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Maternal Thyroid Autoimmunity During Pregnancy and the Risk of Attention Deficit/Hyperactivity Problems in Children: The Generation R Study

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Background: Maternal thyroid status and autoimmunity during pregnancy have been associated with impaired development of the offspring in animal and human studies. Our objective was to examine whether elevated titers of maternal thyroid peroxidase antibodies (TPOAbs) in early pregnancy increased the risk of cognitive impairment and problem behavior in preschool children. Second, we aimed at exploring to what extent any effect on child behavior was mediated by maternal thyroid parameters during pregnancy.

Methods: In the Generation R Study, a population-based cohort of 3139 children and their mothers, we measured maternal thyroid parameters (thyrotropin [TSH], free Thyroxine, and TPOAbs) at 13.5 ± 1.8 weeks of gestation. Children's verbal and nonverbal cognitive functioning was measured at 2.5 years using the Language Development Survey and the Parent Report of Children Abilities. At 3 years, children's behavior was assessed using the Child Behavior Checklist.

Results: Elevated titers of TPOAbs during pregnancy did not predict the verbal and nonverbal cognitive functioning of the children. However, elevated titers of TPOAbs in mothers were associated with externalizing problems in children (odds ratio [OR] = 1.64, 95% confidence interval [CI]: 1.17–2.29, $p = 0.004$). In particular, children of TPOAb-positive mothers were at a higher risk of attention deficit/hyperactivity problems (OR = 1.77, 95% CI: 1.15–2.72, $p = 0.01$). To explore whether the effect of maternal TPOAbs on child problem behavior was mediated by maternal thyroid parameters, we added maternal TSH to the model. After correcting for TSH, the effect of TPOAbs on externalizing problems was attenuated slightly but remained significant (OR = 1.56, 95% CI: 1.14, 2.14, $p = 0.005$).

Conclusions: Our findings imply that the elevated titers of TPOAbs during pregnancy impact children's risk of problem behavior, in particular, attention deficit/hyperactivity. The observed effect is only partially explained by maternal TSH levels. These findings may point to a specific mechanism of Attention Deficit/Hyperactivity Disorder in children. Nevertheless, we can only speculate about public health implication of the study, as there is no specific treatment for TPOAb-positive pregnant women with normal thyroid function. Further investigation is needed to explore whether TPOAb-positive pregnant women and their children can benefit from close monitoring and early detection of developmental delay in populations at risk.

Introduction

In women of reproductive age, autoimmunity is the most common cause of thyroid dysfunction in iodine-sufficient areas. Although slightly down-regulated during pregnancy (1), thyroid autoantibodies are seen in 10% of women even with normal thyroid function (2). Among thyroid autoantibodies, thyroid peroxidase antibodies (TPOAbs) are considered the most sensitive and specific marker of thyroid autoimmunity (3). Previous studies showed that, in women

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with normal thyrotropin (TSH) and free thyroxine (fT4), elevated titers of TPOAbs are associated with pregnancy complications (4), preterm birth (5), abnormal fetal growth (6), and prenatal/postnatal depression symptoms (7,8).

An effect of maternal thyroid dysfunction during pregnancy on the cognitive function of the child such as intelligence and language is well recognized (9,10). In addition, there is evidence for the association between thyroid function and attention deficit/hyperactivity disorder (ADHD) (11–13). Previously, we reported that maternal thyroid dysfunction in early pregnancy predicted impaired cognition and attention deficit/hyperactivity problems in preschool children (14,15). This led us to explore whether elevated titers of TPOAbs in pregnant women underlie the relation of maternal thyroid dysfunction with the offspring's cognitive and behavioral development. Few studies address the role of maternal TPOAbs during pregnancy and the association with cognitive functioning of the child (16,17). Within a sample of about 300 pregnant women, Pop *et al.* reported that the children of TPOAb-positive women with normal thyroid status are at a risk of cognitive dysfunction (17). They argued that it is "autoimmunity rather than thyroid hormone insufficiency" which affects the child's development. Recent evidence on the role of the autoimmune process in the psychiatric disorders in childhood supports this theory (18,19). In a retrospective study within a general population sample, Li *et al.* found that maternal thyroid hormones and TPOAbs were associated with intelligence and motor scores in young children. It is less clear whether maternal thyroid autoimmunity during pregnancy can affect the child's problem behavior, in particular, attention deficit/hyperactivity problems. Elevated titer of TPOAbs in pregnant woman may primarily affect the child's cognition and behavior by causing some degree of thyroid dysfunction in the mother or the child. However, down-regulated general autoimmune condition of the mother during pregnancy may also be a possible explanatory pathway for the association of maternal TPOAbs with child behavior and cognition (for conceptual model, see Supplementary Fig. S1; Supplementary Data are available online at www.liebertonline.com/thy).

Against this background, we designed the present study to examine whether maternal thyroid autoimmunity during the first half of pregnancy increases the risk of cognitive impairment and problem behavior in preschool children. First, we studied whether maternal thyroid autoimmunity predicted the risk of verbal and nonverbal cognitive impairment and problem behavior, in particular, attention deficit/hyperacidity problems. Second, we explored whether any effect is mediated by maternal plasma TSH during pregnancy.

Materials and Methods

Study design and participants

This study was carried out within the Generation R Study, a population-based cohort from early fetal life onward in Rotterdam, the Netherlands. Pregnant women with expected delivery date between April 2002 and January 2006 in the city of Rotterdam were eligible and were invited to participate in the study during their first prenatal visit. While enrollment ideally took place in early pregnancy, it was also possible until after the birth of the child. In total, 9778 mothers and their children were enrolled in the study (participation rate 61%). Blood sampling was performed in about 70% of the participants in early pregnancy (<18 weeks of gestation), from which 4804 pregnant women had their thyroid parameters measured in the blood and gave consent for postnatal assessment. The design and cohort profile of the Generation R Study has been described in detail by Jaddoe *et al.* (20–23). In our sample, 34 women had a history of thyroid medication during pregnancy and were excluded from all analyses. The remaining 4770 pregnant women were eligible for analyses. Of these, we obtained the follow-up data of the behavior in 3139 children.

Although maternal blood sampling had been performed during pregnancy, the measurement of thyroid parameters was performed after the delivery of the child. The parents, as the information source for all outcome measures, were not informed about the results of the tests (except one clinical case that was excluded from this study). The anonymity of respondents was preserved within all steps of data gathering and analyses. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, approved the study. Written informed consent was obtained from adult participants.

Problem behavior, verbal and nonverbal cognition were assessed using two mailed questionnaires. The questionnaires were available in three languages (Dutch, English, and Turkish). If needed, further support for verbal translation of questionnaires was available in Arabic, Portuguese (Cape Verdeans), and French. The parents chose the language of the questionnaire they received.

Thyroid parameters

Maternal blood samples were collected in early pregnancy (mean = 13.5 ± 1.8 weeks of gestation, range: 7.9–17.9 weeks). In addition, the cord blood was obtained immediately after birth in 2121 neonates of the study population. Within 24 hours after sampling, the maternal and cord blood samples were stored at −70°C. The TSH, fT4, and TPOAb were determined in batches, which had been stored for 6 months, using chemiluminescence assays (Vitros ECI Immunodiagnostic System Ortho Clinical Diagnostics, Rochester, NY). The interassay and intra-assay coefficients of variation for TSH were 2.5%–4.1% and 1.0%–1.2% as previously reported (14). The respective coefficients for fT4 were 4.7%–5.4% and 1.4%–2.7%. Maternal TPOAbs were measured using the Phadia 250 immunoassay (Phadia AB, Uppsala, Sweden). TPOAb status was defined as positive when the plasma concentrations were ≥100 IU/mL using the laboratory's reference values. To rule out the effect of cut-off choice, we also tested an alternative cut-off of 60 IU/mL to define TPOAb-positive women. We used the reference values for maternal thyroid parameters as recommended by The Endocrine Society Clinical Practice Guideline (2007) (24).

Verbal and nonverbal cognitive functioning

We used the Language Development Survey (LDS) to identify children with language delay (25). From a checklist of 310 words, the caregivers (mostly mothers) were asked to circle the words that the child used spontaneously and indicate whether the child combined two or more words together. The LDS could be filled out for a child whose first language was not Dutch, English, or Turkish if one of the parents could read one of these languages in which we provided the

questionnaires. For words the child said in another language, the parents could add a letter to the Dutch version of the test. The LDS is an instrument with excellent test-retest reliability and extremely high validity. It has high sensitivity and specificity, which makes it a proper instrument for the identification of language delay in toddlers (25,26). It has been shown to predict language and language-related problems later in life (27). We obtained a vocabulary sum score by adding the number of words and a total score of phrase length from the average number of words in a phrase. Gender- and age-specific percentiles were derived in our sample as described in the Manual for ASEBA Preschool Forms and Profiles (25). Vocabulary scores ≤15th percentile and phrase scores ≤20th percentile suggest delayed language development as recommended by Achenbach and Rescorla (25). These age- and gender-specific cut-offs were derived by calculating a cumulative frequency distribution of vocabulary scores and lengths of phrase in a normalized sample of boys and girls. The Cronbach's alpha coefficient of the LDS in our sample was 0.99.

Nonverbal cognitive functioning of the children was assessed using the parent-administered and the parent-report parts of the Parent Report of Children's Ability (PARCA) (28). The parent-administered part has 22 items and assesses three subsets of functioning in children: matching-to-sample, block building, and imitation. The parent-report part consists of 26 questions on qualitative abilities, symbolic play, planning and organizing, adaptive behaviors, and memory. The PARCA can provide valid estimates of the child's nonverbal cognitive abilities at the age of two. The parent-administered and parent-report components of PARCA are good predictors of the child's cognitive performance by tester-administered assessments (28). Using PARCA, the parents have the chance to assess their children's performance in a natural environment, whereas standard cognitive testing requires young children to perform in the presence of a stranger. Nonverbal cognitive delay was defined as nonverbal cognitive scores below the 15th age- and gender-specific percentile as previously described in another study of this cohort (15).

Among the group with data on TPOAbs, the parents of the 3020 children filled out the questionnaires when their children were at 2.5 years (31 ± 2 months).

Problem behavior

We used the Child Behavior Checklist 1½–5 (CBCL/1½–5) for preschoolers to obtain a standardized rating of the child's problem behavior by parents (25). The CBCL/1½–5 contains 99 problem items, scored on seven empirically based syndromes that were derived by factor analyses: emotionally reactive, anxious/depressed, somatic complaints, withdrawn, sleep problems, attention problems, and aggressive behavior. Two broad groupings of syndromes can be derived from CBCL/1½–5: internalizing and externalizing problem scores. The internalizing score is derived by summing the following subscales: emotionally reactive, anxious/depressed, somatic complains, and withdrawn. The externalizing scale consists of the attention problems and aggressive behavior scales. The parents were asked to rate emotions and behavior of their child based on the preceding 2 months on a 3-point scale: 0 = not true, 1 = somewhat or sometimes true, and 2 = very true or often true. The 8-day stability estimate and the internal consistency (Cronbach's alpha) of internalizing and externalizing syndrome scales of CBCL/1½–5 vary between 0.88 and 0.92 (29). The reliability and validity of the Dutch translation has been demonstrated (30), and the syndrome scales derived from CBCL/1½–5 had a good fit in 23 studies across diverse societies (31).

In the present study, we also used the scales consistent with diagnostic categories of the 4th edition of Diagnostic and Statistical Manual (DSM-IV): affective problems, anxiety problems, attention deficit/hyperactivity problems, and oppositional defiant problems, in order to translate the findings to the standard psychiatric classification.

The CBCL/1½–5 was completed by 3139 mothers (mean age of children = 37 ± 1 months) and 2624 fathers (mean age of children = 37 ± 1 months). We assessed problem behavior in children at the age of 3 years when attention can be assessed reliably. The correlation coefficients (r) between mother and father ratings of internalizing and externalizing scales were 0.54 ($p < 0.001$) and 0.56 ($p < 0.001$), respectively. These correlations are in line with the mean correlation ($r = 0.60$) between parents, reported in a review of multi-informant assessment (32). The IntraClass Correlation Coefficient for mother and father reported behavior at 3 years was 0.70 for internalizing and 0.71 for externalizing problems.

Covariates

We selected the possible confounders on the basis of literature (5,17,33,34). Information on maternal age, educational level, and ethnicity were asked via questionnaires at the time of enrollment. Educational level was the highest education completed and was classified as primary (no or only primary education), secondary (lower or intermediate vocational education), and higher education (higher vocational education or university). The child's national origin was defined on the basis of origin of mother, father, and grandparents. We classified national origin as Western or Nonwestern. Maternal smoking was assessed twice, once at the time of enrollment and the second time during the 30th week of gestation, to record whether mothers had never smoked, stopped smoking when pregnant, or continued smoking during pregnancy. We used the Brief Symptom Inventory (BSI) to measure maternal depressive symptoms during pregnancy (35). The BSI is a validated self-report questionnaire with 53 items that define a spectrum of psychiatric disorders in the previous 7 days. High validity and reliability has been reported for the Dutch translation of the instrument (36). The six-item scale of depressive symptoms was derived from the BSI. The ability of BSI to identify clinical depression (using cut-off scores) has been demonstrated within a subsample of Generation R study [see publication by Henrichs *et al.* (37)]. The child's gender and birth weight were derived from medical records. To define gestational age at birth, we used the last menstrual period of the mother and ultrasound examination of the fetus at the first prenatal visit.

Statistical analysis

We used independent sample t-test, Mann–Whitney U tests, and chi-square statistics to explore whether the nonresponse was selective. In the association analyses, the determinants were maternal TPOAb status or plasma levels of TSH. Maternal TSH was used as continuous variable in the models and divided by its standard deviation to enable comparison.

The associations between maternal TPOAb status and the child cognitive function and problem behavior were explored using logistic regressions. Subsequently, we examined whether any observed effect of TPOAbs on internalizing and externalizing scales was accounted for by more specific symptoms using the DSM-oriented scales. A Generalized Estimating Equation (GEE) approach was used to precisely estimate the overall effect of TPOAbs on problem behavior as repeatedly rated by mothers and by fathers (pooled effect). Moreover, such an overall estimate reduces the errors derived from multiple comparisons.

The CBCL/1½–5 scores were dichotomized, as the scores were not normally distributed and results based on dichotomized scores can then be interpreted as problem behavior. Achenbach and Rescorla suggest using the scores for borderline cut-off (83rd percentile for the broad band syndrome scales and 93rd percentile for the DSM-oriented scales) if the users wished to dichotomize children's scores as being clearly in the normal range versus high enough to warrant concern (25). These cut-offs have been widely used by other researchers to define children with problems (29,38). The population-based study of Dutch norms showed that Dutch children score lower on the behavioral/emotional problems than the American norms (30). Therefore, in the present study, we used the scores for 83rd (for broad band syndrome scale) and 93rd (for DSM-oriented subscales) derived from Dutch norm sample as cut-off points to define children with problems. We also tested an alternative cut-off (80th percentile) that was previously used in another study of the Generation R cohort (39) to examine consistency and whether any effect was observed only due to the choice of the cut-off.

Elevated titers of TPOAbs in pregnant women may be related to the child's behavior, because antibodies affect the thyroid status of the mother, which, in turn, is associated with child neurodevelopment. To test this pathway from TPOAbs to child behavior, we additionally adjusted the models for maternal TSH as a marker of thyroid status.

The models were adjusted for gender (except if gender-specific scores were used) and ethnicity of the child, maternal age, smoking habits, and gestational age at the time of blood sampling for thyroid measurements when appropriate. Confounders were selected if the effect estimates of maternal TPOAbs changed more than 5% in the models (40). Furthermore, we additionally controlled for maternal depressive symptoms to investigate to what extent any association between elevated titers of TPOAbs in mother and child behavior is explained by an effect on maternal psychopathology (7,41).

We applied a Bonferroni adjustment to correct for multiple comparisons in the association between maternal thyroid antibody status and four child outcome measures (verbal and nonverbal cognitive development and internalizing and externalizing problem behavior): α level $0.05/4 = 0.0125$.

To handle the missing values in maternal TSH levels in the mediation analyses, we used multiple imputations. Imputations were based on the relationships between information on the other thyroid parameters and all covariates measured. Five independent datasets were generated, and pooled estimates for those datasets were calculated.

Nonresponse analyses

Nonresponse analysis showed some differences between 3139 participants included in the analyses and the eligible individuals who were excluded because of missing information on behavior ($n = 1631$). The children who were excluded from the analyses had lower birth weight (mean difference: 117 g, $p < 0.001$). The women whose children were not included because of missing behavioral data were younger (mean age difference = 2.9 years, $p < 0.001$), less educated (14.1% vs. 33.3% higher education, $p < 0.001$), and were more likely to continue smoking during pregnancy (24.9% vs. 13.8%, $p < 0.001$).

The mothers of the children who were excluded from the analyses because of missing data on CBCL/1½–5 had higher TSH levels than those included (mean difference = 0.13, $p = 0.002$). The TPOAb status of the mothers during early pregnancy was not associated with responsiveness to CBCL/1½–5.

To investigate whether the missing cord blood data introduced bias in the association between maternal TPOAb status and child cord blood thyroid parameters, we ran a nonresponse analysis. Among the study population, there was no significant difference between the group with cord blood data (TSH or fT4) and those with missing information of cord blood with regard to maternal TPOAb status ($p = 0.27$).

Results

In total, 147 (4.7%) women had TPOAb levels higher than 100 IU/mL and were defined as TPOAb-positive. Of the remainder, 40 had TPOAb levels between 60–100 IU/mL. Plasma TSH was higher in TPOAb-positive than TPOAb-negative women (3.81 ± 4.13 vs. 1.53 ± 1.04, mean difference = 2.28 ± 0.12, $p < 0.001$). fT4 did not differ between the TPOAb-negative and positive women (15.40 ± 3.72 vs. 15.06 ± 4.80, mean difference = 0.34 ± 0.33, $p = 0.29$).

Next, we examined whether maternal TPOAbs were associated with neonatal thyroid status. The fT4 and TSH levels in child cord blood did not differ between TPOAb-positive and TPOAb-negative women (mean difference for fT4 = 0.55 ± 0.22, $p = 0.55$, and mean difference for TSH = 0.26 ± 0.92, $p = 0.78$).

Maternal and child characteristics are shown in Table 1. Pregnant women who were TPOAb negative or TPOAb positive had relatively similar education levels (33.3% vs. 35.4% for higher levels of education). About 14% of the TPOAb-negative women continued smoking during pregnancy. This percentage was 10.2% in TPOAb-positive women.

We found that elevated titers of TPOAbs during the first half of pregnancy did not predict language development in children at 2.5 years (OR = 0.99 for delayed vocabulary development, 95% CI: 0.39–2.50, $p = 0.98$ and OR = 1.49 for delayed phrase development, 95% CI: 0.71–3.12, $p = 0.28$). Children of TPOAb-positive mothers did not have a higher risk than children of TPOAb-negative mothers to develop nonverbal cognitive delay (OR = 1.09, 95% CI: 0.67–1.77, $p = 0.74$).

Table 2 summarizes the association between maternal TPOAbs and internalizing problem scores in children at 3 years. Elevated titers of TPOAbs in pregnant women were not associated with internalizing problems in the offspring (whether problems were rated by mother or father). In line with this finding, there was no association between maternal TPOAbs and affective or anxiety problems.

The relation between elevated titers of TPOAbs in mothers during early pregnancy and externalizing problem scores in

Table 1. Participants' Characteristics (N=3139)

	Maternal TPOAb status[a]		
	Negative (n=*2992*)	*Positive* (n=*147*)	p
Maternal characteristics			
Age at the time of enrollment (years)	31.2 (4.3)	31.1 (4.3)	0.84
Education (%)			
Primary	13.7	13.9	
Secondary	53.1	50.7	
High	33.3	35.4	0.87
Smoking (%)			
Never	76.9	76.5	
Until pregnancy was known	9.1	13.2	
Continued during pregnancy	14.0	10.3	0.16
Depressive symptoms during pregnancy[b]	0.00 (0.00, 0.17)	0.00 (0.00, 0.17)	0.71
Maternal thyroid parameters			
TSH (mIU/L)	1.33 (0.82, 2.02)	3.15 (1.76, 4.28)	<0.001
fT4 (nM)	15.00 (13.28, 16.85)	14.82 (12.67, 16.48)	0.29
Child characteristics			
Female gender (%)	50.5	49.0	0.68
Ethnicity (%)			
Western	80.1	76.0	
Nonwestern	19.9	24.0	0.28
Birth weight (g)	3449 (562)	3483 (589)	0.49
Gestational age at birth, week	40.1 (39.1, 41.0)	40.0 (39.3, 41.1)	0.79
Cord blood thyroid parameters[c]			
TSH (mIU/L)	9.60 (6.60, 14.73)	10.45 (6.52, 14.58)	0.78
fT4 (nM)	20.54 (18.57, 22.76)	20.48 (18.94, 22.02)	0.55
Delayed vocabulary development (%)	3.5	4.3	0.63
Delayed phrase development (%)	4.9	8.2	0.16
Nonverbal cognitive delay (%)	14.0	16.2	0.51
The Child Behavior Checklist score			
Internalizing scores, mother-rated at 3 years	4.9 (4.8)	5.1 (4.4)	0.58
Internalizing scores, father-rated at 3 years	5.3 (5.0)	5.9 (5.1)	0.14
Externalizing scores, mother-rated at 3 years	8.3 (6.1)	9.2 (6.4)	0.09
Externalizing scores, father-rated at 3 years	9.2 (6.5)	10.3 (6.8)	0.04

Data values report children included in one or more analyses. Numbers are means (SD) unless otherwise indicated.
[a]The plasma levels > 100 IU/mL were defined as positive.
[b]Measured by Brief Symptom Inventory.
[c]Neonatal thyroid parameters were available in 2121 individuals.
TPOAbs, thyroid peroxidase antibodies; TSH, thyrotropin; fT4, free thyroxine.

the children are presented in Table 3. Children of TPOAb-positive women had about 60% higher risk of developing externalizing problems than children of TPOAb-negative women (OR = 1.60 for problems rated by mothers, 95% CI: 1.08–2.38, $p = 0.02$). A very similar association was found with father-rating problems (OR = 1.61, 95% CI: 1.04–2.49, $p = 0.03$). Using a GEE approach to pool mother and father-rating problem behavior, we found that children of TPOAb-positive mothers were at an increased risk for externalizing problems at 3 years (OR = 1.64 for mother- and father-rating problems, 95% CI: 1.17–2.29, $p = 0.004$). This association remained significant after correction for multiple comparisons (α level 0.0125). Next, in the subsequent analyses of the DSM-oriented scales, we found that children of TPOAb-positive mothers were at an increased risk to obtain high scores on the attention deficit/hyperactivity problems as rated by fathers (OR = 1.89, 95% CI: 1.16–3.07, $p = 0.01$). The findings were similar for attention deficit/hyperactivity problems rated by mothers, but did not reach significance (OR = 1.60, 95% CI: 0.90–2.87, $p = 0.11$). To show the effect of elevated titers of TPOAbs on child problem behavior independent of the rater, we performed further analyses, using the GEE approach to pool mother and father rating of behavior. We observed an association between elevated titers of TPOAbs and the risk of attention deficit/hyperactivity problems (OR = 1.77, 95% CI: 1.15–2.72, $p = 0.01$). The association between maternal TPOAbs and the risk of attention deficit/hyperactivity in children remained significant after adjustment for multiple comparisons. No association was found between elevated titers of TPOAbs and the oppositional deviant problems scores.

The results were essentially unchanged if we added maternal depressive symptoms to the analyses (data not shown).

Next, we explored whether altered thyroid status of the mother explained the relation between TPOAbs and externalizing behavior in the offspring. First, we found that TPOAb status was associated with TSH levels in pregnant women (mean plasma TSH in TPOAb-positive women = 3.83 ± 4.13 vs. mean plasma TSH in TPOAb-negative women = 1.53 ± 1.04, $p <$ 0.001). Second, as previously reported (14), maternal TSH was associated with children's externalizing problems rated by

Table 2. Maternal Thyroid Peroxidase Antibodies During Pregnancy and Child's Internalizing Problem, the Generation R Study

	Maternal rating		*Paternal rating*		*Maternal and paternal rating*	
Determinant: TPOAb-positive[a]	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*	p
Outcome measures:						
Internalizing problems	1.20 (0.78–1.85)	0.40	1.17 (0.73–1.88)	0.50	1.21 (0.84–1.74)	0.31
DSM-oriented subscales						
Affective Problems	0.96 (0.51–1.80)	0.90	1.31 (0.66–2.59)	0.44	1.02 (0.63–1.65)	0.95
Anxiety Problems	1.27 (0.74–2.17)	0.39	1.14 (0.61–2.13)	0.69	1.27 (0.81–2.00)	0.29

Total of children in one or more analyses is 3139. The models were adjusted for child's gender and ethnicity, maternal age, cigarette smoking, and time of thyroid sampling during pregnancy.
[a]The TPOAbs levels > 100 IU/mL were defined as positive.
OR, odds ratio; CI, confidence interval.

mothers and by fathers ($B = 0.18$ per SD of TSH, 95% CI: 0.02–0.34, $p = 0.03$). Third, after adding maternal TSH to the model, the effect of maternal TPOAbs on externalizing problems of the children was attenuated by 8% only and remained significant (OR = 1.56, 95% CI: 1.14, 2.14, $p = 0.005$).

In similar analyses, the alternative cut-off for TPOAb levels and CBCL/1½–5 scores was used, but the results remained essentially unchanged.

Discussion

In the present study, we found no association between elevated titers of TPOAbs in the mother during early pregnancy and cognitive functioning in the offspring. However, elevated titers of TPOAbs during early pregnancy increased the risk of externalizing problems in preschool children. Further analysis indicated that this effect was largely accounted for by problems tapped by the CBCL/1½–5 attention deficit/hyperactivity problem scale. Interestingly, the association between TPOAb status of the mother and externalizing problems in the children was largely independent of maternal thyroid status.

Elevated titers of TPOAbs in mothers during early pregnancy increased the risk of problem behavior in children, in particular, attention deficit/hyperactivity problems. There are several possible explanations for the observed association. First, high titers of TPOAbs are commonly seen with elevated serum TSH (4,17). We also showed that TPOAb-positive women had higher TSH than TPOAb-negative women. Thyroid autoimmunity is not always associated with low fT4 and clinical consequences. However, in pregnancy, autoimmune damage to the thyroid gland affects its capacity to compensate for high demand. Therefore, maternal autoimmunity can lead to insufficient supply of maternal thyroid hormones to the child, and, subsequently, cause neuropsychological problems. In the present study, the effect of elevated titers of TPOAbs on the child's behavior was not exclusively mediated by plasma levels of TSH as a marker of maternal thyroid status. In other words, it is unlikely that the change in maternal thyroid function during pregnancy is the only explanatory factor behind the observed association of TPOAbs and child behavior. Thus, other explanations should be discussed. Second, maternal autoimmunity (specifically maternal TSH receptor-blocking antibody) is a common cause of transient hypothyroidism in their infants, because maternal antibodies pass the placenta (42). The effect of maternal antibodies on the thyroid function of the child may persist until the antibodies disappear from the child's circulation a few months after birth. Elevated titers of maternal TPOAbs and TSH receptor-blocking antibodies may co-exist and lead to transient subclinical hypothyroidism in infants. In the present study, we did not find any differences in the thyroid parameters of child cord blood between TPOAb-positive and TPOAb-negative women. Therefore, our data do not support a role of the child's thyroid parameters in the association between maternal TPOAb status and the child's behavior.

Table 3. Maternal Thyroid Peroxidase Antibodies During Pregnancy and Child's Externalizing Problem, the Generation R Study

	Maternal rating		*Paternal rating*		*Maternal and paternal rating*	
Determinant: TPOAb-positive[a]	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*	p
Outcome measures:						
Externalizing problems	1.60 (1.08–2.38)	0.02	1.61 (1.04–2.49)	0.03	1.64 (1.17–2.29)	0.004
DSM-oriented subscales						
Attention Deficit/Hyperactivity Problems	1.60 (0.90–2.87)	0.11	1.89 (1.16–3.07)	0.01	1.77 (1.15–2.72)	0.01
Oppositional Deviant Problems	1.46 (0.91–2.34)	0.12	1.36 (0.73–2.52)	0.33	1.39 (0.95–2.03)	0.09

Total of children in one or more analyses is 3139. The models were adjusted for child's gender and ethnicity, maternal age, cigarette smoking, and time of thyroid sampling during pregnancy.
[a]The TPOAbs levels > 100 IU/mL were defined as positive.

However, measurement of child's thyroid parameters in the cord blood may not be the optimal way to assess the thyroid function of neonates. Third, an autoimmune process could explain the relation of maternal TPOAbs with problem behavior in the children. Thyroid autoimmunity may be a marker of a preexisting subclinical autoimmune condition of the mothers; such an immune process could cause the developmental problems of the offspring, as maternal antibodies pass through the placenta. There is growing evidence for the role of the autoimmune process in neuropsychiatric symptoms in children (18,19). Lastly, external factors such as genetic risk factors and maternal depressive symptoms could explain the relation between maternal TPOAbs and child behavior. However, in the present study, we adjusted for the last risk factor. Further investigations are needed to elucidate the possible mechanisms by which maternal thyroid autoimmunity affects the child's problem behavior.

Elevated titers of maternal TPOAbs were not associated with verbal or nonverbal cognitive function in this large cohort of children. Possibly, brain regions crucial to behavior and emotion control such as amygdala and thalamus are more susceptible to thyroid autoimmunity or other immune processes as compared with those crucial to cognitive abilities. This mechanism, however, is speculative and should be tested by animal or imaging studies. Moreover, our negative findings are not consistent with previous studies of thyroid antibodies and child intelligence (16,17). While our negative results are based on parent report of cognition, the other studies used observational measurements of cognition that are not feasible in large population-based studies.

This study had several strengths. It is a population-based study with a large sample size. Antibody levels were measured as the major etiology of thyroid dysfunction in iodine-sufficient areas. The information on numerous potential confounders was available. The child's problem behavior was rated by both mother and father to obtain the effect of maternal thyroid autoimmunity on the child's behavior independent of the rater. The correlation coefficient (r) between mother and father rating of behavior in our sample was in line with the data reported in the review on cross-informants' correlations of child behavior (32). We asked both mother and father to rate their child's problem behavior as recommended by the experts. Since we studied preschool children, teacher reports on child's behavior were not available. Multi-informant rating scales of the child's behavior are highly recommended and widely used in routine clinical practice to assist clinicians in decision making (43).

Several possible limitations of this study should also be discussed. First, we measured maternal thyroid parameters only once. Therefore, any interpretation about the interaction between the thyroid parameters and steroid hormones throughout the pregnancy and after delivery was not possible. Second, the children's cognition and behavior were parent-report information. However, a high validity of parent-report measures on child's ability has been previously described (25,28). It is very unlikely that the use of parent-report measures introduced systematic bias, as parents were blind to the results of thyroid measurement. In addition, we cannot rule out the effect of non-response to our questionnaires and loss to follow-up on the possible relationship between thyroid autoimmunity and the child's behavior.

The findings of this large population-based study have clinical and public health implications. They may point to a specific mechanism of ADHD in children. Currently, we can only speculate about a public health implication, as there is no specific treatment for TPOAb-positive pregnant women with normal thyroid function. Possible suggestions are to screen for thyroid antibodies during pregnancy and to adopt a low threshold for thyroid parameters in women who were antibody positive if interventions are considered. Further investigation is needed to explore whether TPOAb-positive pregnant women and their children can benefit from close monitoring and early detection of developmental delay in the populations at risk.

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