Cancer Research

Human Chorionic Gonadotropin Does Not Correlate with Risk for Maternal Breast Cancer: Results from the Finnish Maternity Cohort

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Abstract

Human chorionic gonadotropin (hCG) is necessary for the maintenance of early pregnancy and promotes normal breast cell differentiation. Administered hCG reduces risk of carcinogen-induced breast cancer in animal models, and higher circulating hCG concentrations were associated with significantly lower long-term risk of breast cancer in a prior nested case–control study. In this study, we investigated early-pregnancy hCG concentrations and subsequent breast cancer risk. We conducted a nested case–control study with 1,191 cases and 2,257 controls (matched on age and date at blood collection) in the Finnish Maternity Cohort, a cohort with serum samples from 98% of pregnancies registered in Finland since 1983. This study included women with a serum sample collected early (<140 days gestation) in their first pregnancy resulting in a live, term birth. Breast cancer cases were identified via the Finnish Cancer Registry. Age at breast cancer diagnosis ranged from 22 to 58 years (mean: 41 years). hCG was measured using a solid-phase competitive chemiluminescence assay. Odds ratios (OR) were calculated using conditional logistic regression. We observed no association between hCG and breast cancer risk, overall [Quartile 4 vs. 1, OR, 1.14; 95% confidence interval (CI), 0.94–1.39], by estrogen and progesterone receptor status, or by ages at first-term birth or diagnosis. Associations did not differ by time between pregnancy and diagnosis (e.g., <5 years, $OR_{Q4\ vs.\ Q1}$, 1.10; 95% CI, 0.64–1.89; ≥15 years, $OR_{Q4\ vs.\ Q1}$, 1.36; 95% CI, 0.86–2.13; $p_{heterogeneity} = 0.62$). This large prospective study does not support an inverse relationship between early pregnancy serum hCG concentrations and breast cancer risk. *Cancer Res; 77(1); 134–41. ©2016 AACR.*

Introduction

A full-term pregnancy before age 25 provides a well-established long-term protective effect against hormone receptor–positive breast cancer, following a transient increase in risk that is evident in the years immediately following pregnancy (1). Human chorionic gonadotropin (hCG), a key hormone in early pregnancy, has been explored as a potential biologic mediator underpinning the long-term protective effect of parity on breast cancer risk (2–5). Circulating hCG concentrations are very low in healthy non-pregnant women, increase rapidly and peak in early pregnancy, during the first 60 to 90 days of gestation, and then decrease to relatively low concentrations until the end of pregnancy, returning to pre-pregnant concentrations after delivery. In murine models, administered hCG (i.e., mimicking pregnancy) resulted in mammary tissue differentiation similar to that observed after pregnancy (4) and a dose-dependent reduction in the frequency of carcinogen-induced mammary tumors (6–9). hCG is antiproliferative and proapoptotic in breast cancer cells (10–12), and promotes differentiation in normal breast tissue (13).

On this basis, it has been hypothesized that comparatively high circulating concentrations of hCG during human pregnancy may confer greater protection against long-term breast cancer risk. However, given the relative rarity of large maternity cohorts with biospecimens and cancer follow-up data, as required to investigate this hypothesis, circulating early pregnancy hCG and breast cancer risk in the mother has been only minimally explored. Two studies in the Northern Sweden Maternity Cohort (NSMC; $n \leq$ 242 cases) reported inverse associations between early pregnancy (<20 weeks gestation) serum hCG concentrations and maternal breast cancer risk (2, 3). High serum hCG in a primiparous pregnancy (i.e., first pregnancy resulting in a live or stillborn

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birth) was associated with significantly lower breast cancer risk among women younger than age 25 at first-term birth, or diagnosed at age 40 or older or at least 10 years after first-term birth, with suggestive increases in risk among women diagnosed less than 10 years after first-term birth (2). However, these previous investigations were relatively small, and, to date, these findings have not been replicated. Furthermore, potential heterogeneity by tumor hormone receptor status (i.e., estrogen (ER) and progesterone (PR) receptors) has not previously been evaluated.

We conducted the first large-scale investigation ($n = 1{,}191$ cases) on early pregnancy hCG and breast cancer risk, overall and by hormone receptor subtype, in a nested case-control study in the Finnish Maternity Cohort (FMC) to address the hypothesis that higher circulating hCG concentrations in early pregnancy are associated with lower breast cancer risk diagnosed more than 5 to 10 years post-pregnancy in the mother, and that this association is strongest for ER^+/PR^+ disease.

Materials and Methods

The FMC is a nationwide initiative to store blood samples collected during early pregnancy for research; the cohort includes 98% of all registered pregnancies in Finland since 1983. The FMC (14) and case and control selection for this investigation have been described in detail previously (15, 16). Briefly, early in pregnancy, women enrolled in prenatal care at municipal maternity care centers that provide free prenatal care throughout Finland. Peripheral venous blood samples were collected for routine screening tests in early pregnancy, and remaining serum was stored for research purposes at −25°C, and shipped frozen to a central biorepository located in Oulu, Finland. Follow-up and covariate data were available through linkages with the Finnish Population, Birth, and Cancer Registries. The Finnish Cancer Registry (1952-present) has close to 100% coverage for diagnosed cancers (17). Tumor hormone receptor was not available from the cancer registry; therefore, data were obtained via linkage with hospital pathology records where available (ER and PR status available for 56% of cases).

Women eligible for inclusion in this study had serum sample available from a singleton primiparous pregnancy with term delivery, were age <40 years at time of blood collection, had no prior history of invasive cancer or *in situ* breast cancer before breast cancer diagnosis/selection as a control, and had data available on gestational age (GA) at time of blood collection. Incident invasive

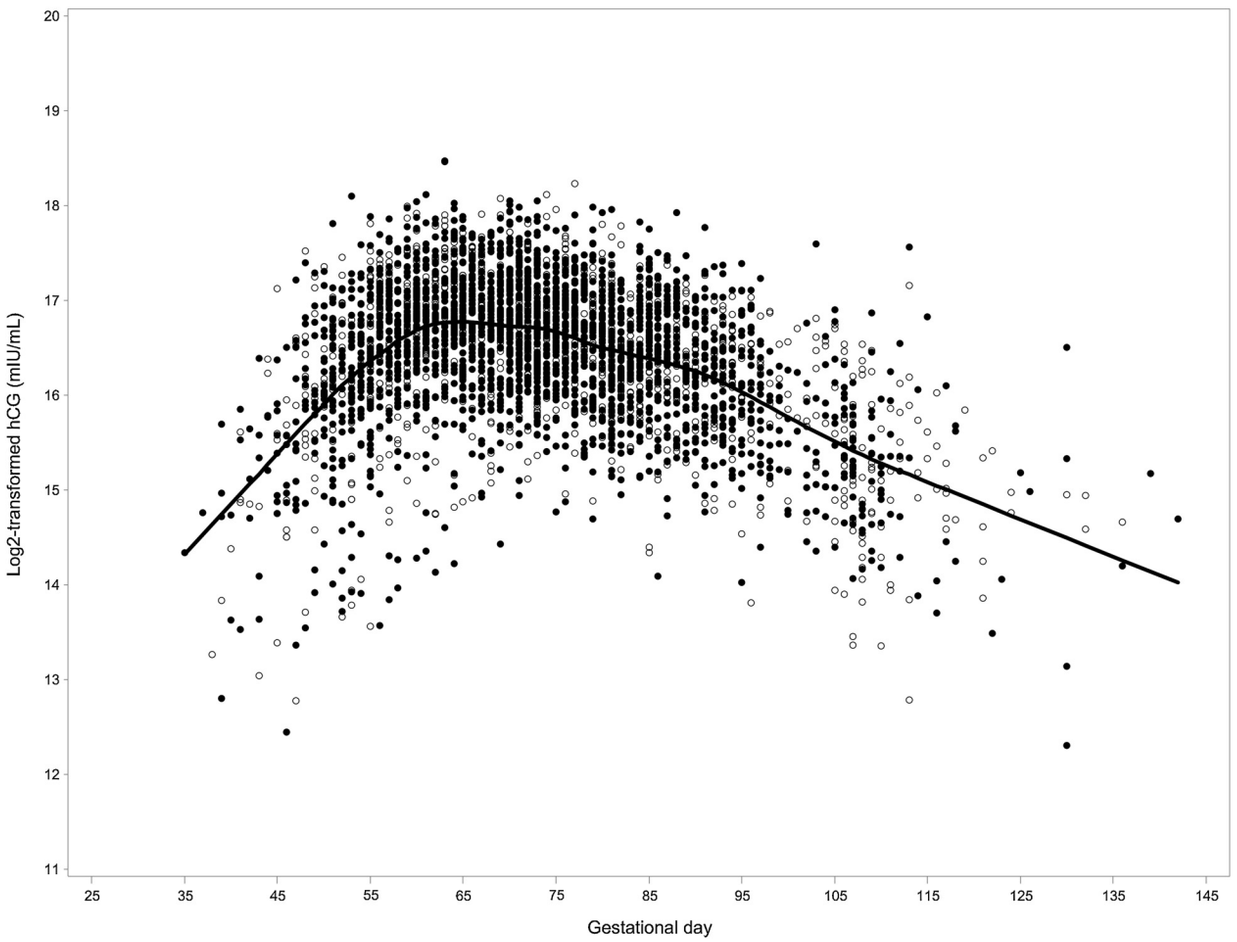

Figure 1.
hCG concentrations by gestational age at blood collection. Solid line represents mean hCG by gestational day and cases are represented by unfilled circles and controls by filled circles: FMC.

breast cancer cases diagnosed between 1988 and 2007 were identified through the Finnish Cancer Registry. Cases were matched to up to 2 controls on age (±6 months) and date (±3 months) at blood collection. The final study population included 1,191 cases and 2,257 controls (1,066 cases with 2 controls; for 125 cases only 1 eligible control was identified). The ethical committee of the National Institute for Health and Welfare, Finland approved the study.

Serum hCG and estradiol assays

Serum total hCG was quantified using a solid-phase competitive chemiluminescence assay and an Immulite 2000 Siemens analyzer. Serum estradiol concentrations were measured using high performance liquid chromatography-tandem mass spectrometry, performed with an Applied Biosystems API4000 triple-stage quadrupole mass spectrometer. Assays were performed at the Department of Clinical Chemistry at Umeå University Sweden. Laboratory personnel were blinded to the case, control, or quality control status of the samples, and case and matched control samples were analyzed in the same laboratory batch. Quality control samples were included in each laboratory batch; mean inter- and intra-batch CVs were ≤9.3% for both hCG and estradiol.

Statistical analysis

Hormone concentrations were $\log_2$ transformed to normalize distributions. Two outliers were identified for hCG (values >3 times the interquartile range), and excluded in sensitivity analyses. GA at blood collection was calculated using the formula: 280-(date of expected delivery minus date of blood draw); 280 days in a 40-week pregnancy. We adjusted for variation in hCG by GA at blood collection by using hCG residuals, as has been done previously (2, 3). The residual (difference) was calculated as: assay value minus gestational day-specific mean value from a local linear regression model. hCG residuals are henceforth referred to as "adjusted hCG." Results from analyses adjusting for gestational age as a covariate were not meaningfully different from those using adjusted hCG from the residual method. Therefore, we present results from analyses using hCG residuals. We evaluated cross-sectional relationships between maternal and pregnancy-related characteristics and early pregnancy hCG concentrations in controls using generalized linear models. Quartiles were defined using the hCG distribution in controls. We modeled the quartile median concentration to test for trend. Odds ratios (OR) were calculated using conditional logistic regression models. Statistical adjustment for maternal or pregnancy characteristics (e.g. parity at index date, family history of breast cancer, smoking, sex of the neonate) or circulating estradiol changed ORs by less than 5%. Therefore, we present the unadjusted results. We evaluated risk overall, by ER and PR status (ER^+/PR^+ vs. ER^-/PR^-), and stratified by age at first-term birth (i.e., age at blood collection), age at diagnosis, and time between first term birth and diagnosis. Because of previously observed associations between early pregnancy serum estradiol and breast cancer risk (15), we cross-classified hCG and estradiol concentrations (i.e., hCG/estradiol split at median: low/low, high/low, low/high, high/high). In sensitivity analyses, we restricted the study population to women who were primigravid (i.e., first ever pregnancy) at blood collection ($n = 794$ complete sets) and who provided blood samples between gestational days 60 to 90 ($n = 679$ complete sets), the period during which hCG peaks and is more stable (Fig. 1).

Heterogeneity (P_{het}) between breast cancer subtypes was assessed using a likelihood ratio test comparing a model assuming the same association between hCG and breast cancer overall (e.g., all hormone receptor subtypes) to one assuming different associations for ER^+/PR^+ and ER^-/PR^- disease in a conditional polytomous logistic regression model (18). Interaction (e.g., by age at first term pregnancy) was tested by including a multiplicative interaction term in the models and evaluating the Wald p value. We examined the possibility of non-linearity with restricted cubic splines (19). Tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. We did not observe significant deviation from linearity (P>0.05), with the exception of the association for ER^+/PR^+ breast cancer among women <30 at first-term birth and ≥40 at diagnosis. Given the sample size of 1,191 cases and 2,257 controls, the minimum detectable ORs contrasting extreme quartiles (or for continuous 1-unit increase in hCG) with 80% power and 95% confidence were: overall, 0.74 (0.89); ER^+/PR^+, 0.57 (0.81); ER^-/PR^-, 0.40 (0.71); age at first-term birth, range: 0.40 (0.71) for <25 years to 0.57 (0.81) for 25–29 years; time between first term birth and diagnosis, range: 0.40 (0.71) for <5 years to 0.60 (0.83) for 10 to 14 years; age at diagnosis, range: 0.31 (0.65) for ≥50 years to 0.65 (0.85) for 40–49 years. The minimum detectable OR for a positive association is the reciprocal of that for inverse association.

Statistical analyses were conducted using SAS 9.3. Two-sided P values <0.05 were considered statistically significant.

Results

Baseline characteristics of the study population have been published previously (15). Briefly, mean age at first birth was 30 years (range, 18–40 years), and the majority were multiparous at diagnosis (64%) or selection as a control (67%). Blood samples were collected at mean 74 days GA in cases and controls (median 73 days; range, 35–142 days), and mean hCG concentrations

Table 1. Mean[a] maternal hCG levels by maternal characteristics and child sex in among 2,257 controls: FMC

Characteristic	N	hCG (mIU/mL)	P
Age at first-term birth (y)			0.08
<25 years	386	111,678	
25–30 years	694	117,132	
30–35 years	669	118,528	
≥35 years	508	115,927	
Maternal smoking			<0.01
Yes	292	95,301	
No	1,900	119,489	
Gravidity			0.47
Primigravid	1,606	116,641	
Multigravid	600	115,192	
Neonate sex			<0.01
Boy	1,118	111,820	
Girl	1,132	120,708	
Time to 2nd birth (y)			0.55
No additional pregnancy	750	114,560	
<2 years	535	116,743	
2–5 years	837	117,548	
>5 years	130	116,615	

[a]Calculated using adjusted hCG; mean corresponds to concentrations at gestational day 73.

were similar in cases and controls (residuals, 0.02 versus 0.01, $P = 0.83$; corresponds to concentrations of 115,717 and 114,962 mIU/mL at median gestational day 73). Cases were diagnosed with breast cancer at mean age 41 years (range, 22–58 years), a mean of 11 years after first pregnancy. In cross-sectional analyses, maternal smoking and male embryo/fetus were associated with lower hCG concentrations (<0.01), whereas maternal age and gravidity did not impact concentrations (Table 1; Supplementary Table S1). Serum hCG concentrations peaked at approximately gestational day 60 and declined thereafter (Fig. 1).

Serum hCG concentration was not associated with breast cancer overall [Quartile 4 vs. 1, OR, 1.14; 95% confidence interval (CI), 0.94–1.39] or by hormone receptor subtype (ER^+/PR^+ vs. ER^-/PR^-), age at first-term birth (<25, 25–29, 30–34, ≥35 years), age at diagnosis (<40, 40–49, ≥50 years), or time between blood collection and diagnosis (<5, 5–9, 10–14, ≥15 years; Table 2). For example, comparing extreme quartiles, high hCG was associated with an OR of 1.10 (95% CI, 0.64–1.89) among cases diagnosed within 5 years of first-term birth ($n = 143$) and an OR of 1.36 (95% CI, 0.86–2.13) for women diagnosed 15 or more years after first-term birth ($n = 254$). Higher concentrations of hCG were suggestively associated with increased risk among women <25 years at first term birth (Quartile 4 vs. 1, OR, 1.67; 95% CI, 0.99–2.80).

We examined the effect of a 1-unit increase in adjusted hCG concentration and breast cancer risk by ages at first-term birth and diagnosis, and time between first-term birth and diagnosis, overall and among ER^+/PR^+ or ER^-/PR^- cases (Table 3). hCG was not associated with breast cancer risk in any of the examined subgroups, and we observed no heterogeneity between ER^+/PR^+ and ER^-/PR^- disease in these analyses (e.g., age at diagnosis <40, OR_{cont}: ER^+/PR^+, 1.05; 95% CI, 0.76–1.44; ER^-/PR^-, 1.19; 95%

Table 2. Early pregnancy hCG concentrations and breast cancer risk: FMC

	Quartiles					
	Q1	**Q2**	**Q3**	**Q4**	P_{trend}	P_{het}
Overall						
Cases/Controls	282/565	317/564	272/565	320/563		
OR (95% CI)	ref.	1.13 (0.92–1.37)	0.96 (0.78–1.17)	1.14 (0.94–1.39)	0.38	
Hormone receptor status						0.31
ER^+/PR^+						
Cases/Controls	99/193	113/212	108/206	114/201		
OR (95% CI)	ref.	1.04 (0.75–1.45)	1.01 (0.72–1.42)	1.12 (0.80–1.57)	0.54	
ER^-/PR^-						
Cases/Controls	38/96	49/72	35/82	38/58		
OR (95% CI)	ref.	1.74 (1.02–2.98)	1.07 (0.60–1.88)	1.62 (0.92–2.83)	0.21	
Age at first term birth						0.62
<25 years						
Cases/Controls	41/114	55/80	52/96	47/78		
OR (95% CI)	ref.	1.88 (1.14–3.08)	1.47 (0.89–2.43)	1.67 (0.99–2.80)	0.08	
25–29 years						
Cases/Controls	77/157	113/190	68/175	105/168		
OR (95% CI)	ref.	1.19 (0.83–1.70)	0.76 (0.52–1.13)	1.25 (0.87–1.79)	0.55	
30–34 years						
Cases/Controls	90/166	86/151	80/158	95/183		
OR (95% CI)	ref.	1.05 (0.73–1.52)	0.93 (0.64–1.34)	0.97 (0.68–1.38)	0.74	
≥35 years						
Cases/Controls	72/124	62/140	71/129	72/130		
OR (95% CI)	ref.	0.77 (0.51–1.18)	0.97 (0.64–1.47)	0.98 (0.65–1.49)	0.86	
Age at diagnosis						0.65
<40 years						
Cases/Controls	129/257	137/254	103/230	139/232		
OR (95% CI)	ref.	1.06 (0.79–1.43)	0.89 (0.65–1.22)	1.19 (0.88–1.61)	0.42	
40–49 years						
Cases/Controls	129/270	157/264	148/292	158/293		
OR (95% CI)	ref.	1.25 (0.93–1.67)	1.05 (0.78–1.40)	1.12 (0.85–1.49)	0.63	
≥50 years						
Cases/Controls	24/38	23/46	21/43	23/38		
OR (95% CI)	ref.	0.84 (0.41–1.75)	0.80 (0.38–1.67)	1.01 (0.47–2.18)	0.99	
Time between first-term birth and diagnosis						0.62
<5 years						
Cases/Controls	39/73	35/62	26/66	43/74		
OR (95% CI)	ref.	1.05 (0.59–1.86)	0.75 (0.41–1.38)	1.10 (0.64–1.89)	0.90	
5–9 years						
Cases/Controls	98/177	93/202	86/167	93/159		
OR (95% CI)	ref.	0.83 (0.59–1.17)	0.92 (0.64–1.33)	1.04 (0.73–1.48)	0.73	
10–15 years						
Cases/Controls	89/195	122/186	97/210	116/220		
OR (95% CI)	ref.	1.47 (1.05–2.07)	1.00 (0.71–1.43)	1.15 (0.82–1.62)	0.81	
≥15 years						
Cases/Controls	56/120	67/114	63/122	68/110		
OR (95% CI)	ref.	1.24 (0.80–1.91)	1.08 (0.70–1.67)	1.36 (0.86–2.13)	0.26	

Table 3. Early pregnancy hCG concentrations (residuals, continuous) and breast cancer risk by age at first-term birth and diagnosis, and time between first birth and diagnosis: FMC

			ER^+/PR^+			ER^-/PR^-			
	Cases/Controls	OR[a]	Cases/Controls	OR[a]	P_{het}[b]	Cases/Controls	OR [a]	P_{het}[b]	P_{het}[c]
Overall	1,191/2,257	1.01 (0.91–1.12)	434/812	1.09 (0.90–1.34)		160/308	1.09 (0.79–1.50)		0.31
Age at first-term birth					0.52			0.84	
<25 years	195/368	1.24 (0.96–1.61)	45/82	1.38 (0.79–2.41)		37/70	1.36 (0.77–2.38)		0.87
25–29 years	355/651	1.01 (0.83–1.23)	128/237	0.94 (0.67–1.32)		60/111	1.26 (0.76–2.07)		0.28
30–34 years	341/619	0.92 (0.76–1.13)	144/262	0.95 (0.69–1.31)		35/64	0.95 (0.47–1.94)		0.80
≥ 35 years	267/494	0.95 (0.75–1.21)	102/184	0.82 (0.54–1.24)		25/47	0.92 (0.40–2.13)		0.82
Age at diagnosis					0.41			0.80	
<40 years	508/973	0.96 (0.71–1.29)	148/280	1.05 (0.76–1.44)		96/186	1.19 (0.83–1.70)		0.38
40–49 years	592/1,119	0.99 (0.77–1.27)	244/459	1.10 (0.83–1.44)		58/111	1.17 (0.68–2.00)		0.58
≥50 years	91/165	1.32 (0.65–2.70)	42/73	1.41 (0.65–3.04)		6/11	3.02 (0.17–53.41)		0.57
Time between first-term birth and diagnosis					0.25			0.38	
<5 years	143/275	0.89 (0.66–1.20)	41/76	0.82 (0.48–1.41)		27/52	1.31 (0.53–3.26)		0.38
5–9 years	370/705	1.01 (0.84–1.22)	131/247	0.94 (0.67–1.31)		55/108	1.04 (0.68–1.60)		0.70
10–14 years	424/811	0.99 (0.83–1.18)	152/292	0.94 (0.70–1.27)		57/109	1.32 (0.78–2.23)		0.27
≥15 years	254/465	1.15 (0.89–1.49)	110/197	1.34 (0.89–2.03)		21/39	1.54 (0.51–4.62)		0.82

[a]Per 1-unit increase in hCG residuals.
[b]*P* for heterogeneity across age at blood draw, age at diagnosis, or lag-time categories.
[c]*P* for heterogeneity comparing ER^+/PR^+ and ER^-/PR^- subtypes.

CI, 0.83–1.70). Results were similar using alternative cutoff points (i.e., <10 vs. ≥10 years between first-term birth and diagnosis; data not shown).

Overall, results were similar in analyses cross-classifying age at first term birth and age at diagnosis or time between first birth and diagnosis (Table 4). In analyses cross-classifying serum hCG and estradiol concentrations, low hCG in the context of high estradiol was suggestively associated with increased risk of breast cancer diagnosed <5 years after first-term birth (hCG/estradiol: low/high vs. low/low, OR, 1.81; 95% CI, 0.98–3.33), but not in any other subgroup (Table 5). Results were similar in analyses cross-classifying age at diagnosis and time between first birth and diagnosis, restricting the study sample to primigravid women, or to samples collected between 60 and 90 days gestation (data not shown).

Discussion

This large, prospective investigation does not support an important role for early pregnancy hCG concentrations in the etiology of breast cancer.

The long-term protective effect of an early pregnancy (i.e., <25 years at first birth) on risk of ER^+/PR^+ breast cancer is well established (1). Pregnancy results in differentiation of the breast terminal ductal lobular unit (TDLU; 20) and gene-expression profiling studies have identified pregnancy-related "signatures" in the normal breast tissue of parous pre- (21, 22) and postmenopausal (22–24) women, underscoring the long-term molecular changes in breast tissue decades after pregnancy. hCG administration in virgin rats promotes mammary differentiation and alters mammary gland gene expression (4), and shields the

Table 4. Early pregnancy hCG concentrations (residuals, continuous) and breast cancer risk: cross-classification by age at first-term birth and age at diagnosis and time between first birth and diagnosis: FMC

	Age at first-term birth <30 years			Age at first-term birth ≥30 years			
	Ca/Co	OR	P_{het}[a]	Ca/Co	OR	P_{het}[a]	P_{het}[b]
Age at diagnosis							
<40 years							
Overall	382/734	1.05 (0.88–1.26)		126/239	0.97 (0.71–1.32)		0.68
ER^+/PR^+	107/203	0.97 (0.69–1.37)	0.26	41/77	0.93 (0.52–1.66)	0.51	0.89
ER^-/PR^-	76/148	1.32 (0.88–1.97)		20/38	0.63 (0.23–1.73)		0.26
≥40 years							
Overall	181/342	1.12 (0.83–1.51)		502/942	0.95 (0.80–1.13)		0.39
ER^+/PR^+	68/129	1.36 (0.83–2.23)[c]	0.81	218/403	0.93 (0.71–1.22)	0.68	0.24
ER^-/PR^-	22/42	1.56 (0.58–4.16)		42/80	1.08 (0.57–2.05)		0.79
Time between blood collection and diagnosis							
<10 years							
Overall	208/398	0.96 (0.75–1.22)		355/678	1.15 (0.94–1.40)		0.79
ER^+/PR^+	55/103	1.02 (0.61–1.68)	0.68	117/220	0.85 (0.60–1.21)	0.77	0.56
ER^-/PR^-	45/89	1.18 (0.72–1.94)		37/71	0.95 (0.50–1.81)		0.76
≥10 years							
Overall	305/582	0.99 (0.80–1.22)		323/599	0.92 (0.74–1.14)		0.15
ER^+/PR^+	120/229	1.13 (0.80–1.59)	0.30	142/260	1.01 (0.72–1.43)	0.74	0.76
ER^-/PR^-	53/101	1.59 (0.90–2.80)		25/47	0.85 (0.33–2.18)		0.44

[a]*P* for heterogeneity comparing ER^+/PR^+ and ER^-/PR^-.
[b]*P* for heterogeneity comparing age at first-term birth <30 versus ≥30 years; no significant heterogeneity by age at diagnosis ($P > 0.27$) or time between first-term birth and diagnosis ($P > 0.26$).
[c]Non-linear association ($P < 0.05$). No association observed in spline model.

Table 5. Early pregnancy hCG and estradiol concentrations and breast cancer risk: FMC

	hCG/Estradiol concentrations			
	Low/low	**High/low**	**Low/high**	**High/high**
Overall				
Cases/Controls	350/681	230/446	248/446	361/682
OR (95% CI)	1.00	0.99 (0.81–1.21)	1.08 (0.88–1.32)	1.03 (0.86–1.24)
Hormone receptor status				
ER^+/PR^+				
Cases/Controls	125/241	95/166	88/166	125/238
OR (95% CI)	1.00	1.09 (0.78–1.53)	1.02 (0.72–1.43)	1.02 (0.74–1.40)
ER^-/PR^-				
Cases/Controls	47/101	29/49	41/65	43/93
OR (95% CI)	1.00	1.25 (0.70–2.22)	1.33 (0.78–2.27)	0.97 (0.58–1.61)
Age at first-term birth				
<25 years				
Cases/Controls	53/110	24/57	46/92	77/127
OR (95% CI)	1.00	0.87 (0.50–1.53)	1.07 (0.66–1.75)	1.27 (0.81–1.98)
25–29 years				
Cases/Controls	105/208	61/131	80/139	116/211
OR (95% CI)	1.00	0.90 (0.61–1.32)	1.12 (0.78–1.60)	1.08 (0.78–1.51)
30–34 years				
Cases/Controls	104/200	78/136	72/118	97/204
OR (95% CI)	1.00	1.10 (0.77–1.59)	1.16 (0.80–1.68)	0.92 (0.65–1.29)
≥35 years				
Cases/Controls	88/163	67/122	50/97	71/140
OR (95% CI)	1.00	1.02 (0.68–1.51)	0.94 (0.61–1.45)	0.93 (0.63–1.39)
Age at diagnosis				
<40 years				
Cases/Controls	148/312	86/185	118/200	156/275
OR (95% CI)	1.00	0.97 (0.71–1.34)	1.24 (0.91–1.67)	1.22 (0.91–1.62)
40–49 years				
Cases/Controls	169/320	128/227	114/213	179/358
OR (95% CI)	1.00	1.04 (0.78–1.39)	1.01 (0.75–1.35)	0.94 (0.72–1.22)
≥50 years				
Cases/Controls	33/49	16/34	16/33	26/49
OR (95% CI)	1.00	0.69 (0.32–1.49)	0.76 (0.37–1.58)	0.79 (0.40–1.54)
Time between first-term birth and diagnosis				
<5 years				
Cases/Controls	41/95	36/60	32/42	33/78
OR (95% CI)	1.00	1.39 (0.79–2.42)	1.81 (0.98–3.33)	0.99 (0.57–1.69)
5–9 years				
Cases/Controls	124/231	77/155	71/144	98/173
OR (95% CI)	1.00	0.90 (0.63–1.28)	0.89 (0.62–1.28)	1.05 (0.75–1.47)
10–14 years				
Cases/Controls	117/229	74/148	91/154	141/280
OR (95% CI)	1.00	0.97 (0.68–1.38)	1.15 (0.82–1.63)	0.97 (0.71–1.31)
≥15 years				
Cases/Controls	68/126	43/83	54/106	89/151
OR (95% CI)	1.00	0.94 (0.58–1.52)	1.00 (0.65–1.53)	1.13 (0.76–1.69)

mammary gland from carcinogenic transformation before or after DMBA administration (6–9). This effect is dose-dependent (8, 9), leading to the hypothesis that relatively high concentrations of hCG in early pregnancy would decrease long-term breast cancer risk in women.

Epidemiologic data on hCG and breast cancer risk are sparse (2, 3, 25). A retrospective case–control study reported reduced breast cancer risk among women administered hCG for weight control or fertility treatments, however, this association was limited to nulliparous women with BMI $\leq$27.4 kg/m^2 (ever vs. never hCG use, OR, 0.30; 95% CI, 0.10–0.96; ref. 25). Two studies, both in the NSMC, have assessed hCG in pregnancy and breast cancer risk. The first investigation included both primiparous and multiparous (i.e., births before the index pregnancy) women (3), whereas the second was restricted to primiparous women (2), as in the current study. Both investigations used hCG residuals to account for gestational age at blood collection; we used the same approach in this investigation. In the initial investigation, Lukanova and colleagues (3) observed suggestive associations between hCG and breast cancer risk among women diagnosed 14 or more years after the index pregnancy ($n = 91$ cases; 3rd vs. 1st tertile OR, 0.53; 95% CI, 0.27–1.03); however, no statistically significant associations were observed. In contrast, Toniolo and colleagues (2) observed a significant inverse association between circulating hCG and breast cancer risk in the investigation limited to primiparous women ($n = 242$ cases; 3rd vs. 1st tertile OR, 0.67; 95% CI, 0.46–0.99). The inverse association was strongest among women <25 years at first birth (3rd vs. 1st tertile OR, 0.41; 95% CI, 0.21–0.80), and observed among women 40 or older at diagnosis or with 10 or more years between pregnancy and diagnosis. We examined risk in the same subgroups as published previously, and observed no significant association between hCG and breast cancer, overall or in any subgroup.

We have previously shown high early pregnancy estradiol concentrations to be inversely associated with breast cancer diagnosed at age 40 or older, and positively associated with ER^-/PR^- disease among women diagnosed before age 40, in this study population (15). Therefore, we controlled for and stratified by circulating estradiol concentrations in this investigation. Results from these analyses were consistent with the overall results, with the exception of a suggestive positive association among women with low hCG and high estradiol (concentrations below/above median relative to both below median), and diagnosed within 5 years of first term birth. It could be speculated that early pregnancy estradiol increases breast cancer risk in the short-term after pregnancy only in the context of less complete differentiation (i.e., low hCG). However, these results should be interpreted with caution as the association did not reach statistical significance, and there were a limited number of cases in this subgroup ($n = 142$).

Both the NSMC and FMC investigations used samples from well-established biorepositories with similar storage conditions (NSMC: −20°C; FMC: −25°C). Furthermore, the same laboratory conducted the hCG assays for both studies. Considering the NSMC study most similar to the current study, restricted to primiparous women (2), blood samples were collected at similar gestational ages (means, NSMC: 70 days; FMC: 74 days), and both studies used the same matching factors. The inverse relationship between smoking and hCG observed in this study is consistent with a prior investigation in the NSMC (26). hCG concentrations at mean GA at blood collection were somewhat lower in the NSMC (geometric means, among controls, NSMC: 97,416 mIU/mL at 70 days GA vs. FMC: 114,962 mIU/mL at 73 days GA). Furthermore, relative to women in the FMC, women in the NSMC were younger at first-term birth (means, NSMC vs. FMC: 27 vs. 30 years), cases were older at diagnosis (means, NSMC vs. FMC: 46 vs. 41 years), and a longer time elapsed between age at first birth and diagnosis (means, NSMC vs. FMC: 19 vs. 11 years). However, hCG concentrations were not correlated with storage time in either investigation (current study, Spearman $r = 0.06$; previous study $r \leq 0.07$). Finally, women in the FMC had lower parity at diagnosis/selection as control (e.g., in controls, NSMC vs. FMC: 19% vs. 33% with parity of 1). Despite these differences in the study populations, given the size of the current study, we were able to evaluate risk in all subgroups investigated in the NSMC investigation.

Our study has important strengths and limitations. We conducted the largest study to date on early pregnancy hCG and maternal breast cancer risk in a nested case–control study using prospectively collected serum samples from a population with extensive, registry-based follow-up. A limitation of our study is incomplete tumor hormone receptor status, as a total of 56% of cases had ER and PR data available. Therefore, analyses in some subgroups, particularly for ER^-/PR^- cases, were limited by sample size. Nonetheless, we provide the first data on early pregnancy hCG and breast cancer risk by hormone receptor status. We evaluated hCG in a single blood sample collected during the first 20 weeks of a primiparous pregnancy. The extent to which a single hCG measure more broadly represents early pregnancy exposure is not known. The current study included breast cancer cases diagnosed between ages 22 and 58 years. Therefore, our results may not be generalizable to women diagnosed at older ages. Finally, consistent with the comparable NSMC study (2), we measured total hCG, a composite of intact and free hCG subforms. Total, free ß-hCG, and the ratio of total to free ß-hCG were similarly associated with risk in a small ($n = 159$ cases), subsequent investigation among a subset of the cases in the NSMC (27), suggesting no meaningful difference by isoform.

We investigated early pregnancy hCG and risk of breast cancer, given strong experimental evidence linking higher doses of hCG to lower incidence of mammary tumors in virgin animal models (i.e. "nulliparous"). Our investigation does not address whether hCG mediates the protective effect of pregnancy on breast cancer risk in women, given that our study population was parous by design. However, in contrast with the experimental literature, and limited epidemiologic data, our large investigation, with more than quadruple the case numbers of prior studies, provides no evidence supporting an association between comparatively high early pregnancy hCG and subsequent breast cancer risk in the mother.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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