Neurobiological Pathways to Childhood Psychopathology

Population-based studies of cognition and behavior

Akhgar Ghassabian

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NEUROBIOLOGICAL PATHWAYS TO CHILDHOOD PSYCHOPATHOLOGY

Population-based studies of cognition and behavior

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psychopathologie op de kinderleeftijd

Onderzoek naar gedrag en cognitie in de algemene bevolking

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To Mom and Dad who taught me to think

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Chapter 2.4

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Chapter 1

Introduction

INTRODUCTION

In the past few decades, considerable advances have been made in understanding childhood psychopathology. This progress is the result of four primary developments in the field. First, both in the research and in the clinical framework, psychopathology has been conceptualized across a spectrum of severity of symptoms and impairment.[1] Second, psychopathology has been studied in the context of young children's real life parallel to referral settings.[2] Third, studying child psychopathology in large-scale prospective epidemiological studies offers new insight into the etiology of child psychiatric disorders.[3] And fourth, enormous progress has been made in understanding the nature of psychopathology and its biological underpinnings.[4]

Dimensions in behavior assessment

The advantage of combining dimensions and categories to assess psychopathology has been long recognized.[5] Dimensions assume that behavior appears across a continuum and that psychopathology is a deviation from normal development of behavior at an extreme end of the continuum. It is well accepted that premature application of behavioral scores and profiles in clinical diagnostic settings should be avoided. Nevertheless, empirical data indicate that most risk factors for mental disorders operate dimensionally.[6] Thus, in this thesis we will use dimensions in the study of the biological bases of psychopathology, in particular because their application provides additional information and increased power.

Behavior in naturalistic settings

Over the years, a variety of behavior checklists or brief observation scales have been developed to assist in the assessment of behavior in children with developmental psychopathology in their naturalistic settings.[7-8] Although behavior in very deviant children may be less affected by situation, assessment of behavioral and emotional problems in most children must take account of variance in the situations on which assessment depends. To assess children's behavioral and emotional problems in different situations, ratings from different informants (parent, teacher, and the child) should be used.[9] All studies presented in this thesis are population-based and focus on child behavior as assessed in a natural setting. We used, in particular, parent ratings of children's behavior.

Prospective assessment of population-based samples

For a long time, the potential advantages of epidemiological studies to clinical-based investigations of childhood psychiatric disorders were debated. Although valuable, clinical-based studies face major limitations. First, they may not be unrepresentative of the population, as referred cases can differ systematically from those not referred.[10] Second, clinical-based

studies depend largely on retrospective recalls from children with psychiatrics disorders that restrict them in pinpointing the onset of the cascade of neurodevelopmental abnormalities.

A major development in psychiatric epidemiology has been the use of the longitudinal design. Population-based prospective studies address the question of why some children do not develop psychopathology over time, while others do. Furthermore, the longitudinal component enables the investigator to assess a child's behavior repeatedly and establish within-individual differences over time. In the present thesis we discriminated children who transiently show deviations from normal development from those who persist in problem behavior by the repeated assessment of behavior.

Neurobiological risk factors

Large-scale epidemiological studies have produced a wealth of useful information on the determination of environmental risk factors as well as early predictors of prospective psychopathology.

In the prenatal period, many factors are involved in the normal development of the fetal brain. Among them, thyroid hormones are known to be an essential regulator of early brain development.[11] Severe thyroid hormone deficiency causes irreversible neural damages and contributes to intellectual disabilities in the child.[12] Animal models provide evidence for the specific abnormalities in brain cytoarchitecture as a response to thyroid hormone insufficiency, i.e. disrupted synaptogenesis, decreased myelination and abnormal cell migrations.[13-14] During early fetal life, the fetus relies entirely on maternal thyroid hormones. In pregnant women with normal thyrotropin levels, low thyroxine concentration is associated with abnormal cognitive functioning in children.[15] However, the behavioral consequences of prenatal exposure to mild maternal thyroid insufficiency have been scarcely studied. Furthermore, autoimmunity and iodine deficiency are the two major causes of thyroid dysfunction in women of reproductive age. Before randomized trails can address the question of the potential benefit of intervention, observational studies are needed to establish a relation between the two underlying factors of thyroid dysfunction and maternal/child outcomes.

Behavioral assessment of young children, e.g. younger than 3 years, is characterized by difficulties in defining the criteria for deviance and a lack of differentiated concept of disorders for these ages.[16] The term 'disorder' is often used with caution in these children due to lack of strong evidence for stability and prognostic significance of preschool problems.[17] During the period of rapid development, there are concerns about distinguishing normal from abnormal behavior, and about labeling very young children with disorders. In young children, behavioral outcome can be better assessed within the two broad-bands of internalizing and externalizing problems, rather than specific psychopathology.[17] Therefore, we first explored whether maternal thyroid function, within the normal range, is related to any behavioral problems in children up to age 3 years (including internalizing and externalizing problems). Based on the previous report from iodine deficient areas,[18] we expected to observe an effect

of maternal thyroid deficiency on children's risk of externalizing problems. As the children become older and enter school age, specific psychopathology is feasible to study in relation to various exposures. Concerning the histological similarities between the neuropathology of autism,[19] and the brain lesions of experimental animal models with maternal mild thyroid hormone deficiency,[13-14, 20] we hypothesized that maternal thyroid deficiency in early gestation could be an important risk factor of autistic symptoms by disrupting critical stages of neuronal migration in brain and cerebellum.

Developmental psychopathologies are disorders of the brain.[21] An extensive part of our information about brain abnormalities in psychopathology comes from studies of children with clinical disorders. However, recent studies show that developmental trajectories rather than absolute change in the brain structures underlie childhood psychopathology such as Attention Deficit/Hyperactivity Disorders (ADHD).[22-23] In postnatal period, the brain has a great plasticity and also undergoes numerous developmental processes, including synaptic pruning and myelination.[24] Assessment of postnatal brain structural differences, preceding psychopathology, can provide valuable information about developmental processes before psychopathology emerges. This goal is only achieved in studies that combine brain imaging with a population-based design that follows children from early childhood through adolescence. In this thesis, we examined the brain morphological abnormalities preceding ADHD, a common childhood disorder with predominant executive dysfunction.

At preschool age temperamental traits, defined as biologically based individual differences in affection and self-regulation, are in close interconnection with psychopathology.[25] While some researchers propose that certain temperamental traits may reflect a vulnerability to psychopathology, others suggest that psychopathology may be the extreme presentation of a temperamental trait that exists across a continuum.[26] Longitudinal studies of temperament and psychopathology, along with exploring possible mechanisms underlying their intimate relation, would contribute to a better understanding of both. Despite extensive work on the relation between temperamental traits and mood disorders,[27-28] mechanistic studies that attempt to explore the nature of this relation in early childhood are largely lacking. In this thesis, we explored the possible pathways through which children's temperamental traits increase the risk for later internalizing problems.

Executive function is described as a group of "top-down" cognitive processes that are responsible for flexible, goal-directed behavior.[25] Neuropsychological differences in the various domains of executive function are found in children with developmental psychopathologies, such as autism spectrum disorders and ADHD.[29-30] However, it is debated whether executive function is a single unitary process or a diverse array of higher order cognitive processes. Also, it remains unclear if domains of executive function contribute differently to predisposing individuals to psychopathology.

In this thesis we present studies of individual biological differences in a prospective cohort of young children without psychopathology at baseline to elucidate the cause and nature of deviations from normal development.

Aim

The aim of this thesis was to addresses risk factors and predictors of major developmental psychopathologies in childhood. Specifically, three aims were defined: 1) to investigate the intrauterine adverse effect of maternal thyroid function on cognition and behavior during childhood, 2) to examine the early predictors of child problem behavior 3) to explore the longitudinal course of childhood behavior and cognition from preschool age through age 6 years.

Setting

The studies in this thesis were embedded within the Generation R Study, a population-based birth cohort, in Rotterdam the Netherlands.[31-32] This prospective cohort with tracking of children from fetal life onwards provides the unique opportunity to examine intrauterine adverse effects, as well as postnatal and early-life predictors of developmental psychopathology. Furthermore, the longitudinal design of the study allowed us to explore the developmental course of cognition and behavior up to early school age.

Briefly, all pregnant women living in Rotterdam with an expected delivery date between April 2002 and January 2006 were invited to participate. In total, 9778 pregnant women participated in the study, including 8879 women with enrollment in pregnancy and 899 at birth of the child. During the two postnatal phases of the study (0-4 and 5 years), information was obtained in 7893 and 8305 children, respectively. Detailed assessments were performed in a subgroup of children with a Dutch national origin, defined as having two parents and four grandparents born in the Netherlands. The measurements in this subgroup included the fetal and postnatal growth and development up to age 4 years.

Outline

In chapter 2, studies on the intrauterine adverse effects of thyroid hormone insufficiency on behavior and cognition in childhood are described. Maternal thyroid function, i.e. thyroid hormone status, thyroid autoimmunity, and iodine levels, are investigated because of their crucial role in normal brain development during early fetal life.

Chapter 3 presents the results of the studies on risk factors for psychopathology. These potential risk factors are postnatal brain morphology, preschool age temperament and executive functioning in the children.

Chapter 4 summarizes the longitudinal course and stability of two major mental health problems in childhood; autistic symptoms and language impairment.

Finally, in chapter 5, we summarize the main findings, address the methodological aspects of the study, and provide a general discussion. The public health and clinical implications of the findings are presented, along with the challenges for future studies.

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Chapter 2

Intrauterine influences: Maternal thyroid function

Chapter 2.1

Maternal thyroid function and child's problem behavior

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ABSTRACT

Maternal thyroid function during pregnancy is implicated in the neurodevelopment of the offspring, yet little is known about the effect of maternal thyroid parameters on the behavior of children. We investigated the association of maternal thyroid function during the first half of pregnancy with parent reported problem behavior of the offspring up to age three years. In the Generation R study, a population-based cohort of 3736 children and their mothers, data on maternal thyroid function and child's behavior were examined. The degree of internalizing and externalizing problems in the children was assessed with the Child Behavior Checklist at ages 1½ and 3 years. Higher levels of maternal TSH during pregnancy predicted a higher externalizing scores in children at 1½ and 3 years (B=0.22 per SD of TSH, 95%CI: 0.04, 0.40; B=0.10 per SD for internalizing scores, 95%CI: -0.01, 0.21). Maternal free thyroxine (T4) and total T4 were not associated with internalizing or externalizing scores of children. The linear relation with more externalizing scores was across the range of TSH; this implies that subtle impairments of maternal thyroid function may affect the child. The results suggest that thyroid function is crucial for fetal brain development, which determines problem behavior later in life.

INTRODUCTION

Thyroid hormones are crucial for the development of the fetal brain. More than 30 years ago, Man and Jones and Man[1] and Serunian[2] suggested that maternal thyroid hormones during pregnancy play an important role in the neuropsychological development of the child. Animal studies provided evidence for the role of thyroid hormones in normal cytoarchitecture of the brain.[3] During early gestation, the fetus depends entirely on maternal thyroid hormones as the fetal thyroid function does not begin before 12-14 weeks of pregnancy.[4-5] Even after the onset of fetal thyroid hormone production, the fetus continues to rely upon maternal thyroid function.[5]

Against this background, several groups investigated the effect of maternal thyroid dysfunction in pregnancy on neurodevelopment of the offspring.[6-8] Haddow et al. showed that during early pregnancy hypothyroidism is associated with poor cognitive function and low IQ in the offspring.[7] Pop indicated that subtle changes in maternal thyroid function during pregnancy may predict poor psychomotor and cognitive development in children.[9-10] However, there is little information on the role of maternal thyroid function in pregnancy for child's behavior. Vermiglio et al. investigated the behavioral problems of children in the context of iodine insufficiency.[8] In a study of 27 subjects, they reported an abnormally high frequency of Attention Deficit/Hyperactivity Disorders in children whose mothers were hypothyroxinemic during pregnancy. In addition, they showed that children's IQ score was inversely related to maternal TSH levels in mid-gestation.

We performed a prospective population-based study with repeated measurement of problem behavior in children up to age three years. Maternal thyroid function [free T4 (thyroxine), total T4 and TSH] was assessed before the 18th week of gestation to investigate the association of maternal thyroid function with internalizing and externalizing problem scores as indicators of offspring's neurodevelopment later in life.

METHODS

Setting

The present study was embedded in Generation R, a population-based cohort in Rotterdam from early fetal life onwards.[11-12] Mothers with a delivery date between April 2002 and January 2006 were enrolled. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all adult participants.

Subjects

We measured one or more thyroid parameters in 4892 pregnant women before 18th week of gestation. Thirty-six pregnant women were excluded because of the current use of thyroid medication. Of the 4856 remaining women, 3369 mothers completed the CBCL (The Child Behavior Checklist) for their children at age 1½ years. At age 3 years, the CBCL was completed by 3177 mothers and 2658 fathers. In total, 3736 (77%) children with maternal thyroid and behavioral data were included in one or more analysis.

Measurements

Maternal thyroid parameters

We assessed maternal thyroid parameters in pregnancy at the first prenatal visit. The gestational age at the time of blood sampling was less than 18 weeks in all participants (mean=13.3 weeks, SD=1.7). Within 24 hours after sampling, the plasma was stored at -70°C. The TSH, free and total T4 were determined in the stored samples in batches over a 6-month period, using chemiluminescence assays (Vitros ECI Immunodiagnostic System Ortho Clinical Diagnostics, Rochester, NY). Reference values of free and total T4 for non-pregnant women in our laboratory were 11-25 pmol/l and 58-128 nmol/l. The interassay and intra-assay coefficients of variation for free T4 were 4.7-5.4% and 1.4-2.7%. The respective coefficients for total T4 were 4.6-6.4% and 2.6-2.7%. To obtain pregnancy reference ranges, the normal population reference ranges for total T4 were multiplied by 1.5 as recommended by Endocrine Society Clinical Practice Guideline.[6] The interassay and intra-assay coefficients of variation for TSH were 2.5-4.1% and 1.0-1.2%. Hypothyroxinemia was defined as TSH levels within the reference range for pregnancy (higher than 0.03 mIU/l and lower than 2.5 mIU/l) and a free T4 below the 10th percentile.[6,13] This percentile corresponded to 11.76 pmol/l. In line with previous studies, we tested an alternative range to define normal maternal TSH plasma levels in the assessment of hypothyroxinemia (TSH levels higher than 0.4 mIU/l or lower than 4.0 mIU/l).[6,8] Thyroid parameters were measured after delivery and parents were not informed about the results of the tests, except one clinical case that was excluded from this study.

Child's problem behavior

To assess the child's problem behavior, the CBCL for ages 1½-5 was used.[14] The CBCL consists of 99 items by which a standardized rating of behavioral and emotional problems of the children can be obtained. Two broad groupings of syndromes are scored: internalizing (anxiety, sadness and withdrawn) and externalizing (attention problems and aggressive behavior). Five Diagnostic and Statistical Manual of Mental Disorder (DSM)-oriented scales consistent with DSM diagnostic categories were derived and used for additional analyses: affective, anxiety, pervasive developmental, attention deficit/hyperactivity, and oppositional defiant problems. The CBCL is a valid instrument to measure the degree of behavioral and

emotional problems of children at young age. The version used in present study is a revision of CBCL/2-3 and is specific for preschoolers but aims at a slightly wider age range than the previous version. It can be used to define the externalizing problems in children as young as 12 months[15] and has been previously used to define the internalizing and externalizing problems at the age of 18 months.[16] In addition, there is a longitudinal correlation between scales of the CBCL/1½-5 and the CBCL/4-18 (14).[14] Mesman et al. showed that the internalizing and externalizing problems at 2-3 years increased the risk of similar problems at 10-11 years and externalizing problems predicted DSM diagnosis of Attention Deficit/Hyperactivity Disorders and Oppositional Defiant Disorders.[17] According to the CBCL manual, 98th percentile of the sample was used to define clinical cut-off (corresponding scores at 3 years: 8.4 for attention deficit/hyperactivity, and 7.2 for oppositional defiant problems). Based on this cut-off, 79 (2.1%) children had Attention Deficit/Hyperactivity Problems at 1½ years. Only 18 children (0.5%) had scores above clinical cut-off for Oppositional Deviant Problems at 1½ years. These numbers were 72 (2.3%) and 81 (2.2%) at 36 months. These categories define children with high levels of attention deficit/hyperactivity and oppositional defiant problems and are not clinical diagnoses.

The CBCL was completed mostly by mothers when the children were 1½ years (mean age=18.4 months, SD=1.0). Both mothers and fathers completed the CBCL again at the age of 3 years (mean age=36.7 months, SD=1.4 and mean age=36.9 months, SD=1.4). The correlation coefficients (r) between mother and father ratings of internalizing and externalizing scales at 3 years were 0.56 and 0.57. These numbers were very close to the mean correlation (r=0.60) between two parents assessing the child's emotion and behavior in the same setting reported in a review.[18] The IntraClass Correlation Coefficient (ICC) for mother's reports on CBCL at 1½ and 3 years was 0.72. The ICC for mother and father reported CBCL at 3 years was 0.73.

Covariates

We considered the following variables as potential confounders: Apgar score, gestational age and weight at birth, maternal psychopathology, cigarette smoking during pregnancy and educational level.[10,19-21] We also controlled for the gestational age at the time of thyroid assessments and child's age when the CBCL was completed.

During enrollment, information on maternal age, parity, maternal educational level, and ethnicity of the child were obtained. Maternal smoking was assessed twice, at the time of enrollment and the 30th week of gestation. The Brief Symptom Inventory, a validated self-report questionnaire with 53 items, was applied to assess maternal psychopathology in mid-pregnancy. We used the Global Severity Index as an indicator of psychopathological problems.[22]

The child's national origin was defined based on the origin of parents and grandparents and categorized into Dutch, Moroccan, Turkish, Surinamese, Antillean, other Western or other

non-Western ethnicity. Information on Apgar scores 1 and 5 minutes after birth, birth weight and mode of delivery was derived from medical records. Gestational age of the child at birth was defined based on fetal ultrasound measures.

Data analysis

We used independent t-tests, Mann-Whitney U tests and chi-square statistics to compare characteristics of children included in the analysis to those who were excluded.

CBCL internalizing and externalizing scores were the dependent variables and analyzed primarily as continuous variables. First, we performed multiple linear regressions to assess if maternal thyroid function is associated with the child's internalizing and externalizing scores at the age of 1½ and 3 years. The determinants were maternal plasma levels of TSH, free and total T4. We divided them by their standard deviation to enable comparison. We tested the normal distribution of the residuals in the analyses with TSH.[23] To avoid multiple testing, we further explored our results only in case of an association with broadband scale (post hoc analyses). To this aim we used the following DSM-oriented subscales of the CBCL: attention deficit/hyperactivity and oppositional defiant Problems. Because of the small number of children with clinical attention deficit/hyperactivity and oppositional defiant problems in general population both DSM-oriented subscales were analyzed as continuous variable. Of the tested variables, only maternal age, educational level and psychopathology, child's gender and ethnicity, mode of delivery and gestational age at the time of thyroid sampling were retained as confounders, based on the change-in-estimate method (5% change criterion).[24] Significance of quadratic terms of thyroid determinants was also examined because of possible non-linear relationship.[7,25]

CBCL scores, reported by two informants and at two time points, are correlated and assess the same construct. Therefore, we analyzed the overall outcome (mother- and father-report internalizing and externalizing scores at 1½ and 3 years), using a GEE procedure (Generalized Estimating Equations) to get to a more precise effect estimate and to reduce the error derived from multiple comparisons (type I error).[26] Any difference between two informants and a possible time trend are not easily interpretable in such a combined model.

Non-response Analyses

Of 4856 pregnant women with data on thyroid parameters, 3736 completed the CBCL for their children. The children whose mother did not complete the CBCL (n=1120, 23.1%) were more likely to have non-Dutch national origins [64.4% versus 34.5% for the children with CBCL data, x^2= 290.2 (1), p <0.001]. Mothers of nonresponders group were younger than mothers of the children with CBCL data [mean difference 3.2 years, 95%CI=2.9, 3.5, p<0.001], less educated [42.3% primary level versus 16.0%, x^2=363.5 (2), p<0.001), and were more likely to continue smoking during pregnancy [25.9 % versus 14.9%, x^2=64.1 (2), p<0.001].

RESULTS

The characteristics of the children and their mothers are summarized in Table 1. 8.8% of the mothers fulfilled the criteria for hypothyroxinemia during early pregnancy. Using the alternative range for maternal TSH (0.4 mIU/l - 4.0 mIU/l), this percentage changed to 9.8. The range for the thyroid parameters were: TSH: 0.0-33.9 mIU/l, total T4: 63.3-380.0 nmol/l, free T4: 6.4-94.6 pmol/l. The mean (SD) of attention deficit/hyperactivity and oppositional defiant problem scores at 3 years were .3.0 (2.3) and 2.9 (2.2). Maternal age was negatively associated with CBCL externalizing scores at 3 years (B=-3.7 per year of maternal age, 95%CI: -0.14, -0.05, p<0.001). Non-Dutch national origin was associated with an increased risk of externalizing scores at 3 years (B=1.93, 95%CI: 1.35, 2.51, p<0.001). Mothers with only primary education had children with higher externalizing scores at 3 years (B=1.79, 95%CI: 1.10, 2.49, p<0.001).

In univariate analysis, there were no differences between the mean values of maternal plasma levels of TSH, free T4 and total T4 in groups of children with and without attention deficit/hyperactivity or oppositional defiant problems (numbers are not shown).

Table 2 summarizes the association between maternal thyroid function and internalizing scores in the offspring. Maternal TSH was not associated with internalizing scores reported by mothers. Higher levels of TSH did not increase the risk of a high internalizing score in children, as demonstrated by the GEE approach using internalizing scores reported by father and mother at 1½ and 3 years (B=0.10 per SD of TSH, 95%CI: -0.01, 0.21, p=0.07). Likewise, free and total T4 did not predict the internalizing scores of children. Looking at 1½ and 3 years separately, we found that TSH levels were not associated with higher internalizing scores at 1½ and 3 years, reported by mothers (B= 0.05 per SD of TSH, 95%CI: -0.10, 0.20, p=0.52 and B=0.01 per SD of TSH, 95%CI: -0.16, 0.16, p=0.96).

Table 3 shows the associations between maternal thyroid parameters and externalizing scores in children. Higher plasma levels of TSH were consistently associated with externalizing scores. Higher TSH levels increased the risk of a high externalizing score at 3 years as reported by fathers (B=0.26 per SD of TSH, 95%CI: 0.02, 0.50, p=0.03). But more importantly, analyses with GEE confirmed a positive association between TSH levels and externalizing scores (B=0.22 per SD of TSH, 95%CI: 0.04, 0.40, p=0.02), combining mother- and father-report externalizing scores at 1½ and 3 years. Maternal free and total T4 were not associated with children's externalizing scores at 1½ and 3 years. Analyzing mother-report externalizing scores at 1½ and 3 years separately showed that maternal TSH levels were associated with higher externalizing scores, but neither of these association was statistically significant (B= 0.20 per SD of TSH, 95%CI: -00.02, 0.42 and B=0.14 per SD of TSH, 95%CI: -0.07, 0.36). Comparing these findings with the results of GEE methods confirmed that pooling the scores from the reports of different informants in different times increases the precision of estimate as reflected by narrower confidence intervals. The posthoc analyses showed that higher plasma levels of TSH were related to higher scores on attention deficit/hyperactivity (B=0.08 per SD

TABLE 1 Baseline characteristics of subjects (n =3736)

	Mean (SD) *
Maternal characteristics	
Age at the time of enrollment, years	30.9 (4.5)
Parity, primipara (%)	60.0
Educational level (%)	
Primary	16.0
Secondary	52.4
High	31.6
Smoking during pregnancy (%)	
Never	75.6
Until pregnancy was known	9.5
Continued in pregnancy	14.9
Overall psychopathology score ** (QR)	0.13 (0.06, 0.29)
TSH, mIU/l	1.6 (1.4)
Total T4, nmol/l	145.6 (31.5)
Free T4, pmol/l	15.3 (3.7)
Hypothyroxninemia (%)	8.8
Child characteristics	
Male gender (%)	49.9
Ethnicity (%)	
Dutch	65.5
Moroccan/ Turkish	9.7
Surinamese/ Antillean	7.3
Other Western	10.0
Other non Western	7.5
Birth weight, grams (QR)	3460 (3100, 3800)
Gestational age at birth, weeks (QR)	40.1 (39.1, 41.0)
Apgar score 1 min (QR)	9 (8, 9)
Apgar score 5 min (QR)	10 (9, 10)
Mode of delivery (%)	
Spontaneous vaginal	71.1
Instrumental vaginal	15.9
Cesarean section	13.0
Internalizing behavioral scores	
Mother-report, 1 ½ years	5.0 (4.6)
Mother-report, 3 years	5.0 (4.8)
Father-report, 3 years	5.2 (4.9)
Externalizing behavioral scores	
Mother-report, 1 ½ years	10.6 (6.6)
Mother-report, 3 years	8.4 (6.2)
Father-report, 3 years	9.2 (6.4)

Note: Numbers denotes children included in one or more analyses.

QR = quartile range

* Unless otherwise is indicated

** Global Severity Index as measured by the Brief Symptom Inventory

TABLE 2 Maternal thyroid function during pregnancy and internalizing scores in children within Generation R Cohort

	Internalizing Scores					
		One parent report			Both parents report	
	Total	Mother report * (1½ and 3 years)	Total	Father report (3 years)	Total	Mother- & Father-report (1½ and 3 years)
Thyroid Parameters	n	B (95%CI), P	n	B (95%CI), P	n	B (95%CI), P
TSH (per SD)**	3677	0.02 (-0.09, 0.11), 0.71	2618	0.24 (0.07, 0.42), 0.01	3682	0.10 (-0.01, 0.21), 0.07
Free T4 (per SD)**	3702	0.03 (-0.11, 0.17), 0.70	2635	-0.07 (-0.25, 0.12), 0.47	3707	0.01 (-0.13, 0.15), 0.91
Total T4 (per SD)**	3719	0.11 (-0.02, 0.24), 0.09	2652	0.06 (-0.12, 0.25), 0.51	3724	0.09 (-0.04,0.22), 0.19

Note: Total of children in one or more analyses is 3736.

B gives the estimate of increase in CBCL score per SD of thyroid parameters.

Models were adjusted for maternal age, educational level and psychopathology, child's gender, ethnicity, mode of delivery and gestational age at the time of maternal thyroid sampling. Other variables were tested as potential confounders but did not change the effect estimate (see the Methods and Materials section)

* At the 1½ year assessment, less than 10% of informants were the primary caregivers other than mothers.

** SD of TSH=1.43, SD of free T4=3.48, SD of total T4=31.31 (SDs are calculated in whole sample)

TABLE 3 Maternal thyroid function during pregnancy and externalizing scores in children within Generation R Cohort

	Externalizing Scores					
		One parent report			Both parents report	
	Total	Mother report * (1½ and 3 years)	Total	Father report (3 years)	Total	Mother & father report (1½ and 3 years)
Thyroid Parameters	n	B (95%CI), P	n	B (95%CI), P	n	B (95%CI), P
TSH (per SD)**	3681	0.15 (-0.03, 0.33), 0.10	2616	0.26 (0.02, 0.50), 0.03*	3687	0.22 (0.04, 0.40), 0.02*
Free T4 (per SD)**	3706	0.02 (-0.16, 0.20), 0.81	2633	-0.14 (-0.39, 0.11), 0.27	3712	-0.02 (-0.16, 0.20), 0.80
Total T4 (per SD)**	3723	0.15 (-0.04, 0.34), 0.13	2650	0.08 (-0.17, 0.33), 0.55	3729	0.12 (-0.06, 0.30), 0.19

Note: Total of children in one or more analyses is 3736.

B gives the estimate of increase in CBCL score per SD of thyroid parameters.

Models were adjusted for maternal age, educational level and psychopathology, child's gender, ethnicity, mode of delivery and gestational age at the time of maternal thyroid sampling. Other variables were tested as potential confounders but did not change the effect estimate (see the Methods and Materials section)

* At the 1½ year assessment, less than 10% of informants were the primary caregivers other than mothers.

** SD of TSH=1.43, SD of free T4=3.48, SD of total T4=31.31 (SDs are calculated in whole sample)

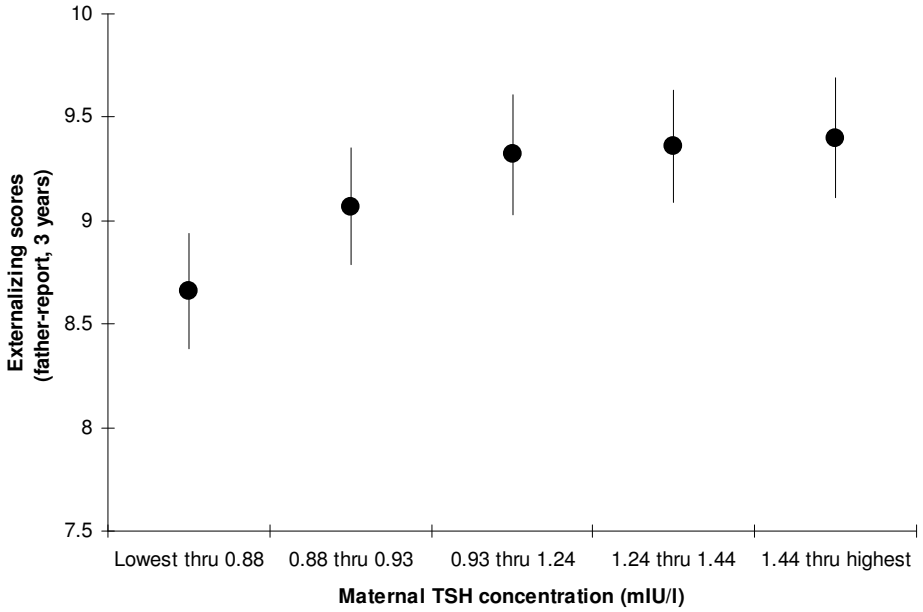
of TSH, 95%CI: 0.01, 0.15, 0.05) and oppositional defiant problems in children (B=0.08 per SD of TSH, 95%CI: 0.02, 0.14, 0.01) in combined analyses.

Figure 1 illustrates the unadjusted association between TSH and externalizing scores at 36 month, reported by fathers. The children were divided in 5 equal groups based on 20th, 40th, 60th and 80th percentile of the maternal TSH. As illustrated in figure 1, externalizing mean scores increased with higher TSH levels. However, there is little evidence for a clear threshold for TSH in the association with externalizing scores.

Maternal hypothyroxinemia was not associated with higher internalizing scores at 1½ and 3 years (B = -0.19 for hypothyroxinemic mothers, 95% CI -0.75, 0.37, p=0.51). Similarly, there was no association between maternal hypothyroxinemia and externalizing scores (B = 0.17 for hypothyroxinemic mothers 95% CI -0.53, 0.87, p=0.64). With the alternative cut-off for the definition of hypothyroxinemia, we obtained very similar results.

Adding quadratic terms of thyroid parameters to the model did not improve the model's fit and not support the notion of a non-linear association between maternal thyroid parameters and problem behavior of children (data not shown).

FIGURE 1 Maternal TSH and externalizing behavioral mean scores in children at 3 years (father-report), The Generation R Study



Note: Error bars are SE.

The children were divided in 5 equal groups based on 20th, 40th, 60th and 80th percentile of maternal TSH.

DISCUSSION

In the present study, higher plasma levels of maternal TSH in the first half of pregnancy predicted the externalizing scores in the offspring. An effect of maternal TSH on the internalizing scores was less clear, but cannot be ruled out in the present study. Plasma levels of free and total T4 in the mothers were not associated with internalizing and externalizing scores in their children.

Results from molecular[4-5] and clinical[7] observations provide evidence for a prominent role of thyroid hormones in brain development. Vermiglio showed that maternal hypothyroxinemia and TSH levels during pregnancy are associated with Attention-Deficit Hyperactivity Disorder in children.[8] The retrospective design of the study and the small sample size make it difficult to infer a causal relation from these results. Kooistra et al. demonstrated that maternal free T4 but not TSH predicts the behavior of neonates.[20] They assessed the behavior of the child at the age of 3 weeks which is too early to interpret the outcome as behavioral problems. In the present study, we showed that maternal TSH can predict children's externalizing scores. Further exploratory analyses extended this by showing an association between maternal thyroid parameters and Attention Deficit/Hyperactivity and Oppositional Deviant problems in children.

Our results support the evidence that TSH is a good indicator of thyroid function problems, due to the delicate feedback mechanism of pituitary. Mild increases in TSH, as a stimulatory mechanism for thyroid hormone secretion, can signal low levels of maternal thyroid hormones.[27] These may lead to impaired fetal brain development and subsequent externalizing problems. However, from the results, we cannot infer that maternal TSH affects internalizing and externalizing scores differently. Firstly, the effect estimates were very similar with largely overlapping confidence intervals. Second, the overall association between TSH and internalizing scores just failed to reach the significant level. We must be careful not to rule out an association with internalizing scores. On the other hand, the role of thyroid hormones in the normal development of neural structures in the cerebral cortex[3] makes an effect on the externalizing problem scores particularly plausible since cortical structures are responsible for the regulation of inhibitory processes.[28] Impairments in the control system may lead to deficits in executive functions as seen in externalizing problems.[29] Our data showed that maternal TSH was consistently associated with higher externalizing scores as reported by mother and father at 1½ and 3 years, with similar B and overlapping confidence interval. When we combined this data, using GEE approach, we gained power. This is comparable to a meta-analysis of Randomized Clinical Trials in which non-significant findings from different trials are combined to obtain one overall effect estimate, which can be significant. However, in the present study we combined repeated measures of behavior in the same children.

Despite some evidence from previous epidemiological studies,[8-9] a valid indicator of maternal thyroid function during pregnancy remains a challenge. As T3 in the brain of the

fetus is directly derived from T4,[27] its measurement in maternal plasma has limited value in the diagnosis of thyroid dysfunction and the active biologic agent is not the best marker of the underlying dysfunction. Commonly, maternal thyroid insufficiency during pregnancy is defined by high plasma levels of TSH; but Pop and colleagues presented free T4 as a predictor of externalizing scores in the child. Other studies suggest that TSH, although not the biologically active hormone in the brain, is a more sensitive indicator of maternal thyroid dysfunction.[7-8] This is consistent with our results showing that maternal TSH predicted problem behavior of the child; whereas, we found no evidence for an effect of low maternal free and total T4 during pregnancy. This may imply that the best surrogate indicator for maternal thyroid dysfunction can be different in the general population than in clinical samples.

In the present study, we found similar effect sizes for maternal education and TSH levels on externalizing scores (both r^2=0.03). For comparison, in medical science, the effect sizes of a magnitude between 0.01 and 0.04 are sometimes considered as "dramatic",[30] whereas the same effect sizes in the field of child's behavior are typically considered "small".

Our study has several strengths. Few population-based studies investigated prospectively the effect of maternal thyroid function on problem behavior of the offspring. We measured maternal thyroid parameters before the 18th week of gestation, as recommended in the literature (7, 10).[7,9] The child's behavior was assessed at two time points and by both parents to reduce the informant effect.[31]

The participation rate in the present study was high but the possibility of selection bias remains, as the non-respondents differed from participants. We can only speculate whether the relation between TSH and externalizing scores was different in mothers lost to follow-up. However, we certainly lost some statistical power. Another limitation of the present study was that the full effect of both time and informant on the outcome could not be shown, as the fathers were approached only when the children were 3 years to minimize the burden on parents. Also, not all items of the DSM scales of CBCL are applicable to all ages. But, an instrument like CBCL which covers a relatively wide age range makes findings across ages easily comparable. In addition, these scales do not provide a diagnostic category but address a continuous trait in children. We did not measure the iodine levels in maternal blood and in the diet, a factor which can affect both thyroid hormones and the brain structure of the child.[8]

CONCLUSION

The positive linear association between maternal plasma TSH levels and externalizing scores of children suggests that subtle variation of maternal thyroid function in the general population impacts on fetal brain development which determines behavioral and emotional problems later in life.

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Chapter 2.2

Maternal autoimmunity and Attention Deficit/Hyperactivity Problems in children

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ABSTRACT

Background Maternal thyroid status and autoimmunity during pregnancy have been associated to 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. Secondly, we aimed to explore 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 Thyroixine, and TPOAbs) at 13.5±1.8 weeks of gestation. Children's verbal and non-verbal cognitive functioning were 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 non-verbal 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 intervals [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 on 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 Disorders 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 to be the most sensitive and specific marker of thyroid autoimmunity.[3] Previous studies showed that, in women 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 addressed 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 risk of cognitive dysfunction.[17] They argued that it is "autoimmunity rather than thyroid hormone insufficiency" which affects child development. Recent evidence on the role of 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. (2010) found that maternal thyroid hormones and TPOAbs were associated with intelligence and motor scores in young children.[16] It is less clear whether maternal thyroid autoimmunity during pregnancy can affect child problem behavior, in particular, attention deficit/hyperactivity problems. Elevated titer of TPOAbs in pregnant woman may primarily affect 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 Figure 1).

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 non-verbal cognitive impairment and problem behavior, in particular, attention deficit/hyperacidity problems. Second, we explored if 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 onwards 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 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 the 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 details by Jaddoe et al.[20-23] In our sample, thirty-four 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 was performed during pregnancy, the measurement of thyroid parameters was performed after 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 parents, the informant for all outcome measures, were not aware of 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 non-verbal 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 for 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 wks). 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 reported previously.[15] 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 non-verbal 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 2 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 norm sample of boys and girls. The Cronbach's alpha coefficient of the LDS in our sample was 0.99.

Non-verbal 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 child's non-verbal cognitive abilities at the age of two. The parents-administered and parent-report components of PARCA are good predictors of 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. Non-verbal cognitive delay was defined as non-verbal cognitive scores below the 15th age- and gender-specific percentile as previously described in another study of this cohort.[14]

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 which 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 emotion and behavior of their child based on the preceding two 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 three 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 (ICC) 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 Non-Western. 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 preceding 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]). 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 non-response 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 since the scores were not normally distributed and results based on dichotomized scores can then be interpreted as problem behavior. Achenbach and Rescorla suggest to use 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 wish to dichotomize children's scores as being clearly in the normal range vs. high enough to warrant concern.[25] These cut-offs have been used widely 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) which was used previously in another study of the Generation R cohort[39] to examine consistency and if any effect was observed only due to the choice of the cut-off.

Elevated titers of TPOAbs in pregnant women may be related to child behavior because antibodies affect on 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, and 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 non-verbal 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.

Non-response analyses

Non-response 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 grams, $p<0.001$). The women whose child was not included because of missing behavioral data were younger (mean age difference=2.9 years, $p<0.001$), lower 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 on cord blood data introduced bias in the association between maternal TPOAb status and child cord blood thyroid parameters, we ran a non-response 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 in 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 non-verbal cognitive delay (OR=1.09, 95%CI: 0.67-1.77, p=0.74).

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	0.87
High	33.3	35.4	
Smoking (%)			
Never	76.9	76.5	
Until pregnancy was known	9.1	13.2	0.16
Continued during pregnancy	14.0	10.3	
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 , nmol/l	15.00 (13.28, 16.85)	14.82 (12.67, 16.48)	0.29

TABLE 1 Participants characteristics (n=3139) *(continued)*

	Maternal TPOAb status [a]		
	Negative (n=2992)	Positive (n=147)	p
Child characteristics			
Female gender (%)	50.5	49.0	0.68
Ethnicity (%)			
Western	80.1	76.0	0.28
Non-Western	19.9	24.0	
Birth weight, g	3449 (562)	3483 (589)	0.49
Gestational age at birth, wk	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 , nmol/l	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
Non-verbal cognitive delay (%)	14.0	16.2	0.51
The Child Behavior Checklist score			
Internalizing scores, Mother-rated at 3 yrs	4.9 (4.8)	5.1 (4.4)	0.58
Internalizing scores, Father-rated at 3 yrs	5.3 (5.0)	5.9 (5.1)	0.14
Externalizing scores, Mother-rated at 3 yrs	8.3 (6.1)	9.2 (6.4)	0.09
Externalizing scores, Father-rated at 3 yrs	9.2 (6.5)	10.3 (6.8)	0.04

Note: 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

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

Note: 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.

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 mother during early pregnancy and externalizing problem scores in 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). 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.

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.

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 we reported previously,[15] maternal TSH was associated with children's externalizing problems rated by 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 were used, but the results remained essentially unchanged.

DISCUSSION

In the present study, we found no association between elevated titers of TPOAbs in 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 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 child's behavior was not exclusively mediated by plasma levels of TSH as a marker of maternal thyroid status. In other word, 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 must be discussed. Second, maternal autoimmunity (specifically maternal TSH receptor-blocking antibody) is a common cause of transient hypothyroidism in the infants, because maternal antibodies pass the placenta.[42]

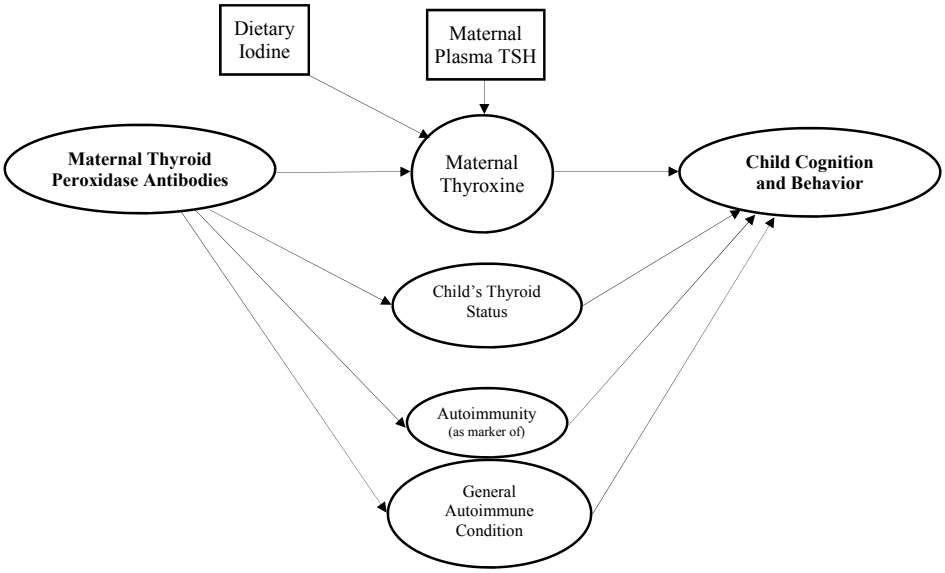
The effect of maternal antibodies on the thyroid function of the child may persist until the antibodies disappear from child's circulation a few months after birth. Elevated titers of maternal TPOAbs and TSH receptor-blocking antibodies may co-exist and lead to transient subclinical hypothyroidism in 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 does not support a role of child's thyroid parameters in the association between maternal TPOAb status and child's behavior. However, measurement of child's thyroid parameters in the cord blood may not be the optimal way to assess 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 pre-existing subclinical autoimmune condition of the mothers; such an immune process could cause the developmental problems of the offspring, as maternal antibodies pass through placenta. There is growing evidence for the role of 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 child's problem behavior.

Elevated titers of maternal TPOAbs were not associated with verbal or non-verbal 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 to those crucial to cognitive abilities. This mechanism, however, is speculative and must 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 which 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 area. 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 child's behavior independent of 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 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 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 once only. 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 described previously.[25, 28] Also, it is very unlikely that the use of parent-report measures introduced systematic bias since parents were blind to the results of thyroid measurement. In addition, we can not rule out the effect of non-responsive and loss to follow-up on the possible relationship between thyroid autoimmunity and 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 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 populations at risk.



SUPPLEMENTARY FIGURE 1 Theoretical model of the role of thyroid autoimmunity in child's problem behavior and cognition.

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Chapter 2.3

Maternal hypothyroxinemia and autistic symptoms in children

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Chapter 2.4

Maternal urinary iodine excretion and executive functioning problems in children

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ABSTRACT

The rare but deleterious effects of severe iodine deficiency during pregnancy on cognitive functioning of children are well known. Reports on possible associations between mild-to-moderate maternal iodine deficiency and child development, however, are scarce. In a population based-cohort we examined the association between maternal urinary iodine during early pregnancy and executive functioning in children at 4 years of age. In addition, we investigated modification of this association by maternal diet and thyroid function.

During pregnancy, we measured urinary iodine and thyroid hormones in 1156 women. In 692 of their children impairment of executive functioning was assessed by the Behaviour Rating Inventory of Executive Function. Five hundred mothers of Dutch national origin filled out a Food Frequency Questionnaire. Analyses were performed by using regression models. The children of mothers with low urinary iodine showed higher scores on the problem scales of inhibition [β=0.05 (95%CI = 0.01; 0.10) P=0.03] and working memory [β=0.07 (95%CI = 0.02; 0.12) P=0.003]. While maternal dietary intake and thyroid hormones did not significantly modify these associations, the associations between urinary iodine and problems of inhibition was attenuated after adjustment for maternal psychological symptoms. In addition, the consumption of bread [β=0.61 (95%CI = 0.27; 0.95) P<0.001] and eggs [β=1.87 (95%CI = 0.13; 3.62) P=0.04] was associated with higher urinary iodine.

Thus, low maternal urinary iodine during pregnancy is associated with impaired executive functioning in children. Because these symptoms are subclinical and occurred at an early age, future studies are needed to show whether these children are more vulnerable to develop later clinical disorders.

INTRODUCTION

Iodine is required for the synthesis of thyroid hormones, which play an essential role in fetal and early postnatal growth and development of most organs, especially of the brain.[1] This micronutrient is mainly obtained by the consumption of foods that contain natural or synthetic iodine. Because during pregnancy the production of thyroxine physiologically increases substantially, this increased need has to be compensated with an increase in daily iodine requirement.

Despite considerable progress over the last decades in developing countries, the prevalence of inadequate iodine intake is estimated at > 20% in industrialized countries previously considered to be iodine sufficient.[2-3] Surveys indicate that especially girls and women of reproductive age, may have deficient iodine consumption.[4-5] This also raises concern about a poor iodine intake during pregnancy in the USA and Europe for which changing dietary habits, especially low fish and milk consumption, are suggested to be responsible.

Severe iodine deficiency during pregnancy detrimentally affects maternal thyroid function and child neurobehavioral development.[6] The severity of maternal iodine deficiency during pregnancy is related to the degree of impaired functioning in children.[7] It is unknown, however, whether the increasing mild-to-moderate iodine-deficiencies during pregnancy especially in industrialized countries detrimentally affects maternal thyroid function and neurodevelopment in offspring.[8-9]

Whereas cognition gives global insight of brain functioning, executive functioning represents different structures and functions of the brain involved in the cognitive regulation of behaviour.[10] Executive function is defined as a group of processes, e.g., inhibition, working memory, and the ability to plan and organize, that are dependent on and influence more basic cognitive abilities, such as attention, language, and perception.[11]

Iodine concentration in urine and excreted by the kidneys is a good marker of the dietary intake of iodine during the previous days. It is the measure of choice for assessment of iodine status, because of its non-invasiveness.[12] In epidemiological studies the urinary iodine concentration of spot samples are used to define the iodine status in individuals and in populations.[13]

Against this background the aims of our study were to examine in a population-based cohort with available assessments of maternal diet and urinary iodine in early pregnancy the associations between: 1) maternal diet and urinary iodine; 2) maternal urinary iodine and thyroid function, and 3) maternal urinary iodine, diet and executive functioning in children at the age of 4 years.

MATERIALS AND METHODS

Design and study population

This study was embedded in the Generation R Study, an ongoing population-based birth cohort from fetal life onwards. Mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. The Generation R Study has been designed to identify early environmental and genetic determinants of growth, development and health. The data obtained comprised detailed questionnaires, ultrasonography, and biological samples. The study has been previously described in detail.[14]

The flow chart of the study population is presented in figure 1. For this study we selected all mother–child pairs (n=1316) with available measurements of urinary iodine concentration and thyroid hormones in early pregnancy. The sample for iodine measurements was selected semi-randomly from the total cohort with thyroid hormones measured (n=5831) with oversampling of women with free thyroxin 4 (FT4) levels below the 10th percentile: Of the sample, 21.4% (n=282) of the women had low FT4 levels, while 78.6% (n=1035) of the sample consists of women with higher FT4 levels. To account for this oversampling, cases were weighted on the ratio of the population proportion on the sample proportion.

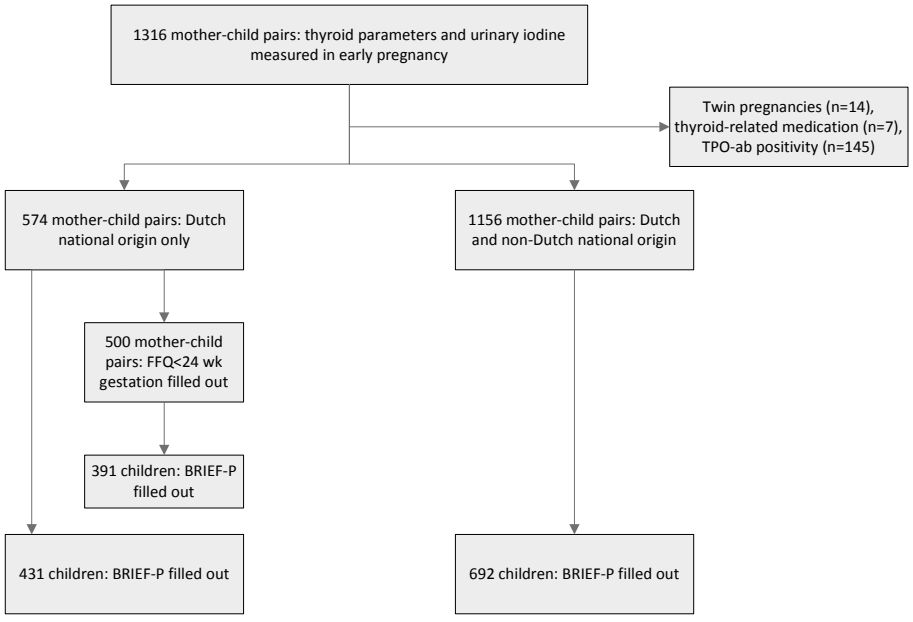

FIGURE 1 Selected characteristics of mothers and children by iodine intake.
Note: Thirty-seven mothers with ≥2 children participated. BRIEF-P, Behavior Rating Inventory of Executive Function for Preschoolers; TPO-ab, thyroid peroxidase antibody.

No instance of fertility treatment was reported in this sample. Twin pregnancies (n=14) were excluded since thyroid parameters in multiple pregnancies are different from singleton pregnancies.[15] In addition, we excluded mother-child pairs in which mothers received any thyroid-related medication including thyroxin (n=7) or who were Thyroid peroxidase antibodies (TPOAb)-positive (n=145), which left 1156 mother-child pairs. A total of 574 mothers were of Dutch national origin; 500 of these completed a food frequency questionnaire (FFQ), 431 completed the Behavior Rating Inventory of Executive Function for Preschoolers (BRIEF-P) for the child, and 391 completed both an FFQ and the BRIEF-P. Of women of non-Dutch national origin (n=583), 354 completed an FFQ, 261 completed the BRIEF-P and 185 filled out both questionnaires.

The study has been approved by the Medical Ethics Committee of the Erasmus MC, University Medical Centre, Rotterdam in the Netherlands. Written informed consent was obtained from all participants before participation.

Maternal dietary intake

In early pregnancy (median 13.2 weeks, 95% range 10.2-17.6) the nutritional intake of the previous three months was assessed by using a modified version of a validated semi-quantitative food frequency questionnaire (FFQ).[16] The FFQ consists of 293 food items and is structured according to meal patterns. Questions in the FFQ include consumption frequency, portion size, preparation method and additions of the foods. Portion sizes were estimated using household measures and photographs.[17] To calculate average daily nutritional values the Dutch food composition table 2006 was used.[18]

The 293 food items were reduced to 19 nineteen food groups, according to The European Prospective Investigation into Cancer and Nutrition (EPIC)-soft classification, based on origin, culinary usage and nutrient profiles.[19]

To extract dietary patterns from food consumption data in the selected study population, we used Principal Component Analysis (PCA) as previously described by Hu[20] and applied in a number of recent studies of dietary patterns and fetal and child development.[21-22] PCA was performed in the total Generation R cohort of women with a Dutch origin (n=3463). Because of the larger number of cases this gives a more accurate estimate.

Each woman was given a score for each of the factor or dietary patterns, calculated as the product of the food group value and its factor loadings summed across foods. For convenience we termed this score 'adherence to dietary pattern'. The 3 most prevalent dietary patterns were selected for further analysis.

Spearman correlation coefficients were used to correlate the dietary patterns following the PCA with the original food groups.

Maternal urinary iodine and thyroid function

At the same time of the assessment of nutritional intake, maternal single voided urine samples were collected at random moments over the day. Urinary iodine was measured through the ceri-arsenite reaction following destruction by means of ammoniumpersulphate. After brief centrifugation, sodium arsenite solution (0.1 mol/L in 1 mol/L of sulphuric acid) was added. Subsequently, ceriammonium sulphate was added and colour was allowed to develop at 250 ◦ C during 60 minutes. Optical density was assessed at 405 nm. At a level of 1.7 µmol/L iodine the within-assay coefficient of variation (CV) was 5.1% and the between-assay CV was 14.3%.

To adjust for total urinary volume we used the iodine to creatinine ratio. Spot urine sampling is considered a reliable and practical laboratory technique available to quantify iodine excretion in individuals.[23] Because >90% of iodine intake is excreted in the urine, urinary iodine excretion is considered the most appropriate indicator of iodine intake of the previous days as well as of iodine status.[12] We defined low urinary iodine as iodine/creatinine ratio below the 10th percentile of the study sample [0.04-0.12 mmol /mol creatinine (48.6-136.1 µg/g creatinine)].

To assess maternal thyroid function, at the same moment of urine sampling venous blood samples were drawn in plain tubes. Serum was transported to the regional laboratory for storage at -80°C within 3 hours after sampling.[24] Thyroid stimulating hormone (TSH) and FT4 from the stored samples were assayed in batches of 50-150 over a 6-month period using a chemoluminescence assay on the Vitros ECI Immunodiagnostic System (ORTHO Clinical Diagnostics, Rochester, NY). The inter-assay coefficients of variation for TSH and FT4 were <4.1% and <5.4%, respectively, the intra-assay coefficients of variation for TSH and FT4 were <1.2% and <2.7%, respectively.

Thyroid Peroxidase antibodies (TPOAb) were measured using ImmunoCAP 250-assays (Phadia AB) and regarded as positive when >0.06 IU/L.

Assessment of executive functioning

We measured impairment of executive functioning in children at 4 years of age using the Behavior Rating Inventory of Executive Function for Preschoolers (BRIEF-P).[25] The BRIEF-P is a standardized rating scale developed to provide a window into behaviors associated with specific domains of executive functioning in children aged 2 to 5 years.

The BRIEF-P consists of a single rating form, completed by parents or other caregivers, with 63 items in five scales: inhibition (to stop own behavior), shifting (to make transition, and change focus from one mindset to another), emotional control (to modulate emotional responses), working memory (to hold information in mind for the purpose of completing a task), and planning/organization (to manage current and future-oriented task demands within the situational context). The scales can be combined into the global executive composite. Raw scale scores are transformed to age- and gender-normed T-scores [50±10 (mean ± SD)] to make scores comparable. Higher scores indicate more problems with executive functioning.

In the present study, the parents were asked to rate how often a particular behavior of the child was problematic in the preceding month.

Other researchers have demonstrated the content validity of the BRIEF-P.[26] The subscales of BRIEF-P show adequate to high test-retest reliability indicating suitability for research purposes.

Covariates

Information was obtained by questionnaires during pregnancy on maternal age, national origin, education, parity, prenatal tobacco and alcohol use, and the use of folic acid supplements or (iodine and non-iodine containing) multivitamin supplement. The season of completing the FFQ was registered. National origin of the mother was based on the country of birth of the parents. The educational level of the mother was assessed by the highest completed education and reclassified into 3 categories: primary school, secondary school and higher education (e.g. higher vocational education or higher).

Maternal smoking and alcohol use were classified as "no use", "use until pregnancy was confirmed" and "continued use during pregnancy". Women were asked about the use of any multivitamin supplement or folic acid supplement during the past 6 months.

Height and weight were measured without shoes and heavy clothing; body mass index (BMI) was calculated from height and weight (kg/m^2). At 20 weeks pregnancy, we measured maternal psychological problems using the Brief Symptom Inventory.[27]

Child gender, birth weight, Apgar-scores 1 minute after birth, and the mode of delivery were derived from the medical records completed by gynecologists and midwives. To define gestational age at birth, we used the last menstrual period of the mother and the ultrasound examination at the first prenatal visit. In case these methods disagreed, pregnancy was dated on the ultrasound data.

The following covariates were considered as potential confounders; maternal age, national origin, education, BMI, parity, prenatal psychological problem score, smoking, alcohol use, energy intake, and the use of any multivitamin, folic acid supplement use, season of filling out FFQ, and child's gender, gestational age at birth, birth weight, Apgar-scores 1 minute after birth, and the mode of delivery.

Statistical analysis

Because the FFQ used is only validated in Dutch populations, all analyses with food intake were primarily restricted to mothers of Dutch national origin (n=500).

First, associations between separate food groups as independent variables and urinary iodine as outcome variable were analyzed using multivariable regression analyses. Second, the Pearson correlation coefficients between urinary iodine and maternal TSH and FT4 were calculated. Prior to analyses, TSH levels were transformed by the natural logarithm to achieve normal distribution.

Third, associations between urinary iodine as categorical determinant (below and above 10th percentile) and BRIEF-P problem scores were calculated with multivariate regression analyses. Because BRIEF-P scores were non-normally distributed, scores were transformed by the natural logarithm. Associations between urinary iodine and BRIEF-P problem scores were further explored by adding FT4 in the model. Maternal psychological problems were added separately in the model to assess the change of estimate due to psychological problems. In addition, we stratified the analysis on executive functioning for gender, and tested interactions between gender and low urinary iodine. For any observed association between urinary iodine and executive functioning the model was further explored by adjustment for maternal intake of food groups. To reduce the number of comparisons, we tested only food groups that were associated with urinary iodine as mediators. To test whether the estimates were influenced by maternal national origin, all analyses were rerun among children of pooled Dutch and non-Dutch mothers (n=692). Finally, to test whether results depended on the choice of the 10% cut-off for urinary iodine, all analyses were rerun using 5th- and 15th-percentile threshold.

All analyses were adjusted for gestational age at blood and urine sampling and estimated protein intake. A covariate was selected as confounding variable if the effect estimates changed ≥5% in the exploratory regression analyses. By using this criterion, maternal age, national origin, education, prenatal smoking, child's birth weight and gestational age at blood sampling were included as confounders in the final multivariable analyses.

Differences in characteristics of mothers and children were tested using Student's T-test or Mann-Whitney U-test for continuous variables and Pearson's Chi-square test for categorical variables.

Missing data of covariables were completed using the Markov Chain Monte Carlo multiple imputation technique creating five datasets. Subsequently multivariable regression analyses were performed separately on each completed dataset and thereafter combined to one pooled estimate.[28] For all analyses, results including imputed missing data are presented.

All analyses were performed using SPSS software, version 17.0 (SPSS Inc.).

RESULTS

Characteristics of mothers and children categorized by urinary iodine are presented in Table 1. In comparison to mothers with urinary iodine above the 10th percentile, mothers with low urinary iodine (mothers of Dutch national origin only n=56; all mothers n=117) were younger, had a higher BMI and experienced less often an instrumental delivery. They presented more often with psychological symptoms and showed lower TSH levels.

Associations between the separate food groups and urinary iodine are analyzed using multivariable regression analyses. In mothers of Dutch national origin, cereal products [β

=0.61 (95% CI = 0.27, 0.95) P<0.001] and eggs [β =1.87 (95% CI = 0.13, 3.62) P=0.04] were significantly associated with higher urinary iodine (Supplementary Table 1).

Three factors were derived from the PCA as the most prominent dietary patterns used in the study group of women with Dutch national origin. The first factor was labeled the Mediterranean dietary pattern and explained 8.1% of the variance of dietary intake of the total study group. It comprised high intakes of vegetables, fruits, cereal products, vegetable oil

TABLE 1 selected characteristics of mothers and children by maternal urinary iodine secretion

	Low urinary iodine <10th percentile (n=117)	Urinary iodine ≥ 10th percentile (n=1,039)	P-value
Mothers			
Age, y	27.4 ± 5.4	30.2 ± 5.0	<0.001
Gestational age at enrolment, wk	13.2 (9.2; 17.7)	13.2 (10.2; 17.6)	0.55
National origin, %			0.19
Dutch	48.2	50.7	
Western, other	8.9	14.0	
Non Western	42.9	35.3	
Parity, % primiparous	61.1	62.8	0.95
BMI, kg/m^2	25.4 ± 5.6	24.4 ± 4.3	0.05
Educational level, %			0.18
Primary school	31.3	23.9	
Secondary school	48.2	52.5	
High education	20.5	23.6	
Psychological symptoms	0.2 (0.0; 1.6)	0.2 (0.0; 1.3)	0.003
Smoking during pregnancy, %			0.30
Never	67.6	74.9	
Until pregnancy was confirmed	12.0	9.9	
Continued	20.4	15.2	
Multivitamin use, % yes	27.1	30.8	0.50
TSH, mU/L	1.3±0.8	1.5 ±1.0	0.001
FT4, pmol/L	15.0 ± 3.3	14.6 ± 3.4	0.14
Children			
Gender, % male	47.0	50.8	0.33
Birth weight, kg	3.4 ± 0.5	3.5 ± 0.5	0.31
Gestational age, wk	40.1 ± 1.6	40.1 ± 1.6	1.00
Apgar score 1 min after birth	8.5 ± 1.1	8.6 ± 1.1	0.44
Mode of delivery, %			0.04
Spontaneous vaginal	87.2	77.4	
Instrumental vaginal	9.2	14.9	
Cesarean section	3.7	7.7	

Note: Values are means ± SD, medians and 95% range, or percentages; total n=1156, FT4, free thyroxine, TSH, thyroid stimulating hormone.

TABLE 2 Associations between low maternal urinary iodine and children's score on BRIEF-P stratified by maternal national origin.[1]

	Dutch women (n=431)				All women (n=692)			
BRIEF-P problem scale	Adjusted[2]		Additionally adjusted[2,3]		Adjusted[2,4]		Additionally adjusted[2-4]	
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value[3]	β (95% CI)	P value
Inhibition	0.08 (0.02; 0.14)	0.008	0.06 (0.00; 0.01)	0.05	0.05 (0.01; 0.10)	0.03	0.04 (-0.00; 0.09)	0.07
Shifting	-0.01 (-0.06; 0.04)	0.76	-0.02 (-0.07; 0.03)	0.51	-0.01 (-0.05; 0.03)	0.64	-0.02 (-0.06; 0.02)	0.41
Emotional Control	0.02 (-0.05; 0.08)	0.59	0.00 (-0.07; 0.07)	0.99	0.01 (-0.04; 0.06)	0.63	0.00 (-0.05; 0.05)	0.94
Working Memory	0.07 (0.01; 0.12)	0.03	0.05 (-0.01; 0.11)	0.11	0.07 (0.03; 0.12)	0.003	0.06 (0.01; 0.10)	0.01
Planning/ Organization	0.05 (-0.01; 0.11)	0.11	0.03 (-0.03; 0.10)	0.28	0.03 (-0.02; 0.08)	0.19	0.02 (-0.03; 0.07)	0.43
Global Executive Composite	0.06 (0.00; 0.12)	0.04	0.04 (-0.02; 0.10)	0.19	0.05 (0.00; 0.10)	0.05	0.03 (-0.01; 0.08)	0.16

Note: [1]Results from multivariable regression analyses. The 5 scales of executive function were analyzed using log-transformed standardized scores (T-scores) to achieve normal distribution. BRIEF-P, Behavior Rating Inventory of Executive Function for Preschoolers.

[2]Adjusted for gestational age at blood and urine sampling, maternal age, education, BMI, and smoking, alcohol use, protein intake, and child's birth weight.

[3]Additionally adjusted for maternal psychological symptoms.

[4]Additionally adjusted for maternal national origin.

and fish and shellfish. The second factor, explaining 6.9% of the total variance, was labeled a traditionally Dutch dietary pattern because it was characterized by high intakes of potatoes, fresh and processed meat, margarine, and low intake of fruit. Pattern three, a Confectionary dietary pattern, explained 6.1% of the variance and was characterized by high intake of cakes, sugar and confectionary and tea (all r≥0.20 and P -value <0.05) (Supplementary Table 2). No significant association was established between adherence to the dietary patterns and urinary iodine (Supplementary Table 3).

Urinary iodine showed no correlation with FT4 and a borderline correlation with TSH [Pearson's Rank correlation coefficients: -0.04 (P=0.17) and 0.06 (P =0.05), respectively].

Children from mothers of Dutch national origin with the lowest decile of urinary iodine, the problems scores on inhibition [β =0.08 (95% CI = 0.02; 0.14) P =0.008], working memory [β =0.07 (95% CI = 0.01; 0.12) P=0.03] and global executive composite [β =0.06 (95% CI = 0.00; 0.12) P=0.04] were significantly higher than those from mothers with urinary iodine at or above 10th percentile (Table 2). After adjustment for maternal psychological problems in pregnancy associations between urinary iodine and problems of child executive functioning became smaller [inhibition β = 0.06 (95% CI = 0.00, 0.12), P =0.046, working memory β =0.05 (95% CI = -0.01, 0.11), P =0.11 and global executive composite β = 0.04 (95% CI =-0.02, 0.10), P =0.19]. As expected, adjustment of the association between urinary iodine and executive functioning for maternal FT4 did not change the effect estimates (data not shown).

When analyses were stratified by gender, the association between urinary iodine on inhibition did not reach significance in these smaller subpopulations. The effect on working memory and global executive composite was, if anything, more prominent in girls [β = 0.12 (95% CI = 0.05, 0.20), P =0.002, and β = 0.09 (95% CI = 0.009, 0.17), P =0.03, respectively] and was not significant in boys. However, an interaction effect of gender was not found (data not shown).

Because of the association between urinary iodine and cereals, bread and eggs, we tested whether maternal intake of these separate food groups modified the association between urinary iodine and executive functioning. Addition of these food groups did not significantly change the effect estimates (data not shown).

Finally, after pooling of mothers of Dutch and non-Dutch national origin (n=692) we showed associations between urinary iodine and higher problem scores of inhibition [β = 0.05 (95% CI = 0.005, 0.10), P =0.03], working memory [β = 0.07 (95% CI = 0.02, 0.12), P =0.003] and global executive composite [β = 0.05 (95% CI = 0.00, 0.10), P =0.05] in children (Table 2). These results changed slightly after adjustment for maternal psychological symptoms [inhibition β 0.04 (95% CI = -0.004, 0.09), P =0.07, working memory β 0.06 (95% CI = 0.01, 0.10), P =0.02, and global executive composite β 0.03 (95% CI = -0.01, 0.08), P =0.16].

All analyses were rerun using 5th and 15th percentile cut-offs instead of the 10th percentile cut-off as indicator of low urinary iodine excretion. Results were essentially the same (data not shown).

DISCUSSION

This study shows that children of mothers with low urinary iodine, a marker of low iodine status, and independent of maternal thyroid levels in early pregnancy have higher scores of impaired executive functioning at 4 years of age. Although maternal urinary iodine was positively associated with maternal intake of specific food groups, these intakes could not explain the association between urinary iodine and impaired executive functioning in children.

Food groups of which the intake was associated with higher urinary iodine in early pregnancy were cereals, bread and eggs. In the Netherlands, consumption of bread, meat, vegetables, potatoes, but also of eggs is relatively high.[19] Our results suggest therefore that in the Dutch population the major sources of iodine are bread and bread-replacements, which are voluntary fortified with iodized salt, and eggs. This is in line with other Western countries where dairy products, bread, seafood, eggs, meat and poultry are the main sources of iodine.[9]

The issue of iodine deficiency during pregnancy is also related to the advisability of iodine supplementation of women as related to the need for fortification of the food supply. Worldwide, the use of iodized salt is the most important method for preventing iodine deficiencies. Before 2008 the most important source of iodine in the Netherlands was bread providing 50% of the average iodine intake.[29] After 2008 the number of foods containing iodized salt has been extended, because of the decreasing consumption of bread, especially among teenagers and adolescents. At the same time, however, the iodine content in iodized salt was reduced to avoid overintake, the use of salt in processed foods was reduced to prevent hypertension, and food producers limited the use of iodized salt. This resulted in a 25% decrease in iodine intake as compared to before 2008.[30] Because our data sampling was performed between 2001 and 2006, iodine deficiency might currently be even more prevalent in this population.

In contrast to studies performed in other western countries,[31-32] dairy foods were not associated with urinary iodine, which might be due to the Dutch legislation on the limitation of iodine in these foods. The content of iodine in milk, poultry and meat depends on the iodine supplementation of animal foods. In addition, the use of iodophor disinfectants in milking equipment contributes to the iodine concentration of dairy products.[33-34] In the Netherlands only small regional differences in iodine content of milk were observed that were explained by the type of soil.[35] This might explain that. in a study of children of 6-18 years of age no differences in urinary iodine were observed.[36] Fish, fruits and vegetables are other iodine rich sources due to the iodine content of soil, fertilizers and irrigation practices.[37]

The intake of these foods, however, is low.[38] Because urinary iodine reflects the short-term iodine status, foods with a low frequency of intake are less reflected by urinary iodine. In addition, we did not establish effect modification by iodine-rich food groups of the association between maternal iodine status and executive functioning in children. This may also be explained by the low frequency of intake of iodine-rich foods.

The amount of variance (21.1%) explained by the dietary patterns are suggested to be rather small, but the estimates are comparable with previous dietary studies in pregnant women.[39-40] Moreover, the explained variance of dietary patterns by definition is dependent on the number of included food groups for the factor analyses.[20] We used 19 predefined food groups which allowed more variance in the model, but at the same time reduced the explained variance of the identified dietary patterns.

Because iodine is released from the body through the urine, the measurement of the amounts of iodine in urine samples is a reliable method to determine iodine deficiency across a large population. The median urinary iodine concentration in our population was 203 µg/L (1.6mmol/L), which meets the WHO recommendations for pregnant women of 150–249µg/L(1.2-2.0mmol/L).[41] However, our estimated range of 9.3-1743.5 µg/L (0.07-13.7mmol/L) for iodine was very large, which supports its high variability.[42]

In the same population-based cohort, we previously reported an association between mothers' hypothyroxinemia in early pregnancy and cognitive delay in their children at age 3 years.[43] In the current analysis, the association between low maternal urinary iodine and impairment of executive function could not be explained by derangements of the biomarkers of hypothyroxinaemia; FT4 and TSH were both low. Different explanations may help to understand this finding. The current analysis is performed in the study population of the same cohort who had a very low expected frequency of impairment in thyroid function, because women using thyroid medication were excluded for analysis. This implies that we examined associations in mothers with a relatively mild iodine deficiency, as one would expect in an iodine-sufficient area. Our findings are supported by others, which show no relationship between urinary iodine and TSH and FT4.[44] A shortage of maternal iodine intake may result in iodine deficiency in the mother and fetus, but both respond differently, with the mother preserving euthyroidism and the fetus becoming hypothyroid.[7] This may explain why the fetus is more affected by iodine deficiency during pregnancy than the mother, resulting in impaired executive functioning of the child and normal maternal biomarkers of thyroid function.

In our study low maternal urinary iodine was associated with problems of inhibition, working memory and global executive composite in children at 4 years of age. Impairments of executive functioning are consistently associated with attention-deficit hyperactivity disorder (ADHD).[45] Children with ADHD are rated higher than controls on all scales of executive functioning with the largest effect sizes on inhibition and working memory.[46] However, deficits in inhibition are not uniquely associated with ADHD, but also with oppositional defiant disorder and conduct disorder.[45] The children in our study population, however, are too young to be diagnosed with ADHD. Although hyperactive and impulsive symptoms typically are observed by the time the child is 4 years of age, they peak in severity at school age.[47] Therefore, follow-up of executive functioning in these children may show interesting associations.

A relationship between maternal iodine deficiency and poor mental and psychomotor development in the offspring has been described repeatedly.[48] This association is suggested among others to be due to the induced derangement in maternal thyroid function. This is supported by the associations between maternal iodine deficiency, congenital hypothyroidism and ADHD.[49-50] This is further substantiated by the reported higher incidence (70%) of ADHD in individuals with generalized resistance to thyroid hormones.[51-52] However, also in these studies maternal thyroid dysfunctions were not due to iodine deficiency, because they were conducted in iodine-sufficient populations.[53] Because the full causal chain that links iodine and thyroid hormone to risk of developmental problems has not been established, the indirect evidence has to be considered carefully.

Part of the effect of low urinary iodine on executive functioning in our study was explained by maternal psychological symptoms. Maternal psychological distress during and after pregnancy is known to be a strong determinant of behavioral and cognitive functioning of the child.[54] After adjustment for this important confounder only the association between urinary iodine and working memory remained. The correlation between diet and mental health is possibly bidirectional. Depression and stress may promote unhealthy dietary preference[55] whereas an unhealthy diet, in turn, may affect the mental health of the mother.[56]

Human studies showed that iodine supplementation trials in iodine-deficient areas were associated with more prominent cognitive improvement among girls.[57-58] Recently Murcia et al. reported potentially deleterious effect of maternal iodine supplement use during pregnancy on psychomotor achievement, especially in girls.[59] This is in line with our data showing a more prominent effect of low urinary iodine on executive functioning in girls as compared with boys. However, because no interaction effect was found, these finding should be interpreted with caution.

A strength of our study is that we examined the relationship between mild iodine deficiency during early pregnancy and executive functioning in children at 4 years of age, thereby including maternal nutrition and thyroid function as determinants of the same pathway. In addition, the large population-based prospective cohort enabled us to control for important confounding factors, including lifestyle factors, socioeconomic factors, and known determinants of fetal and infant development. However, this does not completely exclude residual confounding. Because data were more complete in more highly educated mothers, we cannot rule out that selective nonresponse influenced our findings.

The effect sizes in our study were rather small because executive functions were measured instead of clinical diagnosis of behavioral problems. Nevertheless, the continuous traits of executive functioning provide better statistical power since exposure and outcome are rare. More importantly, the BRIEF-P scale converges with a variety of clinical groups including traumatic brain injury, autism spectrum disorders[60], ADHD and Tourette-syndrome.[61]

In conclusion, low maternal urinary iodine status during early pregnancy is associated with impairment of executive functioning in children at 4 years of age. This finding could not be

explained by low nutritional iodine intake during pregnancy and maternal thyroid function and should be confirmed by others.

The observed impairments in executive function at an early age are considered subclinical symptoms. Only future studies may demonstrate whether these children have an increased vulnerability for developing clinical disorders later in life.

SUPPLEMENTARY TABLE 1 Associations between the intake of food groups and urinary iodine in early pregnancy in mothers of Dutch national origin (n=500).[1]

Food groups	β (95% CI)	P value[2]
Vegetables	0.02 (-0.28; 0.33)	0.89
Fruits	0.05 (-0.08; 0.17)	0.48
Potatoes	0.21 (-0.22; 0.64)	0.34
Legumes	2.08 (-0.46; 4.62)	0.11
Cereals, bread and other cereal products	0.61 (0.27; 0.95)	0.001
Cakes	-0.33 (-1; 0.33)	0.33
Sugar and confectionery	0.09 (-0.46; 0.64)	0.75
Vegetable oils	-2.31 (-6.02; 1.41)	0.22
Margarines	0.53 (-0.73; 1.79)	0.41
Butter	1.65 (-0.81; 4.11)	0.19
Milk	0.06 (-0.02; 0.14)	0.12
Dairy products	0.03 (-0.09; 0.15)	0.61
Fresh meat	-0.13 (-0.73; 0.47)	0.67
Processed meat	0.36 (-0.44; 1.15)	0.38
Eggs	1.87 (0.13; 3.62)	0.04
Fish and shellfish	-0.84 (-2.23; 0.55)	0.24
Sauces	0.01 (-0.82; 0.84)	0.99
Tea	0.02 (-0.03; 0.07)	0.36
Coffee	0.01 (-0.07; 0.09)	0.82
Soft drinks	-0.04 (-0.11; 0.04)	0.34
Fruit/vegetable juices	0.01 (-0.08; 0.1)	0.89
Alcoholic beverages	0.44 (-0.42; 1.3)	0.31
Soups and bouillon	-0.01 (-0.2; 0.17)	0.90
Miscellaneous	-0.04 (-0.87; 0.79)	0.92

Note: [1]Results from multivariable regression analyses.
[2]Adjusted for gestational age at urine sampling, maternal age, education, BMI, prenatal psychological problems and smoking and alcohol use.
BMI; Body Mass Index.

SUPPLEMENTARY TABLE 2 Factor loadings of food groups in dietary patterns in mothers of Dutch national origin (*n*=500).[1]

Food groups according EPIC	Mediterranean Pattern	P-value	Traditionally Dutch Pattern	P-value	Confectionary Pattern	P-value
	r_s		r_s		r_s	
Vegetables	0.65	<0.001	-0.10	0.02	0.01	0.77
Fruits	0.47	<0.001	-0.16	<0.001	0.34	<0.001
Potatoes	-0.15	<0.001	0.42	<0.001	-0.19	<0.001
Legumes	0.09	0.04	-0.21	<0.001	-0.08	0.06
Cereals, bread and other cereal products	0.23	<0.001	0.02	0.67	0.36	<0.001
Cakes	0.07	0.12	-0.08	0.08	0.72	<0.001
Sugar and confectionery	-0.18	<0.001	0.02	0.70	0.55	<0.001
Vegetable oils	0.56	<0.001	-0.01	0.77	-0.08	0.07
Margarines	-0.18	<0.001	0.24	<0.001	0.17	<0.001
Butter	0.12	0.01	-0.08	0.08	0.23	<0.001
Milk	-0.07	0.10	0.14	<0.001	-0.09	0.05
Dairy products	0.12	0.01	0.00	0.93	0.28	<0.001
Fresh meat	0.00	0.97	0.69	<0.001	-0.07	0.10
Processed meat	-0.21	<0.001	0.67	<0.001	0.10	0.02
Eggs	0.26	<0.001	-0.09	0.04	0.05	0.22
Fish and shellfish	0.61	<0.001	-0.16	<0.001	-0.01	0.86
Sauces	0.20	<0.001	0.15	<0.001	0.09	0.04
Tea	0.25	<0.001	-0.05	0.22	0.49	<0.001
Coffee	0.01	0.90	0.04	0.36	-0.02	0.59
Soft drinks	-0.03	0.51	0.19	<0.001	0.02	0.60
Fruit/vegetable juices	-0.19	<0.001	0.04	0.40	-0.05	0.24
Alcoholic beverages	0.11	0.01	-0.04	0.33	0.08	0.06
Soups and bouillon	0.10	0.02	0.10	0.03	-0.03	0.55
Miscellaneous	-0.05	0.23	-0.73	<0.001	-0.01	0.85

Note: [1]PCA was used as an extraction method in which the Spearman's Rank correlation coefficients represent the relative contribution of that food group to the identified dietary pattern. EPIC, European Prospective Investigation into Cancer and Nutrition; PCA, Principle Component Analysis.

SUPPLEMENTARY TABLE 3 Associations between adherence to major dietary patterns, maternal thyroid hormones and urinary iodine in mothers of Dutch national origin (n=500). [1]

	Urinary iodine, mmol/mol creatinine		FT4, pmol/L		TSH, mU/L	
Dietary pattern	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
Mediterranean	-3.09 (-18.3; 12.2)	0.69	0.07 (-0.2; 0.3)	0.64	-0.02 (-0.1; 0.1)	0.73
Traditional Dutch	10.89 (-3.6; 25.3)	0.14	-0.26 (-0.5; 0.0)	0.06	0.08 (-0.0; 0.2)	0.12
Confectionary	5.6 (-6.9; 18.1)	0.38	-0.06 (-0.3; 0.2)	0.58	0.03 (-0.1; 0.1)	0.95

Note: [1]Adjusted for gestational age at blood sampling, maternal age, education, BMI, psychological symptoms, smoking, alcohol use and protein intake. BMI, Body Mass Index; T4, free thyroxine; TSH, Thyroid stimulating hormone.

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Chapter 3

Brain morphology, temperament and executive function

Chapter 3.1

Infant structures, executive and Attention Deficit/Hyperactivity Problems

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ABSTRACT

Background Neuroimaging findings have provided evidence for a relation between variations in brain structures and Attention Deficit/Hyperactivity Disorder (ADHD). However, longitudinal neuroimaging studies are typically confined to children who have already been diagnosed with ADHD. In a population-based study, we aimed to characterize the prospective association between brain structures measured during infancy and executive function and attention deficit/hyperactivity problems assessed at preschool age.

Methods In the Generation R Study, the corpus callosum length, the gangliothalamic ovoid diameter (encompassing the basal ganglia and thalamus), and the ventricular volume were measured in 784 six-week-old children using cranial postnatal ultrasounds. Parents rated executive functioning at 4 years using the Behavior Rating Inventory of Executive Function-Preschool Version in five dimensions: inhibition, shifting, emotional control, working memory, and planning/organizing. Attention Deficit/Hyperactivity Problems were assessed at ages 3 and 5 years using the Child Behavior Checklist.

Results A smaller corpus callosum length during infancy was associated with greater deficits in executive functioning at 4 years. This was accounted for by higher problem scores on inhibition and emotional control. The corpus callosum length during infancy did not predict Attention Deficit/Hyperactivity Problem at 3 and 5 years, when controlling for the confounders. We did not find any relation between gangliothalamic ovoid diameter and executive function or Attention Deficit/Hyperactivity Problem.

Conclusions Variations in brain structures detectible in infants predicted subtle impairments in inhibition and emotional control. However, in this population-based study, we could not demonstrate that early structural brain variations precede symptoms of ADHD.

INTRODUCTION

Neuroimaging studies have provided evidence for a relation between variations in brain structures and neuropsychiatric disorders in children.[1] In children with Attention Deficit/Hyperactivity Disorder (ADHD) various morphological changes have been reported in brain structures such as the thalamus[2], striatum[3], ventricular volumes[3], right prefrontal cortex[4], and the corpus callosum[5]. Recently, longitudinal Magnetic Resonance Imaging (MRI) studies showed that growth trajectories of anterior corpus callosum were different in adolescents with ADHD, indicating anomalies in developmental brain trajectories in these children.[6] Few studies investigated the morphological variations in the brain structures related to ADHD symptoms in normally-developing children.[7] However, most prospective neuroimaging studies are confined to children who have already been diagnosed with ADHD.

Neuropsychological assessments of children with ADHD show that some have deficits in the meta-cognitive processes that control behaviour, known as executive functioning.[8] Although not a sufficient factor, executive dysfunction is an important component of the complex psychopathology underlying ADHD. Prospective neuroimaging studies of premature infants with white matter abnormalities followed until school age showed that executive function was affected even in the absence of ADHD.[9] However, it is unclear whether, within the general population, brain structural variations can be detected in infants who will show symptoms of executive dysfunction or ADHD later in life.

To elucidate whether brain structural differences precede psychopathology, we need neuroimaging studies of very young children free of behavioural problems. If brain structural differences precede symptoms of ADHD, children at risk for ADHD could be identified during infancy. In early childhood, the brain has a great plasticity and myelination is in progress.[10] Therefore, any intervention in that sensitive period could be more effective. Within this context, our goal was to characterize the prospective relation of variations in brain structures during infancy with executive function and attention deficit/hyperactivity problems assessed at preschool age. We measured the following brain structures using cranial ultrasounds at approximately seven weeks of postnatal life: the corpus callosum length, the gangliothalamic ovoid diameter (encompassing the basal ganglia and thalamus), and the cerebral ventricular volume. The choice of brain structures was based on the anatomical abnormalities reported in ADHD children and specific functions of brain structures. The corpus callosum is the largest white matter structure with a role of inter-hemisphere connectivity.[11] The size of corpus callosum may reflect the topographically connected cortical area relevant for higher-order cognitive function or a more efficient inter-hemispheric information transfer [12]. Basal ganglia have a role in disinhibition and planning and are partially involved in the regulation of attention and cognitive function.[11] Forming cortico-striato-thalamo-cortical loops, thalamus is involved in the behaviour regulation.[13] The cerebral ventricular volume has been associated with foetal maturation during gestation, and is a general parameter of global brain develop-

ment.[14] We hypothesized that the variations in the above-mentioned brain structures, which have been shown in children with ADHD, may already exist during infancy.

METHODS

Participants

The present study was embedded within the Generation R Study, a population-based cohort from foetal life onwards in Rotterdam, the Netherlands.[15] A subsample of 1106 children and their parents were assessed in detail postnatally. The eligibility criterion was Dutch ethnicity, defined as four grandparents born in the Netherlands, to exclude the confounding or effect modification by ethnicity. The study received approval from the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participating parents and anonymity was guaranteed.

Approximately at seven weeks, 904 (of 1106) neonates and their parents visited the research centre for detailed postnatal assessments. Because of potential differences in brain development of foetuses born to multiple and singleton pregnancies, we excluded 10 twin pairs. Of the remaining (n=884), we obtained ultrasound images with sufficient quality of one or more structures in 784 infants. Follow-up information on executive function was available in 655 children (83.5%). The corresponding numbers for Attention Deficit/Hyperactivity Problems at 3 and 5 years were 667 (85.1%) and 649 (82.8%), respectively.

Cranial Ultrasound Measurements

Postnatal cranial ultrasounds were performed in infants at the age of 6.8±1.9 wks (age range: 3.6-20.7 wks) with a commercially available multifrequency electronic transducer (3.7-9.3 MHz) with a scan angle of 146°, usable for 3-dimensional volume acquisition (Voluson 730 Expert, GE Healthcare, Waukesha, WI, USA). The details of ultrasound measurements have been described previously.[16-17] The probe was positioned on the anterior fontanel and a volume box was placed at the level of the foramen of Monro in a symmetrical coronal section. A pyramid-shaped volume of the brain tissue was scanned and the diameter of brain structures were measured offline. Two raters, trained by a neonatologist with expertise in neonatal cranial ultrasound imaging (P.G.), independently measured every image. Raters also coded the quality of the ultrasound image on a three-point scale, based on the ability to clearly delineate the boundaries of the structures. We excluded images with a quality rating of zero by both raters.

In the best mid-sagittal view, we defined the corpus callosum length as the largest diameter from rostrum to splenium (see Figure 1). Commonly, the thickness of corpus callosum, as measured by MRI, is used in neuroimaging studies [18]. However, with ultrasound techniques variations in the thickness of corpus callosum cannot be reliably measured [19]. Therefore,

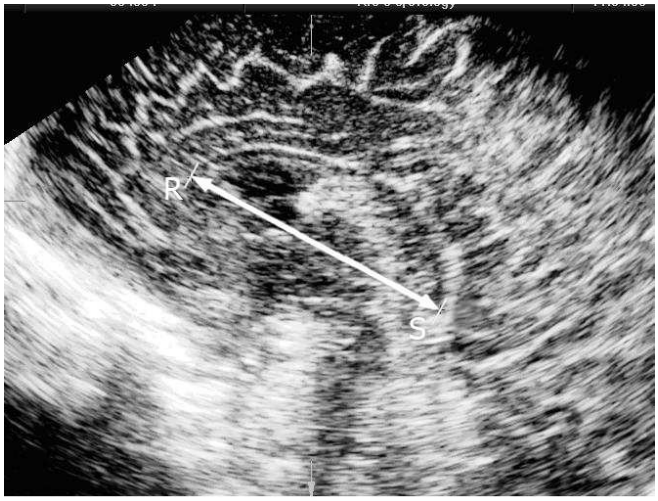

FIGURE 1 The corpus callosum length.
Note: The largest diameter was measured from rostum (R) to splenium (S).

we used the measurement along the entire body of the corpus callosum and obtained an average corpus callosum length using measurements from the two raters. The interrater reliability of the corpus callosum length was good (Cronbach's α=0.85 and, IntraClass Coefficient [ICC] =0.85).

The gangliothalamic ovoid diameter encompassed the following structures: basal ganglia (caudate nucleus, putamen, globus pallidus) and thalamus. Further details about the boundaries of these structures have been described previously.[20-21] The gangliothalamic ovoid is readily identified using ultrasound technology, largely because a parasagittal standard section through lateral ventricular and deep gray nuclei is reproducibly found. The interrater reliability of right and left gangliothalamic ovoid diameter was good (Cronbach's α=0.80 and, ICC =0.80).

To measure the ventricular system, the volume of the ventricular frontal horns, ventricular body, and trigone on both sides was quantified in millilitres. Further details about the measurement of ventricular system have been described elsewhere.[16] Four raters manually traced the left and right cerebral ventricles using a mouse driven cursor. Across the four raters, ICC for the right and left ventricle varied between 0.989-0.993 and 0.992-0.997, respectively.

Executive Functioning

When the children were 4.0 years (SD=1.0 month), the Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) was used to measure executive functioning.[22] The BRIEF-P is a parent-completed questionnaire to assess executive function behaviours in a broad age range of preschoolers. It contains 63 items within five related but non-overlapping theoretically and empirically derived clinical scales that measure children's ability in different

aspects of executive functioning: inhibition (16 items), to stop his/her own behaviour; shifting (10 items), to change focus from one mindset to another; emotional control (10 items), to modulate emotional responses; working memory (17 items), to hold information in mind to complete a task; planning/organization (10 items), to manage a current and future-oriented task demands within the situational context.

A sum score (the Global Executive Composite) can be derived by adding the scores of five domains. The clinical raw scores and the composite scores yield T scores based on gender and age. Higher scores indicate more problems with executive functioning.

The BRIEF-P measures executive functioning within a naturalistic setting and does not have the limitations of performance-based tests and environmental effects during the administration. Mahone and Hoffman compared scales of the BRIEF-P and performance-based executive function measures. They showed positive and consistent but non-significant correlations between parent-report and performance-based scales.[23] The subscales of BRIEF-P show adequate to high test-retest reliability and content validity indicating suitability for research purposes.[24]

Attention Deficit/Hyperactivity Problems

We used the Attention Deficit/Hyperactivity Problem subscale of the Child Behavior Checklist for toddlers (CBCL/1½-5) to acquire a standardized parent report of ADHD-like behaviour in children.[25] Attention Deficit/Hyperactivity Problem subscale has six items: 1) Cannot concentrate, cannot pay attention for long 2) Cannot sit still, restless, or hyperactive 3) Cannot stand waiting, wants everything now 4) Demands must be met immediately 5) Gets into everything and 6) Quickly shifts from one activity to another. The CBCL/1½-5 can be used to evaluate children suspected of having ADHD and determine the extent of a child's problems across a broad spectrum of syndromes.[26] The reliability and validity of the Dutch version of CBCL/1½-5 had been demonstrated previously.[27]

In the present study, the CBCL/1½-5 was completed, in the vast majority, by the mothers when the children were 3 years (36.4±1.0 months) and 5 years (70.5±2.4 months).

Covariates

Potential confounders were selected on the basis of background knowledge about the causal structure of the study question.[17, 28-29] Information on date of birth, gender and birth weight was obtained from midwives and hospital registries. Gestational age at birth was established using the ultrasound examination during pregnancy. Subsequent to brain ultrasound assessment, we measured fronto-occipital head circumference. Parity, maternal age, smoking, and education were assessed by questionnaires at enrolment. Maternal education was defined by the highest completed education and classified as primary (no or only primary education), secondary (lower or intermediate vocational education), and higher education (higher vocational education or university). We used the Brief Symptom Inventory to measure maternal

psychopathology during pregnancy.[30] This is a validated self-report questionnaire with 53 items that define a spectrum of psychiatric disorders. High validity and reliability have been reported for the Dutch translation.[30] We used the Edinburgh Postnatal Depression Scale, a widely used 10-item self-report scale, to assess symptoms of postnatal emotional distress.[31]

Statistical Analysis

Children with information on one or more brain structures were included in the analyses. The percentages of missing for the outcome variables were between 15% and 18%. Missing values of the covariates and the outcomes were imputed using multiple imputations. Ten 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. The analyses were repeated in the original and ten independent imputed datasets. Effect size and confidence intervals were estimated by taking the average effect size of the ten imputed datasets considering the uncertainty associated with the missing data (Supplementary table 1S and 2S present the results of the original dataset). We used independent sample t-test and chi-square statistics to explore whether the response was selective.

The executive function and Attention Deficit/Hyperactivity Problem scores were transformed (natural logarithm and square root, respectively) to satisfy the assumptions of normality. For descriptive purposes, we used the 98th percentile of a Dutch norm group as cut-off score to classify children as having behavioural problems within the clinical range.[27] Z scores were derived for the determinants to allow comparability of the regression coefficients. The associations between brain measurements and executive function or Attention Deficit/Hyperactivity Problem scores were analysed using multivariable linear regression. To avoid multiple comparisons, we first tested the association of brain structures with the Global Executive Composite score. Consequently, we explored whether any observed effect was accounted for by specific domains of executive function using the five subscales. As meta-analyses showed gender differences in ADHD symptoms,[32] we explored the statistical interaction between gender and brain measures.

We adjusted all analyses for head circumference at the time of ultrasound to ensure that the effects did not reflect the association with head size. The final models were adjusted for child's gender, gestational age at birth, age and head circumference at the time of ultrasound, maternal age, education, and smoking history. We ran the models additionally adjusted for maternal psychopathology during pregnancy and postnatal emotional distress. The results of the two latter models were reported separately to allow evaluation of the possible effect of adjustment.

We applied a Bonferroni adjustment to correct for multiple comparisons of three brain structures with the outcomes.

RESULTS

The children without successful brain ultrasounds (n=100, 11.3%) had a slightly greater birth weight than the children included (mean difference=126 grams, 95%CI: 17, 234, p=0.02). Non-response was not associated with gestational age at birth, parity, maternal age or maternal education. However, mothers of children excluded from the analyses were more likely to have smoked during pregnancy (21% vs. 11.8%; x^2=6.8(2), p=0.03). From hundred children excluded from analyses, follow-up data on the Attention Deficit/Hyperactivity Problems and executive function were available in 78 and 84 individuals, respectively. The scores on Attention Deficit/Hyperactivity Problem did not differ between children included in the analyses and those with unsuccessful ultrasound (mean difference=-0.24, 95%CI:-0.80, 0.33, p=0.79). Similar non-significant differences were observed for executive function scores (data not shown).

As expected, only few children scored above the 98th percentile (clinical range) of a Dutch norm for Attention Deficit/Hyperactivity Problems (n=10 at age 3 and n= 22 at 5 years). Table 1 summarizes the participants' characteristics. Boys had a slightly larger head than girls (mean difference=0.7 cm, 95%CI: 0.5, 0.9, p<0.001). There was no gender difference in the size of brain structures after co-varying for head circumference. The corpus callosum length and fronto-occipital head circumference were correlated, r=0.31 (p<0.001). The correlation between head circumference and the gangliothalamic ovoid diameter was r=0.52 (p<0.001); that between head circumference and the cerebral ventricular volume was r=0.27 (p<0.001).

TABLE 1 Participants characteristics

	Total Valid Observation (n=784)	Boys (n=401)	Girls (n=383)
Child			
Gestational age at birth, wk	784	40.0±1.8	40.1±1.5
Birth weight, g	784	3536±535	3468±509
Firstborn, %	782	63.1	60.1
Global Executive Composite Problem Score	655	88.4±17.1	82.5±13.5
Attention Deficit/Hyperactivity Problem scores, 3 yrs	649	2.6±2.1	2.8±2.2
Attention Deficit/Hyperactivity Problem scores, 5 yrs	667	3.0±2.5	2.3±2.2
Brain ultrasound measurements			
Age, wk	784	6.8±1.8	6.9±2.0
Fronto-occipital head circumference, cm	753	39.0±1.4	38.3±1.4
Corpus callosum length, cm	784	4.6±0.3	4.7±0.3
Gangliothalamic ovoid diameter, cm	784	4.3±0.2	4.3±0.2
Cerebral ventricular volume, ml	744	1.0±0.8	0.9±0.7

TABLE 1 Participants characteristics *(continued)*

	Total Valid Observation (n=784)	Boys (n=401)	Girls (n=383)
Mother			
Age at enrolment, yr	784	31.6±4.1	32.1±3.7
Education, %	773		
Primary		10.6	10.1
Secondary		53.3	49.9
High		36.1	40.1
Smoking, %	782		
Never		79.5	79.6
Until pregnancy was known		7.0	10.5
Continued during pregnancy		13.5	9.9
Psychopathology during pregnancy	731	0.20±0.3	0.17±0.2

Note: Numbers are mean ± SD unless otherwise is indicated.

There was a substantial correlation between Attention Deficit/Hyperactivity Problem scores at 5 years and domains of executive function: inhibition: r=0.58, shifting: r=0.21, emotional control: r=0.36, working memory: r=0.49, planning/organization: r=0.38, and the Global Executive Composite score: r=0.53. All the correlations were significant at p<0.001.

Table 2 presents the associations of postnatal brain measurements with executive function and Attention Deficit/Hyperactivity Problem scores in preschoolers. A smaller corpus callosum length predicted a higher Global Executive Composite problem score (adjusted B=-0.02, 95%CI: -0.04, -0.004, Bonferroni corrected p=0.05). In contrast, the gangliothalamic ovoid diameter and the cerebral ventricular volume were not related to executive function (adjusted B=0.002, 95%CI: -0.02, 0.032, p=0.88, and adjusted B=0.004, 95%CI: -0.02, 0.03, p=0.73, respectively). Next, we explored the association between brain measurement and Attention Deficit/Hyperactivity Problem scores. The corpus callosum length and the gangliothalamic ovoid diameter were not related to Attention Deficit/Hyperactivity Problem scores at 3 years (adjusted B=-0.03, 95%CI: -0.09, 0.03, p=0.40 and adjusted B=0.02, 95%CI: -0.05, 0.09, p=0.65, respectively). The corpus callosum length and the gangliothalamic ovoid diameter were associated with Attention Deficit/Hyperactivity Problems at 3 and 5 years. However, after adjustment for confounders, the corpus callosum length and gangliothalamic ovoid diameter did not predict Attention Deficit/Hyperactivity Problem scores at 5 years (B=-0.04, 95%CI: -0.11, 0.02, p=0.21; and B=-0.02, 95%CI: -0.10, 0.05, p=0.54, respectively). The cerebral ventricular volume was not related to Attention Deficit/Hyperactivity Problems.

TABLE 2 Postnatal brain ultrasound measurements, executive functioning, and attention deficit/hyperactivity problems at preschool age.

	Executive Functioning at 4 yrs			Attention Deficit/Hyperactivity Problem Scores at 3 yrs			Attention Deficit/Hyperactivity Problem Scores at 5 yrs		
Ultrasound Measurements (per SD)[a]	Beta	B (95% CI)	p	Beta	B (95% CI)	p	Beta	B (95% CI)	p
Corpus callosum length									
Unadjusted	-0.10	-0.02 (-0.04, -0.005)	0.01	-0.08	-0.06 (-0.12, -0.004)	0.04	-0.10	-0.08 (-0.15, -0.02)	0.01
Adjusted	-0.09	-0.02 (-0.04, -0.004)	0.02	-0.03	-0.03 (-0.09, 0.03)	0.40	-0.05	-0.04 (-0.11, 0.02)	0.21
Gangliothalamic ovoid diameter									
Unadjusted	-0.04	-0.01 (-0.03, 0.01)	0.43	-0.08	-0.06 (-0.12, -0.003)	0.04	-0.08	-0.07 (-0.13, -0.01)	0.03
Adjusted	-0.01	0.002 (-0.02, 0.03)	0.88	0.02	0.02 (-0.05, 0.09)	0.65	-0.03	-0.02 (-0.10, 0.05)	0.54
Cerebral ventricular volume									
Unadjusted	0.01	0.002 (-0.02, 0.02)	0.87	0.01	0.003 (-0.06, 0.07)	0.92	0.01	0.01 (-0.06, 0.08)	0.79
Adjusted	0.02	0.004 (-0.02, 0.03)	0.73	0.03	0.03 (-0.04, 0.09)	0.41	0.02	0.02 (-0.05, 0.08)	0.65

Note: [a] Z scores were derived to make regression coefficient comparable.

Models were adjusted for child's gender, gestational age at birth, age and head circumference at the time of brain ultrasound, maternal age, education, and smoking during pregnancy.

The B's are not interpretable since the mathematically transformed scores were used in the analyses.

TABLE 3 Postnatal corpus callosum length and executive function at 4 years.

	Executive Function								
Ultrasound Measurements (per SD)[a]									
	Inhibition			Shifting			Emotional Control		
	Beta	B (95% CI)	p	Beta	B (95% CI)	p	Beta	B (95% CI)	p
Corpus callosum length									
Unadjusted	-0.10	-0.02 (-0.04, -0.004)	0.02				-0.08	-0.02 (-0.04, -0.001)	0.05
				-0.04	-0.01 (-0.03, 0.01)	0.27			
Adjusted	-0.09	-0.02 (-0.04, -0.002)	0.03	-0.07	-0.02 (-0.03, 0.004)	0.12	-0.08	-0.02 (-0.04, 0.00)	0.05
	Working Memory			Planning/Organization					
	Beta	B (95% CI)	p	Beta	B (95% CI)	p			
Corpus callosum length									
Unadjusted	-0.10	-0.02 (-0.04, -0.01)	0.01	-0.08	-0.02 (-0.03, -0.002)	0.03			
Adjusted	-0.07	-0.01 (-0.03, 0.001)	0.07	-0.07	-0.01 (-0.03, 0.002)	0.10			

Note: [a] Z scores were derived to make regression coefficient comparable.

Models were adjusted for child's gender, gestational age at birth, age and head circumference at the time of brain ultrasound, maternal age, education, and smoking during pregnancy.

The B's are not interpretable since the mathematically transformed scores were used in the analyses.

In further analyses, we explored the associations between the corpus callosum length and five domains of executive function to see which domain accounted for the observed association (Table 3). A smaller corpus callosum length predicted a higher problem score on inhibition (adjusted B=-0.02, 95%CI: -0.04, -0.002, p=0.03) and emotional control (adjusted B=-0.02, 95%CI: -0.04, 0.00, p=0.05).

There was no significant interaction between gender and brain structures in predicting Attention Deficit/Hyperactivity Problems and executive functioning (data not shown).

To explore whether postnatal emotional stress affects the relation of postnatal corpus callosum length with executive function or the Attention Deficit/Hyperactivity Problems, we adjusted all models for maternal postnatal emotional stress. After adjustment, the corpus callosum length was related to the Global Executive Composite scores (B additionally adjusted for maternal emotional stress=-0.02, 95%CI: -0.04, -0.002, p=0.03). The relation between the corpus callosum length and the Attention Deficit/Hyperactivity Problems at 3 and 5 years remained non-significant after additional adjustment.

Next, we reran the analyses between the corpus callosum length and executive function additionally adjusted for maternal psychopathology during pregnancy. When adjusted for maternal psychopathology, the association between the corpus callosum length and the Global Executive Composite scores remained unchanged (B=-0.02, 95%CI: -0.05, 0.00, p=0.05).

DISCUSSION

This study presents the population-based prospective data of a large number of infants followed until preschool age. We found an association between a smaller corpus callosum length in infancy and impaired executive function at 4 years. This association was accounted for by higher scores of inhibition and emotional control, indicating more problems. However, we found no indication for a relation between infant brain structures and Attention Deficit/Hyperactivity Problems.

Our study provides no support for an association between postnatal corpus callosum length and Attention Deficit/Hyperactivity Problems at preschool years. This is not consistent with findings of previous studies in children with clinical diagnosis of ADHD showing abnormalities in the corpus callosum size.[5, 33] In our general population sample, although some children showed symptoms of inattention or hyperactivity, the degree of problem behaviours were in general well below the clinical threshold. Furthermore, as opposed to the above-mentioned studies with concurrent assessment of brain structures and ADHD symptoms, our study had a prospective design. Postnatal brain measurements precede the Attention Deficit/Hyperactivity symptoms. Therefore, any interpretation of these negative findings should be done cautiously.

We found that a smaller corpus callosum length during infancy predicted poorer executive functioning, in particular, inhibition and emotional control. This is compatible with findings of studies in preterm or very low birth weight infants that show corpus callosum abnormalities predict executive dysfunction in all domains.[9] The corpus callosum length in early postnatal period largely reflects the development of this structure during foetal life. However, in our relatively healthy sample of neonates indicators of prematurity such as birth weight did not account for the relation between postnatal corpus callosum length and executive dysfunction. A shorter corpus callosum length may reflect a global reduction in white matter size, either primary or secondary to grey matter size alternation. The length of corpus callosum is an indicator of axon numbers and extent of myelination, both crucial for information transfer and connectivity in the brain. Additionally, variations in the corpus callosum size may influence the downstream pruning in the very plastic brain during infancy, e.g. the fibres connected to the corpus callosum may be more likely to be pruned. Although this is speculative, the possible cascade of events in brain during early development preceding the symptoms of ADHD will increase our understanding the pathophysiology underlying the disorder.

In our sample, postnatal gangliothalamic ovoid diameter was not associated with executive dysfunction or Attention Deficit/Hyperactivity Problem in preschoolers. This is in contrast with previous findings on the key role of prefrontal-basal ganglia-thalamic loop in executive dysfunction of ADHD children.[33] However, the explanation may lie in the fact that different sub-circuits are responsible for symptoms of ADHD. [2] Thus, the absence of a significant association may derive from the fact that ultrasound is not the optimal technique to detect structural variations if abnormalities are restricted to the small substructures. Additionally, after postnatal age, through the process of learning, the brain undergoes changes in numbers of neurons and synapses; which may explain the absence of expected variations during infancy.

In our study, cerebral ventricular volume was not significantly associated with executive function and Attention Deficit/Hyperactivity Problems. Previous longitudinal studies in preterm children showed that ventricular enlargement during infancy predicts executive function at 4 years.[9] However, epidemiological studies in the general population demonstrated that the size of the ventricular volume within the normal range is determined by the maturation during foetal life.[14] Cerebral ventricular volume in healthy newborns and ventricular enlargement in the clinical population may have a different underlying pathophysiology. Furthermore, prior studies reveal that the increase in cerebral ventricular volume, as seen in the normal children with increase in age, diminishes in ADHD children.[3] This may indicate that the growth pattern rather than the size of ventricles is predictive of future behaviour impairment.

Findings from anatomical MRI studies in ADHD children provide evidence for involvement of different brain structures.[33-34] However, findings are consistent only for involvement of striatum.[35] Results are mixed regarding other brain regions. For example, children with

ADHD do not differ from controls in absolute corpus callosum size[3], despite different growth trajectories of corpus callosum[6]. Or, whole thalamic volumes are not different in ADHD children and controls; although mapping of the thalamic surface showed that ADHD children had smaller regional volumes bilaterally than controls[2]. These findings imply that defining a robust neuroanatomical marker for ADHD is complex and may not be easy to achieve.

Although our study has several strengths such as large population-based sample, the unique prospective design, and measurement of brain structures in infants, we were faced with certain methodological limitations. First, we measured the brain structures using cranial ultrasound that cannot provide detailed images of specific substructures in the brain. Although cranial ultrasound in neonates has limited value in reflecting variations in the brain structures as compared to MRI[19], it is a reliable, non-invasive, and cost-effective technique to image infants and can be used in follow-up studies of healthy infants[36]. Second, we measured the corpus callosum across the entire length; whereas the corpus callosum area may be a better indicator of size. The corpus callosum area cannot be measured reliably by cranial ultrasound. However, studies have reported a good correlation between the corpus callosum length and thinness.[37] Third, we used parent report of executive function and Attention Deficit/Hyperactivity Problems. Parents may be affected by many factors in reporting child's behavior which introduce bias.[38] However, parent's reports on child behavior are based on their observation in a naturalistic setting and for a long period, and serves as an inexpensive and easy-to-administer method suitable for research purposes. Fourth, we did not have a clinical diagnosis of ADHD in our sample of children. However, it is very likely that the symptoms of hyperactivity or inattention during preschool period stay persistent to older age, when ADHD can be validly diagnosed in children.[39] Considering the fact that we had a population-based sample, with relatively small number of children meeting the criteria for clinical diagnosis of ADHD, and used ultrasound as measurement technique for brain structures, null findings regarding to attention deficit/hyperactivity problems do not rule out a possible relation between early structural differences in the brain and ADHD. On the other hand, the association of the corpus callosum length with executive functioning shows that the study was well-powered and the measures were sensitive enough to detect an expected association.

SUPPLEMENTARY TABLE 1 Postnatal brain ultrasound measurements, executive functioning, and attention deficit/hyperactivity problems at preschool age (Complete case analyses).

	Executive Functioning at 4 yrs			Attention Deficit/Hyperactivity Problem Scores at 3 yrs			Attention Deficit/Hyperactivity Problem Scores at 5 yrs		
Ultrasound Measurements (per SD)[a]	Beta	B (95% CI)	p	Beta	B (95% CI)	p	Beta	B (95% CI)	p
Corpus callosum length									
Unadjusted	-0.11	-0.02 (-0.04, -0.01)	0.01	-0.07	-0.16 (-0.33, 0.01)	0.07	-0.10	-0.24 (-0.43, -0.05)	0.01
Adjusted	-0.09	-0.02 (-0.03, -0.002)	0.03	-0.02	-0.05 (-0.23, 0.13)	0.59	-0.05	-0.13 (-0.33, 0.07)	0.20
Gangliothalamic ovoid diameter									
Unadjusted	-0.04	-0.01 (-0.02, 0.01)	0.39	-0.08	-0.17 (-0.34, -0.001)	0.05	-0.07	-0.17 (-0.36, 0.02)	0.09
Adjusted	0.02	0.004 (-0.01, 0.02)	0.63	0.01	0.02 (-0.18, 0.22)	0.84	-0.02	-0.05 (-0.27, 0.18)	0.68
Cerebral ventricular volume									
Unadjusted	0.00	0.00 (-0.02, 0.02)	0.99	-0.03	-0.06 (-0.24, 0.11)	0.48	-0.02	-0.05 (-0.26, 0.15)	0.60
Adjusted	0.02	0.004 (-0.01, 0.02)	0.60	0.01	0.03 (-0.16, 0.21)	0.77	-0.01	-0.03 (-0.23, 0.18)	0.80

Note: [a]Z scores were derived to make regression coefficient comparable.

Models were adjusted for child's gender, gestational age at birth, age and head circumference at the time of brain ultrasound, maternal age, education, and smoking during pregnancy.

The B's are not interpretable since the mathematically transformed scores were used in the analyses.

SUPPLEMENTARY TABLE 2 Postnatal corpus callosum length and executive function at 4 years (Complete-case analyses).

	Executive Function								
Ultrasound Measurements (per SD)[a]									
	Inhibition			Shifting			Emotional Control		
	Beta	B (95% CI)	p	Beta	B (95% CI)	p	Beta	B (95% CI)	p
Corpus callosum length									
Unadjusted	-0.11	-0.02 (-0.03, -0.005)	0.01	-0.03	-0.01 (-0.02, 0.01)	0.42	-0.09	-0.02 (-0.03, -0.001)	0.03
Adjusted	-0.09	-0.02 (-0.03, -0.001)	0.04	-0.06	-0.01 (-0.02, 0.004)	0.17	-0.09	-0.02 (-0.04, -0.001)	0.04
	Working Memory			Planning/Organization					
	Beta	B (95% CI)	p	Beta	B (95% CI)	p			
Corpus callosum length									
Unadjusted	-0.10	-0.02 (-0.03, -0.03)	0.02	-0.07	-0.01 (-0.03, 0.001)	0.07			
Adjusted	-0.06	-0.01 (-0.03, 0.004)	0.16	-0.05	-0.01 (-0.03, 0.005)	0.20			

Note: [a] Z scores were derived to make regression coefficient comparable.

Models were adjusted for child's gender, gestational age at birth, age and head circumference at the time of brain ultrasound, maternal age, education, and smoking during pregnancy.

The B's are not interpretable since the mathematically transformed scores were used in the analyses.

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Chapter 3.2

Positive emotionality, executive functioning, and internalizing problems

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Chapter 4

Longitudinal course of behavior and cognition in childhood

Chapter 4.1

The stability of autistic symptoms in the general population

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Chapter 4.2

Early language development and risk of verbal and nonverbal cognitive delay at school age

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Chapter 5

Discussion

Chapter 5.1

Is measurement of maternal serum TSH sufficient screening in early pregnancy?

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SUMMARY

During the past decades, observational studies have demonstrated a relation between thyroid dysfunction in pregnancy and a range of adverse outcomes in mother and offspring. However, results of the few performed randomized trials of screening for thyroid dysfunction in pregnant women did not show any benefit for women and their children. Before implementing screening in pregnant women at population level, randomized trials are needed to show that screening with subsequent intervention is effective for mothers or children. Here, we review the literature and argue that the findings from existing trials are not conclusive. Until conclusive evidence from randomized trials is available, screening of high risk pregnant women only is the best advice to the clinician. Only high risk women, i.e. those with symptoms or a history of thyroid problems should be screened using trimester-specific reference ranges for TSH levels. We recommend new prospective randomized trials that combine different thyroid parameters as screening tool, apply trimester-specific ranges for thyroid parameters, and examine whether screening and intervention during the first trimester of pregnancy will improve neuropsychological abilities in the offspring.

INTRODUCTION

During the past decades, observational studies have demonstrated a relation of thyroid dysfunction in pregnant women with a range of adverse pregnancy outcomes and late child neurodevelopmental problems.[1-2] In view of these findings, many researchers have discussed a need to implement early screening for thyroid dysfunction during pregnancy.[3-6] Although screening for thyroid dysfunction in high risk pregnant women has recently been recommended by some experts, it has not yet been approved for application at population level (universal screening).[7] There are many concerns about the necessity of universal and high risk population screening, the best screening criteria, optimal time for screening, and possible interventions in screen-positive pregnant women.

CURRENT EVIDENCE

Maternal overt thyroid dysfunction during pregnancy, if untreated, has serious adverse effects on mother and child.[1, 8] These effects are more prominent if thyroid dysfunction occurs during first trimester of pregnancy.[9] The consequences of milder forms of thyroid dysfunction such as subclinical hypothyroidism (TSH levels above and a free T4 level within the normal range) and hypothyroxinaemia (free T4 in the lower 5th or 10th percentile of trimester-specific reference values in conjunction with normal TSH) are less clear.[10] Observational studies reported

that women with hypothyroxinaemia in early pregnancy are at higher risk of having children with expressive language delay[11], delayed mental and motor function[12], low IQ[13], and poor neonatal orientation.[14] However, other studies failed to show a relation between non-clinical dysfunction of maternal thyroid gland and child cognitive and behavioural problems.[2, 15-16] Women with thyroid autoimmunity are also at higher risk of pregnancy complications[17], depression[16], or having a child with behavioural and cognitive problems.[18-20] The adverse effect of thyroid autoimmunity during pregnancy is probably independent of maternal thyroid status.

Up to date, there have been two large randomized trials that investigated the effect of early pregnancy screening and intervention for thyroid dysfunction on various child outcomes.[21-22] In 4562 pregnant women, Negro and colleagues compared two approaches of universal screening and case-finding to detect thyroid dysfunction during pregnancy.[21] In this study, all women in the universal screening group and high-risk women in the case-finding group underwent immediate screening for thyroid dysfunction. The criteria for being screen-positive was defined as either 1) TSH above 2.5 mIU/L plus Thyroid Peroxidase Antibodies (TPOAbs)-positive or 2) undetectable TSH plus elevated free T4 in the first trimester of pregnancy. The treatment goal for hypothyroid cases was to maintain plasma TSH less than 2.5 mIU/L in the first trimester and less than 3.0 mIU/L in the following trimesters of pregnancy. Treatment of hyperthyroid cases was performed based on clinical judgement. The study was conducted in the south of Italy, which is considered a mildly iodine-deficient area. In this study, performing universal screening did not decrease the rate of obstetric and neonatal complications as compared to case-findings approach. However, post-hoc analyses showed that women at low risk for thyroid dysfunction in the universal screening group (who had abnormal thyroid function and received treatment because of the trial) had lower rate of adverse effects than the same women in case-finding group (who were not detected during pregnancy and, thus, had no treatment).

In a second large randomized trial in 21846 pregnant women and their children, Lazarus and colleagues examined the effect of antenatal screening and treatment for thyroid dysfunction on child cognitive functioning.[22] They chose cut-offs for positive screening as TSH above 97.5th percentile, free T4 below 2.5th percentile or both. Treatment in screen-positive women was adjusted to achieve the TSH levels between 0.1 to 1.0 mIU/L. They performed an intention-to treat analysis and showed that maternal screening for thyroid dysfunction and early treatment did not improve cognitive outcomes of the child at age three years.

CURRENT RECOMMENDATION FOR THYROID SCREENING

The guideline of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease during Pregnancy[7] recommend using trimester-specific reference values

for thyroid parameters (i.e. free T4 and TSH) to diagnose any dysfunction. In this guideline, the authors describe serum TSH as the most accurate indicator of thyroid dysfunction in pregnancy. The guideline includes recommendations for screening of thyroid dysfunction during pregnancy. They suggest no universal screening using plasma TSH or detection of isolated hypothyroxinaemia because of lack of evidence. Only verbal screening for a history of thyroid dysfunction and thyroid medication in the first prenatal visit is recommended. Some authors of the guideline suggest that serum TSH should be obtained to screen for overt hypothyroidism in high risk pregnant women. They give several criteria to define high risk for thyroid dysfunction such as 1) symptoms or positive history for thyroid disease/surgery, 2) TPOAbs-positivity, 3) autoimmune diseases, 4) past head and neck radiation, 5) family history of thyroid diseases and 6) use of specific drugs.[7] The suggested criteria were based on clinical consensus with no clear evidence for the effectiveness of a high risk approach. The more lenient criteria that were also recommended, e.g. maternal age above 30 years or morbid obesity, are very debatable.

Although the American Thyroid Association provides the clinicians with a comprehensive guideline on management of thyroid dysfunction in pregnant women, there remains a knowledge gap regarding the screening of thyroid dysfunction during pregnancy.

FUTURE INVESTIGATION

Here are some careful suggestions how future randomized trials can address these issues.

First, criteria for being screen-positive should be well defined. Recently, the American Thyroid Association guideline set a specific reference value for TSH during pregnancy and recommended that serum TSH is the best indicator of thyroid dysfunction. Nonetheless, findings from observational studies imply the importance of maternal subclinical hypothyroidism, hypothyroxinaemia and thyroid autoimmunity during pregnancy. In these conditions, measurement of serum TSH is not sufficient for diagnosis. We recommend that the future randomized trials apply the combination of three thyroid parameters (serum TSH, free T4, and TPOAbs) as screening tool in order to optimize the criteria for screen-positive thyroid dysfunction during pregnancy. Second, the best period during pregnancy to perform screening for maternal thyroid dysfunction should be defined. The foetal consequences are more pronounced if thyroid dysfunction occurs during early pregnancy. We suggest that future randomized studies perform screening in the most critical period (that means include only in the first trimester of pregnancy), since pregnant women and their children have a higher chance of benefiting from any intervention and early treatment during this period. Third, observational studies have suggested that thyroid dysfunction is related to different maternal and child adverse outcomes. [2, 17, 20, 23-24] There is not enough evidence for specificity of neuropsychological measures in relation to thyroid dysfunction. Thus, we recommend using

IQ or an overall score in neuropsychological batteries (such as NEPSY II) as the main child outcomes to avoid multiple testing. Fifth, recommendations for using cut-off values to define different status of thyroid function is based on information from women with sufficient iodine intake during pregnancy.[10] Therefore, we recommend studies in iodine-sufficient area and in women with adequate iodine supplementation to reduce the effect of iodine deficiency. Levothyroxine has been accepted as the treatment choice of low thyroid function during pregnancy and adjusted dose should be applied to reach a trimester-specific reference range for TSH.[7] The last, but not the least, trials with multiple arms should be avoided because of multiple comparisons and the decrease of power in the study.

The findings randomized trials will allow experts to answer the existing question on the efficacy of population level screening for thyroid dysfunction during pregnancy. Trials will also provide further evidence for the best thyroid parameter to be used as screening tool and the optimal timing of screening in pregnancy.

MESSAGE TO THE CLINICIAN

Up to now, two large randomized studies did not show any advantage of screening for thyroid dysfunction at population level. These findings, however, do not rule out that pregnant women could benefit from early detection of thyroid dysfunction and intervention. Until conclusive evidence from randomized trials, the recommendation of American Thyroid Association on screening of only high risk pregnant women is the best advice to the clinician. Based on exiting evidence, trimester-specific reference ranges for TSH levels should to be used as screening tool in the high risk group. New prospective randomized trials are recommended which combine different thyroid parameters as screening tool, apply trimester-specific ranges for thyroid parameters and examine whether screening and intervention during first trimester of pregnancy in women with adequate iodine supplementation will improve neuropsychological abilities in the offspring.

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Chapter 5.2

General Discussion

GENERAL DISCUSSION

The prenatal period and early childhood are considered as "windows of plasticity" for the brain. During this period, negative environmental factors can adversely influence the brain's structure and function, which may consequently lead to psychopathology in children.[1] Understanding neurobiological pathways to child psychopathology is important in order to determine modifiable risk factors and to provide information for preventive interventions.

This thesis aimed to examine the main pre and postnatal risk factors for children's problem behavior and cognitive impairment. Furthermore, the longitudinal course of autistic symptoms and language skills from the preschool period to school age are described. The studies presented in this thesis were embedded within the Generation R, a population-based prospective study that tracks children from fetal life onwards in Rotterdam, the Netherlands. In this chapter, the main findings of this research are discussed and major methodological issues in longitudinal studies of childhood psychopathology are addressed. The clinical implications of findings are presented along with considerations for future studies.

MAIN FINDINGS

Intrauterine effect: thyroid hormones

Adverse consequences of maternal thyroid dysfunction in pregnancy on brain development during childhood have been widely studied in animals and humans.[2-4] Studies in animals revealed that thyroid hormone insufficiency during prenatal life affects the fetal brain in different ways (i.e. a decline in numbers of neural cell; abnormal synaptogenesis, dendritic arborizations, and cell migration patterns; and a decrease in neural myelination).[5-6] In early pregnancy, the fetal thyroid gland is not fully mature, thus maternal free thyroxine (T4) is the only source of thyroid hormones for the developing fetus.[7] Previous studies showed that low levels of maternal free T4, even within the normal range, adversely influence a child's normal cognitive development.[8] Here, we focus on the behavioral outcomes of maternal thyroid insufficiency during pregnancy and discuss the role of autoimmunity and iodine insufficiency in this relation.

Molecular studies in animals suggest that abnormalities resulting from thyroid hormone insufficiency occur in multiple brain structures including the cerebellum, the neocortex, the hippocampus and myelinated white matter tracts such as the corpus callosum.[9-10] Few imaging studies in humans have confirmed these findings.[11] Based on the similarities between the neuropathy observed in children with autism and abnormalities in brain cytoarchitecture reported in animals due to thyroid hormone insufficiency, some researchers have postulated a possible effect of maternal thyroid insufficiency on the risk of autism.[12-13] Furthermore, clinical studies have shown that individuals with a generalized resistance to thyroid hormones

are at a greater risk of Attention Deficit/Hyperactivity Disorders (ADHD).[14] Prospective studies of the association between prenatal thyroid hormone insufficiency (due to autoimmunity or iodine insufficiency) and a child's risk of ADHD or autism are sparse.[15]

In Chapter 2, we showed that higher levels of maternal Thyroid Stimulating Hormones (TSH) during pregnancy predicted a higher externalizing score in the offspring up to age 3 years. The linear relation between maternal TSH and externalizing problems was present across the range of TSH, indicating that subtle impairments of maternal thyroid function may adversely affect normal brain development. Post-hoc analyses revealed a relation between maternal TSH during pregnancy and odds of having attention deficit/hyperactivity problems up to age 3 years. An effect of subtle variations in thyroid function on the child's risk of externalizing problems is plausible considering the role of thyroid hormones in the normal development of cortical layers that are responsible for regulation of inhibitory processes. Our finding that low maternal urinary iodine levels during pregnancy are related to the child's inhibition problems support the role of thyroid hormones in regulation of inhibitory processes underlying ADHD. We also found a relation between maternal TPOAbs status during pregnancy and the child's risk of attention deficit/hyperactivity problems. The relation between maternal TPOAbs and a child's problem behavior was only partially explained by maternal thyroid function. This finding may indicate that autoimmunity plays a role in the etiology of ADHD, above and beyond the effect of thyroid hormone insufficiency.

We found a relation between maternal severe hypothyroxinemia in pregnancy and different measures of autistic symptoms in children at age 6 years. During midgestation, late-born neurons migrate past early-born neurons and take their positions in the cortex in an 'inside-out' sequence, forming six cortical layers. Cajal-Retzius neurons in layer I are the first neurons to populate the mantle of the cortex; these neurons produce and secrete reelin. Reelin is an extracellular glycoprotein that binds to membrane receptors on migrating neurons, which phosphorylates the Disabled homolog-1 (dab1) to stop neuronal migration.[16] This reelin-dab signaling system is dependent on thyroid hormones.[17] Any disruption in the reelin-dab signaling system may lead to neuropathological abnormalities in the brain, and consequently autistic symptoms in the child.[18]

To summarize, we found relations between different indicators of low thyroid function in mother during pregnancy (i.e. high TSH levels, hypothyroxinemia, positive TPOAbs, or low urinary iodine levels) and child's outcomes such as executive function, attention deficit/hyperactivity problems and autistic symptoms. The consistent findings on the relation of low maternal thyroid function, and cognition and behavior during childhood indicate the importance of thyroid hormones in brain development during fetal life. Importantly, these indicators were not simultaneously related to the outcomes in every single study. This suggests that there is no specific marker of low thyroid function in pregnancy. Nevertheless, it is suggested that free T4 values can be affected by albumin and Thyroid Binding Globulin levels during pregnancy. Thus, free T4 levels may not be a reliable measure of maternal thyroid

dysfunction during pregnancy. Serum TSH, in contrast, can be a better marker of maternal thyroid function status because of the delicate feedback mechanism of the pituitary gland. In practice, serum TSH is recommended for screening in pregnant women at high risk of thyroid dysfunction.[19]

Child factors: postnatal brain morphology, temperament, and executive function

Individual differences in temperament and executive functioning define a child's interaction with the environment and may eventually lead to the development of psychopathology.[20-21] In Chapter 3, childhood characteristics, including temperament and executive functioning, were studied in relation to psychopathology.

We investigated the prospective relation of corpus callosum length during infancy and executive function and attention deficit/hyperactivity problems during childhood. A smaller corpus callosum length predicted inhibition and emotional control problems at preschool age. Despite moderate-to-strong correlations between scores of executive functioning problems and attention deficit/hyperactivity problems, postnatal brain morphology did not predict attention deficit/hyperactivity problems at age 6 years. To our knowledge, this study has been the first to measure brain morphology before the onset of any symptoms of attention deficit/hyperactivity. Previous studies used concurrent assessment of brain structures and ADHD and reported abnormalities in the corpus callosum in samples of children with clinical diagnosis of ADHD.[22-23] The mixed findings regarding abnormalities in the absolute size of brain structures such as the thalamus[24] or different growth patterns (e.g. in the corpus callosum) in children with ADHD compared to normally developing controls[25] imply that defining a robust neuroanatomical marker for ADHD is complex and not a trivial undertaking. Using a prospective design with brain imaging before the development of ADHD is a way to tackle the challenge. In particular, studies that investigate anatomical brain abnormalities in relation to incident cases of psychopathology in the general population are important to unravel the predictive values of neuroanatomical markers for ADHD.

In an attempt to explain the complex pathway between temperament at young age and future psychopathology, we studied the longitudinal relation of positive emotionality at preschool age with executive functioning and internalizing problems at 4 and 6 years of age. The prospective relation was controlled for psychopathology at baseline. We hypothesized that a child's low positive emotionality predicts internalizing problems during school period. Executive function was anticipated to be one possible pathway for this relation. Children with lower levels of positive emotionality had a higher risk of having withdrawn problems. Problems in shifting domains but not other executive function domains mediated this relation. Low levels of positive emotionality in young children can result in children's inflexibility and rigidity later in life. The inflexibility and rigidity are likely to affect the child's drive to engage with the environment, and thereby lead to withdrawn problems. This finding is consistent with the vulnerability hypothesis behind the association between temperament and

psychopathology.[21] However, we cannot entirely rule out that low positive emotionality and withdrawn problems are the extremes of a trait continuum (spectrum hypothesis). For in an extensive discussion, see below under section Cake or comorbid bread and fudge?

Longitudinal course of developmental psychopathology

Early symptoms of social and communication problems are always a concern for parents and health care providers.[26] If parents recognize problems in social and communication skills in their children, they may seek mental health services. In theory, some children may benefit from early detection and intervention of such communication problems.[27] However, in very young children, the degree of stability in social and communication problems is not well documented. In particular, the stability of social and communication delay in the general population is understudied. Within the general population, many children with social and communication problems at very young age catch up with their peers at school age. For example, studies of preschoolers with isolated expressive language delay have shown that the majority of children are not classified as delayed anymore when they enter school.[28] Few reports from the general population have demonstrated a relatively high stability for autistic symptoms from childhood through early adolescence.[29] To our knowledge, no population-based study has examined the stability of autistic symptoms in very young children (i.e. younger than two years at first symptoms). In addition, to investigate the longitudinal course and stability of developmental delays associated with communication at young age, we followed up children from age of 1½ years through age 6 years and examined both their language skills and their autistics symptoms (Chapter 4.1 and 4.2). We observed that vocabulary skills at 2½ years were better predictors of language comprehension at school age when compared to expressive and receptive language skills at 1½ years. When the children enter school, demographic factors such as maternal education or family income appear to play a more important role in predicting language skills than early developmental factors.

Regarding autistic symptoms, we identified four groups of children based on autistic symptom profiles: the children with 1) No/few problems 2) Flexibility problems 3) Social/communication problems and 4) social/communication problems, fixated interest, flat affection and speech problems. The last group was termed as 'Pervasive developmental problems'. We found that the stability of the symptom profile 'pervasive developmental problems' was only moderate during the preschool period. In other words, children in this group with pervasive developmental problems displayed different symptoms profiles from ages 1½-to-6 years. It is important to note that despite moderate stability of the symptoms profile 'pervasive developmental problems', very few children from the group 'Pervasive developmental problems' at 1½ years presented with 'No/few problems' at 6 years. Rather, most of these children presented with symptoms profiles such as social communication problems or flexibility problems at 6 years. In line with findings from clinical studies in toddlers, which showed that symptoms of social impairments at early age are likely to persist, we found that the symptom

profile 'Social/communication problems' was the most stable parent reported symptom profile. In our sample, the least stable symptom profile was 'Inflexibility problems'. The latter finding may well reflect normal development. Many normally developing children aged 1½ years are upset or disturbed in a new situation or in contact with strangers, whereas, this pattern of behavior is much less typical in similar situations for older children.

METHODOLOGICAL CONSIDERATIONS

Analysis of correlated data in longitudinal studies

Assessing behavior longitudinally in epidemiological studies has had a dramatic impact on child psychiatric research. Assessment of children's behavior and cognition at different time points allow the researcher to study changes within individuals over time under a variety of different conditions.[30] Behavioral development in children is an ongoing process over time, with trajectories that vary individually. Studying within individual differences in childhood behavior without the noise arising from between individual differences can be highly informative, if the interest lies in a change in response to time or under a certain condition.[31] Nevertheless, the correlation between multiple observations of a certain behavior can be challenging from a data analysis prospective. Here, I discuss two statistical methods to analyze longitudinal data with considerable covariance: the Generalized Estimating Equation (GEE) and Latent Transition Analysis (LTA) approaches. The advantages of both techniques are discussed for specific study questions.

Generalized Estimating Equation (GEE) is an extension of generalized linear models, for example simple regression, which allows the correlation of outcomes within an individual to be estimated and taken into account for calculation of the coefficients and standard errors.[32] Most statistical techniques such as simple regressions do not account for this correlation. With longitudinal data, failing to account for the covariance among multiple observations within a subject can lead to incorrectly estimated parameters and thus misleading results. One approach to study repeated measurement data is to create a single summary statistic. Good examples of this approach are using the mean if the purpose of repeated measurement is to average out within individual variability, or applying a difference if the change in a variable over time is of interest to the researcher. However, single summary statistics use only part of the information in the dataset. Generalized Estimating Equation (GEE) is an approach that, when compared to more traditional models, allows one to extract additional information from the data without changing or ignoring the inherent correlation structure. GEE models create robust estimates for standard errors of regression coefficients and ensure that the regression inferences are consistent regardless of which correlation structure exists in the data.[31] Initially a GEE model fits a standard regression model, assuming the independence of observations. Next, the residuals are used to estimate the parameters that quantify

the correlation between observations in the same individual. The model is then refit with a modified algorithm using a matrix which reflects the magnitude of the correlations in the previous step.[31] This process will continue to stabilize the estimate parameters. In GEE models, where there are fewer constraints on the distribution of the dependent variable, normal, binomial, and Poisson distributions are permitted. The main difference between the GEE models and Multi-level Modeling (another approach to analyze repeated measurement data) is that GEE focuses on estimating a non-varying coefficient in the presence of clustering (fixed effect), whereas in Multi-levels Modeling, the focus is on estimating the aspects of the model that vary by group (random effect). Like any statistical technique, users of the GEE should be cautious about certain points, such as number of individuals within each cluster of observation or the nature of missing data in the analysis.[33] In the studies presented in Chapter 2, GEE models were applied to repeated measurements of externalizing scores of children at age 1½ and 3 years, reported by mothers and by fathers. This analysis resulted in a robust effect estimate of maternal thyroid dysfunction on a child's externalizing scores over time and across multiple informants. GEE models are known to be useful techniques for analysis of correlated data and have been extensively used in different disciplines such as political sciences. However the utility of GEE models with fixed effect in medicine and behavioral science remained limited. In many conditions, the researchers rather apply Multi-level Modeling technique to analyze cluster data. Nevertheless, many simple questions can be answered using marginal models with fixed effect if one is interested in the average effect of covariates on the response in a population.

In Chapter 4, the results of a study on the longitudinal course of autistic symptoms from 1½ to 6 years are presented. In this study, we aimed to investigate the stability of autistic symptoms from the preschool period to school age. It is possible to compare the mean of autistic symptom scores at the group level at t and t+1, or to compare the number of children with autistic symptoms within the clinical range at t and t+1 (variable-centered approach). In a variable-centered approach, the emphasis is on identifying a relation between variables, and the assumption is that this relation applies to everyone. Alternatively, a person-centered approach focuses on the individual as a whole and looks for subtypes of individuals that exhibit similar patterns of individual characteristics.[34] Latent Class Analysis (LCA) and **Latent Transition Analysis (LTA)** are two types of multivariate categorical latent variable models that apply a person-centered approach.[35] Both LCA and LTA have been used widely in behavioral science to study behaviors with multiple dimensions.[36] In LCA, a set of observed variables is used to infer an underlying, unobserved grouping variable (the latent variable). The latent classes derived from LCA represent groups of individuals with a specific set of features at a certain time. A good example of this approach is to define groups of adolescents according to their motivations for their drinking (unobserved latent variable) using information from their answers to a list of questions on their drinking behavior in the past three months (observed variables). In LTA (a longitudinal extension of LCA), the latent classes are

obtained using information from observed variables at different time points. Using the same example from above, the adolescents can be asked to fill out questionnaires on their drinking behavior at three waves over time. LTA models estimate the transition probabilities from a particular class at time t to another class at time t+1 (e.g. the probability that an adolescent moves from the latent class 'early experimenter in drinking' at t to the class 'binge drinker' at t+1). The advantage of LTA is that it allows for the uncertainty associated with latent class membership to be taken into account. In the study presented in this thesis, we examined the stability of autistic symptoms in children at three time points: 1½ years, 3 years, and 6 years. LTA was selected as the method of choice since the research question addresses the discrete change in a certain behavior over time and the observed data (as reported by the parents) were categorical in nature.

Despite widespread use of latent analysis approaches in the behavioral science research, limitations of this technique should be discussed. First, model selection in LTA remains a challenge. Different statistical criteria have been suggested to determine the number of classes derived from latent models.[37] However, the statistical criteria may yield different results (in respect to number of classes or class characteristics) in different samples. This illustrates a major problem, the limited generalizability of findings obtained by latent models. More importantly, latent analysis approaches always involve some degree of subjectivity, either in the a priori selection of variables included in the analysis or clinical interpretability of classes derived from latent models.[36, 38]

Methodological development in statistics helps psychiatric epidemiologists work with complex data; nonetheless, the complicated statistical techniques should be applied only if the study question cannot be answered by simpler methods. Using the example from our study on maternal thyroid function and child behavior, extending the choice of model from a simple regression to a GEE model with fixed effect simply increased our power by using the information from mother and father considering the correlation between observations within an individual. Statistical techniques are lenses that an investigator uses to examine empirical data. The worth of such a lens lies in the extent to which it reveals something not only interesting but also scientifically valid. To ignore the required assumptions of a statistical method or to undervalue the interpretability of complicated models may lead to false reports or implausible findings. As Andrew Pickles has stated, "Statistical science is not a set of recipes but rather a set of concepts and principles whose application delivers better science."

Cake or comorbid bread and fudge?*

Temperament is a relatively young concept in child psychiatry and has only recently received more recognition. In the fourth edition of Rutter's Child and Adolescent Psychiatry, temperament was briefly described in 8 pages of a chapter titled Personality and Illness.[39] In the latest edition of the book, temperament in infancy and childhood has been extensively

discussed by Caspi and Shiner, and nuanced questions over its measurement and outcomes are addressed (pages 182-198).[21] The history of temperament research in child psychiatry has started with different proposed models. From these, the models suggested by Thomas and Chess (nine trait model) and by Cloninger (four dimension model) attracted widespread interest.[40-41] Debates exist over these models and, for example, Shiner and Caspi argue that the questionnaires suggested by Thomas and Chess to measure the nine-trait structures address a smaller set of traits (the same traits central to other models of temperament).[42] More recently, Rothbart and Bates have pointed to a model of temperament in preschool and school age children with the main focus on three higher-order domains: positive emotionality, neuroticism or negative emotionality, and conscientiousness.[43] Using this taxonomy for temperamental traits, many researchers have attempted to explain the interplay between temperament, personality and psychopathology. In these attempts, the assessment of temperamental traits in young children remained a challenge.[42] Many instruments have been developed to evaluate temperamental traits in childhood, from parent-report and self-report questionnaires to naturalistic observation of children in home and laboratory assessments. Advantages and limitations are present for each technique (for an extensive comparison, see Rothbart and Bates, 2006).[43] Ideally, more than one method of observation is recommended to be used; or minimum more than one informant when possible.[21, 43] Furthermore, many conceptual models were suggested for the association of temperamental differences and the emergence of psychopathology in children. All of these models can be considered as possible associations from a developmental perspective.[21] Few dispute the fact that a child's temperament and personality play an important role in the development of psychopathology.[42] Nevertheless, establishing a one-to-one relation between a temperamental trait and a certain psychopathology is unlikely to adequately explain the complex relation between the two. Temperament and psychopathology may come to be linked through a range of processes. In the Spectrum association, psychopathology is suggested to represent the extreme end of a continuously distributed temperamental trait. Although psychiatric disorders are typically defined categorically, it is possible that some disorders are the extreme end across a dimension of temperament. Despite advances in the utility of this theory in molecular genetic studies of behavior that support spectrum association between temperament and psychopathology, the Spectrum association has remained an understudied topic in child psychiatry.[42] In the Vulnerability association, temperamental traits predispose individuals to prospective psychopathology. The most compelling evidence for this theory comes from longitudinal studies that measure temperament prior to the development of psychopathology. For example, longitudinal studies have shown that an early history of high negative emotionality and poor self-control predict a higher risk of conduct disorders and severe antisocial behavior at later age.[44] Although the Spectrum and Vulnerability associations seem distinct, many correlations between temperament and psychopathology can be explained well by either of the models.[21] In this thesis, we attempted to address this issue and explore

the longitudinal relation between positive emotionality and internalizing problems with consideration of possible mechanisms. To minimize shared-method bias common to studies relying solely on parental reports, we measured positive emotionality at age 3 years using a laboratory-based observation. Parents reported on internalizing problems of their child at age 6 years. We showed that low positive emotionality at preschool age increases the risk of being withdrawn later, independent of internalizing problems at baseline. This finding is consistent with the Vulnerability association of temperament and psychopathology. However, we cannot entirely rule out that low positive emotionality, observed within a laboratory setting, and parent reported withdrawn problems are the extremes of a trait continuum (spectrum hypothesis).

The link between temperament and psychopathology is also described through a Maintenance association, a Resilience association and a Scarring association.[21] In the Maintenance (or pathoplastic) association, temperament is suggested to influence the form and prognosis of the psychopathology. Further, a certain temperamental trait may protect the individual from developing psychopathology in stress or adversity (Resilience association). Finally, the experience of a psychopathology may change a child's temperamental traits permanently (Scarring association). Further explanation of these associations is beyond the scope of this thesis.

Existing literature on temperament and psychopathology during childhood illustrates how difficult it is to explain symptom patterns using factors that appear very similar to what is labeled as psychopathology. The problem of similarities in content can be solved effectively by eliminating the overlapping items from measures of temperament and psychopathology. Furthermore, using a longitudinal design and controlling for baseline psychopathology helps to better clarify how the two may be connected. Temperament research can largely benefit from epidemiological studies to establish the biological basis of temperamental differences, and to elucidate the course and evolution of temperament from early age through adulthood. Finally, an approach that may help researchers to overcome the obstacle is to remember that temperamental traits, psychopathology or disorders all have to reside in the same brain. Instead of searching for diversity in terminology, our aim should be to figure out how these pieces fit together. David Rettew has used food analogies to describe "it should not be our debate whether a chocolate cake is better understood as comorbid bread and fudge, but to uncover the key ingredients and the ways those ingredients are combined to produce a culinary master, a kitchen disaster, and everything."[45]

Categories or dimensions: the solution of sub-threshold disorders

With little doubt, standardized diagnostic distinction of disorders with Diagnostic and Statistical Manual of Mental Disorders (DSM) and International Statistical Classification of Diseases (ICD) has been a great step in classification of mental health problems in the 20th century.[46] The categorical thinking, reflected in the classification system, has roots in medicine. Blood

pressure is a dimension distributed in the population, but "hypertension" is a diagnostic category, defined as a condition when blood pressure reaches or passes a certain threshold. Despite practical advantages (and thus extensive utility) of categorizations in mental health problems, there are drawbacks to discretizing continuous behavioral phenomena. The main disadvantage of such categorical thinking in the clinical setting is that only those who stand above the threshold receive treatment (a major concern that has been raised after publication of the proposed new DSM-5 definition of autism).[47] Additionally, the choice of an arbitrary cut-off has remained problematic. Dimensional approaches do not have these limitations, can easily be used for monitoring of behavior over time, and are more practical at population level.[46-47] Furthermore, dimensional approaches apply instruments with reliable psychometric properties and tap different aspects of a heterogeneous disorder.[48-49] The disadvantages of dimensional approaches are difficulties in communication with parents, choosing a source of information, and training requirements (in case of standardized instruments). Nevertheless, the distinction between dimensions and categories should not be exaggerated. Both in the clinical settings and for research purposes, the combination of both approaches can be helpful. An alternative approach is using sub-threshold disorders. Studying individuals who have some symptoms but do not meet the criteria for a diagnosis has advanced our understanding of the etiology of psychopathology. Examples of such an application can be found in studies of mood disorders and autism.[50-51] While the introduction of sub-threshold disorders may result in a better clinical referral or appropriate services to a group of children, clear criteria are still needed to establish a distinct diagnosis of psychopathology, and in particular to avoid overtreatment. Additionally, studying genetic and environmental bases of psychopathology is not possible if the complex phenotype of the disorder is not fully understood. Applying any or both of these approaches, the aim should be to increase our knowledge on the phenotype of psychopathology and simplifying the complex nature of it.

CLINICAL IMPLICATIONS

Results from observational longitudinal studies, such as those presented in this thesis, can answer questions of causality in mental health problems only with difficulty. However, they may shed new light on the shared risk factors for common psychopathology and provide exciting possibilities for new approaches to prevention and treatment. Some of these possibilities are presented here.

First, maternal low thyroid function, even within the normal range, is shown to have adverse consequences for a child's mental wellness. Until now, two large randomized studies did not show any advantage of screening for thyroid dysfunction at the population level.[52-53] These findings, however, do not rule out that pregnant women could benefit from early detection of thyroid dysfunction and intervention. Specifically, this is because the best possible

intervention has not been defined yet. Until conclusive evidence from randomized trials is found, the recommendation of the American Thyroid Association for clinicians is screening only high-risk pregnant women.[19] Trimester-specific reference ranges for TSH levels is recommended as a screening tool in the high-risk group only. Recommendations for future studies are presented in the section challenges for the future.

Second, communication problems and language impairment at very young age (i.e. as young as two years) are not good predictors of school-age problems within the general population. Although many children with severe impairments may benefit from intervention, parents and clinicians should be aware of the low-to-moderate stability of early delay in children who are not part of the high-risk group (such as those with a family history). It is recommended that young children undergo further investigation for social and communication impairment (even as young as 1½ years) if the parents are concerned. However, clinicians should inform the parents about the very moderate stability of these problems in toddlers, and monitor the development of the child over time. For language impairments, preventive intervention programs are suggested to focus on demographic factors and improvement of a child's environment. Currently, screening for communication impairments in children younger than 2 years is not justified within the general population due to lack of evidence.

CHALLENGES FOR THE FUTURE

The findings from observational population-based studies presented in this thesis provide further evidence for the role of maternal thyroid hormones in normal brain development of the offspring. Further studies are needed to define the role of maternal nutrition (specifically iodine intake) in thyroid hormone insufficiency and consequent problem behavior and cognitive impairment. Prospective neuroimaging studies of children who were exposed to iodine insufficiency or low thyroid function during prenatal life are needed to extend our knowledge (based on animal studies) and to identify the specific functional and structural brain abnormalities in humans. Currently, the results of the few randomized trials of screening for thyroid dysfunction in pregnant women did not show any benefit of intervention for women and their children. Before implementing screening in pregnant women at the population level, randomized trials are needed to show that screening with subsequent intervention is effective for mothers or children. New prospective randomized trials are recommended to combine different thyroid parameters as screening tools, apply trimester-specific ranges for thyroid parameters, and examine whether screening and intervention during the first trimester of pregnancy will improve neuropsychological abilities in the offspring.

Furthermore, the longitudinal studies discussed in this thesis show that developmental delay at very young age (e.g. as young as 2 years) may resolve during development. However, there is also a group of toddlers who persists in their social/communication problems and

language delay. Population-based prospective studies are needed to disentangle early life characteristics of children with persistent delay from children whose symptoms resolve with development.

In this thesis, the biological and environmental risk factors of developmental psychopathology were discussed. Many childhood psychopathologies such as autism are typically treated as a single phenotype while they consist of a heterogeneous group of symptoms. The consensus on broad diagnostic criteria facilitates etiology research and provision of health care to children with psychopathology. However, different classification approaches are currently used for different purposes. Research into the childhood psychopathology requires groups of children who are reasonably homogeneous. This leads to the selection of a strictly defined group of children with psychopathology for research purposes. In clinical practice, a widely accepted diagnostic approach is indispensible, and a broader and vaguer spectrum of diagnosis is preferable to avoid leaving any child unclassified. For research findings to be applicable to clinical practice, the two approaches are needed to come together. New advances in the etiological research provide support for the theory that genes with a general psychopathological effect exist, and underlie the phenotypic overlap between disorders. This is a promising start on the way toward bringing the diagnostic language of the research and clinical setting together in order to define causes and provide treatment for children.

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Chapter 6

Summary/samenvatting

SUMMARY

The prenatal period and early childhood are considered as "windows of plasticity" for the brain. During this period, negative environmental factors can easily influence the brain's structure and function, which may consequently lead to psychopathology in children. Understanding neurobiological pathways to the child psychopathology is important in order to determine modifiable risk factors and to provide information for preventive interventions.

This thesis aimed to define the main pre and postnatal risk factors for children's problem behavior and cognitive impairment. Furthermore, the longitudinal course of autistic symptoms and language skills from the preschool period to school age are described. The studies presented in this thesis were embedded within the Generation R, a population-based prospective study that tracks children from fetal life onwards in Rotterdam, the Netherlands.

In chapter 2, we studied the intrauterine adverse effects of maternal thyroid hormone insufficiency on children's behavior and cognition. In chapter 2.1 we showed that higher levels of maternal Thyroid Stimulating Hormones (TSH) during pregnancy predicted a higher externalizing score in the offspring up to age 3 years. Post-hoc analyses revealed a relation between maternal TSH during pregnancy and odds of having attention deficit/hyperactivity problems up to age 3 years. Chapter 2.2 shows elevated levels of maternal Thyroid Peroxidase Antibodies (TPOAbs) in pregnancy increased the risk of externalizing problems in the children at age 3 years, in particular attention deficit/hyperactivity problems. The relation between maternal TPOAbs and the child's problem behavior was only partially explained by maternal thyroid function. We did not find any relation between maternal TPOAbs in pregnancy and children's cognition. In Chapter 2.3, we found a consistent relation between maternal severe hypothyroxinemia in pregnancy and different measures of autistic symptoms in the children at age 6 years. In chapter 2.4 low maternal urinary iodine levels during pregnancy were related to children's inhibition problems at age 4 years. The consistent findings on the relation between different markers of low maternal thyroid function and child's cognition and behavior indicate the importance of thyroid hormones in brain development during fetal life. Our findings suggest that there is no specific marker of low thyroid function in pregnancy.

In chapter 3 we studied the potential risk factors of psychopathology in childhood. These risk factors were postnatal brain morphology (the corpus callosum length, the gangliothalamic ovoid diameter, and the ventricular volume), preschool age temperament (positive emotionality) and executive functioning. Chapter 3.1 demonstrates a prospective relation between a smaller corpus callosum length during infancy and children's executive function problems at preschool age. Postnatal brain morphology did not predict attention deficit/hyperactivity problems at age 6 years. Our findings support the notion that developmental trajectories rather than absolute change in the brain structures underlie childhood psychopathologies such as Attention Deficit/Hyperactivity Disorders In chapter 3.2**,** we hypothesized that a child's low positive emotionality predicts internalizing problems during school

period. Executive function was anticipated to be one possible pathway for this relation. We found that the children with lower levels of positive emotionality had a higher risk of having withdrawn problems, independence of baseline internalizing problems. Problems in shifting domains but not other executive function domains mediated this relation. This finding is consistent with the vulnerability hypothesis behind the association between temperament and psychopathology.

Chapter 4 included two studies in which the longitudinal course and the stability of two main developmental delays associated with communication at young age were investigated. We followed up children from age of 1½ years through school period and examined both their language skills and their autistics symptoms. In chapter 4.1 we identified four groups of children based on their autistic symptom profiles: the children with 1) No/few problems 2) Flexibility problems 3) Social/communication problems and 4) social/communication problems, fixated interest, flat affection and speech problems, termed as 'Pervasive developmental problems'. We found that the stability of the symptom profile 'pervasive developmental problems' was only moderate during preschool period. Despite moderate stability of the symptoms profile 'pervasive developmental problems', very few children from the group 'Pervasive developmental problems' at 1½ years presented with 'No/few problems' at 6 years. Chapter 4.2 shows that vocabulary skills at 2½ years were better predictors of language comprehension at school age when compared to expressive and receptive language skills at 1½ years. At school age, demographic factors such as maternal education or family income play a more important role in predicting language skills than early developmental factors. Based on these findings, we recommend that very young children undergo further investigation for communication impairment if the parents are concerned. However, clinicians should inform the parents about the moderate stability of these problems in toddlers and thus monitor the development of the child over time.

Chapter 5 provides a general discussion of the main findings. Major methodological issues in longitudinal studies of childhood psychopathology are addressed. The clinical implications of findings are presented along with considerations for future studies.

SAMENVATTING

De prenatale periode en de vroege kindertijd staan bekend als een periode waarin de hersenen zeer plastisch zijn. In deze periode kunnen omgevingsfactoren gemakkelijk de structuur en functie van de hersenen beïnvloeden, en daarmee ook de ontwikkeling van psychopathologie van het kind. Inzicht in de neurobiologische factoren van psychopathologie in de kindertijd is belangrijk om beïnvloedbare risicofactoren vast te stellen en om te bepalen welke informatie van belang is voor preventieve interventies.

Het doel van dit proefschrift was om de belangrijkste pre- en postnatale risicofactoren voor gedragsproblemen en cognitieve achterstand in de kinderleeftijd te definiëren. Verder worden het longitudinale beloop van autistische symptomen en de verbale ontwikkeling vanaf de voorschoolse periode tot de schoolleeftijd beschreven. De studies in dit proefschrift werden uitgevoerd binnen het Generation R onderzoek, een populatie-gebaseerd prospectief onderzoek in Rotterdam, dat kinderen vanaf de foetale fase tot in de vroege volwassenheid volgt.

In hoofdstuk 2 onderzochten we de intra-uteriene nadelige effecten van een tekort in maternale schildklierhormonen op het gedrag en cognitie van het kind. In hoofdstuk 2.1 hebben we aangetoond dat een hoger niveau van het Schildklier Stimulerende Hormonen (TSH) tijdens de zwangerschap een hogere score op externaliserend gedrag van de kinderen tot 3 jarige leeftijd voorspelt. Een post-hoc analyse toonde een relatie aan tussen de TSH van moeder tijdens de zwangerschap en de kans op aandachtstekort en hyperactiviteit problemen tot de leeftijd van 3 jaar. Hoofdstuk 2.2 toont dat verhoogde niveaus van maternale schildklier peroxidase antistoffen (TPOAbs) tijdens de zwangerschap een verhoogd risico op externaliserende problemen bij kinderen op de leeftijd van 3 jaar voorspellen, ze voorspellen vooral aandachtstekort en hyperactiviteit problemen. De relatie tussen TPOAbs van de moeder en gedragproblemen van het kind werd slechts gedeeltelijk verklaard door de schildklierfunctie van de moeder. We vonden geen relatie tussen TPOAbs van de moeder tijdens de zwangerschap en het cognitief functioneren van het kind. In hoofdstuk 2.3, vonden we een consistent verband tussen ernstige hypothyroxinemia van de moeder tijdens de zwangerschap en verschillende maten voor autistische symptomen bij kinderen op de leeftijd van 6 jaar. In hoofdstuk 2.4 waren lage niveaus van jodium in de moeders' urine tijdens de zwangerschap gerelateerd aan inhibitie problemen van kinderen op 4 jarige leeftijd. De consistente bevindingen met betrekking tot de relatie tussen verschillende indicatoren voor de (lage) schildklierfunctie van moeders en het gedrag en cognitie van kinderen, wijzen op het belang van schildklierhormonen in de ontwikkeling van de hersenen tijdens de foetale periode. Onze bevindingen suggereren dat er geen specifieke marker is voor een beperkte schildklierfunctie tijdens de zwangerschap.

In hoofdstuk 3 hebben we de mogelijke risicofactoren van psychopathologie op de kinderleeftijd bestudeerd. Deze risicofactoren waren postnatale morfologie van de hersenen (het

corpus callosum lengte, de diameter van de gangliothalamic ovoid, en het ventriculaire volume), temperament tijdens voorschoolse leeftijd (positieve emoties) en executief functioneren. In hoofdstuk 3.1 tonen we aan dat er een prospectieve relatie bestaat tussen een kleiner corpus callosum tijdens de postnatale periode en problemen in het executief functioneren van kinderen op voorschoolse leeftijd. Postnatale morfologie van de hersenen voorspelde geen aandachtstekort en hyperactiviteit problemen op 6 jarige leeftijd. Onze bevindingen suggereren dat de ontwikkelingstrajecten, en niet de absolute verandering in de structuren van de hersenen, ten grondslag liggen aan psychopathologie in de kindertijd, zoals ADHD (aandachtstekort en hyperactiviteitstoornis). In hoofdstuk 3.2 hadden we de hypothese dat lage positieve emotie van het kind internaliseren problemen tijdens de schoolperiode voorspelt. Deze associatie kon deels door een specifiek domein van het executief functioneren op vier jaar worden verklaard, namelijk door een verminderd vermogen om de aandacht snel van richting te kunnen veranderen. We vonden dat kinderen met lagere niveaus van positieve emotionaliteit een hoger risico op teruggetrokken gedrag hadden. Dit risico was onafhankelijkheid van het baseline niveau van internaliserende problemen. Deze relatie kon deels verklaard worden door een verminderd vermogen om de aandacht snel van richting te veranderen. Deze bevinding is consistent met de kwetsbaarheids hypothese als verklaring voor de associatie tussen temperament en psychopathologie.

Hoofdstuk 4 omvat twee studies waarin het longitudinale beloop en de stabiliteit van de twee belangrijkste ontwikkelingsstoornissen, die communicatie op jonge leeftijd beïnvloeden, werden onderzocht. We volgden kinderen vanaf 1½ jaar tot de schoolperiode en onderzocht hun verbale ontwikkeling en hun autistische symptomen. In hoofdstuk 4.1 hebben we kinderen ingedeeld in vier groepen op basis van hun autistische symptomen: kinderen met 1) Geen/weinig problemen 2) Problemen met flexibiliteit 3) Sociale/ communicatie problemen en 4) sociale/communicatie problemen, gefixeerd interesses, vervlakt affect en spraak problemen, deze laatste groep werd daarom aangeduid als 'pervasieve ontwikkelingsproblemen'. We vonden dat de stabiliteit van het profiel 'pervasieve ontwikkelingsproblemen' tijdens de voorschoolse periode slechts matig was. Ondanks een matige stabiliteit van het profiel 'pervasieve ontwikkelingsproblemen', waren er slechts weinig kinderen in het 'pervasieve ontwikkelingsproblemen' profiel op 1½ jaar, die op 6 jaar 'geen problemen' lieten zien. Hoofdstuk 4.2 laat zien dat een expressieve en receptieve taalachterstand op 1 ½ jaar zwakkere voorspellers waren van taalbegrip op de schoolleeftijd dan een achterstand in woordenschat op 2 ½ jaar. Op schoolse leeftijd zijn demografische factoren, zoals het opleidingsniveau van de moeder of het gezinsinkomen belangrijkere voorspellers van taalachterstand dan vroege ontwikkelingsstoornissen. Op basis van deze bevindingen, is het raadzaam om, als ouders bezorgd zijn, zeer jonge kinderen verder te onderzoek op communicatie problemen. Echter, artsen dienen ouders te informeren over de beperkte stabiliteit van deze problemen bij peuters en dienen de ontwikkeling van deze problemen te monitoren.

Hoofdstuk 5 geeft een algemene discussie van de belangrijkste bevindingen. Belangrijke methodologische zaken die een rol spelen in longitudinale studies naar psychopathologie in de kindertijd worden behandeld. De klinische implicaties van de bevindingen worden gepresenteerd en suggesties voor toekomstige studies worden besproken.

Chapter 7

- Authors and affiliations
- List of publications
- About the author
- PhD portfolio
- Final words

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1. Habibi M, Mortazavi H, Shadianloo S, Balighi K, Ghodsi SZ, Daneshpazhooh M, Valikhani M, Ghassabian A, Pooli AH, Chams-Davatchi C. Nail changes in pemphigus vulgaris. International Journal of Dermatology. 2008; 47(11): 1141-4.
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Visser W, Verhulst FC, de Rijke YB, Steegers-Theunissen RPM. Low urinary iodine excretion during early pregnancy is associated with executive functioning problems of the child. The Journal of Nutrition. 2012; 142(12): 2167-74.

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ABOUT THE AUTHOR

Akhgar Ghassabian was born in the city of Sary, Iran, on August 3rd 1979. At the age of 15, she moved to Tehran with her family. She finished her secondary education in 1998 at Farzenegan high school, a school affiliated with National Organization for Development of Exceptional Talents. In the same year, she passed the university entrance exam scoring in the top 99.9th percentile of the grade rating for all the university applicants (11th in the Biological Science discipline and 5th in the Art discipline out of 500,000 examinees). Having a choice to study either medicine or art, she started studying medicine at Tehran University of Medical Sciences. During her medical study, she was an active member of "Student Research Committee". At the same time, she continued publishing in a student magazine for art and literature "Shekan". In 2005, she graduated as a medical doctor from Tehran University of Medical Sciences with an overall grade A. In the same year, she started working as an emergency room physician in Hashtgerd city. Gradually her interest extended toward the discipline of public health. Therefore, in 2006, she started working as a public health officer for surveillance of Tuberculosis and Thalassemia in Iran University of Medical Sciences. At the same, she worked as a researcher at the Scientific Writing Unit in Tehran University of Medical Sciences.

In 2008, she was admitted to a Master of Science program at the Netherland Institute for Health Sciences in Rotterdam, and moved to the Netherlands. She obtained her Master's degree in Epidemiology from Erasmus University in August 2009.

In July 2009, she began the work described in this thesis under the supervision of Prof. Dr. H. Tiemeier and Prof. Dr. F.C. Verhulst. Since October 2012, she has been working as a post-doctoral fellow in the neuroimaging group of Dr. T. White and Prof. Dr. H. Tiemeier. Her ambition is to proceed with her academic career in the field of child psychiatric epidemiology in one of the top universities in the United States.

PHD PORTFOLIO

Summary of PhD training and teaching activity

Name of PhD Student:	Akhgar Ghassabian
Erasmus MC Department:	Child and Adolescent Psychiatry/Psychology
Research School:	Netherland Institute for Health Sciences (NIHES)
PhD period:	July 2009-June 2013
Promotors:	Prof. dr. Frank C. Verhulst, Prof. dr. Henning Tiemeier

1. PhD training

General academic skills	**Year**	**Workload**
- Research Integrity	2012	2.0
- Biomedical English Writing and Communication	2010	4.0
Research skills		
- Conceptual Foundations of Study Design	2009	0.7
- Principles of Genetic Epidemiology	2009	0.7
- Methodological Topics in Epidemiological Research	2009	1.4
- Advanced diagnostic Research	2009	1.4
In-depth courses		
Netherland Institute for Health Sciences		
- Causal Inference	2011	0.7
- Psychiatry Epidemiology	2009	1.1
- Genomics in Molecular Medicine	2009	0.7
- Analysis of Time-varying Exposure	2009	0.9
- Genetic Analysis in Clinical Research	2009	1.9
Johns Hopkins Bloomberg School of Public health		
- Epidemiology of Major Mental Disorders	2010	2.0
- Longitudinal Analysis with Latent Variables	2010	3.0
Utrecht University		
- Neurocognition of Memory and Attention	2010	7.5
Presentations		
- Developmental Origin of Health and Disease 2012 Satellite Meeting, Rotterdam, the Netherlands (Oral presentation)	2012	0.5
- 59th Annual meeting of American Academy of Child and Adolescent Psychiatry (AACAP), San Francisco, USA	2012	1.0
- AACAP/CACAP Joint Annual meeting, Toronto, ON, Canada	2011	1.0
- Department of Psychiatry, Utrecht Medical Centre (Oral presentation)	2010	0.5

Seminars and Workshops		
- Brain Development and Developmental Disorders, Utrecht University, the Netherlands	2012	0.3
- 8th Nutrimenthe Symposium, Rotterdam, the Netherlands	2012	0.6
- Writing Successful Grant Proposals (Workshop), Erasmus MC	2011	0.4
- Genetics in Child Cohort studies, Rotterdam, the Netherlands	2010	0.3
- 40 Years Epidemiology at Erasmus MC	2009	0.3
- Research Meetings, the Generation R Study Group	2009-2012	1.0
Reviewing papers		
European Journal of Epidemiology	2013	0.2
European Journal of Endocrinology	2013	0.2

2. Teaching activity		
Supervision of medical students		
- Supervising Natasja Kok, medical student, Erasmus MC Project title: Maternal C-Reactive Protein in pregnancy and autism	2012	3.0
- Supervising Jessica van den Brink, medical student, Erasmus MC Project title: Early determinants of executive function in preschoolers	2011	3.0
- Supervising Sehrash Mahmood and Ayse Dogan: medical students, Erasmus Medical Center Project title: Prevalence of autistic traits in the general population	2011	1.0

1 ECTS (European Credit Transfer System equal to workload of 28 hrs

FINAL WORDS

Finally, the trip is over and the port is near. My ship has reached the destination after four years of a journey in a stormy sea. It was a fantastic, adventurous journey that I finished safely with the help of many people. It is time to name them all, and tell them how grateful I am for their support.

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