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Research Article

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Second-Trimester Placental and Thyroid Hormones Are Associated With Cognitive Development From Ages 1 to 3 Years

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Abbreviations: 5-HT, serotonin; BMI, body mass index; BSID III, Bayley Scales of Infant and Toddler Development, Third Edition; CANDLE, Conditions Affecting Neurocognitive Development and Early Learning; CV, coefficient of variation, FT4, free thyroxine; GDF15, growth differentiation factor 15; hCG, human chorionic gonadotropin; hCGα, free α-subunit of human chorionic gonadotropin; hCGβ, free β-subunit of human chorionic gonadotropin; hCG-h, hyperglycosylated human chorionic gonadotropin; IFMA, immunofluorometric assay; LHCGR, luteinizing hormone/chorionic gonadotropin receptor; T4, thyroxine; TH, thyroid hormone; TSH, thyrotropin

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Abstract

Adequate maternal thyroid hormone (TH) is necessary for fetal brain development. The role of placental human chorionic gonadotropin (hCG) in ensuring the production of TH is less well understood. The objective of the study was to evaluate 1) associations of placental hCG and its subunits, and maternal TH in the second trimester, and 2) the single and joint effects of TH and placental hormones on cognitive development and communication at ages 1 and 3 years. Fifty individuals (5%) were selected from the CANDLE (Conditions Affecting Neurocognitive Development and Early Learning) pregnancy cohort in Memphis, Tennessee, with recruitment from 2006 to 2011, to equally represent male and female fetuses. Participants were 68% Black and 32% White. Hormones measured were maternal thyroid (thyrotropin [TSH] and free thyroxine [FT4]) and placental hormones (hCG, its hyperglycosylated form [hCG-h], and free α- [hCGα] and β-subunits [hCGβ]) in maternal serum (17-28 weeks). The primary outcome measurement was the Bayley Scales of Infant and Toddler Development. All forms of hCG were negatively associated with FT4 and not associated with TSH. hCGα was associated with cognitive development at age 1 year and jointly interacted with TSH to predict cognitive development

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at age 3 years. This pilot study added insight into the thyrotropic actions of hCG in the second trimester, and into the significance of this mechanism for brain development. More research is warranted to elucidate differences between hCGα, hCGβ, and hCG-h in relation to TH regulation and child brain function.

Key Words: epidemiology, interaction, thyroid, human chorionic gonadotropin, hCG-α, Bayley scales

Adequate maternal thyroid hormones (THs) are necessary for fetal brain development during early pregnancy [1]. Especially 3,5,3′-triiodothyronine, converted from thyroxine (T4), is associated with this process [2, 3]. Maternal thyroid function in the first trimester, measured as circulating free T4 (FT4), was associated with brain function and brain morphology in children age 8 years in a population cohort of more than 3500 mother/child pairs [4]. There were small, measurable differences in brain function and morphometry over a normal range of FT4. Low FT4 was positively associated with IQ and cortical plate thickness, whereas high FT4 had an opposite association.

Because THs are essential for fetal brain development, it is plausible that there is a pregnancy-specific mechanism to ensure their supply. In the first trimester, placental human chorionic gonadotropin (hCG) stimulates the maternal thyroid to increase production of FT4 [5]. An extreme scenario that corroborates this is choriocarcinoma (trophoblastic tumor), which secretes high levels of hCG and is a cause of hyperthyroidism in pregnant women [6].

Over the last decades, more mechanistic details have been added to support this concept. The hCG-induced rise in FT4 is associated with a concomitant decrease in thyrotropin (TSH) [7, 8]. This suggests that the placenta may suppress maternal TSH in favor of using its own gonadotropin (hCG) to drive production of T4 by the maternal thyroid, effectively "hijacking" the maternal thyroid gland [7]. The placenta may also use its own enzyme system to regulate the quantities of T4 that enter the placental-fetal circulation and become available to the embryo [9]. TH receptors have been observed in fetal cerebral cortex at gestational week 5; the TH conversion enzyme iodothyronine deiodinase 2 (*DIO2*) is observed in fetal cerebral cortex by gestational week 7 [9, 10]. hCG and TSH are structurally similar glycoprotein hormones, containing identical α-subunits, which may account for a shared molecular mechanism [7, 11]. Furthermore, the maternal thyroid gland expresses, in addition to abundant TSH receptor, small amounts of hCG receptor [12, 13].

We conducted a pilot study using second-trimester maternal serum samples and child cognitive development and communication measures in the Conditions Affecting Neurocognitive Development and Early Learning (CANDLE) study. The first aim was to estimate associations of placental biomarkers and maternal THs with gestational age at the time of blood sample and with each other to generate more specific hypotheses on coregulation. The second aim was to estimate independent and joint associations of these hormones with cognitive development and communication at ages 1 and 3 years.

A unique contribution of this study is the analysis of hCG and its subunits separately, therefore, we analyzed with specific assays the α- and β-subunits of hCG (hCGα and hCGβ, respectively), intact hCG, and a hyperglycosylated form of hCG/hCGβ. Studies to date on the relationship of maternal thyroid and placental hCG have been limited to measures of either hCGβ or intact hCG. Two additional biomarkers that were hypothesized to be relevant to placental regulation of fetal brain development were analyzed. A placental source of the monoamine neurotransmitter serotonin (5-HT) was shown to be essential to early forebrain development in mice [14] and is hypothesized to contribute to forebrain development in humans [15]. Growth differentiation factor 15 (GDF15, also referred to as PLAB) was first characterized to be a "potential mediator of placental control of embryonic development" [16].

Materials and Methods

Participants

CANDLE is a birth cohort study in Shelby County (Memphis), Tennessee, into which pregnant women were recruited from 2006 to 2011 [17]. The primary aim of the cohort study is to identify factors from in utero through early childhood that contribute to child cognitive development. Inclusion criteria were being a resident of Shelby County, Tennessee, able to speak and understand English, aged between 16 and 40 years, and 16 to 28 weeks of gestation with a singleton pregnancy. Exclusion criteria included an existing chronic disease requiring medication, pregnancy complications including maternal red cell alloimmunization (Rh factor incompatibility permitted), prolapsed or ruptured membranes, oligohydramnios, complete placenta previa, and not intending to deliver at 1 of 4 participating hospitals. The study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of the University of Tennessee Health Science

Center. Details of the study cohort are published elsewhere [17, 18]. From the full cohort of 1503 women/infant dyads, we conducted a pilot analysis of 50 mother/infant dyads in which we measured maternal urinary phthalates and serum hormones. The 50 infants were chosen to be 50% male and 50% female and restricted to full-term birth (> 37 weeks) and birth weight greater than or equal to 2500 g. They were oversampled for allergic disease outcomes (20/50 dyads reported childhood asthma, eczema, and allergic rhinitis) to support another pilot study using the same samples. Mothers were age 18 to 42 years.

Circulating Hormones

Maternal second-trimester serum samples, collected at the same time as the urine samples, were stored at –80 °C and shipped to the University of Helsinki Department of Clinical Chemistry, Finland, where they were analyzed for intact hCG, hCGα, hCGβ, the hyperglycosylated form of hCG (hCG-h), TSH, and FT4. Intact hCG was measured using a time-resolved immunofluorometric assay (IFMA) (DELFIA kit A007-101, Perkin-Elmer Wallac [19]), and the other forms were measured using sandwich-type in-house IFMAs [20-23]. For in-house assays, the limit of quantitation was less than 3 pmol/L and the intra-assay coefficient of variation (CV) was less than 10% at concentrations above 10 pmol/L [23]. The intact hCG assay [19] measures only the hCG αβ dimer, irrespective of whether it is hyperglycosylated. The hCGβ assay (antibodies 9C11 [24] and 1B2 [25]) and hCGα assay (antibodies 2G11 [26] and 7E10 [27]) measure essentially only free subunits. The hyperglycosylated hCG assay (hCG-h) (antibodies 1B2 [25] and B152 [28]) measures hyperglycosylated intact hCG and hyperglycosylated hCGβ. TSH and FT4 were measured by commercial IFMAs (DELFIA kits A042-201 [29] and A061-201 [30], respectively, Perkin-Elmer Wallac). For TSH and FT4 assays the sensitivity of the assays, as indicated by the manufacturer, is less than 0.005 μU/mL and less than 2 pmol/L, respectively, and intra-assay and interassay CVs are 8%. Serum levels of 5-HT serotonin and GDF15 were analyzed by enzyme-linked immunosorbent assays (Serotonin kit from Enzo Life Sciences [31] and GDF15 kit from R&D Systems [32], respectively). Mean interassay CVs for these assays were less than or equal to 2.0%.

Infant Cognitive Development and Communication Measures

Child cognitive development was assessed by trained psychologists using the Bayley Scales of Infant and Toddler Development, Third Edition (BSID III) [33-36]. The BSID is a widely used, validated measure of early childhood cognitive development. For the age 1 participants, trained psychologists administered the BSID III Screening Test, which is composed of 5 subtests that assess skills in the cognitive, language, and motor domains. Children were administered the cognitive, receptive communication, and expressive communication subtests. Continuous measures of these 3 subtests were used as primary outcomes. At age 3, children were administered portions of the full BSID III, which consists of 5 scales: cognitive, language, motor, social-emotional, and adaptive behavior. The primary outcome at age 3 was the cognitive composite measured continuously, which is derived from the cognitive scale and consists of 91 items that assess age-appropriate abilities in areas such as sensorimotor development, exploration and manipulation, object relatedness, concept formation, memory, and other aspects of cognitive processing. These 2 outcome measures were chosen to capture a proximal measure of cognitive development to the pregnancy period (ie, age 1 year) as well as a more robust assessment of cognitive development but at a more distal time point (ie, age 3 years). A summary score of cognitive development from years 1 to 3 was created. To achieve this, first the 1- and 3-year cognition scores were log-transformed. Second, the 1-year log score was subtracted from the 3-year log score. This served 2 purposes. First, the 3-year score was normalized by the earlier score. Second, it is a strategy to increase precision and statistical power by combining information per child from the 2 time points. The decision to create this outcome variable was informed and justified by an analysis of the correlation of the 2 scores in the full cohort (N = 896) [37].

Data Analysis

Biomarker, questionnaire, medical record and infant outcome variables were checked for outliers, and for a normal distribution. Where necessary, variables were natural log-transformed. FT4 did not require transformation and was analyzed on the original picomole per liter (pmol/L) scale. Distributions were compared between the full CANDLE cohort and the pilot sample.

Maternal THs and placental hormones were plotted against gestational age using both linear and quadratic terms for gestational age to visually identify patterns of change over the second trimester. First, unadjusted correlations of hormones with gestational age were calculated as Pearson coefficients. Second, linear regression models were fit to evaluate the presence of a curvilinear association of gestational age with the serum hormones. Third, additive interactions were evaluated to identify effect modifiers of gestational age variation (fetal sex, maternal age, body mass index [BMI], race). Effect modifiers were selected according to biologic theory. Fetal sex represents

both genetic and epigenetic sources of variation in development generally and in hormone levels specifically [38, 39]. Maternal age represents epigenetic variation in the transcriptional machinery of the placental and thyroid cells [40, 41]. Maternal race represents a complex mixture of environmental, nutritional, and psychosocial exposures that vary by race because of systemic and historical racism and redlining, often referred to as "weathering" in the case of Black women [42, 43]. Maternal BMI represents a mixture of molecules related to glucose, inflammatory markers, cytokines, and hormones that can alter cellular function at a global level [44]. In all models involving the hormones as exposures or outcomes, we evaluated the curvilinear relationship by including a linear and a quadratic term for the X variable (either gestational age or hormone) in the model, and reported it if the *P* value was less than or equal to .15. This allows for specification of the association that best explains the data empirically and according to the underlying biology [8, 45].

A similar strategy was used to calculate associations of placental biomarkers and maternal THs. Additive interactions were evaluated of the placental hormone with gestational age, with fetal sex, and with confounders (maternal age, BMI, race). A quadratic term for the placental hormone was evaluated. Backward selection was used to identify effect modifiers by noting variables with a product terms with a *P* value less than or equal to .15. The logic behind these models is to reduce confounding bias and to narrow hypotheses regarding placental regulation of maternal TH. Treating the placental biomarkers as outcomes, the same approach was used to evaluate the reverse scenario of the maternal thyroid as the predictor and the placental biomarkers as the outcome.

Adjusted associations of hormones, and infant cognitive development and communication were estimated using multivariable linear regressions. For each combination of TH and placental hormones, single effects of hormones were estimated separately in models with main effects only, from a model that included the additive interaction (joint effects) of the 2 hormones. The interaction was calculated as a product term for the 2 hormones. The β coefficient for the interaction can be interpreted as a change in infant cognitive development for a single-unit increase in the TH for each single-unit increase in hCG. The R^2 was used to compare the models without and with an interaction term and to compare the same model, but with different isoforms of hCG. All models are adjusted for fetal sex, maternal age, maternal race (White vs Black), maternal BMI at the time of the blood sample, marital status (single, partnered), education level (3 categories), and income level (3 categories). Hormones were included in the models as linear and as quadratic given prior knowledge of the shape of these relationships [4, 8]. All analyses and plots were generated in SAS 9.4 (SAS) or in R (R project).

Results

The sample of 50 women selected for this pilot study was similar to the overall CANDLE cohort in most characteristics (Table 1). In the full cohort only, female infants had slightly higher mean scores in infant cognitive development and communication. In the pilot study, maternal BMI was 1.25-fold higher in women carrying females vs women carrying males, and there was a higher incidence of childhood asthma as compared to the full cohort (40% vs 15%) [46]. hCG was slightly higher in women carrying females, consistent with previous knowledge [39, 47]. Levels of FT4, 5-HT, and GDF15 did not differ by fetal sex (see Table 1).

Within the second-/early third-trimester window studied here, FT4, hCG, hCGβ, and hCG-h decreased with increasing gestational age (Fig. 1) (Table 2). hCGα increased slightly. GDF15 and TSH were associated with gestational age according to a concave u-shaped pattern, reaching their lowest level around 22 weeks and then increasing (see Fig. 1). Serotonin did not correlate with gestational age. Associations of gestational age and FT4 were attenuated in women carrying females vs males and were more negative with increasing BMI. Conversely, associations of gestational age and hCGβ and hCG-h were more negative in women carrying females. Higher BMI and being White vs Black race attenuated the decrease of hCG-h with increasing gestational age.

hCGα and hCGβ were more correlated with each other in women carrying females vs males (r = 0.47 vs 0.18) [48]. Intact hCG and hCGβ were almost perfectly correlated [48], and respective associations with gestational age and with other biomarkers were the same. Therefore, intact hCG was omitted from Tables 2 and 3.

hCG was negatively associated with FT4 and not associated with TSH (see Table 3). In a more well-powered study and with control for confounding, hCGα may be positively and hCG-h may be negatively associated with TSH. Notably, maternal age and BMI modified the hCG associations with FT4 (see Table 3). Maternal race was consistently a modifier of the effects of GDF15 on hCG (see Table 3) and the effects of hCG on GDF15 [49]. GDF15 was similar to hCGα in its correlations with gestational age and with TH. Serotonin was not associated with TH or with hCG. In a more highly powered study and with control for confounding, serotonin and hCGα may be negatively associated. FT4 and TSH were not correlated in these data (see Table 3).

Table 1. Characteristics of CANDLE participants in the full study and pilot study sample

Variable	Full cohort	Pilot	Full vs pilot *P*	Male vs female fetus, full study
	No., (%)			*P*
No.	1503 (100)	50 (5)		
Baby sex			.96	.79
Male	736 (50)	25 (50)		
Female	726 (50)	25 (50)		
Education			.30	.49
< High school	184 (12)	3 (6)		
High school/GED	709 (47)	23 (46)		
> High school	608 (40)	24 (48)		
Race			.91	.30
Black	990 (66)	34 (68)		
White	482 (32)	16 (32)		
Income, $			.94	.95
< 24 999	599 (40)	21 (43)		
25 000-54 999	370 (39)	16 (33)		
≥ 55 000	319 (21)	12 (24)		
	Mean (95% CI)			
1-y cognitive score	17.0 (16.9 to 17.1)	17.2 (16.6 to 17.8)	.39	.18
3-y cognitive score	96.7 (96.0 to 97.3)	96.1 (93.8 to 98.2)	.56	< .01
Y 3 (log)-y 1 cognitive score (log)	1.75 (1.70 to 1.80)	1.73 (1.70 to 1.80)	.34	.05
Y 1 expressive communication, score	12.6 (12.5 to 12.8)	12.4 (11.6 to 13.1)	.46	< .01
Y 1 receptive communication, score	11.8 (11.6 to 11.9)	11.5 (10.9 to 12.1)	.35	< .01
Maternal BMI	27.5 (27.1 to 27.9)	28.5 (26.2 to 30.8)	.37	.69
Maternal age, y	26.1 (25.8 to 26.3)	26.9 (25.3 to 28.6)	.30	.37
	Pilot study only			
	Male fetus	Female fetus		
1-y cognitive score	17.2 (16.6 to 17.9)	17.2 (16.2 to 18.2)	.94	
3-y cognitive score	96.1 (93.1 to 99.2)	96.4 (93.6 to 99.2)	.89	
Y 3 (log)-y 1 cognitive score (log)	1.72 (1.70 to 1.80)	1.73 (1.70 to 1.80)	.79	
Y 1 expressive communication, score	12.1 (11.3 to 12.9)	12.6 (11.3 to 14.0)	.46	
Y 1 receptive communication, score	11.4 (10.5 to 12.2)	11.6 (10.7 to 12.5)	.69	
Gestational age at time of blood draw/urine sample, wks	23.9 (22.6 to 25.2)	22.6 (21.2 to 24.0)	.18	
Maternal BMI	25.3 (22.6 to 278)	31.8 (28.3 to 35.2)	< .01	
hCGα, log	10.2 (10.0 to 10.3)	10.4 (10.1 to 10.6)	.15	
hCGβ, log	5.16 (4.84 to 5.49)	5.54 (5.21 to 5.87)	.10	
Hyperglycosylated hCG, log	6.43 (5.98 to 6.87)	6.78 (6.42 to 7.14)	.21	
Intact hCG, log	10.4 (10.0 to 10.8)	10.8 (10.5 to 11.2)	.08	
TSH, log	0.16 (–0.04 to 0.37)	0.25 (0.01 to 0.49)	.57	
FT4, log	2.18 (2.14 to 2.22)	2.19 (2.13 to 2.26)	.68	
Serotonin, log	5.78 (5.52 to 6.05)	5.75 (5.49 to 6.01)	.85	
GDF15, log	6.59 (6.43 to 6.75)	6.50 (6.32 to 6.69)	.48	

Abbreviations: BMI, body mass index; FT4, free thyroxine; GDF15, growth differentiation factor 15; GED, General Educational Development; hCG, human chorionic gonadotropin; TSH, thyrotropin.

When adjusted for levels of TSH, hCGβ, total hCG, or hCG-h, FT4 was associated with cognitive development according to a curvilinear pattern (Table 4). On the other hand, TSH was associated with infant cognitive development only when modeled jointly (ie, interaction) with hCGα, hCGβ, or total hCG (Table 5). The interaction was strongest with hCGα in terms of model fit and precision in the point estimate (see Table 5) [50]. The TSH association was null at age 1 year (Fig. 2D). There was a curvilinear u-shaped association at age 3 years (TSH: –3.9 units cognition per log unit TSH; 95% CI, –8.1 to 0.4; TSH*TSH 8.1 units cognition per log unit TSH; 95% CI. 1.9 to 14) (Fig. 2E).

The association of hCGα with cognitive development at ages 1 and 3 years are presented separately (see Tables 4 and 5, Fig. 2G–2I). hCGα was correlated with cognitive

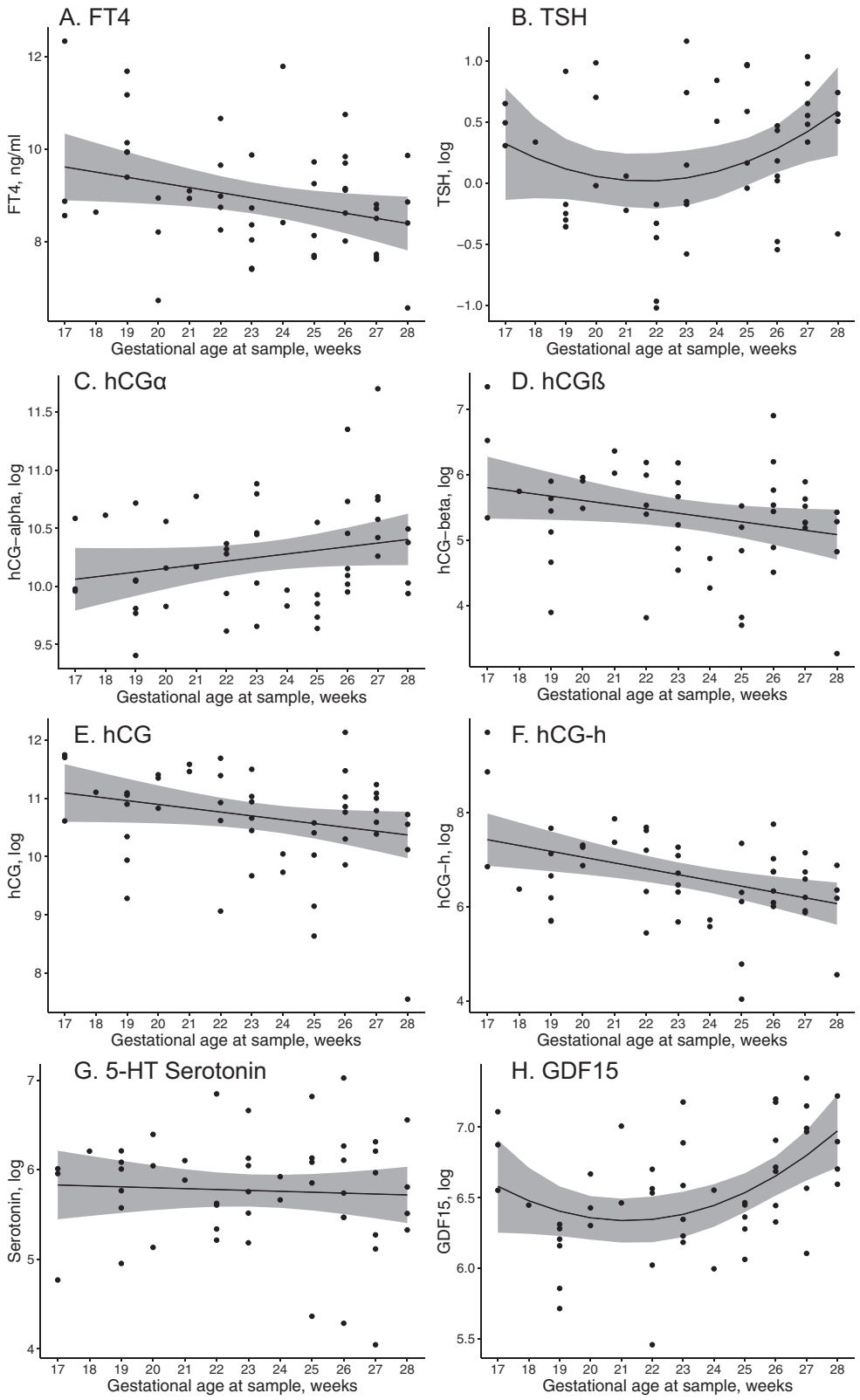

Figure 1. Change over gestational time (17-28 weeks) in maternal serum levels of free thyroxine, thyrotropin (TSH), 4 isoforms of human chorionic gonadotropin (hCG), 5-HT serotonin, and growth differentiation factor (GDF15). The black lines and gray-shaded areas represent the modeled association and 95% CI adjusted for confounding. The black dots represent the observed unadjusted values, not controlled for confounders.

Table 2. Correlations and adjusted linear associations of gestational age at time of blood draw (X) and placental biomarkers and thyroid hormones (Y) in midpregnancy

Biomarker (Y), log	Predictor (X)	Pearson correlation[a] (95% CI)	Linear β[b] (95% CI)	*P*	Quadratic β (95% CI)	*P*	Variables that interacted with gestational age
FT4	Gestational age	−0.32 (−0.55 to −0.05)	−0.01 (−0.02 to 0.00)	.04			Fetal sex (female +, male −), BMI (−)
TSH	Gestational age	0.19 (−0.09 to 0.44)	−0.51 (−1.24 to 0.22)	.17	0.01 (0.00 to 0.03)	.14	None
hCGα	Gestational age	0.23 (−0.05 to 0.48)	0.04 (0.0 to 0.08)	.04			None
hCGβ	Gestational age	−0.28 (−0.51 to 0.01)	−0.06 (−0.13 to 0.01)	.09			BMI (+)
hCG-h	Gestational age	−0.41 (−0.62 to −0.15)	−0.12 (−0.19 to −0.03)	< .01			Fetal sex (female −, male +), race (White +, Black −)
GDF15	Gestational age	0.37 (0.10 to 0.59)	−0.71 (−1.33 to −0.08)	.02	0.02 (0.00 to 0.03)	.02	None
Serotonin	Gestational age	−0.06 (−0.34 to 0.22)	−0.02 (−0.07 to 0.04)	.53			None

A quadratic term for gestational age and interactions of gestational age with variables (fetal sex, maternal age, race, BMI) were reported with a *P* value less than or equal to .15.

Abbreviations: 5-HT, serotonin; BMI, body mass index; FT, free thyroxine; GDF15,growth differentiation factor 15; Gestational age, gestational age at time of blood draw; hCG, human chorionic gonadotropin; hCG-h, hyperglycosylated human chorionic gonadotropin; TSH, thyrotropin.

[a]The Pearson correlation is bounded by 0 and 1.

[b]The β coefficient is not bounded, and it is the change in biomarker (Y) for each week of gestation (X). It is not adjusted for interactions. Interactions were evaluated separately. The linear β is the effect of X and the quadratic β is the effect of X^2.

development at year 1 according to a curvilinear, inverse u-shaped relationship (hCGα: 57.3 units cognition per log unit hCGα; 95% CI, 15.6 to 99; hCGα*hCGα: –2.7; 95% CI, –4.7 to –0.7). hCGα was not associated with year 3 cognition; however, hCGα was associated with the combined 1- to 3-year cognitive development score when coadjusted for TSH (see Fig. 2I).

A comparison of R^2 values was used to identify the unique set of hormones that best explain the variation in each form of infant cognitive development. For each of the 3 outcomes, hCGα combined with TSH offered the best model fit [50]. The highest R^2 was achieved with an interaction model of TSH and hCGα in predicting infant cognitive development at age 3 years (R^2 = 0.55). The interaction effect of TSH and intact hCG had the greatest relative change in R^2 (% change in R^2 = 44%) as compared to a model without interaction.

FT4 was not associated with expressive or receptive communication in infants (see Table 4) [51]. TSH was associated with expressive communication and had a weak negative linear association with receptive communication (see Table 5) [51]. hCGα was associated with expressive communication, but not with receptive communication [51]. 5-HT and GDF15 were not associated with infant cognitive development or communication when modeled independently or jointly with TSH or FT4.

Discussion

This epidemiologic pilot study lends support to and offers novel insight into the theory that the fetal placenta and the maternal thyroid work together in pregnancy to ensure proper fetal brain development [7, 8]. The link we address here between the placenta and thyroid is placental hormone hCG, which reaches high serum levels during pregnancy and has thyrotropic action [52]. We found that brain development, assessed as infant cognitive development and communication at ages 1 and 3 years, was associated with maternal serum levels of FT4, whereas TSH was associated with cognitive development only when modeled jointly with hCG. Importantly, hCGα (common to TSH and other glycoprotein hormones) showed an independent association with cognitive development. To our knowledge, such an association has not been previously reported. Another novel key finding here is that these associations extend beyond the first trimester into the second and early third trimesters.

Based on previous findings in the Generation R birth cohort study, FT4 is positively correlated and TSH is negatively correlated with hCG in the first trimester [8]. We report evidence here that these relationships might switch direction in the second trimester whereby FT4 and hCG are inversely

Table 3. Correlations and adjusted linear associations of placental biomarkers (X) and thyroid hormones (Y) in midpregnancy

Hormone (Y), log	Biomarker (X), log	Pearson correlation[a] (95% CI)	β Coefficient[b] (95% CI)	*P*	Variables that interacted with biomarker (X) (direction of interaction β coefficient)
FT4	TSH	–0.14 (–0.40 to 0.15)	–0.03 (–0.10 to 0.04)	.44	Maternal age (–)
FT4	hCGα	–0.32 (–0.55 to –0.05)	–0.08 (–0.17 to 0.01)	.09	Maternal BMI (–)
FT4	hCGβ	–0.26 (–0.50 to 0.02)	–0.07 (–0.12 to –0.03)	< .01	Maternal age (+), BMI (–)
FT4	hCG-h	–0.13 (–0.39 to 0.16)	–0.05 (–0.09 to –0.01)	.01	Maternal age (+), BMI (–)
FT4	GDF15	–0.34 (–0.57 to –0.07)	–0.06 (–0.14 to 0.02)	.14	None
FT4	Serotonin	–0.03 (–0.30 to 0.26)	–0.03 (–0.09 to 0.04)	.40	Maternal age (–), BMI (+), race (White –, Black +)
TSH	FT4	–0.14 (–0.40 to 0.15)	–0.51 (–1.84 to 0.82)	.44	None
TSH	hCGα	0.21 (–0.07 to 0.46)	0.21 (–0.19 to 0.60)	.31	None
TSH	hCGβ	–0.14 (–0.40 to 0.15)	–0.05 (–0.27 to 0.16)	.60	Gestational age (–)
TSH	hCG-h	–0.22 (–0.47 to 0.06)	–0.11 (–0.29 to 0.08)	.24	Gestational age (–)
TSH	GDF15	0.20 (–0.09 to 0.46)	0.19 (–0.16 to 0.54)	.28	None
TSH	Serotonin	–0.07 (–0.34 to 0.22)	0.02 (–0.25 to 0.29)	.91	None

A quadratic term for placental biomarkers and interactions of placental biomarkers with confounding variables (fetal sex, maternal age, race, BMI) were reported with a *P* value less than or equal to .15.

Abbreviations: BMI, body mass index; FT4, free thyroxine; GDF15, growth differentiation factor 15; hCG, human chorionic gonadotropin; hCG-h, hyperglycosylated human chorionic gonadotropin; TSH, thyrotropin.

[a]The Pearson correlation is bounded by 0 and 1.

[b]The β coefficient is not bounded, and it is the change in hormone (Y) per log unit increase in the biomarker (X), after adjustment for linear gestational age (plus quadratic gestational age for TSH), maternal age, BMI, and race. The interactions with the biomarker (X) were evaluated in a separate model.

correlated. TSH and hCG were not correlated with each other. Overall, the associations suggest thyrotropic actions of hCG differed slightly by the form of hCG, and these actions occurred only at the level of the maternal thyroid and not at the level of the maternal pituitary as no associations were detected with TSH levels. This finding presents a challenge to the overall interpretation of our results, given hCG interacted with TSH and not with FT4 in the estimation of child neurodevelopmental outcomes.

Thyrotropic actions of hCG were weaker with increasing maternal age, and stronger with increasing BMI. Because of the small sample size, and evidence of effect modification, it is not possible to establish here what is "normal" or indicative of "normal" development. However, these data motivate future efforts to measure these hormones in larger data sets and to calculate and report unadjusted correlations and associations with adjustment for confounding and consideration of effect modification.

HCGβ and hCG-h may regulate TSH levels, and that relationship varied by gestational week in the second trimester. We leave open the possibility of the reverse relationship whereby the maternal or fetal thyroid is stimulating production of placental hCG in the second trimester. In Generation R and in our study (before adjustment for confounding), the thyrotropic actions, based on FT4 levels, of hCG were stronger in women carrying females [8].

Findings reported from Generation R evaluated THs during early pregnancy (weeks 9-18) in the regulation of brain development, when the fetal thyroid is not fully functional yet and fetal brain development is dependent on maternal THs [1-4]. They reported that both low and high maternal FT4 concentrations during pregnancy are associated with lower child IQ [4]. Our aim was to expand this to later stages of the pregnancy (17-28 weeks' gestation) and more thoroughly investigate placental contribution by addressing circulating levels of hCG and its subunits. During this period, the fetal hypothalamus matures and fetal TSH production begins [53]. This may explain why TSH levels started to increase at 22 weeks. The cerebellum, which is involved in higher cognitive processes including language, undergoes major growth and expansion [54]. There is new evidence in rodents that the neocortex continues to develop upper-layer cortical neurons throughout gestation [55].

Previous birth cohort studies have not measured hCG subunits (hCGα and hCGβ) or hCG-h (this assay recognizes also hyperglycosylated hCGβ) separately. In doing this, we wanted to address hCG variants and subunits more specifically. This may relate to important differences in binding and activation at the level of the luteinizing hormone/chorionic gonadotropin receptor (LHCGR) [21, 22, 52, 56]. The findings here yield hypotheses that can be explored in functional studies of the receptor activation and regulation of hormone expression. The relative expression of hCG subunits differs in different stages of pregnancy, which may also reflect specific yet unknown roles of these hormones in fetal development [57-60].

Table 4. Adjusted linear associations of second-trimester maternal serum free thyroxine and human chorionic gonadotropin and infant cognition and communication measures at age 1 and 3 years, presented as single effects and as joint effects (interactions)

	Cognition scale, 1 y		Cognition scale, 3 y		Summary score, 1-3 y cognition scale		Expressive communication scale		Receptive communication scale	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
FT4, pmol/L	–5.31[a] (–12.4 to 1.79)	.14	16.4[a] (–4.33 to 37.1)	.12	**0.50[a] (–0.03 to 1.02)**	**.06**	1.02 (–8.49 to 10.5)	.83	–0.24 (–7.79 to 7.31)	.95
TSH, log	0.17 (–1.27 to 1.62)	.81	–3.00[a] (–7.21 to 1.22)	.16	–0.04[a] (–0.15 to 0.07)	.45	–0.20 (–2.14 to 1.74)	.84	–1.20 (–2.74 to 0.34)	.12
FT4 × TSH	0.35 (–0.75 to 1.45)	.52	–0.79 (–4.00 to 2.42)	.62	–0.03 (–0.11 to 0.05)	.42	0.20 (–1.28 to 1.68)	.78	–0.31 (–1.48 to 0.86)	.59
FT4, pmol/L	–3.61 (–10.2 to 2.96)	.27	13.0 (–9.26 to 35.4)	.24	0.35 (–0.15 to 0.86)	.16	4.02 (–4.27 to 12.3)	.33	1.06 (–6.44 to 8.55)	.78
hCGα, log	**52.9[a] (11.4 to 94.3)**	**.01**	–42.0 (–183 to 98.6)	.55	**–3.82[a] (–7.00 to –0.64)**	**.02**	**89.8[a] (37.5 to 142)**	**< .01**	27.3 (–20.0 to 74.5)	.25
FT4 × hCGα	0.20 (–1.51 to 1.90)	.82	–2.50 (–8.23 to 3.22)	.38	–0.04 (–0.17 to 0.09)	.56	0.10 (–2.05 to 2.25)	.92	–1.19 (–3.10 to 0.71)	.21
FT4	**–5.71[a] (–12.6 to 1.21)**	**.10**	16.2[a] (–6.43 to 38.8)	.16	**0.52[a] (0.00 to 1.05)**	**.05**	0.82 (–8.55 to 10.2)	.86	0.95 (–6.72 to 8.63)	.80
hCGβ, log	–0.50 (–7.13 to 6.12)	.88	9.07 (–12.6 to 30.8)	.40	0.13 (–0.37 to 0.64)	.60	–0.34 (–9.32 to 8.63)	.94	4.42 (–2.93 to 11.8)	.23
FT4 × hCGβ	0.62 (–0.21 to 1.45)	.14	–1.33 (–4.09 to 1.43)	.33	–0.05 (–0.11 to 0.01)	.11	0.31 (–0.84 to 1.46)	.59	0.53 (–0.40 to 1.46)	.25
FT4, pmol/L	–5.42[a] (–12.5 to 1.64)	.13	15.2 (–7.69 to 38.1)	.19	**0.49[a] (–0.05 to 1.03)**	**.07**	1.15 (–8.28 to 10.6)	.81	0.75 (–7.01 to 8.52)	.84
hCG-h, log	–0.31 (–5.22 to 4.60)	.90	4.57 (–11.4 to 20.5)	.56	0.07 (–0.31 to 0.45)	.71	0.45 (–6.10 to 7.01)	.89	1.94 (–3.46 to 7.34)	.47
FT4 × hCG-h	0.45 (–0.29 to 1.19)	.23	–1.02 (–3.44 to 1.41)	.40	–0.04 (–0.09 to 0.02)	.19	0.21 (–0.79 to 1.22)	.67	0.42 (–0.39 to 1.24)	.30
FT4, pmol/L	–5.62[a] (–12.6 to 1.35)	.11	13.62 (–9.42 to 36.7)	.24	**0.49[a] (–0.05 to 1.03)**	**.07**	0.64 (–8.73 to 10.0)	.89	0.78 (–7.02 to 8.59)	.84
hCG, log	–3.26 (–14.0 to 7.44)	.54	–3.91 (–39.2 to 31.4)	.82	0.18 (–0.65 to 1.00)	.67	–5.67 (–20.0 to 8.70)	.43	3.49 (–8.48 to 15.5)	.56
FT4 × hCG	0.55 (–0.23 to 1.34)	.16	–0.97 (–3.62 to 1.67)	.46	–0.04 (–0.10 to 0.02)	.15	0.35 (–0.73 to 1.43)	.51	0.48 (–0.41 to 1.37)	.28
FT4	–5.22[a] (–12.4 to 1.95)	.15	12.14 (–10.6 to 34.9)	.29	**0.45[a] (–0.10 to 1.00)**	**.10**	1.49 (–8.09 to 11.1)	.75	1.24 (–6.11 to 8.59)	.73
Serotonin, log	–1.53 (–14.7 to 11.6)	.81	3.80 (–37.8 to 45.3)	.85	0.15 (–0.85 to 1.16)	.76	–3.43 (–21.0 to 14.1)	.69	–4.35 (–17.8 to 9.11)	.52
FT4 × serotonin	0.27 (–0.91 to 1.45)	.65	–1.04 (–4.76 to 2.69)	.58	–0.03 (–0.12 to 0.06)	.50	0.81 (–0.75 to 2.36)	.30	0.77 (–0.41 to 1.95)	.20
FT4, pmol/L	–4.78 (–11.7 to 2.14)	.17	11.9 (–10.9 to 34.7)	.30	0.42[a] (–0.11 to 0.95)	.12	2.52 (–6.77 to 11.8)	.59	1.28 (–6.36 to 8.91)	.74
GDF15, log	14.7 (–24.5 to 53.8)	.45	–64.4 (–193 to 64.7)	.32	–1.63 (–4.62 to 1.37)	.28	36.4 (–16.1 to 88.9)	.17	24.7 (–18.5 to 67.9)	.25
FT4 × GDF15	0.43 (–1.34, 2.19)	.63	–0.26 (–6.09, 5.57)	.93	–0.03 (–0.16, 0.11)	.67	0.98 (–1.37, 3.33)	.40	0.87 (–1.06, 2.80)	.37

Associations with a *P* value less than or equal to .1 appear in bold. All models are adjusted for the other biomarker immediately below or above it, fetal sex, maternal age, maternal race, maternal BMI, marital status, education level, income level, and hormones (linear quadratic). The additive interaction of the 2 hormones was estimated from a linear regression and reported as the β coefficient for the product term.

Abbreviations: BMI, body mass index; FT4, free thyroxine; GDF15, growth differentiation factor 15; hCG, human chorionic gonadotropin; hCG-h, hyperglycosylated human chorionic gonadotropin; TSH, thyrotropin.

[a]Curvilinear association is present as indicated by a quadratic term in the model with a *P* value less than .15. Only the linear coefficient is presented in Table 4.

Table 5. Adjusted linear associations of second-trimester maternal serum thyrotropin and human chorionic gonadotropin and infant cognition and communication measures at age 1 and 3 years, presented as single effects and as joint effects (interactions)

	Cognition scale, 1 y		Cognition scale, 3 y		Summary score, 1-3 y cognition scale		Expressive communication scale		Receptive communication scale	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
TSH, log	0.43 (–0.94 to 1.80)	.53	**–3.88[a] (–8.12 to 0.36)**	**.07**	–0.07 (–0.17 to 0.04)	.20	–0.03 (–1.75 to 1.69)	.97	–1.00 (–2.51 to 0.5)	.19
hCGα, log	**57.3[a] (15.6 to 99.0)**	**.01**	–47.5 (–177 to 81.7)	.46	**–4.15[a] (–7.30 to –1.01)**	**.01**	**83.9[a] (31.4 to 136)**	**< .01**	23.9 (–22.0 to 69.7)	.30
TSH × hCGα	–1.65 (–4.56 to 1.25)	.26	**9.50 (0.92 to 18.1)**	**.03**	**0.21 (0.00 to 0.42)**	**.05**	–1.06 (–4.78 to 2.65)	.57	1.77 (–1.44 to 4.97)	.27
TSH, log	0.37 (–1.13 to 1.88)	.62	–3.28[a] (–7.78 to 1.23)	.15	–0.05 (–0.17 to 0.06)	.34	–0.13 (–2.10 to 1.84)	.89	–1.02 (–2.6 to 0.55)	.20
hCGβ, log	0.51 (–6.51 to 7.52)	.88	1.11 (–19.8 to 22.1)	.91	–0.01 (–0.54 to 0.52)	.98	–0.53 (–9.68 to 8.62)	.91	3.19 (–4.13 to 10.5)	.38
TSH × hCGβ	**–1.86 (–3.61 to –0.12)**	**.04**	0.74 (–4.80 to 6.27)	.79	**0.13 (0.00 to 0.26)**	**.06**	**–2.15 (–4.45 to 0.15)**	**.07**	–0.92 (–2.83 to 0.98)	.33
TSH, log	0.40 (–1.14 to 1.95)	.60	–3.22[a] (–7.77 to 1.34)	.16	–0.06 (–0.17 to 0.06)	.34	0.00 (–2.00 to 2.01)	1.0	–1.19 (–2.79 to 0.41)	.14
hCG–h, log	0.40 (–4.68 to 5.48)	.87	1.48 (–13.5 to 16.5)	.84	0.00 (–0.39 to 0.38)	.98	0.36 (–6.23 to 6.94)	.91	0.99 (–4.27 to 6.25)	.71
TSH × hCG–h	–1.17 (–2.88 to 0.54)	.17	–0.41 (–5.59 to 4.77)	.87	0.07 (–0.06 to 0.2)	.26	–1.21 (–3.45 to 1.03)	.28	–0.41 (–2.23, 1.40)	.65
TSH, log	0.38 (–1.11 to 1.88)	.61	**–3.71[a] (–8.08 to 0.65)**	**.09**	–0.06 (–0.17 to 0.05)	.29	–0.15 (–2.09 to 1.80)	.88	–1.16 (–2.73 to 0.40)	.14
hCG, log	–1.10 (–12.4 to 10.2)	.84	–20.3 (–53.1 to 12.5)	.22	–0.12 (–0.98 to 0.73)	.77	–5.62 (–20.2 to 8.99)	.44	2.74 (–9.04 to 14.5)	.64
TSH × hCG	**–2.06 (–3.82 to –0.31)**	**.02**	2.06 (–3.41 to 7.53)	.45	**0.15 (0.02 to 0.29)**	**.02**	–2.19 (–4.53 to 0.14)	.07	–1.18 (–3.12 to 0.75)	.22
TSH, log	0.26 (–1.23 to 1.74)	.73	–3.31[a] (–7.54 to 0.93)	.12	–0.05 (–0.16 to 0.06)	.38	–0.20 (–2.13 to 1.73)	.83	–1.16 (–2.59 to 0.28)	.11
Serotonin, log	–0.04 (–13.5 to 13.5)	1.0	–4.90 (–43.3 to 33.5)	.80	–0.03 (–1.04 to 0.99)	.96	–3.20 (–20.7 to 14.3)	.71	–4.22 (–17.3, 8.80)	.51
TSH × serotonin	2.00 (–0.68 to 4.68)	.14	–2.11 (–9.96 to 5.75)	.59	–0.15 (–0.35 to 0.05)	.13	0.54 (–3.05 to 4.13)	.76	0.57 (–2.09 to 3.24)	.67
TSH, log	0.12 (–1.35 to 1.59)	.87	**–3.61[a] (–7.89 to 0.66)**	**.10**	–0.04[a] (–0.15 to 0.07)	.43	–0.28 (–2.19 to 1.64)	.77	–1.06 (–2.6 to 0.47)	.17
GDF15, log	20.0 (–19.7 to 59.7)	.31	**–98.9[a] (–214 to 16.5)**	**.09**	–2.30[a] (–5.27 to 0.66)	.12	36.1 (–15.6 to 87.7)	.17	23.17 (–18.29 to 64.6)	.26
TSH × GDF15	–1.25 (–4.61 to 2.10)	.45	6.06 (–3.56 to 15.7)	.21	0.15 (–0.09 to 0.40)	.22	–2.25 (–6.59 to 2.09)	.30	0.38 (–3.15 to 3.92)	.83

Associations with a *P* value less than or equal to .1 appear in bold. All models are adjusted for the other biomarker immediately below or above it, fetal sex, maternal age, maternal race, maternal BMI, marital status, education level, income level, and hormones (linear quadratic). The additive interaction of the 2 hormones was estimated from a linear regression and reported as the β coefficient for the product term.

Abbreviations: BMI, body mass index; FT4, free thyroxine; GDF15, growth differentiation factor 15; hCG, human chorionic gonadotropin; hCG-h, hyperglycosylated human chorionic gonadotropin; TSH, thyrotropin.

[a]Curvilinear association is present as indicated by a quadratic term in the model with a *P* value less than .15. Only the linear coefficient is presented in Table 5.

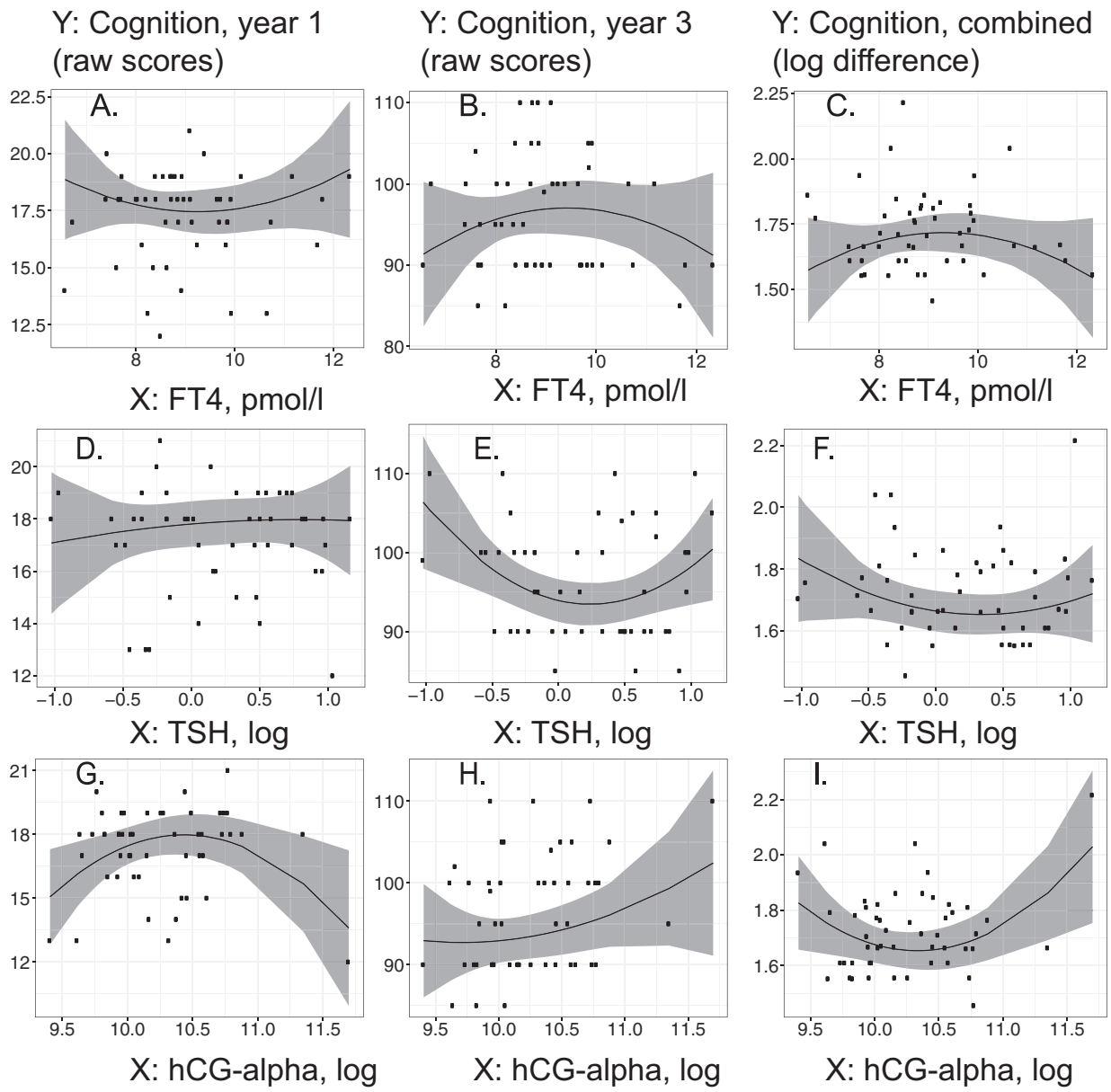

Figure 2. Adjusted associations of second-trimester maternal serum thyroid and placental hormones and infant cognition at years 1 and 3. The black lines and gray-shaded areas represent the modeled association and 95% CI, adjusted for confounding. The black dots represent the observed unadjusted values, not controlled for confounders.

One of the major findings in the present study was the independent association of hCGα with cognitive development. hCGα levels did not correlate strongly with hCGβ levels and generally showed different correlation patterns as compared to the other forms of hCG. While hCGα levels likely reflect the expression in placenta, which is the dominant source of both subunits of hCG, it is noteworthy that hCGα is also expressed in the pituitary gland [61-64]. Thus, it is possible that maternal and/or fetal pituitary contribute to the hCGα in maternal blood.

There is no existing biological theory to explain the specific association between hCGα and infant cognitive development and communication reported here. This association was stronger than that of THs or hCG, hCG-h, or hCGβ. One explanation could be simply that hCGα is in higher abundance in the second trimester [65]. While hCG may be involved indirectly in brain development by way of modulating placental functions, such as nutrient transfer and other mechanisms [66, 67], there is no prior evidence that hCG subunits would be differentially associated with placental size or nutrient transfer [68].

Serotonin and GDF15 were also included as candidate biomarkers that contribute to fetal brain development [69-71]. Serotonin in this context has been studied only in a mouse model and therefore may not translate to human pregnancy. Neither 5-HT nor GDF15 were informative or

supportive of our hypothesis on placental regulation of brain development. Of note, GDF15 and hCG levels were highly correlated, especially hCGα and GDF15. Curiously, the direction of the association of hCGα and hCGβ with GDF15 were in the same direction overall, but the associations were in a different direction when modified by race. One speculation, to be explored in future studies, is that hCGα reflects information different from hCGβ regarding maternal-placental mechanisms of adversity and/or stress. Other important circulating factors, not addressed here, are likely to reflect fetal thyroid function, which also contributes to cognitive development [72].

Despite the small sample size, we increased statistical power by collecting highly precise, highly quantitative, and multiple measures of a common mechanism. A limitation of the sample size was that we were not able to consider sex-specific effects of hormones on infant cognitive development in our final models. The decision to evaluate quadratic terms in our models for all hormones was based on the prior knowledge that these relationships follow nonmonotonic trends [4, 73]. To avoid overadjustment, we limited adjustment for confounding to 7 variables that were constant throughout all the models: fetal sex, maternal age, race, BMI, income, education, and marital status. Maternal stress would have been an important confounder [1], but data were not available for that. Two reasons why these results may not be generalizable to a larger cohort may stem from the fetal sex imbalance in maternal BMI and the high prevalence of childhood asthma, indicating a potentially abnormal maternal and/or fetal environment.

Conclusions

Pilot studies serve an important function in generating new ideas and approaches to be scaled up to larger studies. This study, especially the observed association of hCGα with cognitive development and TH levels, allowed several new hypotheses to be generated. These may open up new functions for hCGα, generally considered to be active when it forms a dimer with β subunits of glycoprotein hormones. Such specific hypothesis include 1) hCG replaces TSH in the second trimester to stimulate FT4 production through negative feedback, and this mechanism is modified by increasing maternal adiposity and maternal age; 2) hCGα has a specific receptor-mediated action on fetal brain cells; and 3) differences in correlations among the hCG forms may relate to the unique characteristics of LHCGR binding; or 4) they may reflect different tissue sources of hCGα (placenta, maternal and fetal pituitary) and TSH (fetal, maternal) that are changing over the second trimester.

These hypotheses can be explored in well-powered, biomarker-based birth cohort studies, or in functional studies at the cellular level. The hormonal pathway outlined here may be a target for environmental exposures, like endocrine-disrupting chemicals and maternal stress, to affect brain development, and will be explored further in that context [1, 74, 75].

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Disclosures: The authors have nothing to disclose.

Data Availability: Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided. CANDLE is a participating cohort in the National Institutes of Health program Environmental Influences on Child Health Outcomes (ECHO), a mechanism for sharing birth cohort data.

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