Thyroid Function and Premature Delivery in TPO Antibody−Negative Women: The Added Value of hCG

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Context: Human chorionic gonadotropin (hCG) stimulates thyroid function during pregnancy. We recently showed that thyroid autoimmunity severely attenuated the thyroidal response to hCG stimulation and that this may underlie the higher risk of premature delivery in thyroperoxidase antibody (TPOAb)-positive women. We hypothesized that a lower thyroidal response to hCG stimulation in TPOAb-negative women is also associated with a higher risk of premature delivery and preterm premature rupture of membranes (pPROM).

Design, Setting, and Participants: Thyrotropin (TSH), free thyroxine (FT4), and hCG concentrations were available in 5644 TPOAb-negative women from a prospective cohort. We tested for interaction between TSH or FT4 and hCG in linear regression models for duration of pregnancy and logistic regression models for premature delivery/pPROM. Accordingly, analyses were stratified per TSH percentile (TSH $\geq$ 85th percentile) and hCG per 10,000 IU/L.

Results: Women with high TSH and low hCG concentrations did not have a higher risk of premature delivery or pPROM, with protective effect estimates. In contrast, women with a high TSH concentration despite a high hCG concentration had twofold to 10-fold higher risk of premature delivery ($P_{difference}$ = 0.022) and an up to fourfold higher risk of pPROM ($P_{difference}$ = 0.079). hCG concentrations were not associated with premature delivery or pPROM.

Conclusion: In TPOAb-negative women with high-normal TSH concentrations, only women with high hCG concentrations had a higher risk of premature delivery or pPROM. These results suggest a lower thyroidal response to hCG stimulation is also associated with premature delivery in TPOAb-negative women and that an additional measurement of hCG may improve thyroid-related risk assessments during pregnancy. **(*J Clin Endocrinol Metab* 102: 3360–3367, 2017)**

Thyroid hormone (TH) regulates numerous metabolic processes that are important for an uncomplicated pregnancy course. During early pregnancy, human chorionic gonadotropin (hCG) stimulates the thyrotropin (TSH) receptor, which leads to an increase in free thyroxine (FT4) concentrations and a subsequent decrease in TSH concentrations (1, 2). We recently demonstrated that thyroperoxidase antibody (TPOAb)-positive women had an impaired response to thyroidal stimulation by hCG (3). In that study, we also showed that TPOAb-positive women

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Abbreviations: BMI, body mass index; FT4, free thyroxine; hCG, human chorionic gonadotropin; pPROM, preterm premature rupture of membranes; SNP, single nucleotide polymorphism; TH, thyroid hormone; TPOAb, thyroperoxidase antibody; TSH, thyrotropin.

with a lower thyroidal response to hCG stimulation had a higher risk of premature delivery. However, even in TPOAb-negative women, subgroups of women may have an impaired response to hCG. This may include women with isolated thyroglobulin antibody positivity, as occurs in up to 25% of women with thyroid autoimmunity (4). Furthermore, the presence of TSH receptor-blocking antibodies, a history of thyroid infection, or exposure to thyrotoxic treatments (*i.e.,* specific drugs or previous irradiation of the neck) may affect thyroid functional capacity in TPOAb-negative women.

hCG is a major determinant of gestational thyroid function but has a notoriously versatile pattern throughout early pregnancy. hCG is undetectable before conception, but after conception, hCG rapidly rises to high concentrations until approximately the ninth to the 12th week, after which it steadily declines during the remainder of pregnancy (5). Gestational hCG concentrations have a large within-individual variability and also exhibit clear between-individual differences when measured at the same time point (5). This leads to high inter- and intraindividual variabilities in the extent of thyroidal stimulation by hCG. Although a recent study from our group indicated that an impaired thyroidal response to hCG in TPOAb-positive women is a risk factor for premature delivery, it remains unknown whether a suboptimal thyroidal response to hCG stimulation in TPOAb-negative women is of clinical relevance and/or is a risk factor for premature delivery.

Overt as well as mild forms of maternal thyroid dysfunction have been associated with a higher risk of premature delivery (6–10). Risk estimates for premature delivery in women with subclinical hypothyroidism and hypothyroxinemia differ widely between studies and vary from a 30% lower risk to a 3.3-fold higher risk (11–16). It has been hypothesized that these between-study differences are a consequence of variations in population characteristics, such as TPOAb positivity, iron status, ethnicity, body mass index (BMI), parity, and smoking status (17–23). Interestingly, all of these population characteristics are important determinants of hCG concentrations during early pregnancy (5). Moreover, we recently showed that women with subclinical hypothyroidism had a lower thyroidal response to hCG stimulation regardless of their TPOAb status or BMI (24). Therefore, we hypothesized that even in TPOAb-negative women, lower thyroidal stimulation by hCG is associated with a higher risk for premature delivery and preterm premature rupture of membranes (pPROM), a major risk factor for premature delivery.

Materials and Methods

To investigate this hypothesis, we determined hCG concentrations in the Generation R Study, a population-based prospective cohort from early fetal life onward in Rotterdam, The Netherlands (25). We previously reported on the association between TSH or FT4 concentrations and the risk of premature delivery in this population (26). In the same population, we investigated whether the addition of hCG during early pregnancy improved the interpretation of maternal TSH and FT4 concentrations for the risk assessment of premature delivery (26). Populations for analyses, covariates, and definitions of outcomes have been described previously (26). In short, 6264 pregnant women had data available during early pregnancy on TSH, FT4, or TPOAb concentration and pregnancy duration or pPROM. We excluded women with twin pregnancies (N = 128), preexisting thyroid disease or thyroid-interfering medication usage (N = 89), women who underwent fertility treatment (N = 76), TPOAb-positive women (N = 312), and women with hCG measurements that were not performed within the same week as thyroid function measurements (N = 15). Premature delivery was defined as a pregnancy duration <37 weeks, and spontaneous premature delivery was defined as not having had a delivery after induction of labor or by an elective caesarean section. pPROM was defined as ruptured membranes before 37 weeks' gestation. All analyses on premature delivery were adjusted for maternal age, BMI, smoking, parity, education level, ethnicity, height, and fetal sex.

We performed various sensitivity analyses: (1) Because the cutoff for the effects of thyroid autoimmunity on thyroidal stimulation by hCG may be lower than currently used TPOAb-positivity cutoffs (*e.g.,* 60 IU/L for our assay), we additionally excluded women with TPOAbs >10 IU/L. (2) Preeclampsia is a risk factor for premature delivery and may be associated with specific combinations of hCG and FT4 (27); therefore, we also excluded women with preeclampsia. (3) Spontaneous premature delivery may be a better reflection of the underlying biology; therefore, we also excluded women with nonspontaneous premature delivery.

During the peer review process, additional analyses were performed in the full data set (including TPOAb-positive women) to investigate whether TSH is a less sensitive marker in TPOAb-positive women than in TPOAb-negative women. To do so, we compared the association of FT4 with TSH concentrations in TPOAb-positive vs TPOAb-negative women. These analyses were subsequently added to the supplemental material.

Statistical analysis

To fulfill model assumptions, TSH values were logarithmically transformed. We used linear or logistic regression models with restricted cubic splines with three knots to assess nonlinearity of the associations between TSH, FT4, hCG, and gestational age or (spontaneous) premature delivery and pPROM. Subsequently, linear or logistic regression models were built accordingly. To test the hypothesis that a high hCG level helps to distinguish women with a high TSH or low FT4 concentration and a high risk of premature delivery, we stratified the association between high TSH and/or low FT4 concentration and premature delivery according to hCG level. To investigate whether the association between continuous TSH and/or FT4 concentration and premature delivery or pPROM differs according to hCG concentrations, we tested for interaction by adding a product term of TSH or FT4 and hCG to the model. The identification of clinically relevant effect modification requires more statistical power; therefore, we

considered interaction terms with a P value <0.15 for assessment of clinical relevance by subsequently stratifying analyses. We performed a similar interaction analysis in which hCG was replaced with gestational age at blood sampling because gestational age is considered a marker of hCG concentrations and has therefore been used in other clinical studies on gestational thyroid function. Removal of outliers for TSH, FT4, or hCG did not change the results.

For variables with missing data, multiple imputations according to the Markov chain Monte Carlo method were used (28). Five imputed data sets were created and pooled for analyses. For the prematurity database, TSH, FT4, hCG, smoking, socioeconomic status, ethnicity, parity, BMI, fetal sex, and gestational age at blood sampling were added to the model (missing because of nonresponse/nonrecording in 6.3%, 5.7%, 3.5%, 12.8%, 7.2%, 5.7%, 1.9%, and all other variables <2.0%, respectively). Furthermore, we added maternal age, total T4, and TPOAb concentrations as prediction variables during the multiple imputation procedure only. No significant differences in descriptive characteristics were found between the original and imputed data sets. All statistical analyses were performed using R statistical software v3.03 (package *rms*, *visreg*) or Statistical Package of Social Sciences v20.0 for Windows (SPSS v22.0; IBM Corp, Armonk, NY).

Results

The final study population comprised 5644 women (Fig. 1), descriptive characteristics of whom are shown in Table 1. The association of TSH concentrations with mean duration of pregnancy, premature delivery, and pPROM differed between women with low hCG concentration and those with high hCG concentration at the time of TSH measurement ($P_{interaction}$ = 0.039, 0.022, and 0.079, respectively; Supplemental Table 1). Heatmaps graphically illustrate the differences in mean gestational age [Fig. 2(a)], the risk of premature delivery [Fig. 2(b)], and the risk of pPROM [Fig. 2(c)] according to the combination of hCG and TSH in the whole study population (blue indicates a lower mean gestational age, lower risk of premature delivery, or lower risk of pPROM, and red indicates a higher mean gestational age, higher risk of premature delivery, or higher risk of pPROM).

To quantify the differences in risks, we stratified the association of high TSH concentration (per percentile) with premature delivery for hCG concentration (per 10,000 IU/L; Table 2). Women with high TSH and low hCG concentrations did not have a higher risk of premature delivery, and most effect estimates pointed toward a protective effect [Fig. 2(b); Table 2]. In contrast, women with a high TSH concentration despite a high hCG concentration had a twofold to 10-fold higher risk of premature delivery depending on the cutoffs used (Fig. 2; Table 2).

A similar analysis for pPROM showed that women with high TSH and low hCG concentrations did not have a higher risk of pPROM, and most effect estimates pointed toward a protective effect [Fig. 2(c); Table 3]. In contrast, women with a high TSH concentration despite a high hCG concentration had a risk of pPROM that ranged between a protective effect and a fourfold higher risk depending on the cutoffs used (Fig. 2(c); Table 3).

In the whole population, the association of FT4 concentration with premature delivery did not differ between low or high hCG concentrations ($P_{interaction}$ = 0.52,

Table 1. Descriptive Statistics of the Study Population

	Median	(95% Range)
Median TSH, mU/L	1.33	(0.05–4.13)
Median FT4, pmol/L	14.8	(10.3–22.3)
Median hCG, IU/L	44.625	(11.999–106.339)
Median UIC, μg/g[a]	273	(96–777)
Gestational age[b]	13.4	(9.6–17.6)
Maternal age, y	30.3	(19.5–38.8)
Maternal height, cm	168	(153–182)
Maternal BMI, kg/m^2	23.5	(18.5–38.8)
	N	**(%)**
Premature delivery, <37 wk[b]	276	(4.9)
Parity[b]		
0	3226	(57.2)
1	1681	(29.8)
≥2	738	(13.1)
Smoking[b]		
Nonsmokers	4140	(73.4)
Stopped smokers	506	(9.0)
Smokers	997	(17.7)
Education level[b]		
Low	606	(10.7)
Middle	2599	(46.0)
High	2439	(43.4)
Ethnicity[b]		
Dutch	2803	(51.6)
Moroccan	341	(6.3)
Turkish	455	(8.4)
Surinamese	479	(8.8)
Other Western	491	(9.0)
Other non-Western	866	(15.9)
Child sex (boys %)[b]	2853	(50.5)

Abbreviation: UIC, urinary iodine to creatinine ratio.

[a] Based on data from a subset of N = 1031 women.

[b] At time of blood sampling; data shown in weeks.

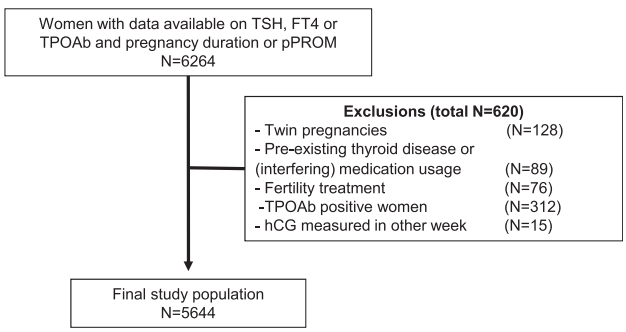

Figure 1. Flowchart exhibiting the selection of the study population.

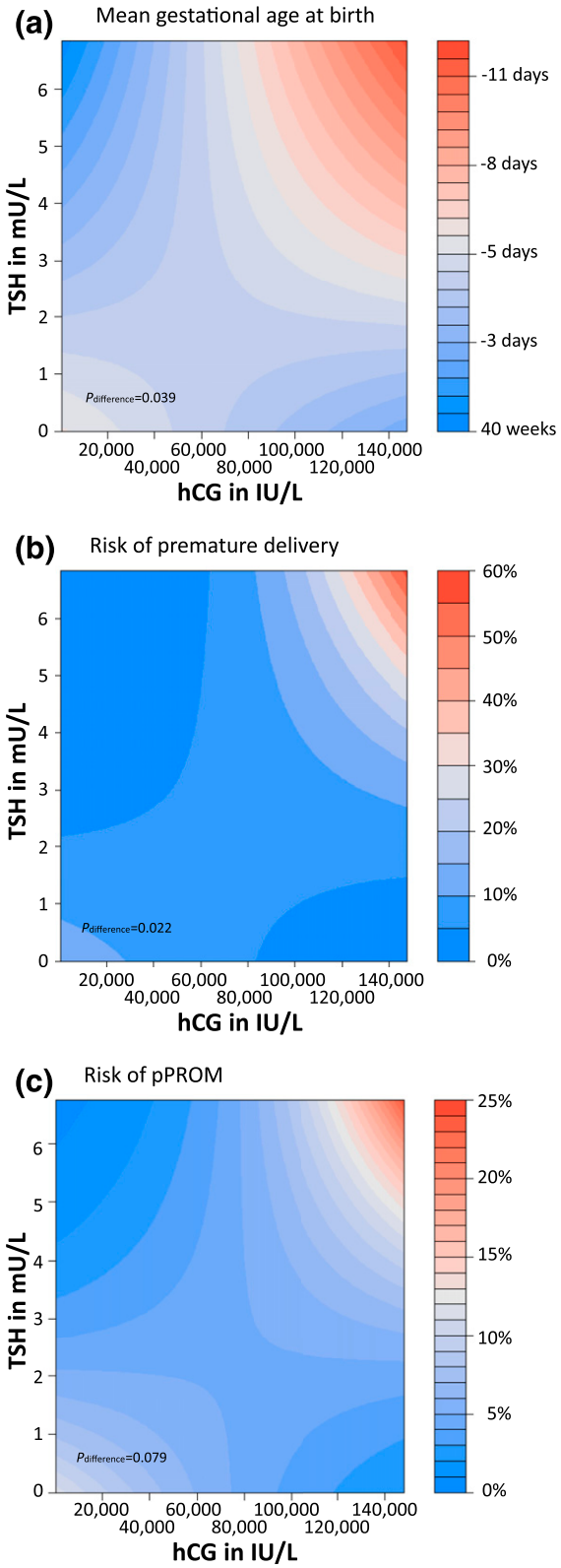

Figure 2. (a) Mean gestational age at birth, (b) risk of premature delivery, and (c) risk of premature rupture of membranes according to TSH and hCG concentrations. Heatmaps show hCG on the *x*-axis, TSH on the *y*-axis, and the mean gestational age of risk of premature delivery on the *z*-axis, which ranges from low values or risks (blue) to high levels or risks (red).

0.69, and 0.68 for mean duration of pregnancy, premature delivery, and pPROM, respectively; Supplemental Table 1). Similar results were obtained when women with TPOAb concentrations above a lower cutoff (*e.g.*, 10 IU/L) were excluded, after women with preeclampsia were excluded, or when analyses were restricted to women with a spontaneous delivery (data not shown). hCG concentrations were not associated with the duration of pregnancy, premature delivery, or pPROM. The association of TSH or FT4 concentration with premature delivery did not differ by gestational age at blood sampling, used as a potential proxy for hCG (data not shown), or by fetal sex (data not shown).

In the total population (including TPOAb-positive women), the association of FT4 with TSH differed between TPOAb-negative and TPOAb-positive women ($P < 0.001$; Supplemental Fig. 1). At similar FT4 concentrations in the lower range, TPOAb-positive women had higher TSH concentrations with a considerably higher spread than TPOAb-negative women (Supplemental Fig. 1).

Discussion

In the current study, we demonstrated that the association of maternal thyroid function during pregnancy with the risk of premature delivery in TPOAb-negative women was modified by hCG concentration. Our main finding is that women with a high TSH concentration despite a high hCG concentration had a twofold to 10-fold higher risk of premature delivery and a 1.6- to 4.2-fold higher risk of pPROM. In contrast, women with high TSH and low hCG concentrations had a lower or normal risk of premature delivery.

Suboptimal placental function is an important risk factor for premature delivery (29–31). TH transporters and receptors are abundantly expressed in the placenta (32–34), and TH regulates the secretion of several growth factors and cytokines implicated in placental development (33, 35–37). Crucial stages of placental development coincide with the peak in hCG concentrations during the late first and early second trimesters (5, 38). Therefore, we speculated that a suboptimal thyroidal response to hCG stimulation was associated with a higher risk of premature delivery because it causes a relative TH shortage during early pregnancy. We previously showed that the thyroidal response to hCG stimulation was considerably impaired in TPOAb-positive women and that TPOAb-positive women with a low thyroidal response to hCG in particular had a higher risk of premature delivery (3). The results of the current study in TPOAb-negative women further strengthen the concept that a lower thyroidal response to hCG stimulation

Table 2. Outline of the Interaction Between hCG and TSH in the Risk of Premature Delivery

	hCG Concentration, IU/L								
TSH Percentile (Value in mU/L)	Overall	<20,000	20,000–30,000	30,000–40,000	40,000–50,000	50,000–60,000	60,000–70,000	70,000–80,000	>80,000
≥96 (4.02)	1.88 (1.14–3.12)	[a]	[a]	[a]	0.74	1.54	3.42	5.91	9.96
≥95 (3.72)	1.62 (0.98–2.67)	0.64	[a]	0.52	0.57	1.15	2.34	4.46	6.84
≥94 (3.49)	1.50 (0.95–2.35)	0.46	[a]	0.53	0.83	1.06	1.93	2.77	6.29
≥93 (3.32)	1.40 (0.92–2.12)	0.40	0.41	0.42	0.73	0.88	1.82	2.48	5.64
≥92 (3.15)	1.28 (0.85–1.92)	0.33	0.38	0.61	0.60	0.67	1.67	2.16	5.05
≥91 (3.01)	1.18 (0.79–1.74)	0.53	0.42	0.54	1.00	1.00	1.60	2.20	4.02
≥90 (2.91)	1.24 (0.85–1.80)	0.48	0.39	0.49	0.93	0.95	1.48	3.36	3.33
≥89 (2.81)	1.16 (0.80–1.69)	0.51	0.37	0.44	0.87	0.89	1.42	2.41	3.05
≥88 (2.73)	1.23 (0.87–1.75)	0.61	0.34	0.39	0.79	0.82	2.60	3.79	2.68
≥87 (2.66)	1.22 (0.85–1.74)	0.80	0.38	0.39	0.88	1.08	2.39	2.59	2.56
≥86 (2.59)	1.16 (0.82–1.64)	0.72	0.50	0.36	0.86	0.91	2.07	2.59	2.29
≥85 (2.53)	1.10 (0.77–1.56)	0.89	0.48	0.35	0.96	0.83	1.89	2.22	1.85

Adjusted odds ratios are shown for premature delivery (<37 weeks) according to different percentile cutoffs for TSH concentrations, stratified by concomitant hCG levels. All analyses were adjusted for maternal age, smoking, education level, ethnicity, parity, BMI, height, and fetal sex. hCG cutoff groups were not associated with the risk of premature delivery.

[a]None of the women in this subgroup had a premature delivery.

increases the risk of premature delivery. Alternatively, an attenuated thyroidal response to hCG stimulation could be associated with premature delivery because it lowers the total TH availability during pregnancy (*e.g.*, a lower area under the curve). Further studies, preferably using repeated measurements of hCG and thyroid function, are required to further elucidate these mechanisms.

As for the cause of a lower thyroidal response to hCG stimulation, the subset of TPOAb-negative women in the current study may still be subject to a form of thyroidal autoimmunity (*e.g.*, reflected by isolated thyroglobulin antibody positivity or because of the presence of blocking TSH receptor antibodies). Unfortunately, these measurements were not available in our pregnancy cohort. Although the current results were not altered after the exclusion of women with very low TPOAb concentrations (*e.g.*, >10 IU/L), further studies are required to identify risk factors for lower thyroid functional capacity in TPOAb-negative women, such as previous thyroiditis, exposure to thyrotoxic treatments, or natural variation in thyroid functional capacity through genetic variability. Regarding the latter, various partial loss-of-function mutations in the TSH receptor have been described (39), and such mutations could potentially also interfere with TSH receptor stimulation by hCG. Furthermore, more commonly occurring single nucleotide polymorphisms (SNPs) in the TSH receptor may

Table 3. Outline of the Interaction Between hCG and TSH in the Risk of Premature Rupture of Membranes

	hCG Concentration, IU/L							
TSH Percentile (Value in mU/L)	Overall	<20,000	20,000–30,000	30,000–40,000	40,000–50,000	50,000–60,000	60,000–70,000	>70,000
≥96 (4.02)	1.24 (0.60–2.55)	[a]	[a]	1.48	0.70	1.31	1.63	4.20
≥95 (3.72)	1.10 (0.54–2.25)	[a]	[a]	1.11	0.62	1.14	1.13	4.31
≥94 (3.49)	0.94 (0.47–1.89)	[a]	[a]	0.90	0.55	0.85	0.96	3.33
≥93 (3.32)	0.97 (0.51–1.84)	[a]	[a]	0.80	1.13	0.75	0.78	3.24
≥92 (3.15)	0.90 (0.51–1.62)	[a]	[a]	0.71	0.96	0.62	0.76	3.83
≥91 (3.01)	0.81 (0.47–1.43)	[a]	0.08	0.70	0.87	0.52	0.68	3.44
≥90 (2.91)	0.84 (0.50–1.44)	[a]	0.09	0.60	0.95	0.54	0.65	3.74
≥89 (2.81)	0.82 (0.48–1.40)	[a]	0.01	0.75	0.82	0.50	0.62	3.54
≥88 (2.73)	0.90 (0.55–1.46)	0.42	0.01	0.68	0.91	0.46	1.55	3.36
≥87 (2.66)	0.97 (0.59–1.59)	0.61	0.39	0.64	0.87	0.69	1.40	3.38
≥86 (2.59)	0.94 (0.57–1.53)	0.58	0.39	0.81	0.81	0.61	1.32	3.25
≥85 (2.53)	0.91 (0.55–1.49)	0.79	0.36	0.77	0.84	0.55	1.22	2.92

Adjusted odds ratios are shown for premature rupture of membranes (<37 weeks) according to different percentile cutoffs for TSH, stratified by concomitant hCG levels. All analyses were adjusted for maternal age, smoking, education level, ethnicity, parity, BMI, height, and fetal sex. hCG cutoff groups were not associated with the risk of premature rupture of membranes.

[a]None of the women in this subgroup had a premature delivery; too little pPROM occurred to do reliable analyses for a cutoff for hCG >80,000 IU/L.

account for some of the variability in TSH receptor stimulation by hCG (40). Although a large genome-wide association study demonstrated that TSH SNPs identified in a population of nonpregnant individuals were also associated with TSH during pregnancy, no SNPs in the TSH receptor were identified (41).

Our results suggest that a measurement of hCG concentrations during pregnancy may improve the interpretation of (high) TSH concentrations, although it is less likely to improve the interpretation of FT4 concentrations. The discrepancy between our results on TSH and FT4 may be explained by the fact that the relative changes in TSH concentrations during pregnancy are larger than the changes in FT4 concentrations, given the log-linear relationship between FT4 and TSH. As such, higher TSH concentrations may better reflect a relative TH shortage.

Intriguingly, in a previous paper, our group demonstrated that FT4 response to hCG may be relevant in determining the risk of premature delivery in TPOAb-positive women (3). In that study, we demonstrated that the risk of premature delivery in TPOAb-positive women was best distinguished by the combination of hCG and FT4, rather than hCG and TSH. Additional analyses in the current paper indicate that TSH was a less-sensitive marker for FT4 in TPOAb-positive women than in TPOAb-negative women. Although both of these studies require replication, the results from the current study on the combination of high TSH and high hCG concentrations also indicated that a suboptimal thyroid response to hCG stimulation (*e.g.,* a deviation from the naturally occurring physiology) is a risk factor for premature delivery.

The risk of premature delivery in women with subclinical thyroid dysfunction has been investigated in many different populations. Taken together, these studies report a wide range in the risk of premature delivery, from a 30% decrease to a 3.3-fold increased risk (11–16). The results from our study suggest that such large between-study differences in the risk of premature delivery for women with subclinical thyroid dysfunction may be due to between-study differences in hCG concentrations at the time of thyroid function measurement. Unfortunately, none of the studies that investigated the association of maternal thyroid function with premature delivery had hCG measurements available.

Although gestational age at blood sampling may be a proxy for hCG, we could not identify a difference in the association of TSH or FT4 concentration with premature delivery according to a different gestational age at blood sampling in our study; this is most likely because of the large interindividual variations in hCG concentrations. Furthermore, we were unable to replicate the results of other studies by selecting only subjects with the same gestational (*i.e.*, 10 to 13 weeks, 10 3/7 to 13 6/7 weeks, and a mean of 14.1 weeks in three other studies) (11, 16, 42). Therefore, gestational age at presentation is unlikely to be a good proxy for hCG given the large interindividual differences in hCG concentrations. Future studies are needed to verify if the observed differences in risk estimates between populations are due to differences in population hCG concentrations or differences in other factors affecting thyroidal stimulation.

In this study, we were able to investigate differences in the association of thyroid function with the risk of premature delivery in a large population with detailed data and pregnancy outcomes that rendered adequate statistical power to detect an overall difference while adjusting for potential confounders. One potential limitation of the current study is that we used an assay that measured total hCG concentrations, including the various isoforms such as nicked, asialo-, and hyperglycosylated hCG. It has been shown that hCG subtypes have different thyrotropic activities (43–45). Although different ratios of hCG isoforms have been reported in pathologic conditions such as preeclampsia (46, 47), we were unable to further distinguish between possible effects of the different isoforms. Such distinctions may prove valuable in future efforts to describe women at high risk for premature delivery and/or pPROM. Also, we were limited because the size of our study population did not allow us to stratify analysis using mutually exclusive groups of women with high TSH concentrations. However, continuous interaction analysis showed that the overall difference between the groups was statistically significant, and we were able to prove heatmaps of these risk estimates.

In conclusion, we showed that the risk of premature delivery according to TSH concentrations was modified by hCG concentrations. Among all TPOAb-negative women with high-normal TSH concentrations, only those with high hCG concentrations at the time of TSH measurement had a higher risk of premature delivery. These data suggest that the assessment of maternal thyroid function together with hCG concentrations can improve the risk assessment of premature delivery and give insights into the pathophysiology of the association between maternal thyroid function and premature delivery. When further replicated, this concept may improve clinical practice by facilitating identification of women at risk for pregnancy complications.

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