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Maternal mild thyroid dysfunction and child behavioral and emotional difficulties at 4 and 6 years of age: The Rhea mother-child cohort study, Crete, Greece

Mariza Kampouri[a,*], Katerina Margetaki[a], Katerina Koutra[a], Andriani Kyriklaki[a], Polyxeni Karakosta[a], Katerina Sarri[a], Despoina Anousaki[a], Georgia Chalkiadaki[a], Marina Vafeiadi[a], Manolis Kogevinas[b,c,d], Leda Chatzi[e,f]

[a] Department of Social Medicine, Faculty of Medicine, University of Crete, Greece
[b] Instituto de Salud Global Barcelona, Spain
[c] Hospital del Mar Medical Research Institute (IMIM), 08003 Barcelona, Spain
[d] Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Spain
[e] Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, USA
[f] Department of Genetics & Cell Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, the Netherlands

1. Introduction

Thyroid hormones are essential for the development of the central nervous system, since they regulate neurodevelopmental processes such as proliferation, migration, synaptogenesis, and myelination (Howdeshell, 2002). The actions of thyroid hormones in the fetal central nervous system occur due to interactions of triiodothyronine with nuclear receptors, a process that triggers signaling cascades and regulates genetic expression of multiple genes involved in corticogenesis (Bernal, 2017). Since fetal thyroid hormones' secretion starts at the 10th gestational week and becomes sufficient between the 18th and 20th gestational week, the fetus depends, almost exclusively, on maternal thyroid hormones' supply until midgestation in order to meet the determinative hormone-dependent neurodevelopmental events of this period (Obregon et al., 2007).

MRI studies have supported that children of mothers with overt hypothyroidism during pregnancy have altered cortical morphology (Lischinsky et al., 2016), abnormal corpus callosum development (Samadi et al., 2015), and smaller hippocampus (Willoughby et al., 2014). Furthermore, animal studies have supported that even subclinically decreased thyroid hormones' concentration during early gestation alters histogenesis and the cytoarchitecture of the somatosensory cortex, the hippocampus, and the cerebellum (Lavado-Autric et al., 2003). While MRI-studies focused on clinical groups with behavioral problems have pinpointed structural and functional alterations in the aforementioned brain regions (Carmona et al., 2015; Duerden et al., 2012; Mous et al., 2014; Uytun et al., 2017; Vieira de Melo et al., 2018; Wang et al., 2018; Wu et al., 2017).

Moreover, a large body of observational studies has demonstrated that even subtle impairments of maternal thyroid function during pregnancy can impede child cognitive development (Finken et al., 2013; Ghassabian et al., 2014; Henrichs et al., 2010; Julvez et al., 2013; Korevaar et al., 2016a; Li et al., 2010; Pop et al., 2003; Pop et al., 1995; Pop et al., 1999; Williams et al., 2012), while at the same time evidence suggests that difficulties in specific cognitive domains underpin behavioral and emotional difficulties. For instance, increased externalizing symptoms have been associated with poor executive functioning (Schoemaker et al., 2013; Woltering et al., 2016), impaired general cognitive ability, and difficulties in learning and memory (Thompson et al., 2018). Conversely, increased internalizing symptoms have been linked with impairments in verbal fluency and memory (Blanken et al., 2017), problems in verbal processing (Toren et al., 2000), and poor executive functioning (Thompson et al., 2018). Even though there is considerable evidence supporting the aforementioned links, studies on the association between maternal thyroid hormones and child externalizing and internalizing problems are relatively scarce.

Findings of a previous population-based study have supported that maternal thyroid stimulating hormone (TSH) concentration levels are positively associated with child externalizing symptoms and that maternal thyroid autoimmunity increases the risk for offspring ADHD symptoms at 3 years of age (Ghassabian et al., 2011; Ghassabian et al., 2012). Results from the same study have detected an association of maternal hypothyroxinemia with child ADHD symptoms at 8 years and no association of maternal TSH with child behavioral development (Modesto et al., 2015). Other observational studies have shown that maternal hypothyroxinemia is associated with child externalizing and internalizing symptoms (Andersen et al., 2017), and child ADHD symptoms' manifestation at 5–6 years of age (Oostenbroek et al., 2017). In addition, low maternal thyroxine (fT4) concentration levels have been related with increased internalizing symptoms at 4 years of age

* Corresponding author at: Department of Social Medicine, Faculty of Medicine, University of Crete, Heraklion 71003, Crete, Greece.
E-mail address: m.kambouri@med.uoc.gr (M. Kampouri).

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(Endendijk et al., 2017).

The objective of this study was to evaluate the impact of maternal thyroid parameters during early pregnancy on child behavioral and emotional development in early childhood, in a population of an iodine sufficient country (Koutras et al., 2003), with high prevalence of iron deficiency (Karanikolaou et al., 1992; Krokidas et al., 1992; Tympa-Psirropoulou et al., 2008). Previous studies have identified various and different indicators of maternal mild thyroid dysfunction as predictors of child behavioral difficulties at different time-points of childhood. To our knowledge, this is the first study that provides a comprehensive behavioral and emotional development assessment in two different time-points of early childhood taking into account the maturational aspect of child development. The assessments were performed at pre-school age and at early elementary school age. These are critical periods due to advances in cognitive and language development that enable emotional and behavioral regulation and due to the increasing academic and social demands that often mark the onset of behavioral difficulties (Steinberg and Drabick, 2015).

We also aim to examine whether child sex moderates the association between maternal thyroid functioning and child behavioral and emotional development. It has been extensively reported that males have increased vulnerability to externalizing symptoms and females to internalizing symptoms (Martel, 2013). Moreover, it has been previously proposed that different prenatal hormonal influences may explain these sex-specific findings (Endendijk et al., 2017). Previous studies regarding the role of child sex on the association of maternal thyroid dysfunction and child behavioral and emotional problems are contradictory. More specifically, results from a previous study supported that maternal TSH is associated with child attention problems in females exclusively (Päkkilä et al., 2013), but other findings suggested that this association is evident in males exclusively (Endendijk et al., 2017).

We hypothesized that increased maternal TSH and decreased maternal fT4 are associated with increased behavioral and emotional symptoms in early childhood. We also hypothesized that maternal subclinical hypothyroidism (TSH above the normal, trimester specific reference interval but below 10 mIU/mL and fT4 within the normal range), maternal hypothyroxinemia (TSH within the normal trimester specific reference range and fT4 below the 5th percentile), and elevated maternal thyroid antibodies (TSH and fT4 within normal trimester specific reference ranges and elevated TPO-Antibodies or/and Tg-Antibodies) during early pregnancy are associated with increased child behavioral and emotional problems. In addition, based on the contradictory previous findings regarding the effect of child sex on the association of maternal thyroid dysfunction and child behavioral development, we aimed to examine whether there are sex differences in the aforementioned associations.

2. Materials and methods

2.1. Participants

The population of this study is derived from the “Rhea” study, a mother-child, birth cohort in Crete, Greece. “Rhea” study was established at 2007 and follows up children from fetal life, in order to explore nutritional, environmental, and psychosocial determinants of children's health, growth, and development. Between February 2007 and February 2008, pregnant women were recruited at the time of the first comprehensive ultrasound examination (12th gestational week approximately) from 4 antenatal clinics (2 public & 2 private) covering the wider Heraklion region. The inclusion criteria were being a resident of the area, older than 16 years, and able to communicate in the Greek language. We contacted two times with the participants during pregnancy (12th and 24th gestational week) to collect information on several psycho-social, dietary, and environmental exposures through face-to-face structured questionnaires and self-administered questionnaires. We obtained additional information from medical records. Parents were invited to participate at child follow up assessments, when the children were 9 months, 18 months, 4 years, and 6 years old. Details on participant recruitment and follow up procedures have been described in detail elsewhere (Chatzi et al., 2017).

The 4- and the 6-year follow-up assessments were completed at the University Hospital of Heraklion, or at health centers for the families residing in rural areas. The study was conducted according to the guidelines of the Declaration of Helsinki and all procedures were approved by the Ethical Committee of the University Hospital in Heraklion. Written informed consent was obtained from all the participants. Of 1363 live singleton births, data on maternal thyroid hormones during pregnancy were available in 1170 women. Information on child behavioral and emotional symptoms were available in 647 participants at 4 years and 489 at 6 years of age. Mothers receiving thyroid medication (N = 95) were included in the main analyses.

2.2. Maternal thyroid hormones' assessment

Maternal blood samples were collected at the first prenatal visit (mean gestational age 14.12 weeks, SD 3.6 weeks). Serum samples were collected in 10 mL vacutainer tubes, were centrifuged and stored in aliquots at −80 °C until assayed. Maternal thyroid functioning was assessed by quantitative analysis of serum thyroid stimulating hormone (TSH), free thyroxine (fT4), thyroid peroxidase antibodies (TPO-Ab), and thyroglobulin antibodies (Tg-Ab), by Immulite 2000 immunoassay system (Siemens Healthcare Diagnostics, Los Angeles, CA). The inter- and intra-assay variability were: TSH < 5.3 and < 6.4 (0.32–39 mIU/mL), fT4 < 7.8% and < 7.1% (0.51–4.82 ng/dL or 6.56–62.03 pmol/L), anti-Tg < 4.9% and < 5.8%, and anti-TPO < 7.4% and 7.2%.

Published, population-based, and trimester-specific reference intervals were used to assign participants into the categories of subclinical hypothyroidism and hypothyroxinemia (Karakosta et al., 2011). Subclinical hypothyroidism was defined as TSH above the normal, trimester specific reference interval but below 10 mIU/mL and fT4 within the normal range (Stagnaro-Green et al., 2002); the comparison group included women with TSH and fT4 concentration levels within the normal trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL & fT4: 0.95–1.53 ng/dL; 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4: 0.87–1.45 ng/dL). Hypothyroxinemia was defined as TSH within the normal trimester specific reference range and fT4 below the 5th percentile (corresponding to fT4 = 0.95 ng/dL); (Stagnaro-Green et al., 2002); the comparison group for hypothyroxinemia included women with TSH within the normal trimester specific reference range and fT4 above the 5th percentile. The 10th percentile of fT4 (corresponding to fT4 = 0.99 ng/dL) was used as an alternative cut-off point for the definition of hypothyroxinemia, in sensitivity analyses. Euthyroid women (TSH and fT4 within the normal population based and trimester-specific reference ranges) with elevated thyroid antibodies were compared to euthyroid women with low concentration levels of thyroid antibodies; thyroid-antibodies' status was considered elevated if thyroid peroxidase antibodies ≥ 35 IU/mL and/or thyroglobulin antibodies > 40 IU/mL.

2.3. Behavioral and emotional symptoms' assessment

Parents reported children's behavioral symptoms through validated and widely used questionnaires. The mean age of the participating children at the first behavioral assessment was 4.2 years and at the second 6.6 years. At the 4-year follow-up parents completed the Attention Deficit Hyperactivity Disorder Test (ADHDT) (Gilliam, 1995) and the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997). The ADHDT is based on ADHD criteria of DSM-IV. It is consisted of 36 items and constructed to identify and assess ADHD related symptoms in ages 3–23 years. The instrument provides 4 indexes, one for each of its three subscales (Hyperactivity, Inattention, and Impulsivity) and an index for total ADHD difficulties (possible range

0–72), for which higher scores indicate more and more severe ADHD symptoms. The ADHDT has been translated and adapted for the Greek population (Maniadaki and Kakouros, 2002). The SDQ is a brief screening questionnaire designed to assess behavioral strengths and difficulties in children from 3 to 16 years of age (Goodman, 1997). The questionnaire includes 25 items that are divided to five subcales: (i) Emotional symptoms, (ii) Conduct problems, (iii) Hyperactivity/Inattention, (iv) Peer-relationship problems, and (v) Prosocial behavior. The two broad band scales of Internalizing problems (emotional symptoms + peer-relationship problems) and Externalizing problems (conduct problems + hyperactivity/inattention) were also used in the current analyses. The SDQ was translated and adapted to the Greek population (Mpimpou-Nakou et al., 2001).

At the 6-year follow up assessment, parents completed the Child Behavior Checklist – Parent Report Form (CBCL) and the Conner's Parent Rating Scale, Revised, Short Form (CPRS-R: S) (Conners et al., 1998). The CBCL is a widely used parent-report questionnaire composed of 113 items and designed to assess behavioral and emotional difficulties in children between 6 and 18 years of age. The CBCL offers two alternative ways to summarize its items, the empirically-based syndrome scales and the DSM-oriented scales. The DSM-oriented scales, which were used in these analyses, include 6 scales that correspond to different diagnostic categories of the DSM-IV (American Psychiatric Association, 1994) (Affective problems, Anxiety problems, Somatic problems, Attention Deficit/Hyperactivity problems, Oppositional Defiant problems, and Conduct problems). The two broad-band scales of Internalizing problems & Externalizing problems were also used in these analyses. The CBCL is translated, adapted, and standardized for the Greek population (Roussos et al., 1999). The *CPRS-R: S* is designed to assess ADHD symptoms and is composed by 27 items, resulting in 3 subscales (Oppositional problems, Cognitive problems/Inattention, Hyperactivity) and an index for total ADHD symptoms (possible range 0–36), for which higher scores indicate more and more severe symptoms. The translation and cross-cultural adaptation of the CPRS-R: S was performed according to the recommended methodology, including the stages of forward translation by two independent translators and synthesis of one translation, back-translation by bilingual expert in psychology, review of the original and the translated version of the manuscript by expert-panel, and pre-testing to conclude to the final version of the questionnaire (Beaton et al., 2000).

2.4. Covariates

Potential covariates were selected on the basis of literature regarding the study's hypotheses (Ghassabian et al., 2012; Ghassabian et al., 2011; Modesto et al., 2015; Oostenbroek et al., 2017) and included maternal age, maternal educational level, maternal marital status at pregnancy, maternal origin, maternal smoking at pregnancy, maternal alcohol intake during pregnancy, maternal BMI, maternal IQ, parity, child's gestational age and weight at birth, child's sex, and child's age at the behavioral assessment. After the application of the change-in-estimate method (applied cut-off point: estimates' change > 10%) the following variables were retained as confounders: maternal age (continuous), maternal educational status (low/medium/high), parity (primiparous/multiparous), maternal smoking during pregnancy (yes/no), and maternal marital status at pregnancy (single, married). Breastfeeding duration (continuous) and birthweight (continuous) were also included in the models, since participants and non-participants significantly differed in these covariates (non-response analyses). This information was collected through questionnaires administered by interviewers at pregnancy and through medical records. The models were also adjusted for child's sex and child's age at the behavioral assessment (a priori selection).

2.5. Statistical analysis

Descriptive analyses on the characteristics of the study population, and the distribution of the exposures and the outcomes of interest were conducted. Multiple imputations were conducted to handle missing data in the ADHDT, SDQ, and CBCL questionnaires. Due to high item non-response, the percentage of missing values in the ADHDT, SDQ, and CBCL scales reached 24%. Thus, 20 complete data sets were generated using multiple imputations with chained equations (MICE) (White et al., 2011). In the imputation model, all the questionnaire items (raw data) were regressed on all the other items (Shrive et al., 2006). For the imputation of ADHDT and SDQ items, ordinal regression models were applied. This method was not feasible for the imputation of CBCL due to empty cells. Thus, Predictive Mean Matching (PMM) was applied. Although PMM is widely used for continuous variables, it has been shown that PMM can yield plausible inference for ordered categorical data as well (Vink et al., 2015). After obtaining the full datasets, all sub-scales were calculated separately for each imputed data set. No such problem occurred with the CPRS-R: S and with covariate information, where missing data did not exceed 2.1%. Estimations of the imputed data sets were combined using Rubin's rules (Rubin, 2004). To explore potential differences between imputed and observed values, complete-case analysis was conducted. No meaningful change of the estimates was observed, thus we present effect estimates based on the multiply imputed data.

Generalized additive models (GAMs) were applied to test for the linearity of the association between maternal thyroid hormones and ADHDT, SDQ, CPRS-R: S, and CBCL scores. Linearity was assumed if p-gain, defined as the difference in normalized deviance between the GAM model and the linear model for the same exposure and outcome (Royston and Ambler, 1998) was > 0.1. Since there was evidence for non-linearity between maternal thyroid data and the outcome (Supplementary Figs. 1–4), maternal thyroid data were categorized into tertiles (low/medium/high). Maternal subclinical hypothyroidism, maternal hypothyroxinemia, and maternal euthyroidism accompanied with elevated thyroid antibodies (TPO-antibodies and/or Tg-antibodies) were also examined as possible predictors of child behavioral and emotional symptoms at 4 and 6 years of age. In order to examine the role of thyroid-antibodies' presence in subclinical hypothyroidism, stratified analyses by maternal thyroid-antibodies' status were also conducted. Multivariate linear regression models were used to estimate crude and adjusted beta coefficients and the corresponding 95% confidence intervals (95% CI's) for the exposure-outcome associations.

In order to assess the potential modifying effect of child sex, additional analyses were conducted. We included interaction terms in the respective regression models (p for interaction < 0.05). Sensitivity analyses were conducted excluding mothers who were under thyroid medication during pregnancy. Furthermore, the analyses were repeated to a subsample with available child thyroid hormones' assessment at 4 years (TSH), to adjust for any potential effect of child thyroid functioning on the identified associations. Sensitivity analyses with additional adjustment for maternal iodine and iron status was also conducted in a sub-sample with available information on these covariates. The multivariate models regarding maternal hypothyroxinemia were repeated using an alternative cut-off point for maternal fT4 (fT4 < 10th percentile).

The statistical analyses were conducted using STATA 13.1 (Statacorp, College Station, TX) and the threshold for statistical significance was set at the 5% level.

2.6. Non-response analysis

Non-response analyses showed no differences between participants and non-participants in terms of maternal thyroid functioning (TSH, fT4), smoking status during pregnancy, marital status, and in gestational age at birth. However non-participant mothers were younger

Table 1
Maternal and child characteristics of the study population at 4 and 6 years of age, Rhea mother-child study, Crete, Greece.

	Population at 4 years (N = 647)		Population at 6 years (N = 489)	
	N	Data[a]	N	Data[a]
Maternal characteristics				
Maternal age at child birth	646	29.89 (5.0)	488	30.15 (4.7)
Maternal education				
Low	105	16.4	69	14.2
Medium	329	51.3	247	50.9
High	208	32.4	169	34.9
Maternal origin				
Greek	612	95	460	95.8
Non-Greek	33	5.1	20	4.2
Parity				
Primiparous	288	44.5	218	44.9
Multiparous	359	55.5	268	55.1
Maternal smoking (early pregnancy)				
Yes	217	34.2	166	34.7
No	418	65.8	313	65.3
Maternal iodine at pregnancy (median) (μg/L)	447	168.4	366	168.4
Maternal iron at pregnancy (μg/dl)	443	72.5 (37.6)	356	72.0 (38.3)
Maternal TSH (μIU/mL)	647	1.34 (1.4)	489	1.32 (1.5)
Maternal ft4 (ng/dL)	646	1.22 (0.2)	489	1.23 (0.2)
Maternal subclinical hypothyroidism (SCH)[b]	41	7.1	30	6.9
Maternal hypothyroxinemia[c]	25	4.6	23	5.6
Maternal TPO-Abs and/or Tg-Abs +[d]	82	15.5	62	15.7
Maternal thyroid medication				
No medication	552	85.3	415	84.9
Thyroxine	90	13.9	69	14.1
Anti-thyroid medication	4	0.6	4	0.8
Yes, no defined	1	0.2	1	0.2
Child characteristics				
Child's sex (female)	309	47.8	218	44.6
Child age at behavioral assessment	647	4.2 (0.2)	489	6.6 (0.3)
Gestational age at birth (weeks)	639	38.2 (1.6)	483	38.1 (1.6)
Birth weight (grams)	634	3211 (446.2)	477	3193 (452.4)
Breastfeeding duration (months)	615	4.1 (4.3)	470	4.0 (4.1)
ADHDT – total score[e]	529	14.21 (12.35)	–	–
SDQ – total score[f]	572	8.7 (4.8)	–	–
CPRS-R: S – total score[g]	–	–	471	8.9 (5.7)
CBCL – internalizing problems score[h]	–	–	423	6.2 (4.5)
CBCL – externalizing problems score[h]	–	–	462	8.7 (6.6)

[a] Data presented as mean (standard deviation) for continuous variables (unless mentioned otherwise) and as frequency (%) on each category for categorical variables.
[b] fT4 concentration levels within the population-based, trimester-specific reference ranges (1st trimester: fT4: 0.95–1.53 ng/dL; 2nd trimester & fT4: 0.87–1.45 ng/dL) and TSH above the upper TSH trimester-specific limit (TSH > 2.53 μIU/mL & TSH > 2.73 μIU/mL for the 1st and the 2nd trimester respectively) and below 10 μIU/mL.
[c] TSH concentration levels within the trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4 < 5th percentile (fT4 < 0.95 ng/dL)).
[d] TSH and fT4 concentration levels within the population-based, trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL & fT4: 0.95–1.53 ng/dL 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4: 0.87–1.45 ng/dL) & TPO-Abs ≥ 35 IU/mL &/or Tg-Abs > 40 IU/mL.
[e] Attention Deficit Hyperactivity Disorder Test
[f] Strengths and Difficulties Questionnaire.
[g] Conners' Parent Rating Scale-Revised: Short form.
[h] Child Behavior Checklist, Parent report form.

[4 years assessment: mean difference = 1.1 years; 95% CI (0.53, 1.69) p < .001, 6 years assessment: mean difference = 1.2 years; 95% CI (0.70, 1.88) p < .001] and less educated [4 years assessment: low educational level 28% versus 16%, $x^2 = 25.48(2)$, p < .000, 6 years assessment: low educational level 27% versus 14%, $x^2 = 31.74(2)$, p < .000] while their children had lower birth weight in comparison with participants [4 years assessment: mean difference = −58.63; 95% CI (−4.92, −112.33) p = .032] and were breastfed for less time [4 years assessment: mean difference = −1.1 months; 95% CI (−0.62, −1.62) p < .001, 6 years assessment: mean difference: −0.5 months; 95% CI (−0.03, −1.03), p = .034].

3. Results

Table 1 presents participants' characteristics at 4 and 6 years of age, respectively. The median urine iodine was 168.4 μg/L, which indicates adequate iodine intake (WHO, 2001; Zimmermann and Andersson, 2012) and the mean serum iron was 72 μg/dl, which indicates marginally normal iron status (Abbassi-Ghanavati et al., 2009). No associations were found between maternal TSH and fT4 concentration levels and child behavioral and emotional difficulties at 4 and 6 years of age (Supplementary Table 1).

3.1. Subclinical hypothyroidism

Children exposed to maternal subclinical hypothyroidism during pregnancy had higher hyperactivity, impulsivity, and internalizing problems at 4 years of age, compared to children of euthyroid mothers (Table 2). Post-hoc analyses showed that increased internalizing scores were primarily driven by emotional problems: SDQ-Emotional problems: adjusted-β coefficient 0.6, 95%CI [0.0, 1.1]. In addition, children exposed to maternal subclinical hypothyroidism had higher oppositional-defiant symptoms and externalizing scores at 6 years of age, compared to children of euthyroid mothers (Table 3). Post-hoc analyses showed that increased externalizing scores were primarily driven by oppositional-defiant and conduct problems: CBCL-Oppositional-Defiant problems: adjusted-β coefficient 1.3, 95%CI [0.6, 2.1] CBCL-Conduct problems: adjusted-β coefficient 1.6, 95%CI [0.6, 2.6].

3.2. Subclinical hypothyroidism & thyroid autoimmunity

Children of subclinically hypothyroid mothers with elevated thyroid antibodies had higher hyperactivity, inattention, impulsivity, externalizing, and internalizing symptoms at 4 years, compared to children of euthyroid mothers with low concentration levels of thyroid antibodies (Table 2). Post-hoc analyses showed that increased internalizing scores were primarily driven by emotional problems: SDQ-Emotional problems: adjusted-β coefficient 1.3, 95%CI [0.4, 2.1] and increased externalizing scores by conduct problems: SDQ-Conduct problems: adjusted-β coefficient 1.1, 95%CI [0.2, 1.9]. In addition, children of subclinically hypothyroid mothers with elevated thyroid antibodies had higher hyperactivity, oppositional, internalizing, and externalizing problems at 6 years, compared to children of euthyroid mothers with low concentration levels of thyroid antibodies (Table 3). Post-hoc analyses indicated that increased externalizing symptoms were primarily the result of hyperactivity/inattention, oppositional-defiant, and conduct problems: CBCL-Hyperactivity/Inattention: adjusted-β coefficient 1.8, 95%CI [0.3, 3.3], CBCL-Oppositional-Defiant: adjusted-β coefficient 2.0, 95%CI [0.9, 3.2], CBCL Conduct: adjusted-β coefficient 2.4, 95%CI [0.8, 4.0] and increased internalizing scores the result of somatic symptoms: CBCL-Somatic Symptoms: adjusted-β coefficient 2.0, 95%CI [0.9, 3.2]. Children of subclinically hypothyroid mothers with low concentration of thyroid antibodies had higher externalizing scores at 6 years, compared to children of euthyroid mothers (Table 3). Post-hoc analyses showed that increased externalizing problems score was primarily driven by oppositional problems: CBCL-

Table 2
Maternal subclinical hypothyroidism, maternal autoimmunity, and maternal hypothyroxinemia during gestation and children's behavioral symptoms at 4 years of age [*Attention Deficit Hyperactivity Disorder Test* (*ADHDT*) and *Strengths and Difficulties Questionnaire* (*SDQ*)]

	Subclinical hypothyroidism (SCH)[a,c] (N = 41)		SCH & thyroid antibodies + status[a,c] (N = 15)		SCH & thyroid antibodies − status[a,c] (N = 26)		Euthyroidism & Thyroid antibodies + status[a,d] (N = 82)		Hypothyroxinemia (N = 25)[a,e]	
	β	95% CI	β	95% CI	β	95% CI	β	95%CI	β	95% CI
Attention Deficit Hyperactivity Disorder Test										
Hyperactivity	**2.4**	**(0.7, 4.1)**[b]	**4.6**	**(1.8, 7.4)**[b]	0.9	(−1.2, 3.1)	−0.1	(−1.4, 1.1)	0.7	(−1.4, 2.8)
Inattention	1.0	(−0.5, 2.4)	**3.1**	**(0.7, 5.4)**[b]	0.0	(−1.7,1.8)	**1.1**	**(0.0, 2.2)**[b]	1.0	(−0.8, 2.8)
Impulsivity	**1.5**	**(0.1, 2.8)**[b]	**2.9**	**(0.7, 5.1)**[b]	0.6	(−1.1, 2.3)	0.4	(−0.6, 1.4)	1.7	(−0.0, 3.3)
ADHDT - total	**4.8**	**(0.8, 8.8)**[b]	**10.6**	**(4.2, 17.1)**[b]	1.6	(−3.3, 6.5)	1.4	(−1.5, 4.3)	3.4	(−1.5, 8.2)
Strengths and Difficulties Questionnaire										
Internalizing score	**0.9**	**(0.1, 1.7)**[b]	**1.5**	**(0.3, 2.8)**[b]	0.5	(−0.4, 1.5)	0.3	(−0.3, 0.9)	−0.0	(−1.0, 0.9)
Externalizing score	0.8	(−0.2, 1.8)	**2.2**	**(0.5, 3.8)**[b]	0.1	(−1.2, 1.4)	0.4	(−0.4, 1.1)	0.5	(−0.8, 1.8)

[a] Adjusted for child age at the behavioral assessment, child sex, birthweight, breastfeeding duration, maternal age, maternal education, parity, marital status during pregnancy and maternal smoking status during pregnancy.
[b] $p < .05$.
[c] Reference group: TSH and fT4 concentration levels within the population-based, trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL & fT4: 0.95–1.53 ng/dL; 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4: 0.87–1.45 ng/dL).
[d] Reference group: TSH and fT4 concentration levels within the population-based, trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL & fT4: 0.95–1.53 ng/dL 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4: 0.87–1.45 ng/dL) & TPO-Abs < 35 IU/mL & Tg ≤ 40 IU/mL.
[e] Reference group: TSH concentration levels within the trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4 > 5th percentile (fT4 ≥ 0.95 ng/dL)).

Oppositional-Defiant: adjusted-β coefficient 1.0, 95%CI [0.1, 1.9]. Subclinically hypothyroid women with elevated thyroid antibodies had higher TSH concentration levels (M = 4.23, SD = ± 1.60) compared to subclinically hypothyroid mothers with low thyroid antibodies concentration levels [(M = 3.44, SD = ± 0.62): t (39) = −2.23, $p < .032$].

3.3. Thyroid autoimmunity

Children of euthyroid mothers with elevated thyroid antibodies had higher inattention scores at 4 years and externalizing scores at 6 years, compared to children of euthyroid mothers with low concentration of thyroid antibodies (Tables 2 & 3). Post-hoc analyses showed that increased externalizing scores were primarily driven by hyperactivity/inattention symptoms: CBCL-Hyperactivity/Inattention score: adjusted-β coefficient 0.8, 95%CI [0.1, 1.5].

3.4. Hypothyroxinemia

No associations of maternal hypothyroxinemia with child behavioral and emotional symptoms at 4 and 6 years of age were observed (Tables 2 & 3); the same results were obtained applying an alternative

Table 3
Maternal subclinical hypothyroidism, maternal autoimmunity, and maternal hypothyroxinemia during gestation and children's behavioral symptoms at 6 years of age [*Conners' Parent Rating Scale-Revised: Short form* (*CPRS-R:S*) and *Child Behavior Checklist* (*CBCL*), *Parent Report Form*].

	Subclinical hypothyroidism (SCH)[a,c] (N = 30)		SCH & thyroid antibodies + status[a,c] (N = 11)		SCH & thyroid antibodies − status[a,c] (N = 19)		Euthyroidism & thyroid antibodies + status[a,d] (N = 62)		Hypothyroxinemia[a,e] (N = 23)	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Conners' Parent Rating Scale-Revised: Short form scales										
Oppositional	**1.2**	**(0.0, 2.5)**[b]	**3.1**	**(1.2, 4.9)**[b]	0.2	(−1.3, 1.7)	0.8	(−0.1, 1.7)	−0.8	(−2.2, 0.6)
Inattention	0.8	(−0.5, 2.0)	1.9	(−0.1, 3.9)	0.6	(−1.6, 2.9)	0.6	(−0.3, 1.6)	−0.8	(−2.2, 0.6)
Hyperactivity	0.4	(−0.7, 1.6)	**1.8**	**(0.0, 3.7)**[b]	−0.5	(−2.0, 0.9)	0.2	(−0.7, 1.0)	0.6	(−0.8, 1.9)
CPRS-R:S: total	0.5	(−1.7, 2.6)	2.7	(−0.7, 6.0)	−0.8	(−3.4, 1.7)	0.6	(−0.9, 2.1)	−0.2	(−2.6, 2.1)
Child Behavior Checklist - Parent report form										
Internalizing score	1.6	(−0.1, 3.2)	**2.8**	**(0.1, 5.4)**[b]	1.0	(−1.1, 3.1)	1.2	(−0.1, 2.4)	0.9	(−1.1, 2.8)
Externalizing score	**4.6**	**(2.2, 6.9)**[b]	**6.5**	**(2.9, 10.2)**[b]	**3.5**	**(0.7, 6.4)**[b]	**1.8**	**(0.1, 3.5)**[b]	−0.9	(−3.4, 1.7)

[a] Adjusted for child age at the behavioral assessment, child sex, birthweight, breastfeeding duration, maternal age, maternal education, parity, marital status during pregnancy and maternal smoking status during pregnancy.
[b] $p < .05$.
[c] Reference group: TSH and fT4 concentration levels within the population-based, trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL & fT4: 0.95–1.53 ng/dL; 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4: 0.87–1.45 ng/dL).
[d] Reference group: TSH and fT4 concentration levels within the population-based, trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL & fT4: 0.95–1.53 ng/dL 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4: 0.87–1.45 ng/dL) & TPO-Abs < 35 IU/mL & Tg ≤ 40 IU/mL.
[e] Reference group: TSH concentration levels within the trimester-specific reference ranges (1st trimester: TSH: 0.05–2.53 μIU/mL 2nd trimester: TSH: 0.18–2.73 μIU/mL & fT4 > 5th percentile (fT4 ≥ 0.95 ng/dL)).

fT4 cut-off point (10th percentile) for the definition of hypothyroxinemia (data not presented).

3.5. Additional analyses

No interaction effect of child sex was identified. The exclusion of the mothers who took thyroid medication during pregnancy did not cause any meaningful change of the results (Supplementary Table 2). Sensitivity analyses were conducted to a subsample with available child thyroid data (assessed at 4 years of age), including an additional adjustment for child TSH in the models; the results further supported the associations of maternal subclinical hypothyroidism during pregnancy and child behavioral and emotional difficulties at 6 years of age (Supplementary Table 3). The analyses were also repeated on a subsample with available data on maternal iron and iodine status at the 13th gestational week; the additional adjustments for maternal iron and iodine status further strengthened the identified associations (Supplementary Table 4 & Supplementary Table 5).

4. Discussion

In this prospective, population-based, mother-child cohort study maternal subclinical hypothyroidism during early pregnancy predicted increased hyperactivity, impulsivity, and emotional difficulties in children at 4 years and increased oppositional-defiant and conduct symptoms at 6 years of age. Maternal thyroid autoimmunity was associated with increased inattention at 4 years and increased hyperactivity & inattention at 6 years of age. We did not identify any association between maternal hypothyroxinemia and child behavioral and emotional symptoms at 4 or at 6 years of age. We did not find any child sex effect in the association between maternal thyroid functioning and child behavioral and emotional development at 4 and 6 years of age.

Findings that indirectly support the role of maternal thyroid dysfunction in child behavioral problems acknowledged a link between generalized resistance to thyroid hormones and more ADHD symptoms in adults (Hauser et al., 1993) as well as a greater risk for ADHD symptoms' manifestation for children exposed to mild iodine deficiency (Vermiglio et al., 2004). More directly related results have supported that maternal mild thyroid dysfunction is associated with child behavior problems, with various indicators as predictors of child behavioral development (Andersen et al., 2017; Endendijk et al., 2017; Ghassabian et al., 2012; Ghassabian et al., 2011; Modesto et al., 2015; Oostenbroek et al., 2017; Päkkilä et al., 2013)

Several previous studies regarding maternal thyroid function and child behavioral development have supported that maternal TSH is a valid predictor of child behavioral problems (Endendijk et al., 2017; Ghassabian et al., 2011; Päkkilä et al., 2013). Our findings suggest that subclinical hypothyroidism predicts child behavioral and emotional problems, which indirectly supports that TSH is a sensitive indicator of maternal thyroid dysfunction. It has been previously suggested that although TSH is not the biologically active hormone in the fetal brain, it might be a reliable indicator of thyroid dysfunction because of the pituitary feedback mechanism and its regulatory role for thyroid hormones secretion (Ghassabian et al., 2011). In addition, the detected associations were stronger and evident in more scales in children of subclinically hypothyroid mothers with elevated thyroid antibodies. Although these findings might be attributed to the additional increase of TSH concentration, which was evident in the present results, an additional direct impact of thyroid antibodies cannot be excluded, since there is evidence that TPO-Abs positivity impairs thyroid stimulation by hCG (Korevaar et al., 2016b)

Maternal thyroid autoimmunity (elevated TPO-antibodies) has been previously related with decreased motor and intellectual development (Li et al., 2010; Pop et al., 1995), greater autism risk (Brown et al., 2015), and more attention deficit/hyperactivity problems at 3 years of age (Ghassabian et al., 2012). The present findings support an association of elevated thyroid antibodies in euthyroid women with child inattention problems at 4 years and hyperactivity/inattention at 6 years. These associations might be attributed to further alterations of thyroid hormones' levels between the two groups in later pregnancy, which were small but already evident in the present results. Additional possible explanations involve an adverse effect of maternal thyroid antibodies on fetal thyroid functioning that may result in transient fetal hypothyroidism (Dussault and Fisher, 1999) and an impact of a pre-existing maternal subclinical autoimmune condition, which is indicated by elevated thyroid antibodies, on child behavioral problems development (Ghassabian et al., 2012).

Interestingly, the observed associations of this study involve both externalizing and internalizing symptoms. Deficits in cognitive control, behavioral inhibition, and emotional regulation are common in clinical disorders that are relevant with the detected symptoms (Amstadter, 2008; Arnsten and Rubia, 2012; Barkley, 2011; Mullin and Hinshaw, 2007; Steinberg and Drabick, 2015; Suveg and Zeman, 2004). These cognitive abilities implicate the prefrontal cortex, the hippocampus, and the cerebellum (Arnsten and Rubia, 2012; Miller and Cohen, 2001; Phillips et al., 2008; Schutter and van Honk, 2009); neural regions that are morphologically affected by insufficient levels of thyroid hormones during gestation (Lavado-Autric et al., 2003; Lischinsky et al., 2016; Willoughby et al., 2014). Moreover, insufficiency of thyroid hormones may result in long-lasting neurophysiological changes in the dopaminergic and the noradrenergic systems, which are implicated in emotional and behavioral problems manifestation (Anand and Charney, 2000; Dunlop and Nemeroff, 2007; Moog et al., 2017; Solanto, 2002).

The null findings regarding the association between maternal hypothyroxinemia and child behavioral development are consistent with several previous studies (Ghassabian et al., 2011; Päkkilä et al., 2013) but don't replicate the findings of others (Andersen et al., 2017; Modesto et al., 2015; Oostenbroek et al., 2017). Although it has been previously suggested that the thyroid stimulating effect of hCG during the first trimester of pregnancy makes maternal TSH the most sensitive predictor of child behavioral development (Endendijk et al., 2017), the evidence provided by this study is not strong enough to support this assumption, since our null findings might be the result of the small number of mothers with hypothyroxinemia in our population.

We did not observe any sex specific effect on the association of maternal TSH and child behavioral development, supporting the findings of one previous study (Ghassabian et al., 2011) but not confirming sex specific associations that have been previously detected by others (Endendijk et al., 2017; Päkkilä et al., 2013). However, there are several methodological differences between the aforementioned studies and the current one that may cause the difference in the results (e.g. different age of the participants, outcome in clinical categories/outcome continuous, trajectories of thyroid hormones/single time-point measurement).

The strengths of the present study include the population-based, prospective design of the study and the opportunity to control for the potential confounding effect of several maternal and child factors. Maternal thyroid antibodies were assessed as an important cause of thyroid dysfunction in iodine sufficient populations. A possible limitation of this study is that thyroid hormones were measured at a single early point during pregnancy; as a result these measurements might reflect a transient thyroid dysfunction. However, there is evidence that longitudinal and single-point thyroid assessments are highly correlated (Ekinci et al., 2013). Although the selection of reliable and valid questionnaires to assess child behavioral and emotional development is considered another strength of this study, it also consists of a limitation of the findings, since questionnaires cannot replace direct developmental assessment and clinical diagnosis of any specific disorder. Moreover, we cannot interpret differences in scores between the two time-points as developmental changes, since differences between the questionnaires may be the cause of the observed variations. Imputation of the missing values of ADHDT, SDQ, and CBCL questionnaires were

applied in order to avoid any bias due to selective response to specific items of the questionnaires. Participants and non-participants did not differ in relevance with the exposure status but they differed in other socio-demographic characteristics. Therefore, bias due to non-participation and loss to follow up cannot be excluded. In addition, we had no available information on offspring congenital hypothyroidism, and although sensitivity analyses with adjustment for child thyroid functioning at 4 years did not significantly affect the identified associations, we have to consider this as a possible limitation of the present results. Furthermore, residual confounding cannot be excluded, although multiple confounders were included in the analyses and additional sensitivity analyses were conducted to control for the potential confounding effect.

5. Conclusions

The results support the conclusion that maternal subclinical hypothyroidism in early pregnancy is associated with externalizing and internalizing problems in early childhood and that maternal thyroid autoimmunity further strengthens the aforementioned associations. Additionally, it is supported that elevated thyroid antibodies in euthyroid pregnant women are associated with adverse behavioral outcomes. However, no association between maternal hypothyroxinemia and child behavioral development was observed. Current findings extend previous evidence on the impact of maternal non-clinical thyroid-hormone dysfunction on later child neuropsychological and suggest that subclinical hypothyroidism and thyroid antibodies concentration levels need to be considered as predictors of child behavioral and emotional development. Future studies combining neuropsychological assessment in multiple domains and neuroimaging techniques are necessary to explore the role of thyroid mild dysfunction in child development, to pinpoint the exact mechanisms underlying such associations, and explore whether the effect of maternal thyroid dysfunction is area specific within the brain.

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Declaration of competing interest

The authors have no potential conflicts of interest to disclose.

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Appendix A. Supplementary data

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