Maternal Thyroid Autoantibody and Elevated Risk of Autism in a National Birth Cohort

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Abstract

Objective—Autoimmune disruption may contribute to risk for autism; however, since previous studies relied upon clinical diagnoses, exposure misclassification and recall bias are limitations. Thyroid peroxidase antibody (TPO-Ab) is an autoantibody involved in autoimmune thyroiditis. We aimed to test the *a priori* hypothesis that positivity to maternal serum TPO-Ab (TPO-Ab+) (defined as > 156 IU/ml) during pregnancy is related to childhood autism.

Method—The study was based on a nested case-control design of the Finnish Prenatal Study of Autism (FiPS-A), a national birth cohort that includes prospectively drawn archived maternal serum specimens from virtually the entire pregnant population of Finland. Cases of childhood autism (ICD-10 F84.0) born from 1987–2005 were ascertained by performing linkages between national birth and inpatient/outpatient registries. All diagnosed cases of childhood autism in Finland over the birth years, and comparison subjects without ASD or severe/profound intellectual disability were matched 1:1 on date of birth, sex, birthplace, and residence in Finland. Maternal

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serum specimens were assayed in 967 matched case-control pairs for TPO-Ab by a chemiluminescent microparticle immunoassay blind to case/control status. Data were analyzed by conditional logistic regression for matched sets.

Results—The prevalence of maternal TPO-Ab+ was significantly increased in pregnancies giving rise to autism cases (6.15%) compared to controls (3.54%). The odds of autism were increased by nearly 80% among offspring of mothers who were TPO-Ab+ during pregnancy (OR=1.78, 95% CI=1.16–2.75, p=0.009), compared to mothers negative for this autoantibody. There was also a significant relationship between maternal TPO-Ab defined as a continuous variable and odds of autism (OR=1.09, 95% CI=1.01, 1.17, p=0.02). Measures of maternal thyroid hormones did not differ between groups.

Conclusions—These findings provide the first biomarker-based evidence that a class of known maternal autoimmune disorders is related to autism in offspring.

Keywords

thyroid; autoantibody; autism; birth cohort; autoimmune; epidemiology

Introduction

Autism is a complex neurodevelopmental disorder characterized by impaired language, disrupted reciprocal social interactions, and stereotyped behaviors and interests(1). Genetic factors are known to play a major role in autism though its etiology is still largely unknown(2). Recent evidence has also implicated an emerging role for environmental factors (3–11).

Thyroid peroxidase (TPO), a thyrocyte apical plasma membrane glycoprotein, is an antigenic epitope that, in susceptible individuals, may induce formation of thyroid peroxidase antibody (TPO-Ab), an autoantibody involved in autoimmune thyroiditis including Hashimoto's thyroiditis(12, 13). Maternal TPO-Ab positivity (TPO-Ab+) has been associated with sensorineural hearing loss in children(14). In addition, five year old offspring of mothers with TPO-Ab+ during late gestation had diminished verbal, perceptual, cognitive, and motor performance(15).

Moreover, some autoimmune disorders may be more frequent in mothers and other relatives of autism probands. Early studies, based on questionnaires of family members, reported that the prevalence of any autoimmune disorder, and one or more of a number of specific autoimmune disorders was significantly higher in families of autism probands than comparison subjects(16, 17). With regard to autoimmune thyroid disorders, the frequency of "hypothyroidism/Hashimoto's thyroiditis" was greater in family members of probands with pervasive developmental disorder (PDD) and probands with autoimmune disorders than healthy comparison subjects(17). In another study, autoimmune thyroiditis in only the maternal lineage was significantly related to regressive autism(18). Other specific autoimmune diagnoses associated with ASD included parental rheumatoid arthritis(16) and rheumatic fever (first degree relatives)(17). These studies were limited, however, by use of diagnoses from family member self-reports and lack of validation of responses predisposing

to diagnostic misclassification, by recall bias, and by low response rates to questionnaires, increasing the likelihood of selection bias.

More recent studies utilizing health plan databases and registries have demonstrated associations between ASD and maternal psoriasis, type I diabetes(19–21), ulcerative colitis, and celiac disease(22). Overall, maternal autoimmune disorders were more commonly associated with autism than paternal autoimmune disorders, suggesting effects during pregnancy on autism risk, though the type of autoimmune disorders related to autism varied between studies.

In a previous study, plasma from 11.5% of mothers of children with ASD, but no mothers of comparison subjects, demonstrated IgG-reactivity against fetal brain proteins at 37 kDa and 73 kDa(23). This finding was extended in a larger sample(24). In a further study, a band reactive to brain protein in the Rhesus macaque was found at 39kDa(25). Prenatal exposure to these antibodies was related to whole body stereotypies and hyperactivity in nonhuman primates and rodents supporting a potential pathogenic role for these antibodies in autism(26). More recently, maternal anti-brain antibodies were shown to be related to a fourfold increased risk of ASD, and mothers with these antibodies exhibited an increased prevalence of anti-nuclear antibodies and certain autoimmune diseases(27).

In the present study, we directly quantified maternal TPO-Ab, a biomarker utilized in the diagnosis of autoimmune thyroiditis. TPO-Ab was ascertained in cases and comparison subjects from a national birth cohort. A definitive diagnosis of autoimmune thyroiditis relies on the demonstration of not only circulating antibodies to thyroid antigens but also reduced echogenicity on thyroid sonogram in a patient with clinical features(28). However, compared to other epidemiologic studies of autoimmune disorders and ASD, this reduces the possibility of inaccurate diagnoses of autoimmune disorders, bias due to preferential recall and treatment seeking behavior, and lack of inclusion of asymptomatic subjects. The large number of cases and comparison subjects enhanced statistical power to detect an association.

We tested the hypothesis that the odds of autism in offspring is related to maternal TPO-Ab+ exposure documented in archived maternal prenatal sera. The investigation was conducted in the Finnish Prenatal Study of Autism (FiPS-A), which capitalizes on a large number of pregnancies from a national birth cohort with prospectively collected, archived maternal serum specimens in the Finnish Maternity Cohort (FMC), an extensive, centralized biobank. Virtually all childhood autism cases in Finland identified from national computerized registries of hospital admissions and outpatient treatment, and validated by a structured research interview, were included.

There is previous evidence that maternal hypothyroidism is associated with adverse cognitive outcomes(29, 30). Although the main focus of this paper was to address maternal thyroid autoimmunity and autism, in order to assess whether associations between maternal TPO-Ab+ and autism were accounted for by thyroid dysfunction, we conducted secondary analyses of maternal clinical and subclinical hypothyroidism, maternal thyroid stimulating hormone (TSH), and free T4 (fT4) levels, and autism.

Methods

The methods are described in detail in Lampi et al(31), and will be summarized here. The FiPS-A is based on a nested case-control design. The sampling frame was defined such that all members of this national birth cohort were within the age of risk of autism. Toward this end, the sampling frame consisted of all offspring born in Finland from 1987–2005, and subjects were followed up until 2007 (see "Case and comparison subject identification").

Description of the birth cohort, biobank, and national registries

All offspring in the FiPS-A were derived from the Finnish Maternity Cohort (FMC), which consists of greater than 1 million pregnancies with archived prenatal serum specimens drawn beginning in 1983. Sera were obtained during the first and early second trimesters from over 98% of pregnant women in Finland. One maternal serum sample was acquired for each pregnancy. Following the screening, serum samples were stored as one aliquot at minus 25°C in a single biorepository at THL in Oulu, Finland. All samples in the FMC can be linked with offspring using a unique personal identification number (PIN), which has been assigned to all residents of Finland since 1971.

Case and comparison subject identification

The Finnish Hospital and Outpatient Discharge Registry (FHDR) was utilized to identify all recorded diagnoses from psychiatric hospital admissions and outpatient visits of childhood autism (ICD-10 F84.0) among members of the FMC. We restricted the outcome to this diagnosis given that only childhood autism, not Asperger disorder or PDD NOS, were validated by interview (see next paragraph). Computerized data are available from January 1, 1987 to the present. Only singleton births were included. Cases diagnosed over the sampling frame were identified from registry linkages between the FMC and the FHDR from January 1, 1987 to December 31, 2007. During this time period, there were 1.2 million births. The total number of childhood autism cases in the FiPS-A study sample was 1,132.

In order to validate the registry diagnoses, 80 cases of infantile autism from the FHDR were assessed with the Autism Diagnostic Interview-Revised (ADI-R). Among these cases, 77 (96%) met the criteria for childhood autism by this instrument(32).

The cases were matched 1:1 to comparison subjects (singleton births only) drawn from the birth cohort who were without ASD (no F84 diagnosis) or severe/profound intellectual disability on date of birth, sex, birthplace, and residence in Finland. Serum samples were assayed for TPO-Ab (see "Laboratory assays") in the 967 matched cases with adequate quantities of sera for this assay from among the 1,132 cases, and in 967 matched comparison subjects. We utilized 1:1 matching of cases to comparison subjects given limited resources for assaying sera on a larger number of comparison subjects. For a secondary analysis aimed at testing whether a putative association between TPO-Ab and autism was accounted for by thyroid hormone abnormalities, assays were also conducted for thyroid stimulating hormone (TSH) and free thyroxine (fT4) in these same 967 matched case-control pairs. Measurements were available on 960 pairs for TPO-Ab, 954 pairs for TSH, and 958 pairs for fT4; these

sample sizes differed slightly from those assayed for TPO-Ab due to sample volume constraints in a small proportion of subjects.

The study was approved by the ethical committees of the hospital district of Southwest Finland, THL, and the Institutional Review Board of the New York State Psychiatric Institute. Informed consent was obtained at the time of donation of all maternal serum specimens after the nature and possible consequences of the procedure and data derived from serum analyses were explained.

Laboratory assays

Quantitative analyses of TPO-Ab and thyroid hormones (TSH and fT4) were performed blind to case and comparison subject status using chemiluminescent microparticle immunoassays with an Architect i2000 automatic analyzer (Abbott Diagnostics, Abbott Park, IL). Intra- and interassay variation, respectively, were as follows: TPO-Ab (IU/ml) (2.5% and 9.8%), TSH (μ IU/ml) (1.7% and 5.3%), and fT4 (pmol/l) (3.6% and 7.8%). The lowest limits of detection for TPO-Ab, TSH, and fT4, respectively, are as follows: 1.0 IU/ml, 0.0025 μIU/ml, and 5.1 pmol/L.

Statistical analysis

In the main analysis, we examined the relationship between maternal TPO-Ab+ and autism in offspring. A dichotomous measure of TPO-Ab was used in keeping with the standard approach of prior research studies and routine clinical practice(14, 15, 33). TPO-Ab+ was defined as >95th percentile in controls for the present study; TPO-Ab negativity was defined as 95th percentile in controls. This percentile cut-off was based on the assay manufacturer's recommendation for the determination of TPO-Ab in a healthy population (Abbott Diagnostics, Wiesbaden, Germany). This cut-point for TPO-Ab+ corresponded to >156 IU/ml. Appropriate to the nested case-control study design, point and interval estimates of odds ratios were obtained by fitting conditional logistic regression models for matched sets. Statistical significance was judged at $P < 0.05$.

In order to assess whether a putative relationship between TPO-Ab+ and autism was related to thyroid hormone disorders, we conducted secondary analyses of relationships between autism and: 1) maternal clinical hypothyroidism; 2) maternal subclinical hypothyroidism; 3) maternal clinical hyperthyroidism; 4) maternal subclinical hyperthyroidism. These conditions, and the reference group, were defined as described in Table 3, based on standard methods used to detect these conditions from TSH and fT4 levels, which were also utilized in the FMC cohort in Männistö et al(34). To further examine the relationship between maternal TSH, fT4, and autism, these hormones were also examined as continuous variables.

Potential confounders were selected based either on previous relationships with tests of thyroid function or autism (35–41). They are presented in Table 1, and included maternal age, paternal age, number of previous births (0, >1), socioeconomic status (upper white collar, lower white collar, blue collar, other), preterm birth (<37 weeks), low birthweight (<2500 g), small for gestational age (SGA) status (defined as birthweight for gestational age based on national sex-specific weight distribution standards in Finland, see Table 1)(42),

maternal/parental history of psychiatric disorders, and gestational week of the blood draw(33). Data on maternal age, paternal age, socioeconomic status, and maternal/paternal history of psychiatric disorders were acquired from the FHDR and the Finnish Population Registry. Data on previous births, preterm birth, low birthweight, and small for gestational age status were acquired from the FMBR. Data on gestational week of the blood draw were obtained from the FMC. Covariates were included in the adjusted model based on the change in estimate method (43), with a cut-off value of a 10% change in the unadjusted odds ratio.

In secondary analyses, we examined maternal TPO Ab+ stratified by sex and by comorbid intellectual disability. The rationale for this includes the well-known sex differences in autism(44) and extensive evidence of comorbidity between autism and intellectual disability(45). This begs the question of whether there may be subgroups of cases stratified by these respective variables in which TPO Ab+ shows greater associations with autism. ICD-10/9 codes for ID are provided in Table 2. Stratified analyses were also conducted by birth year. Statistical analyses were performed with SAS software (SAS 9.2, SAS Institute, Cary, NC, USA). Given that the study tested only one primary hypothesis, the relationship between maternal TPO Ab + and autism, Bonferroni correction was not performed.

Statistical power was calculated using PS software (Power and Sample Size Calculation version 3.0(46)).

Results

Covariates and TPO-Ab+

The relationship between the covariates and TPO-Ab+, and the covariates and childhood autism are presented, respectively, in Tables 1 and 2. None of the covariates met criteria for confounding based on the change in estimate method(43). Only one covariate, paternal age, led to a change in estimate of the odds ratio >5% (5.6%). Hence, we did not adjust for any of the covariates.

Thyroid peroxidase antibody positivity (TPO-Ab+) and autism (Table 3)

The odds of autism were increased by nearly 80% among offspring of mothers who were TPO-Ab+ during pregnancy (OR=1.78, 95% CI=1.16–2.75, p=0.009), compared to mothers negative for this autoantibody.

Results of secondary analyses

There was also a significant relationship between maternal TPO-Ab+ defined as a log transformed continuous variable and odds of autism (OR=1.09, 95% CI=1.01–1.17, p=0.02). The effect sizes were similar in males and females and there was no interaction between maternal TPO-Ab+ and sex (p=0.32) (Table 3). The association was increased greater than twofold for cases without comorbid ID; there was no significant association for cases with intellectual disability (Table 3); however, there was no interaction between TPO-Ab+ and ID (p=0.98).

In order to assess whether birth year played a role in the association between TPO Ab+ and autism, we stratified the sample into older born (1987–1996) and younger born (1997–2005) groups. Associations between maternal TPO-Ab+ and autism were observed in both strata (born 1987–1996: OR=1.60, 95% CI=0.97–2.64, p=0.065; born 1997–2005: OR=2.43, 95% CI=1.007–5.86; p=0.048).

There were no associations between maternal clinical hypothyroidism or subclinical hypothyroidism and autism (Table 4). There were also no relationships between maternal clinical hyperthyroidism or subclinical hyperthyroidism and autism (Table 4). Moreover, we found no association between maternal TSH defined as a continuous variable and autism [cases, mean (SD)=1.36 (1.39); comparison subjects, mean (SD)=1.38 (1.89), OR=0.99, 95% CI=0.91–1.09, p=0.97)], nor between fT4 defined as a continuous variable and autism [cases, mean (SD) =14.7 (2.0); comparison subjects, mean (SD)=14.7 (1.92), OR=1.09, 95% CI=0.52–12.26, p=0.83)]. As expected, there were relationships between maternal TPO-Ab+ and maternal clinical and subclinical hypothyroidism in pregnancies giving rise to both case (p < 0.001) and comparison subject (p < 0.001) offspring.

Discussion

We demonstrated a significant increase in prevalence of maternal TPO-Ab positivity in pregnancies that gave rise to cases of childhood autism compared to control pregnancies in a national birth cohort of over 900 matched case-control pairs. We did not observe any association between maternal hypo- or hyperthyroidism, nor between maternal TSH or fT4 levels and autism, indicating that the relationship between maternal TPO-Ab+ and autism in offspring was not accounted for by maternal thyroid hormones. We note that maternal thyroid hormone levels were consistent with previous studies on the birth cohort of the present study(47). Iodine deficiency, including during pregnancy, is rare in Finland given that iodine supplementation through iodized salt, dairy products, and other means, is routine(48).

Although we did not observe an association between maternal hypothyroidism and autism in the present study, three recent studies suggest an association. In the first, very low neonatal T4 levels (<3rd percentile) were associated with ASD in one of two birth years(49); in a second study, which was the only other previous study to investigate TPO-Ab, severe maternal hypothyroxinemia but not TPO-Ab positivity was related to parent-reported autistic symptoms(50), but was limited to some degree by a lack of clinical diagnoses and a relatively small number of cases with "probable autism" (n=81). In a third study, based on a large Danish sample, maternal hypothyroidism determined via registry diagnosis or treatment, and diagnosed mainly after the birth of the child was associated with an approximately 30% increased odds of ASD(51). The inconsistency in findings across studies may result from methodological differences including sample sizes, methods of ascertainment of hypothyroidism and autism, or from true differences between populations. However, given that only one prior study measured TPO-Ab, it is not known to what extent our findings with regard to TPO-Ab+ would agree with those from other populations.

We also did not demonstrate associations between maternal TPO Ab+ and preterm birth, low birthweight and weight for gestational age. This suggests that these peri-/neonatal complications did not mediate the association between TPO Ab+ and autism.

The study has several strengths. First, the cases and comparison subjects were derived from a large, population-based national birth cohort including all childhood autism cases diagnosed in Finland through psychiatric registries which cover the entire population. The cases with maternal sera represented over 85% of all childhood autism cases in the population and their selection was not predicated on the exposure, or related factors. This indicates that the potential for selection bias was small. Second, diagnoses were restricted to the childhood autism subtype, which was well-validated(32). Third, the sample size was large, with over 900 matched case-control pairs. Fourth, the study had the ability to demonstrate no association between thyroid hormone and autism, which suggests that the findings were not mediated by thyroid hormone abnormalities.

We note some caveats. First, the sera were stored at minus 25°C, which is far from ideal (typical storage temperatures are minus 80°C), and most sera were stored for long intervals of time. This may have accounted for loss of antibody reactivity. However, since cases and comparison subjects were matched on date of birth, this is most likely to have resulted in non-differential misclassification, which generally biases the findings toward the null, rather than introducing spurious associations. Second, although extensive testing of many covariates did not indicate confounding, there is the possibility of residual confounding. Third, maternal TPO-Ab+ may not be specific to childhood autism among ASD and other developmental disorders. Specifically, we did not investigate Asperger's syndrome and pervasive developmental disorder other/NOS in this study because these registry diagnoses have not yet undergone testing for validation. With regard to other developmental disorders, our subsequent analysis revealed that the association between maternal TPO-Ab+ and autism was significantly increased in cases *without*, but not with, comorbid ID. While we have no ready explanation for this, previous work has suggested differences in the genetic and environmental factors contributing to ASD with versus without ID. For example, loss of function mutations were associated with lower IQ levels among boys with ASD from the Simons Simplex Collection(52) and pregnancy complications were associated with risk for ASD comorbid with ID, but not ASD alone, in an Australian cohort(53). Fourth, given that the median age of first diagnosis in the sample was 4 years, and the youngest subjects were 2 years old, misclassification of subjects in the control group who later developed autism may have occurred. However, since such misclassification would have only affected subjects born during the latest years of births, and given the relatively low prevalence of autism, the number of controls who would have developed autism had they been followed longer is very small compared to the total number of controls. Fifth, data are not available on TPO-Ab or thyroid hormones in the offspring, or on other environmental factors present during infancy or childhood that may have influenced the risk of autism.

Although a precise mechanism by which maternal thyroid autoantibody increases risk of autism is not known, the process might involve maternal-fetal transfer of these autoantibodies. Maternal-fetal transmission of IgG antibodies is an active Fc-γ-receptor-mediated process that commences at approximately week 17 of gestation and results in an

IgG concentration above that of the mother at term(54). Cord blood from forty percent of newborns of mothers with TPO-Ab+ evidenced elevated levels of this antibody, and the maternal-neonatal correlation was strong (r=0.96) and highly significant (p < 0.001). In addition, autoimmune thyroiditis in children is significantly related to TPO-Ab+ in both cord blood and maternal sera during pregnancy, suggesting the possibility of longer term effects of fetal exposure(55). Offspring of mothers who were TPO-Ab+ during pregnancy were more likely to be TPO-Ab+ during adolescence (56). Finally, newborns who had transient hypothyroxinemia of prematurity evidenced an increase in ASD risk as young adults, though findings were only significant among those whose mothers did not have hypertension during pregnancy(57).

It is also possible that maternal TPO-Ab+ may serve as a marker for other factors that increase offspring risk of autism. It may be that heritable factors associated with maternal TPO-Ab+ are related to autism, given associations noted above between parental autoimmune thyroiditis, and autism in probands(16–19, 21, 22). Consequently, the association observed in the present study may be accounted for by genes that predispose to autoimmune thyroiditis in family members, which are then inherited by offspring. Consistent with this hypothesis, variation in the human leukocyte antigen (HLA) system of genes has been associated with both autoimmune disease, including autoimmune thyroid disease(58), and with autism(59, 60). Alternatively, an underlying maternal immune dysfunction may involve both TPO-Ab+ and the maternal production and transfer to the fetus of anti-brain antibodies. This would be in accord with findings from a previous study that found maternal anti-brain antibodies to be associated with both ASD among their offspring and an increased prevalence of autoimmune diseases(27). Measuring anti-brain antibodies in subjects from the current study and determining their correlation with TPO-Ab + could allow for assessment of this hypothesis in the future.

Conclusion

Maternal TPO-Ab antibody positivity was significantly associated with childhood autism in a large, national birth cohort. To our knowledge this is the first association between a biomarker of a class of known maternal autoimmune disorders and autism. This finding may have important implications for understanding the pathogenesis of this disorder. In our view, animal models will be necessary to understand the molecular mechanisms by which elevated maternal TPO antibody alters fetal brain development in such a way as to increase risk of autism. Another important future potential implication of this work, if replicated, is to identify neonates at risk for ASD, perhaps by using a scale that incorporates TPO antibody with other biomarkers. These babies could be targeted for early intervention if the scale is demonstrated to have a relatively good positive predictive value.

Acknowledgements

Abbreviations

TPO	Thyroid peroxidase
TPO-Ab	Thyroid peroxidase antibody
FiPS-A	Finnish Prenatal Study of Autism
PDD	Pervasive developmental disorder
FHDR	Finnish Hospital and Outpatient Discharge Registry
ADI-R	Autism Diagnostic Interview-Revised
TSH	Thyroid stimulating hormone
fT4	free thyroxine
ASD	Autism spectrum disorder

References

1. Fombonne E. Epidemiology of pervasive developmental disorders. Pediatr Res. 2009; 65:591–598. [PubMed: 19218885]

2. Persico AM, Napolioni V. Autism Genetics. Behav Brain Res. 2013; 251:95–112. [PubMed: 23769996]

3. Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R. Traffic-related air pollution, particulate matter, and autism. JAMA Psychiatry. 2013; 70:71–77. [PubMed: 23404082]

4. Patterson PH. Maternal infection and immune involvement in autism. Trends Mol Med. 2011; 117:389–394. [PubMed: 21482187]

5. Newschaeffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, Levy SE, Mandell DS, Miller LA, Pinto-Martin J, Reaven J, Reynolds AM, Rice CE, Schendel D, Windham GC. The epidemiology of autism spectrum disorder. Annu Rev Public Health. 2007; 28:235–238. [PubMed: 17367287]

6. Braunschweig D, van De Water J. Maternal autoantibodies in autism. Arch Neurol. 2012; 69:693–699. [PubMed: 22689191]

7. Croen LA, Grether JK, Yoshida CK, Odouli R, Hendrick V. Antidepressant use during pregnancy and childhood autism spectrum disorders. Arch Gen Psychiatry. 2011; 68:1104–1112. [PubMed: 21727247]

8. Atladóttir HO, Thorsen P, Schendel DE, Østengaard L, Lemcke S, Parner ET. Association of hospitalization for infection in childhood with diagnosis of autism spectrum disorder. Arch Pediatr Adolesc Med. 2010; 164:470–477. [PubMed: 20439799]

9. Reichenberger A, Gross R, Weiser M, Bresnahan M, Silverman J, Harlap S, Rabinowitz J, Shulman C, Malaspina D, Lubin G, Knobler HY, Davidson M, Susser E. Advancing paternal age in autism. Arch Gen Psychiatry. 2006; 63:1026–1032. [PubMed: 16953005]

10. Hamlyn J, Duhig M, McGrath J, Scott J. Modifiable risk factors for schizophrenia and autism-shared risk factors impacting on brain development. Neurobiol Dis. 2013:53.

11. Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Miller J, Fedele A, Collins J, Smith K, Lotspeich L, Croen LA, Ozonoff S, Lajonchere C, Grether JK, Risch N. Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry. 2011; 68:1095–1102. [PubMed: 21727249]

12. Ruf J, Carayon P. Structural and functional aspects of thyroid peroxidase. Arch Biochem Biophys. 2006; 445:269–277. [PubMed: 16098474]

13. Hadj-Kacem H, Rebuffat S, Mnif-Féki M, Belguith-Maleej S, Ayadi H, Péraldo-Roux S. Autoimmune thyroid disease: genetic susceptibility of thyroid-specific genes and thyroid autoantigens contributions. Int J Immunogenet. 2009; 36:85–96. [PubMed: 19284442]

14. Wasserman EE, Nelson K, Rose NR, Eaton W, Pillion JP, Seaberg E, Talor MV, Burek L, Duggan A, Yolken RH. Maternal thyroid autoantibodies during the third trimester and hearing deficits in children: an epidemiologic assessment. Am J Epidemiol. 2008; 167:701–710. [PubMed: 18156602]

15. Pop VJ, de Vries E, van Baar AL, Waelkens JJ, de Rooy HA, Horsten M, Donkers MM, Komproe IH, van Son MM, Vader HL. Maternal thyroid peroxidase antibodies during pregnancy: a marker of impaired child development? J Clin Endocrinol Metab. 1995; 80:3561–3566. [PubMed: 8530599]

16. Comi AM, Zimmerman AW, Frye VH, Law PA, Peeden JN. Familiar clustering of autoimmune disorders and evaluation of medical risk factors in autism. J Child Neurol. 1999; 14:388–394. [PubMed: 10385847]

17. Sweeten TL, Bowyer SL, Posey DJ, Halberstadt GM, McDougle CJ. Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. Pediatrics. 2003; 112:e420. [PubMed: 14595086]

18. Molloy CA, Morrow AL, Meinzen-Derr J, Dawson G, Bernier R, Dunn M, Hyman SL, McMahon WM, Goudie-Nice J, Hepburn S, Minshew N, Rogers S, Sigman M, Spence MA, Tager-Flusberg H, Volmark FR, Lord C. Familial autoimmune thyroid disase as a risk factor for regression in children with Autism Spectrum Disorder: a CPEA Study. J Autism Dev Disord. 2006; 36:317–324. [PubMed: 16598435]

19. Croen LA, Grether JK, Yoshida CK, Odouli R, van De Water J. Maternal autoimmune disease, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch Pediatr Adolesc Med. 2005; 159:151–157. [PubMed: 15699309]

20. Mourdisen SE, Rich B, Isager T, Nedergaard NJ. Autoimmune diseases in parents of children with infantile autism: a case-control study. Dev Med Child Neurol. 2007; 49:429–432. [PubMed: 17518928]

21. Keil A, Daniels JL, Forssen U, Hultman C, Cnattingius S, Söderberg KC, Feychting M, Sparen P. Parental autoimmune diseases associated with autism spectrum disorder in offspring. Epidemiology. 2010; 21:805–808. [PubMed: 20798635]

22. Atladóttir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, Parner ET. Association of family hisotry of autoimmune diseases and autism spectrum disorders. Pediatrics. 2009; 124:687–694. [PubMed: 19581261]

23. Braunschweig D, Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Croen LA, Pessah IN, van De Water J. Autism: maternally derived antibodies specific for fetal brain proteins. Neurotoxicology. 2008; 29:226–231. [PubMed: 18078998]

24. Singer HS, Morris CM, Gause CD, Gillin PK, Crawford S, Zimmerman AW. Antibodies against fetal brain in sera of mothers with autistic children. J Neuroimmunol. 2008; 194:165–172. [PubMed: 18093664]

25. Braunschweig D, Duncanson P, Boyce R, Hansen R, Ashwood P, Pessah IN, Hertz-Picciotto I, van De Water J. Behavioral correlates of maternal antibody stauts among children with autism. J Autism Dev Disord. 2012; 42:1435–1445. [PubMed: 22012245]

26. Martin LA, Ashwood P, Braunschweig D, Cabanlit M, van De Water J, Amaral DG. Stereotypies and hyperactivity in rhesus monkeys exposed to IgG from mothers of children with autism. Brain Behav Immun. 2008; 22:806–816. [PubMed: 18262386]

27. Brimberg L, Sadiq A, Gregersen PK, Diamond B. Brain-reactive IgG correlates with autoimmunity in mothers of a child with autism spectrum disorder. Mol Psychiatry. 2013; 18:1171–1177. [PubMed: 23958959]

28. Dayan CM, Daniels GH. Chronic autoimmune thyroiditis. N Engl J Med. 1996; 335:99–107. [PubMed: 8649497]

29. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999; 341:549–555. [PubMed: 10451459]

30. Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, Teng X, Guo R, Wang H, Li J, Chen Y, Wang W, Chawinga M, Zhang L, Yang L, Zhao Y, Hua T. Abnormalities of maternal thyroid function

during pregnancy affect neuropsyhological development of their children at 25–30 months. Clin Endocrinol (Oxf). 2010; 72:825–829. [PubMed: 19878506]

31. Lampi KM, Banerjee PN, Gissler M, Hinkka-Yli-Salomäki S, Huttunen J, Kulmala U, Lindroos J, Niemelä S, Rihko M, Ristkari T, Saanakorpi K, Sarlin T, Sillanmäki L, McKeague IW, Surcel HM, Helenius H, Brown AS, Sourander A. Finnish Prenatal Study of Autism and Autism Spectrum Disorders (FIPS-A): overview and design. J Autism Dev Disord. 2011; 41:1090–1096. [PubMed: 21082229]

32. Lampi KM, Sourander A, Gissler M, Niemelä S, Rehnström K, Pulkkinen E, Peltonen L, Von Wendt L. Brief report: validity of Finnish registry-based diagnoses of autism with the ADI-R. Acta Paediatr. 2010; 99:1425–1428. [PubMed: 20412100]

33. Hershman JM. Hashimoto's Thyroiditis. The Merck Manuals: The Merck Manual for Healthcare Professionals. 2012

34. Männistö T, Väärämäki M, Pouta A. Thyroid dysfunction and autoantibodies during pregnancy as predictive factors of pregnancy complications and maternal morbidity in later life. J Clin Endocrinol Metab. 2010; 95:1084–1094. [PubMed: 20080846]

35. Cheslack-Postava K, Jokiranta E, Suominen A, Lehti V, Sourander AS, Brown AS. Variation by diagnostic subtype in risk for autism spectrum disorders associated with maternal parity among Finnish births. Paediatr Perinat Epidemiol. 2014; 28:58–66. [PubMed: 24313668]

36. Lampi KM, Hinkka-Yli-Salomäki S, Lehti V, Helenius H, Gissler M, Brown AS, Sourander A. Parental age and risk of autism spectrum disorders in a Finnish national birth cohort. J Autism Dev Disord. 2013; 43:2526–2535. [PubMed: 23479075]

37. Sandin S, Hultman CM, Kolevzon A, Gross R, MacCabe JH, Reichenberg A. Advancing maternal age is associated with increasing risk for autism: a review and meta-analysis. J Am Acad Child Adolesc Psychiatry. 2012; 51:477–486. [PubMed: 22525954]

38. Lampi KM, Lehtonen L, Tran PL, Suominen A, Lehti V, Banerjee PN, Gissler M, Brown AS, Sourander A. Risk of autism spectrum disorders in low birth weight and small for gestational age infants. J Pediatr. 2012; 161:830–836. [PubMed: 22677565]

39. Jokiranta E, Brown AS, Heinimaa M, Cheslack-Postava K, Suominen A, Sourander A. Parental psychiatric disorders and autism spectrum disorders. Psychiatry Res. 2013; 207:203–211. [PubMed: 23391634]

40. Rai D, Lewis G, Lundberg M, Araya R, Svensson A, Dalman C, Carpenter P, Magnusson C. Parental socioeconomic status and risk of offspring autism spectrum disorders in a Swedish population-based study. J Am Acad Child Adolesc Psychiatry. 2012; 51:467–476. [PubMed: 22525953]

41. Abel KM, Dalman C, Svensson AC, Susser E, Dal H, Idring S, Webb RT, Rai D, Magnusson C. Deviance in fetal growth and risk of autism spectrum disorder. Am J Psychiatry. 2013; 170:391–398. [PubMed: 23545793]

42. Männistö T, Surcel HM, Bioigu A, Ruokonen A, Hartikainen AL, Järvelin MR, Pouta A, Väärämäki M, Suvanto-Luukkonen E. The effects of freezing, thawing, and short- and long-term storage on serum thyrotropin, thyroid hormones, and thyroid antibodies: implications for analyzing samples stored in serum banks. Clin Chem. 2007; 53:1986–1987. [PubMed: 17954505]

43. Greenland, S.; Rothman, KJ. Introduction to stratified analysis. In: Rothman, KJ.; Greenland, S., editors. Modern Epidemiology. 2 ed. Philadelphia, PA: Lippincott Williams & Wilkins; 1998. p. 253-279.

44. Werling DM, Geschwind DH. Sex differences in autism spectrum disorder. Curr Opin Neurol. 2013; 26:146–153. [PubMed: 23406909]

45. Investigators DDMNSYP, Prevention CfDCa. Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ. 2014; 63:1–21.

46. Dupont WD. Power calculations for matched case-control studies. Biometrics. 1988; 44:1157–1168. [PubMed: 3233252]

47. Männistö T, Surcel HM, Ruokonen A, Väärämäki M, Pouta A, Bloigui A, Järvelin MR, Hartikainen AL, Suvanto E. Early pregnancy reference intervals of thyroid hormone

concentrations in a thyroid antibody-negative pregnant population. Thyroid. 2011; 21:291–298. [PubMed: 21254924]

48. Erkkola M, Karppinen M, Järvelin MR, Knip M, Virtanen SM. Folate, vitamin D, and iron intakes are low among pregnant Finnish women. Eur J Clin Nutr. 1998; 52:742–748. [PubMed: 9805222]

49. Hoshiko S, Grether JK, Windham GC, Smith D, Fessel K. Are thyroid hormone concentrations at birth associated with subsequent autism diagnosis? Autism Res. 2011; 4:456–463. [PubMed: 21882364]

50. Román GC, Ghassabian A, Bongers-Schokking JJ, Jaddoe VW, Hofman A, de Rijke YB, Verhulst FC, Tiemeier H. Association of gestational maternal hypothyroxinemia and increased autism risk. Ann Neurol. 2013; 74:733–742. [PubMed: 23943579]

51. Andersen SL, Laurberg P, Wu CS, Olsen J. Attention deficit hyperactivity disorder and autism spectrum disorder in children born to mothers with thyroid dysfunction: a Danish nationwide cohort study. BJOG. 2014 in press.

52. Ronemus M, Iossifov I, Levy D, Wigler M. The role of de novo mutations in the genetics of autism spectrum disorders. Nat Rev Genet. 2014; 15:133–141. [PubMed: 24430941]

53. Langridge AT, Glasson EJ, Nassar N, Jacoby P, Pennell C, Hagan R, Bourke J, Leonard H, Stanley FJ. Maternal conditions and perinatal characteristics associated with autism spectrum disorder and intellectual disability. PLoS One. 2013; 8:e50963. [PubMed: 23308096]

54. Simister NE. Placental transport of immunoglobulin G. Vaccine. 2003; 21:3365–3369. [PubMed: 12850341]

55. Svensson J, Lindberg B, Ericsson UB, Olofsson P, Johnsson B, Ivarsson SA. Thyroid autoantibodies in cord blood sera from children and adolescents with autoimmune thyroiditis. Thyroid. 2006; 16:79–83. [PubMed: 16487018]

56. Päkkilä F, Männistö T, Surcel HM, Ruokonen A, Bloigui A, Pouta A, Hartikainen AL, Väärämäki M, Järvelin MR, Suvanto E. Maternal thyroid dysfunction during pregnancy and thyroid function of her child in adolescence. J Clin Endocrinol Metab. 2013; 93:965–972. [PubMed: 23408571]

57. Korzeniewski SJ, Pinto-Martin JA, Whitaker AH, Feldman JF, Lorenz JM, Levy SE, Movsas TZ, Pappas A, Paneth N. Association between transient hypothyroxinaemia of prematurity and adult autism spectrum disorder in a low-birthweight cohort: an exploratory study. Paediatr Perinat Epidemiol. 2013; 27:182–187. [PubMed: 23374063]

58. Hasham A, Tomer Y. Genetic and epigenetic mechanisms in thyroid autoimmunity. Immunol Res. 2012; 54:204–213. [PubMed: 22457094]

59. Mostafa GA, Shehab AA, Al-Ayadi LY. The link between some alleles on human leukocyte antigen system and autism in children. J Neuroimmunol. 2013; 255:70–74. [PubMed: 23110937]

60. Crespi BJ, Thiselton DL. Comparative immunogenetics of autism and schizophrenia. Genes Brain Behav. 2011; 10:689–701. [PubMed: 21649858]

61. Pihkala J, Hakala T, Voutilainen P, Raivo K. Characteristics of recent fetal growth curves in Finland. Duodecim. 1989; 105:1540–1546. [PubMed: 2680445]

Highlights

-- Odds of autism were raised after prenatal thyroid peroxidase antibody exposure

-- The finding was observed in a large national birth cohort

-- There were no associations between maternal hypothyroidism and autism

-- This is the first biomarker-based prenatal autoimmune association with autism

-- Further prospective studies are needed to confirm this finding

Table 1

Relationship between covariates and maternal thyroid peroxidase antibody positivity (TPO-Ab+) in comparison subjects (N=960).

	TPO-Ab status[1]		
	Positive	**Negative**	**P**[2,3]
Maternal age, Mean (SD)	29.62 (4.65)	29.22 (5.30)	0.67
Paternal age, Mean, (SD)	33.21 (7.56)	31.58 (5.98)	0.22
Previous births, N (%)[4]			0.60
0	16 (3.90)	394 (96.10)	
1	18 (3.27)	532 (96.73)	
Maternal socioeconomic status[5]**, N (%)**			0.95
Upper white collar	5 (4.17)	115 (95.83)	
Lower white collar	11 (3.18)	335 (96.82)	
Blue collar	5 (3.14)	154 (96.86)	
Other[6]	5 (3.68)	131 (96.32)	
Preterm birth, N (%)[7]			0.41
<37 weeks	0 (0)	47 (100)	
37 weeks	34 (3.72)	879 (96.28)	
Low birthweight, N (%)[8]			0.23
<2500 g	2 (7.69)	24 (92.31)	
2500 g	32 (3.43)	902 (96.57)	
Weight for gestational age, N (%)[7,9]			0.57
SGA	2 (7.14)	26 (92.86)	
AGA	31 (3.42)	876 (96.58)	
LGA	1 (4.00)	24 (96.00)	
Family history, N (%)			
Any maternal psychiatric disorder			0.66
Yes	2 (4.65)	41 (95.35)	
No	32 (3.49)	885 (96.51)	
Any parental psychiatric disorder			0.79
Yes	3 (2.75)	106 (97.25)	
No	31 (3.64)	820 (96.36)	

	TPO-Ab status[1]		
	Positive	Negative	P[2,3]
Gestational week blood draw, Mean (SD)	11.03 (3.14)	10.82 (3.38)	0.73

[1] Percentages expressed as row percents.

[2] P values for maternal age, paternal age, and gestational week calculated by T-test; P values for previous birth, maternal socioeconomic status, preterm birth, low birthweight, weight for gestational age, and family history were calculated using Chi-square test

[3] P values for categorical variables calculated by Fisher's exact test except where indicated

[4] Frequency missing: N= 6

[5] Frequency missing: N=199

[6] Includes individuals both within and outside the documented labor force (housewives, unemployed, students, retired, and self employed entrepreneurs).

[7] Frequency missing: N=6

[8] Frequency missing: N=4

[9] SGA = Small for gestational age (< 2 SD); AGA = Average for gestational age (−2 SD to +2 SD); LGA = Large for gestational age (>2 SD). Calculated according to national sex-specific Finnish weight distribution standards at a given gestational age(61).

Table 2

Relationship between covariates and autism diagnosis (N=960 cases, 960 comparison subjects)

	Cases	Comparison Subjects	P[1]
Maternal age, Mean (SD)	30.15 (5.34)	29.24 (5.28)	0.001
Paternal age, Mean, (SD)[2]	32.98 (6.47)	31.64 (6.04)	<0.01
Previous births, N (%)			0.001
0	328 (34.17)	410 (42.71)	
1	632 (65.83)	550 (57.29)	
Maternal socioeconomic status[3], N (%)			0.83
Upper white collar	111 (14.23)	120 (15.77)	
Lower white collar	354 (45.38)	346 (45.47)	
Blue collar	169 (21.67)	159 (20.89)	
Other[4]	146 (18.72)	136 (17.87)	
Preterm birth, N (%)[5]			0.17
<37 weeks	61 (6.35)	47 (4.9)	
37 weeks	899 (93.65)	913 (95.1)	
Low birthweight, N (%)[6]			0.02
<2500 g	45 (4.69)	26 (2.71)	
2500 g	915 (95.31)	934 (97.29)	
Weight for gestational age, N (%)[7]			0.45
SGA	38 (3.96)	28 (2.92)	
AGA	896 (93.33)	907 (94.48)	
LGA	26 (2.71)	25 (2.60)	
Family history, N (%)			
Any maternal psychiatric disorder			0.001
Yes	78 (8.13)	43 (4.48)	
No	882 (91.88)	917 (95.52)	
Any parental psychiatric disorder			0.009
Yes	148 (15.42)	109 (11.35)	
No	812 (84.58)	851 (88.65)	
Gestational week blood draw, Mean (SD)	11.06 (3.06)	10.93 (3.52)	0.74

[1] P values for maternal age, paternal age, and gestational week calculated by T-test; P values for previous birth, maternal socioeconomic status, preterm birth, low birthweight, weight for gestational age, and family history were calculated using Chi-square test

[2] Frequency missing: cases: N=17; comparison subjects: N=5

[3] Frequency missing: cases: N=180; comparison subjects: N=199

[4] Includes individuals both within and outside the documented labor force (housewives, unemployed, students, retired, and self employed entrepreneurs).

[5] Frequency missing: cases: N=4; comparison subjects: N=6

[6] Frequency missing: cases: N=2; comparison subjects: N=4

[7] SGA = Small for gestational age (< 2 SD); AGA = Average for gestational age (−2 SD to +2 SD); LGA = Large for gestational age (>2 SD). Calculated according to national sex-specific Finnish weight distribution standards at a given gestational age.

Table 3

Maternal thyroid peroxidase antibody positivity (TPO-Ab+) and childhood autism[1]

| | Cases | | Comparison Subjects | | | | |
| | TPO-Ab+ | | TPO-Ab + | | | | |
	N	%	N	%	OR	95% CI	P
All subjects[2]							
(N=960 matched case-comparison subject pairs)	59	6.15	34	3.54	1.78	1.16–2.75	0.009
By Sex							
Males							
(N=759 matched case-comparison subject pairs)	49	6.46	28	3.69	1.81	1.12–2.92	0.015
Females							
(N=201 matched case-comparison subject pairs)	10	4.98	6	2.99	1.67	0.61–4.59	0.32
By co-morbid intellectual disability[3]							
With intellectual disability							
(N=273 matched case-comparison subject pairs)	17	6.23	15	5.49	1.13	0.57–2.27	0.72
Without intellectual disability							
(N=687 matched case-comparison subject pairs)	42	6.11	19	2.77	2.35	1.33–4.15	0.003

[1] The findings are unadjusted given that no covariate was associated with both TPO-Ab and autism

[2] Results of adjusted analysis

[3] ICD 10/9 codes for intellectual disability are as follows: F70/317 (mild), F71/318.0 (moderate), F72/318.1 (severe), F73/318.2 (profound), F78 (no ICD-9 code) (other), and F79/319 (unspecified).

Table 4

Clinical/subclinical maternal hypothyroidism/hyperthyroidism and childhood autism

	N (%) Cases	N (%) Comparison Subjects	OR[6]	95% CI	P
Clinical hypothyroidism[1]	8 (1.01)	12 (1.52)	0.67	0.27 – 1.63	0.37
Subclinical hypothyroidism[2]	36 (4.01)	31 (3.46)	1.17	0.72 – 1.90	0.54
Clinical hyperthyroidism[3]	17 (1.83)	16 (1.72)	1.06	0.54 – 2.10	0.86
Subclinical hyperthyroidism[4,5]	33 (3.68)	28 (3.12)	1.10	0.67 – 1.83	0.71

[1] Defined as TSH>3.10 uIU/mL (>95th percentile) and fT4<12.16 pmol/L (<5th percentile)

[2] Defined as TSH>3.10 uIU/mL (>95th percentile) and fT4=12.16–17.94 pmol/L (5th to 95th percentile)

[3] Defined as TSH<0.178 IU/mL (<5th percentile) and fT4>17.94pmol/L (>95th percentile)

[4] Defined as TSH<0.178 IU/mL (<5th percentile) and fT4=12.16–17.94 pmol/L (5th to 95th percentile)

[5] Adjusted for number of previous births

[6] Odds ratios are calculated separately for each group of thyroid disorders compared to the respective unexposed groups defined as: TSH=0.178–3.10 uIU/mL (5th to 95th percentile) and fT4=12.16–17.94 pmol/L (5th to 95th percentile)

TSH = Thyroid stimulating hormone; fT4 = free thyroxine

N matched case-comparison subject pairs = Clinical hypothyroidism: 792; Subclinical hypothyroidism: 897; Clinical hyperthyroidism: 930; Subclinical hyperthyroidism: 897