. https://doi.org/10.1186/s12884-017-1357-x

Cox, J. L., Holdenand, J. M., & Sagovsky, R. (1987). Detection of Postnatal Depression Development of the 10-item Edinburgh Postnatal Depression Scale. In *British Journal of Psychiatry* (Vol. 150). Retrieved from https://pdfs.semanticscholar.org/a5ab/73bca06b9592eba2f6bc79cd8b5eb0543c5c.pdf

DiGuiseppi, C., Crume, T., Van Dyke, J., Sabourin, K. R., Soke, G. N., Croen, L. A., Daniels, J.L., Lee, L.C., Schieve, L.A., Windham, G.C. and Friedman, S. & Robinson Rosenberg, C. (2021). Peri-

pregnancy cannabis use and autism spectrum disorder in the offspring: Findings from the study to explore early development. *Journal of Autism and Developmental Disorders*, 1-8.

Durkin, M. S., Maenner, M. J., Baio, J., Christensen, D., Daniels, J., Fitzgerald, R., Imm, P., Lee, L.C., Schieve, L.A., Van Naarden Braun, K. and Wingate, M.S. & Yeargin-Allsopp, M. (2017). Autism spectrum disorder among US children (2002–2010): socioeconomic, racial, and ethnic disparities. American Journal of Public Health, 107(11), 1818-1826.

Ertel, K. A., James-Todd, T., Kleinman, K., Krieger, N., Gillman, M., Wright, R., & Rich-Edwards, J. (2012). Racial discrimination, response to unfair treatment, and depressive symptoms among pregnant black and African American women in the United States. *Annals of Epidemiology, 22*(12), 840–846. https://doi.org/10.1016/j.annepidem.2012.10.001

Frau, R., Miczán, V., Traccis, F., Aroni, S., Pongor, C. I., Saba, P., Serra, V., Sagheddu, C., Fanni, S., Congiu, M., Devoto, P., Cheer, J., Katona, I., & Melis, M. (2019). Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone. Nature neuroscience, 22(12), 1975-1985.

Gaudry, E., Vagg, P., & Spielberger, C. D. (1975). Validation of the state-trait distinction in anxiety research. *Multivariate behavioral research*, *10*(3), 331-341.

Hertz-Picciotto, I., Korrick, S. A., Ladd-Acosta, C., Karagas, M. R., Lyall, K., Schmidt, R. J., & program collaborators for Environmental influences on Child Health Outcomes (ECHO). (2022). Maternal tobacco smoking and offspring autism spectrum disorder or traits in ECHO cohorts. *Autism Research*.

Hertz-Picciotto, I., Schmidt, R. J., & Krakowiak, P. (2018). Understanding environmental contributions to autism: Causal concepts and the state of science. *Autism Research, 11*(4), 554-586.

Imm, P., White, T., & Durkin, M. S. (2019). Assessment of racial and ethnic bias in autism spectrum disorder prevalence estimates from a US surveillance system. Autism, 23(8), 1927-1935.

Insel, T., Cuthbert, B., Garvey, M., Heinssen, R., Pine, D. S., Quinn, K., Sanislow, C., & Wang, P. (2010). Research domain criteria (RDoC): toward a new classification framework for research on mental disorders.

Jackson, F. M. (2005). The development of a race and gender-specific stress measure for African-American women: Jackson, Hogue, Phillips contextualized stress measure. *Ethnicity & disease*, *15*(4), 594-600.

Jarlenski, M., Koma, J. W., Zank, J., Bodnar, L. M., Bogen, D. L., & Chang, J. C. (2017). Trends in perception of risk of regular marijuana use among US pregnant and nonpregnant reproductive-aged women. *American Journal of Obstetrics and Gynecology*, *217*(6), 705.

Khoury, J. E., Jamieson, B., & Milligan, K. (2018). Risk for childhood internalizing and externalizing behavior problems in the context of prenatal alcohol exposure: A meta-analysis and comprehensive examination of moderators. *Alcoholism: Clinical and Experimental Research*, *42*(8), 1358-1377.

Khowaja, M. K., Hazzard, A. P., & Robins, D. L. (2015). Sociodemographic barriers to early detection of autism: screening and evaluation using the M-CHAT, M-CHAT-R, and follow-up. *Journal of Autism and Developmental Disorders*, *45*(6), 1797-1808.

Kinney, D. K., Munir, K. M., Crowley, D. J., & Miller, A. M. (2008). Prenatal stress and risk for autism. *Neuroscience & Biobehavioral Reviews*, *32*(8), 1519-1532.

Ko, J. Y., Tong, V. T., Bombard, J. M., Hayes, D. K., Davy, J., & Perham-Hester, K. A. (2018). Marijuana use during and after pregnancy and association of prenatal use on birth outcomes: A population-based study. *Drug and Alcohol Dependence, 187*, 72-78.

Krieger, N. (1990). Racial and gender discrimination: risk factors for high blood pressure?. *Social Science & Medicine*, *30*(12), 1273-1281.

Krieger, N., Smith, K., Naishadham, D., Hartman, C., & Barbeau, E. M. (2005). Experiences of discrimination: validity and reliability of a self-report measure for population health research on racism and health. *Social Science & Medicine*, *61*(7), 1576-1596.

Lefmann, T., & Combs-Orme, T. (2014). Prenatal stress, poverty, and child outcomes. *Child and Adolescent Social Work Journal*, *31*(6), 577-590.

Lord, C., Risi, S., Lambrecht, L., Cook, E. H., Leventhal, B. L., DiLavore, P. C., Pickles, A., & Rutter, M. (2000). The Autism Diagnostic Observation Schedule—Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*, *30*(3), 205-223.

Maenner M.J., Shaw K.A., Baio J, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. MMWR Surveill Summ 2020;69(No. SS-4):1–12. DOI:

Mehmedic, Z., Chandra, S., Slade, D., Denham, H., Foster, S., Patel, A. S., Ross, S.A., Khan, I.A., & ElSohly, M. A. (2010). Potency trends of Δ9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. *Journal of Forensic Sciences*, *55*(5), 1209-1217.

Myers, H. F. (2009). Ethnicity-and socio-economic status-related stresses in context: an integrative review and conceptual model. *Journal of Behavioral Medicine*, *32(*1), 9-19.

Modabbernia, A., Velthorst, E., & Reichenberg, A. (2017). Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. *Molecular Autism*, *8*(1), 13.

Murry, V. M., Brown, P. A., Brody, G. H., Cutrona, C. E., & Simons, R. L. (2001). Racial discrimination as a moderator of the links among stress, maternal psychological functioning, and family relationships. *Journal of Marriage and Family*, *63*(4), 915-926.

Nashed, M. G., Hardy, D. B., & Laviolette, S. R. (2021). Prenatal cannabinoid exposure: Emerging Evidence of Physiological and Neuropsychiatric Abnormalities. *Front. Psychiatry*, *11*, 624275.

Oh, S., Salas-Wright, C. P., Vaughn, M. G., & DiNitto, D. M. (2017). Marijuana use during pregnancy: A comparison of trends and correlates among married and unmarried pregnant women. *Drug and Alcohol Dependence*, *181*, 229-233.

Padula, A.M., Monk, C., Brennan, P.A., Borders, A., Barrett, E.S., McEvoy, C.T., Foss, S., Desai, P., Alshawabkeh, A., Wurth, R. and Salafia, C. (2020). A review of maternal prenatal exposures to environmental chemicals and psychosocial stressors—implications for research on perinatal outcomes in the ECHO program. *Journal of Perinatology*, *40*(1), pp.10-24.

Reece, A. S., & Hulse, G.K. (2020). Cannabis in pregnancy–rejoinder, exposition and cautionary tales.

Reece, A. S., & Hulse, G. K. (2019). Epidemiological associations of various substances and multiple cannabinoids with autism in USA. *Clinical Pediatrics OA*, *4*(2), 155.

Rescorla, L. A., Winder-Patel, B. M., Paterson, S. J., Pandey, J., Wolff, J. J., Schultz, R. T., & Piven, J. (2019). Autism spectrum disorder screening with the CBCL/1½–5: Findings for young children at high risk for autism spectrum disorder. *Autism*, *23*(1), 29-38.

Robins, D. L., Fein, D., Barton, M. L., & Green, J. A. (2001). The Modified Checklist for Autism in Toddlers: an initial study investigating the early detection of autism and pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *31*(2), 131-144.

Rompala, G., Nomura, Y., & Hurd, Y. L. (2021). Maternal cannabis use is associated with suppression of immune gene networks in placenta and increased anxiety phenotypes in offspring. *Proceedings of the National Academy of Sciences*, *118*(47).

Santiago, C. D., Wadsworth, M. E., & Stump, J. (2011). Socioeconomic status, neighborhood disadvantage, and poverty-related stress: Prospective effects on psychological syndromes among diverse low-income families. *Journal of Economic Psychology, 32*(2), 218-230.

Sobell, L. C., & Sobell, M. B. (1992). Timeline follow-back. In *Measuring Alcohol Consumption* (pp. 41-72). Humana Press, Totowa, NJ.

Smith, A., Kaufman, F., Sandy, M. S., & Cardenas, A. (2020). Cannabis exposure during critical windows of development: Epigenetic and molecular pathways implicated in neuropsychiatric disease. *Current Environmental Health Reports*, *7*(3), 325-342.

Spielberger, C. D., Gorsuch, R. L., & Lushene, R. E. (1970). Stai. *Manual for the State-Trait Anxiety Inventory (Self Evaluation Questionnaire). Palo Alto California: Consulting Psychologist*, *22*, 1-24.

Traccis, F., Frau, R., & Melis, M. (2020). Gender differences in the outcome of offspring prenatally exposed to drugs of abuse. *Frontiers in Behavioral Neuroscience*, 14.

Turner, R. J., & Avison, W. R. (2003). Status variations in stress exposure: Implications for the interpretation of research on race, socioeconomic status, and gender. *Journal of Health and Social Behavior*, 488-505.

Wood, A. G., Nadebaum, C., Anderson, V., Reutens, D., Barton, S., O'Brien, T. J., & Vajda, F. (2015). Prospective assessment of autism traits in children exposed to antiepileptic drugs during pregnancy. *Epilepsia*, *56*(7), 1047-1055.

Wall, K. M., & Crawford, S. A. (2019). Is the Use of Cannabis During Pregnancy a Risk Factor for Autism. *Autism Open Access, 9*, 245.

Westfall, R. E., Janssen, P. A., Lucas, P., & Capler, R. (2006). Survey of medicinal cannabis use among childbearing women: patterns of its use in pregnancy and retroactive self-assessment of its efficacy against 'morning sickness'. *Complementary Therapies in Clinical Practice, 12*(1), 27-33.

Young-Wolff, K. C., Sarovar, V., Tucker, L. Y., Goler, N. C., Alexeeff, S. E., Ridout, K. K., & Avalos, L. A. (2020). Association of depression, anxiety, and trauma with cannabis use during pregnancy. *JAMA Network Open, 3*(2), e1921333-e1921333.

Tables

Table 1.

Bivariate Correlations Between Measures of Autism Behaviors

		CBCL	ADOS	M-CHAT
CBCL	Pearson Correlation	1	.16	.31
	Sig. (2-tailed)		**.031***	**<.001****
ADOS	Pearson Correlation		1	.21
	Sig. (2-tailed)			**.005****
MCHAT	Pearson Correlation			1
	Sig. (2-tailed)			

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

Table 2.

Descriptive Charactristics of Sample Divided By Prenatal Cannabis Use

Total Sample N=172	***Cannabis Users N=60***	***Non-Cannabis Users N=112***	***Significance (p-values)***
Sex, N(%) Male	32 (53)	56(50)	.68
Gestational Age, M(SD)	38.40(2.47)	38.70 (1.65)	.35
Birth Weight, M(SD)	2991.22(567.21)	3134.10(491.38)	.09
Maternal Age, M(SD)	24.60(4.67)	25.54(4.61)	.20
Marital Status, N(%) Single	58(97)	89(80)	**.002****
Education, N(%) High School Diploma or less	43(72)	48(43)	**<.001****
Income, N(%) Below the poverty level	32(53)	45(40)	.10
Insurance Type, N(%) Medicaid	27(45)	40(36)	.23
Prenatal Tobacco Use, N(%)	22(71)	9(8)	**<.001****
Prenatal Alcohol Use, N(%)	8(47)	9(8)	.27
Prenatal Other Drug Use, N(%)	1(2)	0(0)	.17

**Correlation is significant at the 0.01 level (2-tailed).

Table 3.

Bivariate Correlations Between Covariates, Moderators, and Autism Measures

*Correlation is significant at the 0.05 level (2-tailed).

		Sex	Alcohol	Tobacco	Other Drugs	SES	Racism
ADOS	Pearson Correlation	**-.22**	.07	**.16**	.13	-.11	-.05
	Sig. (2-tailed)	**.003****	.37	**.04***	.09	.16	.48
CBCL	Pearson Correlation	-.10	-.11	.06	.08	-.14	.05
	Sig. (2-tailed)	.18	.17	.47	.30	.07	.55
M-CHAT	Pearson Correlation	-.09	.02	.08	.06	-.08	-.04
	Sig. (2-tailed)	.24	.80	.28	.43	.33	.59

**Correlation is significant at the 0.01 level (2-tailed).

Table 4

Main Effects ANCOVA Between Prenatal Cannabis Use and Autism Behaviors

	Partial Eta Squared	**F**	**p-value**
ADOS	.00006	0.01	.92
M-CHAT	.004	0.71	.40
CBCL	.021	3.62	.06

Table 5

Results of ANCOVA Between Prenatal Cannabis Use and Autism Behaviors with Covariates

	Partial Eta Squared	**F**	**p-value**
ADOS	.009	1.50	.22
M-CHAT	.017	2.78	.10
CBCL	.009	1.50	.22

Table 6

Main Effects Linear Regression Between Prenatal Distress and Autism Behaviors

	R-Squared Change	Standardized Beta	p-value
ADOS	0	-.01	.87
M-CHAT	.01	.08	.33
CBCL	.07	.27	**<.001****

Table 7

Results of Linear Regression Between Prenatal Distress and Autism Behaviors with Covariates

	R-Squared Change	Standardized Beta	p-value
ADOS	<.01	-.03	.74
M-CHAT	.01	.07	.37
CBCL	.07	.27	**<.001****

Table 8

Interactions Between Proposed Predictors, Prenatal Cannabis Use, and Autism Behaviors

	Interaction Coefficient	Standard Error	t	p-value	Confidence Interval
SES-ADOS	.02	.10	.17	.86	-.19-.22
SES-MCHAT	-.14	.04	-1.10	.27	-.39 — .11
SES-CBCL	-.20	.17	-1.18	.24	-.54 — .14
Distress-ADOS	<.01	.07	.07	.95	-.13 — .14
Distress-MCHAT	-.10	.08	-1.18	.24	-.26 — .06
Distress-CBCL	-.01	.11	-.07	.95	-.22— .20
Racism-ADOS	<.01	.04	.10	.92	-.08 — .08
Racism-MCHAT	.02	.05	.46	.64	-.08 — .12
Racism-CBCL	-.07	.07	-.96	.34	-.20 — .07