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Maternal Folate Status and Risk Factors for Autism Spectrum Disorders

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This is to certify that the doctoral dissertation by

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Walden University
2023

Abstract

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Michael M. Haniff

MS, Rush University Medical Center, 2015

BS, Florida Atlantic University, 2010

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Epidemiology

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Abstract

Autism spectrum disorders (ASD) are thought to be influenced by environmental, genetic, and maternal factors, including potential links to periconceptional folic acid. This quantitative case-control study, guided by the life course theory, explored the association of maternal risk factors (age, race, education, income, folate status, gestational diabetes, gestational hypertension, and MTHFR mutation) on the precipitation of ASD in offspring. The sample comprised 96 mothers of children with ASD and 143 mothers of children without ASD. Logistic regression analysis indicated no significant association between maternal folate status and ASD (OR = 1.00, 95% CI [1.000, 0.001], p = .755). Mothers with higher education were significantly less likely to have offspring with ASD than mothers with a high school diploma, GED, or less (OR = 0.251, 95% CI [0.103, 0.61], p = .002). Univariate analysis found the presence of gestational diabetes to be significantly associated (OR = 5.776, 95% CI [2.357, 14.156], p = < .001) and preeclampsia to be insignificantly associated (OR = 1.564, 95% CI [0.705, 3.469], p = .272) with ASD. However, an inverse relationship was observed between gestational diabetes and ASD in the final model. Further research is recommended to explore the relationship between ASD and maternal conditions, namely gestational diabetes and preeclampsia. These findings underscore the need for comprehensive prenatal care to promote optimal supplementation, nutrition, and strategies targeting modifiable risk factors in ASD risk during pregnancy. Within this context, researchers can better understand ASD etiology, develop targeted interventions, and contribute to more effective prevention efforts.

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Dedication

This work is dedicated to my loving mother. You will always be my foundation for strength, courage, and wisdom. Thank you for this incredible opportunity. And, to my family, close relatives, and friends, I am especially thankful to have you all in my life—you give me hope and joy, encourage positivity, and push me along my journey.

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I want to thank my committee members—Drs. Tawfik and Osoba; my best friends and colleagues—James, Michelle, Jason, and Matt; and my dogs—Knox, Polo, and Charlie. You each give unconditional love, joy, and happiness each day, helping me to keep my vibration high. Thank you all for your support throughout this process and in my life.

Table of Contents

List of Tables

Chapter 1: Introduction to the Study

Introduction

Autism spectrum disorder (ASD) has become a significant public health concern. Dietz et al. (2020) estimated that approximately 5.4 million individuals (2.21% of the US population) live with ASD. Multidisciplinary researchers have agreed on an upward trend of ASD diagnoses in the last 25 years (Cave, 2008; Dawson & Bernier, 2013; Maenner et al., 2020). Further, the World Health Organization (WHO, 2021) estimated that 1 in 270 people struggle with social interaction or communication, which indicates ASD may become a global concern.

The prevalence of ASD in the United States has nearly tripled from 1 in 150 (0.67%) in the early 2000s to 1 in 54 (1.85%) in 2016 (Maenner et al., 2020), to 1 in 44 (2.3%) in 2018. Updated prevalence estimates suggest that ASD now impacts 1 in 36 children 8 years or older, with a significant likelihood of boys developing ASD surpassing that of girls (1 in 145) by 4.3 times (Centers for Disease Control and Prevention [CDC], 2021; Maenner et al., 2020; Maenner et al., 2023).

Although no known cause exists, speculation implies this disorder curtails interactions between genetics and the environment. Researchers now postulate that ASD occurs in utero, possibly during the pre- and perinatal periods (Gardener et al., 2009). This postulation may stem from an interesting finding where patches of disorganization of the neocortical complex were found in the brains of 91% of children diagnosed with ASD—suggesting a high likelihood of in-utero development (Stoner et al., 2014).

Offering more conviction, the areas in which the patches were found highlight the regions that mediate the functions disturbed by autism.

In recent years, epidemiological studies have shifted the potential cause of this disorder to maternal dietary risk factors during pregnancy. Most of the studies underscore the crucial role of adequate folate (Vitamin B9) levels derived from diet and supplementation, particularly its overconsumption (Baio et al., 2018; Brimberg et al., 2013; Brown et al., 2017; Goldstein & Ozonoff, 2018). The rationale behind investigating folate is its crucial role in the proper neurodevelopment of a fetus and its potential to serve as a biomarker for early detection or diagnosis, improve symptoms, and prevent ASD (Frye et al., 2017). But research lacks an understanding of the mechanisms involved in the consumption, absorption, maintenance, and eventual transfer to the fetus during pregnancy. In addition to folate bioavailability, studies have indicated that ASD etiology may be regulated by comorbidities such as gestational diabetes (Roberts & Nguyen, 2015) and preeclampsia (Jenabi et al., 2019), underscoring the association between chronic conditions and increased ASD risk. In their study, Kodesh et al. (2021) described an association with an increased risk of ASD among mothers with metabolic, genitourinary, and psychiatric or depressive disorders, among other diagnoses. Given that chronic conditions are positively associated with an ASD risk, considerations for the presence of gestational diabetes and preeclampsia weighed as correlated factors in this study. To understand population-based characteristics, this study included social factors of race, income, maternal age at childbirth, and education.

The Autism Speaks Foundation (2020) indicated that the economic impact of ASD would surge to $461 billion (a 42% increase) by 2025. This projection correlates with concurrent estimates of an upward trend in ASD prevalence (Baio et al., 2018) and an increased need for utilization, provided no effective intervention is adopted. Contributing to this rise in spending is the difficulty involved in diagnosis.

ASD in early childhood may present with symptoms relating to cognition, communication, and behavior that are often overlooked as a child-like quality or normal childhood behavior, which often leads to a late diagnosis (American Psychiatric Association, 2013; National Institute of Mental Health [NIMH], n.d.). Children diagnosed with ASD often require support from a caregiver across their entire lifespan, further contributing to the lifelong financial burden and increased utilization costs. Improving the time to diagnosis and intervention would significantly impact effective prevention, management, and treatment strategies and help reverse rising ASD incidence and the costs associated with lifelong care treatment and management.

The rise in prevalence and lifelong care costs, along with the number of etiological factors potentially associated with a folate link, presents the opportunity to explore the nutrient as a possible biomarker to understand ASD etiology and early detection (Frye et al., 2017). In this study, I investigated the role of folate on ASD risk while adjusting for maternal age, race, income, education, and comorbidities during pregnancy, including preeclampsia and gestational diabetes.

Background

The specific cause of ASD remains elusive and is theorized as an interplay between environmental, behavioral, gestational, and genetic factors (Baio et al., 2018; Goldstein & Ozonoff, 2018). However, the role of maternal periconceptional and prenatal nutrition and health status is vastly understudied. During pregnancy, demands for folate, among other nutrients, increase as they are vital for normal fetal development and growth. Folic acid has been a critical ingredient in many foods and multivitamins since 1998 because of the food fortification program implemented by the US Food and Drug Administration (FDA) to counteract the rising incidence of spina bifida (Raghavan et al., 2018). Since the program's inception, the prevalence and incidence of spina bifida have decreased. But evidence from a prospective cohort study indicates that the fortification program may increase ASD risk. The study found a U-shaped correlation between ASD occurrence and maternal folate levels during pregnancy. Some studies contraindicate folate's protective effect against ASD (Braun et al., 2014; Li et al., 2018; Raghavan et al., 2018), while others suggest a decreased risk of ASD with periconceptional multivitamin supplementation and folic acid intake (Braun et al., 2014; Li et al., 2018; Raghavan et al., 2018). Surén et al. (2013) reported potential harm from folic acid to a developing fetus. Nonetheless, most of these studies rely heavily on self-reported data that may be prone to recall bias, warranting an investigation into adequate folate intake better to understand folate's crucial role in proper neurodevelopment.

Folate, also known as Vitamin B9, is an essential nutrient required for DNA replication and serves as a critical substrate for various enzymatic reactions involved in

amino acid synthesis and vitamin metabolism (Greenberg, Bell, Guan, & Yu, 2011). Normal gut bacteria cannot synthesize folate, so it must be incorporated into diet. Folate-rich food sources include green, leafy vegetables, sprouts, fruits, brewer's yeast, and liver (Scaglione & Panzavolta, 2014). Despite an evident ability to ensure these demands are met through diet alone, approximately 60% of the population cannot metabolize folate adequately (Greenberg, Bell, Guan, & Yu, 2011).

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is critical in the biosynthesis of active folate form and dictates the intracellular levels of bioactive metabolite. Greenberg, Bell, Guan, and Yu (2011) found that 60% of the US population may have an MTHFR mutation, which translates to a deficiency in the effective absorption of available folate from diet alone, rendering low to deficient levels of serum folate levels (Vidmar Golja et al., 2020). Folate deficiency is associated with many abnormalities among mothers and is the leading cause of neural tube defects (NTDs) in offspring (Raghavan et al., 2018). Thus, the relevance of polymorphisms in MTHFR is a topic of interest in this study.

Because ASDs are multifactorial neurodevelopmental disorders, there are several factors to consider for study. Some comorbidities during pregnancy have been positively linked with an association of neurodevelopmental disorders, including ASD: maternal mental illness, thyroid disease, oral contraception, and hypertensive disorders during pregnancy (Magdalena et al., 2020; Maher et al., 2018). For this reason, I also examined pregnancy-based hypertensive disorders, including preeclampsia and gestational diabetes. Some researchers found race associated with differences in ASD prevalence, namely

greater among Caucasian and Black East African women, and maternal age at birth also associated with ASD (Fairthorne et al., 2017; Sandin et al., 2016).

Problem Statement

ASD is an umbrella term for developmental delay conditions characterized by persistent deficits in social interaction, complemented by a range of restrictive, repetitive behavioral patterns, activities, or interests (American Psychiatric Association, 2013). Despite a wealth of research indicating maternal, genetic, dietary, and environmental risk factors (Baio et al., 2018; Brimberg et al., 2013; Brown et al., 2017; Goldstein & Ozonoff, 2018), the exact etiology of ASD remains elusive. In this section, I discuss the research gaps and describe the problem.

Folate supplementation is commonly recommended to pregnant women by a healthcare professional during pregnancy to prevent NTDs and other abnormalities (Mayo Clinic, 2018; Raghavan, 2018). However, recent evidence points to a possible link to ASD due to over-supplementation or improper use (Raghavan et al., 2018). Children diagnosed with ASD typically exhibit behaviors that diverge from the norm in cognitive functioning. Common traits include difficulties in social interaction, affective engagement, motor skill development, and communication (Goldstein & Ozonoff, 2018). Additionally, several children on the spectrum have one or more comorbidities, including but not limited to attention–deficit hyperactivity disorder, anxiety, depression, chronic sleep issues, epilepsy, or schizophrenia (Autism Speaks Foundation, 2021). In some cases, children with ASD require caregiver support. This can place a heavy burden on families in areas of daily living, including housekeeping, finances, the emotional and

mental health of parents and siblings, marital relationships, and physical health, limiting the response to the needs of other children within the family (Begum & Mamin, 2019).

Children diagnosed with ASD often mature into adults facing significant hurdles in social, educational, and occupational attainments and requiring external resources and emotional support to navigate life's various stages (Begum & Mamin, 2019). Annually, 70,700 to 111,600 children with ASD transition to adulthood (Autism Speaks Foundation, 2021). Over half of these individuals are often underserved in terms of healthcare, with many unemployed and not involved in higher education. To date, adults with ASD have limited resources and programs to assist in achieving a fulfilling life.

Early intervention can improve learning, communication, social skills, and underlying brain development among children with ASD. A clinician may reliably diagnose children with ASD as early as age 2, but most children receive a diagnosis after 4 years (Autism Speaks Foundation, 2021). Early intervention provides the best opportunity to support healthy development and benefits across the life course of this condition, underscoring the need for effective measures for early detection. However, diagnosing ASD can be clinically difficult as there is no serological diagnostic test, requiring a complete developmental and behavioral history to make a definitive diagnosis. This approach often leads to a delay in diagnosis or a missed diagnosis.

Given the severe nature of ASD and its profound impact across an individual's lifespan, this study is significant by evaluating the association between maternal risk factors relative to nutrition and folate, a particularly understudied area. This study aims to explore the associations of possible maternal characteristics from demographic

information, past medical history, and nutritional survey data to estimate maternal folate status from dietary intake and possible association with ASD in offspring associated with comorbidities and social factors. The implications of this study are considerable as I sought to elucidate the role of folate and other maternal characteristics in ASD risk, potentially informing the development of educational and dietary programs, policy recommendations, and interventions to mitigate the financial and social burden associated with ASD. Identifying folate and other maternal factors can expedite the ASD diagnosis and intervention process, ultimately improving outcomes for those affected.

Purpose of the Study

This study explored the potential associations between maternal risk factors, such as folate status, age, income, race, education, and certain conditions (preeclampsia, gestational diabetes, MTHFR mutation) and the risk of ASD in offspring. Findings from this study can aid in predicting whether a child will have an ASD diagnosis and can may help inform the development of educational and dietary programs and policies that address the cost burden and lead to positive social change. The findings of this study add to the literature the position that folate and other maternal risk factors may serve as determinants of an increased likelihood of ASD in children.

Folate is a critical one-carbon metabolism component vital in DNA methylation, synthesis, and repair (Crider et al., 2012). Insufficient folate intake during pregnancy has been linked to an increased risk of NTDs and other congenital abnormalities (Raghavan, 2018). Moreover, evidence suggests that abnormal folate metabolism may also be implicated in ASD risk (Raghavan, 2018). Therefore, examining the relationship between

folate and ASD risk is crucial to understanding etiologic potential. By investigating the associations between maternal risk factors, including folate status, and ASD risk, this study may provide valuable insights for developing targeted prevention and early intervention strategies that could reduce ASD-related healthcare costs and improve social change.

Research Questions and Hypotheses

RQ1: To what extent are maternal demographics (age, education, income, and race) associated with ASD?

$H_0$1: Maternal demographics (age, education, income, and race) have no association with ASD compared to controls.

H_A1: Maternal demographics (age, education, income, and race) are associated with ASD compared to controls.

Maternal demographics are categorical variables, including age, education, income, and race.

RQ2: To what extent are maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) associated with ASD compared to controls after adjusting for maternal age, education, income, and race?

$H_0$2: Maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) are not associated with ASD compared to controls after adjusting for maternal age, education, income, and race.

H_A2: Maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) are associated with ASD compared to controls after adjusting for maternal age, education, income, and race.

Maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) are binomial variables.

RQ3: To what extent is estimated maternal daily folate equivalent (DFE) status during pregnancy associated with ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes?

H_03: Estimated maternal DFE status has no association with ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes.

H_A3: Estimated maternal DFE status is associated with ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes.

Estimated DFE status was measured as a scale.

Theoretical Foundation

The life-course health development theory (LCHD) helps organize several theories and conceptual models to address a complex question (Halfon & Forrest, 2017). LCHD provides an understanding of the origins of health and biological and behavioral plasticity in facilitating different adaptations, mismatches between biological propensity, environmental context, and their interactions to produce disease (Halfon & Forrest, 2017). This study employed the LCHD framework to examine the potential associations

between maternal folate intake, MTHFR enzyme polymorphisms, and the likelihood of a child developing ASD.

The *critical period* within life-course epidemiology references a timeframe during which exposure may have adverse or protective effects on development and disease outcomes (Kuh et al., 2003). The critical period causation model defines an exposure—in this case, folate—acting during a crucial development period. This period affects the structure or function of organs, tissues, or body systems that are not modified by later experience, precipitating disease later in life (Kuh et al., 2003). The study's focus was on maternal nutrition. The critical period to consider would be the time of conception and the first trimester of pregnancy, when neural tube closure occurs, and the foundation for brain development is established (Raghavan et al., 2018).

On the other hand, the *sensitive period* refers to a timeframe during which exposure to a factor, such as folate supplementation, has a more substantial effect on development and subsequent disease risk (in this study, ASD) than it would at any other time (Kuh et al., 2003). In this study, the sensitive period was defined as the entire pregnancy, as maternal nutrition and health status during this time may have significant implications for fetal development and later disease risk (Braun et al., 2014; Li et al., 2018; Raghavan et al., 2018).

Critical and sensitive period concepts were applied to this study to elucidate the potential associations between maternal folate status, age, income, race, education, comorbidities (preeclampsia and gestational diabetes), and the risk of ASD in the child.

Nature of the Study

The nature of this study was to conduct a comprehensive investigation into the potential association between folate status and maternal risk factors, such as folate status, age, income, race, education, and the presence of certain conditions (preeclampsia, gestational diabetes, and MTHFR mutation) and the risk of ASD in offspring. I employed a case-control observational approach and incorporated principles from LCHD to examine these effects (Halfon & Forrest, 2017). In this case-control study, mothers of children with ASD (cases) were compared to a control group of mothers of children without ASD, in which potential confounders were not matched.

Using a robust study design with an unmatched control group, I aimed to minimize biases and provide greater reliability and validity than previous investigations. Furthermore, the findings of this study may have implications for public health policy and clinical practice by informing strategies to promote optimal nutrition and supplementation in minimizing the risk of ASD in future generations. In the next section, I introduce keywords used throughout the study.

Definitions

There exist keywords in this study that are appropriate to define:

Autism: "A developmental disorder that appears in the first 3 years of life and affects the brain's normal development of social and communications skills" (US National Library of Medicine, 2014, p. 1).

Autism spectrum disorder (ASD): A spectrum of developmental delay conditions that affect communication and behavior characterized by persistent impairments in social

interaction and the presence of restrictive, repetitive behavioral patterns, interests, or activities (American Psychiatric Association, 2013; NIMH, n.d.). The *Diagnostic and Statistical Manual of Mental Disorders* (5th ed.; *DSM-5*) considers ASD a developmental disorder, as symptoms generally appear within the first 2 years of life (American Psychiatric Association, 2013). Additionally, autism is a spectrum disorder due to the wide variation in the type and severity of symptoms a child or adult may experience (NIMH, n.d.). Still, ASD is not limited to clinical presentations of signs of excess, deficit, and delays in achieving expected milestones (American Psychiatric Association, 2013).

American Psychiatric Association: The primary professional organization of psychiatrists and psychiatrists in training in the United States.

Daily folate equivalent (DFE): A metric used to account for the differences in bioavailability and absorption of naturally occurring food folate and synthetic folic acid. One DFE is equivalent to 1 microgram of dietary folate, 0.6 micrograms of folic acid from fortified foods, or 0.5 micrograms of supplemental folic acid taken on an empty stomach (National Institutes of Health [NIH] Office of Dietary Supplements, 2021).

Diagnostic Statistical Manual of Mental Disorders (5th ed., DSM-5): A taxonomic and diagnostic tool published by the American Psychiatric Association.

Folate: Commonly used interchangeably with *folic acid*, a nutrient the body requires to function and stay healthy. The naturally occurring form of Vitamin B9 is found in whole grain bread and cereals, liver, green vegetables, orange juice, lentils, beans, and yeast. Folate deficiency can cause brain and spinal defects in a fetus.

Folic acid: The synthetic version of Vitamin B9 derived in a laboratory and found in supplements and fortified foods such as rice, pasta, bread, and some breakfast cereals.

National Institute of Mental Health (NIMH): Part of the NIH, the US Department of Health and Human Services agency.

Assumptions

This study included several assumptions that should be noted. The primary assumption was that ASD characteristics are similar across cultural, social, and environmental contexts in individuals worldwide. However, I assumed that mothers of specific ethnicities, genetic profiles, socioeconomic status, educations, folate status, or having a hypertensive disorder (preeclampsia and gestational diabetes) might have a greater likelihood of giving birth to a child with an ASD due to these factors (Fairthorne et al., 2017; Maher et al., 2018). The generalizability of the findings to different cultural and environmental contexts was considered, given the known variability in ASD prevalence and risk factors across populations.

Estimated dietary folate and folic acid from foods were assumed to come from three meals per day and did not include snacking or other dietary patterns, including portion size. Estimated supplementation was assumed to be taken under a strict regimen. Values for multivitamins and folate-only supplements were calculated from the top eight selling supplements available over the counter from online retailers and stores such as grocery stores. However, I acknowledge that access to these supplements may differ across geographic regions, affecting consumption patterns, values of estimation, and access.

The assessment of maternal dietary intake relied on participants' self-reported consumption of specific foods during pregnancy. Due to the study's retrospective nature, it was challenging to quantify the exact amount of folic acid or folate consumed from food and supplement sources, as participants may have consumed different portion sizes and frequencies (see Scaglione & Panzavolta, 2014). In this study, I asked participants to respond to a questionnaire and assumed that respondents would provide truthful information with unbiased recall during data collection.

I also assumed that, on a population level, a varied diet could only partially address nutritional requirements and folate consumption. Therefore, assuming that populations experience multiple daily dietary consumption levels and various uncertainty levels was reasonable. Furthermore, I acknowledge that the bioavailability of folate and folic acid might vary among participants due to malabsorption and other factors such as genetic polymorphisms, including MTHFR mutations, or the use of medications that could affect absorption (Greenberg, Bell, Guan, & Yu, 2011; Vidmar Golja et al., 2020). I also assumed that individual differences in folate absorption and processing might exist, which could impact the generalizability of the study findings.

Scope and Delimitations

The scope of this study was to investigate the potential associations between maternal folate status, maternal characteristics (age, income, education, race, and comorbidities such as gestational diabetes, preeclampsia, and MTHFR mutation), and the risk of ASD in offspring. I collected data by asking mothers of children with ASD and mothers of children without ASD to provide self-reported information about their

pregnancy through an online survey I developed. The survey gathered information on maternal demographic characteristics, lifestyle choices, dietary habits, and pregnancy history.

I recruited participants across the United States to gather data from multiple geographic areas. Still, recruitment efforts were limited to individuals who responded to online ads from Google Ads, social media, or an email list. Because all recruitment occurred online, this approach limited the ability to participate to only individuals who had access to a computer or smart device with an internet connection. Moreover, the use of specified terms and the use of only Google Ads limited the ability for access to the survey only to those who used the specific or related terms to elicit an ad display and further limited the reach of potential participants to only those who used Google as their primary search engine.

The data collected were saved electronically in an online spreadsheet password protected on the HIPAA-compliant Google Drive platform and limited to only me, the primary investigator. All study records must be kept in this secure database for 5 years per university requirements. Data were also limited to participants receiving services or participating in online groups related to mothers of children or a child with an active ASD diagnosis, potentially creating a selection effect.

Limitations

This study was limited in several ways. First, the study was observational, and its objective was to analyze an association between ASD diagnosis, supplementation, and the mother's characteristics. Thus, only the exploration of associations and patterns using

statistical models was postulated and explored. Next, the study population was limited to mothers with access to a computer or the internet and, thus, did not include all mothers. The study population was recruited from multiple online advertisements and recruitment materials and, therefore, was limited to only mothers interested in participating and exposed to the specific recruitment materials (email, ad, referral).

Potential confounding elements are common in etiological and observational studies. Therefore, possible confounding factors may include the misclassification of exposure and the inability to control or differentiate between environmental exposures. Additionally, there may have been errors relative to coding and minor errors in data collection, capture, or manipulation due to primary data collection efforts.

Other limitations of this study include confounding factors that could influence the interpretation of the results, such as the misclassification of exposure, the inability to control for or differentiate between various environmental exposures (toxins, pollutants, family history), and errors in coding or data collection. Although efforts were made to limit confounding factors cautiously, some unmeasured or residual confounding may remain. I also relied on self-reported data for children up to 12 years old, which may have introduced recall bias. This study was further limited in estimating folate from dietary sources, supplementation from recall, and any calculations involved in assessing daily folate equivalents. The variability in maternal characteristics, behaviors, and beliefs about supplementation may have introduced uncertainty and heterogeneity in the observed associations.

Selection Biases

Selection bias is a distortion of a measure of association, such as the risk ratio, due to selecting a sample that does not accurately reflect the target population (Alexander et al., 2015). This study boasted a robust recruitment plan that minimized the risk of selection bias. However, some sources of bias were considered, including participant willingness, motivations, and technology access.

Participants who chose to enroll in the study may have been more motivated and engaged, possibly resulting in a sample that does not represent the broader population of cases and controls. As previously mentioned, the study may be biased toward mothers with access to a computer or the internet, which could affect the generalizability of the findings. To address these potential biases, I applied the following strategies: aiming to include mothers with various backgrounds and experiences, simplifying questionnaires through a user-friendly and familiar platform, and making the questionnaire easy to complete while encouraging and maintaining participation from a broader range of individuals.

Significance

ASDs have varying presentation degrees and often result in delayed diagnoses. Studies indicate that parents report developmental concerns by about 36 months (approximately three years) of age; however, the median age at diagnosis in the United States is 52 months (Abbas et al., 2020). Zuckerman et al. (2017) found that the mean age at diagnosis in the United States was 4.4 years, with a mean diagnostic delay of 2.2 years. Like many chronic conditions, delays in diagnosis and treatment have a greater likelihood

of long-term management challenges. Therefore, early intervention is the most significant factor in determining long-term outcomes for children with ASD (Zuckerman et al., 2017).

The role of periconceptional or prenatal nutrition in the development of ASD is a particularly understudied area. This research contributes to emerging evidence on the etiology of ASD and supplements the literature on the possible impact on ASD risk. Previous studies have linked ASD to several factors related to the womb, including pregnancy and complications, diet and nutrition, medication history (Brown et al., 2017), mental health (Viktorin et al., 2017), and genetic, immunologic (Brimberg et al., 2013), metabolic, and comorbid associations. In this study, I assessed diet and nutritional information provided by mothers of children with ASD and mothers of children without ASD, aiming to provide empirical and evidence-based data and to establish known and unknown patterns of predictors.

By gaining a better understanding of the associations between maternal nutrition, particularly folate intake, and ASD risk, this study may inform strategies that promote optimal nutrition and supplementation across a variety of countries and cultures beyond the United States. The findings may aid in developing targeted public health policies and educational programs on the importance of adequate maternal nutrition during pregnancy, potentially minimizing the risk of ASD in future generations. Additionally, such insights may enable healthcare professionals to be more engaged in providing culturally appropriate recommendations for prenatal nutrition, addressing potential barriers to adequate folate intake among diverse populations.

Summary

This study investigated the potential associations between maternal folate status, maternal characteristics (age, income, education, race, and certain conditions such as gestational diabetes, preeclampsia, and MTHFR mutation), and the risk of ASD in offspring. As the incidence and prevalence rates of ASD continue to rise, understanding the mechanisms involved in the complex interplay between genetic and environmental factors and the role folate may play is becoming increasingly crucial for informing potential interventions and prevention strategies (Lyall et al., 2017).

Existing research has indicated that maternal nutrition, particularly folate intake, may be associated with the risk of ASD in offspring (Raghavan et al., 2018). However, the relationship between folate intake and ASD remains unclear, warranting further investigation (Schmidt et al., 2012). By examining maternal characteristics and periconceptional folate intake, I sought to contribute to the literature on ASD etiology and inform future research and clinical practice by working toward achieving early intervention for children needing healthcare services, lowering the financial burden associated with ASD.

Chapter 2: Literature Review

Introduction

ASD is a complex neurodevelopmental condition characterized by impairments in social interaction and communication and can present with repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). The incidence and prevalence of ASD have increased dramatically over the past few decades, making it a significant public health concern (Lyall et al., 2017). Although the etiology of ASD is not yet fully understood, it is widely accepted that genetic and environmental factors play a role in its development (Modabbernia et al., 2017). One area of ecological research that has gained increasing attention is the potential association between maternal nutrition, specifically maternal folate intake, and the risk of ASD in offspring (Raghavan et al., 2018).

In this chapter, I provide a comprehensive overview of the current knowledge regarding folate, maternal demographics, and the associated risk of ASD in children. Here I present findings and gaps in the literature that this study was conducted to address. I begin with a history and definition of ASD, provide epidemiological data on ASD, the significance of maternal nutrition in ASD etiology, the impact of maternal characteristics on ASD risk, and information on the LCHD framework.

Literature Search Strategy

The literature review supporting this study included a review of all peer-reviewed literature published from 2009 to 2023. I located sources through databases, including EBSCO, Thoreau, PubMed, Embase, Medline, and Google Scholar. The key search terms

included singularly or in combination were: *autism, autism spectrum disorder(s), ASD, Asperger's syndrome, folate, folic acid, supplementation, pregnancy, mothers, ethnicity, maternal folate, maternal diet, trimester, nutrition, folate or folic acid intake, family history, diet, in-utero,* and *mother*, with an emphasis on studies in which dietary supplementation, particularly with folate or folic acid or diet, was associated with an ASD among the offspring of mothers. Among these articles, there was little research specific to the impact of dietary folic acid or folate on a potential ASD diagnosis in offspring, and no papers were related to the long-term effects of ASD.

Theoretical Foundation

LCHD is a framework that organizes several theories and conceptual models to make sense of a challenging question (Halfon & Forrest, 2017). The framework addresses the developmental origins of health, the role of biological and behavioral plasticity in facilitating different adaptations, and mismatches between biological propensity, environmental context, and their interactions to produce disease (Halfon & Forrest, 2017). LCHD served as the theoretical framework for this research study. I examined potential associations between maternal characteristics during pregnancy, estimated folate status, MTHFR enzyme polymorphisms, and the precipitation of ASD in offspring.

LCHD is best suited to provide a valuable means of elucidating potential harms to a fetus regarding dietary, behavioral, and social experiences during pregnancy and subsequent health outcomes. Within the LCHD framework, the concepts of *critical and sensitive periods* are particularly relevant for understanding how prenatal exposures, such as folate intake, may contribute to ASD risk. The critical period references a window

when exposure (e.g., folate) may cause adverse or protective effects on development and disease outcomes (Kuh et al., 2003). The critical period causation model defines an exposure (e.g., folate) acting during a crucial development period. This period affects the structure or function of organs, tissues, or body systems that are not modified by later experience, precipitating disease (ASD) later in life (Kuh et al., 2003). In contrast, the sensitive period is a time when an exposure (e.g., folate) has a more substantial effect on development and subsequent disease risk (ASD) than it would at other times (Kuh et al., 2003).

The critical and sensitive periods are pertinent to this study as previous research on ASD within the LCHD framework has identified these periods as gestation and early postnatal life, at which specific environmental exposures and epigenetic factors interact to increase ASD risk (Modabbernia et al., 2017). For example, Raghavan et al. (2018) demonstrated that maternal folate intake during pregnancy, particularly during the periconceptional period, is critical for preventing NTDs and may reduce ASD risk. Cementing that the critical and sensitive periods may be defined as a time that neurodevelopment occurs.

Many first-time mothers internalize information during pregnancy (critical period) received from outside influences, such as recommendations from family members, care professionals, or friends. In contrast, during subsequent pregnancies, mothers may use the same or alternate methods related to supplementation, possibly altering their natural life course and the offspring's (Halfon & Forrest, 2017). The application of the principles of LCHD may unveil the health development process, prior experiences, and environmental

interactions (unfolding), the reciprocal interactions between mothers and their physical, natural, and social environments (complexity), sensitivity to pregnancy, and supplementation (timing). These principles lead to adaptive dietary changes due to pregnancy (plasticity) to promote a healthy pregnancy (thriving) while balancing diet, exercise, and behavioral and cultural beliefs (harmony; Halfon & Forrest, 2017).

Applying the LCHD framework and the concepts of critical and sensitive periods may inform potential preventive and intervention strategies to address the rising incidence and prevalence of ASD and ASD-associated healthcare costs. For example, understanding the timing and nature of the associations between maternal folate intake, MTHFR polymorphisms, and ASD risk may enable targeted interventions that optimize maternal folate supplementation during specified windows of vulnerability to minimize ASD risk in future generations. In the next section, I discuss the literature on ASD and associated maternal risk factors that may mediate risk.

Literature Review

ASD is a developmental disability that can lead to significant social, communication, and behavioral challenges. The condition varies in severity, hence the term *spectrum*. ASD is difficult to notice as there is often nothing visual that sets apart people with ASD from people who do not have ASD. Typical signs and symptoms of ASD present in early childhood and typically last throughout a person's life. These symptoms include problems with learning, paying attention, or reacting to things. Thus, early diagnosis and intervention are crucial to allowing one with an ASD diagnosis to perceive a normal life.

Compounding a problematic diagnosis is the elusive etiology. Many studies on this condition have narrowed genetic, dietary, and social factors. However, a consensus on the exact cause still needs to be developed. Recent studies have suggested a maternal folic acid concentration link where over-supplementation, improper processing, or malabsorptive disruptions due to polymorphisms may lead to disease in the offspring, especially during pregnancy when demands for folate are most significant. In this chapter, I provide findings from an exhaustive review of potential contributing factors related to folate and an ASD diagnosis.

Increasing Prevalence of ASD

There has been an unprecedented rise in the prevalence of ASD worldwide. In 2023, the CDC estimated the prevalence of ASD to affect 1 in 36 children living in the United States, a significant increase from 1 in 150 children estimated two decades earlier (Maenner et al., 2023). This increase has been observed in multiple countries and across different ethnic groups and socioeconomic backgrounds (Baxter et al., 2015). The reasons for this increase are not fully understood. Still, it is suggested that changes in diagnostic criteria, increased awareness, improved screening methods, or a combination of these factors may contribute to this rise (Baxter et al., 2015; Maenner et al., 2020). However, some researchers argue that these factors alone may not fully explain the observed increase, pointing to a potential role for a combination of environmental factors in ASD etiology (Modabbernia et al., 2017).

The global impact of ASD has increased rapidly over the last decade, mirroring the findings in the United States. Kang et al. (2013) indicated that ASD is a worldwide

epidemic as most cases are idiopathic, inherent, and lack any known cure. The WHO (2021) estimated that about 1 in 160 children had an ASD. However, St-Hilaire et al. (2012) purported that the increased prevalence among countries was associated with a higher socioeconomic status, indicating ASD is a condition of wealthy nations. The steepest rise in cases worldwide was seen in South Korea, Europe, and some areas of North America (Johnson et al., 2012).

Diagnosis of ASD

The *DSM-5* describes ASD as a neurodevelopmental delay disorder characterized by persistent deficits in social communication and social interaction and restricted, repetitive patterns of behavior, interests, or activities (American Psychiatric Association, 2013). This is updated from the previous description, which combined previously separate diagnoses, such as autistic disorder, Asperger's syndrome, and pervasive developmental disorder-not otherwise specified, into a single ASD diagnosis (American Psychiatric Association, 2013). The *DSM-5* criteria are based on a two-domain framework—social communication and interaction and restricted and repetitive behaviors—that replaced the previously used three-domain model in the *DSM-IV* (American Psychiatric Association, 2013). This change aimed to reflect the shared features of ASD better and improve diagnostic reliability (Coury, 2013).

Diagnosing ASD proves challenging as no medical or serological diagnostic tests exist. A definitive diagnosis requires a complete developmental and behavioral history. Children with ASD often present with one or more comorbidities, including but not limited to attention–deficit hyperactivity disorder, anxiety, depression, chronic sleep

issues, epilepsy, or schizophrenia (Autism Speaks Foundation, 2021). Diagnosis can be difficult for a practitioner to make alone as there are varying degrees of presentation of symptoms that can evolve with time. Thus, parents, educators, and physicians are essential in recognizing various signs and symptoms of this group of disorders (Faras et al., 2010).

Etiology

ASD is a condition of unknown etiology involving both genetic and environmental factors. Twin concordance and familial studies have demonstrated a strong correlation in genetic heritability, with estimates ranging from 64% to 91% (Sandin et al., 2014; Tick et al., 2016). Researchers have pinpointed several hundred genes associated with ASD risk, many of which are involved in neurodevelopment (Geschwind, 2011; Iossifov et al., 2014). However, genetic factors alone may not fully explain the occurrence of ASD. Mounting evidence suggests an interplay between genetics and environmental factors in ASD etiology (CDC, 2021; Lyall et al., 2017; Modabbernia et al., 2017).

Established environmental factors in ASD etiology include prenatal exposure to air pollution, maternal infections, and certain medications, such as valproic acid and selective serotonin reuptake inhibitors (Lyall et al., 2017; Modabbernia et al., 2017). However, additional considerations have gained attention as potential environmental factors influencing ASD risk, including maternal nutrition, particularly micronutrient intake (Schmidt et al., 2011). These findings solidify that interactions between genetic,

environmental, and maternal nutrition and other characteristics are factors in the development of ASD (Hallmayer et al., 2011; Lyall et al., 2017).

ASDs are multifactorial neurodevelopmental disorders, and there are several avenues for etiological research. Studies show an association between ASD and maternal mental illness, thyroid disease, and oral contraception (Magdalena et al., 2020). Moreover, hypertensive disorders during pregnancy have been liked to consequential neurodevelopmental disorders in offspring, including ASDs (Maher et al., 2018).

Concerning maternal demographics, Fairthorne et al. (2017) cited maternal race and ethnicity as associated with ASD, with greater prevalence among Caucasian and Black East African women. Finally, in a large cohort study (n = 5,766,794), Sandin et al. (2016) found that maternal age was positively linked to ASD in offspring. Therefore, research suggests links to maternal, genetic, dietary, and environmental risk factors (Baio et al., 2018; Brimberg et al., 2013; Brown et al., 2017; Goldstein & Ozonoff, 2018). Approximately 10% of ASD cases are secondary to tuberous sclerosis, fragile X syndrome, and phenylketonuria. Other issues may arise secondary to congenital infections, such as rubella and cytomegalovirus (Faras et al., 2010).

Researchers now believe that the development of ASD occurs most likely in utero, suggesting the pathogenesis may begin during the prenatal and perinatal periods (Gardener et al., 2009). Stoner et al. (2014) examined the brains of 11 postmortem children ages 2–15 with an ASD diagnosis. The researchers found disorganization of the neocortical architecture among fetal brains of 19–32 weeks of gestation in 10 of the 11

brains examined. This information further indicates the development of ASD in utero while the cortex is forming because folate is an essential component.

Some studies recognize folate (Vitamin B9) as a risk factor associated with the prevalence of ASD (Baio et al., 2018; Raghavan et al., 2018). Other studies suggest an association between a mother's folate status and a child's risk for developing ASD (Raghavan et al., 2018), affording it a vital investigational risk factor for in-utero studies. Despite evidence supporting the protective role of maternal folate intake against ASD, other studies have reported conflicting findings or have failed to establish a clear association (DeVilbiss et al., 2017; Raghavan et al., 2018). However, these inconsistencies could be attributed to differences in study designs, sample sizes, exposure assessment methods, and potential confounding factors (Raghavan et al., 2016). Further research is needed to elucidate the relationship between maternal folate intake and ASD risk and to identify possible critical windows of exposure and susceptible populations.

Maternal Nutrition During Pregnancy

Maternal nutrition plays a crucial role in fetal development and the overall health outcomes of the fetus (Gernand et al., 2016). Cetin & Laoreti (2015) indicated that the pre-conception and pregnancy periods are critical for ensuring adequate nutrient intake, as the mother's nutritional status can significantly impact the fetus's growth, development, and future health. During pregnancy, the demand for essential nutrients is increased and required for proper fetal brain development, including proteins, lipids, vitamins, and minerals (Georgieff, 2007). Inadequate maternal nutrition during pregnancy

has been associated with various adverse outcomes, such as preterm birth, low birth weight, and developmental delays (Ramakrishnan et al., 2012).

Folate and Fetal Development

Folate, a B vitamin, plays a significant role in fetal development, particularly in neural tube formation and closure, DNA synthesis, methylation, and repair (Greenberg & Bell, 2011). Folate is also involved in one-carbon metabolism, a crucial step in the methylation reactions and synthesizing neurotransmitters, proteins, and lipids necessary for proper brain development (Selhub, 2002). Therefore, the bioavailability of folate is essential during the pre-conception and pregnancy periods, as it prevents NTDs, including spina bifida and anencephaly (CDC, 2023; Czeizel & Dudas, 1992). Folic acid supplementation before conception and during early pregnancy has been shown to reduce the risk of NTDs by up to 70% (Wald et al., 2001).

Folate in DNA Replication

Folate is an essential precursor for various enzymatic reactions involved in amino acid synthesis, vitamin metabolism required for DNA replication, and a necessary coenzyme in DNA and RNA synthesis (Greenberg, Bell, Guan, & Yu, 2011). Critical folate-dependent reactions include the conversion of homocysteine to methionine and the methylation of deoxyuridylate to thymidylate (NIH Office of Dietary Supplements, 2021). Folate is also necessary for forming red blood cells and average healthy cell growth and function (Mayo Clinic, 2018). Thus, humans must always consume at least the recommended amount daily to maintain healthy folate levels.

Maternal Folate Intake and ASD Risk

Folate Supplementation and Dietary Intake. Several studies have investigated the relationship between maternal folate intake and ASD risk, with mixed findings. Some research has demonstrated that adequate maternal folate intake, through supplementation or dietary sources, is associated with a reduced risk of ASD (Schmidt et al., 2012; Surén et al., 2013). For example, Schmidt et al. (2012) found that mothers who used prenatal vitamins containing folic acid were less likely to have children with ASD than mothers who did not use such supplements. Evidence supports maternal folate intake and plasma concentration regulation by diet and genetic factors, which also have an elusive role during pregnancy and the development of ASD while in-utero (DeVilbiss et al., 2015).

Potential Mechanisms Linking Folate to ASD Risk. The exact mechanisms underlying the relationship between folate and ASD risk are unknown. However, some theories suggest that folate's role in one-carbon metabolism, epigenetic regulation, and neurotransmitter synthesis may contribute to ASD development (Raghavan et al., 2016). Disruptions in these processes may lead to alterations in gene expression, brain development, and neural connectivity, potentially increasing the risk of ASD.

Folate Versus Folic Acid. Folate, known as Vitamin B9, *folic acid*, and *folate*, is often used interchangeably. However, the main difference is that folic acid is the synthetic form of the naturally occurring form of Vitamin B9 (folate). Folate is a water-soluble compound that humans must consume as gut bacteria cannot synthesize it. Folate-rich sources in the diet include green, leafy vegetables, sprouts, fruits, brewer's yeast, and

liver (Scaglione & Panzavolta, 2014). Folates derived from foods occur naturally in the tetrahydrofolate (THF) form (NIH Office of Dietary Supplements, 2021).

In contrast, supplementary folic acid is the fully oxidized monoglutamate form used in food fortification and found in most dietary supplements. However, some nutritional supplements containing folate exist in the monoglutamyl form, 5-methyl-THF (NIH Office of Dietary Supplements, 2021). The molecular differences between natural folate and synthetic folic acid molecules warrant additional investigation in maternal absorption, bioavailability, maintenance, and excretion.

Recommended Folate Intake. The National Academies of Sciences, Engineering, and Medicine's expert panel committees of the Food and Nutrition Board (FNB) maintain a list of dietary reference intakes (DRI), which set reference values used for planning and assessing healthy nutrient intakes (NIH Office of Dietary Supplements, 2021). The recommended dietary allowance for folate is based on age range, gender, pregnancy, and lactation (see Table 1).

The FNB defines dietary folate equivalents (DFEs) as follows:

- One mcg DFE = 1 mcg food folate
- One mcg DFE = 0.6 mcg folic acid from fortified foods or dietary supplements consumed with foods
- One mcg DFE = 0.5 mcg folic acid from dietary supplements taken on an empty stomach" (NIH Office of Dietary Supplements, 2021).

Table 1

Recommended Dietary Allowances for Folate

Age	Male (mcg DFE)	Female	Pregnancy	Lactation
Birth to 6 months*	65*	65*		
7–12 months*	80*	80*		
1–3 years	150	150		
4–8 years	200	200		
9–13 years	300	300		
14–18 years	400	400	600	500
19+ years	400	400	600	500

- *Note*. * = Adequate intake. Adapted from "Folate: Fact Sheet for Health Professionals," by NIH Office of Dietary Supplements, 2021, https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/#en2. Copyright 2019 by the National Institutes of Health.

Folate Deficiency

Cases of isolated folate deficiency are uncommon due to coexistence with other nutrient deficiencies and strong associations with diet, alcoholism, and malabsorptive disorders (NIH Office of Dietary Supplements, 2021). Women with insufficient folate levels are at an elevated risk of giving birth to children with NTDs or severe mental illness (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline, 1998; NIH Office of Dietary Supplements, 2021). The NIH Office of Dietary Supplements (2021) describes folate deficiency as a rare occurrence in the US. However, folate inadequacy may exist among certain groups, as presented in Table 2.

Table 2

Groups at Risk of Folate Inadequacy

Group	Description
Alcohol use disorder	Alcohol interferes with folate absorption and hepatic uptake, accelerates folate breakdown, and increases renal excretion >60% of people with chronic alcoholism have low folate status Folate inadequacy can be caused by moderate consumption of alcohol (8 fl. oz. red wine, 2.7 fl. oz. vodka) per day for 2 weeks
Women of childbearing age	All women capable of becoming pregnant should obtain an adequate amount of folate to reduce NTDs Women of childbearing age should obtain 400mcg/day folic acid from dietary supplements
Pregnant women	Demand for folic acid increase during pregnancy because of its role in nucleic acid synthesis Recommended daily adequacy is 400mcg DFE for nonpregnant women to 600mcg DFE during pregnancy
People with malabsorptive disorders	Malabsorptive disorders (tropical sprue, celiac disease, and inflammatory bowel disease) increase the risk of folate deficiency People with diminished gastric acid secretion (atrophic gastritis, gastric surgery, and other conditions) can reduce folate absorption
People with MTHFR polymorphism	Individuals with the polymorphism 677 C>T in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene have an impaired ability to convert folate to its active form.

Note. Adapted from "Folate: Fact Sheet for Health Professionals," by NIH Office of Dietary Supplements, 2021, https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/#en2. Copyright 2019 by the National Institutes of Health.

Folate-Modifying Drugs

Folate deficiency can present as megaloblastic anemia but may be caused by several medications (Hariz & Bhattacharya, 2023). Methotrexate (Rheumatrex, Trexall) is indicated for anti-cancer, autoimmune disease, and inflammatory conditions (FDA,

2009). It works by inhibiting a key enzyme activating folic acid, dihydrofolate reductase (FDA, 2009). Other drugs that may hinder folate absorption and lead to depleted plasma folate levels include Phenytoin (Dilantin), Carbamazepine (Carbatrol, Tegretol, Equetro, Epitol), Valproate (Depacon), and Sulfasalazine (Azulfidine).

Sources of Folate

Vitamin B9 or *folate* occurs naturally in various ranges, depending on the food type. The most significant amount of naturally occurring folate is in beef liver, boasting up to 215 mcg per serving (NIH Office of Dietary Supplements, 2021). However, some of the highest folate levels may be found in spinach, asparagus, and Brussels sprouts—containing between 78–105 mcg per serving. Fruits, fruit juices, nuts, beans, peas, seafood, eggs, dairy products, poultry, and grains had adequate folic acid (NIH Office of Dietary Supplements, 2021).

The FDA required food manufacturers to add 140 mcg of folic acid to every 100 grams of enriched bread, cereals, flours, cornmeal, pasta, rice, and other grain products in 1998 to adjust for rising incidence and prevalence of neural tube defects, including spina bifida (NIH Office of Dietary Supplements, 2021). Since the food fortification program's implementation, the mean folic acid intake in the United States has increased by about 190 mcg daily. The Canadian, Costa Rican, Chilean, and South African governments have established similar mandatory folic acid fortification programs following the FDA's decision.

Folic acid is found in multivitamins and prenatal vitamins, supplements containing other B-complex vitamins, and stand-alone supplements. Doses range from

400–800 mcg in adult supplements, depending on the purpose of the supplement (NIH Office of Dietary Supplements, 2021). Thus, dietary supplements may provide adequate amounts of folic acid to enhance circulating levels, especially during pregnancy when demands for folate are increased.

Folate Absorption

Folic acid in foods consumed orally provides between 50–85% bioavailability compared to folic acid supplements that provide close to 100% bioavailability (NIH Office of Dietary Supplements, 2021). However, genetic polymorphisms leave approximately 60% of the population unable to metabolize folate adequately (Greenberg, Bell, Guan, & Yu, 2011). According to the National Health and Nutrition Examination Survey (NHANES), most people in the United States consume adequate amounts of folate from food sources (NIH Office of Dietary Supplements, 2021). The same NHANES survey found that the mean dietary intake of folate from foods ranged from 417–547 mcg of DFE for women daily—enough daily folate for the population.

Although most individuals consume adequate amounts of folate, women of childbearing age and non-Hispanic Black women are at risk of insufficient folate intake even after factoring in folic acid intake via dietary supplements (NIH Office of Dietary Supplements, 2021). Additionally, another NHANES study found that 23% of non-Hispanic Black women have inadequate total folate intake compared to 13% of non-Hispanic White women (NIH Office of Dietary Supplements, 2021).

Food Fortification Program

A large proportion of the population experiences a lower socioeconomic status—leaving many groups with limited access to folate supplements and folate-rich foods during pregnancy, resulting in folate deficiency (Scaglione & Panzavolta, 2014). For this reason, many established countries increased recommended intake of folates through mandatory fortification with folic acid. The timing of the implementation of the food fortification program suggests a possible link to the rise in ASD cases, and concerns about their possible link have been questioned. Currently, the FDA recommends 400 mcg of daily supplemental folic acid for women of childbearing age set by the CDC in 1992 (Baio et al., 2018).

Foods such as cereal have been fortified with folate, immediately increasing median serum levels in women. Findings suggest this was causal for ASD as the program should have decreased the median serum folate levels (Baio et al., 2018). Indicating a positive association between the mother's folate status and a child's risk for developing ASD, rendering it a significant risk factor for investigating developmental disorders, including ASD (Raghavan et al., 2018).

However, the findings contraindicated reports of folate's protective effect against ASD (Braun et al., 2014; Li et al., 2018; Raghavan et al., 2018). Some studies suggest a decreased risk of ASD with periconceptional multivitamin supplementation and folic acid intake (Braun et al., 2014; Li et al., 2018; Raghavan et al., 2018). However, Surén et al. (2013) assert that folic acid harms the developing fetus, and another warrants caution on

over-supplementation (Wiens & DeSoto, 2017). However, these studies relied on self-reported data.

Folate in Pregnancy

Folate supplementation is commonly recommended to pregnant women by a healthcare professional during pregnancy to prevent NTDs and other abnormalities (Mayo Clinic, 2018; Raghavan, 2018). Studies show that maternal folate status may affect the deoxyribonucleic acid methylation capacity in the brain during the periconceptional period and early pregnancy (DeVilbiss et al., 2015). This highlights early pregnancy as a critical period that may influence key neurodevelopmental processes. The methylation pathway is vital for normal offspring brain development, but environmental exposures can influence this period, which may result in hypomethylation (DeVilbiss et al., 2015).

Maternal Demographics and ASD Risk

The risk of ASD varies by maternal race, ethnicity, immigration status, income, socioeconomic status, and region of birth (Fairthorne et al., 2019).

Maternal Race

Fairthorne et al. (2017) found that Indigenous women were 50% less likely to have children with ASD than Caucasian non-immigrant women. The authors concluded that women who identified as an immigrant were 40% less likely to have children with ASD. Black women from East Africa had 3.5 times more odds of birthing children with ASD than Caucasian non-immigrant women. Suggesting there may be a link between ASD and maternal race or ethnicity.

Socioeconomic Status

Socioeconomic status is an individual or group's social standing or class measured as a combination of education, income, and occupation (American Psychological Association, n.d.). According to Kelly et al. (2017), socioeconomic status and ASD risk in the United States is proportional. Lower rates of ASD are associated with lower socioeconomic status and vice versa, while other countries report the inverse. Cementing these findings, Durkin et al. (2017) found that the prevalence of ASD increased with increasing socioeconomic status among all racial groups (Black, White, and Hispanic) in the United States. The authors conclude that a positive socioeconomic status gradient was associated with a persistent prevalence of racial and ethnic disparities among children of low-socioeconomic status (Durkin et al., 2017).

In a study examining socioeconomic status and ASD (n = 13,857) in Great Britain, Kelly et al. (2017) found that children born to mothers with a greater level of education had twice the rate of an ASD diagnosis, 1.5% of children (95% CI [1.1, 1.9]), compared to children of mothers with lower levels of education (95% CI [0.5, 0.9]). However, income and neighborhood had no significant relationship (Kelly et al., 2017). In contrast, a recent study examined the effects of socioeconomic status and ASD risk in a Taiwanese cohort. Among 706,111 singleton births, Yu et al. (2021) identified 7,323 (17%) ASD cases after adjusting for income, urbanization levels, child sex, parental age, and other covariates. Multivariate Cox regression analysis determined that a higher socioeconomic status was independently associated with a lower risk of ASD (Yu et al., 2021).

Maternal Age and ASD Risk

Maternal age has been established as a factor associated with an increased risk of having children with ASD. Several studies have reported an increased risk of ASD among children born to mothers of advanced age (Croen et al., 2007; Durkin et al., 2008). In their meta-analysis, Sandin et al. (2012) found that the risk of ASD increased with maternal age, with the highest risk among mothers aged 35 years or older. In a later meta-analysis, Sandin et al. (2016) found maternal age to be significantly associated with ASD risk. The meta-analysis consisted of 25,687 cases of ASD and over 8 million control subjects and compared mothers >35 years of age with mothers 25–29. Crude RR for ASD in the offspring was 1.52 (95% CI [1.12, 1.92]). Comparing mothers ≥ 35 with mothers 25–29, [corrected] the adjusted relative risk (ARR) for autism in the offspring was 1.31 (95% CI [1.19, 1.45]), [corrected] for mothers <20 compared with mothers 25–29 years old, there was a statistically significant decrease in risk (RR = 0.76, 95% CI [0.60, 0.97]).

Maternal Conditions

Preeclampsia and ASD

Preeclampsia is the new onset of high blood pressure and at least one associated symptom, such as protein in urine during or after the delivery of offspring (Herndon, 2021). Herndon (2021) maintained that the cause of preeclampsia is idiopathic but may be associated with genetic factors, vascular abnormalities, autoimmune disorders, and other risk factors. Herndon (2021) identified the following risk factors associated with developing preeclampsia: (a) having multiple pregnancies, like twins or triplets; (b) being over the age of 40; (c) being pregnant for the first time; (d) having preeclampsia in a

previous pregnancy; (e) having a family history of preeclampsia; (f) having obesity; (g) having a history of health conditions like high blood pressure, diabetes, kidney disease, lupus or other autoimmune disorders, and sickle cell disease; and (h) becoming pregnant using in vitro fertilization. In a recent meta-analysis of 16 complete studies and 13 journal articles, Jenabi et al. (2019) found a significant association between preeclampsia and ASD [(OR, 1.36; 95% CI [1.12, 1.60]) and (RR, 1.30; 95% CI [1.20, 1.41]), affording it a critical investigative risk factor for this study.

Gestational Diabetes and ASD Risk

Gestational diabetes is defined as glucose intolerance that begins during pregnancy and is associated with several adverse outcomes for both the mother and fetus and may lead to obstetric complications, including emergency cesarean delivery and risk of Type 2 diabetes mellitus in the mother and metabolic syndrome in the offspring (Rowland & Wilson, 2021). In their meta-analysis, the researchers examined the association between gestational diabetes and ASD. They found a significantly increased risk of ASD (OR = 1.42; 95% CI [1.22, 1.65]) among mothers with gestational diabetes. Although the researchers found an association, various levels of risk and mechanistic pathways may lead to gestational diabetes, and its mechanisms in the risk of ASD are poorly understood (Rowland & Wilson, 2021).

Summary and Conclusions

ASD is a lifelong condition with wide variability in clinical presentation, making it difficult to diagnose. Due to its idiopathic nature, no current treatment or mechanism of symptom management exists in the community. A clinician can diagnose a child with

ASD as early as 2 years, but most children receive a diagnosis after 4 years (The Autism Speaks Foundation, 2021). Children with ASD become adults with several social and comprehension incapacities, which may require a caregiver. Early intervention is the most effective method to ensure enhanced quality of life and lessen the financial burden. Recent evidence suggests a significant increase in both the incidence and prevalence of ASD globally. Although, some insist the condition has already reached epidemic proportions.

Maternal characteristics, including age, race, education, income, and the presence of certain conditions such as preeclampsia, gestational diabetes, and MTHFR mutations, may be associated with ASD risk. Researchers now believe that the mechanism underlying autism etiology is most likely in utero—suggesting the pathogenesis may begin during the pre-and peri-natal periods (Gardener et al., 2009).

Some studies recognize folate (Vitamin B9) as having a coincidental relationship with the prevalence of ASD (Baio et al., 2018; Raghavan et al., 2018) following the recommendation by the FDA to fortify foods with synthetic folate analog, folic acid. Studies suggest an association between the mother's folate status and a child's risk for developing ASD (Raghavan et al., 2018), affording it a vital investigational risk factor for in-utero studies.

Chapter 3: Research Method

Introduction

ASD is becoming a common diagnosis among children in the United States and worldwide. Unknown etiology and investigations on a possible biomarker during pregnancy have the potential to slow the ongoing rise of cases. This case-control study examined the relationship between maternal risk factors (maternal age, race, education, income, gestational diabetes, gestational hypertension, MTHFR mutation, and folate status) and an ASD diagnosis in offspring. In this chapter, I discuss research design and methods, target population, sampling procedures, survey instruments, recruitment and data collection procedures, and data analysis. I also explain the study's reliability, validity, and ethical practices.

Research Design and Rationale

In this case-control study, I collected primary data from mothers of children with ASD and mothers of children without ASD. The rationale for this study was to analyze the factors that play a role in ASD etiology, which remains unknown. Research on this topic has indicated that folate has both protective and harmful effects on neurodevelopment, aligning with findings of a U-shaped correlation with ASD (Raghavan et al., 2018). Considering the timing in which ASD diagnosis has peaked, it is uncertain whether a relationship exists between an increased folate status during pregnancy due to food fortification and supplementation. This study aimed to aid in advancing the literature on folic acid as a potential biomarker for ASD diagnosis while in utero, so families and their children can receive intervention early on with the hope of

living a long, healthy life. This investigation also aimed to characterize maternal socioeconomic and demographic trends, including age, race, education, income, pregnancy-related factors (preeclampsia, gestational diabetes, MTHFR mutation), and estimated folate status to help profile potential future cases.

Carlson and Morrison (2009) described a case-control study approach as appropriate for diseases with a long latency where the case and the exposure have already occurred. Therefore, I used a case-control approach to explore the potential associations between maternal characteristics and ASD risk. The target population for this study was US resident women who gave live birth to an infant and responded to an advertisement for study participation. This study design was appropriate because case-control studies select participants based on an outcome of interest (mothers of children with autism, mothers of children without autism) and allow for examining a previous exposure associated with the outcome (folate).

The primary exposure of interest in this study was estimated daily folate status and ASD risk as assessed by the consumption of folate supplements or diet reported in retrospect by mothers of children with or without an ASD diagnosis. This study also examined maternal age, race, education, income, and the presence of certain conditions during pregnancy, including preeclampsia, MTHFR mutation, and gestational diabetes. The data and results may be available for effective methods for future studies as a unique data set not previously available. The main research question asserts the independence of a variable and lacks a relationship between the variables. The research questions aim to

test the association between folate status and the dependent variables associated with ASD in the offspring.

Methodology

Population

The inclusion criteria for this study were mothers ages 18 or older with a young child (between 3–12 years old) who had an official ASD diagnosis (cases) from a clinician and mothers of children who (between 3–12 years old) did not have an ASD diagnosis (controls) born in the United States. This study included mothers with varying degrees of parity, gravida, socioeconomic status, race, ethnicity, and age, and participants provided informed consent to participate in the study. The inability to consent, less than 18 years of age, male sex, and inability or unwillingness to participate in surveys limited participation in this study. This study also analyzed diverse mothers with varying diets, cultures, and habits because previous studies have attempted to determine the association between folate status and multivitamin use on the risk of ASD. However, they lacked diversity and could not generalize their findings to the population.

Sampling and Sampling Procedures

Social media outreach and advertisements for participation using keywords were employed to elicit responses from mothers of children with ASD and mothers without an ASD diagnosis. Recruitment was conducted from June 2022 to January 2023 on social media platforms, including Facebook, Instagram, LinkedIn, and Reddit. The keywords and phrases used in the recruitment process included autism, autism awareness, autism mom, *autism acceptance, autism love, autism family, autism life, autism rocks, autism*

spectrum, autism parent, autism parents, autism support, autism advocate, autism spectrum disorder, autism warrior, autism community, autism proud, autism teacher, autism parenting, autism education, autism journey, autism stars, autism mama, autism kids, autism world, autism strong, and *Asperger syndrome*.

Table 3

Inclusion and Exclusion Criteria

Inclusion criteria
• Mothers 18+ with a young child who has an official ASD diagnosis (cases) from a clinician • Mothers of children who do not have an ASD diagnosis (controls) • Mothers with varying degrees of parity/gravida • Mothers of diverse socioeconomic status, race/ethnicity, age • Willingness and capacity to complete the study survey and questionnaires • Willingness and capacity to provide informed consent to the study
Exclusion criteria
• Inability to consent • Less than 18 years of age • Male • Inability or unwillingness to participate in surveys

Statistical Power

I used OpenEpi to calculate the estimated sample size using logistic regression as the statistical method. The power (1-error probability) was set to 80% CI to detect a difference, if any, between the exposed and unexposed groups and an alpha of 0.05 (Chasan-Taber, 2014). Surén et al. (2013) examined the association between maternal use of prenatal folic acid supplements and the subsequent risk of ASD in children. The study established that the adjusted odds ratio for autistic disorder in children of folic acid users was 0.61 (95% CI [0.41, 0.90]). The calculated sample size was 180 participants, with 90 controls and 90 cases (OpenEpi, n.d.). The estimated ratio of controls to cases was 1. The

hypothetical proportion of controls with exposure was 61, while the hypothetical proportion of cases with exposure of 39. The least extreme odds ratio to be detected was 0.41 (OpenEpi, n.d.). The OpenEpi output is available in Appendix A.

The control group was selected from the same population as the cases but had no ASD diagnosis. The controls were not matched with the cases for potential confounders such as age and sex. However, data on these variables were collected for both groups.

The response rate was calculated as the number of participants who completed the survey divided by the number of individuals who initially accessed the survey link. A total of 458 responses were recorded, and the survey was accessed approximately 6,800 times; thus, the approximate response rate was 6.73%.

Procedures for Recruitment, Participation, and Data Collection

Recruitment was conducted online through social media and Google Ads. Keywords used to recruit participants included *autism symptoms, test for autism, take an autism test, autism test for adults, signs of autism in babies, early autism signs,* and *children with autism*. Themes included autism research, autism spectrum disorders, autism parenting, advocacy, and ads survey. The ads were available 24 hours a day, 7 days a week. A domain name was created to improve search engine optimization. During recruitment, the survey was open to respondents at the domain http://www.asdsurvey.com.

Other recruitment means included posting the study information and links on websites, including the Walden University participant pool and ClinicalTrials.gov (NCT05453708). The study recruitment flyer was posted to group-appropriate Facebook

and Instagram pages and community forums for autism, including Reddit and specialized forums. Targeted emails to schools, universities, and programs were sent and disseminated via lists available to me. Known clinical investigators and influencers also shared the flyer via email with their personal or professional contacts.

The landing page of the survey explained the study requirements, including its voluntary nature, risks, and benefits. Additionally, potential participants were ensured that withdrawal from the study would not impact current or future care at their primary care provider or affiliates. All participants who elected to participate assumed implied consent by taking the survey—standards on informed consent strictly adhered to Walden University's Institutional Review Board (IRB) requirements.

Respondent data came from interested mothers of children with ASD and mothers of children without ASD. Respondents were directed to the domain to participate via Google Forms. Participants who shared their data manually uploaded responses to the survey via the secured hypertext transfer protocol using the Google Forms platform. Upon completing the survey, participants were sent to a conclusion page indicating they had concluded all study activities. The study did not require respondents to participate in any follow-up procedures. No identifying information except the date of birth was asked. All survey responses were stored on Google Drive, a valid (HIPAA)-compliant web-based cloud software accessible only to me. I kept all data in Google Drive to maintain HIPPA provisions for adequate protection and privacy of patient information.

The data were available in a Google Sheets file and were downloaded for exploration in Microsoft Excel. Data were examined and processed for consistency. The

inclusion criteria were referenced to ensure they were met, and all data were removed if they did not. Some data were removed if they did not fit the requested data type. For example, any text in place of a number was replaced with that number (if provided) or removed if it did not qualify as a valid response. There were some outliers removed. The data were then exported to Statistical Package for the Social Sciences (SPSS) software for analysis.

Instrumentation and Operationalization of Constructs

I created the study survey as no explicitly validated instruments were available. However, to elicit a valid response from respondents, the survey followed guidelines from Surveymonkey.com (n.d.). The survey was also developed in concert with mothers of children with ASD and mothers of children without ASD.

The survey utilized in this study was designed based on a review of the relevant literature and aimed to capture pertinent information related to maternal diet, social standing, and supplementation using folate or folic acid during pregnancy and the potential association with ASD in children (Liu et al., 2022; Surén et al., 2013). Although no validated instrument nor secondary dataset existed to address the research questions, the literature provided a foundation for developing the survey questions. A small pilot study with five mothers helped assess the survey's feasibility and effectiveness in eliciting a response (Creswell & Creswell, 2018).

Operationalization of Variables

The survey responses were provided by recruited mothers who completed the survey. The primary outcome variable was a diagnosis of ASD or not. The independent

variables included age, education, race, income, preeclampsia, MTHFR mutation, gestational diabetes, and folate status (daily folate equivalents).

- Maternal age: The mother's age at her child's birth was derived from the reported month and year of her birth and the month and year of delivery of the child. Age was kept as a scale variable for robust data evaluation.
- Maternal race: The mother's self-reported race was available for selection during the survey. Race categories in the survey included Caucasian, African American, Hispanic, Asian, mixed race (2 or more), or other. Due to low distribution, race was recoded to Caucasian for white respondents and non-Caucasian for all others (African American, Hispanic, Asian, mixed race (2 or more), or other). Maternal race was measured as a categorical variable.
- Education: The mother's self-reported highest education was available for selection during the survey. Categories for education were classified into four groups: a) None to some high school, high school graduate or GED, b) some college/AA/technical school, c) college graduate (BS/BA), and d) master's, doctorate, or professional degree. Education was measured as a categorical variable.
- Income: Respondents provided self-reported income while pregnant. Income levels at $20,000 increments were available for selection in the survey. Due to distribution, income was classified as either low-income ($40,000 or less), moderate-income ($40,001 - $79,999), or high-income ($80,000 or more). Maternal income was measured as a categorical variable.

- Preeclampsia: This variable refers to having elevated blood pressure while pregnant or other pregnancy-induced hypertensive disorders otherwise classified as preeclampsia. Preeclampsia was measured as a categorical variable.
- MTHFR mutation: This variable refers to having a diagnosed MTHFR mutation before pregnancy.
- Gestational diabetes: This variable refers to having diabetes diagnosed during pregnancy. Gestational diabetes was measured as a categorical variable.
- Folate supplementation: This variable refers to taking folate supplements during pregnancy. Folate supplementation was measured as a categorical variable.
- Multivitamin supplementation: This variable refers to taking multivitamin supplements during pregnancy. Multivitamin supplementation was measured as a categorical variable.
- Frequency: This variable refers to the frequency the mother chose to supplement. Options available included none, daily, every other day, once weekly, monthly, or I don't remember. Frequency was measured as a categorical variable.
- Fruits and vegetables: This variable refers to consuming vegetables during pregnancy measured as a categorical variable.
- Enriched foods: This variable refers to consuming enriched foods during pregnancy, measured as a categorical variable.
- Cereals: This variable refers to consuming cereals during pregnancy. If respondents answered yes, 170 micrograms of food folate were added to the estimated DFE. Cereals were measured as categorical variables.

- Meat percent: This variable refers to consuming meat, poultry, fish, dry beans, eggs, & nuts during pregnancy. Mothers were asked to estimate the amount of meat in their diet during pregnancy as a percentage range (none, 1-25%, 26-50%, 51-75%, 76-99%, and I don't remember) measured as a categorical variable.
- Fruits and vegetable percent: This variable refers to consuming fruits and vegetables during pregnancy. Mothers were asked to estimate the number of fruits and vegetables in their diet during pregnancy as a percentage range (none, 1-25%, 26-50%, 51-75%, 76-99%, and I don't remember) measured as a categorical variable.
- Grains percent: This variable refers to consuming grains and enriched foods during pregnancy. Mothers were asked to estimate the number of grains and enriched foods that their diet consisted of while pregnant expressed as a percentage range (none, 1-25%, 26-50%, 51-75%, 76-99%, and I don't remember) measured as a categorical variable.
- Daily folate from supplements: This variable refers to the estimated daily folate equivalents consumed from folate only and multivitamin supplement sources multiplied by the intake frequency during pregnancy measured as a scale variable.
- Daily folate from food sources: This variable refers to the estimated daily folate equivalents consumed from food sources (meats, fruits, vegetables, grains, enriched foods, and cereals) multiplied by the intake frequency during pregnancy measured as a scale variable.

- Folate: This variable refers to the estimated daily folate equivalents consumed from food and supplement sources measured as a scale variable.
- DFE category: This variable refers to the total estimated daily folate equivalents consumed from food and supplement sources measured as a scale variable.

Table 4 shows the operationalization of the variables based on the data collected.

Table 4

Operationalization of Variables

Name	Description	Type
AGE	Mother's age at child's birth	Scale
RACE	Mother's race 0 = Caucasian 1 = Non-Caucasian (African American, Hispanic, Asian, 2 or more races, other)	Categorical
EDUCATION	Education 0 = None to some high school, high school graduate or GED 1 = Some college/AA/technical school 2 = College graduate (BS/BA) 3 = Master's, doctorate or professional degree	Categorical
INCOME	Income 1 =Low (< $40,000) 2 = Moderate ($40,001–$79,999) 3 = High (> $80,000)	Categorical
	Table continues	
PREG_PREECLAMPS	Hypertension while pregnant 0 = No 1 = Yes	Categorical
PREG_DIABETES	Diabetes while pregnant (gestational diabetes) 0 = No 1 = Yes	Categorical
PREG_MTHFR	MTHFR disorder while pregnant 0 = No 1 = Yes	Categorical
FOLATE_SPP	Folate supplementation during pregnancy 0 = No 1 = Yes	Categorical
MULTIV_SPP	Multivitamin use during pregnancy 0 = No 1 = Yes	Categorical

Name	Description	Type
FOLATESPP_FREQ	Folate//folic acid supplements 0 = None 1 = Daily 2 = Every other day 3 = Once weekly 4 = Monthly 9999 = I don't remember	Categorical
VEGETABLES	Ate fruits and vegetables during pregnancy 0 = No 1 = Yes	Categorical
ENRICHED	Ate folate-enriched foods during pregnancy 0 = No 1 = Yes	Categorical
CEREAL	Ate cereal during pregnancy 0 = No 1 = Yes	Categorical
MEAT_PCT	Meats consumed 0 = 0% 1 = 1–25% 2 = 26–50% 3 = 51–75% 4 = 76–99% 888 = I don't recall	Categorical
FRUITVEG_PCT	Fruits and vegetables consumed 0 = 0% 1 = 1–25% 2 = 26–50% 3 = 51–75% 4 = 76–99% 888 = I don't recall	Categorical
	Table continues	
GRAINS_PCT	Enriched or grains foods consumed 0 = 0% 1 = 1–25% 2 = 26–50% 3 = 51–75% 4 = 76–99% 888 = I don't recall	Categorical
	Child's health and ASD	
ASD (dependent)	Has an ASD diagnosis 0 = No 1 = Yes	Categorical
	Daily folate equivalents	
DFE_{fspp}	Estimated folate equivalents based on folate supplementation and frequency	Scale
DFE_{multi}	Estimated folate equivalents based on multivitamin supplementation and frequency	Scale
DFE_{spp}	Estimated folate equivalents from the sum of (DFE_{fspp} + DFEmulti)/frequency	Scale

Name	Description	Type
DFE_{cereal}	Add 170 micrograms folic acid equivalents for diets containing cereals	Scale
$DFE_{fruitsvegs}$	Estimated folic acid equivalents based on percent fruits and vegetables consumed daily	Scale
DFE_{grains}	Estimated folic acid equivalents based on percent grains consumed daily	Scale
DFE_{meats}	Estimated folic acid equivalents based on percent meats consumed daily	Scale
DFE_{food}	Estimated folic acid equivalents based on sum of DFE_{cereal}, $DFE_{fruitsvegs}$, DFE_{grains}, and DFE_{meats} consumed daily	Scale
FOLATE	Estimated daily folic acid equivalents consumed from food and supplement sources multiplied by bioavailability	Scale
DFE_CAT	Daily folate equivalents categorized by group 0 = No supplementation 1 = Below recommendation (<600mg) 2 = Above recommendation (>600mg)	Categorical

Estimating Daily Folate Equivalent

Maternal folate status was estimated based on diet and supplementation data to determine inadequate, adequate, and over-supplementation. Maternal folic acid was quantified through the estimated consumption of foods reported in diet and supplementation.

Calculating DFE

Daily folate and folic acid consumption were calculated using the values found in Table 7 using an average of three meals per day using the formula (NIH Office of Dietary Supplementation, 2021), DFE = mcg naturally occurring folate + (1.7 x mcg folic acid).

Estimating DFE From Supplementation

I estimated the amount of folic acid consumed from supplementation, a multivitamin containing folic acid, or both during pregnancy. The estimated folic acid found in folic acid-only supplements (900 mcg) and multivitamins (800 mcg) was taken using the average of the top 8 selling supplements in each category available over the counter from various convenience stores, pharmacies, and grocery store shelves (shown

in Table 5). I assumed these supplements are available nationwide and for all mothers in the United States. Frequency was categorized as daily, every other day, weekly, or monthly to produce a daily folate equivalent from supplementation value. For example, if a respondent replied that she took both a folic acid supplement (900 mcg) and a multivitamin (800 mcg) daily throughout her pregnancy will have an estimated DFE from supplements of 1,700 mcg ($DFE_{spp} = (DFE_{fspp} + DFE_{multi})/\text{frequency} = (900 + 800)/1) =$ 1,700 mcg.

Table 5

Values for Estimating Daily Folate Equivalents

Source	Folic acid (mcg)
Foods	
Grain foods	105
Fruits and vegetables	40
Meat, poultry, fish, dry beans, eggs, and nuts	31
High-folic acid foods	
Cereal	170
Enriched foods (breads, etc.)	159
Supplementation	
Folic acid only*	900
Multivitamin containing folic acid*	800

Note. *Folate and multivitamin supplementation were estimated based on the average of the top 8 selling folic acid supplements or multivitamins in 2022.

Estimating DFE From Food Sources

I estimated the amount of folic acid consumed from food sources using information given by the respondent's diet and frequency. Food sources were categorized and quantified into grain foods, fruits and vegetables, and meat, poultry, fish, dry beans, eggs, and nuts using the median of each category. The categories include grain foods, fruits and vegetables, meat, poultry, fish, dry beans, eggs, and nuts. The median values

for each food type used in the analysis are listed in Table 6. A complete list of the values used is referenced in Appendix B.

To estimate consumption of each category, I asked mothers to provide the estimate of the amount of each as a percentage (1-25%, 26-50%, 51-75%, and 76-100%). For estimating close-to-true consumption estimates, consideration was taken for serving size (see Appendix B) and an average consumption of 3 meals per day. An additional consideration was taken for foods with high folic acid levels, including cereals and grains once daily as breakfast items. For example, a respondent answered that throughout pregnancy, she consumed cereals ($DFE_{cereal} = 170$), she consumed 76-100% meats ($DFE_{meats} = 83$), 57-75% fruits and vegetables ($DFE_{fruitsveg} = 76$), and 57-75% grain foods ($DFE_{grains} = 198$), her estimated daily folate equivalent from foods was estimated as $DFE_{foods} = 1(DFE_{cereal}) + (DFE_{meats}) + (DFE_{fruitsvegs}) + (DFE_{grains}) = 1(170) + 83 + 76 + 198 = 527$ mcg.

Table 6

Estimates of Food Folate in Micrograms

Daily Food Folate Values	1-25%	26-50%	51-75%	76-100%
Grain foods	41	120	198	277
Fruits & vegetables	16	46	76	106
Meat, poultry, fish, dry beans, eggs, & nuts	12	36	59	83

Note. The average consumption of each food category was estimated as 3x per day. Bioavailability is 85% for supplements and 50% for food folate.

Estimating Total DFE

The sum of the respondents' daily folic acid from supplements and daily folic acid from food sources were used to estimate daily folate intake during pregnancy. It was

assumed that the mother stayed on this regimen throughout her entire pregnancy, and thus estimated that the daily folic acid equivalent achieves 85% bioavailability for supplements (Caudill, 2010) and up to 50% for food sources (Caudill, 2010; Ohrvik & Witthoft, 2011). Therefore, the daily folate equivalent was estimated as DFE = (DFE_{spp} x 0.85) + (DFE_{foods} x 0.5). Continuing the above example, for this respondent, the estimated DFE equals (1700 mcg x 0.85) + (527 mcg x 0.50) = 1,445 mcg + 263.5 mcg = 1,708.5 mcg. This value aided in determining the category to which respondents belong (none, below recommendation, above recommendation) according to gross estimates. Continuing the example, the above-referenced respondent was categorized as "above recommendation" as their estimated DFE is 1,708.5 mcg, above the daily recommended value of 600 mcg per day for pregnant women.

Data Analysis Plan

For this study, IBM SPSS 28 for Apple Mac aided all analyses. Survey data were stored on Google Drive and exported to Microsoft Excel for Mac. Surveys were conducted online using the Google Forms platform.

The data collected were examined to ensure the quality entry of data by respondents. Any data found to be duplicative or irrelevant were removed from the dataset. I examined the data set for quality by removing any outliers and mismatched or irrelevant data points. Missing data were absent in the study data set but would have undergone procedures for multiple imputation or maximum likelihood estimation.

This study posed minimal risk to study participants. All subjects remained de-identified and assigned a serial number in order of survey completion. The only potential

identifying information was the date of birth and birth location. The study followed Walden University Institutional Review Board (IRB) oversight for ethical matters. The Walden University IRB number for this study was 06-16-22-0412467. All requirements ensure the proper protection of human subjects with minimal to zero harm.

This study was a case-control observational study to determine if maternal characteristics, including folate status, were associated with an ASD diagnosis in offspring. Participants were enrolled to participate in the survey and assigned to one of the two groups: (1) cases, mothers of children with a confirmed ASD diagnosis, or (2) controls, mothers of children without an ASD diagnosis. No vulnerable populations were involved in this study, including fetuses, neonates, pregnant women, children (under 18), prisoners, institutionalized individuals, and other vulnerable populations.

The data provided by respondents were collected retrospectively as part of the study. All collected data were de-identified and stored on Google Drive, a password-protected platform. Only I had access to the collected survey data.

Participants completed a self-reported survey to assess demographics (age, education, income, and race), folate consumption from food and supplemental intake, conditions (preeclampsia, MTHFR mutation, and gestational diabetes), and ASD diagnosis in the child. I will destroy all recorded and collected data from my Google Drive in 2028.

The landing page of the survey explained the study requirements, its complete voluntary nature, risks, and benefits. Additionally, potential participants were ensured that withdrawal from the study did not impact current or future care at their primary care

provider or affiliates. All participants who elected to participate assumed implied consent by taking the survey—standards on informed consent strictly adhered to Walden University's IRB.

Significant steps were taken to protect patient confidentiality, such as de-identifying study records. All computerized data are password-protected and stored on a secure HIPAA-compliant cloud-based server. Access to data were limited to only me. There were no paper records.

An adverse event (AE) or serious adverse event (SAE) was not a concern for this study, as participants answered survey questions. Should any AEs or SAEs related to this study have arisen, I would have adequately reported to the IRB, followed, and assessed for severity, suspected relationship to the regimen, and provided alleviating factors to the participant.

Participants were asked to continue the survey if they felt they met the inclusion/exclusion criteria on the landing page. I was available by phone or email to discuss written information on the participant requirements, their complete voluntary nature, risks, and benefits and address any participant questions. A respondent was assumed to consent after proceeding past the study information home page.

Data were collected using the secure Google Forms platform. The feature to capture the participant's email address was turned off, and any data were kept confidential by me, the (Principal Investigator) in a secured file on a password-protected computer.

The main source of this study's data comes from primary data collection methods. This method captures and deciphers data points pertinent to the research questions. However, there was a small risk of inaccurate data entry and susceptibility to errors in data collection.

Considerations for premature stopping of the study pertain to robustly conclusive evidence of futility and efficacy. The proposed observes initial effect size estimates. It is unlikely that the intervention will generate sufficient conclusive data of benefits or no benefits associated with the superior outcomes. Study data were analyzed preliminary at approximately 50% enrollment to review progress and outcomes. There was no evidence of futility or conclusive results, so early stoppage of the study and modifications to its procedures were unnecessary.

Summary

This study used a case-control approach and a survey design to elicit responses from mothers of children with ASD and mothers of children without ASD in the United States. Respondents were recruited through online methods, including social media and Google Ads. Keywords for finding the study included *autism symptoms, test for autism, take an autism test, autism test for adults, signs of autism in babies, early autism signs,* and *children with autism*. Other recruitment means included posting the study information and links on websites, including the Walden University participant pool and ClinicalTrials.gov. The landing page of the survey explained the study requirements. Respondents were directed to the domain (http://www.asdsurvey.com) to participate via Google Forms. The data were available in a Google Sheets file and downloaded for

exploration in Microsoft Excel. All data were kept private, and Walden University IRB provided oversight to ensure adequate protection of human subjects.

Chapter 4: Results

Introduction

In this study, I explored cases and controls among mothers in the United States to identify risk factors associated with demographics and supplementing with folate or folic acid supplements during pregnancy and ASD. Data were obtained via an online survey that asked questions on maternal health during pregnancy, sociodemographic information, and information on a child's health. Recruitment took place via Google Ads, clinicaltrials.gov (NCT05453708), social media, and outreach to autism centers throughout the United States from June 2022 to January 2023.

Data Collection

The study included 239 mothers of children with ASD (n = 96) and mothers of children without ASD (n = 143). The data set contained self-reported information about the mother's demographics (age, education, income, and race), conditions while pregnant (gestational diabetes, MTHFR mutation, preeclampsia), folate status, and an ASD diagnosis in their child.

Demographic Information

I split the file by cases and controls to obtain frequencies and percentages for each of the variables used in the study. Demographic information is presented in Table 7.

Table 7

Frequency of Variables

	N (%)		
	Cases	Controls	Total
Race			
Caucasian	79 (82.3%)	100 (69.9%)	179
Non-Caucasian	17 (17.7%)	43 (30.1%)	60
Education			
High school diploma, GED or less	10 (10.4%)	47 (32.9%)	57
Some college, AA, technical school	14 (14.6%)	41 (28.7%)	55
Bachelor's degree	29 (30.2%)	31 (21.7%)	60
Master's, doctorate, or professional degree	43 (44.8%)	24 (16.8%)	67
Income			
Low (< $40,000)	27 (28.1%)	65 (45.5%)	92
Moderate ($40,001–$79,999)	27 (28.1%)	46 (32.2%)	73
High (> $80,000)	42 (43.8%)	29 (20.3%)	71
Conditions			
Preeclampsia	10 (10.4%)	22 (15.4%)	32
Gestational diabetes	22 (22.9%)	7 (4.9%)	29
MTHFR mutation	1 (1.0%)	2 (1.4%)	3
Folate status			
No supplements	2 (2.1%)	7 (4.9%)	9
Below recommendation (< 600 mcg)	41 (42.7%)	69 (48.3%)	110
Above recommendation (> 600 mcg)	53 (55.2%)	67 (46.9%)	120

Race

Respondents provided self-reported data on race and ethnicity. Due to poor distribution, mothers were classified as either Caucasian or non-Caucasian. Most mothers of children with ASD were Caucasian (n = 179, 75%). All other respondents were African American, Hispanic, Asian, mixed (2 or more), or other, classified as non-Caucasian (n = 60, 25%).

Education

Most respondents held a master's, doctorate, or professional degree (n = 67, 28%). Sixty (25%) respondents held a bachelor's degree; 57 (24%) respondents had a

high school diploma, GED, or less; and 55 (23%) had some college, an associate's degree, or technical school education.

Annual Income

Most mothers reported making an annual salary of $40,000 or less (n = 92, 39%), followed by moderate-income ($40,001–$79,999; n = 73, 31%) and high-income (>$80,000; n = 71, 30%).

Maternal Conditions

Thirty-two (13%) respondents had pregnancy-induced hypertension. Twenty-nine (12%) had gestational diabetes, and three (1%) reported an MTHFR mutation.

Folate Status

The average DFE (mcg) was higher among mothers of children without ASD (mean = 952.10, SD = 814.10) compared to mothers of children with ASD. Most respondents (n = 120, 49%) exceeded the FDA recommendation of 600 mcg. Data on the average daily folate intake are presented in Table 8.

Table 8

Descriptive Statistics of Scale Variables

	Cases (N = 96)		Controls (N = 143)	
	Mean (SD)	Range	Mean (SD)	Range
Age (years)	27.74 (6.11)	18-47	27.73 (5.81)	18-43
Folate (mcg)	984.05 (725.99)	0-2295	952.10 (814.10)	0-2356

Univariate Analysis

Univariate analysis revealed a significant ($p < .05$) relationship between ASD and race, education (high school diploma, GED or less, some college, an associate's degree or

technical school, and bachelor's degree), moderate income, high income, and gestational diabetes.

Results

Research Question 1

RQ1: To what extent are maternal demographics (age, education, income, and race) associated with ASD?

$H_0$1: Maternal demographics (age, education, income, and race) have no association with ASD compared to controls.

H_A1: Maternal demographics (age, education, income, and race) have an association with ASD compared to controls.

In Approach 1, the number and percent distribution of education, income, and race were calculated. Binomial logistic regression was performed to test the association of maternal demographics (age, education, income, and race) and the occurrence of ASD compared to controls. The odds ratio, 95% confidence intervals, and adjusted odds ratios were reported. All models were run with and without a suspected confounder. A covariate that changes the estimate for comorbidity by 10% or greater was considered a confounder.

I used binomial logistic regression as a model to assess the association of several factors on respondents' likelihood of having children with ASD. The model contained seven independent variables (age, race [Caucasian, non-Caucasian], income [low, moderate, high], and education [high school diploma, GED, or less; some college, an associate's degree or technical school; college graduate (BS/BA); master's, doctorate, or

professional degree]). The logistic regression model (see Table 9) demonstrated statistically significant results (χ^2 (7) = 38.067, p < .001). The Nagelkerke R^2 for the model was .201, indicating the model explained about 20.1% of the variance in ASD occurrence. The Hosmer-Lemeshow test, which assesses goodness-of-fit, was insignificant (χ^2 (8) = 9.208, p = .325), suggesting the model adequately fit the data. Model diagnostics, such as the variance inflation factor (VIF), were examined to ensure the model's assumptions were met and assess multicollinearity. VIF values were below the threshold of 10, indicating multicollinearity was not a concern.

Significant effects were found for education (χ^2 (3) = 20.386, p = <.001). As shown in Table 9, only two independent variables made a unique statistically significant contribution to the model (some college, an associate's degree, or technical school and college graduate [BS/BA]). The findings suggest that respondents with some college, an associate's degree, or technical school education were less likely to have children with ASD compared to respondents with a high school diploma, GED, or less (B = –2.005, p < .001, OR = 0.135, 95% CI [0.051, 0.356]). Additionally, respondents who held a bachelor's degree were also less likely to have children with ASD compared to those with a high school diploma, GED, or less (B = –1.528, p < .001, OR = 0.217, 95% CI [0.092, 0.513]). However, no significant associations were found between ASD occurrence and maternal age, income, or race.

Table 9

Binomial Logistic Regression Predicting Likelihood of ASD Based on Age, Education, Income, and Race

	B	SE	*p*	OR	Adjusted 95% CI for OR	
					Lower	Upper
Age	-0.017	0.025	0.494	0.983	0.935	1.033
Race						
Caucasian	ref	ref	ref	ref	ref	ref
Non-Caucasian	-0.27	0.369	0.465	0.764	0.37	1.574
Education						
High school diploma, GED or less	ref	ref	ref	ref	ref	ref
Some college, AA, technical school	-2.005	0.497	<.001*	0.135	0.051	0.356
College graduate (BS/BA)	-1.528	0.439	<.001*	0.217	0.092	0.513
Masters, doctorate, or professional degree	-0.588	0.38	0.122	0.556	0.264	1.17
Income						
Low (< $40,000)	ref	ref	ref	ref	ref	ref
Moderate ($40,001–$79,999)	-0.233	0.419	0.578	0.792	0.349	1.799
High (> $80,000)	-0.3	0.386	0.436	0.741	0.348	1.577

Research Question 2

RQ2: To what extent are maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) associated with ASD compared to controls after adjusting for maternal age, education, income, and race?

$H_0$2: Maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) are not associated with ASD compared to controls after adjusting for maternal age, education, income, and race.

H_A2: Maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) are associated with ASD compared to controls after adjusting for maternal age, education, income, and race.

In Approach 2, the frequency and percent distribution of preeclampsia, MTHFR mutation, and gestational diabetes were calculated. Binomial logistic regression was

performed to test the association of maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) and the occurrence of ASD compared to controls after adjusting for maternal age, education, income, and race. The odds ratio, 95% confidence intervals, and adjusted odds ratios were reported. All models were run with and without a suspected confounder. A covariate that changed the estimate for conditions by 10% or greater was considered a confounder.

I used binomial logistic regression as a model to test the association of several maternal conditions with the likelihood that respondents would have children with ASD. The model contained ten independent variables (age, race [Caucasian, non-Caucasian], income [low, moderate, high], and education [high school diploma, GED, or less; some college, an associate's degree or technical school; college graduate (BS/BA); master's, doctorate, or professional degree]) and the presence of maternal conditions during pregnancy (preeclampsia, gestational diabetes, and MTHFR mutation).

The logistic regression model was statistically significant, χ^2 (10) = 54.309, p < .001. The model explained 20.6% (Cox & Snell R^2) and 27.7% (Nagelkerke R^2) of the variance in ASD diagnosis and correctly classified 69.9% of the cases. The Hosmer and Lemeshow test was insignificant (χ^2 (8) = 6.152, p = .630), indicating a good fit of the model to the data. Model diagnostics, such as VIF, were examined to ensure the model's assumptions were met and assess multicollinearity. VIF values were below the threshold of 10, indicating that multicollinearity was not a concern. As shown in Table 10, after adjusting for maternal demographic variables, the model with maternal conditions (preeclampsia, MTHFR mutation, and gestational diabetes) was significant, χ^2(10) =

54.309, $p < .001$. This model explained 20.6% (Cox & Snell R^2) to 27.7% (Nagelkerke R^2) of the variance in ASD occurrence and correctly classified 69.9% of cases. Gestational diabetes was found to be a significant predictor of ASD occurrence. Respondents with gestational diabetes were significantly less likely to have children with ASD ($B = –1.878$, $p < .001$, OR = 0.153, 95% CI [0.055, 0.426]). However, preeclampsia and MTHFR mutation were not significantly associated with ASD occurrence.

Table 10

Binomial Logistic Regression Predicting Likelihood of ASD Based on Presence of Preeclampsia, Gestational Diabetes, or MTHFR Mutation, Adjusting for Age, Race, Education, and Income

	B	SE	*p*	OR	Adjusted 95% CI for OR	
					Lower	Upper
Age	-0.013	0.026	0.612	0.987	0.937	1.039
Race						
Caucasian	ref	ref	ref	ref	ref	ref
Non-Caucasian	-0.41	0.391	0.295	0.664	0.308	1.43
Education						
High school diploma, GED or less	ref	ref	ref	ref	ref	ref
Some college to AA	-1.853	0.517	<.001*	0.157	0.057	0.432
College graduate (BS/BA)	-1.378	0.452	0.002*	0.252	0.104	0.612
Masters, doctorate, or professional degree	-0.6	0.397	0.131	0.549	0.252	1.195
Income						
Low (< $40,000)	ref	ref	ref	ref	ref	ref
Moderate ($40,001–$79,999)	-0.469	0.441	0.287	0.625	0.264	1.484
High (> $80,000)	-0.217	0.400	0.587	0.805	0.368	1.761
Preeclampsia						
No	ref	ref	ref	ref	ref	ref
Yes	0.306	0.465	0.511	1.357	0.546	3.377
Gestational diabetes						
No	ref	ref	ref	ref	ref	ref
Yes	-1.878	0.523	<.001*	0.153	0.055	0.426
MTHFR mutation						
No	ref	ref	ref	ref	ref	ref
Yes	1.669	1.378	0.226	5.308	0.356	79.046

Research Question 3

RQ3: To what extent is estimated maternal daily folate equivalent (DFE) status during pregnancy associated with ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes?

$H_0$3: Estimated maternal DFE status has no association with ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes.

H_A3: Estimated maternal DFE status is associated with ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes.

In Approach 3, the number and percent distribution of folate status were calculated. Binomial logistic regression was performed to test the association of maternal folate status during pregnancy and the occurrence of ASD compared to controls after adjusting for age, education, income, race, preeclampsia, MTHFR mutation, and gestational diabetes. The odds ratio, 95% confidence intervals, and adjusted odds ratios were reported. All models were run with and without a suspected confounder. A covariate that changed the estimate for conditions by 10% or greater was considered a confounder.

I used binomial logistic regression as a model to assess whether estimated maternal folate status has an association with the occurrence of ASD. The model contained 12 independent variables (age, race [Caucasian, non-Caucasian], income [low, moderate, high], and education [high school diploma, GED, or less; some college, an

associate's degree, or technical school; college graduate (BS/BA); master's, doctorate, or professional degree]) and the presence of maternal conditions during pregnancy (preeclampsia, gestational diabetes, and MTHFR mutation) and folate status. After adjusting for maternal demographic variables and conditions, adding maternal folate status to the model did not significantly improve, $\chi^2(1) = 0.429$, $p = .512$. Maternal folate status during pregnancy was not significantly associated with ASD in the adjusted model (B = 0, p = .512, OR = 1.000, 95% CI [1.000, 1.001]), as shown in Table 11.

Table 11

Binomial Logistic Regression Predicting Likelihood of ASD Based on Folate Status During Pregnancy After Adjusting for Age, Race, Education, Income, Presence of Preeclampsia, Gestational Diabetes, or MTHFR Mutation

	B	SE	*p*	OR	Adjusted 95% CI for OR	
					Lower	Upper
Age	-0.017	0.027	0.523	0.983	0.932	1.037
Race						
Caucasian	ref	ref	ref	ref	ref	ref
Non-Caucasian	-0.401	0.392	0.306	0.669	0.31	1.445
Education						
High school diploma, GED or less	ref	ref	ref	ref	ref	ref
Some college to AA	-1.831	0.518	<.001*	0.16	0.058	0.443
College graduate (BS/BA)	-1.383	0.454	0.002*	0.251	0.103	0.61
Masters, doctorate, or professional degree	-0.601	0.397	0.131	0.548	0.252	1.195
Income						
Low (< $40,000)	ref	ref	ref	ref	ref	ref
Moderate ($40,001–$79,999)	-0.474	0.441	0.283	0.623	0.262	1.479
High (> $80,000)	-0.239	0.402	0.551	0.787	0.358	1.731
Preeclampsia						
No	ref	ref	ref	ref	ref	ref
Yes	0.328	0.469	0.484	1.389	0.554	3.483
Gestational diabetes						
No	ref	ref	ref	ref	ref	ref
Yes	-1.925	0.531	<.001*	0.146	0.052	0.413
MTHFR mutation						
No	ref	ref	ref	ref	ref	ref
Yes	1.725	1.375	0.21	5.611	0.379	83.08

Folate Status	0	0	0.512	1.000	1.000	1.001

The logistic regression model was fitted using a three-step hierarchical method. The first block included demographic variables (age, race [Caucasian, non-Caucasian], income [low, moderate, high], and education [high school diploma, GED, or less; some college, an associate's degree, or technical school; college graduate (BS/BA); master's, doctorate, or professional degree]); and the third block contained estimated folate status. In the adjusted model, the odds ratio for the non-Caucasian race was 0.669 (95% CI [0.31, 1.445], p = .306), indicating that the effect of race on ASD is reduced after controlling for other variables in the model. Similarly, the adjusted odds ratios for some college, an associate's degree, technical school (OR = 0.16, 95% CI [0.058, 0.443], p< .001), and bachelor's degree (OR = 0.251, 95% CI [0.103, 0.61], p = .002), are still significantly lower than 1, indicating a lower odds of having children with ASD compared to those with a high school diploma or less, after adjusting for other variables. The odds ratio for estimated folate status was not statistically significant in either the unadjusted (OR = 1.000, 95% CI [1.000, 1.000], p = .755) or adjusted models (OR = 1.000, 95% CI [1.000, 1.000], p = .512), indicating that folate status was not associated with ASD risk in this population.

Overall, an increase in maternal age showed a slight, non-significant decrease in ASD risk (OR = 0.983, 95% CI [0.932, 1.037], p = .523). Non-Caucasian mothers were less likely to have children with ASD than Caucasian mothers, although the effect was not statistically significant (OR = 0.669, 95% CI [0.31, 1.445], p = .306). In terms of education, mothers with some college to an associate's degree had significantly lower

odds of having children with ASD compared to those with a high school diploma or GED (OR = 0.16, 95% CI [0.058, 0.443], p < .001). Similarly, college graduates (BS/BA) had a lower risk of having children with ASD (OR = 0.251, 95% CI [0.103, 0.61], p = .002). Still, the difference was insignificant for those with a master's, doctorate, or professional degree (OR = 0.548, 95% CI [0.252, 1.195], p = .131).

Income showed no significant association with ASD risk across different levels compared to the low-income group (Moderate: OR = 0.623, 95% CI [0.262, 1.479], p = .283; High: OR = 0.787, 95% CI [0.358, 1.731], p = .551). Preeclampsia did not significantly affect the likelihood of ASD (OR = 1.389, 95% CI [0.554, 3.483], p = .484). However, the presence of gestational diabetes had a significant effect on ASD (OR = 0.146, 95% CI [0.052, 0.413], p < .001). The MTHFR mutation showed an increased but non-significant risk of ASD (OR = 5.611, 95% CI [0.379, 83.08], p = .210). Folate status did not significantly correlate with ASD risk (OR = 1.000, 95% CI [1.000, 1.001], p = .512). Table 12 presents the unadjusted and adjusted odds ratios for all study variables, including age, race, education, income, preeclampsia, gestational diabetes, MTHFR mutation, and folate status, in relation to the risk of ASD in this study.

Table 12

Unadjusted and Adjusted Odds Ratios for All Study Variables

	Unadjusted			Adjusted		
	OR	95% CI	p-value	OR	95% CI	p-value
Age	1.00	0.957, 1.045	0.995	0.983	.932, 1.037	.523
Race						
Caucasian	ref	ref	ref	ref	ref	ref
Non-Caucasian	0.500	0.265, 0.944	0.032*	0.669	0.31, 1.445	0.306
Education						
High school diploma, GED or less	ref	ref	ref	ref	ref	ref
Some college, AA, technical school	0.119	0.051, 0.277	<.001*	0.16	0.058, 0.443	<.001*
Bachelor's degree	0.191	0.087, 0.418	<.001*	0.251	0.103, 0.61	0.002*
Master's, doctorate, or professional degree	0.522	0.256, 1.063	0.073	0.548	0.252, 1.195	0.131
Annual Income						
Low (< $40,000)	ref	ref	ref	ref	ref	ref
Moderate ($40,001–$79,999)	0.287	0.149, 0.551	<.001*	0.623	0.262, 1.479	0.283
High (> $80,000)	0.405	0.207, 0.792	.008*	0.787	0.358, 1.731	0.551
Conditions						
Preeclampsia						
No	ref	ref	ref	ref	ref	ref
Yes	1.564	0.705, 3.469	0.272	1.389	0.554, 3.483	0.484
Gestational Diabetes						
No	ref	ref	ref	ref	ref	ref
Yes	5.776	2.357, 14.156	<.001*	0.146	0.052, 0.413	<.001*
MTHFR mutation						
No	ref	ref	ref	ref	ref	ref
Yes	1.348	0.120, 15.071	0.809	5.611	0.379, 83.08	0.210
Folate Status	1.000	1.000, 1.000	0.755	1.000	1.000, 1.001	0.512

Note. *Statistically significant predictor ($p < .05$). Unadjusted odds ratios for each variable on ASD diagnosis. Adjusted odds ratios and significance for each variable in the final model (age, race, income, conditions, and folate status). Reference categories include Caucasian race, high school diploma, GED or less, low income, no preeclampsia, no gestational diabetes, no MTHFR mutation, and folate status.

Summary

The purpose of this study was to examine the association of maternal characteristics, including age, education, income, race, the presence of gestational diabetes, preeclampsia, MTHFR mutation, and folate status on the risk of ASD among

mothers of children with an ASD diagnosis and mothers of children without an ASD diagnosis.

The analysis revealed that education significantly predicted the occurrence of ASD ($p < .001$). Respondents with some college, an associate's degree, or technical school and college graduates (BS/BA) are significantly less likely to have children with ASD (OR = 0.251, 95% CI [0.103, 0.61], $p = .002$) than respondents with a high school diploma, GED or less. After adding maternal conditions (preeclampsia, gestational diabetes, and MTHFR mutation) into the model, education remained a significant predictor indicating that respondents who held some college, an associate's degree, or technical school education or less, and respondents who held a bachelor's degree were significantly less likely (OR = 0.157, 95% CI [0.057, 0.432], $p < .001$; OR = 0.252, 95% CI [0.104, 0.612], $p < .001$) respectively, to report having children with ASD compared to respondents who held a high school diploma, GED or less.

The presence of gestational diabetes was also found to have a significant association with ASD. In the initial model, respondents with gestational diabetes were found more likely to have children with ASD in the unadjusted model (OR = 5.776, 95% CI [2.357, 14.156], $p < .001$), but in the adjusted model, the association was inverted, such that mothers with gestational diabetes were less likely to have children with ASD, (OR = 0.146, 95% CI [0.052, 0.413], $p < .001$). The presence of preeclampsia (OR = 1.389, 95% CI [0.554, 3.483], $p = 0.484$) and MTHFR mutation (OR = 5.611, 95% CI [0.379, 83.08], $p = 0.210$) were not found to be significantly associated with ASD occurrence, laying the groundwork for further exploration. After adding folate status into

the model, the findings remained consistent with the second research question, where education and gestational diabetes remained significantly associated with ASD occurrence. However, folate status did not show a significant effect (OR = 1.00, 95% CI [1.000, 1.001], $p < .512$).

The findings of this study suggest that maternal education and the presence of gestational diabetes have a significantly inverse association with ASD. Mothers with higher education and experienced gestational diabetes were less likely to have children with ASD after adjusting for maternal age, race, income, preeclampsia, MTHFR mutation, and folate status. In the next chapter, I discuss this study's findings, draw conclusions, and recommend social change.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

This case-control study aimed to examine the association of maternal risk factors (maternal age, race, education, income, estimated folate status, gestational diabetes, preeclampsia, and MTHFR mutation) on the precipitation of ASD in offspring. The study relied on responses from mothers of children born in the United States with ASD and without ASD. I employed an observational approach and incorporated principles from LCHD to examine the effects of the independent variables (see Halfon & Forrest, 2017). Utilizing a robust study design that included a control group, I aimed to minimize biases and provide greater reliability and validity than previous investigations. ASD was found to be dependent on education and gestational diabetes but independent of all other factors, including age, race, income, race, MTHFR mutation, preeclampsia, or folate status.

Interpretation of the Findings

In this study, I aimed to examine the association of maternal characteristics, including folate status during pregnancy, on the occurrence of ASD compared to controls, after adjusting for age, education, income, race, and conditions (preeclampsia, MTHFR mutation, and gestational diabetes). Binomial logistic regression analysis was employed to address the research questions. The logistic regression model was fitted using a three-step hierarchical method. The first block tested the association between ASD and maternal demographic variables (age, race, education, and income). The second block added the association between ASD and pregnancy-related factors (preeclampsia,

gestational diabetes, and MTHFR mutation). The third block added estimated folate status to the model to test its association with ASD.

Maternal Age

In this study, maternal age was not a significant predictor of ASD in the model. This was an unexpected result as maternal age is a considerable risk factor in ASD etiology in previous investigations on age and ASD risk (Croen et al., 2007; Durkin et al., 2008). However, the findings are inconsistent with prior research. Given that risk was found to have a linear relationship with age, Sandin et al. (2012) underscored that the highest risk was among mothers aged 35 years or older. Wu et al. (2019) found that both advanced and early maternal age were associated with an increased risk of ASD, suggesting a potential U-shaped relationship between maternal age and ASD risk. Application of LCHD suggests advanced maternal age may be associated with increased genetic mutation and chromosomal abnormalities, which may increase ASD risk in offspring (Halfon et al., 2014).

This study's findings could be attributed to the average age of respondents. The mean age of respondents was 27.74 years. However, only 21 (15%) respondents aged 35 years or older were mothers of children without ASD, and 17 (18%) were mothers of children with ASD. The small sample of mothers may have been inadequate to detect a difference between cases and controls. Future research efforts should account for this limitation, as advanced maternal age is an established risk factor for ASD.

Maternal Race

This study indicates that maternal race may play a role in the development of ASD in offspring (unadjusted OR = 0.500, 95% CI [0.265, 0.944], p = 0.032). However, the mechanisms underlying these relationships are not yet fully understood. Maternal race was not a significant predictor of ASD occurrence in children in the final model (adjusted OR = 0.669, 95% CI [0.31, 1.445], p = 0.306), providing insights that confirm and extend previous findings.

A growing body of literature has investigated the potential mediating effects of maternal race on ASD risk. Becerra et al. (2014) found that children born to non-Hispanic Black mothers had greater odds of developing ASD than those born to non-Hispanic White mothers, indicating race as an established risk factor in ASD risk. This difference could result from racial disparities, risk factors, environmental exposures, or genetic susceptibilities (Bölte et al., 2011; Lyall et al., 2017; Modabbernia et al., 2017). The association of maternal race and ASD in offspring is further supported by Fairthorne et al. (2017), who demonstrated that maternal comorbidities and socioeconomic status might contribute to racial disparities in ASD risk, possibly due to a combination of environmental, genetic, social, and economic factors. In their study, Wallace-Watkin et al. (2023) highlighted the potential impact of access to healthcare and diagnostic services on racial disparities in ASD prevalence may be due to health literacy levels, available services, financial pressures, geographic barriers, and cultural differences or a combination of any of these.

The results of this study extend the knowledge base in the discipline, as they encourage further research efforts as to the potential pathways through which maternal race may contribute to ASD risk. As proposed by the LCHD framework (Halfon et al., 2014), a better understanding of these pathways may be achieved by examining how various biological, social, and environmental factors interact over time to shape children's developmental trajectories at risk of developing ASD. The findings do not suggest a causal relationship between maternal race and ASD risk. Instead, the associations observed may be attributed to genetic, epigenetics, and environmental factors that vary among racial and ethnic groups. Also, the findings of this study cannot be generalized to the population because racial and ethnic minorities only accounted for 25% of the study population. Therefore, this study's findings on the association between maternal race and ASD occurrence should be taken cautiously and emphasize the significance of examining how the interplay between individual and environmental factors is associated with ASD risk.

Maternal Education

Education was a significant predictor of ASD, highlighting that children born to mothers with higher education may have a lower risk of developing ASD. These findings support the literature despite discrepancies. Some studies have supported the significance of the association between maternal education on ASD occurrence (Durkin et al., 2010; Lung et al., 2018). Durkin et al. (2010) reported that higher maternal education is associated with an increased risk of ASD, possibly attributed to increased awareness and access to diagnostic services and care among mothers with higher education. Lung et al.

(2018) found that more children of mothers with a higher-than-average education were diagnosed with ASD. Still, more children of mothers with lower-than-average education were found to be positive on screening. Further supporting Durkin et al. (2010), this underscores the possibility that mothers of higher education may seek a diagnosis or have access to better resources to obtain a diagnosis than mothers of lower education. On a global scale, mothers of children with ASD are twice as likely to report having a greater level of education in the United Kingdom and Asia (Kelly et al., 2017; Yu et al., 2021).

Education, race (as described above), and income are often included in an accounting of socioeconomic status, which also considers other factors. In previous research, socioeconomic status is significantly associated with ASD. Most notably, Durkin et al. (2017) found that the prevalence of ASD increased with increasing socioeconomic status in an 8-year period among White, Black, and Hispanic children. However, prevalence persisted during this period among children of lower socioeconomic status. Zhou et al. (2019) explained that socioeconomic status is an established risk factor for ASD and lower mental health and thus leaves a greater propensity to precipitate ASD as lower maternal socioeconomic status is also correlated with greater anxiety and depressive symptoms than mothers from higher educational backgrounds.

Race and income were also found to be insignificant in this study. This is on par with information found in the literature, where some studies have reported significant associations between ASD prevalence when race or income are factored in (Becerra et al., 2014; Durkin et al., 2017), while others have not found significant relationships

(Croen et al., 2002). The discrepancies may be attributed to sample characteristics, methodology, or variable measurement differences.

The LCHD framework (Halfon et al., 2014) may be used to interpret the findings and guide further research as it emphasizes the role of early-life experiences and exposures in shaping long-term health and developmental outcomes. Within this context, maternal education may be considered a proxy for various factors, including socioeconomic status, access to care, personal health behaviors, and beliefs that could influence ASD risk in children. Higher maternal education is often linked to better health outcomes, possibly contributing to lower ASD risk. For example, highly educated mothers may be more likely to engage in prenatal care, adhere to provider recommendations, and access healthcare resources that may impact their offspring's health and development.

Maternal Income

Literature on the role of maternal income in ASD risk has yielded mixed findings. This study suggests that children born to higher-income mothers may have a decreased risk of ASD. However, this finding does not negate the potential association between maternal income on ASD risk, as previous research reported mixed results on the association between socioeconomic status and ASD prevalence (Durkin et al., 2010; Rai et al., 2012). Some studies have suggested that higher socioeconomic status and income levels may be associated with increased ASD diagnosis due to better access to healthcare and diagnostic services (Durkin et al., 2010), while others have found no association between socioeconomic status and ASD risk (Rai et al., 2012). However, any

discrepancies may be due to educational differences (Lung et al., 2018), which may also influence income.

Lower maternal income may be associated with increased exposure to environmental risk factors, such as air pollution, toxicants, psychosocial stressors, maternal stress, poor nutrition, and limited access to prenatal care, that have been positively linked to ASD risk (Kalkbrenner et al., 2014; Roberts et al., 2013). These studies supported the LCHD framework (Halfon et al., 2014). Further, they highlighted that higher income may contribute to better access to resources, improved living conditions, and reduced exposure to adverse environmental factors, which may influence the risk of ASD in offspring.

Maternal Conditions

Maternal conditions, including preeclampsia, gestational diabetes, and MTHFR mutation, were considered covariates to assess whether there was an association with precipitating ASD among offspring. After adjusting for maternal age, education, income, and race, the presence of gestational diabetes had a significant inverse effect against ASD (OR = 0.146, 95% CI [0.052, 0.413], $p < .001$). Because of this unexpected finding that contradicts existing literature, I recommend future research examining the link between maternal conditions and ASD risk in the offspring. This study's findings confirm these associations, providing insight that maternal conditions such as preeclampsia, gestational diabetes, and the MTHFR mutation may contribute to ASD risk. For instance, preeclampsia has been associated with altered placental functioning and reduced nutrient supply to the fetus, contributing to suboptimal brain development and increased ASD risk

(Burstyn et al., 2010). While the results did not show a significant association between preeclampsia or MTHFR mutation and ASD, evidence suggests a link between these conditions does exist, indicating a possible sampling error (Aviel-Shekler et al., 2020; Jenabi et al., 2019; Li et al., 2020; Sener et al., 2014). The sample consisted of 32 (13%) participants who reported having preeclampsia or gestational hypertension. Despite a positive link in previous research, preeclampsia affects 2%–8% of all pregnancies (Jenabi et al., 2019). Therefore, there was a minimal chance of detecting statistical significance. Future research should consider a larger sample size more representative of the population.

The LCHD framework (Halfon et al., 2014) offers a valuable perspective for interpreting the findings, emphasizing the role of early-life exposures in shaping future child development. The presence of certain maternal conditions such as preeclampsia, gestational diabetes, and MTHFR mutation can be considered a prenatal exposure that may influence offspring health and development through various biological pathways. This underscores the need for prospective longitudinal studies to examine the dynamic interplay between maternal conditions, genetics, the environment, and social factors during the life course to understand the complex etiology of ASD better.

Folate Status

This study's findings align with the knowledge gained from previous research that suggests a potential protective role of maternal folate status in ASD development (Raghavan, 2018; Virk et al., 2016). Previous research also alluded to folate and

homocysteine levels as a risk factor for ASD (Main et al., 2010; Sener, 2014), which implicated the involvement of MTHFR mutation in ASD.

The MTHFR gene provides instructions for folate processing and is necessary for DNA methylation. This polymorphism has been implicated as a risk factor for ASD, congenital disabilities, neurological disorders, and cancers due to impaired folic acid metabolism (Li et al., 2020; Pu et al., 2013; Schmidt et al., 2012). In a recent meta-analysis, Li et al. (2020) discovered that the MTHFR C677T polymorphism in either mother or inherited by the child was positively associated with ASD, but the exact role remains inconclusive. However, in their meta-analysis, Pu et al. (2013) found that the MTHFR C677T polymorphism was not only associated with an increased risk of ASD but was prevalent only in countries without mandatory folic acid fortification, which placed a great emphasis on the potential association between folic acid prenatal supplementation in modulating ASD risk. A deficiency in folic acid or having an MTHFR C677T polymorphism may result in low folate-dependent DNA hypomethylation activity and an altered methylation process. These effects can be the consequences of high concentrations of unmetabolized folic acid.

From an LCHD (Halfon et al., 2014) lens, the findings support maternal nutrition, particularly folate status, is crucial in shaping offspring health and development as it is an essential catalyst for DNA synthesis, repair, and methylation. During pregnancy, demands for folic acid increase as it plays an integral role in tissue growth and cell division during fetal development (CDC, 2022). It has been hypothesized that disruptions

in these processes may be due to folate deficiency that may contribute to an increased ASD risk (Raghavan et al., 2018; Schmidt et al., 2012).

According to FDA recommendations, folate supplementation is currently indicated in early pregnancy to reduce the risk of NTDs at 600 mcg daily (CDC, 2022; Hoxha et al., 2021). Fortified foods such as bread, cereals, flour, pasta, rice, and other grain products with folic acid are aimed to reduce the risk of NTDs because it has had a profound effect on the American diet, leading to increased mean folic acid intake of more than 190 mcg per day (Choumenkovitch et al., 2002). In this study, ASD risk was not significantly associated with maternal folate status.

Maternal folate status was estimated by the approximate amount of folic acid found in food sources as estimated by the respondent's diet and supplementation. Folate status was not a significant predictor of ASD in either mothers of children with or without ASD. This finding aligns with previous studies that maternal folic acid supplementation is associated with either a reduced ASD risk, enhanced ASD risk, or no risk (Hoxha et al., 2021). Furthermore, Hoxha et al. (2021) added that there may be an enhanced risk of ASD following high doses of folic acid intake before or during pregnancy, which may give rise to unmetabolized folic acid. In another study, high concentrations of unmetabolized folic acid were found to be associated with neurological and cognitive disorders.

Limitations of the Study

Despite the valuable insights gained from this study, several limitations must be acknowledged. First, using a case-control design limited the ability to establish a causal

relationship between maternal factors examined and ASD risk in offspring. While case-control studies can provide evidence of associations, they are vulnerable to recall and selection biases, which could confound the observed relationship (Mann, 2003).

Second, the study relied on self-reported data collection through an online survey. That may have introduced measurement errors and recall biases. Mothers may have had difficulty accurately recalling and reporting past behaviors, experiences, and conditions during pregnancy as participants were asked to recall their diet and supplementation regimen. This phenomenon may have increased the likelihood of recall bias based on the child's age (Althubaiti, 2016). This possibly leads to differential misclassification, as mothers of children with ASD may be more likely to recall specific exposures or conditions, given the potential psychological impact of having a child diagnosed with ASD (Liew et al., 2014). Future studies should consider employing prospective data collection methods to minimize recall bias and improve the accuracy of the reported information. Furthermore, using validated questionnaires and objective measures, including those from medical records or biological samples, significantly improves the accuracy and reliability of data in future studies, particularly with diagnostic information, confirmation of maternal health, and laboratory values of serum folate.

Third, the recruitment strategy employed in this study may have introduced selection bias. Recruitment occurred solely through online platforms such as Google Ads, Facebook posts, email invitations, online forums, and social media. Some demographic groups less likely to access or engage with these platforms may have been inadvertently excluded (Topolovec-Vranic & Natarajan, 2016). This could have limited the

generalizability of the findings to a broader population. Future research should consider employing diverse recruitment strategies to minimize potential biases and enhance the sample's representativeness.

Finally, the current study's sample size and demographic characteristics may have limited the ability to detect significant associations and reliability (Biau et al., 2018). Insufficient sample size may increase the risk of Type II error, meaning genuine associations may not be detected. Conversely, a larger sample size may increase the likelihood of detecting small, potentially irrelevant associations (Type I error). Future research should consider the appropriate sample size needed to achieve adequate statistical power while minimizing the risks of Type I and Type II errors. Further research with more extensive and diverse samples may help to clarify the relationships between these maternal demographic factors and ASD occurrence.

Recommendations

Considering the findings, future research must continue examining the complex interplay of maternal demographic factors and ASD occurrence. Additionally, identifying potential mechanisms underlying the relationship between maternal education and ASD occurrence may inform targeted interventions and support strategies for families affected by ASD. Moreover, considering other factors such as paternal demographics, family history of neurodevelopmental disorders, neurotypical siblings, and environmental exposures could contribute to a more comprehensive understanding of the factors influencing ASD occurrence. The following recommendations can be made for future research and clinical practice.

Future research should employ a prospective longitudinal or a randomized case-control study design to address the potential recall bias and improve the validity of the data and collection of information from mothers during pregnancy over time. Future research should also consider neurotypical siblings in the study design to understand better familial factors contributing to ASD risk and resilience (Levy et al., 2011). Incorporating additional potential confounding factors, such as paternal age, family history of neurodevelopmental disorders, and environmental exposures, may also give future researchers a better understanding of the complex interactions of genetics, epigenetics, and environmental factors contributing to ASD risk (Modabbernia et al., 2017).

To enhance the reliability and validity of the findings, researchers should use validated survey instruments for data collection (Bölte et al., 2011). This approach would ensure that the information collected is accurate, reliable, and relevant to the research questions being investigated. To improve the generalizability and impact of the findings, future research should involve collaborations with clinical professionals and stakeholders, such as pediatricians, psychologists, and advocacy groups (Pellicano et al., 2014). This collaborative approach can help inform clinical practice guidelines, promote early identification and intervention, and ultimately improve outcomes for children with ASD and their families.

Based on the findings of this study, healthcare professionals should consider incorporating the assessment and monitoring of maternal factors associated with ASD into their routine prenatal care and follow-up practices (Lavelle et al., 2014). This

approach can facilitate early identification and intervention, improve the quality of care provided to both mother and child, and ultimately contribute to better health outcomes for families affected by ASD.

The implications of this study's findings for practice and positive social change suggest that healthcare professionals should consider multiple factors when assessing ASD risk in children. Because there was a positive association in the unadjusted model, there should be an emphasis on future research of modifiable risk factors such as gestational diabetes and preeclampsia supporting pregnant or expectant mothers in maintaining optimal health during pregnancy. Therefore, care providers should consider providing resources and education about the potential risks and benefits of various factors during pregnancy, including the appropriate use of folic acid supplementation during critical and sensitive periods, such as in the first trimester when neurodevelopment occurs.

The findings of this study may still have implications for further investigation into folate as a risk factor for ASD and, subsequently, public health policy and clinical practice by informing strategies to promote optimal nutrition and supplementation in minimizing the risk of ASD in future generations. Improving the time to diagnosis and intervention would significantly impact effective prevention, management, and treatment strategies and help reverse rising ASD incidence and the costs associated with life-long care treatment and management.

Implications

The findings from this study contribute to the growing body of literature on maternal factors associated with ASD and have several implications for positive social change at multiple levels, including the individual, organizational, and societal & policy levels. This study highlights the importance of utilizing an LCHD framework to understand the complex interplay of maternal factors in ASD (Halfon & Forrest, 2017). By examining the associations between various maternal factors and ASD within this context, researchers can better understand ASD etiology, develop targeted interventions, and contribute to more effective prevention efforts.

At the individual or familial level, understanding the role of maternal factors in ASD can empower families to make informed decisions regarding pregnancy planning, prenatal care, and early interventions for their children (Lavelle et al., 2014). This information may reduce anxiety and increase resilience among families affected by ASD. Moreover, increased awareness of certain maternal factors can encourage more effective communication between families and healthcare providers, leading to tailored care plans and improved outcomes for the mother and her children.

The findings can also inform the development of evidence-based training programs for healthcare professionals, such as obstetricians, pediatricians, and psychologists, to better understand, identify, and address maternal factors associated with ASD (Wiggins et al., 2019). The training program can enhance healthcare providers' understanding of the complex interplay between genetics, epigenetics, and environmental factors in ASD etiology and foster a more comprehensive approach to care.

The results may also contribute to developing and refining public health policies and guidelines to improve prenatal care, early identification of ASD, and access to early intervention services (Baio et al., 2018). By addressing these maternal factors, policymakers can make more informed decisions regarding resource and service allocation for ASD-affected populations, ultimately promoting more equitable access to care and improved services.

Conclusion

This study sought to examine the association between maternal risk factors (maternal age, race, education, income, folate status, gestational diabetes, gestational hypertension, and MTHFR mutation) on the precipitation of ASD in offspring among mothers of children born in the United States. Although no significant association was found between maternal folate status and ASD diagnosis, the study sheds light on other important factors that may be associated with ASD development, including maternal education and gestational diabetes.

The findings from this study have implications for healthcare providers, policymakers, and families. They highlight the need to consider multiple factors when assessing ASD risk in children and emphasize the importance of addressing modifiable risk factors, such as gestational diabetes, and support for pregnant and expectant mothers in maintaining optimal health during pregnancy (Bailey et al., 2019). Furthermore, the study calls attention to the potential inaccuracies in self-reported maternal education, which could impact the development of targeted interventions and resources for affected families (Kogan et al., 2018).

This study also highlights the need for further research to understand better the complex relationships between maternal factors and ASD etiology. Future studies should explore potential genetic and environmental interactions, including the role of bioavailability and optimal consumption of folic acid or folate from food and supplemental sources (Bailey et al., 2019). In addition, refining methodologies for improved accuracy, reliability, and validity, such as using validated instruments and comprehensive, objective data collection, proves crucial for future research.

In conclusion, this study adds to the growing body of evidence on the complex etiology of ASD. It emphasizes the importance of considering multiple maternal factors when assessing ASD risk and guiding prevention and intervention strategies. Through continued research, healthcare professionals and policymakers can collaborate to develop evidence-based solutions and resources that promote optimal health during pregnancy and ultimately improve the lives of individuals with ASD and their families. Further research is required to elucidate the mechanisms through which maternal characteristics may be associated with ASD risk and to determine the optimal level and timing of folate supplementation during pregnancy to minimize the risk of ASD.

References

Abbas, H., Garberson, F., Liu-Mayo, S., Glover, E., & Wall, D. P. (2020). Multi-modular ai approach to streamline autism diagnosis in young children. *Scientific Reports, 10*(1). https://doi.org/10.1038/s41598-020-61213-w

Alexander, L. K., Lopes, B., Ricchetti-Masterson, K., & Yeatts, K. B. (2015). Selection bias. *Eric notebook* (2nd ed.). University of North Carolina at Chapel Hill, Department of Epidemiology. https://sph.unc.edu/wp-content/uploads/sites/112/2015/07/nciph_ERIC13.pdf

Althubaiti, A. (2016). Information bias in health research: Definition, pitfalls, and adjustment methods. *Journal of Multidisciplinary Healthcare, 9*, 211–217. https://doi.org/10.2147/jmdh.s104807

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

American Psychological Association. (n.d.). Socioeconomic status. Adapted from *APA dictionary of psychology*. https://www.apa.org/topics/socioeconomic-status

Aviel-Shekler, K., Hamshawi, Y., Sirhan, W., Getselter, D., Srikanth, K. D., Malka, A., Piran, R., & Elliott, E. (2020). Gestational diabetes induces behavioral and brain gene transcription dysregulation in adult offspring. *Translational Psychiatry, 10*(1), 412. https://doi.org/10.1038/s41398-020-01096-7

Bailey, R. L., Pac, S. G., Fulgoni, V. L. , Reidy, K. C., & Catalano, P. M. (2019). Estimation of total usual dietary intakes of pregnant women in the United States. *JAMA Network Open, 2*(6), e195967.

https://doi.org/10.1001/jamanetworkopen.2019.5967

Baio, J., Wiggins, L., Christensen, D. L., Maenner, M. J., Daniels, J., Warren, Z., Kurzius-Spencer, M., Zahorodny, W., Robinson Rosenberg, C., White, T., Durkin, M. S., Imm, P., Nikolaou, L., Yeargin-Allsopp, M., Lee, L. C., Harrington, R., Lopez, M., Fitzgerald, R. T., Hewitt, A., Pettygrove, S., … Dowling, N. F. (2018). Prevalence of autism spectrum disorder among children aged 8 years. *Morbidity and Mortality Weekly Report Surveillance Summaries, 67*(6), 1–23. https://doi.org/10.15585/mmwr.ss6706a1

Baxter, A. J., Brugha, T. S., Erskine, H. E., Scheurer, R. W., Vos, T., & Scott, J. G. (2015). The epidemiology and global burden of autism spectrum disorders. *Psychological Medicine, 45*(3), 601–613. https://doi.org/10.1017/S003329171400172X

Becerra, T. A., von Ehrenstein, O. S., Heck, J. E., Olsen, J., Arah, O. A., Jeste, S. S., Rodriguez, M., & Ritz, B. (2014). Autism spectrum disorders and race, ethnicity, and nativity: a population-based study. *Pediatrics, 134*(1), e63–71. https://doi.org/10.1542/peds.2013-3928

Begum, R., & Mamin, F. A. (2019). Impact of autism spectrum disorder on family. *Autism Open Access, 9*(244). DOI: 10.35248/2165-7890.19.09.244

Biau, D. J., Kernéis, S., & Porcher, R. (2008). Statistics in brief: The importance of sample size in the planning and interpretation of medical research. *Clinical Orthopaedics and Related Research, 466*(9), 2282–2288. https://doi.org/10.1007/s11999-008-0346-9

Bölte, S., Westerwald, E., Holtmann, M., Freitag, C., & Poustka, F. (2011). Autistic traits and autism spectrum disorders: The clinical validity of two measures presuming a continuum of social communication skills. *Journal of Autism and Developmental Disorders, 41*(1), 66–72. https://doi.org/10.1007/s10803-010-1024-9

Braun, J. M., Froehlich, T., Kalkbrenner, A., Pfeiffer, C. M., Fazili, Z., Yolton, K., & Lanphear, B. P. (2014). Brief report: Are autistic-behaviors in children related to prenatal vitamin use and maternal whole blood folate concentrations? *Journal of Autism and Developmental Disorders, 44*(10), 2602–2607. https://doi.org/10.1007/s10803-014-2114-x

Brimberg, L., Sadiq, A., Gregersen, P. K., & Diamond, B. (2013). Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. *Molecular Psychiatry, 18*(11), 1171–1177. https://doi.org/10.1038/mp.2013.101

Brown, H. K., Ray, J. G., Wilton, A. S., Lunsky, Y., Gomes, T., & Vigod, S. N. (2017). Association between serotonergic antidepressant use during pregnancy and autism spectrum disorder in children. *Journal of the American Medical Association, 317*(15), 1544–1552. DOI: 10.1001/jama.2017.3415

Burstyn, I., Sithole, F., & Zwaigenbaum, L. (2010). Autism spectrum disorders, maternal char vacteristics and obstetric complications among singletons born in Alberta, Canada. *Chronic Diseases and Injuries in Canada, 30*(4), 125–134. Internet Archive. https://doi.org/10.24095/hpcdp.30.4.04

Carlson, M. D., & Morrison, R. S. (2009). Study design, precision, and validity in

observational studies. *Journal of Palliative Medicine, 12*(1), 77–82. https://doi.org/10.1089/jpm.2008.9690

Cave, S. F. (2008). The history of vaccinations in the light of the autism epidemic. *Alternative Therapies in Health & Medicine, 14*(6), 54–57.

Caudill, M. A. (2010). Folate bioavailability: implications for establishing dietary recommendations and optimizing status. *The American Journal of Clinical Nutrition, 91*(5), 1455S–1460S. https://doi.org/10.3945/ajcn.2010.28674E

Centers for Disease Control & Prevention. (2021). Autism prevalence higher in CDC's ADDM network: Improvements being made in identifying children with autism early [Press Release]. *Morbidity and Mortality Weekly Report* (MMWR). https://www.cdc.gov/media/releases/2021/p1202-autism.html

Centers for Disease Control & Prevention. (2022). *MTHFR gene and folic acid*. https://www.cdc.gov/ncbddd/folicacid/mthfr-gene-and-folic-acid.html

Centers for Disease Control & Prevention. (2023). *Facts about neural tube defects*. https://www.cdc.gov/ncbddd/birthdefects/facts-about-neural-tube-defects

Cetin, I., & Laoreti, A. (2015). The importance of maternal nutrition for health. *Journal of Pediatric and Neonatal Individualized Medicine, 4*(2), e040267. DOI: 10.7363/040267

Chasan-Taber, L. (2014). *Writing dissertation and grant proposals: Epidemiology preventative medicine and biostatistics*. CRC Press.

Choumenkovitch, S. F., Selhub, J., Wilson, P. W., Rader, J. I., Rosenberg, I. H., & Jacques, P. F. (2002). Folic acid intake from fortification in United States exceeds

predictions. *The Journal of Nutrition, 132*(9), 2792–2798. https://doi.org/10.1093/jn/132.9.2792

Coury, D. L. (2013). DSM-5 and autism spectrum disorders. *Journal of Developmental & Behavioral Pediatrics, 34*(7), 494–496. https://doi.org/10.1097/dbp.0b013e31829cac3e

Creswell, J. W., & Creswell, J. D. (2018). *Research design: qualitative, quantitative, and mixed methods approaches*. (5th ed.). SAGE.

Crider, K. S., Yang, T. P., Berry, R. J., & Bailey, L. B. (2012). Folate and DNA methylation: A review of molecular mechanisms and the evidence for folate's role. *Advances in Nutrition, 3*(1), 21–38. https://doi.org/10.3945/an.111.000992

Croen, L. A., Najjar, D. V., Fireman, B., & Grether, J. K. (2007). Maternal and paternal age and risk of autism spectrum disorders. *Archives of Pediatrics & Adolescent Medicine, 161(*4), 334–340. https://doi.org/10.1001/archpedi.161.4.334

Czeizel, A. E., & Dudas, I. (1992). Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *The New England Journal of Medicine, 327*(26), 1832–1835. DOI: 10.1056/NEJM199212243272602

Dawson, G. & Bernier, R. (2013). A quarter century of progress on the early detection and treatment of autism spectrum disorder. *Development and Psychopathology, 25(4pt2)*, 1455–1472. https://doi.org/10.1017/S0954579413000710

DeVilbiss, E. A., Gardner, R. M., Newschaffer, C. J., & Lee, B. K. (2015). Maternal folate status as a risk factor for autism spectrum disorders: A review of existing evidence. *The British Journal of Nutrition, 114*(5), 663–672.

https://doi.org/10.1017/S0007114515002470

Dietz, P. M., Rose, C. E., McArthur, D., & Maenner, M. (2020). National and State Estimates of Adults with Autism Spectrum Disorder. *Journal of Autism and Developmental Disorders, 50*(12), 4258–4266. https://doi.org/10.1007/s10803-020-04494-4

Durkin, M. S., Maenner, M. J., Newschaffer, C. J., Lee, L. C., Cunniff, C. M., Daniels, J. L., Kirby, R. S., Leavitt, L., Miller, L., Zahorodny, W., & Schieve, L. A. (2008). Advanced parental age and the risk of autism spectrum disorder. *American Journal of Epidemiology, 168*(11), 1268–1276. https://doi.org/10.1093/aje/kwn250

Durkin, M. S., Maenner, M. J., Meaney, F. J., Levy, S. E., DiGuiseppi, C., Nicholas, J. S., Kirby, R. S., Pinto-Martin, J. A., & Schieve, L. A. (2010). Socioeconomic inequality in the prevalence of autism spectrum disorder: Evidence from a U.S. cross-sectional study. *PloS One, 5*(7), e11551. https://doi.org/10.1371/journal.pone.0011551

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., 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. https://doi.org/10.2105/AJPH.2017.304032

Fairthorne, J., de Klerk, N., Leonard, H. M., Schieve, L. A., & Yeargin-Allsopp, M. (2017). Maternal race-ethnicity, immigrant status, country of birth, and the odds

of a child with autism. *Child Neurology Open, 4*, 2329048X1668812. https://doi.org/10.1177/2329048x16688125

Faras, H., Al Ateeqi, N., & Tidmarsh, L. (2010). Autism spectrum disorders. *Annals of Saudi Medicine, 30*(4), 295–300. https://doi.org/10.4103/0256-4947.65261

Food and Drug Administration. (2009). Methotrexate tablets label. [PDF]. https://www.accessdata.fda.gov/drugsatfda_docs/label/2009/008085s063lbl.pdf

Frye, R. E., Slattery, J. C., & Quadros, E. V. (2017). Folate metabolism abnormalities in autism: potential biomarkers. *Biomarkers in Medicine, 11*(8), 687–699. https://doi.org/10.2217/bmm-2017-0109

Gardener, H., Spiegelman, D. & Buka, S. L. (2009). Prenatal risk factors for autism: A comprehensive meta-analysis. *British Journal of Psychiatry, 195*(1), 7–14. DOI: 10.1192/bjp.bp.108.051672

Gernand, A. D., Christian, P., Schulze, K. J., Shaikh, S., Labrique, A. B., Shamim, A. A., & West, K. P., Jr (2012). Maternal nutritional status in early pregnancy is associated with body water and plasma volume changes in a pregnancy cohort in rural Bangladesh. *The Journal of Nutrition, 142*(6), 1109–1115. https://doi.org/10.3945/jn.111.155978

Georgieff, M. K. (2007). Nutrition and the developing brain: Nutrient priorities and measurement. *The American Journal of Clinical Nutrition, 85*(2), 614S–620S. DOI: 10.1093/ajcn/85.2.614S

Geschwind, D. H. (2011). Genetics of autism spectrum disorders. *Trends in Cognitive Sciences, 15*(9), 409–416. https://doi.org/10.1016/j.tics.2011.07.003

Goldstein, S., & Ozonoff, S. (2018). *Assessment of autism spectrum disorder* (2nd ed.). Guilford Press.

Greenberg, J. A., Bell, S. J., Guan, Y., & Yu, Y. H. (2011). Folic acid supplementation and pregnancy: More than just neural tube defect prevention. *Reviews in Obstetrics & Gynecology, 4*(2), 52–59.

Greenberg, J. A., & Bell, S. J. (2011). Multivitamin supplementation during pregnancy: Emphasis on folic acid and l-methylfolate. *Reviews in Obstetrics and Gynecology, 4*(3–4), 126–127.

Halfon, N. & Forrest, C. B. (2017). The emerging theoretical framework of life course health development. In *Handbook of Life Course Health Development,* pp 19–43. DOI: 10.1007/978-3-319-47143-3_2

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., Miller, J., Fedele, A., Collins, J., Smith, K., Lotspeich, L., Croen, L. A., Ozonoff, S., Lajonchere, C., Grether, J. K., & Risch, N. (2011). Genetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry, 68*(11), 1095–1102. https://doi.org/10.1001/archgenpsychiatry.2011.76

Hariz, A., Bhattacharya, P. T. (2023). Megaloblastic Anemia. In: *StatPearls* [Internet]. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK537254/

Herndon, J. (2021). Preeclampsia: Causes, diagnosis, and treatments. *Healthline*. https://www.healthline.com/health/preeclampsia

Hoxha, B., Hoxha, M., Domi, E., Gervasoni, J., Persichilli, S., Malaj, V. & Zappacosta,

B. (2021). Folic acid and autism: A systematic review of the current state of knowledge. *Cells, 10*(8). DOI: 10.3390/cells10081976

Institute of Medicine Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline. (1998). *Dietary reference intakes for thiamin, riboflavin, niacin, vitamin b6, folate, vitamin b12, pantothenic acid, biotin, and choline*. National Academies Press (US). DOI: 10.17226/6015

Iossifov, I., O'Roak, B. J., Sanders, S. J., Ronemus, M., Krumm, N., Levy, D., ... Wigler, M. (2014). The contribution of de novo coding mutations to autism spectrum disorder. *Nature, 515*(7526), 216–221. https://doi.org/10.1038/nature13908

Jenabi, E., Karami, M., Khazaei, S., & Bashirian, S. (2019). The association between preeclampsia and autism spectrum disorders among children: A meta-analysis. *Korean Journal of Pediatrics, 62*(4), 126–130. https://doi.org/10.3345/kjp.2018.07010

Johnson, N. L., Giarelli, E., Lewis, C., & Rice, C. E. (2012). Genomics and autism spectrum disorder. *Journal of Nursing Scholarship, 45*(1), 69–78. DOI: 10.1111/j.1547-5069.2012.01483.x

Kalkbrenner, A. E., Windham, G. C., Serre, M. L., Akita, Y., Wang, X., Hoffman, K., ... & Daniels, J. L. (2014). Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders. *Epidemiology, 26*(1), 30–42.

Kang, D. W., Park, J. G., Ilhan, Z. E., Wallstrom, G., Labaer, J., Adams, J. B., &

Krajmalnik-Brown, R. (2013). Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. *PloS One, 8*(7), e68322. https://doi.org/10.1371/journal.pone.0068322

Kelly, B., Williams, S., Collins, S., Mushtaq, F., Mon-Williams, M., Wright, B., Mason, D. & Wright, J. (2017). The association between socioeconomic status and autism diagnosis in the United Kingdom for children aged 5–8 years of age: Findings from the born in Bradford cohort. *Autism, 23*(1), 131–140. DOI: 10.1177/1362361317733182

Kodesh, A., Levine, S. Z., Khachadourian, V., Rahman, R., Schlessinger, A., O'Reilly, P. F., Grove, J., Schendel, D., Buxbaum, J. D., Croen, L., Reichenberg, A., Sandin, S., & Janecka, M. (2021). Maternal health around pregnancy and autism risk: A diagnosis-wide, population-based study. *Psychological Medicine*, 1–9. Advance online publication. https://doi.org/10.1017/S0033291721001021

Kogan, M. D., Vladutiu, C. J., Schieve, L. A., Ghandour, R. M., Blumberg, S. J., Zablotsky, B., Perrin, J. M., Shattuck, P., Kuhlthau, K. A., Harwood, R. L., & Lu, M. C. (2018). The prevalence of parent-reported autism spectrum disorder among US children. *Pediatrics, 142*(6), e20174161. https://doi.org/10.1542/peds.2017-4161

Kuh, D., Ben-Shlomo, Y., Lynch, J., Hallqvist, J., & Power, C. (2003). Life course epidemiology. *Journal of Epidemiological Community Health, 57*, 778–783. DOI: 10.1136/jech.57.10.778

Lavelle, T. A., Weinstein, M. C., Newhouse, J. P., Munir, K., Kuhlthau, K. A., & Prosser,

L. A. (2014). Economic burden of childhood autism spectrum disorders. *Pediatrics, 133*(3), e520–e529. https://doi.org/10.1542/peds.2013-0763

Levy, D., Ronemus, M., Yamrom, B., Lee, Y. H., Leotta, A., Kendall, J., Marks, S., Lakshmi, B., Pai, D., Ye, K., Buja, A., Krieger, A., Yoon, S., Troge, J., Rodgers, L., Iossifov, I., & Wigler, M. (2011). Rare de novo and transmitted copy-number variation in autistic spectrum disorders. *Neuron, 70*(5), 886–897. https://doi.org/10.1016/j.neuron.2011.05.015

Li, Y. M., Shen, Y. D., Li, Y., J., Xun, G., L., Liu, H., Wu, R. R., Xia, K., Zhao, J. P., & Ou, J. J. (2018). Maternal dietary patterns, supplements intake and autism spectrum disorders: A preliminary case-control study. *Medicine, 97*(52), e13902. DOI: 10.1097/MD.0000000000013902

Li, Y., Pei, Y. X., Wang, L. N., Liang, C., Tang, Y. L., Zhang, X. L., Huang, L. B., Luo, X. Q., & Ke, Z. Y. (2020). MTHFR-C677T gene polymorphism and susceptibility to acute lymphoblastic leukemia in children: A meta-analysis. *Critical Reviews in Eukaryotic Gene Expression, 30*(2), 125–136. https://doi.org/10.1615/CritRevEukaryotGeneExpr.2020033468

Liew, Z., Ritz, B., Rebordosa, C., Lee, P. C., & Olsen, J. (2014). Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatrics, 168*(4), 313–320. https://doi.org/10.1001/jamapediatrics.2013.4914

Liu, X., Zou, M., Sun, C., Wu, L., & Chen, W. X. (2022). prenatal folic acid supplements and offspring's autism spectrum disorder: A meta-analysis and meta-regression. *Journal of Autism and Developmental Disorders, 52*(2), 522–539.

https://doi.org/10.1007/s10803-021-04951-8

Lung, F.-W., 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. https://doi.org/10.1007/s10995-018-2470-9

Lyall, K., Croen, L., Daniels, J., Fallin, M. D., Ladd-Acosta, C., Lee, B. K., Park, B. Y., Snyder, N. W., Schendel, D., Volk, H., Windham, G. C., & Newschaffer, C. (2017). The changing epidemiology of autism spectrum disorders. *Annual Review of Public Health, 38*, 81–102. https://doi.org/10.1146/annurev-publhealth-031816-044318

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., ... & Dietz, P. M. (2020). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 Sites, United States, 2016. *MMWR. Surveillance Summaries, 72*(SS-2), 1–14. http://dx.doi.org/10.15585/mmwr.ss7202a1

Maenner, M. J., Warren, Z., Robinson Williams, A., Amoakohene, E., Bakian, A. V., Bilder, D. A., ... & Shaw, K.A. (2023). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2020. *MMWR. Surveillance Summaries, 69*(4), 1–12. https://doi.org/10.15585/mmwr.ss6904a1

Magdalena, H., Beata, K., Justyna, P., Agnieszka, K.-G., Szczepara-Fabian, M., Buczek, A. & Ewa, E.-W. (2020). Preconception risk factors for autism spectrum disorder-

A pilot study. *Brain Science, 10*(5):293. DOI: 10.3390/brainsci10050293

Maher, G. M., O'Keeffe, G. W., Kearney, P. M., Kenny, L. C., Dinan, T. G., Mattsson, M. & Khashan, A. S. (2018). Association of hypertensive disorders of pregnancy with risk of neurodevelopmental disorders in offspring: A systematic review and meta-analysis. *Journal of the American Medical Association Psychiatry, 75*(8), 809–819. DOI: 10.1001/jamapsychiatry.2018.0854

Main, P. A., Angley, M. T., Thomas, P., O'Doherty, C. E., & Fenech, M. (2010). Folate and methionine metabolism in autism: A systematic review. *The American Journal of Clinical Nutrition, 91*(6), 1598–1620. https://doi.org/10.3945/ajcn.2009.29002

Mann C. J. (2003). Observational research methods. Research design II: cohort, cross sectional, and case-control studies. *Emergency Medicine Journal, 20*(1), 54–60. https://doi.org/10.1136/emj.20.1.54

Mayo Clinic. (2018). *Folate (folic acid)*. https://www.mayoclinic.org/drugs-supplements-folate/art-20364625

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. https://doi.org/10.1186/s13229-017-0121-4

National Institutes of Health (NIH) Office of Dietary Supplements. (2021). *Folate: Fact sheet for health professionals*. https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/#h3

National Institute of Mental Health. (n.d.). *Autism spectrum disorder*.

https://www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd

Ohrvik, V. E. & Witthoft, C. M. (2011). Human folate bioavailability. *Nutrients, 3*, 475-490. DOI: 10.3390/nu3040475.

OpenEpi. (n.d.). *Sample size for unmatched case-control study calculator*. https://www.openepi.com/SampleSize/SSCC.htm

Pellicano, E., Dinsmore, A., & Charman, T. (2014). What should autism research focus upon? Community views and priorities from the United Kingdom. *Autism, 18*(7), 756–770. https://doi.org/10.1177/1362361314529627

Pu, D., Shen, Y., & Wu, J. (2013). Association between mthfr gene polymorphisms and the risk of autism spectrum disorders: A meta-analysis. *Autism Research, 6*(5), 384–392. https://doi.org/10.1002/aur.1300

Raghavan, R., Fallin, M. D., & Wang, X. (2016). Maternal plasma folate, vitamin B12 levels and multivitamin supplementation during pregnancy and risk of autism spectrum disorder in the Boston Birth Cohort. *Federation of American Societies for Experimental Biology (FASEB) Journal, 30*(1), 151–156. https://faseb.onlinelibrary.wiley.com/doi/abs/10.1096/fasebj.30.1_supplement.151.6

Raghavan, R., Riley, A. W., Volk, H., Caruso, D., Hironaka, L., Sices, L., Hong, X., Wang, G., Ji, Y., Brucato, M., Wahl, A., Stivers, T., Pearson, C., Zuckerman, B., Stuart, E. A., Landa, R., Fallin, M. D., & Wang, X. (2018). Maternal multivitamin intake, plasma folate and vitamin b12 levels and autism spectrum disorder risk in offspring. *Paediatric and Perinatal Epidemiology*, *32*(1), 100–111.

https://doi.org/10.1111/ppe.12414

Rai, D., Lewis, G., Lundberg, M., Araya, R., Svensson, A., Dalman, C., Carpenter, P., & Magnusson, C. (2012). Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. *Journal of the American Academy of Child and Adolescent Psychiatry, 51*(5), 467–476.e6. https://doi.org/10.1016/j.jaac.2012.02.012

Ramakrishnan, U., Grant, F., Goldenberg, T., Zongrone, A., & Martorell, R. (2012). Effect of women's nutrition before and during early pregnancy on maternal and infant outcomes: A systematic review. *Paediatric and Perinatal Epidemiology, 26*(s1), 285–301. DOI: 10.1111/j.1365-3016.2012.01281.x

Roberts, A. L., Lyall, K., Hart, J. E., Laden, F., Just, A. C., Bobb, J. F., Koenen, K. C., Ascherio, A., & Weisskopf, M. G. (2013). Perinatal air pollutant exposures and autism spectrum disorder in the children of nurses' health study II participants. *Environmental Health Perspectives, 121*(8), 978–984. https://doi.org/10.1289/ehp.1206187

Roberts, A. L., & Nguyen, V. T. (2015). Growing evidence that maternal gestational diabetes increases risk of autism in offspring. *Evidence-Based Mental Health, 18*(4), 113. https://doi.org/10.1136/eb-2015-102142

Rowland, J. & Wilson, C. A. (2021). The association between gestational diabetes and ASD and ADHD: A systematic review and meta-analysis. *Scientific Reports, 11*(1), 5136. https://doi.org/10.1038/s41598-021-84573-3

Sandin, S., Hultman, C. M., Kolevzon, A., Gross, R., MacCabe, J. H., & Reichenberg, A.

(2012). Advancing maternal age is associated with increasing risk for autism: a review and meta-analysis. *Journal of the American Academy of Child and Adolescent Psychiatry, 51*(5), 477–486.e1. https://doi.org/10.1016/j.jaac.2012.02.018

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M., & Reichenberg, A. (2014). The familial risk of autism. *Journal of the American Medical Association, 311*(17), 1770–1777. https://doi.org/10.1001/jama.2014.4144

Sandin, S., Schendel, D., Magnusson, P., Hultman, C., Surén, P., Susser, E., Gronborg, T., Gissler, M., Gunnes, N., Gross, R., Henning, M., Bresnahan, M., Sourander, A., Horning, M., Carter, K., Francis, R., Parner, E., Leonard, H., Rosanoff, M., Stoltenberg, C., & Reichenberg, A. (2016). Autism risk associated with parental age and with increasing difference in age between the parents. *Molecular Psychiatry, 21*, 693–700. https://doi.org/10.1038/mp.2015.70

Scaglione, F. & Panzavolta, G. (2014). Folate, folic acid and 5-methyltetrahydrofolate are not the same thing. *Xenobiotica, 44*(5), 480–488. DOI: 10.3109/00498254.2013.845705

Schmidt, R. J., Hansen, R. L., Hartiala, J., Allayee, H., Schmidt, L. C., Tancredi, D. J., Tassone, F., & Hertz-Picciotto, I. (2011). Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. *Epidemiology, 22*(4), 476–485. https://doi.org/10.1097/EDE.0b013e31821d0e30

Schmidt, R. J., Tancredi, D. J., Ozonoff, S., Hansen, R. L., Hartiala, J., Allayee, H., ... &

Hertz-Picciotto, I. (2012). Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (childhood autism risks from genetics and environment) case-control study. *American Journal of Clinical Nutrition, 96*(1), 80–89. DOI: 10.3945/ajcn.110.004416

Selhub, J. (2002). Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *Journal of Nutrition and Health Aging, 6*(1), 39–42.

Sener, E. F., Oztop, D. B., & Ozkul, Y. (2014). MTHFR gene c677t polymorphism in autism spectrum disorders. *Genetics Research International*, 698574. https://doi.org/10.1155/2014/698574

St-Hilaire, S., Ezike, V. O., Stryhn, H., & Thomas, M. A. (2012). An ecological study on childhood autism. *International Journal of Health Geographics, 11*, 44. https://doi.org/10.1186/1476-072X-11-44

Stoner, R., Chow, M. L., Boyle, M. P., Sunkin, S. M., Mouton, P. R., Roy, S., Wynshaw-Boris, A., Colamarino, S. A., Lein, E. S., & Courchesne, E. (2014). Patches of disorganization in the neocortex of children with autism. *New England Journal of Medicine, 370*(13), 1209–1219. https://doi.org/10.1056/NEJMoa1307491

Surén, P., Roth, C., Bresnahan, M., Haugen, M., Hornig, M., Hirtz, D., Lie, K. K., Lipkin, W. I., Magnus, P., Reichborn-Kjennerud, T., Schjølberg, S., Davey Smith, G., Øyen, A. S., Susser, E., & Stoltenberg, C. (2013). Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. *Journal of the American Medical Association, 309*(6), 570–577. https://doi.org/10.1001/jama.2012.155925

Surveymonkey.com. (n.d.). *Demographic survey questions: What they are and why you need them*. https://www.surveymonkey.com/mp/gathering-demographic-information-from-surveys/

Tick, B., Bolton, P., Happé, F., Rutter, M., & Rijsdijk, F. (2016). Heritability of autism spectrum disorders: a meta-analysis of twin studies. *Journal of Child Psychology and Psychiatry, 57*(5), 585–595. https://doi.org/10.1111/jcpp.12499

The Autism Speaks Foundation. (2021). *Autism statistics and facts*. https://www.autismspeaks.org/autism-statistics

Topolovec-Vranic, J., & Natarajan, K. (2016). The use of social media in recruitment for medical research studies: A scoping review. *Journal of Medical Internet Research, 18*(11), e286. https://doi.org/10.2196/jmir.5698

U.S. National Library of Medicine. (2014). *Autism*. Medline Plus. http://www.nlm.nih.gov/medlineplus/ency/article/001526.htm

Vidmar Golja, M., Šmid, A., Karas Kuželički, N., Trontelj, J., Geršak, K., & Mlinarič-Raščan, I. (2020). Folate insufficiency due to mthfr deficiency is bypassed by 5-methyltetrahydrofolate. *Journal of Clinical Medicine, 9*(9), 2836. https://doi.org/10.3390/jcm9092836

Viktorin, A., Uher, R., Reichenberg, A., Levine, S. Z., & Sandin, S. (2017). Autism risk following antidepressant medication during pregnancy. *Psychology Medicine, 47*(16), 2787–2796. DOI: 10.1017/S0033291717001301

Virk, J., Liew, Z., Olsen, J., Nohr, E. A., Catov, J. M., & Ritz, B. (2016). Preconceptional and prenatal supplementary folic acid and multivitamin intake and autism

spectrum disorders. *Autism, 20*(6), 710–718. https://doi.org/10.1177/1362361315604076

Wald, N. J., Law, M. R., Morris, J. K., & Wald, D. S. (2001). Quantifying the effect of folic acid. *Lancet, 358*(9298), 2069–2073. https://doi.org/10.1016/s0140-6736(01)07104-5

Wallace-Watkin, C., Sigafoos, J., & Waddington, H. (2023). Barriers and facilitators for obtaining support services among underserved families with an autistic child: A systematic qualitative review. *Autism, 27*(3), 588–601. https://doi.org/10.1177/13623613221123712

Wiens, D., & DeSoto, M. C. (2017). Is high folic acid intake a risk factor for autism?-a review. *Brain Sciences, 7*(11), 149. https://doi.org/10.3390/brainsci7110149

Wiggins, L. D., Rubenstein, E., Daniels, J., DiGuiseppi, C., Yeargin-Allsopp, M., Schieve, L. A., Tian, L. H., Sabourin, K., Moody, E., Pinto-Martin, J., Reyes, N., & Levy, S. E. (2019). A phenotype of childhood autism is associated with preexisting maternal anxiety and depression. *Journal of Abnormal Child Psychology, 47*(4), 731–740. https://doi.org/10.1007/s10802-018-0469-8

World Health Organization. (2021). *Autism spectrum disorders key facts*. https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders

Yu, T., Lien, Y.-J., Liang, F.-W. & Kuo, P.-L. (2021). Parental socioeconomic status and autism spectrum disorder in offspring: A population-based cohort study in Taiwan. *American Journal of Epidemiology, 190*(5), 807–816. https://doi.org/10.1093/aje/kwaa241

Zhou, W., Liu, D., Xiong, X., & Xu, H. (2019). Emotional problems in mothers of autistic children and their correlation with socioeconomic status and the children's core symptoms. *Medicine, 98*(32), e16794. https://doi.org/10.1097/MD.0000000000016794

Zuckerman, K. E., Lindly, O. J., & Chavez, A. E. (2017). Timeliness of autism spectrum disorder diagnosis and use of services among U.S. elementary school-aged children. *Psychiatric Services, 68*(1), 33–40. DOI: 10.1176/appi.ps.201500549

Appendix A: Sample Size Calculation

Start | Enter | Results | Examples | Help

Sample Size for Unmatched Case-Control Study

For:

Two-sided confidence level(1-alpha)	95
Power(% chance of detecting)	80
Ratio of Controls to Cases	1
Hypothetical proportion of controls with exposure	61
Hypothetical proportion of cases with exposure:	39
Least extreme Odds Ratio to be detected:	0.41

	Kelsey	Fleiss	Fleiss with CC
Sample Size - Cases	82	81	90
Sample Size - Controls	82	81	90
Total sample size:	164	162	180

References

Kelsey et al., Methods in Observational Epidemiology 2nd Edition, Table 12-15
Fleiss, Statistical Methods for Rates and Proportions, formulas 3.18 &3.19

CC = continuity correction
Results are rounded up to the nearest integer.
Print from the browser menu or select, copy, and paste to other programs.

Results from OpenEpi, Version 3, open source calculator--SSCC
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

Appendix B: Common Foods and Folate/Folic Acid Contents

Food	Serving Size	Folic Acid Amount* (µg)	Folate Amount (µg)
GRAIN FOODS†			
Bagel	1, 3-inch diameter	30	70
Bread, roll, biscuit, ½ English muffin	1 piece	15	32.5
Breakfast cereal	1 cup (check label)	250	170
Cookies	1 ounce, 2 medium size cookies	10	17.50
Crackers, round	5 crackers	15	
Crackers, saltine or melba	½ ounce	15	25
Flour tortilla, soft	1, 10-inch diameter	80	140
Grits, cooked	½ cup	40	70
Macaroni, cooked	1 cup	85	150
Noodles, cooked	1 cup	90	160
Oatmeal, instant	1 package	70	125
Oatmeal, regular and quick, cooked	½ cup	0	5
Pretzels	½ ounce, 8 small thin twists	15	30
Spaghetti, cooked	1 cup	90	160
Toaster pastry	1 pastry	40	70
Wheat germ, toasted	2 Tablespoons	0	50
White rice, cooked	1 cup	95	170
FRUITS			
Apple (with skin)	1, medium	5	
Banana	1, medium	20	
Blueberries, fresh	½ cup	5	
Cantaloupe	¼, medium	40	
100% grapefruit juice, ready-to-drink	1 cup	25	
Grapes	1 cup	40	
Orange	1, medium	40	
100% orange juice, ready-to-drink	1 cup	80	
Peaches, canned, juice pack	½ cup	5	
Raisins	½ cup	5	
Strawberries, fresh	8, medium	80	
VEGETABLES			
Asparagus, cooked	5 spears	100	
Broccoli, cooked	½ cup	50	
Brussels sprouts, cooked	½ cup	80	

Carrots, cooked	½ cup	10
Cauliflower, cooked	½ cup	35
Corn on the cob	1 large ear	55
Corn, cooked	½ cup	20
French fries, prepared from frozen	10 fries	5
Green beans, cooked	½ cup	5
Green peas, cooked	½ cup	5
Lettuce, iceberg	1 cup	30
Lettuce, romaine	1 cup	40
Mashed potatoes	½ cup	10
Mustard greens, cooked	½ cup	90
Okra, cooked	½ cup	135
Potato, Idaho, baked (with skin)	1, medium	25
Spinach, cooked	½ cup	100
Spinach, raw	1 cup	110
Squash, yellow, cooked	½ cup	15
Tomato, raw	½ tomato	10
Tomato juice	1 cup	50
Turnip greens, cooked	½ cup	85
MEAT, POULTRY, FISH, DRY BEANS, EGGS, & NUTS		
Beans, cooked (black, navy, pinto, kidney)	½ cup	130
Egg	1, large	25
Meat, fish, poultry (breaded or batter-fried with enriched flour)	3 ounces, size of a deck of cards	37.5
Meat, fish, poultry (not breaded or batter-fried)	3 ounces, size of a deck of cards	12.50
Peanut butter	2 tablespoons	25
Peanuts, dry roasted	1 ounce, ¼ cup	40
DAIRY		
Cheese, American or hard cheeses (cheddar, etc.)	1¼-inch cube	5
Ice cream	½ cup	10
Milk	1 cup	12.5

Adapted from Journal of the American Dietetic Association (2000)

Appendix C: Foods High in Folate or Folic Acid

Food	Micrograms (mcg) DFE per serving
Beef liver, braised, 3 ounces	215
Spinach, boiled, ½ cup	131
Black-eyed peas (cowpeas), boiled, ½ cup	105
Breakfast cereals, fortified with 25% of the DV	100
Rice, white, medium-grain, cooked, ½ cup	90
Asparagus, boiled, 4 spears	89
Brussels sprouts, frozen, boiled, ½ cup	78
Spaghetti, cooked, enriched, ½ cup†	74
Lettuce, romaine, shredded, 1 cup	64
Avocado, raw, sliced, ½ cup	59
Spinach, raw, 1 cup	58
Broccoli, chopped, frozen, cooked, ½ cup	52
Mustard greens, chopped, frozen, boiled, ½ cup	52
Bread, white, 1 slice	50
Green peas, frozen, boiled, ½ cup	47
Kidney beans, canned, ½ cup	46
Wheat germ, 2 tablespoons	40
Tomato juice, canned, ¾ cup	36
Crab, Dungeness, 3 ounces	36
Orange juice, ¾ cup	35
Turnip greens, frozen, boiled, ½ cup	32
Peanuts, dry roasted, 1 ounce	27
Orange, fresh, 1 small	29
Papaya, raw, cubed, ½ cup	27
Banana, 1 medium	24
Yeast, baker's, ¼ teaspoon	23
Egg, whole, hard-boiled, 1 large	22
Cantaloupe, raw, cubed, ½ cup	17
Vegetarian baked beans, canned, ½ cup	15
Fish, halibut, cooked, 3 ounces	12
Milk, 1% fat, 1 cup	12
Ground beef, 85% lean, cooked, 3 ounces	7
Chicken breast, roasted, 3 ounces	3

Appendix D: Survey

* Indicates required question

Mother's Demographics

This section will ask you to provide information about yourself. Mothers of multiple children may submit more than one survey.

1. What is your date of birth?* *Example: January 7, 2019*
2. What is your race?* *Mark only one oval.*
    a. Caucasian
    b. Black/African-American
    c. Asian
    d. Native Hawaiian/other Pacific Islander
    e. American Indian/Alaskan Native
    f. Mixed Race (2 or more)
    g. Other:
3. What is your birthplace?*
4. What is your primary language spoken at home?*
5. Select your height?* *Mark only one oval.*
    a. 4' 5"
    b. 4' 6"
    c. 4' 7"
    d. 4' 8"
    e. 4' 9"
    f. 5' 0"
    g. 5' 1"
    h. 5' 2"
    i. 5' 3"
    j. 5' 4"
    k. 5' 5"
    l. 5' 6"
    m. 5' 7"
    n. 5' 8"
    o. 5' 9"
    p. 5' 10"
    q. 5' 11"
    r. 6' 0"
    s. 6' 1"
    t. 6' 2"
    u. 6' 3"
    v. 6' 4"
    w. 6' 5"

x. 6’ 6”
y. 6’ 7”

6. What was your average weight in pounds prior to pregnancy with this child?*
7. What was your marital status while pregnant with this child?* *Mark only one oval.*
    a. Married
    b. Divorced
    c. Remarried
    d. Single
    e. Other:
8. Do you drink caffeinated beverages daily (such as coffee, tea, colas) before or while pregnant?* *Mark only one oval.*
    a. Yes, before pregnancy
    b. Yes, while pregnant
    c. Yes, before and during pregnancy
    d. No
9. While pregnant, what was your highest level of education?* *Mark only one oval.*
    a. Some high school
    b. High school graduate or GED
    c. Some college/AA/technical school
    d. College Graduate (BS/BA)
    e. Master’s
    f. Doctorate/Professional Degree
    g. None
10. How many people were currently living in your household, including yourself during pregnancy with this child?*
11. Please describe the home where you lived while pregnant with this child:* *Mark only one oval.*
    a. It is owned or being bought by you (or someone in the household)
    b. It is rented for money by you (or someone in the household)
    c. It is occupied without payment or money or rent
    d. I live with friends
    e. I live with family
    f. I have no permanent residence
    g. Other:
12. Prior to pregnancy, would you say, in general, your mental health was (check one):* *Check all that apply.*
    a. Excellent
    b. Good
    c. Fair
    d. Poor

13. Prior to pregnancy, would you say, in general, your physical health was (check one):* *Check all that apply.*
    a. Excellent
    b. Good
    c. Fair
    d. Poor
14. Did you smoke cigarettes/tobacco before, during, or after pregnancy?* *Mark only one oval.*
    a. Current smoker
    b. Former smoker
    c. Never
15. Did you drink alcohol prior to giving birth to this child?* *Mark only one oval.*
    a. Yes
    b. No
16. If yes, how many drinks of alcohol do you consume per week? Please indicate N/A if none. Example, 3 glasses of wine
17. Do you have a history of autism spectrum disorders in your family including Asperger's?* *Mark only one oval.*
    a. Yes
    b. No
    c. I don't know
18. Does the child's father have a history of autism spectrum disorders in his family including Asperger's? *Mark only one oval.*
    a. Yes
    b. No
    c. I don't know
19. Prior to pregnancy, did you have any of the following conditions? (Please select all that apply)* *Check all that apply.*
    a. Anemia
    b. Arthritis
    c. B12 deficiency
    d. Cancer
    e. Diabetes
    f. Epilepsy
    g. Folate (B9) deficiency
    h. High blood pressure (hypertension)
    i. MTHFR mutation or folate metabolism deficiency
    j. Seizures
    k. Skin disorders
    l. Ulcerative Colitis (UC)
    m. Celiac Disease

n. None
o. Other:

20. Did you have health insurance while pregnant with this child?* *Mark only one oval.*
    a. Yes
    b. No
21. How did you pay for your health care and medical expenses while pregnant with this child?* *Mark only one oval.*
    a. Government funding (Medicaid/Medicare)
    b. Private insurance
    c. Self-pay, out of pocket
    d. Other:
22. While pregnant with this child, what was your work status? *Mark only one oval.*
    a. Employed
    b. Self-employed/Freelancer
    c. Part-time
    d. Unemployed- Looking for work
    e. Unemployed – Not looking for work
    f. Homemaker
    g. Military/Forces
    h. Retired
    i. Not able to work
    j. Student
23. While pregnant with this child, what was your household income? *Mark only one oval.*
    a. Under $20,000
    b. $20,001 – $40,000
    c. $40,001 – $60,000
    d. $60,001 – $80,000
    e. $80,001 – $100,000
    f. $100,001 or over

Diet, Nutrition, Exercise & Supplementation During Pregnancy

Please answer the questions about yourself while pregnant with this child. Mothers of multiple children may submit more than one survey.

24. While pregnant with this child, did you have any of the following conditions?* *Check all that apply.*
    a. Anemia
    b. Arthritis
    c. B12 Deficiency

d. Cancer
e. Diabetes
f. Epilepsy
g. Folate (B9) deficiency
h. High blood pressure (hypertension)
i. MTHFR mutation or folate metabolism deficiency
j. Seizures
k. Skin Disorders
l. Ulcerative Colitis (UC)
m. Weight gain
n. Celiac Disease
o. None
p. Other:

25. Did you ever take any of the following medications BEFORE or DURING pregnancy with this child?* *Check all that apply.*
    a. Methotrexate (Rheumatrex®, Trexall®)
    b. Phenytoin (Dilantin®)
    c. Carbamazepine (Carbatrol®, Tegretol®, Equetro®, Epitol®)
    d. Valproate (Depacon®)
    e. Sulfasalazine (Azulfidine®)
    f. None
26. Did you use any folate/folic acid supplements during pregnancy with this child?* *Mark only one oval.*
    a. No
    b. Yes, full term
    c. Yes, partial term
    d. I don't remember
27. If partial, which trimesters did you use folate/folic acid supplements? *Check all that apply.*
    a. First trimester
    b. Second trimester
    c. Third trimester
    d. I don't remember
28. Did you use any multivitamins containing folate/folic acid during pregnancy with this child?* *Mark only one oval.*
    a. No
    b. Yes, full term
    c. Yes, partial term
    d. I don't remember
29. If partial, which trimesters did you use multivitamins containing folate/folic acid? *Check all that apply.*

a. First trimester
b. Second trimester
c. Third trimester
d. I don't remember

30. While pregnant with this child, did the amount of folate/folic acid you consumed change? *Mark only one oval.*
    a. Yes, the dose increased
    b. Yes, the dose decreased
    c. No, I took the same folate/folic acid or multivitamin throughout my pregnancy
    d. Other:
31. How often did you use folate/folic acid supplements while pregnant with this child? If other, please specify.* *Mark only one oval.*
    a. Daily
    b. Every other day
    c. Once weekly
    d. Monthly
    e. I did not use folate/folic acid supplements during pregnancy with this child
    f. Other:
32. Do you recall the name brand of the supplements you took during pregnancy with this child?
33. Do you recall the amount of folate or folic acid found in your supplement or multivitamin while pregnant with this child?
34. While pregnant with this child, did you consume any of the following foods fortified with folic acid?* *Check all that apply.*
    a. Beef liver
    b. Vegetables (especially asparagus, brussels sprouts, and dark green leafy vegetables such as spinach and mustard greens)
    c. Fruits and fruit juices (especially oranges and orange juice)
    d. Nuts, beans, and peas (such as peanuts, black-eyed peas, and kidney beans)
    e. Enriched bread, flour, cornmeal, pasta, and rice
    f. Fortified breakfast cereals
    g. Fortified corn masa flour (used to make corn tortillas and tamales, for example)
    h. None
    i. Other:
35. While pregnant with this child, what percentage of your diet consisted of meat or meat products?* *Mark only one oval.*
    a. 0%

b. 1-25%
c. 26-50%
d. 51-75%
e. 76-99%
f. I don't know

36. While pregnant with this child, what percentage of your diet consisted of vegetables or vegetable products?* *Mark only one oval.*
    a. 0%
    b. 1-25%
    c. 26-50%
    d. 51-75%
    e. 76-99%
    f. I don't know
37. While pregnant with this child, what percentage of your diet consisted of home-cooked meals?* *Mark only one oval.*
    a. 0%
    b. 1-25%
    c. 26-50%
    d. 51-75%
    e. 76-99%
    f. I don't know
38. While pregnant with this child, what percentage of your diet consisted of restaurant meals?* *Mark only one oval.*
    a. 0%
    b. 1-25%
    c. 26-50%
    d. 51-75%
    e. 76-99%
    f. I don't know
39. While pregnant with this child, what percentage of your diet consisted of pre-cooked, microwave or TV dinners?* *Mark only one oval.*
    a. 0%
    b. 1-25%
    c. 26-50%
    d. 51-75%
    e. 76-99%
    f. I don't know
40. While pregnant with this child, did you experience any change in your diet?* *Mark only one oval.*
    a. No
    b. Yes, home-cooked meals to restaurant meals

c. Yes, restaurant meals to pre-cooked, microwave or TV dinners
d. Yes, home-cooked meals to pre-cooked, microwave or TV dinners
e. Yes, restaurant meals to home-cooked meals
f. Yes, pre-cooked, microwave or TV dinners to restaurant meals
g. Yes, pre-cooked, microwave or TV dinners to home-cooked meals
h. Other:

41. While pregnant with this child, what was your estimated exercise in minutes per day?* *Mark only one oval.*
    a. less than 20 minutes/day
    b. 21-30 minutes/day
    c. 31-45 minutes/day
    d. None

Pregnancy Health & History

Please answer the following questions about your child. Mothers of multiple children may submit more than one survey.

42. How many times have you given birth in your lifetime?* *Mark only one oval.*
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5+
43. How many times have you given birth in your lifetime?* *Mark only one oval.*
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5+
44. How many times have you been pregnant in your lifetime?* *Mark only one oval.*
    a. 1
    b. 2
    c. 3
    d. 4
    e. 5+
45. How many births prior to this child?*
46. How many births after this child?*
47. Was this pregnancy planned?* *Mark only one oval.*
    a. Yes
    b. No
48. How old were you when your child was born?*

49. Did you receive routine medical prenatal care?* *Mark only one oval.*
    a. Yes
    b. No
50. What type of setting did you receive prenatal care? *Mark only one oval.*
    a. Doctor's office
    b. Planned Parenthood
    c. Community Health Center
    d. Immediate Care
    e. Other:
51. Please specify any medications used during pregnancy and the reason used:
52. How many weeks did your pregnancy last? Typically, pregnancy lasts 40 weeks.*
53. What was your child's weight at birth (ex, 5lbs 6oz)? *
54. Please check the conditions below that describe the health of the child and mother during pregnancy...* *Check all that apply.*
    a. No complications
    b. Blackouts
    c. Falls
    d. Physical injury
    e. Excessive bleeding
    f. Emotional stress
    g. Alcohol and/or drug use
    h. Use of tobacco
    i. Marijuana use
55. Please check the conditions below that describe the health of the child and mother during your child's delivery...* *Check all that apply.*
    a. Vaginal birth
    b. C-section
    c. Breech birth
    d. Unusually long labor (>12 hours)
    e. Premature
    f. Overdue
    g. Lack of oxygen
    h. Breathing problem
    i. Birth injury/defect
    j. Jaundice
    k. Newborn ICU
    l. Other:
56. How much weight did you gain during pregnancy with this child, if any?

Child's Health & ASD

Please answer these questions based on your child. Mothers of multiple children may submit more than one survey.

57. What is your child's date of birth?* *Example: January 7, 2019*
58. Does your child have a confirmed ASD diagnosis by a physician?* *Mark only one oval.*
    a. Yes
    b. No
59. What is your child's gender?* *Mark only one oval.*
    a. Male
    b. Female
    c. Prefer not to say
60. If yes, how old was your child when he or she was diagnosed with ASD?
61. If yes, how old was your child when you first noticed behavioral differences?
62. How did you hear about the survey? *Mark only one oval.*
    a. Google Ad
    b. Facebook Post/Ad
    c. VT Center for Autism Research
    d. Instagram Post
    e. Other: