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Do Placental Genes Affect Maternal Breast Cancer? Association between Offspring's *CGB5* and *CSH1* Gene Variants and Maternal Breast Cancer Risk

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Abstract

The protective effect of full-term pregnancy on breast cancer is thought to be induced by two placental hormones: human chorionic gonadotropin (hCG) and human chorionic somatotropin hormone (CSH) produced by the placental trophoblastic cells. We hypothesized that variants in placental genes encoding these hormones may alter maternal breast cancer risk subsequent to pregnancy. We conducted a case-control study to examine the association between polymorphisms in a woman's placental (i.e., her offspring's) homologous chorionic gonadotrophin beta 5 (*CGB5*) and *CSH1* genes and her post-pregnancy breast cancer risk. A total of 293 breast cancer cases and 240 controls with at least one offspring with available DNA were selected from the New York site of the Breast Cancer Family Registry. Three single nucleotide polymorphisms (SNPs) in *CGB5* and *CSH1* genes were genotyped for 844 offspring of the cases and controls. Overall, maternal breast cancer risk did not significantly differ by the offspring's carrier status of the three SNPs. Among women with an earlier age at childbirth (< median age 26 years), those with a child carrying the variant C allele of *CGB5* rs726002 SNP had an elevated breast cancer risk (OR = 2.09; 95% CI, 1.17-3.73). Among women with a later age at childbirth, breast cancer risk did not differ by offspring's carrier status of *CGB5* rs726002 SNP (OR = 0.90; 95% CI, 0.53-1.51, p for interaction = 0.04). The findings suggest that placental CGB5 genotype may be predictive of maternal post-pregnancy breast cancer risk among women who give birth early in life.

Introduction and Background

Completion of full-term pregnancy has long been known to reduce breast cancer risk (1-3). It has been hypothesized that the placental hormones underlie this protective effect (4,5). Human chorionic gonadotropin (hCG) and human placental lactogen (hPL) are the two main placental

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hormones. hCG, produced by the placental trophoblastic cells, helps maintain pregnancy by acting on the corpus luteum of maternal ovaries to produce progesterone. hPL, also known as human chorionic somatotropin hormone (CSH), influences a woman's ability to lactate through its lactogenic activity on the mammary gland during pregnancy. The hCG and hPL hormones are produced during pregnancy by placental tissues; therefore, a woman's mammary glands are exposed to these hormones only during pregnancy.

A large number of experimental studies and several epidemiologic studies suggested that the pregnancy-induced protection against breast cancer is mediated by hCG (6-10). hCG, a glycoprotein hormone, has the same alpha chain as other glycoprotein hormones in addition to a hormone-specific beta catalytic subunit (11). The receptor-binding beta-subunit of hCG is encoded by highly homologous chorionic gonadotrophin beta 1, 2, 3, 5, 7, and 8 genes (*CGB1*, *CGB2*, *CGB3*, *CGB5*, *CGB7* and *CGB8*), according to their order in chromosome 19q, respectively (11). In particular, the *CGB5* gene is polymorphic, the most frequently expressed, and a highly conserved gene among the eight genes, and it is the major contributor of hCG function during pregnancy (12-14). Since not all women completing full-term pregnancies receive equal protection against breast cancer, it is possible that the variation in the placental *CGB5* gene may determine levels of hCG hormone and hence the pregnancy-related protection for breast cancer risk (15). Although the effect of hPL on breast cancer risk is not well studied, it is possible that variations in the gene (*CSH1*) encoding hPL may influence breast cancer risk in parous women, because hPL influences a woman's ability to lactate (16) and lactation is a recognized protective factor for breast cancer.

We hypothesized that the placental *CGB5* and *CSH1* genotypes are associated with breast cancer risk among parous women. Because the placental genotype is the same as that of the fetus, we assessed the association between genotypes of offspring and maternal breast cancer risk. The placental genotype may vary across pregnancies, because each pregnancy results from fertilization of gametes with potentially different alleles. Therefore, genotypes of multiple offspring should be considered.

The New York site of Breast cancer Family Registry (B-CFR), one of the six NCI-funded international sites collaborating in the B-CFR,provides a unique opportunity to examine the hypothesis. Using data from the B-CFR, we tested the novel hypothesis that a woman's placental (i.e., her offspring's) *CGB5* and *CSH1* genotypes are associated with her post-pregnancy breast cancer risk.

Materials and Methods

Study population

Details of the B-CFR, a resource for genetic studies of breast cancer, have been presented elsewhere (17,18). Briefly, families with breast and/or ovarian cancers in clinical and community settings within the metropolitan New York area meeting one or more of the following criteria were invited to participate: 1) having a female relative with breast or ovarian cancer diagnosed before age 45 years, 2) having a female relative with both breast and ovarian cancer diagnosed at any age, 3) having ≥ two relatives with breast or ovarian cancer diagnosed after age 45 years, 4) being a male with breast cancer diagnosed at any age, or 5) having a family member with a known *BRCA* mutation. The minimum age for consenting participants is 18 years. Information on demographics, ethnicity, smoking, alcohol consumption, reproductive history, hormone use, weight, height, and physical activity, and history of all cancers was collected using structured questionnaire. Information on maternal preeclampsia status was not available. A blood or buccal sample was collected from each willing participant at the time of recruitment. The study was approved by Columbia University's Institutional Review Board and written informed consent was obtained from all participants.

At the time of selecting women to be included in the current study (September, 2003), the MNYR had enrolled 1,158 families including over 3,900 participants. We identified 293 female breast cancer cases and 240 unaffected women with at least one offspring who gave a blood or buccal sample from the overall MNYR families. The cases and controls selected for the present study came from 437 pedigrees. Although some (n=45) large pedigrees contained more than one case or control, and 7 pairs of controls and 3 pairs of cases were full sisters, no nuclear families included both cases and controls. A total of 844 offspring with available DNA, including 391 offspring of 240 controls and 453 offspring of 293 cases, were genotyped.

Selection of SNPs

In this paper, we report results on two SNPs in the *CGB5* gene and one SNP in the *CSH1* gene in relation to breast cancer risk (Table 1). At the time of genotyping (April 2004), there was only one validated SNP in the *CSH1* gene with available frequency data (rs2955245) and six validated SNPs in the *CGB5* gene, of which two were in the promoter region (rs7260002 and rs7246045) and the remaining four SNPs were in 3′-UTR region. We genotyped both the *CGB5* SNPs in the promoter region and the only validated *CSH1* SNP because these SNPs are more likely to lead to functional changes.

Laboratory Analyses

In the first step, 12.5 ng of the genomic DNA extracted from blood was amplified by polymerase chain reaction (PCR) using appropriate primers as shown in Table 1. For the two *CGB5* SNPs, single set of primers was designed to include both SNPs in a single amplicon. Thermocycling condition for all the SNPs were similar: Initial denaturation for 15 min at 94° C, followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 45 sec at 56°C and 1 min extension at 72°C with a final extension at 72°C for 4 min. After PCR amplification, the primers and dNTPs were digested with the 10 μL Shrimp Alkaline Phosphatase (SAP) cocktail containing 1.0 μL (1 units/μL) of SAP, 0.1 μL of *E. coli* Exonuclease I (10 unit per μL, USB, Cleveland, OH), 0.3 μL of Pyrophosphatase (1 mg/ml), 1.0 μL of 10x SAP buffer and 7.6 μL of DNase and RNase free water for 60 min at 37°C followed by heating at 80°C for 15 min for enzyme deactivation. Single nucleotide extension was then carried out in the presence of the appropriate allele specific ddNTPs deferentially fluorescence-labeled with either R110 or TAMRA (Perkin Elmer Life Science, Boston, MA) as shown in Table 1. The probes used are shown in Table 1. Reaction mixture (13μL/well) containing 0.025 μL Acycloprime enzyme, 0.5 μL terminator dye, 1 μL reaction buffer, 0.25 μL extension probe (10 p mol/μL) and 11.2 water was added to 7 μL of digested PCR product to make 20 μL reaction volume. Thermocycling was done using heating at 95°C for 3 min followed by the optimum number of cycles of 95°C for 15 sec and 55°C for 30 sec, with optimum cycle numbers for different SNPs (Table 1). Finally, fluorescence was measured with a Wallac 1420 Multi-label Counter Victor 3 (PerkinElmer Life & Analytical Sciences, Wallac Oy, Finland). In addition to assay specific quality control samples, 10% of samples were duplicated after re-labeling to keep laboratory researchers blinded to its identity. Concordance based on the Kappa statistics was > 0.96.

Statistical Analyses

We first conducted descriptive analyses to compare cases and controls with regards to demographics and reproductive history. Then unconditional logistic regression models were used to estimate the adjusted odds ratios (OR) and 95% confidence intervals (CI) for the associations between offspring's *CGB5* and *CSH1* genotypes and maternal breast cancer risk.

Because the first pregnancy or earlier pregnancies may be more strongly associated with reduced breast cancer risk compared to the subsequent ones, we focused on the evaluation of the relationship between the genotype status of a child from the earliest available pregnancy

(i.e., genotype of oldest available offspring with DNA) and subsequent maternal breast cancer risk. We also assessed the association between having any child with *CGB5* and *CSH1* variant alleles and maternal breast cancer risk, regardless of the genotyped offspring's birth order. The ORs were adjusted for index age (age at interview or blood donation for controls and age at diagnosis for cases), ethnic background, number of children, and number of children with available genotype data. Although information on maternal preeclampsia status was not available, we did not consider preeclampsia as a potential confounder in this study. Since levels of hCG during pregnancies are significantly associated with preeclampsia (19), preeclampsia may be a causal intermediate in the pathway between offspring's *CGB5* genotypes and maternal breast cancer risk. Additional adjustment for other risk factors including hormone replacement therapy use, oral contraceptive use, body mass index (BMI), age at menarche, lactation history, gender of the oldest available offspring (20), and total number of male offspring did not change estimates appreciably, and therefore results were not presented for the extended model for parsimony.

In addition, we explored whether the associations between the offspring's *CGB5* genotypes and maternal breast cancer risk differed by maternal reproductive risk factors of breast cancer, including maternal menarche and maternal age at childbirth of the genotyped offspring. Potential effect-modifiers were dichotomized based on the median values in the overall study population. Analyses were also performed using different cutpoints (age 10 and 24 years for maternal menarche and maternal age at childbirth, respectively); however the results were similar and therefore were not shown. Cross product terms representing products of genetic status and potential effect-modifier were entered into logistic models to test interaction on the multiplicative scale. We also evaluated the association between offspring's carrier status of the at-risk haplotype, i.e., carrier of both *CGB5* variant alleles of interest, and maternal breast cancer risk. Haplotype pairs (diplotypes) were constructed using the PHASE 2.1 (21,22). Only participants with known genotypes on both the *CGB5* SNPs were included in the haplotype construction. Linkage disequilibrium between the two *CGB5* SNPs was evaluated by normalized disequilibrium (D').

Finally, we conducted logistic regression analyses of clustered data using generalized estimating equations (GEE) (23) to assess whether statistical significance of observed associations was influenced by the possible reduced variance due to familial correlation (23), since some of the cases and controls came from the same pedigrees. Robust variance estimating techniques were used to calculate standard errors and confidence intervals. These results were very similar to those of standard logistic regression analyses and therefore, were not shown. All analyses were conducted using the SAS 9.1.3 statistical package for Windows (SAS Institute Inc., Cary, NC, USA).

Results

Compared with controls, cases were more likely to be Caucasian and African American than Hispanic or of other racial/ethnic groups, and more likely to have an earlier age at menarche, a later age at first birth, and fewer live births (Table 2). Because the MNYR recruited families which included prevalent cases of breast cancer and their unaffected relatives, cases were younger in comparison to controls.

All SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) in cases and controls.Linkage disequilibrium analysis suggests strong linkage disequilibrium among the two SNPs of *CGB5* (D' = 1). Although not statistically significant, there was a positive association between having the oldest available offspring or at least one offspring with a *CGB5* rs7260002 SNP C allele and maternal breast cancer risk (Table 3). No offspring carried the GG genotype for this *CGB5* SNP, and few offspring carried TG genotype. Women with the oldest available offspring

carrying at least one CT haplotype had a non-significant increased risk of breast cancer (OR = 1.32; 95% CI, 0.91-1.94). There was no apparent association between *CSH1* rs2955245 genotypes of the oldest available offspring and maternal breast cancer risk. A total of 109 and 106 of the oldest offspring available in the analyses were the first child of cases and controls, respectively. The associations were not stronger in the subpopulation and therefore the results were not presented.

Among women who gave birth at an earlier age to the genotyped offspring (age < 26), those with a child carrying AC or CC genotypes of the *CGB5* rs726002 SNP had an increased breast cancer risk (OR = 2.09; 95% CI, 1.17-3.73), compared with those with a child carrying AA genotype (Table 4). On the other hand, among women with a later childbirth (age 26+), there was no association between having a child with AC or CC genotype and breast cancer risk (p for interaction = 0.04). In comparison to mothers who gave birth at an earlier age to a child carrying the AA genotype, those who gave birth at an earlier age to a child carrying the AC or CC genotype and those who gave birth at a later age were at a similar level of breast cancer risk, with ORs ranging from 1.94 to 2.26. A similar interaction effect was seen between having an offspring with a *CGB5* CT haplotype and the age at childbirth. The association between the older offspring's genotype of the *CGB5* rs726002 SNP and maternal breast cancer risk was not significantly modifiable by maternal age at menarche.

Discussion

To our knowledge, the present study is the first to test the novel hypothesis that a woman's placental (i.e., her offspring's) *CGB5* and *CSH1* genotypes are associated with her breast cancer risk.

The literature has established that the protection against breast cancer associated with pregnancy is largely age-dependent (24-27) and a reduced breast cancer risk is observed for women with age at first full-term pregnancy by 24 years of age (28,29) or early age at any birth (30,31). The protective effect of early pregnancies is thought to be mediated through hCG. In experimental rodent models, Russo and colleagues have shown that hCG has a dose-related effect on the reduction of the incidence of breast cancer through a variety of mechanisms including: induction of mammary gland differentiation (8,10), inhibition of cell proliferation (7,32,33), decrease in cell invasion (32) , decreased binding of carcinogens to DNA (34), and activation of genes controlling programmed cell death (35,36).

Epidemiologic evidence suggests that women who used hCG for weight loss or as part of an infertility treatment had a significantly lower risk of breast cancer, compared with non-users (37). Less direct epidemiologic evidence supporting the hCG hypothesis comes from case-control and cohort studies, with findings showing that women with preeclampsia (38-41) or experiencing multiple pregnancies (38,42-45), conditions that are associated with an elevated hCG level (19), have a reduced risk of breast cancer.

Miller-Lindholm et al. found that in most normal placentas, *CGB5* was the most highly transcribed gene (12). Another study has found a high level of expression of *CGB5* and *CGB8* in the placenta throughout pregnancy with a minor decrease during the second trimester, and the expression was found to be moderately correlated with hCG level in maternal serum (46). In the present study, overall, maternal breast cancer risk did not differ by having an offspring with different *CGB5* and *CSH1* genotypes. We found that having an offspring with *CGB5* rs7260002 CA or CC genotype was significantly positively predictive of maternal breast cancer risk among women with an early age at childbirth of the genotyped offspring (Table 4). This finding further provides evidence that the variation of the protection against breast cancer risk from early pregnancies may be modified by polymorphisms in placental *CGB5*. Additional

epidemiological studies are needed to evaluate whether the variation in maternal circulating levels of hCG during pregnancy is associated with subsequent breast cancer risk, and whether such variation can be explained by specific polymorphisms in *CGB5* and other *CGB* genes.

Our study has several important implications. First, from the risk assessment point of view, if future studies confirm the findings, subgroups of women who do not receive the same level of protective effect from pregnancy as others may be identified. Our analyses indicate that women who gave birth at an age <26 with a child carrying the C allele of the *CGB5* rs726002 SNP may face a similar level of risk compared with women giving birth at older ages (Table 4). Second, since the genetic status of the placenta is determined by both the mother's genotype and that of the child's father, our findings imply that among parous women, the offspring's genotype and the genotype of the mating partner may be predictive of who would receive the protective effect from a pregnancy at early maternal age. Lastly, because the placenta produces a wide range of hormones and enzymes (in addition to hCG and hPL), results supporting a role of placental genes opens new dimensions to genetic research for diseases beyond breast cancer which may be causally related to the placental products.

Several potential limitations in the present study should be considered when interpreting our results. First, the present study was conducted in families with breast cancer and/or ovarian cases. Therefore, the findings may not be generalizable to other populations. Second, it is possible that the observed effects are due to other variants that are in linkage disequilibrium with the SNPs of interest. Future large-scale genetic association studies using more comprehensive genomic approach with tagging polymorphisms are needed. Third, DNA samples were not available from all offspring. However, it is not likely that the self-selection of participation in the registry differ by genetic status. The number of offspring available for genotyping was similar for cases and controls. Lastly, the information on tumor characteristics was limited, and the number of women with genotyping data available for the first-born offspring was limited. Whether the observed associations differ by subtypes of breast cancer which may have different etiology (47) and whether the interaction of the offspring's genotype and maternal age at birth differs by first-born status of the genotyped offspring need to be investigated in future studies.

In conclusion, in the overall study population, maternal breast cancer risk did not differ by offspring's carrier status of *CGB5/CSH1* genotypes. We found that among women with an earlier age at childbirth, offspring's carrier status of the C allele of the *CGB5* rs7260002 SNP was associated with an elevated maternal breast cancer risk. This finding provides an explanation for the variation of breast cancer risk due to early pregnancies.

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Table 1

Characteristics of the studied SNPs and their primers and probes used for genotyping

Gene	*CGB5*	*CGB5*	*CSH1*
Chromosomal location	chr19: 54237684	chr19: 54237894	chr17: 62453216
Role	Promoter	Promoter	Promoter
SNP ID	rs#7260002	rs#7246045	rs#2955245
Allele	−1595 A/C	−1385 G/T	−5974 G/A
Seq 5′- to SNP	GGC ACT GGG TGT CTG AGT GCA AGG CC	GCC CAG CTG CTC TAC GGA CCC CAG TT	TCA AGG AAA TTG TTT ACA GTC AGA A
Seq 3′- to SNP	AGC AGT CCT ATG TGC GGG CAT TGA C	GGG AAC TCA TCA AAT CAT CAC AAA A	AAA ATA TGCA CTT CTT GAT GAA AAC
Amino acid change			
Forward primer	5′-CGC TGT GGA CTC AGG TGT GCT G-3′	5′-CGC TGT GGA CTC AGG TGT GCT G-3′	5′-AGG ACA GGG AAG GAG GCT GAA C-3′
Reverse primer	5′-TTC AAA ACC CCA TGT GGT G-3′	5′-TTC AAA ACC CCA TGT GGT G-3′	5′-GGG AGC TAA ATT TGT AGT CCA T-3′
PCR product	491 bp	491 bp	566 bp
Probe type	Reverse	Reverse	Forward
Probe sequence	5′-TGC CCG CAC ATA GGA CTG CT-3′	5′-GAT TTT GTG ATG ATT TGA TGA GTT CCC-3′	5′-TCA AGG AAA TTG TTT ACA GTC AGA A-3′
Acycloprime dye used	G/T	C/A	G/A
Number of cycles for base extension	20	15	25

Table 2

Characteristics of Cases and Controls

Characteristics[†]	Breast Cancer Status	
	Cases (N = 293)	Controls (N = 235)
Reference age, mean (SD)[*]	58.2 (13.8)	65.1 (11.9)
Race/ethnic background, %		
Caucasian	82.3	74.6
African American	3.9	0.9
Hispanic	10.6	21.2
Other	3.2	3.4
BMI, mean (SD)	25.9 (5.0)	25.9 (4.5)
Postmenopausal, %	70.0	76.0
Ever use of OC, %	23.9	20.6
Ever lactation, %	41.9	44.5
Age at menarche, mean (SD)	12.5 (1.5)	12.8 (1.6)
Age at first birth, mean (SD)	25.1 (4.1)	24.0 (4.1)
Number of live births, mean (SD)	2.8 (1.3)	3.3 (1.8)
Number of male offspring, mean (SD)	1.3 (0.7)	1.4 (0.8)
Age at the birth of the oldest offspring with genotyping data available, mean (SD)	27.1 (4.7)	26.1 (4.6)
Gender of the oldest offspring with genotyping data available, male, %	15.4	11.5
Number of offspring with genotyping data available, %		
1	58.7	54.2
2	32.1	33.8
3	6.8	9.2
4	2.1	1.7
5	0.0	1.3
6	0.3	0.0
Mean	1.5	1.7

[*] Reference age was defined as age at diagnosis for cases and age at interview or blood donation for controls.

[†] Information on race/ethnic background was unknown for 8 and 9 controls and cases, respectively; on menopausal status for 34 and 39; on OC use for 2 and 4; on age at menarche for 30 and 43; on age at first birth for 4 and 6; and on total number of live births for 3 and 4.

Table 3

Associations between offspring's *CGB5* and *CSH1* genotypes and maternal breast cancer risk

Genotype/haplotype of the offspring	Maternal breast cancer status		Crude OR for mother's breast cancer, 95% CI	Adjusted OR for mother's breast cancer , 95% CI *
	Case	Control		
***CGB5* rs7260002**				
The oldest available offspring				
AA	106		961.00 (Ref)	1.00 (Ref)
AC	139		1051.20 (0.86-1.74)	1.27 (0.84-1.92)
CC	48		341.28 (0.76-2.15)	1.32 (0.75-2.33)
AA	106		961.00 (Ref)	1.00 (Ref)
AC/CC	187		1391.22 (0.86-1.73)	1.28 (0.87-1.89)
AA/AC	245		2011.00 (Ref)	1.00 (Ref)
CC	48		341.16 (0.72-1.87)	1.16 (0.69-1.96)
No offspring with AC/CC	91		841.00 (Ref)	1.00 (Ref)
At least one offspring with AC/CC	202		1511.24 (0.86-1.78)	1.36 (0.90-2.04)
***CGB5* rs7246045**				
The oldest available offspring				
TT	289		2281.00 (Ref)	1.00 (Ref)
TG	7		90.61 (0.23-1.67)	0.60 (0.19-1.97)
***CGB5* diplotypes**				
The oldest available offspring				
Other/other	109		1001.00 (Ref)	1.00 (Ref)
CT/other	140		1061.21 (0.84-1.75)	1.29 (0.87-1.92)
CT/CT	44		291.39 (0.81-2.39)	1.38 (0.77-2.48)
Other/other	109		1001.00 (Ref)	1.00 (Ref)
CT/other, CT/CT	184		1351.25 (0.88-1.78)	1.32 (0.91-1.94)
***CSH1* rs2955245**				
The oldest available offspring				
GG	154		1271.00 (Ref)	1.00 (Ref)
GA	112		940.98 (0.69-1.41)	0.97 (0.65-1.44)
AA	22		141.30 (0.64-2.64)	1.33 (0.62-2.88)
GG	154		1271.00 (Ref)	1.00 (Ref)
GA/AA	134		1081.02 (0.72-1.45)	1.02 (0.69-1.48)
GG/GA	266		2211.00 (Ref)	1.00 (Ref)
AA	22		141.31 (0.65-2.61)	1.35 (0.64-2.87)
No offspring with AC/CC	261		2131.00 (Ref)	1.00 (Ref)
At least one offspring with AC/CC	27		221.00 (0.55-1.81)	1.04 (0.55-1.99)

* ORs were adjusted for index age, race/ethnic background, age at giving the birth, number of children, and number of children with vailable genotype data.

Table 4

Associations between *CGB5* genotype of the oldest available offspring and maternal breast cancer risk, by maternal age at birth, age at first birth, and age at menarche

Genotype/haplotype of the oldest available offspring	Potential effect-modifier	Maternal breast cancer status		Adjusted OR for mother's breast cancer, 95% CI*, Multiplicative scale	Adjusted OR for mother's breast cancer, 95% CI*, Additive scale	p-value for multiplicative interaction
		Case	Control			
***CGB5* rs7260002**						
	Maternal age at birth†					
AA	< 26	40	49	1.00 (Ref)	1.00 (Ref)	0.04
AC/CC	< 26	95	70	2.09 (1.17-3.73)	1.94 (1.11-3.40)	
AA	26+	66	47	1.00 (Ref)	2.26 (1.23-4.17)	
AC/CC	26+	91	69	0.90 (0.53-1.51)	2.08 (1.18-3.66)	
	Maternal age at menarche†					
AA	≤ 12	47	36	1.00 (Ref)	1.00 (Ref)	0.37
AC/CC	≤ 12	79	55	1.10 (0.59-2.04)	1.13 (0.63-2.03)	
AA	12+	39	46	1.00 (Ref)	0.71 (0.38-1.35)	
AC/CC	12+	83	67	1.51 (0.86-2.68)	1.15 (0.64-2.07)	
***CGB5* diplotypes**						
	Maternal age at birth†					
Other/other	< 26	43	51	1.00 (Ref)	1.00 (Ref)	0.11
CT/other, CT/CT	< 26	92	67	1.97 (1.11-3.48)	1.82 (1.05-3.15)	
Other/other	26+	66	49	1.00 (Ref)	2.00 (1.10-3.63)	
CT/other, CT/CT	26+	91	68	0.97 (0.58-1.64)	1.99 (1.14-3.48)	
	Maternal age at menarche†					
Other/other	≤ 12	49	38	1.00 (Ref)	1.00 (Ref)	0.32
CT/other, CT/CT	≤ 12	77	53	1.11 (0.61-2.05)	1.12 (0.63-2.00)	
Other/other	12+	40	47	1.00 (Ref)	0.69 (0.37-1.30)	
CT/other, CT/CT	12+	82	66	1.57 (0.89-2.76)	1.17 (0.66-2.07)	

*ORs were adjusted for index age, race/ethnic background, age at birth, number of children, and number of children with available genotype data.

†Cut points were determined based on the median in the overall study population.