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Association between Transient Hypothyroxinemia of Prematurity and Adult Autism Spectrum Disorder in a Low Birthweight Cohort: An Exploratory Study

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Abstract

Objective—Transient hypothyroxinemia of prematurity (THOP) is associated with increased risk of cerebral palsy and lower IQ in low birthweight infants. This study explores whether THOP is also associated with increased risk of autism spectrum disorders (ASD).

Methods—This secondary analysis uses data from a birth cohort of newborns weighing 500g–2,000g ($n = 1105$) who were followed to age 21 (years), when they were assessed for ASD in the second of a two stage process. Of the 187 assessed at age 21, 14 had ASD. Neonatal thyroxine results were available for 12/14 and 165/173 participants diagnosed with and without ASD, respectively. THOP was defined as thyroxine z-score < -2.6 . Unadjusted relative risks and confidence intervals were calculated.

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Results—The mean neonatal thyroxine z-score in young adults diagnosed with ASD was 0.5 SD lower (95%CI: -0.16-1.06) than in those without ASD. Participants with THOP were at 2.5 fold greater risk of ASD (RR 2.5, 95%CI: 0.7-8.4). While neither of these differences was statistically significant, in a secondary subgroup analysis of those whose mothers did not have hypertension during pregnancy, THOP significantly increased the RR for ASD (5.0; 95%CI 1.2-20.5).

Conclusion—While the primary relation between THOP and ASD found here is not statistically significant, the magnitude of association and significant relationship observed in the subgroup whose mothers did not have hypertension during pregnancy suggest that it is worthy of further investigation.

Keywords

autism spectrum disorders; premature; infants; thyroxine; transient hypothyroxinemia of prematurity

Introduction

Thyroid hormones play important roles in neurogenesis and neuronal migration, both pre- and perinatally.¹ Given that disturbances of neurogenesis and neuronal migration are associated with autism spectrum disorders (ASD),^{2, 3} it is possible that abnormal concentrations of pre- and perinatal thyroid hormone levels,^{4, 5} may play an etiologic role.^{6, 7} Some evidence from animal models is consistent with this possibility. Rats exposed in utero to antithyroid agents thought to induce transient hypothyroxinemia exhibit cerebellar migration defects and behavioral features interpreted as resembling or co-occurring with autism.^{6, 8} Evidence from human studies for a relation between abnormalities of pre- and perinatal thyroid concentrations is intriguing but limited and inconsistent.

There are no prospective studies and only two case-control studies have examined the relationship between neonatal thyroid hormone status and ASD. The first found no difference in neonatal thyroxine concentrations between the 6 autism cases and 23 controls, but it did not include participants with values less than 7 mg/dL (90 nmol/L)- a threshold for defining abnormal values supplied by the assay manufacturer.⁹ In a much larger study, Hoshiko et al. found a significant association between low neonatal thyroxine concentrations and ASD in only one of two pre-existing population-based samples. The significant difference in that group persisted following adjustment for multiple perinatal factors (OR 2.7;95%CI [1.3-5.8]).¹⁰ Both of the above studies were limited exclusively or predominantly to normal birth weight, term infants.

Low birthweight (LBW)/preterm infants may be a more promising population in which to study the relation between hypothyroid states and ASD. In preterm infants abnormally low thyroid levels in the newborn period is a common phenomenon known as transient hypothyroxinemia of prematurity (THOP).¹¹ Two studies report a prevalence of ASD among LBW/preterm survivors that is five times higher than that reported for the general population.^{12, 13} In the present population-representative cohort study of LBW/ preterm infants, we explore the association between THOP and ASD.

Methods

Study Design & Participants

This is a secondary analysis of data from participants of the Central New Jersey Neonatal Brain Hemorrhage Study followed to age 21 (see Figure 1). Study design and methods for the original cohort have been reported previously.^{14, 15} Briefly, this cohort was comprised of 1,105 newborns weighing 500-2000g born-in or transferred to three neonatal intensive care units that cared for 83% of newborns in this weight range born 9/1/1984 – 6/30/1987 in three New Jersey counties.¹⁶

Neonatal Thyroxine Data

Acquisition and processing of neonatal thyroxine concentrations were previously reported.¹⁷ Briefly, thyroxine concentrations obtained from the New Jersey Department of Health's Inborn Errors of Metabolism Laboratory were matched to NBH study participants using hospital of birth, name, date of birth and birth weight by personnel blinded to participants' neurologic and developmental status. Successful matches were achieved for 86% of the 1,105 newborns. Thyroxine concentrations were corrected for inter-assay variation by converting them into z-scores: [(measured thyroxine concentration – mean thyroxine concentration for the assay)/standard deviation (SD) for the assay]. Each assay included approximately 240 unselected New Jersey newborns, predominantly born at term. None of the study participants here had congenital hypothyroidism.

Autism Spectrum Disorders Diagnosis

The ASD screening protocol for this cohort was recently published.¹⁸ Briefly, within a larger follow-up study at age 16,¹⁹ 623 parents of original cohort participants completed at least one of two research-validated ASD screening instruments: the Social Communication Questionnaire (SCQ) and the Autism Spectrum Screening Questionnaire (ASSQ). Nearly 20% (n=117) screened positive for ASD based on either the SCQ, ASSQ, or having been assigned a diagnosis of autism by a professional. At age 21, 60% (n=70) of those with positive screens for ASD at age 16 and a systematic sample of participants with negative screens (n=119; 24%) were assessed for ASD. The diagnosis of ASD was based on the Autism Diagnostic Observation Schedule (ADOS) and/or Autism Diagnostic Interview-Revised (ADIR) using published algorithms. There were no significant differences in mean gestational age, birth weight, or small for gestational age (SGA) status between the ASD-screened cohort (N=623) and those eligible, but not screened (N=239) at age 16.¹⁸ Characteristics of participants who were and were not retained in the sample from 16 to 21 years of age were not significantly associated with screening status except for maternal receipt of public assistance. Aside from a greater risk for suboptimal neurodevelopmental outcomes, participants retained in the sample at age 21 were representative of those not retained based on assessment of demographic and perinatal factors including mean/median thyroxine z-scores.

Statistical Analysis

Normality of continuous parameters was assessed using the Kolmogorov-Smirnov test and inspection of histograms. The t-test, Mann-Whitney U, chi-square or Fisher's exact tests were used to compare continuous and categorical variables, as appropriate. The primary *a priori* threshold for T4 z-score dichotomization was 2.6 standard deviations (SD) below the mean, consistent with the criterion used at the time for congenital hypothyroidism newborn screening in New Jersey. Secondary dichotomizations included 2 and 3 SD thresholds. The potential effect of exposure on average risk is shown in 2×2 contingency tables and unadjusted relative risks (RR) are calculated^{20, 21} with confidence intervals (CI) computed using a modified Poisson regression model with a robust variance estimator.²² Statistical significance is determined using a 5% threshold. Analyses were performed using SAS version 9.3 (Carry, NC, U.S.A).

Results

Of 24 perinatal and developmental characteristics examined (Table 1), only maternal hypertensive disorders and motor impairment at age 16 were significantly associated with ASD diagnosis ($p=0.04$ and $p=0.002$, respectively). Participants diagnosed with ASD had non-significantly lower mean/median thyroxine z-scores than undiagnosed participants (mean difference: 0.5 [95%CI: -0.16, 1.06]; median difference: 0.5; both $p=0.15$) (Figure 2). There was no difference in median age at specimen collection by ASD status ($p=0.50$).

Table 2 shows three 2×2 contingency tables presenting the magnitude of association between different severities of THOP and ASD diagnosis. Children with thyroxine z-scores < -2.6 were at 2.5 fold greater risk of ASD (95%CI [0.7, 8.4]) than children with values at/above this threshold. Use of a less extreme thyroxine z-score threshold (< -2) resulted in a similar and marginally significant association (RR: 2.9; 95%CI [1.0, 8.7]). Application of a more extreme thyroxine z-score threshold (< -3) resulted in a stronger yet still only marginally significant association between THOP and ASD (RR: 3.7; 95%CI [1.0, 14.6]), although values exceeded this threshold in only 2 of 12 ASD cases.

Based on the association of maternal hypertension during pregnancy with ASD in this cohort (Table 1), differences in thyroxine z-scores by maternal hypertension during pregnancy were examined. Participants born to mothers with hypertension during pregnancy had higher mean and median thyroxine z-scores than those born to mothers without hypertension during pregnancy (-1.1 vs. -1.6, $p=0.03$ and -1.2 vs. -1.6, $p=0.02$, respectively). The prevalence of THOP was also lower among those whose mothers had hypertension during pregnancy than among those who did not (2/32 vs. 19/145, respectively), but not significantly so (RR: 0.50; 95%CI [0.08, 1.8]). When the subgroup of participants born to mothers with hypertension during pregnancy were excluded, the prevalence of THOP among participants with ASD was significantly greater than among those without ASD (3/7 vs. 16/138, RR 5.0; 95%CI [1.2, 20.5]).

Discussion

In this comparatively small study, the risk of ASD was 2.5 fold greater in participants with than without THOP. This increase in risk was not statistically significant. Given the sample size and rate of ASD in participants without THOP, this study had only 36% power to detect a true RR of 2.5 with a 5% limit on Type I error. Thus, our findings are inconclusive about whether THOP increases the risk of ASD in LBW/preterm infants.

When pregnancies with hypertension were excluded, however, THOP was associated with a fivefold increase in risk of ASD ($p=0.03$). The rationale for excluding these participants was the significant bivariate relation between maternal hypertension during pregnancy and ASD observed in this low birth weight cohort (Table 1). The higher prevalence of ASD among participants whose mothers had hypertension during pregnancy is consistent with information presented by Buchmayer and colleagues²³, who reported a significantly increased risk of ASD associated with maternal hypertension in a preterm sample.

Limitations of this study include the low power for detecting a relation between THOP and ASD, as discussed above, and the small sample size, which precluded adjustment for potential confounders identified previously.²³ Moreover, the effect of the increased prevalence of motor scores in the top 10% of the Riley Motor Problem Inventory distribution (5) could not be explored by subgroup analysis because only one of the eight participants with ASD who were evaluated with this measure had a score lower than this. However, the type of non-disabling motor problems assessed by the Riley would not affect the validity of the ADI-R or ADOS. The methodological limitations of the assessment of ASD prevalence in this cohort have been previously discussed.¹²

Conclusion

THOP is associated with a non-significant 2.5 fold greater risk of ASD. This magnitude of association in addition to the significant relationship between THOP and ASD in the subgroup born to mothers who did not experience hypertension during pregnancy suggests that further investigation is warranted.

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Abbreviations

ADIR	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
ASD	autism spectrum disorders
ASSQ	Autism Spectrum Screening Questionnaire
LBW	low birth weight
SCQ	Social Communication Questionnaire
THOP	transient hypothyroxinemia of prematurity

Figure 1.
Flow diagram showing the derivation of the final study sample

Figure 2. Distribution of neonatal thyroxine z-Scores by Autism Spectrum Disorders status

[This is a skeletal box style meaning the box represents the interquartile range, the solid line within the box represents the mean value, '+' indicates the median thyroxine z-score, and the whiskers and serifs show the extreme values for each group]

Table 1
Descriptive characteristics by ASD Status

Demographic/Perinatal Characteristics & Developmental Outcomes	No ASD (N=165)	ASD (N=12)	p-value
<i>Continuous Variables- Mean [SD]</i>			
Maternal Age	28.9 [5.1]	29.8 [4.4]	0.75
Gestational Age (weeks)	31.3 [3.2]	31.2 [4.0]	0.68
Birth weight (g)	1477 [371]	1313 [316]	0.09
Head Circumference at Birth (cm)	28.2 [2.6]	27.6 [2.2]	0.27
Duration of Ventilation	12.7 [19.5]	20.4 [25.2]	0.14
Duration of Oxygen	15.9 [28.3]	22.4 [35.1]	0.25
<i>Categorical Variables- %</i>			
White Race	87.0	66.7	0.11
Male	54.6	75.0	0.17
Maternal education beyond high school	62.6	72.7	0.75
Hypertension during pregnancy ^a	16.4	41.2	0.04
Chorioamnionitis	6.1	0%	1.00
Placenta previa or Abruption placenta	10.9	0%	0.61
Prolonged Rupture of Membranes (>18hours)	24.9	16.7	0.73
Cesarean Section Birth	54.6	58.3	0.80
Singleton Birth	66.7	63.6	0.84
Cord Abnormality	9.7	16.7	0.35
Small for Gestational Age	23.0	41.7	0.17
5 minute Apgar < 7	14.4	25.0	0.39
Cord PH < 7.25	18.2	8.3	0.69
First C02 < 30	24.9	16.7	0.73
Mechanical Ventilation (yes/no)	53.9	75.0	0.23
Cerebral Palsy at Age 2	15.3	25.0	0.41
Riley Motor Problems Inventory Score >=5 at age 16 ^b	30.6	87.5	0.002
IQ at age 16 < 70 ^c	4.6	12.5	0.34

^aChronic (preexisting) maternal hypertension, PIH, or preeclampsia.

^b147 and 8 participants without and with ASD, respectively, had data available for analysis.

^c151 and 7 participants without and with ASD, respectively, had data available for analysis.

Table 2
Magnitude of association between THOP and ASD using different thresholds for thyroxine z-scores dichotomization

T4 Z-score Dichotomization		No ASD (n=165)	ASD (n=12)	RR[95%CI]
Conventional	< -2.6	11%	25%	2.5 [0.7, 8.4]
	-2.6	89%	75%	
Less Extreme	< -2	31%	58%	2.9 [1.0, 8.7]
	-2	69%	42%	
More Extreme	< -3	4%	17%	3.7 [1.0, 14.6]
	-3	96%	83%	

Note: RR= relative risk, computed by dividing the risk in the lower z-score grouping by risk observed in the higher z-score grouping; CI= confidence intervals, computed using a modified Poisson regression model with a robust variance estimator.