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Associations between maternal thyroid dysfunction, estimated iodine intake and child autism spectrum disorder and other neurodevelopmental outcomes in U.S. pregnancy cohorts

By

ZOE BAYSDORFER KAPLAN

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

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in the

OFFICE OF GRADUATE STUDIES

of the

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DAVIS

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2025

Abstract

Introduction: The etiology of autism spectrum disorder (ASD) is unknown, though both environmental and genetic factors likely play a role in its development. Research suggests that maternal exposure to thyroid disrupting compounds during pregnancy could increase the likelihood of ASD in the child. Additionally, changes in dietary patterns among women of reproductive age may contribute to a decreased consumption of iodine, a nutrient necessary for thyroid hormone production. Severe maternal iodine deficiency and resulting hypothyroidism are well-documented predictors of neurodevelopmental disability in children, but less is known about the relationship between maternal thyroid function and ASD development or mild to moderate maternal deficiency and ASD.

Objective: 1) Summarize literature on maternal thyroid dysfunction, iodine, and ASD outcomes (diagnosis and traits) and identify gaps 2) Characterize the relationship between maternal thyroid function during pregnancy and ASD outcomes 3) Characterize the relationship between maternal iodine status during pregnancy and ASD outcomes.

Methods: First, scientific publications on maternal thyroid dysfunction and child ASD outcomes in humans were reviewed (through 2023) and synthesized into narrative review. Then, two original research studies were conducted on different study populations. In the first study, maternal thyroid hormone concentrations (thyroid stimulating hormone [TSH] and free thyroxine [free T4]) were measured from pregnancy blood samples collected from participants in Markers of Autism in Babies: Learning Early Signs (MARBLES), a high likelihood autism cohort study. Statistical associations between maternal thyroid dysfunction (hypothyroid conditions or hyperthyroid conditions compared to euthyroid), thyroid hormones, and ASD diagnosis or non-typical development (non-TD) compared typical development (TD) were examined. Autism traits (social responsiveness scale standardized scores [SRS]) and child cognitive ability (Mullen Scales of Early Learning [MSEL]) were also examined as outcomes. The second study included participants from the Environmental Health Influences on Children's Health Outcomes (ECHO) Cohorts program. Individual maternal iodine intake per day was estimated using at least two pregnancy

urinary iodine concentrations per mother. Mothers were classified as deficient based on the National Academy of Sciences, Engineering, and Medicine Estimated Average Requirement (EAR) and upper tolerable limit for iodine for women who are pregnant. Continuous, quintile, and binary (deficient vs. adequate) iodine intake was examined in relation to continuous standardized SRS scores and binary SRS scores (possible ASD or no ASD indicated).

Results: The literature review identified multiple population-based studies that found positive associations between maternal hypothyroidism and child ASD. Relationships between hyperthyroidism or iodine status and ASD were less consistent. Misclassification bias for outcome and exposure may have occurred in studies that used medical records. In MARBLES (n=285 pregnancies from 273 mothers), 9.5% of pregnancies were affected by hypothyroid conditions and 6.7% were affected by hyperthyroid conditions. Maternal hypothyroid conditions during pregnancy were significantly associated with an increased chance of ASD compared to TD (AOR 3.17 95% CI 1.13, 8.93; p=0.03) and non-TD compared to TD (AOR 4.27, 95% CI 1.36, 13.38; p=0.01) after adjusting for confounders. Hyperthyroid conditions during pregnancy were not significantly associated with either outcome. First trimester natural log of TSH was significantly association with some percentiles of the SRS and MSEL. In ECHO, 42.5% of mothers were categorized as having deficient iodine intake with the remaining categorized as adequate intake (n=653 mother-child pairs). In adjusted quintile analysis, the 1st and 4th quintile were significantly associated with about a quarter of a standard deviation increase in SRS scores compared to the 3rd quintile (adjusted coefficient 1st quintile 2.70, 95% CI 0.56, 4.83; p=0.013; adjusted coefficient 4th quintile 2.65, 95% CI 0.52, 4.7; p=0.015). Similar results were found in the binary SRS outcome model for the 4th quintile only (adjusted coefficient 2.64, 95% CI 1.07, 6.55; p=0.04). Neither deficient iodine intake nor continuous iodine intake was significantly associated with binary or continuous SRS scores.

Discussion: This analysis of MARBLES data contributed to the literature by using a sample where maternal thyroid dysfunction was assessed based on laboratory analysis in all pregnancies and all children were assessed for ASD. To our knowledge, this analysis of ECHO participants was the first to examine

maternal iodine intake and ASD outcomes using a sample from the United States. Findings from MARBLES support previous findings that maternal hypothyroidism during pregnancy is associated with an increased likelihood of ASD diagnosis. Because an association was also found for non-typical development, maternal thyroid dysfunction may not be an etiological factor that is specific to ASD development. Findings from the ECHO align with previous research that also found minimal evidence of an association between maternal iodine status during pregnancy and child ASD traits. Both studies may have been limited by a small sample size, smaller number of first trimester pregnancies, and survival bias.

Conclusion: Studies that include multiple urine and blood samples per trimester per person and collect information on consumption of iodine rich foods and vitamins in relation to urine sample timing would be valuable in further exploring this relationship. Because iodine deficiency is suspected to contribute to ASD development by disrupting thyroid function, additional research is needed that examines thyroid function along with maternal iodine status and child ASD. Thyroid dysfunction (especially hypothyroidism) and iodine deficiency among pregnant women should continue to be monitored along with child developmental outcomes.

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I would like to thank my committee for investing their time and intellectual curiosity in to my research career. Thank you, Dr. Rebecca Schmidt for your mentorship throughout my PhD. I appreciate how you gave me the freedom to explore my interests and the independence to develop new skills while still providing guidance when I needed it. I feel very fortunate to have had you as my advisor. Thank you, Dr. Hyeong-Moo Shin for providing me with the opportunity to examine thyroid dysfunction in the MARBLES. Your reliability and clear feedback made the dissertation process easier. Thank you, Dr. Daniel Tancredi for sharing a small piece of your vast statical knowledge with me. Your quick responses and trouble-shooting ability were extremely helpful.

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Introduction

Pregnancy is a critical time for child neurodevelopment. During the prenatal period, the developing brain is exposed to a variety of environmental factors through the mother. This pregnancy "exposome" includes factors that are internal to the mother such as her metabolism, endocrine function or inflammation and external factors such as diet, medication use, or environmental pollutants.[1,2] Factors can be interrelated and environmental exposures can result in epigenetic changes.[1] Gaining a greater understanding of how environmental exposures shape neurodevelopment could enhance what is known about the etiology of autism spectrum disorder (ASD). Core features of ASD diagnosis include difficulty with social interactions, and at least two of the following: repetitive behaviors, rigid adherence to routines, limited interests, and sensory differences.[3] At the same time, ASD is a heterogeneous condition that encompasses individuals with a range of abilities and symptom severities. Though the cause of ASD is not known for most cases, it is believed to have genetic[4,5] epigenic,[6] and environmental underpinnings.[7]

This dissertation examines two related prenatal environmental exposures (maternal thyroid function and iodine intake) in relation to ASD outcomes (diagnosis and traits). Adequate iodine intake is necessary for thyroid hormone production and iodine deficiency is one cause of thyroid dysfunction.[8] The first chapter is a literature review that summarizes existing literature on maternal thyroid dysfunction, iodine deficiency, and ASD outcomes and identified areas of future research. The second and third chapter are original research studies that address some gaps in the literature as determined by the literature review. The second chapter examines the association between maternal thyroid function during pregnancy and ASD and other neurodevelopmental outcomes in participants from the Markers of Autism in Babies: Learning Early Signs (MARBLES) cohort study. The third chapter examines the relationship between maternal iodine status during pregnancy and ASD traits in participants from the Environmental Health Influences on Children's Health Outcomes (ECHO) Cohorts program.

Findings from this dissertation are applicable to other research questions and relevant to prenatal public health interventions. Environmental pollutants such as perchlorate and nitrate, bisphenol A, and PFAS may disrupt thyroid function and increase the chance of ASD in the child when the mother is exposed during pregnancy. [9–15] Characterizing the relationship between maternal thyroid dysfunction and ASD outcomes could shed light on the physiological mechanisms through which thyroid disrupting chemicals might increase the likelihood of ASD. Both maternal thyroid dysfunction and iodine deficiency can be addressed through public health actions if needed. Thyroid conditions can be screened for during pregnancy and can typically be treated when applicable.[16] Iodine deficiency can be prevented through prenatal supplements and increased awareness and access of iodine rich foods.[17] Thus, the findings of the proceeding studies can contribute to an existing body of knowledge that can be used to guide public health recommendations surrounding maternal thyroid dysfunction and iodine intake during pregnancy.

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REVIEWS and SCHOLARLY DIALOG

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Maternal Thyroid Dysfunction During Pregnancy as an Etiologic Factor in Autism Spectrum Disorder: Challenges and Opportunities for Research

Zoe B. Kaplan,[1] Elizabeth N. Pearce,[2] Sun Y. Lee,[2] Hyeong-Moo Shin,[3] and Rebecca J. Schmidt[1,4]

Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition with unknown etiology. Both genetic and environmental factors have been associated with ASD. Environmental exposures during the prenatal period may play an important role in ASD development. This narrative review critically examines the evidence for a relationship between maternal thyroid dysfunction during pregnancy and ASD in the child.
Summary: Studies that assessed the associations of hypothyroidism, hyperthyroidism, hypothyroxinemia, thyroid hormone concentrations, or autoimmune thyroid disease with ASD outcomes were included. Most research focused on the relationship between hypothyroidism and ASD. Multiple population-based studies found that maternal hypothyroidism was associated with higher likelihood of an ASD diagnosis in offspring. Associations with other forms of maternal thyroid dysfunction were less consistent. Findings may have been affected by misclassification bias, survival bias, or publication bias. Studies using medical records may have misclassified subclinical thyroid dysfunction as euthyroidism. Two studies that assessed children at early ages may have misclassified those with ASD as typically developing. Most studies adjusted for maternal body mass index (BMI) and/or mental illness, but not interpregnancy interval or pesticide exposure, all factors associated with fetal survival and ASD. Most studies reported a combination of null and statistically significant findings, although publication bias is still possible.
Conclusions: Overall, evidence supported a positive association between maternal thyroid dysfunction during pregnancy and ASD outcomes in the child, especially for hypothyroidism. Future studies could reduce misclassification bias by using laboratory measures instead of medical records to ascertain thyroid dysfunction and evaluating children for ASD at an age when it can be reliably detected. Survival bias could be further mitigated by adjusting models for more factors associated with fetal survival and ASD. Additional research is needed to comprehensively understand the roles of maternal levothyroxine treatment, iodine deficiency, or exposure to thyroid-disrupting compounds in the relationship between maternal thyroid dysfunction and child ASD outcomes.

Keywords: autism spectrum disorder, thyroid dysfunction, hypothyroidism, hyperthyroidism, prenatal exposure, delayed effects

Introduction

Prevalence and etiology of autism spectrum disorder

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by difficulties in social communication, such as challenges in understanding social cues or lack of interest in social behaviors, and the presence of restricted interests and/or repetitive behaviors.[1] These characteristics usually develop early in life and can be accompanied by differences in intellectual ability, sensory sensitivities, and other copresenting characteristics.[1] Studies suggest that ASD is sometimes associated with heightened

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attention to detail and strong abilities in pattern recognition and visual search tasks.[2–4] In 2020, the U.S. prevalence of ASD was estimated to be 21.5 per 1000 children aged 4 years.[5] Globally, prevalence estimates vary considerably both within and between regions.

Based on the data collected in the last decade, ASD prevalence is estimated to range from 0.24% to 2.68% in Europe, 0.08% to 1.40% in Southeast Asia, 0.01% to 2.50% in the Americas, and 0.01% to 4.36% in the Western Pacific region.[6] Regional differences in prevalence likely reflect differences in reporting and diagnostic practices and may not be indicative of differences in factors that increase the likelihood of ASD.[6] Currently, the gold standard assessments for diagnosing ASD are the Autism Diagnostic Observation Schedule (ADOS), a series of professional observational examinations,[7] and the Autism Diagnostic Interview-Revised (ADI-R),[8] a parental interview.[9]

The etiology of ASD is poorly understood, despite decades of investigation. Beginning in the 1940s, psychiatrist Leo Kanner popularized a theory that autism in children was the result of emotionally distant parenting.[10] Since the 1960s, this theory has been rejected owing to research supporting genetic and environmental origins.[11]

Most cases of ASD are hypothesized to stem from multiple genetic and/or environmental factors that may be interdependent (e.g., gene–environment interactions).[12] Genetic factors associated with ASD include both inherited and *de novo* variants. A meta-analysis of monozygotic and dizygotic twin studies estimated the heritability of ASD to be between 64% and 91%.[13,14] Common single-nucleotide polymorphisms are estimated to collectively contribute 12–52% to ASD development.[15] Novel epigenetic changes, alternations to genetic material that affect how a gene is expressed without changing the DNA sequence, have been found in the early brain development period among children diagnosed with ASD.[16,17] Environmental exposures can lead to epigenetic changes that in turn affect neurodevelopment.[18]

While environmental exposures occur throughout the life course, those that occur during pregnancy may play an especially important role in ASD development.[19] Studies have explored the relationship between ASD and a variety of exposures that have the potential to impact neurodevelopment. Examples of prenatal exposures that may augment the likelihood of ASD include the use of sodium valproate[20] and selective serotonin reuptake inhibitors,[21] maternal health conditions, including obesity,[22] polycystic ovary syndrome,[23] fever,[24] autoimmune conditions,[25] folic acid deficiency,[26] older parental age,[27] shorter interpregnancy intervals,[28] and exposure to air pollutants[29] and pesticides.[30] Multiple cohort studies and meta-analyses have found no statistically significant relationship between maternal prenatal tobacco use and ASD diagnosis,[31–33] but one study found significantly more ASD traits among children whose mothers used prenatal tobacco.[34] Increasingly, thyroid disrupting compounds are being investigated as a possible environmental cause of ASD.[35,36]

Thyroid dysfunction during pregnancy

Hypothyroidism is estimated to affect 4% of pregnancies (3.5% subclinical hypothyroidism and 0.5% overt hypothyroidism), and hyperthyroidism is estimated to affect 2.4% of pregnancies (1.8% subclinical hyperthyroidism and 0.6% overt hyperthyroidism) in the United States.[37,38] Since the 1960s, overt maternal hypothyroidism during pregnancy has been linked to cretinism and other developmental disabilities.[39,40] More recent research has explored relationships between milder forms of thyroid dysfunction and differences in developmental outcomes. Findings from a meta-analysis suggest that maternal subclinical hypothyroidism and hypothyroxinemia may be associated with increased risk for intellectual disability in children.[41] Both high and low maternal free thyroxine (fT4) concentrations have been associated with a lower child intelligence quotient (IQ), lower gray matter, and cortex volume.[42]

Maternal thyroid autoimmunity during pregnancy has been associated with decreased cognitive and motor functioning in offspring compared with children born to mothers without thyroid autoimmunity, although study results are inconsistent.[43,44] In the maternal immune activation model proposed for ASD, maternal autoimmune diseases or other events triggering an immune response during pregnancy are suggested to alter neurodevelopment toward ASD through inflammatory pathways.[45] Thyroid autoimmunity is common during pregnancy, with prevalence of thyroid peroxidase antibody (TPO-Ab) positivity and thyroglobulin antibody (Tg-Ab) positivity estimated to be 5–14% and 3–18%, respectively.[43]

This narrative review focuses on the relationship between maternal thyroid dysfunction during pregnancy and ASD in the child. We aim to: (1) review existing literature on associations between maternal thyroid dysfunction during pregnancy and ASD in the child, (2) evaluate the strengths and limitations of existing literature, and (3) develop strategies for future research to effectively address knowledge gaps.

Review

Scope

The literature search focused on original human research studies that examined the relationship between maternal thyroid dysfunction during pregnancy and child ASD outcomes. ASD outcomes included a clinical ASD diagnosis (as reported in medical records, registry, or research study evaluation) or results from a nondiagnostic ASD assessment (screening tests that may be suggestive of an autism diagnosis or measures of autism traits). We did not consider studies that only assessed maternal thyroid dysfunction outside of pregnancy, only measured thyroid function in the child, did not report thyroid-specific results (e.g., any autoimmune or endocrine diseases), or were not published in scientific journals. In this review, all studies used a significance level of 5% and associations were deemed statistically significant when confidence intervals (CIs) did not include the null value.

Summary

Description of the literature

Articles were published between 2013 and 2022 (Table 1).[46,47] Study designs included case–control,[48–51] cohort,[46,47,52–55] nested case–control,[56] case-cohort,[57] meta-analysis of cohorts,[58] or randomized control trial.[59] One study, a pilot study surveying parents about pregnancy risk factors for ASD diagnosis, used a cross-sectional design (Table 1).[60]

Hypothyroidism was the most frequently investigated type of thyroid dysfunction, compared with hyperthyroidism and hypothyroxinemia. Studies also examined thyroid autoimmunity, continuous measures of thyroid hormones, or levothyroxine use in relation to ASD outcomes. Most studies ascertained maternal thyroid conditions through medical records or laboratory analysis of thyroid hormone concentrations, instead of relying on maternal self-reports. ASD outcomes included probable autism based on screening tests or measures of autism traits[47,54,58,59] and ASD diagnosis. Among studies using an ASD diagnosis as an outcome, less than half specified that gold standard measures, ADOS and/or ADI-R, were used in the ASD diagnostic process (Table 1).[51,53,56,60]

All but two studies [49,60] described the confounders considered for regression models. Most studies controlled for multiple characteristics that may be associated (either directly or as a proxy) with ASD and maternal thyroid function, including maternal age,[27,61] smoking,[34,62] income or socioeconomic status,[63] ethnicity,[5,61] and parity.[28,61] When participants were not matched by sex of the child, regression models were adjusted for sex[48,52–55,57–59] and/or stratified by sex.[46,47,53,56,57]

Discussion of the relationship between maternal thyroid dysfunction and ASD

Across all studies, there was evidence supporting an association between maternal thyroid dysfunction during pregnancy and ASD in children, but much remains unknown. Of the 15 studies, 12 found statistically significant associations between a maternal thyroid abnormality and ASD outcomes, and three studies[46,51,59] reported no significant associations. Of the studies that did not report any associations, Hales et al. and Ge et al. primarily investigated levothyroxine treatment and Lyall et al. examined thyroid autoimmunity. A more detailed summary of findings by each type of thyroid problem follows.

Hypothyroidism and ASD outcomes. Five out of the seven studies examining hypothyroidism found that maternal hypothyroidism during pregnancy significantly increased the likelihood of ASD outcomes. This includes four population-based studies,[52,53,55,57] and a smaller unmatched case–control study (George et al.),[49] all of which used ASD diagnosis as an outcome. Two of these analyzed participants from the Danish National birth registry during similar time periods and for this reason, these studies may not each represent novel findings.[52,57]

In the unmatched case–control study, mothers who reported hypothyroidism during pregnancy had more than four times the odds of having a child diagnosed with ASD compared with mothers who reported not having hypothyroidism[49] (Table 1). However, the sample size was relatively small and CIs were wide. The likelihood of ASD increased by less than twofold in all four population-based studies, but effect estimates from these studies had narrower CIs indicating less uncertainty (Table 1).

Two studies distinguished between subclinical and overt thyroid dysfunction,[56,57] and there were no clear patterns related to the severity of thyroid dysfunction and ASD outcomes. Brown et al. found that the direction of effect differed depending on whether the hypothyroidism was subclinical (increased odds of ASD) or overt (decreased odds of ASD), although none of the relationships between thyroid dysfunction and ASD was statistically significant.[56] Andersen et al. found that subclinical hypothyroidism was significantly associated with ASD, while overt hypothyroidism was not.[57] For each study, after stratifying by hypothyroidism severity, the number of women in each group was small.

Three studies tested interaction terms between child sex and hypothyroidism, but no interactions were statistically significant (reported *p*-values ranged from 0.30 to 0.38).[53,55,57] However, one of these studies (Getahun et al.) observed differences in ASD risk between male and female children depending on when the mother was diagnosed with hypothyroidism. In analyses stratified by child sex, the risk of ASD for female children was greatest when their mother was diagnosed with hypothyroidism before pregnancy (adjusted hazard ratio [HR] = 1.61 [CI: 1.09–2.40]), compared with during pregnancy when the associations were not significant for any trimester (adjusted HR ranging from 0.85 [CI: 0.21–3.38] in the third trimester to 1.60 [CI: 0.51–4.96] in the second trimester).

The risk of ASD for male children remained relatively constant and not significant regardless of whether maternal hypothyroidism was diagnosed before or during pregnancy (adjusted HR ranging from 1.24 [CI: 0.68–2.23] in the second trimester to 1.41 [CI: 0.80–2.48] in the third trimester).[53]

Hyperthyroidism and ASD outcomes. There was less evidence supporting an association between hyperthyroidism during pregnancy and ASD outcomes in the child. Of the six studies that examined hyperthyroidism, two found statistically significant associations. Andersen et al. reported that overt, but not subclinical, hyperthyroidism was significantly associated with ASD diagnosis.[57] Chen et al. reported that hyperthyroidism was significantly associated with ASD diagnosis, but did not differentiate between subclinical and overt cases.[48] Both studies found that ASD likelihood or odds more than doubled among children of affected mothers, but CIs were wide in Chen et al. (odds ratio [OR] 6.62 [CI 1.08–40.47]) (Table 1). The remaining four studies found a positive, but not statistically significant, relationship between maternal hyperthyroidism during pregnancy and child ASD. Given that ASD is a relatively rare condition among the general population,[63] it is possible that effect estimates for hyperthyroidism were underpowered in some studies.

For example, Chen et al. only had three ASD cases with maternal hyperthyroidism[48] and Andersen et al. had nine ASD cases with overt maternal hyperthyroidism and two ASD cases with subclinical maternal hyperthyroidism.[57] However, because the reviewed studies did not provide power calculations, it cannot be confirmed that studies were underpowered.

Hypothyroxinemia and ASD outcomes. Three studies examined hypothyroxinemia. Román et al. reported that maternal hypothyroxinemia (normal thyrotropin [TSH] with fT4 in the lower fifth percentile) increased the odds of probable autism in children nearly fourfold. Using the same definition of hypothyroxinemia, Levie et al. found that maternal hypothyroxinemia nearly doubled the odds of autism

traits (Table 1). Román et al. also tested a broader cutoff point for fT4 and found no association with ''mild'' hypothyroxinemia (normal TSH and fT4 in the lower 10th percentile).[54] Andersen et al. found a significant positive association between maternal hypothyroxinemia and ASD diagnosis, but only in girls (Table 1).[57] Román et al. found no difference by child sex,[54] and sex differences for maternal hypothyroxinemia and ASD outcomes were not examined by Levie et al.[58]

Thyroid hormones as continuous exposure measures and ASD outcomes. Only one study, Yau et al., found a significant association between continuous thyroid hormone measures and ASD outcomes. In this study, log-transformed maternal TSH and child ASD diagnosis were inversely related (Table 1).[50] Two studies found no significant association between fT4 or TSH, treated as continuous variables, and ASD outcomes.[57,58] Brown et al. reported no significant association between continuous measures of fT4 or TSH and ASD but did not state whether a nonlinear relationship was tested.[56]

Thyroid autoimmunity and ASD outcomes. Evidence regarding a relationship between thyroid autoimmunity and autism outcomes was discordant. Teng et al.[47] and Brown et al.[56] found a significant positive association with ASD outcomes, while Lyall et al.[51] and Román et al.[54] found no significant association. Teng et al. stratified children by sex and found that boys whose mothers were TPO-Ab positive and Tg-Ab negative had about double the likelihood of probable ASD compared with boys born to mothers who were negative for both antibodies (Table 1). This association was not found among girls or boys born to Tg-Ab-positive mothers.[47]

Brown et al. found that the odds of having a child diagnosed with ASD were increased by 80% in TPO-Ab-positive mothers compared with TPO-Ab-negative mothers (Table 1). When treated as a continuous measure, TPO-Ab titers were associated with ASD, but the strength of the association was weakened.[56] Brown et al. found no significant interaction between TPO-Ab and child sex.[56]

Challenges and limitations

Several themes emerged surrounding the challenges of this research topic and the limitations of studies included in this review. First, achieving an adequate sample size may have been challenging. Thyroid conditions during pregnancy (especially hyperthyroidism) and ASD are relatively rare among the general population.[37,63] The challenges and impacts of misclassification, selection (including survival), and publication bias warrant further consideration for strengthening the evidence in future studies, as summarized in Table 2.

Misclassification of exposure. In regions without a universal thyroid screening during pregnancy, women with subclinical cases may have been misclassified as euthyroid in medical records. The four studies that used medical records to identify thyroid dysfunction[48,52,53,55] were conducted in countries that do not have universal screening for thyroid dysfunction during pregnancy (Denmark,[64,65] Israel,[66] the United States,[67,68] and Taiwan[48]). Misclassification of the exposure could result in a bias toward the null, although there is not enough research on the relationship between subclinical thyroid outcomes and ASD outcomes to assess the extent to which this would occur. Prospective studies evaluating women using thyroid laboratory tests are critical to learning more about whether the nature or strength of the relationship between thyroid dysfunction and ASD outcomes differs based on thyroid dysfunction severity.

Findings on thyroid autoimmune diseases could have been affected by misclassification bias. In this review, self-reported thyroid autoimmune diseases had no significant association with ASD diagnosis,[51] but TPO-Ab positivity was associated with a significantly increased likelihood of ASD.[47,56] By using self-reported thyroid autoimmunity, mothers with thyroid autoimmunity who were not aware of

their status might have been misclassified as not having a thyroid autoimmune condition and findings may have been biased toward the null.

Misclassification of outcome. Child age at ASD assessment ranged from <36 months in a case–control study[51] to 2–17 years in a retrospective cohort study (Table 1).[53] When children were assessed for ASD at too young an age, misclassification could have arisen. A study conducted through a university health center was able to achieve ASD diagnostic stability by age 14 months (as determined by the 36-month reassessment),[69] but some children with less severe symptoms could be missed,[70,71] and in practice, children are usually diagnosed at a later age. In the United States, the median age of ASD diagnosis is estimated to be 51 months.[72] Globally, the average age of diagnosis among children 10 years or younger is estimated to be 43.2 months (range 30.9–74.7 months).[73] ASD can be diagnosed during adulthood, but this is rare.[74]

Because many of the studies to date relied on clinical diagnosis from medical records or registries rather than research-grade assessments of all enrolled children, ASD would be less likely to be reported for younger children in these studies.

Both Brown et al. and Yau et al. stated that some children may have been misclassified as typically developing controls because the age at classification overlapped with the age at which ASD is most commonly diagnosed.[50,56] If this misclassification was nondifferential with respect to maternal thyroid dysfunction, it could result in a bias toward the null. Despite this, both studies found associations between a thyroid measure and ASD diagnosis, although the strength of the association could have been affected by misclassification bias.

Survival bias. Survival bias is present in pregnancy studies because developmental outcomes such as ASD are only known for children who are conceived and survive.[75] Maternal thyroid dysfunction can reduce the likelihood of conception and survival to the age of ASD diagnosis by decreasing fertility or increasing pregnancy loss. Thyroid dysfunction can cause hormonal disruptions that affect fertility. Women with overt hyperthyroidism or hypothyroidism may have impaired fertility compared with euthyroid women, although this is better documented in hypothyroid women than hyperthyroid women.[76] A meta-analysis found that TPO-Ab- or Tg-Ab-positive women had 1.5 times the odds [CI: 1.1–2.0] of unexplained subfertility compared with antibody-negative women, however, these findings may be confounded by co-occurring autoimmune diseases that affect fertility.[76,77]

Multiple types of thyroid dysfunction are associated with increased likelihood of pregnancy loss. Women with overt or subclinical hypothyroidism are at increased risk of pregnancy loss compared with euthyroid women[78–80] and untreated hypothyroidism may be associated with early pregnancy loss.[37,81] TPO-Ab positivity is also associated with increased likelihood of miscarriage.[43,82,83]

Survival bias can affect results differently depending on the true nature of the relationship between the exposure and outcome and whether models are conditioned on conception or fetal survival. Liew et al. used the relationship between prenatal per-and polyfluoroalkyl substance (PFAS) exposure and child attention-deficit/hyperactivity disorder (ADHD) to demonstrate possible implications of survival bias on ORs.[75] When this relationship was modeled as null and conditioned on fetal survival through 6–12 weeks, PFAS appeared to be protective against ADHD, contradicting biological models. When the relationship between the exposure and outcome was modeled as casual and conditioned on fetal survival to 6–12 weeks, it could result in a bias toward the null.[75] If maternal thyroid dysfunction is a cause of ASD, the full magnitude of this relationship may be masked by survival bias, and studies might report a weaker relationship than is true.

Although survival bias cannot be eliminated from a study, the impact can be mitigated by adjusting for variables that affect both the study outcome and fetal survival.[75] Most studies to date adjusted for maternal body mass index (BMI) or body weight[47,50,55,57,58] and/or maternal mental illness,[46,48,52,54,56,57] two factors that are associated with increased ASD likelihood and reduced fertility and/or fetal loss.[84,85] However, adjustment for additional factors associated with both ASD outcomes and fetal survival such as the interpregnancy interval,[86] polycystic ovarian syndrome,[87] and pesticide exposure, could reduce live birth bias further.

Publication bias. In most areas of medical research, null results are more likely to remain unpublished compared with results showing an association.[88] As a result of publication bias, this review may underrepresent research that found no association between maternal thyroid dysfunction during pregnancy and child ASD outcomes. However, among the articles reviewed, null findings were not uncommon. Most studies examined multiple maternal thyroid conditions or multiple child development outcomes and published a combination of null and statistically significant findings in a single article.

Future directions: knowledge gaps and suggestions for further research

Effect of levothyroxine treatment. Multiple studies examining hypothyroidism stated that their sample included women who were receiving treatment for thyroid dysfunction.[52,53,55,57] Theoretically, if abnormal thyroid hormone levels during pregnancy increased the likelihood of ASD outcomes, treatment to normalize hormone levels would reduce ASD likelihood. This hypothesis was supported by Andersen et al. who found that an association between maternal hypothyroidism and child ASD was present among mothers diagnosed and treated with levothyroxine after birth, but not among mothers diagnosed and treated before birth.[52] However, Rotem et al. found that the odds of ASD were similarly elevated regardless of whether mothers were diagnosed and treated for hypothyroidism before or after giving birth, indicating that levothyroxine treatment may not influence ASD outcomes.[55] Among a subset of the sample who had TSH levels available, mothers treated for hypothyroidism before conception with normal TSH levels during pregnancy still had increased odds of having a child with ASD.[55]

Hales et al. performed a secondary analysis of a randomized clinical trial to examine the effects of levothyroxine treatment during pregnancy and found that treated mothers

were more than twice as likely as controls to have children with a positive ASD screen using the Social Communication Questionnaire.[59] However, the mean test scores did not vary significantly between the treated, untreated, and normal thyroid function groups and about a third of the treatment group was likely overtreated with levothyroxine.[59] Ge et al. found no increased likelihood of child ASD among mothers who used levothyroxine during pregnancy compared with euthyroid mothers, but this study did not include mothers with thyroid dysfunction not using levothyroxine as a comparison group.[46]

The relationship between levothyroxine treatment for hypothyroidism and ASD outcomes was challenging to elucidate for multiple reasons. First, both the adequacy and timing of levothyroxine might affect ASD outcomes. Fetal neurodevelopment is hypothesized to be most strongly affected by maternal thyroid function between 8 and 14 weeks of gestation.[89] Not all studies reported the gestational age at which thyroid function was measured, and many studies focused on the late first and early second trimester.[54,56–59] Also, studies did not describe how treatment for hypothyroidism would have likely affected maternal thyroid hormone levels during this proposed critical period of ASD development. Second, the three studies that examined treatment[52,55,59] had different approaches to measuring thyroid dysfunction (clinical diagnosis of hypothyroidism, suboptimal gestational thyroid function) and ASD (clinical diagnosis, nondiagnostic assessments), which make it difficult to generalize their results.

Prenatal exposure to thyroid-disrupting compounds. It is possible that there is an additional factor that underlies both maternal thyroid dysfunction and ASD. As suggested by Rotem et al., environmental exposures affecting both maternal thyroid function and child neurodevelopment could result in an association between maternal thyroid dysfunction and ASD that is independent of treatment.[55] If maternal hypothyroidism is the result of an environmental exposure that could also increase the likelihood of having a child with ASD, treating the mother with levothyroxine may not reduce the likelihood of child ASD because the environmental exposure could still drive ASD development. Prenatal exposures to many chemical compounds are hypothesized to disrupt thyroid function and increase the likelihood of child ASD. Perchlorate and nitrate interfere with thyroid function by inhibiting the sodium–iodide symporter (NIS) and have been known to increase the likelihood of ASD.[90,91]

Both bisphenol A[92–94] and PFAS[35] are suspected of disrupting thyroid function, and prenatal exposure to either of these is associated with ASD.[95] However, the physiological effects of these compounds are not limited to the thyroid, and prenatal exposure to these compounds may alter neurodevelopment through multiple pathways,[96,97] including pathways that are likely independent of levothyroxine use. For example, prenatal exposure to chlorpyrifos, an organophosphate pesticide, has been found to disrupt thyroid function[98,99] and increase the likelihood of ASD.[100] However, prenatal exposure to chlorpyrifos can also lead to breakage and rearrangement of DNA in the gene *KMT2A*, a gene associated with ASD development.[15] Such genetic damage could occur independent of maternal thyroid hormone concentrations. Additional research is needed to identify the pathways by which prenatal exposure to thyroid-disrupting compounds could lead to ASD and to clarify how abnormal thyroid hormone concentrations resulting from exposure might contribute to ASD development.

Iodine, thyroid dysfunction, and ASD. Iodine is necessary for thyroid hormone synthesis.[40] Inadequate maternal iodine intake during pregnancy has long been tied to developmental disabilities in children.[40] In the United States and Europe, population estimates of inadequate iodine intake during pregnancy have decreased greatly in the last century as a result of food fortification policies, but mild-to-moderate iodine deficiency presently still remains a concern in these regions and globally.[101] Iodine deficiency during pregnancy is common within multiple countries included in this review. A median urinary iodine concentration (UIC) falling below the World Health Organization's recommended adequate range for pregnancy (median UIC 150–499 μg/L) was found in a representative sample of pregnant women in the United States (median UIC 129 μg/L)[102] and in two-thirds of the European countries that have assessed pregnancy UIC.[103]

It is unclear how maternal iodine concentrations are related to ASD outcomes. Two studies have investigated this topic and found no statistically significant association between maternal iodine deficiency and ASD traits[104] or ASD diagnosis.[105] These studies assessed for iodine status before 14 weeks of gestation[104] and between 26 and 28 weeks of gestation,[105] respectively. However, both studies used a single UIC measurement per woman, which is not diagnostic of habitual iodine intakes.[104,105] Because urinary iodine measures fluctuate based on recent consumption of iodine-containing foods, and consumption patterns could change throughout pregnancy, multiple urinary iodine measures per person are needed to estimate individual iodine deficiency status.[106,107]

Conclusions

Of the 15 studies included in this review, 12 studies found statistically significant associations between a maternal thyroid abnormality during pregnancy and increased likelihood of ASD outcomes in the child. The most consistent evidence was for hypothyroidism. The rigor of this research would be enhanced if additional steps were taken to mitigate misclassification bias and survival bias. Research with null findings should be given comparable consideration to research with significant findings to reduce publication bias. This review has limitations. As a narrative review, it does not include summary estimates or systematic assessments of article quality.

Future research is needed to investigate the possibility that the relationship between thyroid dysfunction and ASD outcomes is driven by a third factor. Certain environmental chemical exposures are associated with both ASD and thyroid dysfunction.[90–94] These relationships could be clarified by examining maternal exposure to thyroid-disrupting compounds in relation to both maternal thyroid dysfunction and child ASD outcomes. In addition, it is unclear whether levothyroxine treatment for mothers with mild hypothyroidism or hypothyroxinemia is protective for ASD development. This question would be best investigated using studies that are adequately powered to examine multiple ASD outcomes among mothers with different types and severity of thyroid dysfunction.

Iodine deficiency is a modifiable risk factor for hypothyroidism. Given the possible positive associations between maternal hypothyroidism during pregnancy and ASD outcomes, it is important to investigate the proportion of maternal hypothyroidism cases that are attributable to iodine deficiency. Public health strategies to prevent maternal iodine deficiency should continue to be enacted because these strategies could reduce potential downstream effects of iodine deficiency on maternal thyroid function and child neurodevelopment.

Authors' Contributions

Z.B.K.: conceptualization and writing—original draft; E.N.P.: writing—review and editing and supervision; S.Y.L.: writing—review and editing and supervision; H.-M.S.: writing—review and edition and supervision; R.J.S.: writing—review and editing and supervision.

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Table 1. Summary of Studies Examining Thyroid Dysfunction During Pregnancy and Autism Spectrum Disorder Outcomes

First author (year)[Ref.]	*Study design*	*Sample size and description*	*ASD outcome and ascertainment*	*Child age at assessment*	*Time of thyroid condition evaluation*	*Thyroid conditions and ascertainment*	*Effect estimates with CIs and reference group*
Román (2013)[54]	Population-based birth cohort	4039 Children, included mothers treated for thyroid dysfunction (Gen R, Netherlands)	Parent report of autistic traits ($n = 80$)	Until 6 years	13.4 weeks (SD 1.9, range 5.9–17.9)	Hypothyroxinemia, autoimmunity (Laboratory)	Hypothyroxinemia: OR 3.98 [CI 1.83–8.20] (Ref.: no hypothyroxinemia) TPO-Ab positivity: OR 0.78 [CI 0.28–2.16] (Ref.: TPO-Ab negative)
Lyall (2014)[51]	Case–control	560 ASD cases, 168 DD cases, 391 TD controls, included mothers treated for thyroid dysfunction (CHARGE, California)	Clinician diagnosis (ADOS and ADI-R)	Before 36 months	3 Months before pregnancy through breastfeeding	Maternal thyroid autoimmune disease (Self-report)	OR 0.99 [CI 0.61–1.60] (Ref.: no autoimmune disease reported)
Andersen (2014)[52]	Population-based birth cohort	857,014 Children, included mothers treated for thyroid dysfunction (Danish nationwide cohort study)	Diagnosis in nationwide registries ($n = 5311$)	3 Years	Before, during, and after pregnancy	Hypo- and hyperthyroidism, (ICD-8/10 codes from registry)	Hypothyroidism: HR 1.30 [CI 1.11–1.53] Hyperthyroidism: HR 1.18 [CI 0.96–1.45] (Ref.: no hypo- or hyperthyroidism diagnosis)
George (2014)[49]	Unmatched case–control	143 ASD cases, 200 children without ASD, did not report if treated mothers were included (Kerala, India)	Clinician diagnosis	2–6 Years	Antenatal	Hypothyroidism (Self-report)	OR 4.25 [CI 1.38–13.07] (Ref.: no report of hypothyroidism)
Yau (2015)[50]	Case–control	78 ASD cases, 45 DD cases, 149 general population, did not have data on treatment (EMA, California)	Diagnosis from medical record	3–4 Years	15–19 Weeks	TSH levels only (Laboratory)	Inverse relationship between child ASD diagnosis and log-transformed maternal TSH: OR 0.33 [CI 0.12–0.91] (Ref.: general population)

(*continued*)

Table 1. (Continued)

First author (year)[Ref.]	*Study design*	*Sample size and description*	*ASD outcome and ascertainment*	*Child age at assessment*	*Time of thyroid condition evaluation*	*Thyroid conditions and ascertainment*	*Effect estimates with CIs and reference group*
Brown (2015)[56]	Nested case–control	967 Matched case–control pairs from the Finnish prenatal study of autism, did not report if treated mothers were included	Diagnosis from hospital and outpatient registry	2+ Years, median of 4 years	Case mean 11.1 weeks (SD 3.1); control mean 10.9 weeks (SD 3.5)	Autoimmunity, hypo- and hyperthyroidism, (Laboratory)	TPO-Ab positivity: OR 1.78 [CI 1.16–2.75] (Ref.: TPO-Ab negative) Hypothyroidism: clinical, OR 0.67 [CI 0.27–1.63]; subclinical: OR 1.17 [CI 0.72–1.90] Hyperthyroidism: clinical: OR 1.06 [CI 0.54–2.10]; subclinical, OR 1.10 [CI 0.67–1.83] (Ref.: euthyroid)
Getahun (2018)[53]	Retrospective cohort	397,201 Children, included mothers treated for thyroid dysfunction (Kaiser Permanente members, California)	Diagnosis from medical records ($n = 6475$)	2–17 Years	Included women at any point in pregnancy	Hypothyroidism (ICD-9 codes from medical record)	Before pregnancy: HR 1.30 [CI 1.07–1.58], during pregnancy: HR 1.33 [CI 1.05–1.69] (Ref.: no hypothyroidism)
Andersen (2018)[57]	Case–cohort	7624 Children from subcohort random sample, 302 children with ASD, included mothers treated for thyroid dysfunction (Danish National Birth Cohort)	Diagnosis from medical record	Median age of 5.3 years	9 Weeks of pregnancy (range 5–19 weeks)	Hypo- and hyperthyroidism, Isolated low free thyroxine (Laboratory)	Hypothyroidism: overt, HR 2.03 [CI 0.71–5.77]; subclinical, HR 1.70 [CI 1.04–2.75] Hyperthyroidism: overt, HR 2.18 [CI 1.08–4.39]; subclinical, HR 0.37 [CI 0.09–1.52] Hypothyroxinemia: Girls: HR 4.92 [CI 2.03–11.9]; Boys: HR 0.92 [CI 0.39–2.15] (Ref.: euthyroid)

(*continued*)

Table 1. (Continued)

First author (year)[Ref.]	Study design	Sample size and description	ASD outcome and ascertainment	Child age at assessment	Time of thyroid condition evaluation	Thyroid conditions and ascertainment	Effect estimates with CIs and reference group
Levie (2018)[58]	Meta-analysis of population-based cohorts	9036 Mother–child pairs (Gen R, Netherlands; INMA, Spain; ALSPAC, United Kingdom) Excluded mothers with history of thyroid disease, did not report if any mothers received treatment during study	Parent report of autism traits ($n=339$)	Gen R: median 5.9 years, INMA: median 4.6 years, ALSPAC: median 7.6 years	INMA: 13.1 (SD 1.3 weeks); Gen R: 13.4 (SD 2.0) weeks; ALSPAC: 11 (SD 3.2) weeks	Hypo- and hyperthyroidism, hypothyroxinemia (Laboratory)	Subclinical hypothyroidism: OR 1.2 [CI 0.8–1.9] Subclinical hyperthyroidism: OR 1.3 [CI 0.7–2.6] Hypothyroxinemia: OR 1.8 [CI 1.1–2.8] (Ref.: euthyroid)
Hales (2020)[59]	Follow-up to CATS randomized clinical trial	475 Mother–child pairs (CATS participants, United Kingdom) Excluded mothers with a history of thyroid disease, treated and untreated mothers	Parent report of possible ASD ($n=19$)	Mean 9.5 years (SD 0.8)	Median 12 weeks and 3 days of pregnancy	Suboptimal gestational thyroid function (Laboratory)	No association (Ref.: normal thyroid function)
Rotem (2020)[55]	Retrospective cohort	437,222 Children, included mothers treated for thyroid dysfunction (Maccabi Health Services, Israel)	Diagnosis from medical record ($n=4022$)	Children followed until at least 8 years	Before and after delivery (medical records) First trimester (TSH)	Hypo- and, hyperthyroidism, autoimmunity (ICD-9 codes, dispensing record from medical record) TSH levels (subset of sample)	Hypothyroidism: before delivery: OR 1.28 [CI 1.11–1.49] after delivery: OR 1.23 [CI 1.02–148], ever: OR 1.26 [CI 1.12–1.42] Hyperthyroidism: before delivery: OR 1.39 [CI 0.88–2.18] after deliver OR 1.45 [CI 0.95–2.21] (Ref.: no diagnosis of thyroid dysfunction)
Magdalena (2020)[60]	Cross-sectional survey	221 Children, did not report if treated mothers were included (Poland)	Clinician diagnosis (ADOS-2; $n=121$)	Ages 3–12 years	Not specified—preconception thyroid disease	Thyroid disease (Parent and physician report)	RR 1.56 [CI 1.23–1.98] (Ref.: no report of thyroid disease)

(*continued*)

Table 1. (Continued)

First author (year)[Ref.]	Study design	Sample size and description	ASD outcome and ascertainment	Child age at assessment	Time of thyroid condition evaluation	Thyroid conditions and ascertainment	Effect estimates with CIs and reference group
Chen (2020)[48]	Matched case–control	330 Mothers with hyperthyroidism and their children, 1320 matched control pairs, included mothers treated for thyroid dysfunction (Taiwan Longitudinal Health Insurance Research Database)	Diagnosis from medical record ($n=5$)	Cases: 7.8 years (SD 2.7) Controls: 7.9 years (SD 2.7)	Not specified—diagnosis before birth of child	Hyperthyroidism (ICD codes from medical record)	OR 6.62 [CI 1.08–40.47] (Ref.: no history of thyroid disease)
Ge (2022)[46]	Population-based cohort	422,156 Mother–child pairs (Hong Kong Clinical Data Analysis and Reporting System) Excludes mothers with history of thyroid medication, included mothers who received treatment during study	Diagnosis from medical record ($n=10,892$)	Mean follow-up, exposed: 10.6 years (SD 4.2); control: 10.8 years (SD 4.0)	Started levothyroxine at median of 18 gestational weeks	Levothyroxine use during pregnancy (dispensing records)	HR 1.00 [CI 0.78–1.29] (Ref.: no history of thyroid dysfunction)
Teng (2022)[47]	Retrospective cohort	2455 Mother–child pairs (Ma'anshan Birth Cohort study, China). Excludes mothers with history of thyroid disease, did not report if any mothers received treatment during study	Parent report of possible ASD ($n=142$)	1.5–5 Years	First trimester: mean 10 weeks; second trimester: mean 25 weeks; third trimester: mean 34 weeks	TPO-Ab and Tg-Ab (Laboratory)	TPO-Ab positive and Tg-Ab negative: Boys, first trimester: RR 2.01 [CI 1.24–3.27]; second trimester: RR 2.15 [CI 1.08–4.26]; third trimester: RR 2.13 [CI 1.20–3.79] Girls, first trimester: RR 0.92 [CI 0.45–1.87]; second trimester: RR 1.21 [CI 0.53–2.79]; third trimester: RR 1.08 [CI 0.46–2.52] (Ref.: TPO-Ab and Tg-Ab negative)

ADI-R, Autism Diagnostic Interview-Revised; ADOS, Autism Diagnostic Observation Schedule; ALSPAC, Avon Longitudinal Study of Parents and Children; ASD, Autism Spectrum Disorder; CATS, Controlled Antenatal Thyroid Screening; CHARGE, CHildhood Autism Risk from Genetics and the Environment; CI, confidence interval; DD, developmental disability; EMA, Early Markers for Autism; Gen R, Generation R; HR, hazard ratio; INMA, Infancia Y Medio Ambiente; OR, odds ratio; RR, relative risk; SD, standard deviation; TD, typically developing; Tg-Ab, thyroglobulin antibody; TPO-Ab, thyroid peroxidase antibody; TSH, thyrotropin.

Table 2. Considerations for Examining Associations Between Maternal Thyroid Dysfunction During Pregnancy and Autism Spectrum Disorder Outcomes

Study design	Consider nested designs
Sample size	Adequate power to detect differences for specific types of thyroid dysfunction and autism phenotypes
	Sample size should allow for stratification by child sex
Sample description	Women with well-characterized medical history, including history of thyroid problems, treatment for thyroid problems, and use of thyroid-interfering medications
Thyroid conditions and ascertainment	Hypothyroidism (subclinical and overt), hyperthyroidism (subclinical and overt), hypothyroxinemia, thyroid autoimmunity
	Measure thyroid hormone concentrations across sample and classify thyroid dysfunction using pregnancy-specific reference ranges
	Assess nonlinearity when examining continuous thyroid hormone concentrations
Time of thyroid condition evaluation	Early in pregnancy, when the fetus is most likely to be affected by maternal thyroid levels (~8–14 weeks of gestation)
ASD outcome and ascertainment	Clinician assesses all children in the study for ASD diagnosis or ASD traits
Child age at assessment	Follow until the age when most children are diagnosed for their country of residence
Additional variables to examine	Maternal iodine, maternal exposure to thyroid disrupting compounds hypothesized to increase likelihood of ASD in offspring
	Variables that affect fetal survival and ASD development

Chapter 2: Associations between maternal thyroid dysfunction during pregnancy and ASD and other developmental outcomes in the MARBLES high familial likelihood cohort

Background

Autism is a neurodevelopmental condition associated with unique strengths and weaknesses. The main criteria for an autism spectrum disorder (ASD) diagnosis include challenges in social communication, such as reduced interest in social behaviors or difficulty interpreting social cues.[3] ASD is typically diagnosed during childhood and median age of diagnosis is estimated to be around 49 months in the U.S.[18] Based on the 2022 Centers For Disease Control and Prevention (CDC) surveillance, ASD is estimated to occur in 1 out of 31 children aged 8 years old.[19] About 40% of children diagnosed with ASD are estimated to have co-occurring intellectual disability.[19] Traits associated with autism occur throughout the population on a spectrum, including among people who would not meet criteria for the binary ASD diagnosis. ASD traits may arise from some of the same etiological factors underlying an ASD diagnosis and examining the underpinnings of ASD traits may shed light on the origins of ASD as a clinical entity.[20,21]

ASD is a multifactorial condition, with both genetic and environmental (non-genetic) factors contributing ASD development.[7] Because pregnancy is a sensitive period for child neurodevelopment, maternal environmental exposures during pregnancy warrant careful consideration as etiological factors for ASD. Previous research has identified a variety of prenatal exposures as potential etiological factors for ASD, including several factors that are associated with thyroid function. Exposure to certain endocrine disrupting compounds during pregnancy may be positively associated with the incidence of child ASD outcomes.[22] Perchlorate and nitrate are known to interfere with thyroid function by inhibiting the sodium iodide symporter (NIS) and have been hypothesized to increase the likelihood of ASD.[9,10] Bisphenol A is also suspected of disrupting thyroid function and prenatal exposure is associated with ASD.[11–13] Prenatal exposure to per- and polyfluoroalkyl substances (PFAS) is associated with abnormal thyroid function and child ASD.[22] Smoking tobacco products has an adverse effect on thyroid health[23] and tobacco use during

pregnancy may be associated with increased likelihood of autism traits.[24] Additionally, maternal autoimmune conditions may increase the chance of ASD.[25,26]

Women of reproductive age are at risk for multiple types of thyroid dysfunction. Hypothyroidism is the most prevalent type of thyroid dysfunction among both pregnant and non-pregnant women.[27,28] In the United States, it is estimated that subclinical hypothyroidism [elevated thyroid stimulating hormone (TSH) with normal free thyroxine (FT4)] occurs in 3.5% of pregnancies and overt hypothyroidism (elevated TSH with decreased FT4) in 0.5% of pregnancies.[27,29] Subclinical hyperthyroidism (decreased TSH with normal FT4) is estimated to occur in 1.8% of pregnancies and overt hyperthyroidism (decreased TSH with elevated FT4) in 0.6% of pregnancies.[27,29] Hypothyroxinemia is characterized by normal TSH and low FT4 levels and affects approximately 2% of pregnancies.[29] Thyroid autoimmunity can occur with or without thyroid dysfunction. Thyroid autoimmunity is indicated by the presence of antibodies, such as thyroid peroxidase (TPO-Ab), thyroglobulin (Tg-Ab), or TSH receptor (TR-Ab).[30] Among pregnant women, the prevalence of TPO-Ab positivity and Tg-Ab positivity is 5-14% and 3-18%, respectively.[27,30] Risk factors for thyroid dysfunction that are applicable to women during the reproductive years include iodine deficiency,[8,28,31] use of medications including lithium [32,33] and amiodarone,[34] smoking,[23,35] and pesticide exposure.[36] Thyroid autoimmune disorders such as Hashimoto's thyroiditis and Graves' disease have genetic components.[37–39]

The overall objective of this study is to examine associations between maternal thyroid dysfunction during pregnancy and child ASD. This study tests the hypotheses that: 1) maternal thyroid dysfunction (especially hypothyroidism) during pregnancy is associated with increased likelihood of child ASD diagnosis and ASD traits compared to mothers without thyroid dysfunction; 2) increased concentrations of maternal TSH and decreased concentrations of FT4 are associated with increased likelihood of ASD diagnosis and ASD traits in children; and 3) similar relationships will be observed for thyroid dysfunction, TSH, and FT4 with child cognitive development.

Methods

Study design and eligibility

Study participants were mothers, and their biological children enrolled in the Markers of Autism in Babies: Learning Early Signs (MARBLES) study. MARBLES is a prospective cohort study that aims to identify environmental etiological agents contributing to ASD, understand their mechanisms of action, and recognize early biological markers associated with increased likelihood of ASD.[40] MARBLES participants are recruited from the California Department of Developmental Services (DDS), a state organization that provides services for Californians with developmental disabilities and their families. A small percentage of participants were referred by other research studies or health care providers. Main eligibility criteria for MARBLES included 1) having a biological child with ASD, 2) being currently pregnant or planning a pregnancy and 3) living within a 2.5-hour drive of the Davis/Sacramento area. MARBLES collected self-reported data on maternal diet, lifestyle and health, medical records, survey of cleaning and household products and dust samples from home visits, and biological specimens from parents and children.

To be eligible in the present study, MARBLES mothers needed to have 1) a biological child with a 36-month ASD assessment and 2) at least one blood and urine sample collected during the pregnancy of that child. Only blood samples (to measure thyroid hormones and antibodies) were needed in the present study. Mothers participating in MARBLES for multiple pregnancies were included the analyses, which adjusted for clustering by family. For twin pregnancies, only one twin was selected for the outcome based on lowest ID number. In sensitivity analyses, the main analysis was re-run with the first enrolled pregnancy only and then with the twin who had the higher ID number.

Thyroid measures

For each pregnancy, mothers contributed 1-3 blood samples. For pregnancies with two or three samples, each sample was taken from a different trimester so that there was no more than one sample

from a given pregnancy per trimester. Blood samples were frozen at -80 °C within 24 hours after collection following protocol requirements for future assays.[40] Serum was analyzed for TSH, FT4, total thyroxine (TT4), total triiodothyronine (TT3), TPO-Ab, and TG-antibody at the University of Washington clinical laboratory. For quality assurance, 21 (3.5%) duplicates were analyzed along with the study samples. The average relative percent difference for FT4 samples was 13% (ranged from 1.5% to 38%). For TSH samples, the average was 3% (range from 0.3% to 6.4%). Re-testing did not change TPO or TG antibody status.

Antibody positivity TPO and TG antibodies were measured for each trimester. Cut points for antibody positivity were based on manufacture's recommendations. Pregnancies were categorized as TPO and TG negative if antibodies were negative for both TPO and TG for all trimesters where samples were available. Pregnancies were categorized as antibody positive if they were TPO and/or TG positive for any trimester.

Presence of thyroid dysfunction Trimester-specific TSH and FT4 cut points were used to categorize pregnancies as euthyroid or affected by a type of thyroid dysfunction. TSH and FT4 cut points were ascertained using a subsample of among mothers who were TPO and TG antibody negative for all trimesters with blood draws and did not self-report a thyroid condition during pregnancy. As part of the MARBLES protocol, all mothers complete a questionnaire asking about health conditions and medication use during pregnancy, including specific questions about thyroid conditions. Medical records were not used in this study because they were unavailable for about 30% of pregnancies.

Pregnancies were categorized as euthyroid if TSH and FT4 were between the 2.5th and 97.5th percentiles for every trimester a sample was collected. Pregnancies were categorized as being affected by thyroid dysfunction if any type of thyroid dysfunction was present in at least one trimester. Initially, non-euthyroid pregnancies were categorized into one of these six types of thyroid dysfunction: overt hypothyroidism (TSH greater than the 97.5th percentile and FT4 less than 2.5th percentile), subclinical hypothyroidism (TSH greater than 97.5th percentile and FT4 between 2.5th and 97.5th percentile), overt

hyperthyroidism (TSH less than the 2.5th percentile, FT4 greater than 97.5th percentile), subclinical hyperthyroidism (TSH less than the 2.5th percentile, FT4 between 2.5th and 97.5th percentile), hypothyroxinemia (TSH between 2.5th and 97.5th percentile and FT4 less than 2.5th percentile), and hyperthyroxinemia (TSH between 2.5th and 97.5th percentile and FT4 greater than 2.5th percentile) (Table A2.1). Pregnancies were not affected by multiple types of thyroid dysfunction, so categories were mutually exclusive. Because of small sample sizes, thyroid dysfunction categories were collapsed into two categories for analysis: hypothyroid conditions (subclinical or overt hypothyroidism, or hypothyroxinemia) or hyperthyroid conditions (subclinical or overt hyperthyroidism or hyperthyroxinemia) (Table A2.2).

TSH, FT4 concentrations TSH and FT4 concentrations for each trimester were natural log-transformed to reduce the effect of outliers.

Autism measures

ASD diagnosis status Children were evaluated for ASD symptomology by trained clinicians starting at age six months. Diagnostic outcomes were classified at age 36 months using an algorithm combining results from the Autism Diagnostic Observation Schedule (ADOS) and Mullen Scales of Early Learning (MSEL). Children were classified as having ASD, typical development (TD), or atypical development that is not ASD (non-TD).[40]

Social Responsiveness Scale The social responsiveness scale (SRS) is a validated, caregiver completed measure of dimensional autism phenotypes and intensity of ASD traits.[41] The SRS-2 consists of 65 Likert items, each ranging from not true (1 point), sometimes true (2 points), often true (3 points), to almost always true (4 points). The SRS-2 assesses five different traits associated with ASD: social awareness (8 items), social cognition (12 items), social communication (22 items), social motivation (11 items), restricted interests and repetitive behavior (12 items). Total and trait subscale scores were converted to standardized t-scores. The total score is standardized to a mean of 50 and standard deviation of 10.[41]

In this study, the SRS total scores were examined as continuous measures when available for MARBLES participants (n=228). SRS was not collected from the start of the study. Families who enrolled closer to the start of MARBLES completed their SRS when their children were older compared to families who enrolled after SRS was part of the study protocol. When available, school age SRS scores completed when the child was at least 48 months old were used. For children whose only available score was a pre-school form, this score was used. A small number of children had a school age form completed before 48 months of age. For these children, their score from the pre-school form was used if available. For 5 children whose school age SRS scores were from under 48 months of age, no other form was available. These children remained in the main analysis and were removed in sensitivity analysis.

Mullen Scales of Early Learning The Mullen Scales of Early Learning (MSEL) is a validated assessment of early child development comprised of scales for different developmental domains. Clinicians score children through interactive activities or through caregiver report of child. Items are scored from 0 to 5 points, where a score of 1 indicates a correct response for most items. Within a domain scale, items are presented in increasing order of difficulty and the assessor ends the assessment for a scale after three zero responses.[42] The present study uses MSEL composite scores, which combine the scales for visual reception, fine motor, receptive language, and expressive language into a single measure of overall cognitive functioning. The MSEL composite is standardized to a mean score of 100 with a standard deviation of 15.[42] The MSEL is appropriate for use among minimally verbal children with ASD or development concerns because it incorporates non-verbal measures of cognitive abilities.[42]

Statistical methods

Descriptive statistics Fisher's exact tests were used to test for differences in the distribution of categorical variables by thyroid dysfunction category. T-tests were used to test for differences in normal continuous variables by thyroid dysfunction category (hypothyroid conditions vs. euthyroid or hyperthyroid vs. euthyroid), while Wilcoxon's two sample tests were used for non-normally distributed variables (Table 2.1).

Regression analysis Thyroid measures (maternal thyroid dysfunction, maternal TSH concentrations, and FT4 concentrations) were examined in relationship to child diagnostic outcome, SRS standard score, and MSEL composite score. Models examining maternal thyroid dysfunction were analyzed by pregnancy (each pregnancy was affected by, at most, one type of thyroid dysfunction). Hypothyroid conditions and hyperthyroid conditions were compared to euthyroid (reference). Models examining TSH or FT4 concentrations were analyzed by trimester because concentrations of TSH typically increase after the first trimester and averaging concentrations across pregnancy would not take into account the normal fluctuations in thyroid hormones that occur during pregnancy.[27] TSH and FT4 were modeled separately. TSH and FT4 are typically negatively correlated in large population-based studies.[43] They are typically analyzed separately in relation to child development outcomes because their associations with the outcome may differ.

For categorical outcomes (child diagnostic outcomes), multinomial logistic regression was used to produce relative risk ratios showing the odds of having a child diagnosed with ASD or non-TD compared to typical development. Multinomial models were run in SAS using "proc surveylogistic" with a glogit link for nominal outcomes. Clustering by family was used to account for multiple pregnancies from the same mother when estimating standard errors. For continuous outcomes (SRS scores, MSEL scores), quantile regressions ("proc quantreg") were used to generate coefficients predicting the effect of the thyroid measures on the 25th, 50th and 75th percentile of the score distribution. Quantile regression can be used with non-normally distributed outcomes. [44] Though standardized SRS scores were used in this study, they were not normally distributed in this sample based on Q-Q plots and the Kolmogorov-Smirnov test ($p<0.01$). Additionally, quintile regression can predict the effect of thyroid measures at different percentiles of SRS scores,[44] which is important because the effect of thyroid abnormalities may not be uniform across the distribution of SRS scores.[45] Maternal thyroid dysfunction (or one unit increase in natural log of TSH or FT4 concentrations) is hypothesized to be associated with increased autism traits (increase in SRS score) at each quintile. Regressions predicting SRS scores at the 75th percentile are

hypothesized to show the strongest association between maternal thyroid measures and child autism traits because this percentile represents a more extreme end of the SRS distribution. Confidence intervals were calculated using the resampling method for quintile regressions. Because "proc quantreg" was not compatible with a cluster statement, SRS models were run again using "proc surveyreg", with and without a cluster statement. The ratio of standard errors for thyroid measures with and without clustering was calculated to approximate the change in variance after accounting for multiple pregnancies from the same mother. A ratio of 1 indicated no change. This ratio was used to determine if standard error in quantile models would need to be adjusted. Statistical analysis was conducted in SAS version 9.4.

Covariate selection was based on literature review, a DAG, and empirical screen. In bivariate analysis, potential confounders with p-values of less than 0.20 (by ASD diagnosis outcome or thyroid dysfunction category) were added individually to the unadjusted model (thyroid dysfunction predicting ASD diagnosis) to be further evaluated. Covariates whose introduction to the model resulted in more than about a 5% change in either thyroid dysfunction measure (hypothyroid or hyperthyroid conditions) compared to the unadjusted thyroid measures were then added to the adjusted model. Based on these criteria, models were adjusted for maternal public or private health insurance, whether the pregnancy was planned for that time, maternal education level, inter-pregnancy interval of less than 12 months or greater than 12 months (includes pregnancies of 20 weeks of gestation or more), prenatal vitamin use in first month of pregnancy, and child's race and ethnicity.

Additionally, main models were adjusted for antibody positivity, regular maternal smoking, self-reported thyroid conditions and thyroid medication use, each in separate models. Child sex assigned at birth and number of maternal blood draws were evaluated as effect modifiers. Results were considered statistically significant at $p<0.05$.

Confounders with missing values (maternal health insurance, prenatal vitamin use, intended

pregnancy, inter-pregnancy interval, and history of regular maternal smoking) were imputed using multiple imputation with fully conditional specification and the discriminant function method.[46] All regressions were run with imputed covariates unless other specified. Among the 285 pregnancies analyzed, maternal health insurance had the highest percentage of missing at about 15% followed by prenatal vitamin use which was missing about 10%. Intended pregnancy and inter-pregnancy interval had less than 3% missing. History of regular maternal smoking had less than 3% missing and was included as a confounder in a sensitivity analysis.

Results

Thyroid dysfunction and sample characteristics

285 pregnancies resulting in 289 children from 273 mothers were analyzed. 285 children were analyzed because four pregnancies resulted in twins and only the twin with the lowest ID was included in the main analysis. An additional 9 pregnancies had thyroid measures but did not have child outcomes so were not included in the analysis. The majority of pregnancies (83.9%, n= 239) were euthyroid. The remaining pregnancies were either affected by hypothyroid conditions (9.5% n=27) or hyperthyroid conditions (6.7%, n=19). Hypothyroid conditions included 15 cases of subclinical hypothyroidism and 12 cases of hypothyroxinemia. No pregnancies were affected by overt hypothyroidism. Hyperthyroid conditions included 7 cases of subclinical hyperthyroidism, 3 cases of overt hyperthyroidism, and 9 cases of hyperthyroxinemia (Table A2.2).

Significance testing did not find any significant differences across thyroid groups and participant demographics (Table 2.1). The hypothyroid group had a non-significantly higher prevalence of history of regular maternal smoking at any time (29.17%) and a non-significantly higher prevalence of obesity (40.74%) compared to the other groups (Table 2.1). Mothers with hyperthyroid conditions were non-significantly more likely to have planned pregnancies (78.95%) and more likely to have an interpregnancy interval of less than 12-months (15.79%) compared to the other groups. About 42% of

pregnancies affected by hyperthyroid conditions had a first trimester blood draw, compared to 37% of hypothyroid pregnancies and 33% of euthyroid pregnancies, though those were not significantly different (Table 2.2). The average gestational age of samples provided per pregnancy was similar across thyroid function categories at around 26-28 weeks. Mothers with hypothyroid conditions during pregnancy were significantly more likely to be TG and/or TPO antibody positive (25.93%) compared to euthyroid mothers (11.72%). No hyperthyroid mothers were antibody positive. Less than 4% of euthyroid mothers self-reported a thyroid condition or thyroid medication use compared to about 20-25% of mothers with hypothyroid or hyperthyroid conditions (Table 2.2, Table A2.3). Maternal and child demographic characteristics were similarly distributed across trimesters (Table A2.20).

Thyroid function and child diagnostic categories

From the 285 children analyzed, 56.5% had typical development (n=161; female=77), 28.8% had an ASD diagnosis (n=82; female=27), and 14.7% had non-TD development (n=42; female=19). Distribution of child diagnostic categories differed significantly across thyroid function groups (Fisher's exact p=0.02) (Table 2.2). Children born to mothers with hypothyroid conditions during pregnancy were more likely to have an ASD diagnosis (44.44%) or have non-TD development (25.93%) compared to the other thyroid condition groups. Children born to mothers with hyperthyroid conditions during pregnancy were more likely to have non-TD development (21.05%) compared to children born to euthyroid mothers (12.97%), but prevalence of child ASD was higher among euthyroid mothers (28.03%) than mothers with hyperthyroid conditions. Child's median age at diagnostic assessment was similar across thyroid function groups (36-37 months), though the age range was larger in the euthyroid group (Table 2.2).

SRS and MSEL scores

Of the 285 pregnancies with thyroid measures and child diagnostics, 228 pregnancies had an SRS score Children born to mothers with hypothyroid conditions had a significantly higher median SRS score (median 52.0, range 41-89) compared to children born to euthyroid mothers (median 47.0, range 35-95)

(p=0.047). Median SRS scores for children born to mothers with hyperthyroid conditions did not differ significantly from scores of children born to euthyroid mothers. The median child age at SRS (school age or preschool form, whichever was used) was slightly higher among the hypothyroid group, though these differences were not significant (Table 2.2). MSEL composite scores were available for 264 pregnancies. Children born to mothers with hypothyroid conditions had lower MSEL composite scores (median 92.5, range 50-136) compared to children born to euthyroid mothers (median 99.0, range 49-149) or mothers with hyperthyroid conditions (median 99.0, range 75-130), though these differences were not significant. Child's median age when the MSEL was administered was similar across thyroid function groups (Table 2.2).

Regression analysis

Thyroid dysfunction predicting ASD Maternal hypothyroid conditions during pregnancy were significantly associated with 3.17 times the odds of ASD diagnosis compared to typical development (95% CI: 1.13, 8.93; p=0.03) after adjusting for confounders (health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity) (Table 2.3). Maternal hyperthyroid conditions during pregnancy were non-significantly associated with 0.43 times the odds of ASD diagnosis (95% CI: 0.11, 1.67; p=0.23) after adjustment (Table 2.3). Unadjusted results showed similar findings, where hypothyroid conditions were significantly associated with 3.16 times the odds of ASD (95% CI: 1.21, 8.25; p=0.01) and hyperthyroid conditions were non-significantly associated with 0.53 the odds (95% CI: 0.14, 1.97; p=0.08) (Table A2.4). Prior to covariate imputation, hypothyroid conditions were marginally associated with 3.05 times the odds of ASD (95% CI: 0.98, 9.49; p=0.05) and hyperthyroid conditions were non-significantly associated with 0.67 times the odds (95% CI: 0.17, 2.61; p=0.55) (Table A2.5). Variance inflation factors were below 5.00 for all variables.

Thyroid dysfunction predicting non-TD development Maternal hypothyroid conditions during pregnancy were significantly associated with 4.27 times the odds of non-TD compared to typical development (95% CI: 1.36, 13.38; p=0.01) after adjusting for maternal and child demographic characteristics (Table 2.3).

Maternal hyperthyroid conditions during pregnancy were non-significantly associated with 1.28 times the odds of non-TD (95% CI: 0.28, 5.83; p=0.75) after adjustment (Table 2.3). Unadjusted results show that hypothyroid conditions were non-significantly associated with 3.98 times the odds of non-TD (95% CI: 1.32, 11.96; p=0.051) and hyperthyroid conditions were non-significantly associated with 1.52 the odds (95% CI: 0.45, 5.10; p=0.67) (Table A2.4). Prior to covariate imputation, hypothyroid conditions were non-significantly associated with 1.95 times the odds of non-TD (95% CI: 0.44, 8.58; p=0.38) and hyperthyroid conditions were associated with 1.45 times the odds (95% CI: 0.25, 8.24; p=0.68) (Table A2.5).

Thyroid dysfunction predicting SRS and MSEL scores Quantile regressions were conducted on the 25th, 50th, and 75th percentiles of SRS scores and MSEL scores using models adjusted for maternal and child characteristics. No coefficients were associated with statistically significant changes in SRS scores except for hyperthyroid conditions at the 50th percentile (-5.88, 95% CI: -10.19, -1.56; p=0.01) (Table 2.4). For hypothyroid conditions, effect measures indicated an increase in SRS scores at all percentiles while hyperthyroid conditions indicated a decrease in SRS scores (Table 2.4). No relationships between thyroid dysfunction and MSEL scores were statistically significant and no clear trends in direction of effect were identified (Table 2.5).

TSH and FT4 predicting ASD diagnosis For each trimester, the natural log (ln)-transformed level of maternal TSH was associated with increased adjusted relative risk ratios of child ASD compared to typical development, though this relationship was only significant in the first trimester (Figure 1). A one unit increase in first trimester ln of TSH was associated with 3.24 times the odds of ASD (95% CI: 1.02, 10.31; p=0.046) (Figure 1, Table A2.15). In second trimester, the ln of TSH was non-significantly associated with 1.54 times the odds of ASD (95% CI: 0.86, 2.76, p=0.15) and in third trimester, 1.53 times the odds (95% CI: 0.93, 2.53, p=0.09). The ln of maternal FT4 was not significantly associated with ASD diagnosis in any trimester, though odds ratios indicated decreased odds of child ASD in each

trimester. Neither the ln of TSH nor the ln of FT4 was significantly associated with non-TD development for any trimester (Figure 2.1, Tables A2.15).

TSH and FT4 predicting SRS and MSEL scores First trimester ln of TSH was significantly associated with an increase in SRS scores at the 75th percentile (adjusted coefficient 4.72, 95% CI: 1.38, 8.05; p=0.01), but no other relationships between ln of TSH or FT4 and SRS reached significance (Figure 2.2A, Figure 2.2B, Table A2.17). Within first trimester, all ln of TSH coefficients were positive and increased in magnitude when moving from the 25th to the 75th percentile. The ln of FT4 was associated with a non-significant decrease in SRS scores for all percentiles across all trimesters and there were no clear patterns across percentiles within trimester.

For MSEL scores, the only significant relationship was in the first trimester, between ln of TSH and the 25th percentile of MSEL scores (adjusted coefficient -10.01, 95% CI: -18.33, -1.7; p=0.02) (Figure 2.3A, Figure 2.3B, Tables A2.18). Ln of TSH coefficients for the 25th MSEL percentile were all negative and typically moved towards the null when moving from the 25th to the 75th percentile within trimesters. For each trimester, ln of FT4 was associated with a non-significant increase in MSEL scores at the 25th and 50th percentiles. At the 75th percentile, ln of FT4 was around the null value for all trimesters.

Additional analyses and sensitivity tests Maternal antibody status and history of regular maternal smoking were added separately to the adjusted logistic regression predicting the effect of maternal thyroid dysfunction on ASD diagnosis and non-TD development. Adjusted relative risk ratio (ARRR) were comparable to the main findings presented in Table 2.3. The relationship between hypothyroid conditions and both outcomes remained similar after adding antibody status (ASD: ARRR with antibody status 3.22, 95% CI: 1.13, 9.22; p=0.03, non-TD: ARRR with antibody status 4.15, 95%: 1.28, 13.46; p=0.02) (Table A2.6). No pregnancies affected by hyperthyroid conditions were antibody positive. Adjusted relative risk ratios (ARRR) for hypothyroid conditions attenuated slightly after the addition of history of regular maternal smoking for the ASD outcome (ARRR with smoking 3.02, 95% CI: 1.07-8.53; p=0.04) (Table A2.7) or the non-TD outcome (ARRR with smoking 3.95, 95% CI: 1.26, 12.36; p=0.02) (Table A2.7).

Adjusted relative risk ratios for hyperthyroid conditions remained similar after the addition of the maternal smoking variable for the ASD outcome (ARRR with smoking 0.44, 95% CI: 0.12, 1.69; p=0.23) (Table A2.7) and the non-TD outcome (ARRR with smoking 1.37, 95% CI: 0.29, 6.35; p=0.73) (Table A2.7).

Adjusting for either maternal self-reported thyroid conditions or self-reported thyroid medication use strengthened the association between hypothyroid conditions and ASD (ARRR thyroid conditions 4.15, 95% CI: 1.33, 12.98; p=0.01, ARRR thyroid medications 3.77, 95% CI: 1.22, 11.59, p=0.02) and the relationship between hypothyroid conditions and non-TD (ARRR thyroid conditions 5.90, 95% CI: 1.69, 20.57; p=0.01, ARRR thyroid medication 5.03, 95% CI: 1.50, 16.89; p=0.01) (Tables A2.13 and A14). For hyperthyroid conditions, adjusting for either of these self-report measures attenuated the relative risk ratios for ASD (ARRR thyroid conditions 0.56, 95% CI: 0.14, 2.24; p=0.42, ARRR thyroid medication 0.50, 95% CI: 0.11, 2.15; p=0.35) but slightly strengthened the relationship for the non-TD outcome (ARRR thyroid condition 1.79, 95% CI: 0.35, 9.06; p=0.48, ARRR thyroid medication 1.47, 95% CI: 0.30-7.17; p=0.63) (Tables A2.13 and A2.14).

Thyroid dysfunction did not predict the child diagnostic outcome differently depending on the number of maternal blood draws or the sex of the child. Interactions between the number of maternal blood draws and thyroid dysfunction were non-significant for the ASD outcome (hypothyroid×numbers of blood draws p=0.89; hyperthyroid×numbers of blood draws p=0.90) and for the non-TD outcome (hyperthyroid X numbers of blood draws p=0.97) (Table A2.8). An interaction between hypothyroid conditions and number of blood draws was omitted for the non-TD outcome because no hypothyroid conditions occurred among mothers with three blood draws. Interactions between sex of the child and thyroid dysfunction were non-significant for the ASD outcome (hypothyroid×sex of child p=0.34; hyperthyroid×sex of child p=0.82) (Tables A2.9, A2.11, A2.12) and for the non-TD outcome (hypothyroid×sex of child p=0.33; hyperthyroid X sex of child p=0.13) (Tables A2.9). The adjusted relative risk ratio for hypothyroid conditions and ASD diagnosis among males was 4.30 (95% CI: 1.09,

17.04; p=0.04), a stronger association than what was found for males and females combined (ARRR 3.17, 95% CI: 1.13, 8.93; p=0.03) (Table A2.12, Table 2.3). Adjusted relative risk ratios were not calculated for females because of zero cells for covariates in this group.

The analytic sample of 285 pregnancies included 10 mothers who contributed two pregnancies, and one mother who contributed three pregnancies. The multinomial logistic regression predicting the effect of maternal thyroid dysfunction on ASD diagnosis and non-TD development was run again only including the first enrolled pregnancy for each mother, so that each mother only contributed one pregnancy. For the ASD outcome, the relative risk ratio for hypothyroid conditions attenuated slightly after removing the sibling pregnancies (ARRR no siblings 3.52, 95% CI: 1.27, 9.77; p=0.0157) as did the relative risk ratio for hyperthyroid conditions (ARRR no siblings 0.44, 95% CI: 0.11, 1.82; p=0.2557) (Table A2.10). For the non-TD outcome, the relative risk ratios for both types of thyroid dysfunction strengthen slightly after removing sibling pregnancies (hypothyroid conditions: ARRR no siblings 3.84, 95% CI: 1.11, 13.26; p=0.0333. hyperthyroid conditions: ARRR 1.43, 95% CI: 0.37, 5.50; p=0.6046) (Table A2.10). Results based on including the other twin were similar to as before for both developmental outcomes (Table A2.21).

For five children, their only SRS score was from a school age form completed at less than recommended age of 48 months. The model predicting the effect of thyroid dysfunction on SRS percentiles was re-run without these five children. Results showed a similar trend as the model with these children (hypothyroid conditions indicative of increased SRS score, hyperthyroid conditions indicative of decreased SRS score) and the relationship between hyperthyroid conditions and the 50^{th} percentile of SRS scores remained significant (ARRR age-appropriate forms only -5.94, 95% CI: -10.56, -1.32; p=0.0117) (Table A2.16). The ratios of standard errors for thyroid dysfunction and ln of TSH from linear regressions without and without clustering by family was 0.99 or 1.00 so standard error of quantile regressions was not adjusted.

Discussion

Prevalence of thyroid dysfunction

Compared to pregnancy prevalence estimates from the general population, MARBLES pregnancies were more likely to be affected by thyroid dysfunction. 9.5% of MARBLES pregnancies were categorized as having a hypothyroid condition, whereas the population prevalence of hypothyroid conditions during pregnancy is estimated to be closer to 6% (combined prevalence of subclinical and overt hypothyroxinemia and hypothyroxinemia).[27,29] The most common hypothyroid conditions in the MARBLES sample were subclinical hypothyroidism (5.3% of pregnancies) followed by hypothyroxinemia (4.2% of pregnancies) (Table A2.2). These are also the most commonly occurring conditions in population estimates. Historically, overt (severe) maternal hypothyroidism has been associated with cognitive disabilities in children. However the introduction of iodine supplementation has made this type of thyroid dysfunction uncommon during pregnancy in western countries[8] and overt hypothyroidism was not observed in this study. Overt hypothyroidism is also associated with lower fertility and greater risk of miscarriage. [27,47–49] This could contribute to selection bias in the present study because developmental outcomes are only known for born children.

Hyperthyroid conditions were also overrepresented in the MARBLES sample compared to the population pregnancy estimates. Combined prevalence of subclinical and overt hyperthyroidism during pregnancy in population estimates was about 2.4%[27,29], while about 3.5% of MARBLES pregnancies were categorized with these conditions. Hyperthyroxinemia, which affected 3.2% of pregnancies in this sample, was included in the hyperthyroid conditions group (Table A2.2). There are no existing prevalence estimates for hyperthyroxinemia during pregnancy, which can result from Grave's disease, excess use of thyroid replacement hormones, or may be a transient response to changes in human chorionic gonadotropin (hCG) hormone that is released during pregnancy.[50] When overt hyperthyroidism during pregnancy is transient (gestational transient thyrotoxicosis), it is not typically associated with pregnancy

loss. When part of an on-going condition like Grave's disease, overt hyperthyroidism can adversely affect fertility and increase the risk of miscarriage.[27]

The prevalence of thyroid dysfunction may be higher in this sample compared to population estimates for several reasons. First, average maternal age at delivery in this sample was close to 35 years (Table 2.1) and risk of thyroid dysfunction during pregnancy likely increases after age 30.[16] Second, because MARBLES is a high likelihood ASD cohort, all participating mothers already have a child diagnosed with ASD. If maternal thyroid dysfunction is a causal factor for ASD, then mothers in this cohort might be more likely to have characteristics associated with ASD development compared to mothers in the general population.

Thyroid dysfunction and child diagnostic outcomes

Maternal hypothyroid conditions during pregnancy were associated with over three times the odds of child ASD compared to typical development (ARRR 3.17, 95% CI: 1.13, 8.93; p=0.03) after adjusting for demographic characteristics. Though this finding was statistically significant, it should be interpreted with caution because confidence intervals were wide, and the number of pregnancies affected by hypothyroidism was small (12 cases among the ASD group and 8 among the typically developing group). The significant association between maternal hypothyroid conditions and ASD was maintained after adjusting for TPO and/or TG antibody positivity, history of regular maternal smoking, self-reported history of thyroid conditions, self-reported thyroid medication use, and limiting the sample to one pregnancy per mother. Unlike hypothyroid conditions which were associated with increased likelihood of ASD, hyperthyroid conditions showed estimates in the direction of decreased likelihood, though this relationship was not significant in any models.

Multiple previous studies, but not all, have also identified significant positive associations between maternal hypothyroid conditions and child ASD diagnosis. Four population-based cohort studies based in Denmark, California, and Israel found that mothers with a hypothyroidism diagnosis were more

likely to have a child diagnosed with ASD.[51–54] Three of these studies also examined hyperthyroidism, though only one (a population-based study in Denmark) found a significant association with ASD diagnosis.[52] Another population-based study in Taiwan, which only examined hyperthyroidism, found a significant association between maternal hyperthyroidism and ASD diagnosis.[55] A Finnish-based population study found no association between any type of maternal thyroid dysfunction and ASD diagnosis, though it did find a positive association with maternal antibody positivity,[56] which the present study did not. These studies used medical records to ascertain thyroid status and ASD diagnoses, which could lead to misclassification bias because not all participants are evaluated for the exposure and the outcome. This is of special concern with less severe (subclinical) thyroid conditions which frequently do not cause symptoms. Screening for thyroid conditions during pregnancy is typically recommended based on individual maternal risk factors (e.g. history of thyroid disease or older age) rather than for all pregnancies.[16,57] The present study addresses these limitations by evaluating all pregnancies for thyroid dysfunction and all children for ASD.

Maternal hypothyroid conditions likely play a role in the etiology of other neurodevelopment conditions in addition to ASD. In this study, maternal hypothyroid conditions exhibited a similar positive association with the non-TD outcome, though confidence intervals were wider than for the ASD outcome and even fewer children fell into the non-TD group. Recently, research has focused on the relationship between less severe thyroid conditions and child developmental outcomes. Findings from a meta-analysis suggest hypothyroxinemia and subclinical hypothyroidism may be associated with intellectual impairment in the child.[58]

Thyroid measures and child continuous outcomes

Maternal thyroid dysfunction was not predictive of SRS or MSEL scores at most percentiles. The exception was a significant six-point decrease (a little over half a standard deviation) in median SRS scores (lower ASD traits) among the hyperthyroid conditions group compared to the euthyroid group. ASD diagnosis and SRS outcomes showed a similar trend where hypothyroid conditions suggested an

increase in ASD or SRS scores while hyperthyroid conditions suggested decreases (Table 2.3, Table 2.4). Though hyperthyroid conditions appear to be associated with reduced likelihood of ASD diagnosis or ASD traits in this sample, hyperthyroid conditions might still contribute to other types of non-typical development (hyperthyroid conditions were non-significantly associated with increased odds of non-TD, Table 2.3).

For TSH and FT4 analyzed by trimester, the expectation was that first trimester values would show a greater association with child outcome measures compared to subsequent trimesters. Women are less likely to know that they are pregnant earlier in first trimester and may not have yet had a prenatal visit where thyroid function could be measured. The neonate is more reliant on maternal thyroid hormone production earlier in pregnancy and thus neurodevelopment may be more sensitive to abnormal maternal thyroid hormone concentrations prior to around 14 weeks of gestation.[59] This hypothesis was supported for TSH in some outcomes, but not for FT4 in any outcome. In this study, natural log of TSH was most strongly associated with increased odds of ASD diagnosis in the first trimester, with relative risk ratios attenuating in following trimesters. However, the first trimester had the fewest number of pregnancies with blood draws (n=96) and confidence intervals were widest in this trimester. MSEL scores showed a similar pattern in its relationship with TSH, where a unit increase in the natural log of TSH was most strongly associated with a decreased MSEL composite score at the 25th and 50th percentiles in the first trimester compared to later trimesters. These findings align with some existing research identifying underactive maternal thyroid as a risk factor for hampered cognitive development.[58]

Natural log of FT4 did not appear to be associated with ASD, SRS, or MSEL scores for any trimester. When FT4 levels are low, the pituitary gland responds by increasing production of TSH, which in turn stimulates the thyroid gland to produce T4 and T3 until its levels are high enough to trigger a reduction in TSH production. During pregnancy, increased secretion of hCG can also increase TSH production.[60] Abnormal FT4 levels can indicate more severe thyroid dysfunction compared to when TSH alone is affected (e.g. the body was not able to maintain appropriate FT4 levels through regulating TSH).

In adults, TSH is typically a more sensitive measure of thyroid function compared to FT4. This may make it more likely to identify relationships between TSH and child outcomes in this sample (especially given that most thyroid dysfunction was mild), and women with severe thyroid disfunction would be less likely to become pregnant.[47–49]

In previous studies, findings on TSH or FT4 and child outcomes tend be inconsistent, especially in comparison to research using categorical thyroid dysfunction. One study conducted in Denmark found no association between first trimester maternal TSH or FT4 and ASD or ADHD diagnosis or standardized school test scores.[61] Another Danish study conducted about 10 years earlier found a significant positive association between maternal hypothyroxinemia and SRS scores, but found no association between TSH or FT4 and SRS.[62] Using pooled data from several European cohorts, a study found no association between TSH or FT4 and possible autism based on the combination of several measures (Childhood Autism Spectrum Test, Child Behavior Checklist for Toddlers, and Social Communication Disorder Checklist).[63] Children were considered to have high autism traits when they scored above the clinical cut point for the measure used in their cohort. A study which pooled two U.S. cohorts found significant associations between TSH and FT4 at 16 weeks of gestation and SRS scores at the 95th percentile, but not at the 75th, 50th, 25th, or 5th percentile.[45] Authors reported that the standardized SRS score represented by the 95th percentile was above the clinical cut point for SRS scores of 60 percentile points.[45] However, if the 95th percentile SRS score was higher than the clinical cut point, children classified as having high autistic traits in the U.S. study may have been more severely affected than children classified as having high autistic traits in the European meta-analysis. Lastly, another study based in California found that low levels of TSH in their sample were associated with increased chance of ASD diagnosis or developmental disability (based on MSEL and Vineland scores), though FT4 was not measured.[64]

The present study did not find evidence supporting effect modification of thyroid dysfunction by sex of the child. One previous study found a significant interaction between maternal hypothyroxinemia (grouped as a hypothyroid condition in the present study) and females using ASD diagnosis as the

outcome.[57] The authors did not find evidence for an interaction between sex and subclinical or overt hypothyroidism or hyperthyroidism.[57] However, another study found no interaction between sex of the child and hypothyroxinemia using the SRS as an outcome.[62] Two additional studies tested interactions between sex of the child and maternal hypothyroidism (without distinguishing between overt and subclinical) using ASD diagnosis as an outcome, though neither found a significant interaction.[53,54] Neither of these two studies tested interactions for other types of thyroid dysfunction.

Limitations, strengths, and future research

This study was not without limitations. The relatively small sample size required thyroid conditions to be grouped as hypothyroid or hyperthyroid conditions and individual types of thyroid conditions could not be analyzed separately. Similarly, more extreme ends of SRS or MSEL distributions (5^{th} or 95^{th} percentiles) could not be analyzed as an outcome, though prior research has found significant relationships with thyroid measures at high percentiles of SRS scores.[45] First trimester thyroid measures were not available for most pregnancies (though maternal and child demographic characteristics were similar for each trimester). When first trimester blood draws were available, the average gestational age was 10-11 weeks (SD 2.16) which is around the window when the neonate is most reliant on maternal thyroid function.[59]

Because MARBLES is a high familial likelihood cohort, this sample may not be representative of families in the general population, though findings from population-based studies also support a positive association between maternal hypothyroid conditions and ASD. The prevalence of thyroid dysfunction was higher in this sample than expected and the underlying causes of thyroid dysfunction participants was not evaluated. If some cases of thyroid dysfunction were caused by an exposure that could also drive ASD development (such as exposure to PFAS[22]), this would be important to identify because targeting this exposure could address multiple health conditions. Survival bias is a concern in this study because more severe forms of thyroid dysfunction reduce fertility and increase risk of miscarriage. To participant in this study, women needed to previously give birth to a child who survived until they were old enough to

receive an ASD diagnosis and then plan a second pregnancy. Based on this criteria, women who have on-going fertility challenges from thyroid conditions could be excluded from this study.

This present study adds to a body of research supporting a relationship between maternal hypothyroidism during pregnancy and increased likelihood of ASD diagnosis. Discussions surrounding how these findings should be further integrated into recommendations for thyroid screening and treatment during pregnancy are needed. Currently, screening and treatment guidelines consider the impact of thyroid dysfunction on the mother's health during pregnancy, risk of delivery complications, and the child's development but there is potential to include more updated findings on ASD,[16] including in families already having an ASD-affected child.

Conclusion

This study found that maternal hypothyroid conditions during pregnancy were associated with an increased likelihood of ASD diagnosis in the child. Relationships were also found between maternal hypothyroid conditions and non-TD development and first trimester TSH and some percentiles of SRS and MSEL scores. Maternal thyroid dysfunction may not be an etiological factor that is unique to ASD. Public health implications of maternal hypothyroidism during pregnancy on ASD and other child developmental outcomes should continue to be monitored. Guidelines for pregnancy thyroid screening should take a holistic approach by considering how thyroid dysfunction during pregnancy could affect the development of ASD, other child developmental outcomes, and affect the health of the mother.

Tables and figures

Table 2.1. Mother and child characteristics by maternal thyroid dysfunction condition group[1], n (%) unless otherwise noted (n=285 pregnancies)			
	Euthyroid (n=239)	**Hypothyroid (n=27)**	**Hyperthyroid (n=19)**
Maternal characteristics			
Gestational or type 2 diabetes[2]	47 (19.75)	7 (25.93)	6 (31.58)
Regularly smoker at any time[2]	40 (16.81)	7 (29.17)	2 (10.53)
Prenatal vitamin use in first month of pregnancy[3]	106 (49.07)	11 (50.00)	8 (44.44)
Pregnancy intended at that time[2]	142 (59.66)	12 (48.00)	15 (78.95)
Interpregnancy interval under 12 months[2]	9(3.86)	1(4.17)	3(15.79)
Mean maternal age at delivery, SD	239 (34.63, 4.90)	27 (35.51, 3.65)	19 (34.24, 4.71)
Maternal pre-pregnancy BMI			
Not obese	175 (73.22)	16 (59.260	15 (78.95)
Obese	64 (26.78)	11 (40.74)	4 (21.05)
Highest level of education			
Less than High school or High school/GED	21 (8.79)	2 (7.41)	2 (10.53)
Some college	95 (39.75)	12 (44.44)	13 (68.42)
Bachelor's or higher	123 (51.46)	13 (48.15)	4 (21.05)
Health insurance[4]			
Private insurance	159 (76.81)	17 (80.95)	12 (75.00)
Public insurance	48 (23.19)	4 (19.05)	4 (25.00)
Homeownership[2]			
Renter	105 (44.68)	11 (44.00)	9 (47.37)
Owner	130 (55.32)	14 (56.00)	10 (52.63)
Child characteristics			
Child race and ethnicity			
White, non-Hispanic	82 (34.31)	7 (25.93)	7 (36.84)
Other, non-Hispanic	35(14.64)	6 (22.22)	3 (15.79)
Asian, non-Hispanic	36 (15.06)	3 (11.11)	2 (10.53)
Hispanic, any race	86 (35.98)	11 (40.74)	7 (36.84)
Child sex (female)	102 (42.68)	11 (40.74)	10 (52.63)
Median gestational age at delivery (range), weeks	239 (39.14, 28.29 - 41.43)	27 (39.14, 37.57-41.71)	19 (39.57, 37.71-41.14)
Median child year of birth, range	239 (2014, 2009-2020)	27 (2012, 2009-2019)	19 (2014, 2009-2019)
Child breast feed after 3 weeks	220 (92.05)	25 (92.59)	19 (100.00)
1. Significance testing found no significant differences ($p<0.05$) across thyroid function categories for any covariates 2. Missing less than 3% 3. Missing 10% 4. Missing 14%			

Table 2.2. Maternal thyroid and child development characteristics by maternal thyroid dysfunction condition group, percentage unless otherwise noted			
	Euthyroid (n=239)	**Hypothyroid (n=27)**	**Hyperthyroid (n=19)**
Maternal thyroid-related characteristics			
Number of blood draws in pregnancy			
Once	82 (34.31)	5 (18.52)	3 (15.79)
Twice (two different trimesters)	88 (36.82)	12 (44.44)	9 (47.37)
Three (once in each trimester)	69 (28.87)	10 (37.04)	7 (36.84)
First trimester blood draw	78 (32.64)	10 (37.04)	8 (42.11)
Mean gestational age at blood draw in weeks, SD	239 (27.54, 6.26)	27 (26.25, 4.62)	19 (27.09, 4.73)
Thyroid auto-antibody positivity[1]			
Antibody negative	211 (88.28)	20 (74.07)	19 (100.00)
TPO and/or TG antibody positive	28 (11.72)	7 (25.93)	0 (0.00)
Self-reported thyroid condition[1]	9 (3.77)	7 (25.93)	5 (26.32)
Self-reported thyroid medication[1,2]	4 (1.67)	5 (19.23)	5 (26.32)
Child neurodevelopmental outcome characteristics			
Diagnostic group[1]			
Typically developing	141 (59.00)	8 (29.63)	12 (63.16)
ASD	67 (28.03)	12 (44.44)	3 (15.79)
Non-typical development, not ASD	31 (12.97)	7 (25.93)	4 (21.05)
Diagnostic age (months)			
Median, range	239 (36.0, 15-46)	27 (37.0, 36-47)	19 (36.0, 35-38)
Social responsiveness scale standard total score[3,4]			
Median (range)	195 (47.0, 35-95)	16 (52.0, 41-89)	17 (44.0, 35-83)
Age in months at Social Responsiveness Scale[5]			
Median (range)	194 (72.0, 35-166)	15 (77.0, 36-143)	17 (64.0, 36-138)
Mullen Scales of Early Learning Composite, standard score[6]			
Median, range	221 (99.0, 49 - 149)	24 (92.5, 50-136)	19 (99.0, 75-130)
Age in months at Mullen early learning composite			
Median (range)	221 (37.0, 31-64)	24 (37.0, 36-47)	19 (36.0, 35-38)

1. Fisher's exact p-value<0.05
2. <1% missing
3. Wilcoxon test of euthyroid vs. hypothyroid p-value<0.05
4. 20% missing (n=228)
5. <1% of pregnancies with SRS scores are missing child age at SRS
6. 7.4% missing (n=264)

Table 2.3. Association between thyroid dysfunction and child diagnostic outcome, adjusted relative risk ratio (RRR) and 95% confidence interval[1]			
	ASD (n=82) compared to TD (n=161)		
Variable	RRR	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.17	(1.13, 8.93)	0.0290
Hyperthyroid conditions	0.43	(0.11, 1.67)	0.2249
	Non-TD (n=42) compared to TD (n=161)		
	RRR	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.27	(1.36, 13.38)	(0.0128)
Hyperthyroid conditions	1.28	(0.28, 5.83)	(0.7518)
1. Multinomial model adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity. ASD, Autism Spectrum Disorder Non-TD, non-typical/non-autism spectrum disorder development TD, typically developing			

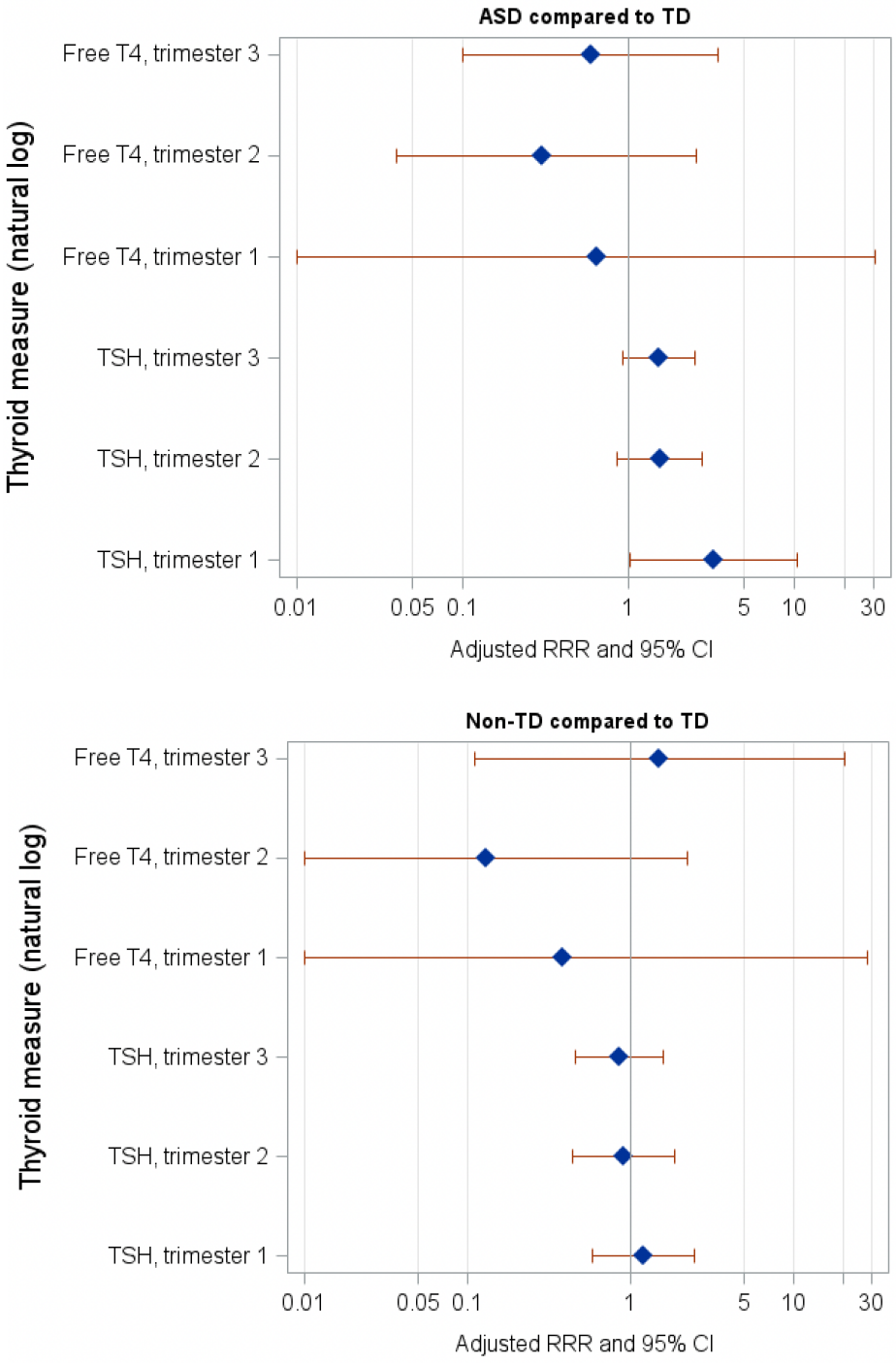
Table 2.4. Associations between maternal thyroid dysfunction and 25th, 50th, and 75th percentile SRS scores, change in percentage point, adjusted coefficients and 95% confidence interval[1] (n=228 pregnancies)

	25th percentile		
Variable	Coeff	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	2.87	(-3.24, 8.98)	0.3569
Hyperthyroid conditions	-2.71	(-6.68, 1.25)	0.1796
	50th percentile		
Variable	Coeff	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.87	(-6.24, 15.98)	0.3904
Hyperthyroid conditions	-5.88	(-10.19, -1.56)	0.0076
	75th percentile		
Variable	Coeff	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	2.97	(-18.25, 24.18)	0.7841
Hyperthyroid conditions	-9.03	(-19.14, 1.09)	0.0803

1. Quantile regressions adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.

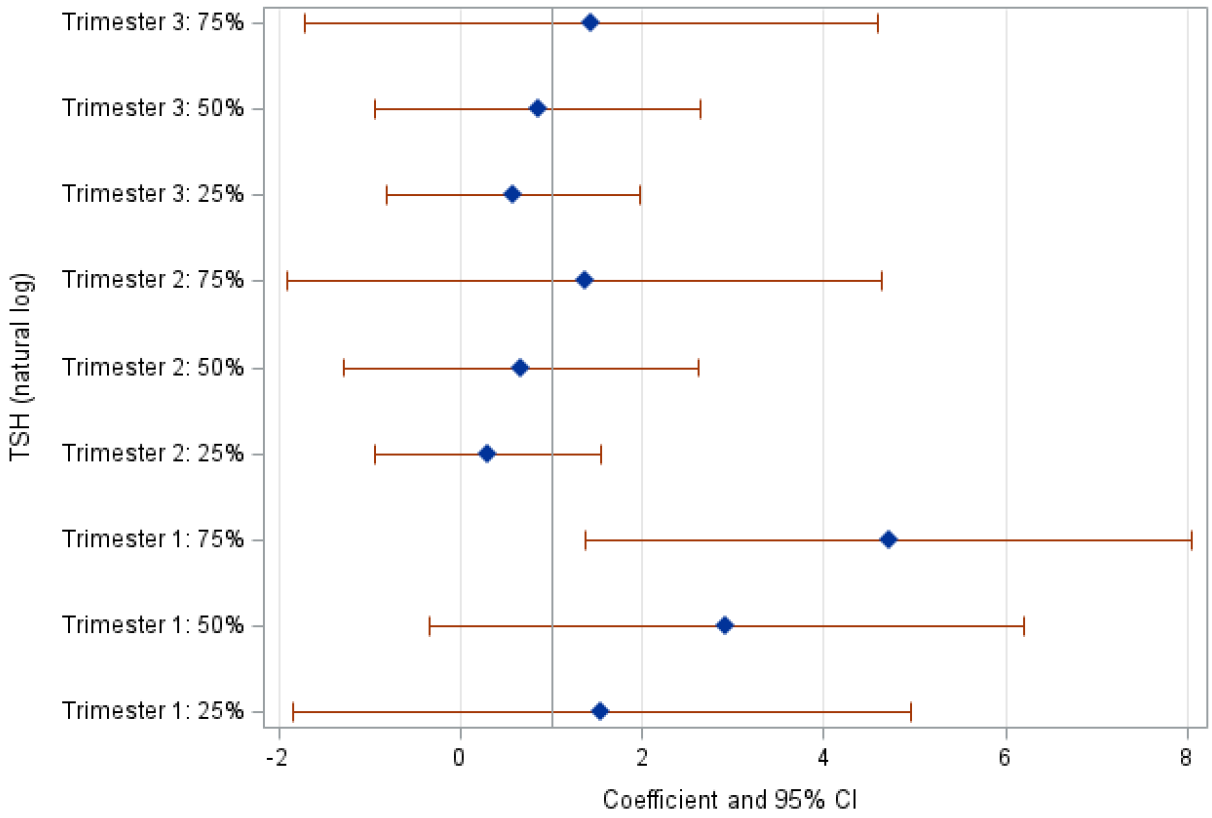
Table 2.5. Associations between maternal thyroid dysfunction and 25th, 50th, and 75th percentile MSEL composite scores, change in percentile point, adjusted coefficients and 95% confidence intervals[1] (n=264 pregnancies)			
	25th percentile		
Variable	Coeff	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.03	(-22.14, 28.21)	0.8133
Hyperthyroid conditions	12.02	(-3.66, 27.69)	0.1330
	50th percentile		
Variable	Coeff	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	-3.46	(-16.97, 10.06)	0.6159
Hyperthyroid conditions	4.86	(-7.18, 16.9)	0.4289
	75th percentile		
Variable	Coeff	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	-2.28	(-15.32, 10.77)	0.7324
Hyperthyroid conditions	-0.03	(-12.62, 12.57)	0.9969
1. Quantile regressions adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.			

Figure 2.1. Association between log of TSH or log for free T4 and diagnostic outcome compared to TD. Analysis of pregnancies by trimester of blood draw.[1] (First trimester n=96, Second trimester n=199, Third trimester n=272).



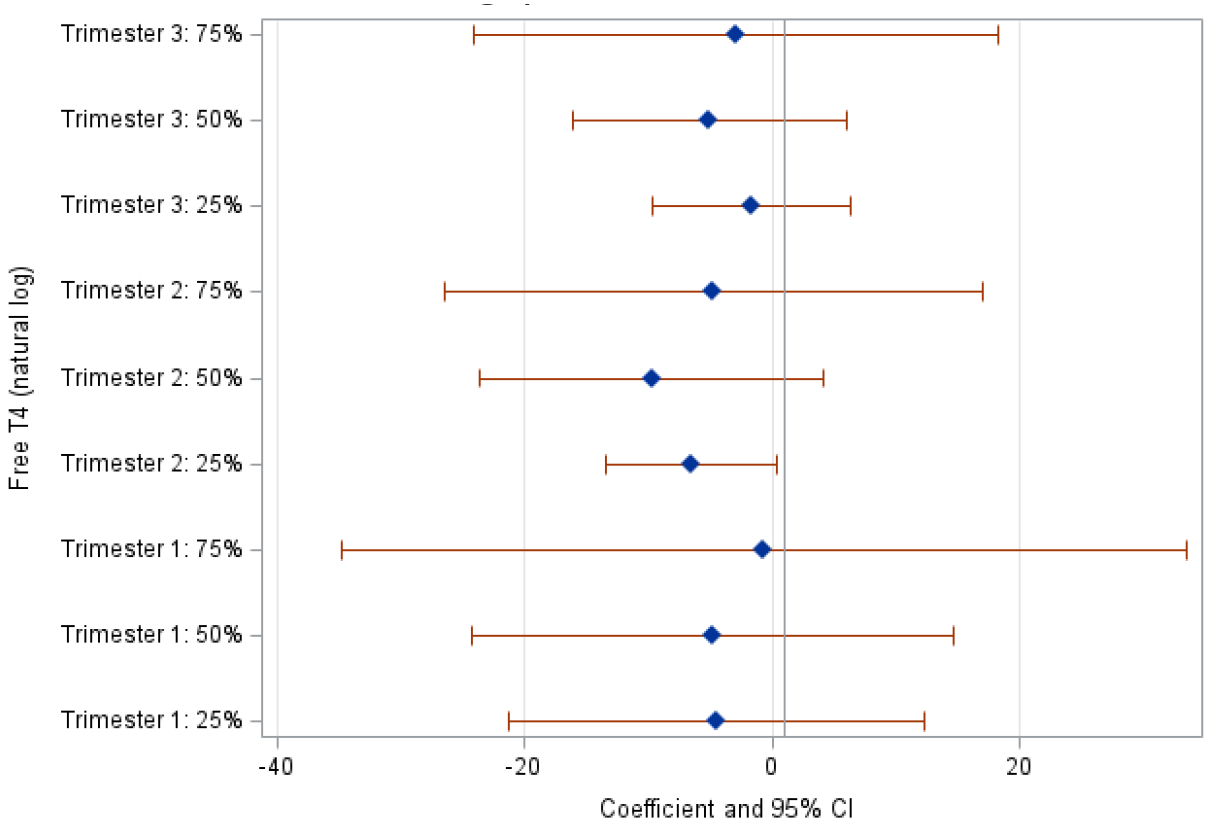
1. Multinomial regression adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.
TSH, thyroid stimulating hormone
Free T4, free thyroxine

Figure 2.2a. Association between natural log of TSH and SRS percentiles. Analysis of pregnancies by trimester of blood draw.[1] First trimester n=85, second trimester n=167, third trimester n=215.



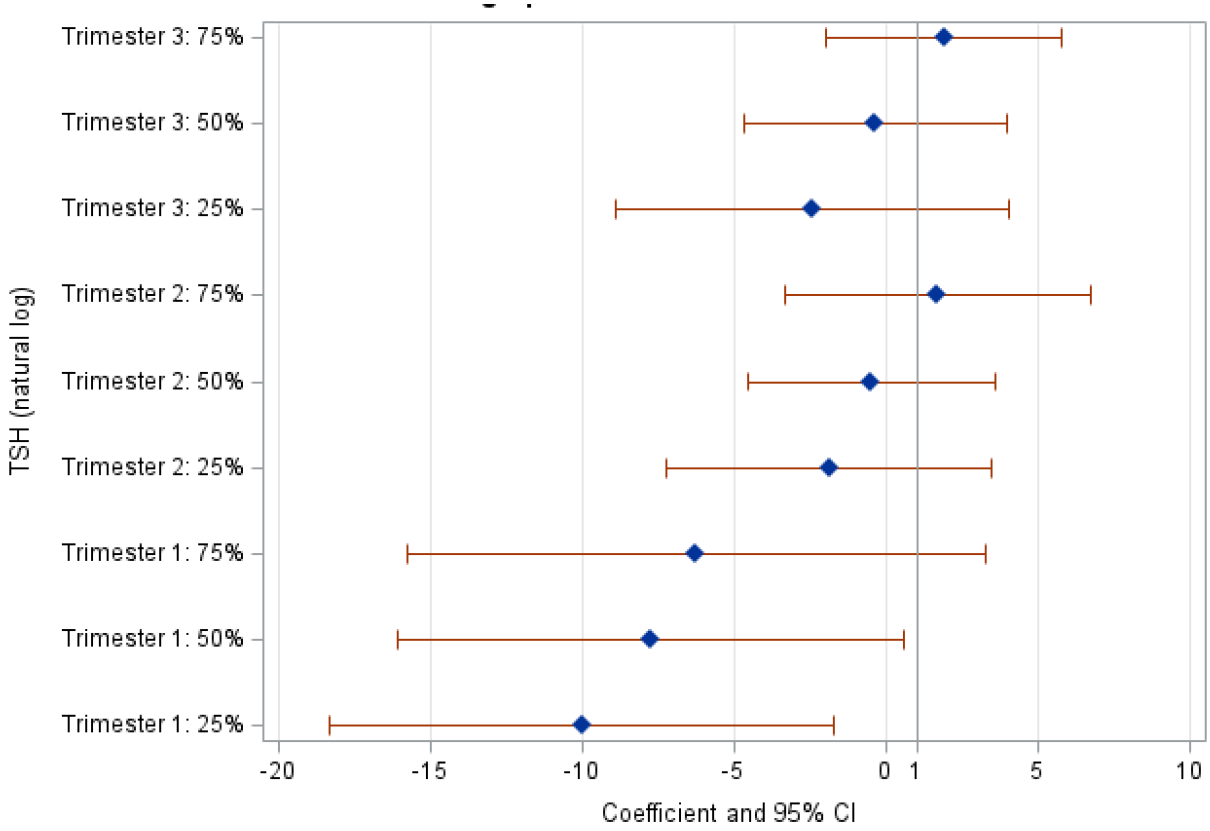
1. Quantile regression adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.
TSH, thyroid stimulating hormone
SRS, social responsiveness scale

Figure 2.2b. Association between natural log of free T4 and SRS percentiles. Analysis of pregnancies by trimester of blood draw.[1] First trimester n=85, second trimester n=167, third trimester n=215.



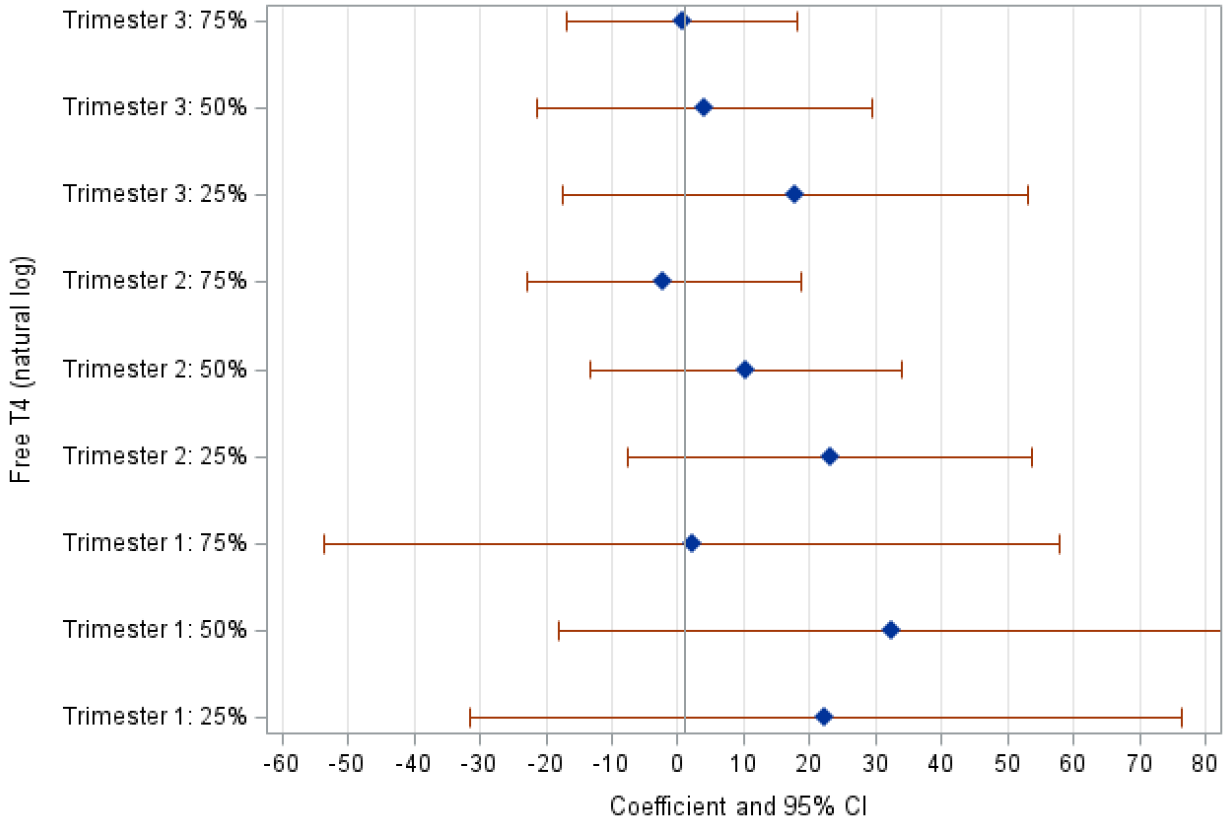
1. Quantile regression adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.
Free T4, free thyroxine
SRS, social responsiveness scale

Figure 2.3a. Association between natural log of TSH and MSEL percentiles. Analysis of pregnancies by trimester of blood draw.[1] First trimester n=85, second trimester n=182, third trimester n=252.



1. Quantile regression adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.
TSH, thyroid stimulating hormone
MSEL, Mullen scales of early learning

Figure 2.3b. Association between natural log of free T4 and MSEL percentiles. Analysis of pregnancies by trimester of blood draw.[1] First trimester n=85, second trimester n=182, third trimester n=252.



1. Quantile regression adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.
Free T4, free thyroxine
MSEL, Mullen scales of early learning

Appendix

Table A2.1. Thyroid stimulating hormone (TSH) and free thyroxine (FT4) values[1] for thyroid function categories in MARBLES cohort.		
First Trimester		
Category	TSH (uIU/mL)	FT4 (ng/dL)
Euthyroid	0.0270-3.8965	0.5900 -1.0400
Subclinical hypothyroidism	>3.8965	0.5900-1.0400
Overt hypothyroidism	>3.8965	<0.5900
Subclinical hyperthyroidism	<0.0270	0.5900-1.0400
Overt hyperthyroidism	<0.0270	>1.0400
Hypothyroxinemia	0.0270-3.8965	<0.5900
Hyperthyroxinemia	0.0270-3.8965	>1.0400
Second Trimester		
Euthyroid	0.2715- 3.4807	0.4500 -0.8700
Subclinical hypothyroidism	>3.4807	0.4500-0.8700
Overt hypothyroidism	>3.4807	<0.4500
Subclinical hyperthyroidism	<0.2715	0.4500-0.8700
Overt hyperthyroidism	<0.2715	>0.8700
Hypothyroxinemia	0.2715-3.4807	<0.4500
Hyperthyroxinemia	0.2715-3.4807	>0.8700
Third Trimester		
Euthyroid	0.3221-3.6709	0.4300 -0.7900
Subclinical hypothyroidism	>3.6709	0.4300-0.7900
Overt hypothyroidism	>3.6709	<0.4300
Subclinical hyperthyroidism	<0.3221	0.4300-0.7900
Overt hyperthyroidism	<0.3221	>0.7900
Hypothyroxinemia	0.3221-3.6709	<0.4300
Hyperthyroxinemia	0.3221-3.6709	>0.7900
1. Cutoff values are 2.5^{th} and 97.5^{th} percentiles of TSH and FT4 in an antibody negative subsample with no self-report of thyroid conditions.		

Table A2.2. Conditions included in hypothyroid and hyperthyroid conditions. Counts and percentage of column total.				
Thyroid function category	Total (n=285)	TD (n=161)	ASD (n=82)	Non-TD (n=42)
Euthyroid	239 (83.86)	141 (87.58)	67 (81.71)	31 (73.81)
Hypothyroid conditions	27 (9.47)	8 (4.97)	12 (14.63)	7 (16.67)
Subclinical hypothyroidism	15 (5.26)	5 (3.11)	7 (8.54)	3 (7.14)
Overt hypothyroidism	0 (0)	0 (0)	0 (0)	0 (0)
Hypothyroxinemia	12 (4.21)	3 (1.86)	5 (6.1)	4 (9.52)
Hyperthyroid conditions	19 (6.67)	12 (7.45)	3 (3.66)	4 (9.52)
Subclinical hyperthyroidism	7 (2.46)	4 (2.48)	1 (1.22)	2 (4.76)
Overt hyperthyroidism	3 (1.05)	2 (1.24)	0 (0)	1 (2.38)
Hyperthyroxinemia	9 (3.16)	6 (3.73)	2 (2.44)	1 (2.38)

Table A2.3. Maternal self-reported thyroid conditions and medication use, by thyroid function group determined by laboratory analysis Counts and percentage of column total. (n=285)			
	Euthyroid (n=239)	Hypothyroid conditions (n=26)[1]	Hyperthyroid conditions (n=19)
No self-report, no medication reported	230 (96.23)	19 (73.08)	4 (73.68)
No self-report, medication was reported	0 (0)	0 (0)	0 (0)
Self-report of thyroid condition, no medication reported	5 (2.09)	2 (7.69)	0 (0)
Self-report of thyroid condition, medication was reported	4 (1.67)	5 (19.23)	5 (26.32)
One pregnancy missing self-reported data for hypothyroid conditions group			

Table A2.4. Association between thyroid dysfunction and child diagnostic outcome compared to typical development Unadjusted multinomial regression			
ASD vs. TD (ASD n=82, TD n= 161)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.16	(1.21, 8.25)	0.0093
Hyperthyroid conditions	0.53	(0.14, 1.97)	0.0782
Non-TD vs. TD (Non-TD n=42, TD n=161)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.98	(1.32, 11.96)	0.0509
Hyperthyroid conditions	1.52	(0.45, 5.10)	0.6678

Table A2.5. Association between thyroid dysfunction and child diagnostic outcome compared to typical development no imputed variables			
ASD vs. TD (n=203)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.05	(0.98, 9.49)	0.0541
Hyperthyroid conditions	0.67	(0.17, 2.61)	0.5465
Non-TD (n=175)			
Variable	RRR	95% confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	1.95	(0.44, 8.58)	0.3765
Hyperthyroid conditions	1.45	(0.25, 8.24)	0.6760
Multinomial regression adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.			

Table A2.6. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, adjusting for TG and/or TPO antibody positivity			
ASD vs. TD (n=243)			
Variable	RRR	95% confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.22	(1.13, 9.22)	0.0291
Hyperthyroid conditions	0.42	(0.11, 1.65)	0.2158
Antibody positive (ref: negative)	0.86	(0.32, 2.33)	0.7631
Non-TD (n=203)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.15	(1.28, 13.46)	0.0179
Hyperthyroid conditions	1.30	(0.28, 6.00)	0.7404
Antibody positivity (ref: negative)	1.18	(0.35, 3.95)	0.7864
Multinomial regression adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.			

Table A2.7. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, adjusting for regular maternal smoking			
ASD vs. TD (n=243)			
Thyroid dysfunction (ref: euthyroid)	RRR	95% confidence interval	P-value
Hypothyroid conditions	3.02	(1.07, 8.53)	0.0366
Hyperthyroid conditions	0.44	(0.12, 1.69)	0.2338
Regular maternal smoking (ref: no)	1.50	(0.67, 3.36)	0.3297
Non-TD vs. TD (n=203)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.95	(1.26, 12.36)	0.0181
Hyperthyroid conditions	1.37	(0.29, 6.35)	0.7270
Regular maternal smoking (ref: no)	1.87	(0.74, 4.69)	0.1838
Multinomial adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.			

Table A2.8. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, interaction between thyroid dysfunction and number of blood draws during pregnancy			
ASD vs. TD (n=243)			
Variable	Odds ratio	95% confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.13	(0.64, 15.25)	0.1589
Hyperthyroid conditions	0.34	(0.03, 3.77)	0.3788
1-2 blood draws (ref: 3 draws)	0.72	(0.36, 1.41)	0.3342
Hypothyroid X Numbers of blood draws	1.17	(0.14, 9.57)	0.8862
Hyperthyroid X Numbers of blood draws	1.22	(0.06, 23.52)	0.8938
Non-TD[1]			
Variable	Odds ratio	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.49	(1.04, 12.39)	0.0333
Hyperthyroid conditions	1.35	(0.11, 14.99)	0.8373
1-2 blood draws (ref: 3 draws)	1.58	(0.6, 4.12)	0.3522
Hyperthyroid X Numbers of blood draws	0.94	(0.03, 26.79)	0.9727
Logistic regressions adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed. 1. Interaction between hypothyroid conditions and blood draws omitted because no hypothyroid conditions occurred among mothers with three blood draws.			

Table A2.9. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, interaction between thyroid dysfunction and sex of the child			
ASD vs. TD (n=243)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.37	(1.17, 16.34)	0.0283
Hyperthyroid conditions	0.36	(0.06, 2.16)	0.2653
Female (ref=male)	0.64	(0.33, 1.24)	0.1873
Hypothyroid X Sex of child	0.31	(0.03, 3.43)	0.3387
Hyperthyroid X Sex of child	1.42	(0.07, 28.02)	0.8194
Non-TD (n=203)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	2.09	(0.35, 12.49)	0.4201
Hyperthyroid conditions	0.37	(0.04, 3.27)	0.3694
Sex of the child (ref=male)	0.68	(0.27, 1.71)	0.4109
Hypothyroid X Sex of child	3.48	(0.29, 41.87)	0.3255
Hyperthyroid X Sex of child	9.00	(0.53, 153.87)	0.1292
Multinomial regression adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.			

Table A2.10. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, First pregnancy enrolled in MARBLES (any subsequent pregnancies excluded)			
ASD vs. TD (n=235)			
Variable	OR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.52	(1.27, 9.77)	0.0157
Hyperthyroid conditions	0.44	(0.11, 1.82)	0.2557
Non-TD (n=196)			
Variable	OR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.84	(1.11, 13.26)	0.0333
Hyperthyroid conditions	1.43	(0.37, 5.50)	0.6046
Adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.			

Table A2.11. Unadjusted association between thyroid dysfunction and child diagnostic outcome compared to typical development, by sex of the child (multinomial regression)						
ASD vs. TD						
	Male (TD=84, ASD=55)			Female (TD=77, ASD=27)		
Variable	RRR	95% CI	P-value	RRR	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)						
Hypothyroid conditions	4.30	(1.23, 15.07)	0.0228	1.40	(0.22, 8.71)	0.7191
Hyperthyroid conditions	0.57	(0.11, 3.11)	0.5168	0.47	(0.05, 4.42)	0.5024
Non-TD						
	Male (TD= 84, Non-TD=23)			Female (TD= 77, Non-TD=19)		
Variable	RRR	95% CI	P-value	RRR	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)						
Hypothyroid conditions	1.85	(0.30, 11.37)	0.5043	7.61	(1.68, 34.60)	0.0090
Hyperthyroid conditions	0.62	(0.07, 5.75)	0.6695	3.05	(0.63, 14.79)	0.1655

Table A2.12. Association between thyroid dysfunction and ASD[1] compared to typical development, **males only** (n=139)			
Variable	OR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.30	(1.09, 17.04)	0.0379
Hyperthyroid conditions	0.32	(0.05, 2.35)	0.2645
Logistic regression adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed. 1. Stratified adjusted odds ratios not calculated for non-TD outcome or ASD females because of small sample size with zero cell covariates			

Table A2.13. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, adjusting for **maternal self-report of thyroid condition**			
ASD vs. TD (n=243)			
Variable	RRR	95% Confidence interval	P-value
Self-report of thyroid condition (ref=no)	0.50	(0.14, 1.80)	0.2869
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.77	(1.22, 11.59)	0.0207
Hyperthyroid conditions	0.50	(0.13, 1.97)	0.3238
Non-TD (n=203)			
Variable	RRR	95% Confidence interval	P-value
Self-reported thyroid conditions (ref=no)	0.50	(0.11, 2.15)	0.3482
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	5.03	(1.50, 16.89)	0.0089
Hyperthyroid conditions	1.47	(0.30, 7.17)	0.6333
Multinomial regression adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.			

Table A2.14. Association between thyroid dysfunction and child diagnostic outcome compared to typical development, adjusting for **self-reported maternal thyroid medication use**			
ASD vs. TD (n=243)			
Variable	RRR	95% Confidence interval	P-value
Self-report of thyroid medication (ref=no)	0.30	(0.06, 1.25)	0.0982
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.15	(1.33, 12.98)	0.0143
Hyperthyroid conditions	0.56	(0.14, 2.24)	0.4166
Non-TD (n=203)			
Variable	RRR	95% Confidence interval	P-value
Self-report of thyroid medication (ref=no)	0.15	(0.02, 1.26)	0.0806
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	5.90	(1.69, 20.57)	0.0054
Hyperthyroid conditions	1.79	(0.35, 9.06)	0.4836
Multinomial regression adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.			

Table A2.15. Association between log of TSH or log for free T4 and diagnostic outcome compared to TD.[1] 1st trimester n=96; 2nd trimester n=199 3rd trimester n=272.[2]			
ASD compared to TD			
Variable	RRR	95% Confidence interval	p-value
First trimester, log of TSH	3.24	(1.02, 10.31)	0.0464
Second trimester, log of TSH	1.54	(0.86, 2.76)	0.1457
Third trimester, log of TSH	1.53	(0.93, 2.53)	0.0931
First trimester, log of free T4	0.64	(0.01, 30.9)	0.8198
Second trimester, log of free T4	0.30	(0.04, 2.55)	0.2704
Third trimester, log of free T4	0.59	(0.10, 3.45)	0.5558
Non-TD compared to TD			
Variable	RRR	95% Confidence interval	p-value
First trimester, log of TSH	1.19	(0.58, 2.44)	0.6368
Second trimester, log of TSH	0.91	(0.44, 1.87)	0.7946
Third trimester, log of TSH	0.85	(0.46, 1.59)	0.6152
First trimester, log of free T4	0.38	(0.01, 28.06)	0.6563
Second trimester, log of free T4	0.13	(0.01, 2.22)	0.1609
Third trimester, log of free T4	1.50	(0.11, 20.32)	0.7626
1. Multinomial model adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed. 2. 1st Tri: TD=54, ASD=32, non-TD=10; 2nd Tri: TD=116, ASD=56, non-TD=27; 3rd Tri: TD=157, ASD=78, non-TD=37			

Table A2.16. Associations between maternal thyroid dysfunction and 25th, 50th, and 75th percentile SRS scores. SRS completed at appropriate age for form.[1] (n=223)									
	25th percentile			50th percentile			75th percentile		
Variable	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value
Thyroid dysfunction (ref: euthyroid)									
Hypothyroid conditions	2.74	(-3.45, 8.94)	0.3855	4.38	(-6.57, 15.32)	0.4331	3.37	(-18.09, 24.82)	0.7584
Hyperthyroid conditions	-2.93	(-6.95, 1.1)	0.1537	-5.94	(-10.56, -1.32)	0.0117	-8.6	(-19.93, 2.73)	0.1370
1. Excludes 5 children whose caregivers completed their school age SRS form when they were below 48 months of age. Models adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.									

Table A2.17. Association between **natural log of TSH or log of free T4** and **SRS score percentiles** (25^{th}, 50^{th}, 75^{th}), by trimester									
	Trimester 1 (n=85)			Trimester 2 (n=167)			Trimester 3 (n=215)		
Variable	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value
25^{th}, natural log of TSH	1.55	(-1.85, 4.95)	0.3727	0.31	(-0.94, 1.55)	0.6298	0.59	(-0.81, 1.98)	0.4110
50^{th}, natural log of TSH	2.93	(-0.35, 6.20)	0.0799	0.66	(-1.29, 2.62)	0.5054	0.85	(-0.94, 2.64)	0.3517
75^{th}, natural log of TSH	4.72	(1.38, 8.05)	0.0056	1.37	(-1.90, 4.64)	0.4123	1.44	(-1.72, 4.59)	0.3733
25^{th}, natural log of free T4	-4.49	(-21.26, 12.29)	0.6001	-6.52	(-13.41, 0.37)	0.0636	-1.66	(-9.60, 6.29)	0.6828
50^{th}, natural log of free T4	-4.76	(-24.23, 14.71)	0.6319	-9.72	(-23.63, 4.20)	0.1710	-5.09	(-16.15, 5.96)	0.3666
75^{th}, natural log of free T4	-0.65	(-34.81, 33.51)	0.9704	-4.73	(-26.44, 16.99)	0.6697	-2.92	(-24.1, 18.26)	0.7868
Models adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.									

Table A2.18. Association between **natural log of TSH** or **free T4** and **MSEL composite score percentiles** (25^{th}, 50^{th}, 75^{th}), by trimester									
	Trimester 1 (n=85)			Trimester 2 (n=182)			Trimester 3 (n=252)		
Variable	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value	Coefficient	95% CI	P-value
25^{th}, natural log of TSH	-10.01	(-18.33, -1.7)	0.0183	-1.88	(-7.25, 3.49)	0.4929	-2.44	(-8.92, 4.05)	0.4613
50^{th}, natural log of TSH	-7.76	(-16.09, 0.57)	0.0679	-0.49	(-4.56, 3.59)	0.8142	-0.36	(-4.69, 3.96)	0.8692
75^{th}, natural log of TSH	-6.25	(-15.78, 3.29)	0.1993	1.69	(-3.35, 6.73)	0.5113	1.91	(-1.98, 5.8)	0.3357
25^{th}, natural log of free T4	22.42	(-31.46, 76.29)	0.4148	23.12	(-7.52, 53.76)	0.139	17.86	(-17.36, 53.07)	0.3203
50^{th}, natural log of free T4	32.6	(-17.99, 83.19)	0.2066	10.43	(-13.2, 34.06)	0.387	4.12	(-21.22, 29.47)	0.7498
75^{th}, natural log of Free T4	2.19	(-53.51, 57.9)	0.9385	-2.10	(-22.96, 18.75)	0.8433	0.72	(-16.75, 18.18)	0.936
Models adjusted for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity and missing covariates are imputed.									

Table A2.19. Standardized SRS scores corresponding to quintile cut points for quantile regression analyses by pregnancy and by trimester				
SRS quintile	By pregnancy (n=228)	1st trimester (n=85)	2nd trimester (n=167)	3rd trimester (n=215)
75th	58	55	54	57
50th	47	48	47	47
25th	43	43	43	43

Table A2.20. Mother and child characteristics by trimester of blood draw[1], n (%) unless otherwise noted			
	1st trimester n=96	2nd trimester n=199	3rd trimester n=272
Maternal characteristics			
Gestational or type 2 diabetes	18 (18.75)	48 (24.12)	57 (21.03)
Regularly smoker at any time	14 (14.74)	36 (18.37)	46 (17.16)
Prenatal vitamin use in first month of pregnancy[3]	53 (58.89)	52.51 (94)	122 (49.19)
Pregnancy intended at that time	65 (68.42)	118 (60.20)	164 (60.97)
Interpregnancy interval under 12 months[2]	8 (8.60)	13 (6.77)	12 (4.56)
Mean maternal age at delivery, SD	96 (35.5, 4.2)	199 (35.0, 4.7)	272 (34.7, 4.8)
Maternal pre-pregnancy BMI			
Not obese	72 (75.00)	1141 (70.85)	199 (73.16)
Obese	24 (25.00)	58 (29.15)	73 (26.84)
Highest level of education			
Less than High school or High school/GED	7 (7.29)	18 (9.05)	23 (8.46)
Some college	33 (34.38)	72 (36.18)	114 (41.91)
Bachelor's or higher	56 (58.33)	109 (54.77)	135 (49.63)
Health insurance			
Private insurance	69 (84.15)	129 (76.33)	182 (77.45)
Public insurance	13 (15.85)	40 (23.67)	53 (22.55)
Homeownership			
Renter	36 (38.71)	87 (44.85)	119 (44.74)
Owner	57 (61.29)	107 (55.15)	147 (55.26)
Child characteristics			
Child race and ethnicity			
White, non-Hispanic	34 (35.42)	73 (36.68)	94 (34.56)
Other, non-Hispanic	15 (15.63)	33 (16.58)	41 (15.07)
Asian, non-Hispanic	18 (18.75)	30 (15.08)	40 (14.71)
Hispanic, any race	29 (30.21)	63 (31.66)	97 (35.66)
Child sex (female)	45 (46.88)	87 (43.72)	117 (43.01)
Median gestational age at delivery (range), weeks	96 (39.1, 33.6-41.1)	199 (39.0, 28.3-41.43)	272 (39.2, 33.6-41.7)
Median child year of birth, range	96 (2014, 2009-2020)	199 (2014, 2009, 2020)	272 (2014, 2009-2020)
Child breast fed after 3 weeks	89 (92.71)	184 (92.46)	252 (92.65)
Diagnostic category			
Typically developing	54 (56.25)	116 (58.29)	157 (57.72)
ASD diagnosis	32 (33.33)	56 (28.14)	78 (28.68)
Non-typically developing	10 (10.42)	27 (13.57)	37 (13.60)
1. Significance testing found no significant differences ($p<0.05$) across trimesters for any covariates			

Table A2.21. Association between thyroid dysfunction and child diagnostic outcome compared to typical development. Second twin included instead of first twin.			
ASD vs. TD (n=242)			
Variable	RRR	95% Confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	3.11	(1.11, 8.70)	0.0309
Hyperthyroid conditions	0.42	(0.11, 1.62)	0.2096
Non-TD (n=202)			
Variable	RRR	95% confidence interval	P-value
Thyroid dysfunction (ref: euthyroid)			
Hypothyroid conditions	4.09	(1.32, 12.65)	0.0146
Hyperthyroid conditions	1.22	(0.27, 5.45)	0.7980
Adjusts for health insurance, pregnancy intentions, maternal education, interpregnancy interval, prenatal vitamin use, and child race/ethnicity.			

Chapter 3: Investigation of maternal iodine status during pregnancy and childhood autism outcomes in U.S. children

Background

Iodine is essential for fetal brain development.[8] The primary physiological role of iodine is the synthesis of thyroid hormone, and inadequate dietary intake of iodine can lead to a reduction of thyroid hormone production. Thyroid hormones are vital to neural migration and brain myelination, processes beginning in the embryo, and continue to play an important role in cognitive functioning throughout the lifespan.[65] Autism spectrum disorder (ASD) is a neurodevelopmental condition thought to involve genetic and environmental etiologies[66–68]. Maternal deficiencies in vitamin D and folic acid during pregnancy are associated with increased likelihood of ASD development and prenatal multivitamin use has been associated with a reduced chance of ASD in the child.[69] Additionally, prenatal nutrients may further shape ASD development through interactions with genetic or multiple environmental exposures.[70]

In 2022, the Centers for Disease Control (CDC) estimated the prevalence of ASD to be 32.2 per 1,000 children aged 8 (ranging between 9.7 to 53.1 cases per 1,000 depending on surveillance site).[19] About 40% of children diagnosed with ASD met criteria for co-occurring intellectual disability.[19] ASD diagnostic criteria is characterized by difficulties with social awareness and communication, reduced motivation for social activities, restricted interests, and repetitive behaviors.[3] Autistic traits exist on a continuum. Among people with an ASD diagnosis, there is considerable variability in type and intensity of autistic traits. Autistic traits are also present in individuals without clinical ASD and the distribution of ASD traits can be measured in non-clinical samples.[41,71,72] ASD traits can be useful in understanding the etiology of ASD because they allow for the inclusion of borderline cases and the identification of more subtle effects of prenatal exposures.[73]

During pregnancy, the National Academies of Sciences, Engineering and Medicine (NASEM) has an estimated average requirement (EAR) for iodine of 160 µg per day and an upper tolerable limit (UTL) of 900 µg per day for those under 18 years old and 1,100 µg per day of those 19 through 50 years

old.[74] For measuring iodine status at a population level, the World Health Organization (WHO) recommends a median urinary iodine concentration (UIC) of 150 µg per liter during pregnancy, with median UIC over 500 µg per liter considered as excess.[75] Both deficient and excess iodine intake are associated with thyroid dysfunction.[76] Food fortification policies have successfully increased iodine intake among pregnant women to the point where severe iodine deficiency in this population is increasingly rare both in Western nations and globally.[77] However, mild or moderate iodine deficiency during pregnancy remains a concern. For women of reproductive age in the U.S., main sources of dietary iodine include iodized salt, dairy products, and iodine containing supplements. In the U.S., the amount of iodine found in retail milk can vary considerably by location because of differences in feed supplementation or iodine sanitation practices in dairies. Milk samples collected during 2021-2022 from 12 retail locations within the U.S. had, on average, 82.5 µg per 240 ml serving (two servings would provide EAR), but milk in some locations fell 47 µg per serving size below this average, suggesting geographic variability in the iodine content of milk.[78] In recent years, women of reproductive age have gravitated towards diets that are lower in iodized salt and use plant-based milk instead of dairy products, thus reducing consumption of important sources of iodine.[17] Lower iodized salt intake may be the result of more women adopting a low sodium diet for health reasons or using non-iodized table or sea salt instead of iodize salt.[17,79] Prenatal vitamins are recommended to contain 150 µg of iodine.[80,81] A 2021 review of prenatal vitamins found that only about 76% of prenatal vitamins are estimated to contain iodine. Among the prenatal vitamins that contained iodine, 69% had at least the recommended amount.[81]

There is some evidence that U.S. pregnant women are not receiving enough iodine. A study of NHANES participants from 2011-2020 found a decrease in the median iodine intake among women of childbearing age for this time period.[82] In the 2017-2020 period, 10.2% of pregnant NHANES participants were estimated to have inadequate iodine intake.[82] A Michigan based cohort study that followed women during pregnancy found that 23% of their participants were iodine deficient.[83]

Severe maternal iodine deficiency during pregnancy is a consistent predictor of intellectual disability in children,[8] however less is known about the effects of mild or moderate iodine deficiency on child development. Existing research suggests that mild or moderate maternal iodine deficiency could be associated with deficiency in reading skills. In a UK-based study (ALSPAC, n= 958 mothers), children of mothers whose UIC concentration was moderately below WHO criteria for iodine deficiency (50-150 µg) had lower reading accuracy and comprehension at age 8 compared to children of mothers with median urinary iodine concentrations at adequate levels or above (greater or equal to 150 µg).[84] This study excluded urine samples with UIC above 500 µg/L due to concerns about sample contamination. Similarly, an Australian study of 266 children born to mothers in a mildly iodine deficient population (median 83.2 µg/L, IQR: 46.0–180.0 µg/L) found that maternal urinary iodine below the WHO criteria was associated with decreased reading skills in children at ages 8-9 year (grade 3).[85] This relationship was observed again when the children were 10-11 years (grade 5), but there was not association at the grade 7 or 9 time points. Both studies adjusted for maternal education, socio-economic status, maternal age, sex of the child[84,85], though the U.K. study adjusted for more variables related to maternal stress and health behaviors (maternal alcohol use, smoking, and food frequency questionnaire).[84]

Child cognitive ability and language may also be negatively impacted by maternal mild or moderate iodine deficiency during pregnancy. Using an iodine-specific food frequency questionnaire, a prospective study of an iodine sufficient population in Australia found that pregnancy iodine intake at the lowest (<220 µg/day) and highest (>391 µg /day) intake quartiles were associated with decreased Bayley-III cognitive and language scores in children (n=699).[86] This was after adjusting for maternal IQ, maternal age, parity, smoking in pregnancy, education, and Home Screening Questionnaire score. The average intake from food and supplements across pregnancy was estimated to be 305 µg/day (SD 115), which is notably higher than NASEM EAR of 160 µg/day. Because iodine intake was based on a self-reported questionnaire, measurement error (participants not accurately reporting what foods or supplements were consumed) could affect these results. Urinary iodine was not associated with any developmental outcomes in this cohort in a previous study.[87] A Norwegian study analyzing 851 mother-child pairs in an iodine

deficient population (median UIC 78 μg/L, IQR 46–130), found that a urinary iodine concentration of 100 μg or less was associated with reductions in Bayley-III expressive and receptive language scores, but not cognitive or motor skills.[88] Expressive language scores had a u-shaped relationship to urinary iodine concentration, while receptive language did not.[88] In an iodine replete population of pregnant women in the Netherlands (median urinary iodine concentration of 203 μg per liter), children born to mothers with urinary iodine concentrations in the lowest 10% were more likely to have problems with working memory compared to children born to mothers with urinary iodine concentrations in the highest 10%. Other areas of executive functioning were not associated with maternal iodine status in the study population.[89]

Three previous studies have examined maternal iodine during pregnancy as predictor of ASD diagnosis or traits, though none have found strong evidence of association. Levie et al (2020) conducted a meta-analysis of three European population-based pregnancy cohorts.[90] The study population included 5,546 mother-child pairs. UIC/creatine was estimated using 1-4 spot urine samples per women collected during pregnancy and binary autistic traits was used as the outcome (high autistic traits was indicated by scoring at or above the 93rd percentile for each cohort's assessment measure). This study found no relationship between maternal UIC and high autistic traits in the pooled analysis, though a UIC to creatine ratio of less than 150 μg per gram was associated with a decreased chance of ASD traits in one cohort.[90]

Using a U.K. based sample of 6639 women contributing 6955 pregnancy urine samples, Cromie et al (2020),[91] found no association between the maternal ratio of iodine to creatine and child ASD diagnosis risk. Children were considered to have ASD if their primary care records indicated a clinical diagnosis.[91] A third study (Berghuis et al, 2025), using data from a Canadian sample (n=399 mother-child pairs), found no association between low maternal iodine (UIC/creatine <150 μg/g) and ASD traits as a continuous measure (standardized social responsiveness scale scores).[92] Mothers had UIC/creatine from first and second trimester.

The present study aims to evaluate the relationship between maternal iodine status during pregnancy and autism in the child across geographically varied U.S. cohorts. This is the first study to examine the relationship between maternal iodine intake during pregnancy and autism in the child using a

U.S. based sample. This is also first study to examine this relationship using estimated iodine intake (rather than UIC) from two or more urine samples per mother. The following hypotheses will be tested: 1) Children whose mothers were iodine deficient during pregnancy will have a higher frequency of autism traits compared to children whose mothers were iodine adequate during pregnancy. 2) When treated continuously, lower maternal iodine concentrations during pregnancy will be associated with a higher likelihood of autism traits in the child. 3) Maternal iodine deficiency during pregnancy will be associated with a higher likelihood of autism traits in the child.

Methods

Data source

Participant data is from the Environmental Health Influences on Children's Health Outcomes (ECHO) Cohorts program. This is an NIH-funded program that brings together individual prospective cohort studies that follow pregnant women, caregivers, and their children over time into a central platform. The focus of the cohorts participating in ECHO is environmental and nutritional risk factors for children's health and development. Though cohorts are individually managed by principal investigators at each site, data is managed and harmonized by ECHO. To be eligible for the present study, mother child-pairs must 1) have at least two urine samples analyzed for iodine concentrations collected during pregnancy, 2) a child from the pregnancy when the urine samples were collected who is enrolled into ECHO, and 3) the enrolled child has an age-appropriate SRS score.

Iodine measures

Because urinary iodine measures fluctuate based on recent consumption of iodine containing foods, multiple urinary iodine measures per person are needed to estimate individual iodine deficiency status.[93] At least two urinary samples were collected and stored as part of individual cohort protocols. Urine samples were shipped on dry ice to the Trace Elements Laboratory at the New York State Department of Health's Wadsworth Center (Albany, NY). Iodine content was analyzed using a PerkinElmer NexION® 300D Inductively Coupled Plasma Mass Spectrometer (ICP-MS). The Wadsworth ICP-MS method is based on the methods used by the U.S. Centers of Disease Control and Prevention

(CDC) to analyze urine for iodine for the National Health and Nutrition Examination Survey (NHANES), which are described elsewhere.[94,95] Internal quality control (QC) pools were produced and characterized for low, median, and high iodine concentrations, using the NHANES population to determine deficient to elevated iodine concentration. Between 86 and 127 internal QC pools (QC low, QC medium, and QC high) were analyzed across 43 analytical batches. This includes urine samples from participants who are not included in the present study because their children did not have SRS scores. Coefficients of variation (CV) for each urine pool were QC Low 4% at 23.3 µg/L, (n=127); QC Medium 5% at 158 µg/L, (n=86); and QC High 5% at 396 µg/L, (n=86). Multielement QC pools were also analyzed alongside internal QC's and yielded comparable CVs.

Individual iodine intake per day was estimated using the UIC from the laboratory analysis. Intake was estimated with two or more urinary iodine concentration (UIC) measures per pregnancy using methods described by Nusser et al (1996)[96] and Zimmerman et al (2016).[97] This method first adjusts the UICs to have an equal mean and variance for the earliest measurement. Next, the adjusted UICs are transformed by a combination of power and grafted polynomial functions to approximate normality. The usual daily intake is estimated using a measurement error framework. Finally, the usual daily intake is estimated in the original scale by applying a back transformation to the normal distribution of the daily intakes. Software for Intake Distribution Estimation (SIDE) was used to compute the estimated usual daily iodine intake per person. These iodine intake estimates were compared to the NASEM estimated average requirement (EAR) of 160 µg per day for pregnant people of any ages and upper tolerable limit (UTL) of 900 µg per day for pregnant people under 18 years old and 1,100 µg per day of those 19 through 50 years old.[74] Mothers whose intake was less than the EAR of 160 µg were categorized as iodine deficient, mothers whose intake was estimated to be over 160 µg but less than the UTL for their age were categorized as iodine adequate, and mothers over the UTL were categorized as iodine excessive. Alternatively, based on the WHO guidelines,[75] sample (or subsample) median UIC was considered inadequate when less than 150 µg/L, adequate when between 150-249 µg/L, and excessive when 500 µg/L or above.[75]

Measure of autism traits

The social responsiveness scale (SRS) was used to measure intensity of ASD traits.[41] Among eligible cohorts, caregivers could complete the SRS-2 pre-school form (for children ages 18-54 months) and/or SRS-2 school age form (for children ages 4-18 years). The school age form could be completed as the full form (65 items) or the short form (16 items). The short form was developed to reduce participant burden while maximizing reliability and content coverage. Responses from the short form have been found to be highly correlated with those from the full form,[98] including among ECHO participants.[99] For children with multiple SRS scores completed at or above the minimum age, we chose the school age form over the preschool form and/or the form completed closest to age 5. In ECHO participants with both forms, the preschool and school age form generally show similar results, but the preschool form tends to have more variability than the school age form.[100] In studies of children with clinical ASD diagnoses, ASD traits or symptoms were more stable around age 5 compared to younger ages.[100–102] Standardized SRS scores were analyzed as continuous scores (population normalized T-scores, mean=50, SD=10) and as a binary measure based on ASD threshold scores for possible ASD (SRS>=60) or no ASD indicated (SRS<60).[103] No participating cohorts were high-likelihood ASD cohorts.

Statistical analysis

Maternal and child characteristics were examined by the mother's iodine status (Table 3.1). Frequencies were calculated for categorical variables. For continuous variables, means with standard deviations were calculated. Median UIC was calculated by trimester and by binary SRS score (Table A3.2).

The first hypothesis concerns a crude association, that children who had iodine deficient mothers will have more autism traits compared to children who had iodine adequate mothers. It was tested in two ways. First, by comparing the mean SRS scores from children between those whose mothers were iodine deficient during pregnancy and those whose mothers were iodine adequate. T-tests were used to determine significant differences between the groups. Secondly, we tested the association of possible autism by maternal iodine status using chi-square tests. Statistical significance is indicated by ($p<0.05$).

The second hypothesis (lower maternal iodine concentrations during pregnancy will be associated with a higher likelihood of autism traits in the child) was tested two different ways. First, continuous usual daily estimated iodine intake was modeled as a predictor of continuous total SRS T-scores. Second, continuous usual daily estimated iodine intake was modeled as a predictor of binary SRS category. The third hypothesis (maternal iodine deficiency during pregnancy will be associated with a higher likelihood of autism traits in the child) was tested four different ways. First, binary usual daily iodine intake (iodine deficiency vs. iodine adequacy) was modeled as a predictor of continuous total SRS T-scores. Second, binary usual daily iodine intake was modeled as a predictor of binary SRS category (possible ASD vs no ASD indicated). Third, usual daily estimated iodine intake quintiles were modeled as a predictor of continuous total SRS T-scores. The 3rd quintile (estimated iodine intake 153.6-195.9 µg/day) was used as the reference because it was closest to the NASEM cut point for deficient iodine intake (160 µg per day) (Table A3.1). Fourth, usual daily estimated iodine intake quintiles were modeled as a predictor of binary SRS category.

Maternal iodine and child ASD were operationalized as both continuous and categorical variables to reflect the ways these are treated in previous studies examining the relationship between maternal UIC and child ASD[90–92], and other studies examining maternal iodine intake specifically and child neurodevelopment outcomes.[86] The models with the continuous total SRS T-scores were estimated using a hierarchical model allowing random intercepts for the cohorts the pregnant participants belonged. These models reported the parameter estimates and 95% confidence intervals. For the binary iodine intake model with a continuous SRS outcome, the coefficient for deficient iodine intake represented the change in SRS score that is associated with deficient intake compared to adequate intake. For the quintile iodine intake model with continuous SRS outcome, the coefficient for a given quintile represented the change in SRS score associated with that intake quintile compared to the third intake quintile (reference). For the continuous iodine intake model with a continuous SRS outcome, the coefficient for iodine intake represented the change in SRS scores for each microgram per day increase in iodine intake. Logistic

regressions were fitted for the binary SRS outcome. These models included a random cohort effect and reported odds ratios and 95% confidence intervals. Odds ratios indicated the odds of possible ASD compared to no ASD indicated. All statistical tests used the significance level 0.05 to indicate statistical significance.

Models were run unadjusted and adjusted. All adjusted models included pre-pregnancy BMI (underweight/normal weight, overweight, or obese, calculated using derived pre-pregnancy weight and maternal height or self-reported pre-pregnancy BMI), maternal education (less than high school, high school degree, some college, bachelor's degree or higher), gestational diabetes (yes if a self-report or medical record diagnosis or treatment of gestational diabetes was indicated), any infections during pregnancy (yes if a self-report or medication record indicated genitourinary tract infections during this pregnancy, including urinary tract infection, infection in uterus, vaginal infection, group B Strep vaginal infection, or other genitourinary tract infection or pelvic inflammatory condition was indicated), child race/ethnicity (Non-Hispanic Black, White, or multiple race or Hispanic of any race) and age at SRS (in years) as covariates. Confounders were selected based on prior literature and DAGs. Sex of the child was tested as an effect modifier of iodine's association with outcomes by adding sex*iodine interaction terms to model 1 and then in any other models with significant findings for iodine.

Results

Analytic sample

ECHO has over 96,000 participants from across the Unites States. Following inclusion criteria, 1,460 mothers had two urine samples collected at different times during pregnancy that were analyzed for iodine. Of these mothers, nearly all (n=1,442) had a child enrolled in ECHO who was from the pregnancy where iodine was measured. SRS was not available for all children whose mothers had pregnancy iodine measures. Of the 33,549 SRS records in ECHO, 30,772 were complete with a total T-score and the child age of completion. Of these, about two thirds (n=19,065) were completed within or above the recommended age range for the form. The overlap between the 1,442 mothers with pregnancy iodine whose child was enrolled in ECHO and the 19,065 children with complete age-appropriate SRS forms

was 780 mother-child pairs. The present analysis includes 653 mother-child pairs, with the remaining participants withheld from the present analysis because of restrictions on data use. (Figure 3.1).

Prevalence of iodine deficiency and descriptive statistics

About 42.5% (n=278/653) of mothers were categorized as having deficient iodine intake while the remainder were categorized as having adequate intake. One person had excessive intake and was excluded from the analysis. Maternal demographic characteristics were distributed similarly within the deficient and adequate intake groups and there were no significant differences between intake groups (Table 3.1). For both groups, mothers were on average about 28 years old at the first urine specimen collection (SD 5.5) and over 40% had a bachelor's degree or higher. For mothers with data available on health insurance, about half had private insurance and over 40% had public insurance. In both iodine intake groups, less than 15% of mothers had hypertensive disorders, less than 10% had gestational diabetes, and around 20% of each group had infections during pregnancy. Nearly a third of each intake group was classified as obese based on BMI and less than 10% used tobacco products during pregnancy (Table 3.1).

Child year of birth differed significantly across iodine intake groups ($p<0.01$) but no other child demographic characteristics differed significantly across iodine intake groups (Table 3.1). About 45% of children born to mothers with iodine deficiency were female compared to about 51% of children born to iodine adequate mothers. Average gestational age at birth was about 39 weeks (SD 1.7 weeks) for both adequate and deficient iodine intake groups and birthweight was similar between the two groups. Maternal and child demographic characteristics that were missing in over 10% of the sample included average maternal age at first specimen (14.5%), prenatal tobacco use (24.8%), and maternal health insurance (43.3%) (Table 3.1).

Average SRS scores were similar for the adequate iodine intake group (mean t-score of 49.5, SD 9.1) and the deficient iodine intake group (mean t-score of 49.1, SD 7.8) (Table 3.2). For mothers with adequate iodine, 12.3% of children had SRS scores that suggested possible ASD (SRS>=60). For mothers

with iodine deficiency, this percentage was 8.6%. Children born to mothers with iodine deficiency were about a year younger when their SRS was completed than children born to mothers with adequate iodine, and this difference was significant ($p<0.01$). About 2-3% of children in each iodine intake group had a parent report of an ASD diagnosis, however about 20% of children were missing a response for this item (Table 3.2).

Median UIC

Median urinary iodine concentration was the highest in the first trimester (160.80 µg/L), followed by the second trimester (152.20 µg/L), then the third trimester (145.40 µg /L) (Table A3.2). Samples missing trimester information had the lowest median (108.80 µg/L). Overall, the median UIC for the sample was 146.30 µg/L (Table A3.2). The possible ASD group had a median UIC of 162.65 µg/L while the no ASD indicated group had a median of 144.70 µg/L. Within each group, first trimester UIC medians were the highest and medians decreased with the remaining trimesters (Table A3.2)

Regressions with continuous SRS outcomes

Deficient iodine intake was not associated with continuous SRS scores in adjusted (adjusted coefficient 0.53; 95% CI -0.84, 1.91) (Table 3.3) or unadjusted analyses (Table A3.3). In the adjusted quintile analysis, quintiles 1 and 4 were significantly associated with about a quarter of a standard deviation increase in SRS scores compared to the 3rd quintile (adjusted coefficient 1st quintile 2.70, 95% CI 0.56, 4.83; p=0.013; adjusted coefficient 4th quintile 2.65, 95% CI 0.52, 4.7; p=0.015) (Table 3.3). No other quintile was significantly associated with continuous SRS scores in the adjusted or unadjusted analyses (Table 3.3, Table A3.3). There was no association between continuous maternal iodine intake and continuous SRS scores (adjusted coefficient 0.00, 95% CI -0.01. 0.01) (Table 3.3). The variance inflation factor was below 2 for all covariates.

Regressions with binary SRS outcome

There was no association between deficient iodine intake and possible ASD in the adjusted (adjusted OR 0.96; 95% CI 0.53, 1.75) (Table 3.4) and unadjusted regressions (Table A3.4). In unadjusted analyses, no iodine intake quintile was significantly associated with possible ASD (Table

A3.4). After adjustment, iodine intake in the 4th quintile was significantly associated with 2.64 times the OR of possible ASD (95% CI 1.07, 6.55; p=0.04). Quintile 1 suggested an increased likelihood of possible ASD, but the odds ratio was not significant (Adjusted OR 1.76, 95% CI 0.66, 4.47, p=0.27) (Table 3.4). There was no association between continuous maternal iodine intake and possible ASD in the adjusted (adjusted OR 1.00; 95% CI 0.99, 1.00) or unadjusted regressions (Table3. 4, Table A3.4).

Effect modification by sex of the child

Interaction terms between iodine intake and sex of the child were tested in the binary iodine and continuous SRS outcome model (interaction p=0.70) and both iodine intake quintile models (continuous SRS outcome overall interaction p=0.80; binary SRS outcome overall interaction p=0.96). (Table A3.5). When the iodine quintile and continuous SRS score model was stratified by child's sex, the coefficient for quintile 4 for males was significantly associated with about a third of a standard deviation increase in SRS scores (adjusted coefficient 3.4, 95% CI 0.3, 6.5; p=0.03), but this association attenuated and non-significant in females (adjusted coefficient 1.6, 95% CI -1.4, 4.5; p=28). Findings for iodine intake in the first quintile and continuous SRS scores were not significant for either sex in the stratified analysis. (Table A3.6).

Discussion

This study found minimal evidence of an association between maternal iodine status during pregnancy and child ASD traits. Average SRS scores of children born to mothers with deficient iodine intake were nearly identical to those from mothers with adequate iodine. Though the median UIC indicated an iodine deficient sample (median UIC 146.3 µg/L) based on the WHO UIC cut off of 150 µg/L, the median UIC in the possible ASD group was above this cut point (162.7 µg /L). Trimester-specific median UIC indicated adequate iodine in the first trimester for the total study population and by binary SRS group.

Iodine intake in the 4th quintile was associated with a small but significant increase in standardized SRS scores and a significantly increased chance of possible autism when SRS scores were treated as binary measure. This was in comparison to the 3rd quintile, which spanned the NASEM cut

point for deficient iodine intake. For continuous SRS scores, the 1st quintile (lowest intake category) was also significantly associated with a small increase in SRS scores compared to the 3rd quintile, but this relationship was no longer significant when SRS scores were analyzed as a binary measure. These findings only partially support the hypothesis that maternal iodine deficiency during pregnancy is associated with increased likelihood of ASD traits in children. The finding that the lowest iodine intake category is associated with greater ASD traits is congruent with this hypothesis, but the associations between the 4th quintile and ASD traits challenge this hypothesis because this quintile represents a range within that of adequate iodine consumption (Table A3.1). Quintile 5 (the highest intake quintile) was not associated with continuous or binary SRS scores. Excess iodine intake is associated with greater risk of thyroid dysfunction,[104] though no participants in the analysis had excess iodine intake. Highest iodine intake included in the analysis was about 720 µg/day (Table A3.1), which is below the NASEM upper tolerable limits (900 µg per day for pregnant people under 18 years old and 1,100 µg per day of those 19 through 50 years old). No evidence of effect modification by sex of the child was found.

Maternal iodine intake and urinary iodine have been previously associated with child cognitive abilities and language skills,[84–88] however past research has not found evidence of a causal relationship between maternal iodine status and child ASD.[90–92] Levie et al (2020) analyzed material UIC/creatine from three population-based pregnancy cohorts: Generation R (Netherlands), Infancia y Medio Ambiente Project (INMA) (Spain), and Avon Longitudinal Study of Parents and Children (ALSPAC) (UK).[90] INMA and ALSPAC had inadequate median UIC based on World Health guidelines (INMA 134 µg/L, ALSPAC 98 µg/L), while intake was adequate for Generation R (178 µg/L). Due to concerns about sample contamination in the ALSPAC cohort, women with UIC of 500 µg/L were excluded from the study for this cohort only. The three cohorts in this study measured ASD traits differently. Generation R used the social responsiveness scale completed by parents (mean child age of 5.9 years). INMA used the Childhood Autism Spectrum Test completed by parents and psychologists (mean child age of 4.5 to 5.8, depending on region). ALSPAC used the Social Communication Disorder Checklist completed by parents (mean child age of 7.6). In one cohort, Generation R, UIC/creatine less than 150 µg/g was significantly

associated with lower likelihood of autistic traits (compared to greater or equal to 150 μg/g,[90] which is contary to the hypothesis that lower maternal iodine would lead to an increased likelihood of ASD. However, in the meta-analysis, no significant associations were identified between binary maternal UIC/creatine and autistic traits across pregnancy or limiting the sample to two time points in early pregnancy (gestational age prior to 14 weeks or prior 18 weeks) A supplemental analysis found that a unit increase in the natural log of UI/Creatine concentrations was associated with a slight increase in child autistic traits across the three cohorts (OR 1.2, 95% CI 1.0, 1.5; P=0.04).[90] Analyses controlled for maternal age, parity, pre-pregnancy BMI, maternal smoking, ethnicity, maternal education, gestational age at time of urine sample, sex of the child, and child age at assessment.

In Cromie et al (2020),[91] samples were provided between 26-28 weeks gestation as part of the Born in Bradford cohort study. The study population was iodine deficient according to World Health guidelines for median UIC (76 μg/L, IQR 45 -120). In regression models, UIC/creatine was fitted using restricted cubic splines to avoid categorizing by iodine concentration. Child ASD status was determined using primary care records. Children were aged 8 years to 12 years during record review,[91] above the median age of ASD diagnosis in the U.K. when the study was conducted.[105] The authors found no association between the maternal UI/ creatine and child ASD diagnosis and no effect modification by child sex. Models were adjusted for maternal age, ethnicity, maternal socioeconomic status, maternal education, smoking or alcohol in pregnancy, pre-pregnancy BMI, and parity. Berghuis et al (2025), using an iodine replete Canadian sample (median UIC 151.9; IQR 75.9-278.5), found no significant associations between UIC/creatine <150 μg/g and ASD traits measured though SRS scores (pre-school form) completed when children were 3-4 years old. This was the case for a single first trimester sample, a single second trimester sample, and the average of first and second trimesters.[92] In this study, 150-500 μg/g served as the reference group UIC/creatine. Analyses were adjusted for maternal age, race, pre-pregnancy body mass index, parity, maternal education level, child sex, maternal alcohol use during pregnancy, second-hand smoke during pregnancy, maternal depression, breastfeeding, study site, and Home Observation Measurement of the Environment score.

Inadequate iodine was hypothesized to contribute to ASD etiology through maternal thyroid dysfunction. Iodine is necessary for the production of thyroid hormones and iodine deficiency is an established cause of hypothyroidism.[8,65] Maternal hypothyroidism during pregnancy has been found to increase the chances of ASD in the child,[106] though thyroid stimulating hormone (TSH) and free thyroxine (free T4) concentrations are often not correlated with iodine status.[93,107] Hypothyroidism can have multiple causes, including ones that are independent of iodine status. The leading cause of hypothyroidism in iodine replete areas is autoimmune thyroiditis (Hashimoto's thyroiditis),[108] which has a genetic basis.[37,38] This study did not measure thyroid function, though two previously mentioned studies, Levie et al. and Berghuis et al measured both maternal iodine, TSH and free T4 and found no difference between thyroid measures based on maternal iodine status (UIC/creatine above or below 150 μg/g).[90,92] Levie et al. found no association between free T4 or TSH and autistic traits in the meta-analysis and the relationship between thyroid measures and autistic traits was not examined in Berghuis et al. In one of the cohorts (INMA) analyzed by Levie et al., UIC/creatine less than 150 μg/g was an effect modifier in the relationship between maternal TSH and autistic traits. This suggests that elevated maternal TSH could be associated with greater autistic traits in children whose mothers have low UIC/creatine during pregnancy, though effect modification by iodine status did not occur in the meta-analysis or other individual cohorts.[90]

Strengths and limitations

To our knowledge, this is the first study to examine the relationship between maternal iodine intake during pregnancy and autism in the child using a U.S. based sample. This study used at least two urine samples per person to estimate iodine intake. However, it is possible this is not enough samples to be representative of usual iodine intake during pregnancy. 24-hour urine collection is the gold standard for measuring iodine from urine and would theoretically provide a better assessment of iodine consumption than a single spot urine sample from that day. It is not practical to collect 24-hour urine

samples for large sample sizes and several 24-hour urine collections from different time points would still be needed to characterize iodine intake throughout pregnancy. The use of two samples per person is supported by a pregnancy study that found little difference in the prevalence of iodine deficiency in their study population regardless of whether they used two or three spot urine samples per person.[83] Some studies suggest that at least 10 spot urine samples are needed to characterize individual iodine status over a period of time, though changes in diet during the time period and timing of urine samples in relationship to the consumption of iodine rich foods could also affect the number of samples needed to generalize iodine status.[93,109,110]

This study did not standardize for differences in fluid volume of urine. Standardizing iodine measures by creatinine concentration, an indicator of kidney function, can reduce intraindividual variation caused by differences in urine volume.[93] However, a Michigan based cohort found that UIC's remained stable throughout pregnancy, suggesting that changes in urine volume throughout pregnancy may not have a large effect on UIC.[83]

Information on prenatal supplement use or dietary iodine intake was not available for participants in this study. When iodine is consumed in food or supplements, it is stored in the thyroid while the excess is excreted in urine.[76] Even if an individual with low iodine stores begins to consume more iodine, urinary iodine concentration will continue be low while the thyroid regains its iodine stores. Because this study estimated iodine intake using urine samples from an iodine deficient population, it is possible that actual dietary iodine intake was higher than was estimated. This could be the case for participants who generally had low iodine diets but then began taking an iodine-containing prenatal supplement. They may be consuming adequate iodine to support their child's thyroid function and neurodevelopment, but there may be little iodine lost in urine as they replenish their stores, which would lead them to be misclassified as iodine-deficient based on UIC. It is not clear that this misclassification would be differential in terms of SRS score. Prenatal vitamin use early in pregnancy is considered to reduce the chances of ASD diagnosis,[111] but many prenatal vitamins do not contain iodine[80] and the use of iodine containing supplements in relation to ASD outcomes is not known.

This study only examined maternal iodine status during pregnancy, though iodine continues to play a role in neurodevelopment after birth. Infants are typically born with low iodine stores and rely on iodine from breastmilk or formula.[112] Few studies have examined adverse neurodevelopmental outcomes associated with mild to moderate iodine deficiency during infancy and maternal iodine deficiency during pregnancy is believed to be a greater risk factor for adverse developmental outcomes compared to post-partum deficiencies.[112] One RCT conducted in an iodine-sufficient population found higher 36-month cognitive scores in child whose mothers received 150 µg per day iodine supplements during lactation compared to those who received a placebo,[113,114] though an observational study did not find differences in cognitive ability based on iodine concentration found in colustrum.[115]

Future studies should collect multiple urine samples from each trimester to allow for a more rigorous analysis of maternal iodine by trimester. Data on consumption of iodine rich foods and prenatal vitamins containing iodine should be collected in relationship to when urine samples are provided to reduce over or underestimation of iodine intake from UIC. Because prenatal vitamins can vary in iodine context,[80] information on brand and dose should be recorded. Future research studies should examine mothers with the lowest iodine intake to see if they are deficient in other micronutrients that could contribute to an increased chance of ASD in their children. Maternal TSH and free T4 should be analyzed from maternal blood samples collected during pregnancy to replicate findings from a previous study[90] that found maternal iodine status was an effect modifier in the relationship between maternal thyroid dysfunction and child ASD.

Conclusion

This study found an inconsistent relationship between maternal iodine intake during pregnancy and increases in ASD traits. It was one of the first studies to examine this relationship in U.S. mothers and their children. Additional research is needed to clarify which levels of maternal iodine intake might be associated with increased likelihood of ASD traits. Studies that include multiple urine samples per trimester per person and collect information on consumption of iodine rich foods and vitamins in relation

to urine sample timing would be valuable in further exploring this relationship. Because iodine deficiency is hypothesized to contribute to ASD development by disrupting thyroid function, additional research is needed that examines thyroid function in conjunction with maternal iodine status and child ASD. Prevalence of iodine deficiency among pregnant and breastfeeding women in the U.S. should continue to be monitored along with child developmental outcomes including ASD.

Tables and figures
Figure 3.1. Diagram for final analytic set

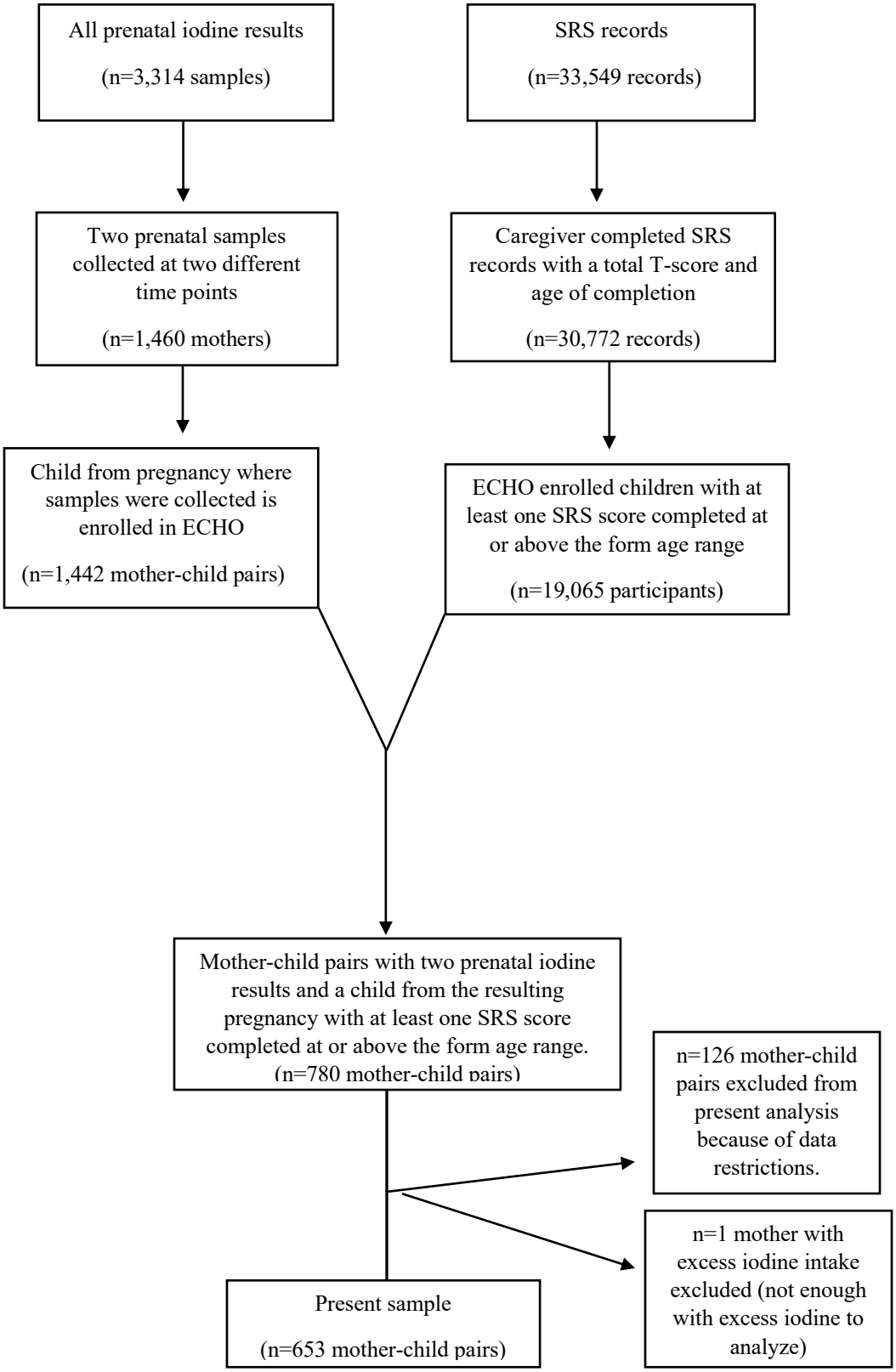


Table 3.1. Sample characteristics by iodine status, n (%) or mean (SD), (n=653).

	Adequate iodine (n=375)	Iodine deficiency (n=278)	P-value	% sample missing
Maternal characteristics				
Mean age at 1st specimen in years (SD)	27.61 (5.43)	28.32 (5.54)	0.1330	14.55%
Highest educational level				
Less than high school	36 (9.89%)	19 (7.01%)		
High school degree	81 (22.25%)	53 (19.51%)	0.3470	2.76%
Some college	88 (24.18%)	64 (23.62%)		
Bachelor's degree or higher	159 (43.68%)	135 (49.82%)		
Pregnancy tobacco or nicotine use (yes)	18 (6.77%)	17 (7.56%)	0.7351	24.81%
Health insurance status				
Private	102 (49.51%)	84 (51.22%)		
Public	91 (44.17%)	69 (42.07%)	0.9193	43.34%
Other	13 (6.31%)	11 (6.71%)		
Hypertensive disorder in pregnancy (yes)	47 (12.57%)	37 (13.36%)	0.7661	0.31%
Gestational diabetes (yes)	31 (8.42%)	26 (9.52%)	0.6285	1.84%
Any infection during pregnancy (yes)	70 (19.72%)	59 (22.61%)	0.3842	5.67%
Pre-pregnancy BMI				
Underweight/Normal weight	136 (39.53%)	128 (48.30%)		
Overweight	91 (26.45%)	62 (23.40%)	0.0926	6.74%
Obese	117 (34.01%)	75 (28.30%)		
Child characteristics				
Sex of child is female	191 (50.93%)	126 (45.32%)	0.1561	0.00%
Mean year of birth (SD)	2013.9 (3.55)	2014.7 (3.47)	0.0027	0.00%
Race/ethnicity				
Black, non-Hispanic	90 (24.13%)	74 (26.62%)		
White, non-Hispanic	185 (49.60%)	142 (51.08%)	0.6860	0.31%
Multiple or other race, non-Hispanic	38 (10.19%)	24 (8.63%)		
Hispanic, any race	60 (16.09%)	38 (13.67%)		
Mean gestational age at birth in weeks (SD)	38.90 (1.69)	38.84 (1.66)	0.6144	0.00%
Mean birthweight in grams (SD)	3335.97 (524.01)	3357.00 (544.91)	0.6214	1.84%

Table 3.2. Child ASD traits by maternal iodine status during pregnancy, n (%) or mean (SD), (n=653).

	Adequate iodine (n=375)	Iodine deficiency (n=278)	P-value	% sample missing
Mean SRS score (SD)	49.47 (9.10)	49.06 (7.75)	0.5361	0.00%
SRS binary category				
No ASD indicated	329 (87.73%)	254 (91.37%)	0.1378	0.00%
Possible ASD	46 (12.27%)	24 (8.63%)		
Mean age at SRS in years (SD)	7.45 (3.23)	6.61 (3.12)	0.0008	0.00%
Parent report of ASD diagnosis				
No	288 (96.97%)	220 (97.78%)	0.5714	20.06%
Yes	9 (3.03%)	5 (2.22%)		

Table 3.3. Adjusted[1] association between maternal iodine and continuous SRS scores (n=556)[2]

Model 1: Binary iodine intake			
	Coefficient	95% CI	P-value
Deficient iodine intake (ref: adequate iodine)	0.53	(-0.84, 1.91)	0.4457
Model 2: Iodine quintiles			
	Coefficient	95% CI	P-value
Iodine intake (μg/day), (ref: Quintile 3)			
Quintile 1*	2.70	(0.56, 4.83)	0.0133
Quintile 2	1.19	(-0.95, 3.32)	0.2763
Quintile 4*	2.65	(0.52, 4.77)	0.0148
Quintile 5	1.73	(-0.40, 3.85)	0.1106
Model 3: Continuous iodine intake			
	Coefficient	95% CI	P-value
Iodine intake (μg/day)	0.00	(-0.01, 0.01)	0.8180

1. Adjusted for maternal BMI, maternal education, gestational diabetes, infections during pregnancy, child race/ethnicity, and child age at SRS.

2. 15% of mother-child pairs are missing because of missing covariates.

*$p<0.05$

Table 3.4. Adjusted[1] odds ratio from logistic regressions testing associations between maternal iodine and possible child ASD based on SRS score (ref: no ASD indicated) (n=556)[2]

Model 1: Binary iodine intake			
	Odds ratio	95% CI	P-value
Deficient iodine intake (ref: adequate iodine)	0.96	(0.53, 1.75)	0.8906
Model 2: Iodine quintiles			
	Odds ratio	95% CI	P-value
Iodine intake (μg /day), (ref: Quintile 3)			
Quintile 1	1.72	(0.66, 4.47)	0.2696
Quintile 2	0.76	(0.25, 2.30)	0.6269
Quintile 4*	2.64	(1.07, 6.55)	0.0358
Quintile 5	0.96	(0.35, 2.62)	0.9388
Model 3: Continuous iodine intake			
	Odds ratio	95% CI	P-value
Iodine intake (μg/day)	1.00	(0.99, 1.00)	0.4360

1. Adjusted for maternal BMI, maternal education, gestational diabetes, infections during pregnancy, child race/ethnicity, and child age at SRS.

2. 15% of mother-child pairs are missing because of missing covariates.

*$p<0.05$

Appendix

Table A3.1. Iodine intake cut points for quintile regression

Quintile	Cut Points
Quintile 1	(30.23 μg/day, 114.68 μg/day)
Quintile 2	(114.91 μg/day, 152.33 μg/day)
Quintile 3 (reference)	(153.57 μg/day, 195.90 μg/day)
Quintile 4	(196.21 μg /day, 260.06 μg/day)
Quintile 5	(260.17 μg/day, 720.70 μg/day)

Table A3.2. Urinary Iodine Concentrations (UIC) μg/L, by trimester and binary SRS category (n=653 mother-child pairs)

Trimester	Samples (count)	Median	Q1	Q3	Min	Max
Total sample						
First Trimester	229	160.80	104.00	252.00	18.00	1167.40
Second Trimester	611	152.20	87.30	253.90	8.40	2113.20
Third Trimester	603	145.30	85.60	250.10	4.00	1995.30
Missing	197	108.80	59.00	221.80	3.40	1248.00
Overall	1640	146.20	85.00	248.05	3.40	2113.20
Possible ASD						
First Trimester	29	208.00	132.50	304.00	47.60	365.20
Second Trimester	65	182.00	123.00	255.00	8.40	493.00
Third Trimester	74	149.00	89.40	247.00	14.40	1338.40
Missing	14	87.45	63.40	281.70	28.10	315.30
Overall	182	162.65	98.40	255.00	8.40	1338.40
No ASD indicated						
First Trimester	200	160.15	103.00	248.25	18.00	1167.40
Second Trimester	546	146.30	85.80	247.90	8.80	2113.20
Third Trimester	529	144.70	85.50	255.20	4.00	1995.30
Missing	183	109.00	59.00	221.80	3.40	1248.00
Overall	1458	144.45	84.70	247.60	3.40	2113.20

Table A3.3. **Unadjusted** coefficients from linear regressions testing associations between maternal iodine and continuous child SRS scores (n=653)

Model 1: Binary iodine intake			
	Coefficient	95% CI	P-value
Deficient iodine intake (ref: adequate iodine)	-0.36	(-1.66, 0.95)	0.5932
Model 2: Iodine quintiles			
	Coefficient	95% CI	P-value
Iodine intake (μg/day) (ref: Quintile 3)			
Quintile 1	0.72	(-1.31, 2.75)	0.4849
Quintile 2	0.05	(-1.98, 2.07)	0.9642
Quintile 4	1.57	(-0.47, 3.60)	0.1306
Quintile 5	0.96	(-1.08, 3.01)	0.3559
Model 3: Continuous iodine intake			
	Coefficient	95% CI	P-value
Iodine intake (μg/day)	0.00	(-0.00, 0.01)	0.5221

Table A3.4. **Unadjusted** odds ratios from logistic regressions testing associations between maternal iodine and possible child ASD based on SRS score (ref: no ASD indicated) (n=653)

Model 1: Binary iodine intake			
	Odds ratio	95% CI	P-value
Deficient iodine intake (ref: adequate iodine)	0.68	(0.40, 1.14)	0.1401
Model 2: Iodine quintiles			
	Odds ratio	95% CI	P-value
Iodine intake (μg/day), (ref: Quintile 3)			
Quintile 1	0.93	(0.43, 2.03)	0.8629
Quintile 2	0.44	(0.17, 1.11)	0.0820
Quintile 4	1.56	(0.77, 3.17)	0.2167
Quintile 5	0.79	(0.35, 1.75)	0.5557
Model 3: Continuous iodine intake			
	Odds ratio	95% CI	P-value
Iodine intake (μg/day)	1.00	(0.99, 1.00)	0.9506

Table A3.5. Adjusted[1] association between maternal iodine intake and SRS scores (continuous or binary), with interaction between iodine and sex of the child

Binary iodine intake and continuous SRS outcome (n=556)[2]			
	Coefficient	95% CI	P-value
Usual Daily Iodine Intake, Adequate Iodine	0.28	(-1.57, 2.14)	0.4381
Child Sex, Male	-0.51	(-2.291, 1.28)	0.7291
Usual Daily Iodine Intake*Child Sex	0.53	(-2.18, 3.24)	0.7006
Quintile iodine intake and continuous SRS outcome (n=556)[2]			
Iodine intake (μg/day), (ref: Quintile 3)	Coefficient	95% CI	P-value
Quintile 1	3.18	(0.24, 6.12)	0.0344
Quintile 2	1.07	(-1.93, 4.08)	0.4838
Quintile 4	3.33	(0.33, 6.33)	0.0299
Quintile 5	2.68	(-0.25, 5.61)	0.0730
Child sex (ref: male)	0.63	(-2.43, 3.68)	0.7589
Overall interaction between iodine intake*child sex			0.8021
Quintile iodine intake and binary SRS outcome (n=556)[2]			
Iodine intake (μg/day), (ref: Quintile 3)	Odds ratio	95% CI	P-value
Quintile 1	1.65	(0.62, 4.40)	0.3750
Quintile 2	0.71	(0.23, 2.25)	0.9365
Quintile 4	2.63	(1.06, 6.56)	0.1419
Quintile 5	0.96	(0.35, 2.65)	0.7809
Child sex (ref: male)	0.69	(0.37, 1.3)	0.2611
Overall interaction between iodine intake*child sex			0.9605

1. Adjusted for maternal BMI, maternal education, gestational diabetes, infections during pregnancy, child race/ethnicity, and child age at SRS.

2. 15% of mother-child pairs are missing because of missing covariates.

Table A3.6. Adjusted[1] association between maternal iodine and continuous SRS scores, stratified by sex

Males only (n=294)[2] Iodine quintiles			
Iodine intake (μg/day), (ref: Quintile 3)	Coefficient	95% CI	P-value
Quintile 1	3.00	(-0.07, 6.07)	0.0556
Quintile 2	0.80	(-2.32, 3.92)	0.6152
Quintile 4*	3.40	(0.27, 6.52)	0.0333
Quintile 5	2.58	(-0.4, 5.62)	0.0953
Females only (n=262)[3] Iodine quintile			
Iodine intake (μg/day), (ref: Quintile 3)	Coefficient	95% CI	P-value
Quintile 1	2.45	(-0.52, 5.42)	0.1052
Quintile 2	1.49	(-1.39, 4.37)	0.3092
Quintile 4	1.58	(-1.35, 4.47)	0.2812
Quintile 5	0.45	(-2.52, 3.41)	0.7674

1. Adjusted for maternal BMI, maternal education, gestational diabetes, infections during pregnancy, child race/ethnicity, and child age at SRS.

2. 13% of mother-child pairs are missing because of missing covariates.

3. 17% of mother-child pairs are missing because of missing covariates.

*$p<0.05$

Conclusion

The etiology of ASD is complex and likely involves a confluence of genetic, epigenetic, and environmental factors. Some exposures might be uniquely associated with ASD development, but many are likely associated with more than one developmental outcome. An additional element to consider is the timing of environmental exposures in relation to critical periods of neurodevelopment. Pregnancy itself is a time of rapid neurodevelopment and within pregnancy, there may be periods of heightened sensitivity to certain exposures. To add another layer, differing amounts (doses) of the exposure could have varying impacts on neurodevelopment. With this framework in mind, this dissertation made a contribution to what is known about two prenatal environmental exposures, maternal thyroid function and iodine intake, and their associations with ASD diagnosis or traits.

The first original research study examined the relationship between maternal thyroid dysfunction, thyroid hormones and child neurodevelopmental outcomes. In the MARBLES cohort, maternal thyroid hormone concentrations were measured from pregnancy blood samples. Based on a TPO and TG negative subsample with no self-report of thyroid conditions, pregnancies were categorized as euthyroid (normal thyroid), affected by a hypothyroid condition (subclinical hypothyroidism or hypothyroxinemia, no one had overt hypothyroidism), or affected by a hyperthyroid condition (subclinical or overt hyperthyroidism, hyperthyroxinemia). From the 285 pregnancies and 273 mothers analyzes, 9.5% of pregnancies were affected by hypothyroid conditions and 6.7% were affected by hyperthyroid conditions. Mothers with hypothyroid conditions had the highest prevalence of children with ASD (44%) or non-typical development (26%) compared to mothers with hyperthyroid conditions (ASD 16%, Non-TD 21%) or mothers with euthyroid function (ASD 28%, Non-TD 13%).

In regression analyses, maternal hypothyroid conditions were significantly associated with about three times the odds of ASD compared to typical development. Maternal hyperthyroid conditions were not significantly associated with ASD. These findings are congruent with existing literature, [51–54] though they may reflect a greater difficulty in researching hyperthyroidism compared to hypothyroidism because

hyperthyroidism is relatively rare during pregnancy. [27] Maternal hypothyroid conditions during pregnancy were also significantly associated with non-TD, which suggests that maternal hypothyroid conditions may not be an etiological factor unique to ASD. A one unit increase in first trimester natural log of TSH was significantly associated with an ASD diagnosis and some percentiles of the SRS and MSEL while second and third trimester were not. This lends support to the hypothesis that prior to 14 weeks in first trimester may be a window of increased sensitivity to thyroid hormones.[59] One limitation of this study was the small number of mothers with each type and severity of thyroid dysfunction. It was not possible to examine subclinical and overt cases separately, which would have been valuable for determining if severity of thyroid dysfunction predicted child neurodevelopmental outcomes differently. Similarly, the lack of mothers with overt hypothyroidism may indicate selection bias because overt hypothyroidism is associated with reduced fertility and increased risk of miscarriage. [27,47–49]

The second original research study examined the relationship between maternal iodine status during pregnancy and autism traits in the child (standardized SRS scores). In the ECHO cohorts that met eligibility, individual maternal iodine intake per day was estimated using at least two pregnancy urinary iodine concentrations per mother. In a sample of 653 mother-child pairs, 42.5% of mothers were categorized as having estimated deficient iodine intake with the remaining categorized as adequate intake. There was no difference in average SRS scores among children born to mothers with estimated deficient intake compared to adequate intake. In regression analyses, the only significant relationships were in quintile analyses, where the 1st and 4th quintile were associated with about a quarter of a standard deviation increase in SRS scores compared to the 3rd quintile. The 4th quintile was similarly associated with an increased likelihood of possible ASD when the SRS was treated as a binary measure. These results are comparable to the small body of existing literature that found little evidence to support a causal relationship between maternal iodine status and ASD outcomes.[90–92] One limitation of this study is that two urine samples per pregnancy may not be enough to generalize iodine intake throughout pregnancy.

Most mothers did not have multiple urine samples from first trimester, which limits the extend that SRS scores can be studied in relation first trimester iodine status.

The MARBLES and ECHO studies suggested a higher prevalence of thyroid dysfunction and iodine deficiency respectively than what is estimated in the general population. This may be because these samples are not representative of the general population. MARBLES is a high-likelihood ASD cohort, and if thyroid dysfunction truly increases the chance of ASD, then this may lead to overrepresentation of thyroid dysfunction in this cohort. The ECHO sample does not include any high-likelihood ASD cohorts, but some cohorts included in this study restricted participation based on maternal age, race or health conditions, which could affect overall generalizability.

In this dissertation, maternal hypothyroid conditions were significantly associated with an increased likelihood of ASD outcomes, but an association between maternal iodine and ASD was not substantiated. Existing literature reflects similar findings- multiple studies have found that maternal hypothyroidism increases the chance of ASD outcomes [106] and only one study partially supports a relationship between maternal iodine and ASD.[90] This study, and others examining maternal iodine and ASD, may not have used enough spot urine samples to be representative of regular iodine intake.[90–92] Because the fetal thyroid gland is not functional until around 14 weeks of gestation, adequate maternal iodine intake is particularly important in first trimester.[59] Women who were iodine deficient prior to pregnancy are less prepared to handle the increased demands of iodine during pregnancy. Estimating preconception iodine status and determining when or if iodine containing prenatal supplements are initiated could provide clearer picture of maternal iodine status early in pregnancy. More studies are needed to monitor the prevalence of maternal thyroid dysfunction and iodine deficiency and investigate the extent that maternal hypothyroidism is caused by deficient iodine intake. Because iodine deficiency is only one possible cause of hypothyroidism, other possible causes (including endocrine disrupting chemicals) should be evaluated alongside child developmental outcomes. Thyroid autoimmunity is a common cause of thyroid dysfunction[27] and like, ASD, has genetic components.[5,37,38] Future research

should investigate whether there are shared genetic risks between autoimmune thyroid conditions and ASD. Additional studies that use a combination of self-reported dietary measures and urine samples collected multiple times during first trimester and again in subsequent trimesters would be help improve accuracy of estimated iodine intake. Though it is challenging, untangling how exposures such as maternal thyroid dysfunction and iodine could contribute to ASD development is important to enriching what is known about the origins of ASD.

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