Association of Gestational Maternal Hypothyroxinemia and Increased Autism Risk

Gustavo C. Román, MD,[1,2] Akhgar Ghassabian, MD, PhD,[3,4]
Jacoba J. Bongers-Schokking, MD, PhD,[5,6] Vincent W. V. Jaddoe, MD, PhD,[3,5,7]
Albert Hofman, MD, PhD,[3,5] Yolanda B. de Rijke, PhD,[8,9]
Frank C. Verhulst, MD, PhD,[4] and Henning Tiemeier, MD, PhD[4,5,10]

Objective: Transient gestational hypothyroxinemia in rodents induces cortical neuronal migration brain lesions resembling those of autism. We investigated the association between maternal hypothyroxinemia (gestational weeks 6–18) and autistic symptoms in children.

Methods: The mother-and-child cohort of the Generation R Study (Rotterdam, the Netherlands) began prenatal enrollment between 2002 and 2006. At a mean gestational age of 13.4 weeks (standard deviation = 1.9, range = 5.9–17.9), maternal thyroid function tests (serum thyrotropin [TSH], free thyroxine [fT_4], and thyroid peroxidase [TPO] antibodies) were assessed in 5,100 women. We defined severe maternal hypothyroxinemia as $fT_4 < 5$th percentile with normal TSH. Six years later, parents reported behavioral and emotional symptoms in 4,039 children (79%) using the Pervasive Developmental Problems (PDP) subscale of the Child Behavior Checklist and/or the Social Responsiveness Scale (SRS). We defined a *probable autistic child* by a PDP score > 98th percentile and SRS score in the top 5% of the sample (n = 81, 2.0%).

Results: Severe maternal hypothyroxinemia (n = 136) was associated with an almost 4-fold increase in the odds of having a probable autistic child (adjusted odds ratio = 3.89, 95% confidence interval [CI] = 1.83–8.20, $p < 0.001$). Using PDP scores, children of mothers with severe hypothyroxinemia had higher scores of autistic symptoms by age 6 years (adjusted B = 0.23, 95% CI = 0.03–0.37); SRS results were similar. No risk was found for children of TPO-antibody–positive mothers (n = 308).

Interpretation: We found a consistent association between severe, early gestation maternal hypothyroxinemia and autistic symptoms in offspring. Findings are concordant with epidemiological, biological, and experimental data on autism. Although these findings cannot establish causality, they open the possibility of preventive interventions.

ANN NEUROL 2013;74:733–742

The cause of autism spectrum disorders (ASD) remains unknown despite advances in genetic research,[1] suggesting a possible etiologic role of environmental factors; autism risk increases with older parental age,[2] prenatal medications, gestational bleeding, and diabetes.[3]

In 1999, Haddow et al[4] demonstrated that maternal hypothyroidism during pregnancy affects cognitive development of the child; Morreale de Escobar and her colleagues further delimited critical thyroid brain effects to first-trimester maternal hypothyroxinemia, defined as low-for-gestational-age circulating maternal free thyroxine

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.23976

Received Mar 19, 2013, and in revised form Jun 21, 2013. Accepted for publication Jul 3, 2013.

Address correspondence to Dr Román, Methodist Neurological Institute, 6560 Fannin Street, Suite 802, Houston, TX 77030.
E-mail: GCRoman@tmhs.org

From the [1]Methodist Neurological Institute, Houston, TX; [2]Department of Neurology, Weill Cornell Medical College, New York, NY; [3]Generation R Study Group, Rotterdam, the Netherlands; [4]Department of Child and Adolescent Psychiatry; [5]Department of Epidemiology; [6]Department of Endocrinology; [7]Department of Pediatrics; [8]Department of Internal Medicine; [9]Department of Clinical Chemistry; and [10]Department of Psychiatry, Erasmus University Medical Center, Rotterdam, the Netherlands.

Additional supporting information can be found in the online version of this article.

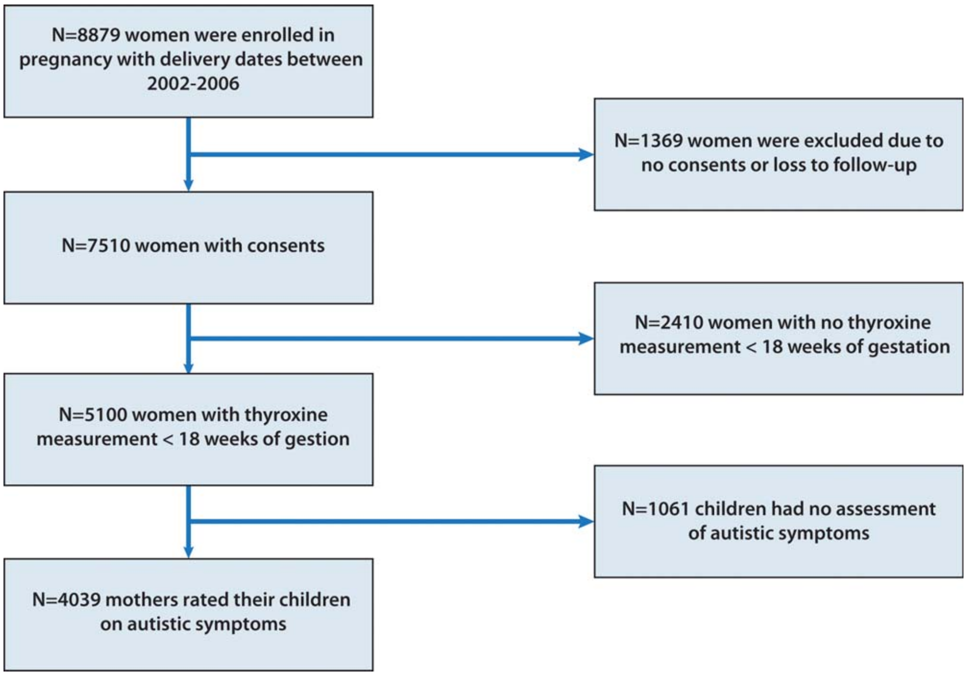

FIGURE 1: Study population.

(fT_4) with normal or increased thyrotropin (TSH).[5,6] A series of elegant animal models from her research group demonstrated that transient and mild fT_4 deficits during early gestation produce permanent abnormalities in cortical development of the progeny, causing faulty neuronal migration in hippocampus and somatosensory cortex.[7–9] Cortical layers were blurred, irregular, and lacking cortical barrels, with neurons in abnormal locations, including white matter.[7,8] Thyroid deficits, as short as 3 days in rodents, induced permanent alterations of cortical brain cytoarchitecture, radial distribution of neurons, and abnormal tangential migration of medial ganglionic-eminence–derived neurons.[9] In humans, neocortical development occurs between weeks 6 and 24 of gestation, although the bulk of cortical cell migration occurs between 8 and 24 weeks, before the onset of fetal thyroid hormone secretion at midgestation (weeks 18–22).

In 2007, Román[10] discerned the remarkable histological similarities existing between the neuropathology of autism[11] and the brain lesions of experimental maternal hypothyroxinemia[7–9]; he postulated that maternal fT_4 deficiency in early gestation could be important in autism by disrupting critical stages of neuronal migration in brain and cerebellum. Recently, Hoshiko et al[12] found that infants born with very low fT_4 had an increased risk of autism.

To investigate the role of maternal hypothyroxinemia, we analyzed maternal thyroid function during pregnancy and autistic symptoms data from the Generation R Study (Rotterdam, the Netherlands), a unique cohort that has already contributed important information on thyroid function and cognitive, behavioral, and psychomotor development.[13–15] We explored the relation between thyroid function tests at 6 to 24 weeks of gestation and parent-reported autistic symptoms in children at 6 years of age, controlling for maternal factors and children's characteristics.

Subjects and Methods

Participants

The Generation R Study is a population-based birth cohort designed to identify early environmental and genetic determinants of growth, development, and health from fetal life onward.[16,17] Erasmus Medical Center, Rotterdam, the Netherlands, and Methodist Research Institute, Houston, Texas approved this study. Written informed consent was obtained from adult participants, and all data were deidentified. Parents were not informed about test results (except 1 case, excluded from this study).

In total (Fig 1), 8,879 pregnant women were enrolled during pregnancy (7,069 women in early pregnancy, 1,594 in midpregnancy, and 216 in late pregnancy), and 7,510 consented for pre- and postnatal participation; fT_4 levels were measured in 5,100 women in early pregnancy (<18 weeks of gestation). Information on autistic symptoms was available in 4,039 children (79%) at 6 years of age. All children were born between April 2002 and January 2006.

Children's Autistic Symptoms

We assessed parent-reported autistic symptoms using 2 instruments: the Pervasive Developmental Problems (PDP) subscale of the Child Behavior Checklist for Toddlers (CBCL1½-5)[18] and the Social Responsiveness Scale (SRS).[19] As part of the Generation R protocol, parents responded to multiple questionnaires from the neonatal period until 6 years of age; therefore, we consider responses at 6 years of age to be accurate.

PDP SUBSCALE OF THE CBCL1½-5. The CBCL1½-5 is a highly validated instrument to measure behavioral and emotional problems of children at young age.[18] The Dutch version is reliable and well validated,[20] and the subscales for syndromes derived from the CBCL1½-5 had good fit in 23 international studies across diverse societies,[21] and are consistent with diagnostic categories of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV).[22] The PDP subscale of the CBCL1½-5 is a useful screening instrument to identify children with ASD,[23] with good predictive validity identifying preschoolers at risk of ASD.[24] Although 40% of children were 6 years or older at the time of assessment (mean age = 72.2 months, standard deviation [SD] = 4.7, range = 57.7–95.9), we used the CBCL1½-5 (preschool version) both for reasons of continuity and because many children were not yet in formal schooling. In our sample, Cronbach alphas for the PDP scale in 5-year-old children and in children older than 5 years were similar (alpha = 0.79 vs 0.73), indicating that autistic symptoms were reliably measured in children older than 5 years.

SRS. To minimize subject burden, the lengthy original questionnaire was reduced to an 18-item short-form SRS for assessment of autistic behaviors based on parents' observations of children's social behavior in a naturalistic setting[19]; items selected encompassed all DSM-IV autism domains (personal communication with SRS test developer; see Supplementary Table 1). Previously, another SRS short version (11 items) was validated to assess autistic behaviors in young Australian twins.[25] Each item is rated from 0 (never true) to 3 (almost always true), covering social, language, and repetitive behaviors; higher scores indicate more problems. Mean age at time of SRS assessment was 73.8 (SD = 5.6) months, with good correlation between SRS and PDP scores ($r = 0.6$, $p < 0.001$, n = 3,141).

Maternal Thyroid Parameters

Maternal thyroid parameters (TSH, fT_4, and thyroid peroxidase [TPO] antibodies) were assessed in blood samples obtained during the first prenatal visit (mean = 13.4 weeks, SD = 1.9, range = 5.9–17.9). Within 24 hours, plasma was stored at −80°C and processed in batches over 6 months using chemiluminescent assays (Vitros ECI Immunodiagnostic System; Ortho Clinical Diagnostics, Rochester, NY). Our laboratory reference value is fT_4 = 11 to 25pmol/l for nonpregnant women. Inter- and intra-assay coefficients of variation for fT_4 were 4.7 to 5.4% and 1.4 to 2.7%. We defined *mild* maternal hypothyroxinemia (n = 295, 73% of the sample) in early pregnancy as normal TSH (>0.03, <2.5mIU/l) and $fT_4 < 11.82$pmol/l (<10th percentile of the sample) and *severe* maternal hypothyroxinemia (n = 136, 3.4% of the sample) as $fT_4 < 10.99$pmol/l (<5th percentile).[15] To explore a dose–effect relation between maternal hypothyroxinemia and autistic symptoms in the children, we defined a group of "only mild hypothyroxinemia" that consisted of pregnant women with normal TSH levels and $10.99 < fT_4 < 11.82$pmol/l (n = 159). We measured maternal TPO antibodies (Phadia 250 immunoassay; Phadia, Uppsala, Sweden) and defined positive TPO antibodies by plasma concentrations ≥ 100IU/ml.

Previous Generation R cohort studies[13–15] demonstrated that maternal TSH is not a good predictor of cognitive and behavioral problems in the offspring.[15] In contrast, low maternal fT_4 predicted delays in nonverbal cognitive functions and expressive language (odds ratio [OR] = 2.03, 95% confidence interval [CI] = 1.22–3.39, $p = 0.007$).[15] Therefore, low maternal fT_4 concentrations appear to affect fetal brain development despite normal TSH levels in pregnant women.[15] We included TPO antibodies because children of TPO antibody–positive mothers were at a higher risk of attention deficit/hyperactivity problems (OR = 1.77, 95% CI = 1.15–2.72, $p = 0.01$), with minimal influence of maternal TSH on the risk.[13]

Covariates

Potential confounders were selected a priori.[14,15,26] Information on birth date, sex, and birth weight was obtained from registries. Gestational age at birth was established using the ultrasound examination during pregnancy. Parity, parental age, education, marital status, ethnicity, and history of smoking were assessed by questionnaires. Each child's ethnic background was defined based on the country of birth of the parents and classified as Western or Non-Western according to Statistics Netherlands.[27]

Maternal education was defined by the highest completed education. Maternal smoking was assessed at enrollment and in mid and late pregnancy. We used the Brief Symptom Inventory (BSI), a 53-item validated self-report questionnaire, to measure maternal psychopathology during pregnancy.[28] High validity and reliability have been reported for the BSI Dutch translation.[29]

Maternal folate[30] and C-reactive protein (CRP)[31] concentrations were analyzed in plasma samples in early pregnancy by using an immunoelectrochemiluminescence assay on the Architect System (Abbott Diagnostics, Hoofddorp, the Netherlands). Intelligence was assessed by having the children complete 2 subtests (Mosaics and Categories) of the Snijders-Oomen Niet-verbale intelligentie Test–Revisie.[32] In an unrelated sample of 626 children (mean age = 6.0 years; SD = 0.85), the correlation between the sum of these 2 subtests and the full intelligence quotient (IQ) battery was very high, $r = 0.86$ (P. Tellegen, personal communication). The raw test scores were converted into nonverbal intelligence score using norms tailored to exact age.[32]

Statistical Analysis

All children with data on maternal fT_4 and 1 or more measures of autistic symptoms were included in the analyses. In our sample, missing values of the covariates ranged between 0 and 10%, except for maternal psychopathology scores (13%) and children's intelligence. The missing values for the PDP and the SRS scores were 3.8% and 18.0%, respectively. We used independent sample *t* tests and chi-square statistics to determine whether the response to the outcome measures of autistic behavior was selective. Missing values were imputed using multiple imputations. Thirty copies of the original data set were generated, with missing values replaced by values randomly generated from the predictive distribution on the basis of the correlation between the variable with missing values and other variables (for complete case analyses, see Supplementary Tables 2 and 3).

Early gestation maternal thyroid functions were determinants in all analyses. Maternal TSH and fT_4 levels were divided by SD so that their associations with children's autistic symptoms could be interpreted via SD increments in the predictor. Children's SRS and PDP scores were used as outcomes in continuous analyses to obtain higher power and categorically to facilitate the clinical interpretation of the findings. In the continuous analyses, the SRS and PDP scores were transformed using square root to satisfy the assumption of normality in the linear regression models.

We used the 93rd (borderline) and 98th (clinical) percentile of a Dutch norm group as cutoff scores to classify children with behavioral problems within the borderline and clinical range of the PDP.[18] On the PDP scores, 263 children were borderline and 123 were in the clinical range. We explored the associations between maternal thyroid function and both borderline and clinical PDP using multivariate logistic regressions.

We defined a *probable autistic child* by stringent criteria that included a PDP score $>$ 98th percentile and also a SRS score in the top 5% of the sample (n = 80, 2.0%). The odds of being a probable autistic child were calculated using multivariate logistic regressions, if the mother had maternal hypothyroxinemia in comparison with the rest of the mother–child cohort.

Furthermore, we performed a sensitivity analysis in a sample of women with fT_4 values measured in the first trimester of pregnancy (gestation age at the time of blood sampling $<$ 13 weeks, n = 1,902).

All models were adjusted for child's sex, ethnicity, gestational age at birth, birth weight, parental age, education, smoking history, prenatal psychopathology, thyroid medication during pregnancy, parity, marital status, time of thyroid sampling, and early pregnancy folate and CRP. All these variables (except parity) were associated with children's autistic symptoms in univariate analyses. Additionally, we adjusted all the analyses for children's intelligence because of the possible impact of IQ differences on the observed association between low maternal thyroid function and autistic symptoms.

Attrition Analysis

We found differences between the 4,039 mother–child pairs included in the analyses and the 1,061 (21% of 5,100) pairs excluded because of missing information on autistic behavior. Excluded children were mostly non-Western (52.2% vs 26.4%, $p < 0.001$), and had a lower birth weight (mean difference = −114 g, $p < 0.001$); their mothers were younger (mean difference = −2.9 years, $p < 0.001$), less educated (10.6% vs 28.9% higher education, $p < 0.001$), more likely to smoke (33.9% vs 22.8%, $p < 0.001$), and had a higher prenatal psychopathology score than those included (mean difference score = 0.14, $p < 0.001$). Maternal TSH levels were higher in the children excluded than those in the analysis (mean difference = −0.19, $p = 0.04$). However, there were no differences in maternal fT_4 between the 2 groups.

Results

In our study, 80 children were defined as a probable autistic child, having a PDP score (>98th percentile) within the clinical range, plus SRS scores in the top 5% of the sample. We found that the odds of being a probable autistic child increased almost 4-fold (adjusted OR = 3.89, 95% CI = 1.83–8.20) when the mother had severe hypothyroxinemia in early gestation.

Maternal and child characteristics in the study sample are presented in Table 1. There was no association between maternal TSH and fT_4 during early pregnancy and children's borderline and clinical PDP. However, we found a consistent association between maternal hypothyroxinemia and parent-reported autistic symptoms in the offspring (Table 2). Severe maternal hypothyroxinemia during early gestation was associated with double risk of borderline PDP at age 6 years (adjusted OR = 2.02, 95% CI = 1.16–3.51). Similarly, children of hypothyroxinemic mothers had higher odds of developing clinical PDP at 6 years (adjusted OR = 2.60, 95% CI = 1.30–5.18). The relation between maternal hypothyroxinemia and children's autistic symptoms, as rated by the PDP subscale of the CBCL1½-5, was not dose dependent, considering the negative association of only mild hypothyroxinemia in mothers during early pregnancy and autistic symptoms.

As shown in Table 3, results of the association analyses with PDP and SRS scores as continuous outcomes showed similar findings (adjusted B = 0.23 for PDP, 95% CI = 0.07–0.37; and adjusted B = 0.05 for SRS, 95% CI = 0.01–0.10). There was no relation between only mild hypothyroxinemia in mothers and children's autistic symptoms. Figure 2 illustrates the mean SRS scores in 3 groups of children based on maternal thyroid function in early pregnancy. Positive maternal TPO antibodies were not associated with autistic symptoms in the children (see Tables 2 and 3).

In analyses additionally adjusted for children's intelligence, the effect estimates decreased but remained significant (OR for a probable autistic child = 3.61, 95% CI = 1.70–7.70, $p = 0.001$). Similarly, we found that, when additionally adjusted for children's intelligence, children exposed prenatally to maternal severe hypothyroxinemia were more likely to have higher scores on both the PDP (B = 0.21, 95% CI = 0.06–0.36) and SRS (B = 0.05, 95% CI = 0.01–0.09).

TABLE 1. Maternal and Child Characteristics, n = 4,039

Characteristic	Values
Maternal	
Age at enrollment, yr, mean (SD)	31.2 (4.7)
Parity, No. primipara [%]	2,419 [59.9]
Marital status, No. single [%]	388 [9.6]
Education, No. [%]	
Low	723 [17.9]
Mid-low	1,200 [29.7]
Mid-high	961 [23.8]
High	1,154 [28.6]
History of smoking, No. any time [%]	924 [22.9]
Prenatal psychopathology score, median {90% range}	0.16 {0.02–0.63}
Serum folate, nmol/l, median {90% range}	17.70 {8.08–31.20}
Serum C-reactive protein, median {90% range}	4.40 {1.20–13.30}
Thyroid parameters	
Age of biological father at enrollment, yr, mean (SD)	33.5 (5.4)
Time of blood sampling, median weeks of gestation {90% range}	13.1 {11.4–16.1}
Thyrotropin, mU/l, median {90% range}	1.37 {0.45–2.95}
fT_4, pmol/l, median {90% range}	14.88 {11.82–18.91}
Thyroid peroxidase antibodies, No. yes [%]	308 [7.6]
Hypothyroxinemia, No. yes [%]	
Mild, $fT_4 < 11.82$ pmol/l	295 [7.3]
Severe, $fT_4 < 10.99$ pmol/l	136 [3.4]
Child	
Age, yr, median {90% range}	5.9 {5.7–6.5}
Male sex, No. [%]	2,026 [50.2]
Ethnicity, No. Western [%]	2,965 [73.4]
Birth weight, g, mean (SD)	3,428 (566)
Gestational age at birth, wk, median {90% range}	39.9 {37.9–41.7}
Intelligence, score (SD)	102.4 (14.7)
Pervasive Developmental Problems score, median {90% range}	2.00 {0.00–5.00}
Social Responsiveness Scale score, median {90% range}	0.17 {0.00–0.45}

fT_4 = free thyroxine; No. = number of cases; SD = standard deviation.

When we restricted the sample to only mother-child pairs with thyroid parameters assessed in the first trimester of pregnancy, similar results emerged (Supplementary Tables 4 and 5). There was no sex difference in the relation between maternal hypothyroxinemia and autistic symptoms in the offspring (data not shown).

Discussion

In the present study, we found that severe maternal hypothyroxinemia in early gestation was associated with significant increase in the risk of parent-reported autistic symptoms in the offspring by 6 years of age. Although these findings cannot establish causality, they suggest that maternal thyroid dysfunction could result in autistic

TABLE 2. Multivariate Logistic Regression Analyses of Early Gestation Maternal Thyroid Function and Autistic Symptoms in the Offspring, n = 4,039

Maternal Thyroid Parameters	Pervasive Developmental Problems at Age 6 Years			
	Borderline Problems n = 263		Clinical Problems n = 123	
	OR (95% CI)	*p*	OR (95% CI)	*p*
TSH per SD	0.91 (0.77–1.07)	0.27	0.92 (0.72–1.17)	0.49
fT_4 per SD	0.93 (0.80–1.09)	0.37	0.95 (0.77–1.17)	0.62
Mild hypothyroxinemia	1.31 (0.84–2.04)	0.23	1.41 (0.78–2.57)	0.26
Only mild hypothyroxinemia	0.77 (0.38–1.55)	0.46	0.51 (0.16–1.64)	0.26
Severe hypothyroxinemia	2.02 (1.16–3.51)	0.01	2.60 (1.30–5.18)	0.01
TPO-Abs^+	1.47 (0.85–2.55)	0.17	0.78 (0.28–2.16)	0.63

Mild hypothyroxinemia (n = 136): 0.03 < TSH < 2.5mIU/l and fT_4 < 11.82pmol/l (<10th percentile of the sample); only mild hypothyroxinemia (n = 295): 0.03 < TSH < 2.5mIU/l and 10.99 < fT_4 < 11.82pmol/l; severe hypothyroxinemia (n = 159): 0.03 < TSH < 2.5mIU/l and fT_4 < 10.99pmol/l (<5th percentile); TPO-Abs^+ (n = 308): ≥100IU/ml.
Models were adjusted for child's sex, ethnicity, gestational age at birth, birth weight, maternal age, educational level, smoking history, prenatal psychopathology, thyroid medication during pregnancy, parity, marital status, maternal folate and C-reactive protein levels in early pregnancy, time of thyroid sampling during pregnancy, and paternal age. For the results of the additional adjustment for child's intelligence, please see the text.
CI = confidence interval; fT_4 = free thyroxine; OR = odds ratio; SD = standard deviation; TPO-Abs = thyroid peroxidase antibodies; TSH = thyrotropin.

TABLE 3. Linear Regression Analyses of Early Gestation Maternal Thyroid Function and Autistic Symptoms in the Offspring, n = 4,039

Maternal Thyroid Parameters	Autistic Symptoms at Age 6 Years, Scores			
	Pervasive Developmental Problems		Social Responsiveness Scale	
	B (95% CI)	*p*	B (95% CI)	*p*
TSH per SD	0.01 (−0.02 to 0.03)	0.68	0.01 (−0.001 to 0.01)	0.12
fT_4 per SD	−0.03 (−0.06 to −0.01)	0.04	−0.002 (−0.01 to 0.01)	0.67
Mild hypothyroxinemia	0.09 (−0.02 to 0.19)	0.10	0.02 (−0.01 to 0.04)	0.33
Only mild hypothyroxinemia	−0.04 (−0.17 to 0.004)	0.62	−0.02 (−0.06 to 0.02)	0.28
Severe hypothyroxinemia	0.23 (0.07 to 0.37)	0.04	0.05 (0.01,0.10)	0.01
TPO-Abs^+	0.05 (−0.08 to 0.18)	0.43	0.03 (−0.01 to 0.06)	0.11

Mild hypothyroxinemia (n = 136): 0.03 < TSH < 2.5mIU/l and fT_4 < 11.82pmol/l (<10th percentile of the sample); only mild hypothyroxinemia (n = 295): 0.03 < TSH < 2.5mIU/l and 10.99 < fT_4 < 11.82pmol/l; severe hypothyroxinemia (n = 159): 0.03 < TSH < 2.5mIU/l and fT_4 < 10.99pmol/l (<5th percentile); TPO-Abs^+ (n = 308): ≥100IU/ml.
Models were adjusted for child's sex, ethnicity, gestational age at birth, birth weight, maternal age, educational level, smoking history, prenatal psychopathology, thyroid medication during pregnancy, parity, marital status, maternal folate and C-reactive protein levels in early pregnancy, time of thyroid sampling during pregnancy, and paternal age. For the results of the additional adjustment for child's intelligence, please see the text.
The B's are not interpretable, as the mathematically transformed scores were used in the analyses.
CI = confidence interval; fT_4 = free thyroxine; SD = standard deviation; TPO-Abs = thyroid peroxidase antibodies; TSH = thyrotropin.

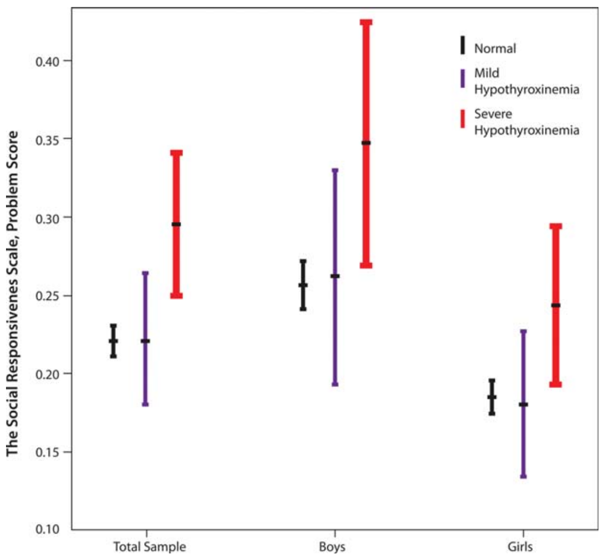

FIGURE 2: Maternal hypothyroxinemia in early pregnancy and autistic symptoms in the children at age 6 years.

symptoms in the child, perhaps as a result of alterations of neuronal migration in the developing brain.

Our study has several strengths, such as the large population-based sample and a unique prospective design, but we were faced with some limitations. First, we measured maternal thyroid parameters once during pregnancy. However, the measurements were performed during early gestation, as recommended.[4] Second, we used parental reports of autistic-like behaviors. Clinical diagnosis based on interviews and direct observations is unfeasible in large samples; instead, parental reports of children's behavior have been used widely in epidemiological studies.[20,21,23–25] There are limitations to parental reports of children's social deficits, including parental inability to detect impaired dyadic interactions.[33] However, parents are ideal observers of certain aspects of social behavior, such as smiles, and are often familiar with their child's functioning in different settings across time.[34] In a sample of 2,719 children from the Interactive Autism Networks,[35] the parent-reported SRS scores were considerably higher in ASD children than in their siblings (see Supplementary Table 1). Additionally, Muratori et al[24] reported a sensitivity of 0.85 and specificity of 0.90 for the CBCL1½-5 PDP subscale to differentiate ASD children from children with typical development. In our study, the number of children with clinical diagnosis of ASD was unavailable; however, by using stringent criteria and combining 2 instruments, we defined 80 children at high risk of developing ASD. Third, as in many epidemiological studies, in Generation R we observed nonresponse (72% participation rate in early pregnancy) and loss to follow-up (21% loss to follow-up at 6 years). The nonresponse may affect the generalizability of the findings and could introduce a selection bias in the study.[36,37] However, we showed that the participation rate at age 6 years was unrelated to maternal fT_4. This finding, together with the relatively low loss to follow-up, makes it less likely that our results were affected by selection bias.

Thyroid hormones are critical during pregnancy. Maternal hypothyroidism causes pregnancy complications, including postpartum hemorrhage, placental abruption, and preterm labor; some of these are risk factors for autism.[38] Thyroid deficiency in utero during critical periods of brain development causes mental retardation, psychomotor delay, and deafness.[5,6,39] It is also associated with other neurodevelopmental outcomes such as cerebral palsy.[40] In the fetal brain, the prohormone T_4 is converted into the active thyroid hormone 3,5,3′-triiodothyronine (T_3) via 5′ deiodination of maternal thyroxine by local brain type 2 iodothyronine deiodinase (D_2).[39] T_3 binds to nuclear thyroid hormone receptors that regulate gene expression in the brain.[39] These receptors appear in the cerebral cortex of the human fetus by the 8th to 9th weeks of gestation and increase about 10-fold between the 10th and 18th weeks of gestation,[39] concurrently with increased D_2 activity to augment T_3 cortical levels. Therefore, moderately low T_4 maternal levels may be damaging to the cortical formation of the fetus.

Children with autism have increased head circumference, excessive brain growth, cortical and white matter abnormalities dated to early pregnancy in brain imaging,[41] and significantly more neurons in the prefrontal cortex, particularly in the dorsolateral region (79% more neurons than control cases, ie, 1.57 vs 0.88 billion; 95% CI = 0.66–1.10).[42] Neuropathologically, autism is a defect of neurogenesis and neuronal migration, causing abnormal cortical laminar patterns, focal cortical dysplasia, excessive neuronal counts, and cell immaturity with densely packed neurons in hippocampus, subiculum, mammillary body, septal nuclei, and amygdala.[11,43]

In humans, neocortical neurons derived from the neuroepithelium migrate from periventricular regions between weeks 12 and 24 of gestation.[44] Migration occurs along radial glia scaffolds, with late born neurons migrating past early born neurons.[44] Cajal-Retzius neurons secrete reelin,[45] an extracellular glycoprotein that binds to membrane receptors on migrating neurons phosphorylating the disabled homolog-1 (Dab1) to stop neuronal migration.

The reelin–dab signaling system is dependent on thyroid hormones.[46] Hypothyroidism reduces reelin expression and enhances Dab1 expression during brain development.[47] Fatemi and her group[45,47,48] demonstrated reelin signaling abnormalities in autism. Alterations included significant reductions in reelin protein, reelin mRNA, and Dab1 mRNA along with elevations in Reln receptor, and VLDL-R mRNA in frontal and cerebellar cortex.[47,48] Recently, liver X receptor beta (LXRβ) interactions with thyroid hormone receptor alpha (THRα) in brain cortical layering were described.[49]

Some studies have found genetic susceptibility polymorphisms of the reelin gene (*RELN*) in autism; however, other comprehensive studies have been negative, suggesting that environmental factors probably play an important role in the disruption of this signaling system.[1]

The neuropathological abnormalities of autism could be explained by disruption of the reelin–dab signaling system resulting from early maternal hypothyroxinemia. Maternal T_4 and T_3 are transported to the fetal brain across the blood–brain barrier, but prior to the formation of the fetal thyroid around midgestation the fetus is unable to produce thyroxine and is, therefore, completely dependent on maternal thyroxine.[5,39] Maternal serum levels of thyrotropin increase progressively during normal pregnancy, causing a corresponding rise of serum thyroglobulin.[5] Transfer of maternal T_4 to the fetus occurs up to birth; therefore, the availability of fT_4 to embryonic and fetal tissues is dependent on circulating maternal T_4. Decreases in fT_4 occur in hypothyroxinemic women even if they are clinically euthyroid.[5,6]

In conclusion, the association of severe maternal hypothyroxinemia early in pregnancy with significant increase in risk of parent-reported autistic symptoms is concordant with epidemiological, clinical, neuropathological, molecular biological, genetic, and experimental data on autism. Currently, it is recommended to treat overt maternal thyroid dysfunction in pregnancy because of potentially serious consequences for the child.[50] However, the evidence is inconclusive as to whether children of hypothyroxinemic mothers would benefit from treatment. Although our findings cannot establish causality, they provide further support for the possible role of low maternal thyroid function in children's brain development. Future studies are recommended to apply long-term follow-up of children to obtain more meaningful performance estimates.[51] If confirmed by future research, this study provides arguments in favor of universal thyroid-function screening in the first trimester of pregnancy[52] and may open the possibility of preventive intervention in autism.

Acknowledgment

The work of G.C.R. is supported by a grant from the Nancy Lurie Marks Family Foundation, Wellesley, Massachusetts. The work of H.T. and A.G. was supported by a research grant from the European Community's 7th Framework Program (FP7/2008–2013) under grant agreement 212652 (Nutrimenthe project, "The Effect of Diet on the Mental Performance of Children"). Measurements of thyroid parameters have been made possible by Ortho Clinical Diagnostics, Jan Dekker/Ludgardine Bouwman Foundation, Fa. Merck, and Foundation Zeist. Funding was also received from the Erasmus University Trust Fund, Janivo Foundation, Merck-Serono, Dutch Research Council, and Ministry of Family Affairs (the Netherlands).

A.G. and H.T. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. The Generation R Study is conducted by Erasmus Medical Center, Rotterdam in close collaboration with the faculty of Social Sciences of Erasmus University, Rotterdam; the Municipal Health Service, Rotterdam area; the Rotterdam Homecare Foundation; and the Stichting Trombosedienst and Artsenlaboratorium Rijnmond, Rotterdam, the Netherlands.

We thank the general practitioners, hospitals, midwifes, and pharmacies in Rotterdam.

Authorship

Conception of the study: G.C.R.; design: J.J.B.-S., A.H., F.C.V., H.T.; acquisition and analysis of the data: G.C.R., A.G., H.T.; interpretation of the data: G.C.R., J.J.B.-S., Y.B.d.R., H.T.; drafting the article: G.C.R., A.G., H.T.; critical revision of the manuscript for important intellectual content: G.C.R., A.G., J.J.B.-S., V.W.V.J., A.H., Y.B.d.R., F.C.V., H.T.

G.C.R. and A.G. contributed equally to this article.

Potential Conflicts of Interest

F.C.V.: remunerated contributing editor of the *Achenbach System of Empirically Based Assessment*.

References

1. Betancur C. Etiological heterogeneity in autism spectrum disorders: more than 100 genetic and genomic disorders and still counting. Brain Res 2011;1380:42–77.
2. Durkin MS, Maenner MJ, Newschaffer CJ, et al. Advanced parental age and the risk of autism spectrum disorder. Am J Epidemiol 2008;168:1268–1276.
3. Gardener H, Spiegelman D, Buka SL. Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry 2009;195:7–14.
4. Haddow JE, Palomaki GE, Allan WC, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999;341:549–555.

5. Morreale de Escobar G, Obregon MJ, Escobar del Rey F. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? J Clin Endocrinol Metab 2000; 85:3975–3987.

6. de Escobar GM, Obregon MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab 2004;18:225–248.

7. Lavado-Autric R, Auso E, Garcia-Velasco JV, et al. Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 2003;111:1073–1082.

8. Auso E, Lavado-Autric R, Cuevas E, et al. A moderate and transient deficiency of maternal thyroid function at the beginning of fetal neocorticogenesis alters neuronal migration. Endocrinology 2004;145:4037–4047.

9. Cuevas E, Auso E, Telefont M, et al. Transient maternal hypothyroxinemia at onset of corticogenesis alters tangential migration of medial ganglionic eminence-derived neurons. Eur J Neurosci 2005;22:541–551.

10. Román GC. Autism: transient in utero hypothyroxinemia related to maternal flavonoid ingestion during pregnancy and to other environmental antithyroid agents. J Neurol Sci 2007;262:15–26.

11. Wegiel J, Kuchna I, Nowicki K, et al. The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol 2010;119:755–770.

12. Hoshiko S, Grether JK, Windham GC, et al. Are thyroid hormone concentrations at birth associated with subsequent autism diagnosis? Autism Res 2011;4:456–463.

13. Ghassabian A, Bongers-Schokking JJ, de Rijke YB, et al. Maternal thyroid autoimmunity during pregnancy and the risk of attention deficit/hyperactivity problems in children: the Generation R Study. Thyroid 2012;22:178–186.

14. Ghassabian A, Bongers-Schokking JJ, Henrichs J, et al. Maternal thyroid function during pregnancy and behavioral problems in the offspring: the Generation R Study. Pediatric Res 2011;69:454–459.

15. Henrichs J, Bongers-Schokking JJ, Schenk JJ, et al. Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: the Generation R study. J Clin Endocrinol Metab 2010;95:4227–4234.

16. Jaddoe VW, Bakker R, van Duijn CM, et al. The Generation R Study Biobank: a resource for epidemiological studies in children and their parents. Eur J Epidemiol 2007;22:917–923.

17. Jaddoe VW, van Duijn CM, van der Heijden AJ, et al. The Generation R Study: design and cohort update 2010. Eur J Epidemiol 2010;25:823–841.

18. Achenbach TM, Rescorla LA. Manual for ASEBA preschool forms & profiles. Burlington, VT: University of Vermont, Research Center for Children, Youth, & Families, 2000.

19. Constantino JN, Todd RD. Genetic structure of reciprocal social behavior. Am J Psychiatry 2000;157:2043–2045.

20. Tick NT, van der Ende J, Koot HM, Verhulst FC. 14-year changes in emotional and behavioral problems of very young Dutch children. J Am Acad Child Adolesc Psychiatry 2007;46:1333–1340.

21. Ivanova MY, Achenbach TM, Rescorla LA, et al. Preschool psychopathology reported by parents in 23 societies: testing the seven-syndrome model of the child behavior checklist for ages 1.5–5. J Am Acad Child Adolesc Psychiatry 2010;49:1215–1224.

22. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (DSM-IV-TR). Washington, DC: American Psychiatric Association, 2000.

23. Sikora DM, Hall TA, Hartley SL, et al. Does parent report of behavior differ across ADOS-G classifications? Analysis of scores from the CBCL and GARS. J Autism Dev Disord 2008;38: 440–448.

24. Muratori F, Narzisi A, Tancredi R, et al. The CBCL 1.5–5 and the identification of preschoolers with autism in Italy. Epidemiol Psychiatr Sci 2011;20:329–338.

25. Reiersen AM, Constantino JN, Grimmer M, et al. Evidence for shared genetic influences on self-reported ADHD and autistic symptoms in young adult Australian twins. Twin Res Hum Genet 2008;11:579–585.

26. Kooistra L, Crawford S, van Baar AL, et al. Neonatal effects of maternal hypothyroxinemia during early pregnancy. Pediatrics 2006;117:161–167.

27. Statistics Netherlands. Allochtonen in Nederland 2004 [Immigrants in the Netherlands]. Statistics Netherlands: Voorburg/Heerlen, 2004.

28. Derogatis LR, ed. The Brief Symptom Inventory (BSI): administration, scoring, and procedures. Manual. 3rd ed. Minneapolis, MN: National Computer Systems, 1993.

29. de Beurs E. Brief Symptom Inventory, handleiding. Leiden, the Netherlands: Pits Publishers, 2004.

30. Steenweg-de Graaff J, Roza SJ, Steegers EAP, et al. Maternal folate status in early pregnancy and child emotional and behavioral problems: the Generation R Study. Am J Clin Nutr 2012;95: 1413–1421.

31. Ernst GDS, de Jonge LL, Hofman A, et al. C-reactive protein levels in early pregnancy, fetal growth patterns, and the risk for neonatal complications: the Generation R Study. Am J Obstet Gynecol 2011;205:132.e1–132.e12.

32. Tellegen PJ, Winkel M, Wijnberg-Williams B, Laros JA. Snijders-Oomen Niet-Verbale Intelligentietest: SON-R 2 1/2-7. Amsterdam, the Netherlands: Boom Testuitgevers, 2005.

33. Chawarska K, Klin A, Paul R, Volkmar F. Autism spectrum disorder in the second year: stability and change in syndrome expression. J Child Psychol Psychiatry 2007;48:128–138.

34. Verhulst FC, Van der Ende J. Using rating scales in a clinical context. In: Rutter M, Bishop D, Pine D, et al, eds. Rutter's child and adolescent psychiatry. Malden, MA: Blackwell Publishing, 2010: 289–298.

35. Daniels A, Rosenberg R, Anderson C, et al. Verification of parent-report of child autism spectrum disorder diagnosis to a Web-based autism registry. J Autism Dev Disord 2012;42:257–265.

36. Greene N, Greenland S, Olsen J, Nohr EA. Estimating bias from loss to follow-up in the Danish National Birth Cohort. Epidemiology 2011;22:815–822.

37. Jacobsen T, Nohr E, Frydenberg M. Selection by socioeconomic factors into the Danish National Birth Cohort. Eur J Epidemiol 2010;25:349–355.

38. Gardener H, Spiegelman D, Buka SL. Perinatal and neonatal risk factors for autism: a comprehensive meta-analysis. Pediatrics 2011;128:344–355.

39. Bernal J, Guadano-Ferraz A, Morte B. Perspectives in the study of thyroid hormone action on brain development and function. Thyroid 2003;13:1005–1012.

40. Nelson KB, Ellenberg JH. Antecedents of cerebral palsy. Multivariate analysis of risk. N Engl J Med 1986;315:81–86.

41. McAlonan GM, Cheung V, Cheung C, et al. Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain 2005;128:268–276.

42. Courchesne E, Mouton PR, Calhoun ME, et al. Neuron number and size in prefrontal cortex of children with autism. JAMA 2011; 306:2001–2010.

43. Kemper TL, Bauman ML. The contribution of neuropathologic studies to the understanding of autism. Neurol Clin 1993;11:175–187.

44. Parnavelas JG. The origin and migration of cortical neurones: new vistas. Trends Neurosci 2000;23:126–131.

45. Fatemi SH. Reelin glycoprotein: structure, biology and roles in health and disease. Mol Psychiatry 2005;10:251–257.

46. Alvarez-Dolado M, Ruiz M, Del Rio JA, et al. Thyroid hormone regulates reelin and dab1 expression during brain development. J Neurosci 1999;19:6979–6993.

47. Fatemi SH, Stary JM, Egan EA. Reduced blood levels of reelin as a vulnerability factor in pathophysiology of autistic disorder. Cell Mol Neurobiol 2002;22:139–152.

48. Fatemi SH, Snow AV, Stary JM, et al. Reelin signaling is impaired in autism. Biol Psychiatry 2005;57:777–787.

49. Tan XJ, Fan XT, Kim HJ, et al. Liver X receptor β and thyroid hormone receptor α in brain cortical layering. Proc Natl Acad Sci U S A 2010;107:12305–12310.

50. Stagnaro-Green A, Abalovich M, Alexander E, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid 2011;21:1081–1125.

51. Haddow JE. Maternal thyroxine and fetal brain development: the latest chapter, a look back, and considerations for the future. J Clin Endocrinol Metab 2013;98:1388–1390.

52. Brent GA. The debate over thyroid-function screening in pregnancy. N Engl J Med 2012;366:562–563.