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Autism-specific maternal anti-fetal brain autoantibodies are associated with metabolic conditions

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Abstract

Lay Abstract—Approximately 23% of mothers of children with autism spectrum disorder (ASD) produce specific patterns of antibodies to fetal brain tissue that have been detected in only 1% of mothers of typically developing children. However, it is unknown what causes these ASD-specific anti-fetal antibodies to be produced. We examined the relationship between ASD-specific anti-fetal antibodies and metabolic conditions during pregnancy in 227 mothers of 2–5 year old children with ASD, enrolled in the CHARGE (Childhood Autism Risk from Genetics and the Environment) Study, and who had blood samples measured for these anti-fetal brain antibodies after study enrollment. Metabolic conditions included diabetes, hypertensive disorders, and prepregnancy obesity or overweight. The presence of ASD-specific anti-fetal brain antibody patterns was more common among mothers diagnosed with diabetes, hypertensive disorders, or overweight during pregnancy compared to healthy mothers, but these differences did not reach statistical significance. In a subset of 145 mothers whose children exhibited severe ASD symptoms, those diagnosed with type 2 or gestational diabetes were nearly 3 times more likely to have ASD-specific anti-fetal antibodies compared to healthy mothers. Further, those diagnosed with gestational diabetes specifically were over 3 times more likely to have these anti-fetal brain antibodies. In this exploratory study, mothers whose children had severe ASD and who were diagnosed with diabetes were more likely to have anti-fetal brain autoantibodies 2–5 years later.

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Conflict of Interest Disclosures: Dr Van de Water has a published patent on the protein targets of the maternal autoantibodies described in this manuscript and is a consultant for Pediatric Bioscience, a company that has licensed this technology from UC Davis. None of these activities are directly related to the hypotheses explored in this manuscript. No other disclosures pertaining to this manuscript were reported.

Scientific Abstract—Approximately 23% of mothers of children with autism spectrum disorder (ASD) produce specific patterns of autoantibodies to fetal brain proteins that have been detected in only 1% of mothers of typically developing children. The biological mechanisms underlying the development of ASD-specific maternal autoantibodies are poorly understood. We sought to determine whether ASD-specific maternal autoantibodies identified postnatally were associated with metabolic conditions (MCs) during gestation. Participants were 227 mothers of 2–5 year old children with confirmed ASD, enrolled in CHARGE (Childhood Autism Risk from Genetics and the Environment) between January 2003 and April 2008, and from whom blood samples were collected and analyzed for anti-fetal brain autoantibodies (Ab+). MCs included diabetes, hypertensive disorders, and prepregnancy obesity or overweight, ascertained from medical records or structured telephone interviews. Log-linear regression models were performed to estimate prevalence ratios (PR) and 95% confidence intervals (CI) based on robust standard errors. Fifty-six (25%) mothers were Ab+. Ab+ prevalence was higher among mothers with diabetes, hypertensive disorders, or overweight compared to healthy mothers, but differences were not statistically significant. In a subset of 145 mothers whose children exhibited severe ASD (31 Ab+), those diagnosed with type 2 or gestational diabetes were 2.7-fold more likely to be Ab+ (95% CI 1.1, 6.6), controlling for delivery payer and smoking. Gestational diabetes specifically was associated with a 3.2-fold increased Ab+ prevalence (95% CI 1.2, 8.6). In this exploratory study, mothers whose children had severe ASD and who experienced diabetes were more likely to have anti-fetal brain autoantibodies 2–5 years later.

Keywords

autism; pregnancy; maternal autoantibodies; anti-fetal brain autoantibodies; metabolic conditions; diabetes

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition that manifests in early childhood and is characterized by impairments in social communication and presence of stereotyped behaviors and restricted interests, with varying degrees of symptom severity and presentation.(American Psychiatric Association, 2013) Approximately 1.5% of U.S. children are affected by ASD.(Centers for Disease Control and Prevention (CDC) & Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators, 2014) Multiple etiologies are likely involved in the pathogenesis of ASD. Maternal immune factors that can cross the placenta have been implicated for a subset of ASD.

During pregnancy, the fetus relies on maternal immunoglobulin G (IgG) antibodies for protection from pathogens. At approximately 13 weeks gestation through term, maternally derived IgG antibodies are transported across the placenta and can persist for up to 6 months postnatally although at lower levels than found at birth. Maternal antibodies are also transferred to the infant through breast milk in the form of IgA.(Palmeira, Quinello, Silveira-Lessa, Zago, & Carneiro-Sampaio, 2012) Although most maternal IgG antibodies are considered beneficial in that they have the capacity to confer passive immunity, others appear to be pathogenic with the potential to trigger compromise in immune and neurodevelopment.(Braunschweig & Van de Water, 2012; Chang, 2012) The blood-brain

barrier is permissive during fetal development, and maternal antibodies are allowed greater access to the brain; consequently, pathogenic antibodies targeting particular fetal brain proteins may alter their function.(Diamond, Huerta, Mina-Osorio, Kowal, & Volpe, 2009; Fox, Amaral, & Van de Water, 2012)

Several groups have identified independently that some mothers of children with ASD produce antibodies reactive to fetal brain tissue.(Braunschweig et al., 2008; Braunschweig et al., 2012; Brimberg, Sadiq, Gregersen, & Diamond, 2013; Dalton et al., 2003; Singer et al., 2008; Zimmerman et al., 2007) Originally, Braunschweig et al(Braunschweig et al., 2008; Braunschweig et al., 2012) observed specific band patterns of IgG reactivity to fetal brain proteins at 37 and 73 kDa in a subset of mothers of children with ASD but not among mothers of typically developing or developmentally delayed children in the current population-based case-control study. These findings have been replicated in other populations, including a nested case-control study that used prospectively collected mid-gestation blood, further implicating transplacental passage of maternal anti-fetal brain autoantibodies in ASD pathogenesis.(Croen et al., 2008; Piras et al., 2014; Rossi, Van de Water, Rogers, & Amaral, 2011)

Recently, Van de Water and colleagues identified the target antigens of these ASD-specific maternal autoantibodies using more precise laboratory methods than those implemented previously: lactate dehydrogenase A and B (LDH), cypin (guanine deaminase), stress-induced phosphoprotein 1 (STIP1), collapsin response mediator proteins 1 and 2 (CRMP1, CRMP2) and Y-box-binding protein 1 (YBX1).(Braunschweig et al., 2013) These proteins are highly expressed in the brain and perform critical functions in neurodevelopment. Using these newly identified proteins, it was found that approximately 23% of mothers of children with ASD had specific combinations of antibody reactivity to these proteins, while these combinations were detected in 2 of 149 mothers of typically developing children; these findings suggested a high specificity for ASD relative to typical development although associations with other neurodevelopmental disabilities were not examined. Furthermore, the original 37/73 kDa banding patterns were associated with more severe ASD symptoms and greater impairments in expressive language.(Braunschweig et al., 2012; Piras et al., 2014) More recently, autoantibodies to LDH, CRMP1 and STIP1 were highly associated with increased stereotypic behavior, a core feature of ASD.(Braunschweig et al., 2013) Although the biological mechanisms underlying the development of anti-fetal brain autoantibodies remain unknown, some have postulated that systemic immune dysregulation, arising from environmental or genetic factors, may lead to a breakdown of maternal immune tolerance and subsequently the production of these autoantibodies.(Braunschweig & Van de Water, 2012; Fox et al., 2012; Heuer, Braunschweig, Ashwood, Van de Water, & Campbell, 2011) We have previously shown a strong correlation between the c-MET ‘C’ allele polymorphism and autoantibodies to the 37/73 kDa bands in fetal brain; the MET signaling pathway is involved in regulating immune response and maintaining tolerance to both self-proteins and the developing fetus.(Heuer et al., 2011)

Mothers of children with ASD are also disproportionately afflicted with metabolic conditions (MCs) during pregnancy, such as obesity, gestational diabetes and hypertensive disorders, compared to mothers of typically developing children.(Connolly et al., 2016;

Krakowiak et al., 2012; Li et al., 2016; Walker et al., 2015; Xiang et al., 2015; Xu, Jing, Bowers, Liu, & Bao, 2014) These conditions are characterized by persistent low-grade inflammation and insulin resistance.(Chaiworapongsa, Chaemsaithong, Yeo, & Romero, 2014; Korkmazer & Solak, 2015; Olefsky & Glass, 2010; Pantham, Aye, & Powell, 2015) Moreover, metabolic function and immune tolerance are controlled by common signaling pathways, suggesting that sufficient disruption in one system may lead to a dysfunction in the other.(Matarese, Procaccini, & De Rosa, 2012; Procaccini et al., 2013) We therefore hypothesized that some mothers of children with ASD and pregnancies complicated by MCs would be susceptible to producing autoantibodies with reactivity to fetal brain proteins, as these antigens are detectable in maternal circulation. Among mothers of children with ASD, we sought to determine whether postnatally detected ASD-specific maternal autoantibodies to proteins highly expressed in fetal brain were more prevalent in women who experienced specific MCs during pregnancy, and whether the relationship between MCs and these autoantibodies differed across strata of ASD symptom severity.

METHODS

Study population

This study included 227 mothers of children with ASD enrolled in the Childhood Autism Risks from Genetics and the Environment (CHARGE) Study between January 29, 2003 and April 4, 2008. This subset consisted of all mothers of children with ASD who had measurements of anti-fetal brain autoantibodies (n=246) and complete data on MCs (n=227). CHARGE is an ongoing population-based case-control study investigating a broad range of risk factors for ASD and other neurodevelopmental disabilities, with participants selected from 3 groups: ASD, developmental delay, and typical development from the general population.(Hertz-Picciotto et al., 2006) Eligible children were 2–5 years old, born in California, living with a biological parent who spoke English or Spanish, and resided in the catchment areas of selected regional centers in California. Children were identified as described previously.(Braunschweig et al., 2013; Hertz-Picciotto et al., 2006; Krakowiak et al., 2012) The CHARGE Study protocol was approved by the institutional review boards of the University of California in Davis and Los Angeles and the State of California Committee for the Protection of Human Subjects. Families provided written informed consent before participating.

Diagnosis

Participants completed a series of standardized assessments administered by research-reliable clinicians at the UC Davis Medical Investigations of Neurodevelopmental Disorders (MIND) Institute to confirm diagnoses. ASD diagnosis was verified with gold standard instruments, the Autism Diagnostic Observation Schedule (ADOS)(Lord, Rutter, DiLavore, & Risi, 2000; Lord et al., 2012) and Autism Diagnostic Interview – Revised (ADI-R)(Le Couteur, Lord, & Rutter, 2003), using criteria described by Risi *et al*(Risi et al., 2006) and in accordance with the *Diagnostic and Statistical Manual of Mental Disorders–5* (DSM-5). (American Psychiatric Association, 2013) ADOS comparison scores(Gotham, Pickles, & Lord, 2009; Lord et al., 2012) (range 1–10) were used to determine ASD intensity, with scores ≥7 indicating severe symptoms.

Exposures

We used medical records or maternal report from structured telephone interviews (with abstractors and interviewers masked to the child's case status) to establish whether mothers experienced any of the following MCs during their index pregnancy: diabetes (type 2 [n=2], gestational [n=17]), hypertensive disorders without diabetes (chronic [n=4], gestational/ preeclampsia [n=18]), and prepregnancy obesity (body mass index [BMI] ≥30 [n=36]) or overweight (moderate [BMI 27–29.9] [n=22], slight [BMI 25–26.9] [n=27]) without other MCs; the referent group consisted of women with no MCs and with a healthy weight (BMI <25 [n=101]; 6 were underweight [BMI <18.5]). MCs were grouped into hierarchical mutually exclusive categories based on a presumed level of metabolic and immune disruption that was expected to be the highest among those with diabetes (the breakdown of metabolic condition categories is presented in eTable 2 in the Supplement). We obtained medical records for >78% of mothers (78% with prenatal and 85% with delivery records); 60% had prepregnancy weights recorded in medical charts. Maternal report was used when medical records were unavailable. Medical record extraction and validation of maternal report are described elsewhere.(Krakowiak, Walker, Tancredi, & Hertz-Picciotto, 2015)

Outcome

Anti-fetal brain autoantibodies were measured in maternal blood samples collected at the CHARGE clinic visit when the child was 2–5 years old. Target antigens were identified using laboratory methods described elsewhere.(Braunschweig et al., 2013) We defined mothers as having ASD-specific autoantibodies (Ab+) if they had one of a set of antigen reactivity patterns that was collectively 99% specific to ASD (eTable 1 in the Supplement, includes statistical methods).

Statistical analysis

Covariates included maternal age at delivery, self-identified race/ethnicity, birth place, education, delivery payer, parity, gestational age (GA), inter-pregnancy interval (IPI), smoking and alcohol consumption during the 3 months before conception and pregnancy. We assessed associations between the outcome (ASD-specific autoantibodies) and our predictor (MCs), as well as with each covariate. Variables broadly associated (P <0.20) with the outcome were included in initial regression models; a change of ≥10% in the β-coefficient of the MCs determined which variables remained in the final models. Log-linear models with robust standard errors(Zou, 2004) were carried out in SAS 9.4 (SAS Institute, Cary NC) to estimate prevalence ratios (PR) and 95% confidence intervals (CI) on the entire study sample (N=227) and subset of mothers of children with severe ASD (n=145). Final models were adjusted for maternal smoking and delivery payer. Figures were created in GraphPad Prism 5.0 (GraphPad Software, La Jolla CA).

Sensitivity analyses

To examine associations between MCs irrespective of co-existing conditions and Ab+ status, we repeated our main analysis using non-mutually exclusive indicators for each condition. Specifically, each MC was modeled separately with no MCs and a healthy weight (BMI <25) as the referent category for all comparisons. For each condition, the predictor variable

consisted of 3 nominal categories: (1) has the MC of interest, (2) has another MC or is overweight, or (3) has a healthy weight with no MCs. We also conducted an analysis using 100% ASD-specific antigen combinations to confirm main analysis findings.

RESULTS

Fifty-six (25%) of 227 mothers were Ab+. Mothers with and without these autoantibodies did not differ with respect to age, education, GA, parity, or IPI; Ab+ mothers were more likely to be Hispanic or multiracial, born outside of U.S., smoke, and use public assistance for delivery (Table 1; 'Severe ASD' subset in eTable 3 in the Supplement).

ASD-specific autoantibodies were more prevalent among women with diabetes, hypertensive disorders, and moderate overweight than healthy women (Figure 1; eTable 4); nevertheless, these differences were not statistically significant (Table 2). Restricting analyses to mothers of children with severe ASD yielded significant associations between diabetes and Ab+ status (Table 2); women diagnosed with diabetes (type 2 or gestational) were 2.7 times more likely to be Ab+ than healthy women, in models adjusted for delivery payer and smoking (PR=2.66, 95% CI 1.07, 6.63). Moderate overweight without co-occurring MCs was marginally associated with Ab+ (PR=2.55, 95% CI 1.00, 6.49), but surprisingly, obesity was not. Excluding women with chronic conditions (type 2 diabetes [n=2] or hypertension without diabetes [n=4]) strengthened the association between gestational diabetes and Ab+ status in the severe ASD subset (PR=3.18, 95% CI 1.17, 8.64).

Sensitivity analyses

As expected, our findings using non-mutually exclusive indicators for individual MCs were similar to those in the main analysis (eTables 5, 6); additionally, preeclampsia was associated with Ab+ status (PR=2.83, 95% CI 1.12, 7.18). Interestingly, all women diagnosed with both gestational diabetes and preeclampsia (n=3) were Ab+ in contrast to ≤25% of those with one condition (2 of 9 with diabetes; 3 of 12 with preeclampsia); by comparison, only 7 (12%) of 58 healthy mothers were Ab+. Using 100% ASD-specific antigen combinations yielded similar results as the main analysis; in the severe ASD subset, associations between Ab+ status and diabetes and also moderate overweight were slightly stronger (eTables 7, 8).

DISCUSSION

Our study revealed that ASD-specific maternal autoantibodies were more prevalent among mothers diagnosed with diabetes, hypertensive disorders, or who were moderately overweight compared to healthy mothers. Among mothers of children exhibiting severe ASD symptoms, we observed a 3-fold higher prevalence of Ab+ in relation to gestational diabetes. Our findings suggest that some MCs may contribute to a breakdown of maternal immune tolerance and subsequent production of autoantibodies to fetal brain, although prospective and mechanistic studies are needed to establish a causal relationship. This is the first study to investigate MCs in relation to maternal anti-fetal brain autoantibodies.

Inflammation and MCs in gestation

It is well established that MCs (obesity; hypertension, preeclampsia; type 2, gestational diabetes) are characterized by decreased sensitivity to insulin signaling and persistent low-grade inflammation.(Chaiworapongsa et al., 2014; Olefsky & Glass, 2010; Pantham et al., 2015) Moreover, elevated BMI is a recognized risk factor for diabetes and hypertensive disorders. Adipose tissue consists of many cell types including immune cells (macrophages, B and T lymphocytes), and it is therefore an abundant source of signaling proteins with metabolic- and immune-modulating functions (adipocytokines).(Makki, Froguel, & Wolowczuk, 2013; Olefsky & Glass, 2010; Pantham et al., 2015) In a state of metabolically-induced inflammation, immune cell profiles shift favoring a proinflammatory environment, chronically maintained through an autocrine and paracrine network of signaling molecules, in tissues responsible for regulating energy metabolism including adipose, liver, and pancreas; pregnancy complications, such as gestational diabetes, ensue when compensatory mechanisms break down. Furthermore, metabolic and immune derangements evident in diabetic and hypertensive pregnancies may alter placental function by stimulating production of inflammatory immune factors and inducing oxidative stress.

The placenta is a complex organ with metabolic and immune regulatory functions that acts as an interface between maternal and fetal circulations. It is composed of both fetally- and maternally-derived cells and can be separated into three regions.(Hsiao & Patterson, 2012; Racicot, Kwon, Aldo, Silasi, & Mor, 2014) The maternal decidua forms the peripheral layer in contact with the uterine wall and is densely populated with immune cells (natural killer cells, macrophages, T lymphocytes); beneath is a layer of fetally-derived trophoblast cells that synthesize hormones and endocrine factors to support the pregnancy; finally, the chorionic villi form intervillous spaces where regulated maternal-fetal blood exchange takes place, including transfer of nutrients, waste products, gases, and IgG antibodies. Consequently, the placenta is influenced by the metabolic and immune milieu present in both the maternal and fetal compartments.

The physiological changes of pregnancy, facilitated by placentally-derived hormones, adipocytokines, and other signaling molecules, naturally lead to an insulin resistant state in the 2nd trimester that persists through term to promote healthy fetal growth and to increase maternal adipose tissue in preparation for lactation.(Barbour et al., 2007; Gabbe et al., 2012) While most women maintain normoglycemia through increased insulin secretion, gestational diabetes develops when the glucose homeostasis is disrupted resulting in maternal and fetal hyperglycemia. Poorly controlled hyperglycemia stimulates inflammatory responses in the adipose tissue and placental immune cells, which can have profound effects on placental vasculature and function, thus leading to reduced uteroplacental or fetoplacental blood flow (i.e., placental dysfunction).(Lin et al., 2005; Magee et al., 2014; Pantham et al., 2015; Radaelli, Varastehpour, Catalano, & Hauguel-de Mouzon, 2003; Sisino et al., 2013) Indeed, women with dysregulated metabolic function are also at an increased risk for preeclampsia, a gestational hypertensive disorder thought to arise from abnormal placentation, possibly due to insufficient immune tolerance.(Chaiworapongsa et al., 2014; Hsiao & Patterson, 2012)

Although trophoblast cells express paternal alloantigens, several tolerogenic mechanisms are in place to prevent an anti-fetal immune response and to maintain an anti-inflammatory environment including: (1) trophoblast secretion of factors and expression of surface ligands to control immune reactivity, (2) fetal cell shedding into maternal circulation to promote tolerance, and (3) trophoblast evasion of maternal immune recognition through a limited expression of specific cell surface molecules that interact with immune cells.(Hsiao & Patterson, 2012) Nevertheless, maternal immune and/or metabolic dysfunction may alter the balance of immune and endocrine factors at the placental level, leading to a breach in immune tolerance and possibly the development of anti-fetal brain autoantibodies.

Autoantibodies to fetal brain proteins

Several potential mechanisms have been proposed to explain maternal production of anti-fetal brain autoantibodies including: (1) immune response to a molecular mimic during an infection, (2) alloimmunization to fetal proteins in an earlier pregnancy, and (3) genetic susceptibility that impairs maternal tolerogenic mechanisms.(Braunschweig & Van de Water, 2012) Both ASD and maternal anti-fetal brain autoantibodies have been independently associated with a functional polymorphism in the *MET* gene that leads to a decreased expression of MET receptor tyrosine kinase, suggesting a genetic susceptibility for immune dysregulation.(Campbell, Li, Sutcliffe, Persico, & Levitt, 2008; Heuer et al., 2011; Jackson et al., 2009) Interestingly, impairments in MET signaling have also been linked to type 2 and gestational diabetes in several animal studies.(Araujo et al., 2012; Demirci et al., 2012; Fafalios et al., 2011; Mellado-Gil et al., 2011) We are currently investigating whether specific MCs are associated with the c-MET ‘C’ allele polymorphism in our study population.

MCs and anti-fetal brain autoantibodies

Our findings revealed an association between MCs, particularly gestational diabetes, and having anti-fetal brain autoantibodies. Furthermore, the magnitude of these associations increased in our subset of mothers whose child exhibited severe ASD symptoms and when we used 100% ASD-specific autoantibody combinations. Anti-fetal brain autoantibodies have been linked to more intense stereotyped behaviors and greater impairments in expressive language in children with ASD.(Braunschweig et al., 2012; Braunschweig et al., 2013; Piras et al., 2014) Additionally, we have previously observed increased expressive language deficits among children with ASD whose mothers had gestational diabetes. (Krakowiak et al., 2012) Thus, our results extend previous findings in a novel direction.

Moderate overweight (without co-occurring MCs) was also associated with Ab+ status, but to our surprise, obesity was not. There are several possible explanations. First, obese mothers may have received different prenatal care interventions than overweight women (e.g., closer monitoring of weight gain, dietary changes). Second, moderately overweight women may have had different exposure profiles of factors or susceptibilities associated with both BMI and maternal autoantibodies. We noted that obese women were more likely to be of Hispanic ethnicity or non-White race and less likely to have a Bachelor degree or IPI <18 months than moderately overweight mothers. Third, obese women may have been misclassified as moderately overweight, diluting the association between obesity and

maternal autoantibodies; however, misclassification alone is unlikely to explain a complete lack of an association. Without examining metabolic or immune biomarkers or obtaining more accurate BMI assessments, it is difficult to determine exactly how these two BMI groups differed; hence, our findings should be interpreted with caution.

Limitations and strengths

Our study had several limitations. First, we had a small sample size of mothers with ASD-specific autoantibodies and were unable to examine the relationship between Ab+ status and combinations of MCs or conditions that were rare (e.g., type 2 diabetes). Our sample size was further reduced when we restricted to mothers of children with severe ASD. Thus, we conducted robust regression analyses (i.e., Poisson regression with robust error variance) to improve the precision of our prevalence ratio estimates and overcome the limitations that burden traditional parametric regression models when applied to small sample sizes (e.g., sensitivity to outliers). Nonetheless, the possibility of these being chance findings cannot be decisively eliminated. Second, maternal samples were collected after the pregnancy of interest; consequently, a clear temporal relationship between MCs and anti-fetal brain autoantibodies could not be established. Third, we used self-reported MCs for a subset of mothers that could have introduced exposure misclassification. However, we relied on medical records to determine diagnosis of MCs for >78% of mothers and structured telephone interviews for maternal report of MCs; medical record abstractors and interviewers were masked to the child's case status to minimize interviewer bias. We have previously shown that maternal report of MCs is highly valid, particularly for diabetes. (Krakowiak et al., 2015) Still, we could not account for factors that might have influenced the physiologic manifestation of these conditions, such as lifestyle (e.g., physical activity, diet) and dietary or pharmaceutical interventions. Each condition represents a broad range of metabolic function uniquely influenced by genetic predisposition as well as lifestyle and pharmaceutical interventions. Therefore, biological markers of metabolic dysregulation (e.g., blood levels of insulin or glucose) are needed to characterize the pathological features of these conditions more accurately. Finally, unmeasured upstream genetic or environmental factors associated with both MCs and ASD-specific autoantibodies may have confounded our findings. Given these weaknesses, our preliminary findings need to be interpreted with caution.

This study also had noteworthy strengths. ASD diagnosis was confirmed with gold standard instruments administered by research-reliable clinicians, thereby minimizing diagnostic misclassification. Maternal anti-fetal brain autoantibodies were validated with multiple laboratory methods and the patterns of reactivity have been replicated in other populations. (Croen et al., 2008; Piras et al., 2014; Rossi et al., 2011)

In this exploratory study, we demonstrate associations between MCs, particularly gestational diabetes, and having ASD-specific maternal autoantibodies to fetal brain. However, the temporal relationship remains unclear between these conditions and maternal production of anti-fetal brain autoantibodies. Other limitations include having no biological markers of metabolic dysregulation and instead relying on the diagnosis of metabolic conditions in

medical records. Prospective and mechanistic studies are needed to elucidate these associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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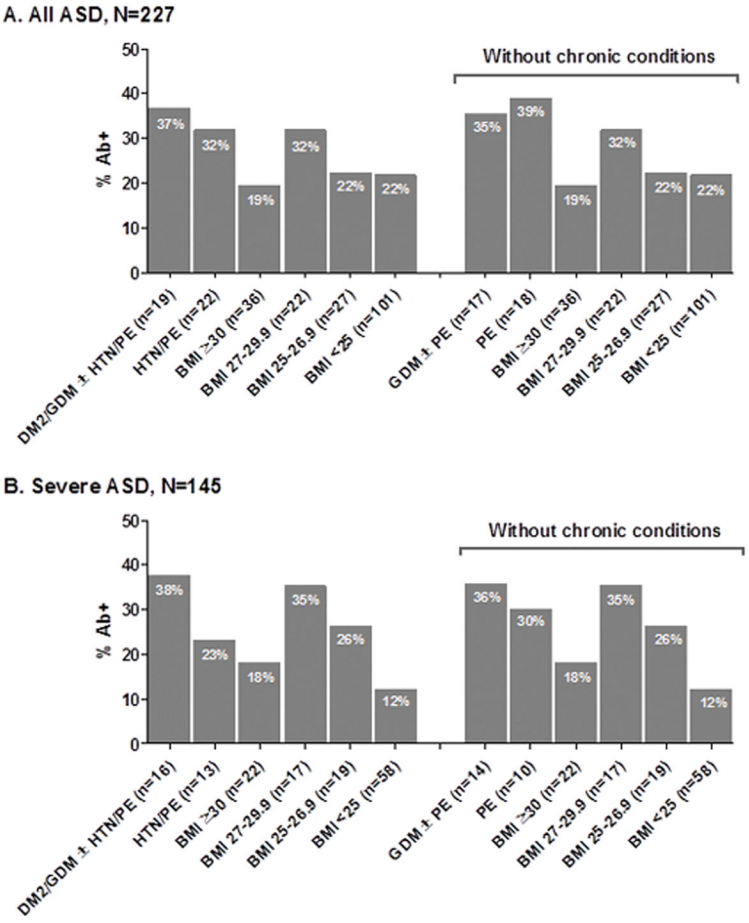

Figure 1. Prevalence of 99% ASD-specific maternal autoantibodies by metabolic condition status in (A) all mothers and (B) the severe ASD subset

Metabolic condition categories are mutually exclusive. Subset 'Without chronic conditions' excludes women diagnosed with type 2 diabetes (n=2) and chronic hypertension (n=4). *Abbreviations:* Ab+ = ASD-specific maternal autoantibodies present; DM2 = Type 2 diabetes, GDM = gestational diabetes, HTN = chronic hypertension, PE = preeclampsia / gestational hypertension, HTN/PE = hypertensive disorders, BMI 30.0 = obese, BMI 27.0–29.9 = moderately overweight, BMI ≥25.0–26.9 = slightly overweight, BMI <25 = healthy weight ('All ASD' sample includes 6 Ab− women with BMI <18.5 [underweight]; 'Severe ASD' subset includes 5 Ab− women with BMI <18.5)

Table 1

Maternal characteristics by 99% ASD-specific autoantibody status[a], N=227

	Ab+ (n=56)	Ab− (n=171)	
	No. (%)	**No. (%)**	**P-value**
Birthplace			0.23
United States	38 (67.9)	130 (76.0)	
Other	18 (32.1)	41 (24.0)	
Race/Ethnicity			0.27
Hispanic	18 (32.1)	44 (25.7)	
Non-Hispanic White	28 (50.0)	106 (62.0)	
Non-Hispanic other[b]	10 (17.9)	21 (12.3)	
Educational attainment			0.95
≤High school	8 (14.3)	21 (12.3)	
Some college	24 (42.9)	79 (46.2)	
Bachelor's degree	16 (28.6)	45 (26.3)	
Graduate degree	8 (14.3)	26 (15.2)	
Delivery payer			0.06
Public	14 (25.0)	24 (14.0)	
Private	42 (75.0)	147 (86.0)	
Parity			0.86
Primipara	26 (46.4)	77 (45.0)	
Multipara	30 (53.6)	94 (55.0)	
Interpregnancy interval			0.90
<18 mo	12 (21.4)	38 (22.2)	
≥18 mo[c]	44 (78.6)	133 (77.8)	
Smoking			0.06
Periconception only[d]	6 (10.7)	4 (2.3)	
Pregnancy	5 (8.9)	12 (7.0)	
No	44 (78.6)	152 (88.9)	
Unknown	1 (1.8)	3 (1.8)	
Alcohol consumption			0.79
Periconception only[d]	10 (17.9)	38 (22.2)	
Pregnancy	14 (25.0)	41 (24.0)	
No	30 (53.6)	89 (52.1)	
Unknown	2 (3.6)	3 (1.8)	
	Mean (SD)	***Mean (SD)***	***P-value***
Age at delivery, y	30.9 (6.3)	31.1 (5.0)	0.80
Gestational age, wk	39.1 (2.0)	38.9 (2.1)	0.63

[a]Maternal autoantibody status was defined as positive (Ab+) or negative (Ab−) using antigen combinations shown to identify ASD cases with 99% specificity

[b] Other race categories include Black or African American, American Indian or Alaska Native, Asian, Pacific Islander or Hawaii Native, and Bi/Multiracial

[c] Interpregnancy interval (IPI) ≥18 months includes primiparas

[d] Periconception includes the 3 month period immediately prior to pregnancy

Table 2

Prevalence ratios relating metabolic conditions with 99% ASD-specific autoantibodies[a] in all mothers and the severe ASD subset

	All ASD (N=227)				Severe ASD (N=145)			
	Crude		Adjusted		Crude		Adjusted	
Metabolic conditions[b]	**PR**	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**	**PR**	**95% CI**
DM2/GDM ± HTN/PE, any BMI	1.67	0.84, 3.35	1.61	0.83, 3.12	**3.05**	**1.19, 7.81**	**2.66**	**1.07, 6.63**
HTN/PE, any BMI, no other MCs	1.45	0.71, 2.95	1.46	0.71, 3.01	1.88	0.56, 6.31	1.59	0.49, 5.20
BMI ≥30, no other MCs	0.80	0.36, 1.81	0.73	0.33, 1.61	1.48	0.48, 4.56	1.36	0.46, 3.99
BMI 27–29.9, no MCs	1.52	0.75, 3.08	1.42	0.72, 2.80	**2.87**	**1.12, 7.40**	**2.55**	**1.00, 6.49**
BMI 25–26.9, no MCs	1.01	0.46, 2.24	1.15	0.51, 2.56	2.14	0.77, 5.96	2.57	0.91, 7.20
BMI <25, no MCs	1.00	--	1.00	--	1.00	--	1.00	--
Without chronic conditions[c]								
GDM ± PE, any BMI	1.60	0.76, 3.37	1.71	0.82, 3.56	**2.95**	**1.10, 7.95**	**3.18**	**1.17, 8.64**
PE, any BMI, no other MCs	1.77	0.89, 3.51	1.80	0.88, 3.69	2.49	0.77, 8.04	2.22	0.66, 7.50
BMI ≥30, no other MCs	0.80	0.36, 1.81	0.73	0.33, 1.61	1.51	0.49, 4.65	1.43	0.47, 4.29
BMI 27–29.9, no MCs	1.52	0.75, 3.08	1.41	0.72, 2.77	**2.92**	**1.13, 7.54**	**2.66**	**1.07, 6.59**
BMI 25–26.9, no MCs	1.01	0.46, 2.24	1.14	0.51, 2.54	2.18	0.78, 6.07	2.49	0.90, 6.89
BMI 18.5–24.9, no MCs	1.00	--	1.00	--	1.00	--	1.00	--

[a] Maternal autoantibody status was defined as positive (Ab+) or negative (Ab−) using antigen combinations shown to identify ASD cases with 99% specificity; prevalence ratios (PR) and 95% confidence intervals (CI) were estimated by log-linear regression models with robust standard errors adjusted for delivery payer (public vs. private) and maternal smoking during periconception or pregnancy (smoking vs. no/unknown)

[b] Metabolic condition (MC) categories are hierarchical mutually exclusive that were based on the presumed level of metabolic disruption, with metabolic disruption expected to be highest in women with type 2 diabetes; mothers with no MCs and with a healthy weight (BMI <25) were the referent for all comparisons; abbreviations: DM2/GDM = Type 2 or Gestational diabetes, HTN/PE = Hypertensive disorders (chronic hypertension, preeclampsia or gestational hypertension), BMI ≥30.0 = obese, BMI 27.0–29.9 = moderately overweight, BMI 25.0–26.9 = slightly overweight, BMI <25 = healthy weight (6 mothers were underweight [BMI <18.5] and all were Ab−)

[c] Excluded 6 women with type 2 diabetes (n=2) or chronic hypertension (n=4) from the 'All ASD' sample; 5 women with type 2 diabetes (n=2) or chronic hypertension (n=3) were excluded from the 'Severe ASD' subset