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Reproductive windows, genetic loci and breast cancer risk.

Shaneda Warren Andersen, PhD^{1,2}, Amy Trentham-Dietz, PhD^{1,2}, Ronald E Gangnon, PhD², John M Hampton, MS¹, Jonine D Figueroa, PhD³, Halcyon G Skinner, PhD², Corinne D Engelman, PhD², Barbara E Klein, MD⁶, Linda J Titus, PhD⁷, Kathleen M Egan, ScD⁸, and Polly A Newcomb, PhD^{1,9}

¹ University of Wisconsin Carbone Cancer Center, Madison, WI 53726, USA

² Department of Population Health Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726, USA

³ Division of Cancer Epidemiology & Genetics, National Cancer Institute, Bethesda, MD 20892, USA

⁶ Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI 53726, USA

⁷ Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756, USA

⁸ Division of Cancer Prevention and Control, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL 33612, USA

⁹ Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Abstract

Purpose—The reproductive windows between age at menarche and first childbirth (standardized AFB) and from menarche to menopause (reproductive lifespan) may interact with genetic variants in association with breast cancer risk.

Methods—We assessed this hypothesis in 6131 breast cancer cases and 7274 controls who participated in the population-based Collaborative Breast Cancer Study. Risk factor information was collected through telephone interviews and DNA samples were collected on a sub-sample (N=1484 cases, 1307 controls) to genotype for 13 genome-wide association study-identified loci. Adjusted odds ratios (OR) and 95% confidence intervals (CI) were calculated and P-values for the interaction between reproductive windows and genotypes were obtained by adding cross-product terms to statistical models.

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Correspondence and reprints: Shaneda Warren Andersen, University of Wisconsin Carbone Cancer Center, 610 Walnut St, WARF Room 307, Madison, WI 53726, USA Phone: (615) 875-4193 Fax: (608) 265-5330 snandersen@uwalumni.com.

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Results—For standardized AFB, the OR was 1.52 (CI:1.36-1.71) comparing the highest to lowest quintile. Carrier status for rs10941679 (5p12) and rs10483813 (*RAD51B*) appeared to modify this relationship ($P=0.04$ and $P=0.02$, respectively). For reproductive lifespan, the OR comparing the highest and lowest quintiles was 1.62 (CI:1.35-1.95). No interactions were detected between genotype and reproductive lifespan (all $P>0.05$). All results were similar regardless of ductal versus lobular breast cancer subtype.

Conclusions—Our results suggest reproductive windows are associated with breast cancer risk, and that associations may vary by genetic variants.

Keywords

breast neoplasms; epidemiology; menarche; menopause; genetic loci; histology

Introduction

Reproductive and menstrual exposures such as the ages at menarche, first full-term birth and menopause have been consistently, but modestly, associated with breast cancer risk. The windows defined by these reproductive experiences have also been associated with risk (1–3), such as the length of time between the initiation of menarche and the age at first full-term birth (AFB), termed the “standardized age at first birth” (1). Standardized age at first birth has been proposed to represent the number of ovulatory cycles a woman experiences with undifferentiated breast tissue, which is hypothesized by Russo and Russo to be more susceptible to the proliferative effects of ovarian hormones (4).

Epidemiologic studies have produced findings consistent with this hypothesis (1,2). In one study postmenopausal nulliparous women and women with 15 or more years between menarche and AFB had an increased breast cancer risk when compared to women with an interval of fewer than 10 years (nulliparous relative risk=2.0, 95% confidence interval(CI): 1.0-3.8; 15 years relative risk=2.4, CI: 1.3-4.3) (1).

A second reproductive window possibly linked to breast cancer risk is defined as the “reproductive lifespan”, and comprises the time between ages at menarche and natural menopause. Several studies report an association between longer reproductive lifespan and increased breast cancer risk, particularly when comparing extremes of the risk factor's spectrum (2,3,5). Approaches to estimating the reproductive lifespan have varied, with some investigators removing anovulatory phases from the calculation of reproductive lifespan (for example, time during pregnancy, lactation, or exogenous hormone use) (2,5), and other investigators including these phases (1). A small case-control study assessed the role of reproductive lifespan excluding time for pregnancy, lactation, and hormone use on postmenopausal breast cancer risk and found a one-year increase in reproductive lifespan was associated with a 10% increased odds of breast cancer risk (CI: 2-19%) (5). However, at least one study found no association between reproductive lifespan and breast cancer risk (6). Further research is needed to confirm the associations between reproductive windows and breast cancer risk.

Reproductive windows may have stronger associations in certain subgroups identified by genetic factors. Many of these single nucleotide polymorphisms (SNPs) identified in genome-wide association studies (GWAS) act through unknown mechanisms but epidemiological evidence suggests that certain breast cancer susceptibility loci modify the effects of hormonal risk factors on breast cancer risk (7–9). We assessed the relationships between reproductive windows, top GWAS-identified loci, and their interactions in relation to invasive breast cancer risk in a population-based case-control study.

Methods

Study Sample

The study data arise from the Collaborative Breast Cancer Study, a previously described population-based case-control study (10–12). Eligible participants were selected from English-speaking female residents of Massachusetts, New Hampshire or Wisconsin. Cases were women age 20–69 with an incident invasive breast cancer reported to a state cancer registry between 1995 and 2000. Community controls were randomly selected in each state from lists of licensed drivers (<age 65) or Medicare beneficiaries (≥ age 65) and were frequency matched to approximate the age distribution of the cases within five-year age strata. Participants gave informed consent during study enrollment. This study was conducted with the approval of the University of Wisconsin Health Sciences Institutional Review Board.

Data collection

Telephone interviews were used to obtain detailed information on reproductive and menstrual experiences. Participant interviews were conducted on average one year after a specified reference date which was defined as the date of cancer diagnosis for the cases. A comparable reference point for control participants was calculated based on their five-year age strata and date of interview (12). Among eligible participants approximately 80% (N=6421) of cases and 76% (N=7673) of controls completed the interview.

Information about the histology and stage of breast cancer was obtained from each state's cancer registry. Cases were grouped by histology using International Classification of Diseases-Oncology codes (ICD-10), ductal (code 8010, 8012, 8021, 8140, 8310, 8323, 8410, 8500, 8502, 8530, 8560, 8571), lobular (code 8520) and mixed ductal-lobular (code 8521, 8522, 8523) (13). All other individual tumor subtypes were excluded from histologic specific analyses (N=463).

DNA extraction and genotyping

For a selected sample interviewed between the years 2000–2001, participants were asked to donate a buccal cell sample for genetic analyses. 70% (N=1717) of approached cases and 61% (N=1524) of approached controls agreed to donate a sample.

Samples were sent by participants through the mail directly to a National Cancer Institute-affiliated laboratory lab under the direction of Dr. Montserrat Garcia-Closas for processing. DNA collection, isolation and storage were conducted according to previously described

protocols (10). DNA was quantitated from frozen aliquots and plated for the genotyping assays. Top significant results from GWAS and follow-up studies were used to identify candidate loci for this analysis (7,14–17). In total 13 SNPs were genotyped: rs4973768, rs10941679, rs2981582, rs3817198, rs3803662, rs13281615, rs11249433, rs889312, rs2046210, rs17468277, rs10483813, rs13387042, rs6504950. Genotyping was conducted using Taqman nuclease assay (Taqman®) with reagents designed by Applied Biosystems (<http://www.appliedbiosystems.com/>) as Assays-by-Design™ and performed using the ABI PRISM 7900HT, 7700 or 7500 Sequence Detection Systems according to the manufacturer's instructions.

To reduce the possibility of population stratification and maintain a study sample with similar ancestry to the GWAS in which the loci were identified, all analyses were limited to participants self-identified as White/Caucasian in race (95.1% of participants). Quality control measures were taken to remove poor quality genetic data. SNPs missing >20% of values or individual participants with a call rate <80% for genotypic data were excluded from the analysis. All 13 SNPs passed quality control measures. 178 cases and 174 controls were removed from genetic analyses due to a high percent of missing genotype data for a total of 1484 breast cancer cases and 1307 community controls with a viable DNA sample for study analyses.

Reproductive window definitions

The first reproductive window, standardized age at first birth, was defined as the interval between age at menarche and age at first full term birth in which duration of oral contraceptive use before a first pregnancy was subtracted from the interval. Participants with negative standardized age at first birth values were set to zero (N=5). A secondary variable was created where the time when participants used oral contraceptives before first pregnancy was included in the interval. Among postmenopausal nulliparous women standardized age at first birth was defined as the difference between age at menarche and menopause. Premenopausal nulliparous women (N=446 cases and 414 controls) were excluded from this analysis.

The second reproductive window examined was the reproductive lifespan and restricted to postmenopausal women. Participants were considered postmenopausal if they reported their menstrual cycles had stopped for at least the last six months prior to reference date. The postmenopausal participants were categorized into two groups: participants with natural menopause or a second group defined as postmenopausal due to other causes. The primary definition of reproductive lifespan was the time between age at menarche and age at natural menopause excluding phases of pregnancy, lactation and oral contraceptive use. A secondary definition was established in which these phases were included in the window. A third reproductive lifespan analysis was conducted among all postmenopausal women irrespective of menopause type.

Population for analysis

A total of 6131 White breast cancer cases and 7274 White controls were included in this analysis. Of these 1484 cases and 1307 controls were involved in the genetic analyses.

Participants with missing data from a component of the reproductive windows (age at menarche N=146, oral contraceptive duration N=162, parity N=15, lactation duration N=38, age at menopause N=1543; among DNA participants: age at menarche N=29, oral contraceptive duration N=27, parity N=15, lactation duration N=13, age at menopause N=390) were excluded from analyses that involved the missing variable.

Statistical Analysis

Frequencies and distributions of reproductive, menstrual and established breast cancer risk factors were evaluated. We calculated odds ratios (OR) and 95% confidence intervals (CI) using logistic regression to assess the associations between reproductive windows and breast cancer risk. All statistical models included a term for age and state of residence.

Reproductive windows were categorized into quintiles according to the variable distribution in control participants. These cut-points were kept throughout for ease of comparison analyses. Reproductive window analyses were further adjusted for the following potential confounders identified *a priori*: family history of breast cancer, body mass index (BMI, kg/m²) at one year prior to reference date, hormone therapy use, recent alcohol use, and education (variables categorized as displayed in Table 1).

Polytomous regression analyses—The relationships between the length of the reproductive windows and ductal, lobular, and mixed ductal-lobular histologic subtypes of breast cancer were evaluated by ORs and 95% CI obtained from multivariate polytomous logistic regression models using controls as the common comparison group (18).

Genetic analysis—Hardy-Weinberg equilibrium was tested among controls with a DNA sample by using chi-squared tests to compare the observed to expected genotype frequencies. Per-allele ORs and 95% CIs were calculated for the association between each SNP and breast cancer risk represented by an ordinal term in the regression model for the number of minor alleles present. Potential effect modification of the associations between reproductive windows and breast cancer risk by individual SNPs were evaluated by including cross-product terms combining the number of minor alleles by the reproductive window in multivariate models. To determine significance, log likelihood values were compared to assess whether the cross-product term contributed significantly to the model. To further elucidate risk pattern differences by genotypes and reproductive windows stratified odds ratios and 95% CI were calculated for interactions that were significant ($P \leq 0.05$). Reproductive windows OR were grouped into non-risk and risk allele categories. Statistical analyses were conducted in SAS software (Cary, NC 9.1).

Results

In the full study sample, cases were more likely to have a family history of breast cancer, earlier age at menarche, fewer children and later age at menopause than controls (Table 1). Cases and controls were equally likely to be premenopausal and the majority of breast cancer cases were diagnosed with ductal carcinoma (78.1%, N=4791). The subgroup of women who donated a DNA sample was similar to the total participant sample in age, family history of breast cancer and other established risk factors for breast cancer. In

analyses restricted to women with a DNA sample, case participants had an earlier age at menarche and later age at menopause than controls. There was no significant difference in parity by case-control status in the DNA sample subgroup.

The range of standardized age at first birth values was 0 to 48 years and a median length of 10.8 years. There was a trend toward increased breast cancer risk with longer duration of the window between age at menarche and first birth ($P < 0.001$). Women in the highest quintile for standardized age at first birth interval (15.3 years) were at a 52% (95% CI: 36%-71%) higher risk of breast cancer compared to women with the shortest intervals (<7.3 years) (Table 2). Results restricted to participants with a DNA sample were similar in magnitude and direction, but confidence intervals were less precise. Standardized age at first birth results were similar in statistical analyses when calculated by including or excluding duration of oral contraceptive use before first pregnancy and when postmenopausal nulliparous women were removed from the interval (data not shown). When potential confounders were added to the model point estimates for the association between standardized age at first birth and breast cancer risk did not change substantially (Table 2).

The median length of reproductive lifespan calculated among postmenopausal women with natural menopause was 33.3 years (range 2 to 48 years). In analyses with anovulatory time removed, longer reproductive lifespan was significantly associated with increased breast cancer risk in women with natural menopause and in those with all-cause menopause (Table 3). Among women with natural menopause, the OR was 1.62 (95% CI: 1.35-1.95) for 37.4 reproductive lifespan years compared to <26.0 reproductive lifespan years. In the larger group with all-cause menopause the analogous comparison yielded an OR of 1.77 (95% CI: 1.51-2.08). Overall, analyses including all postmenopausal women yielded point estimates further from unity. The median value for reproductive lifespan when anovulatory phases were included was 38 years (range, 2-49 years). The findings were similar when restricted to women with a DNA sample, but confidence intervals were less precise (Table 3). We found little evidence of confounding of the association between reproductive lifespan and breast cancer risk by established breast cancer risk factors (Table 3). Analyses that did not subtract anovulatory time due to pregnancy, lactation, and oral contraceptive use produced slightly attenuated results (data not shown).

Histologic-specific subtype results

We found no evidence of heterogeneity in the association between reproductive windows and histological subtype (ductal, lobular and mixed ductal-lobular) (data not shown).

Genetic analyses and interactions

We confirmed associations between 7 of the 13 candidate SNPs and breast cancer risk (rs13387042, rs4973768, rs10941679, rs2981582, rs3817198, rs3803662 and rs6504950) (Table 4). The risk alleles of these SNPs were associated with 11-22% increase in breast cancer risk.

We observed evidence that two breast cancer susceptibility loci modified the association between standardized age at first birth and breast cancer risk (Table 5). Standardized age at

first birth analyses stratified by rs10483813 (*RAD51B*) genotype revealed distinct trends by the presence of risk alleles ($P=0.02$). Women homozygous for the major allele of rs10483813 (TT genotype) and with longer time between menarche and AFB had an increased risk of breast cancer. For example, women homozygous for the T allele and in the quintile for longest interval between age at menarche and age at first birth had two-times the odds of breast cancer (95%CI: 1.47-3.05) compared to women in the quintile for the shortest interval. In the analyses restricted to women with AT or TT genotype the associations between standardized age at first birth and breast cancer risk were null. The risk allele of rs10941679 at 5p12 also modified the relationship between standardized age at first birth and breast cancer risk ($P=0.04$) (Table 5). Women with at least one copy of the G allele and longer length of standardized age at first birth interval had higher breast cancer risk. The effect of standardized age at first birth was effectively null in women homozygous for the major allele (A). No interactions were detected between the 13 evaluated SNPs and reproductive lifespan (all $P>0.05$).

Discussion

In this study women with the longest reproductive windows were 1.5 to 1.7 times more likely to have breast cancer compared to women with the shortest reproductive windows, a result consistent with other studies (1–3,5). Standardized age at first birth may be associated with breast cancer risk as a proxy for breast tissue differentiation and ovarian hormone exposure. Breast tissue fully differentiates in preparation for lactation at first pregnancy and may be less influenced by the harmful effects of hormones in the fully differentiated state (4). Prior studies have not consistently accounted for the use of hormonal contraceptives before first childbirth in calculating the interval between age at menarche and first birth (6), which we were able to do here. Hormonal contraceptives reduce the number of ovulatory cycles experienced while also exposing the woman to exogenous estrogen and/or progestin which may alter the strength of the association between the reproductive window and breast cancer risk. In our study many of the study participants did not use or only used oral contraceptives for a short time before first pregnancy.

Under the reproductive lifespan hypothesis, menarche starts a window whereby estrogen exposure from the ovary can stimulate breast cancer until menopause (19) and, therefore, longer reproductive lifespan intervals are associated with increased breast cancer risk. The breast cancer risk associated with reproductive lifespan may become more substantial considering evidence that menarche and breast development are beginning at earlier ages than in previous birth cohorts (20). Findings from an U.S. Environmental Protection Agency 2008 scientific panel focused on menarche timing, concluded that increasingly women will be exposed to ovarian hormones for a larger proportion of their lifecourse possibly leading to increased breast cancer rates (20). Moreover, at least one study reported the age of menopause is slowly increasing over time (21).

We examined whether associations between standardized age at first birth and reproductive lifespan with breast cancer risk varied according to breast cancer histologic subtype. We found no evidence for heterogeneity overall nor did we observe a stronger association between standardized age at first birth and lobular breast cancer, as reported previously (22).

Inconsistencies between our results and other published results may be related to limited power to detect interactions in our sample.

We considered whether associations between reproductive windows and breast cancer risk may be modified by previously identified breast cancer susceptibility variants. We observed no overall association for rs10483813 (14q24/*RAD51B*); however, the data were consistent with possible interaction by standardized age at first birth ($P=0.02$) in which a positive association with increasing quintile of standardized age at first birth was limited to homozygotes for the wild-type (TT) allele. rs10483813 is located in the intronic region of *RAD51B*, a gene involved in DNA repair. The effect of this SNP on breast cancer risk has been shown to be modified by estrogen-only postmenopausal hormone therapy in past studies (23) which lends plausibility that rs10483813 works through or in conjunction with hormonal risk factor pathways. It is also possible that rs10483813 is in linkage disequilibrium with another variant which is the true source of the association. Our results also suggest potential interaction between standardized age at first birth and rs10941679 (5p12) ($P=0.04$) in which the positive association was mainly observed in women with the variant (AG/GG) allele. rs10941679 has been frequently associated with breast cancer risk (7,24,25) and is in linkage disequilibrium with *MRPS30*, a gene implicated in apoptosis and estrogen-receptor positive breast cancer (26,27). The biologic mechanisms which could cause an interaction between rs10941679 and standardized age at first birth have not been identified. It also must be noted that tests for interaction had modestly significant p-values and given the number of tests conducted the results could have arisen by chance. Functional studies and replication of our analyses in other study samples may provide more information on potential interactions between breast cancer susceptibility loci and reproductive windows.

Our results underscore the importance of two reproductive windows in association with breast cancer risk, and provide evidence that associations are consistent regardless of breast cancer histologic subtype. Our data add further weight to the evidence that mechanisms of genetic susceptibility for breast cancer may operate at least in part through reproductive risk factors. Further research into this area may identify sub-populations of women at higher risk for breast cancer who would benefit from focused prevention efforts.

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List of abbreviations

AFB	age at first birth
BMI	body mass index

CI	confidence interval
GWAS	genome-wide association study
OR	odds ratio
SNP	single nucleotide polymorphism

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Table 1

Reproductive and menstrual characteristics by case status.

Characteristic	All participants		Participants with a DNA sample	
	Cases N (%) (N = 6131)	Controls N (%) (N = 7274)	Cases N (%) (N = 1484)	Controls N (%) (N = 1307)
Age (median, standard deviation)	54.3 (9.5)	54.4 (9.6)	54.5 (9.0)	54.5 (9.4)
Family history of breast cancer				
Absent	4765 (77.7)	6211 (85.4)	1145 (77.2)	1108 (84.8)
Present	1252 (20.4)	924 (12.7)	303 (20.4)	175 (13.4)
Age at menarche (years)				
<12	1276 (20.8)	1394 (19.2)	317 (21.4)	255 (19.5)
12	1474 (24.0)	1680 (23.1)	397 (26.8)	290 (22.2)
13	1750 (28.5)	2051 (28.2)	402 (27.1)	378 (28.9)
14	929 (15.2)	1152 (15.8)	213 (14.4)	215 (16.5)
15	638 (10.4)	918 (12.6)	137 (9.2)	159 (12.2)
Age at first full-term birth (years)				
<20	881 (14.4)	1205 (16.6)	213 (14.4)	211 (16.1)
20-24	2415 (39.4)	3110 (42.8)	609 (41.0)	536 (41.0)
25-29	1323 (21.6)	2374 (32.6)	316 (21.3)	266 (20.4)
30	616 (10.1)	585 (8.0)	143 (9.6)	118 (9.0)
Parity				
Nulliparous	885 (14.4)	891 (12.3)	199 (13.4)	171 (13.1)
1	714 (11.7)	769 (10.6)	173 (11.7)	145 (11.1)
2	1950 (31.8)	2130 (29.3)	483 (32.6)	420 (32.1)
3	1307 (21.3)	1677 (23.1)	324 (21.8)	270 (20.7)
4	1268 (20.7)	1799 (24.7)	303 (20.4)	298 (22.8)
Lactation ^a (months)				
Never	3731 (61.0)	4307 (59.4)	878 (59.6)	756 (57.7)
Ever	2385 (39.0)	2945 (40.6)	602 (40.7)	545 (42.0)
3	702 (11.5)	940 (12.9)	171 (11.5)	158 (12.1)
4-12	883 (14.4)	991 (13.6)	207 (14.0)	202 (15.5)
13-23	467 (7.6)	551 (7.6)	125 (8.4)	104 (8.0)
24	333 (5.4)	464 (6.4)	99 (6.8)	81 (6.2)
Oral contraceptive use (years)				
Never	2785 (45.8)	3434 (47.6)	641 (43.2)	558 (42.7)
Ever	3302 (54.2)	3775 (52.4)	833 (56.3)	740 (56.6)
<3	934 (15.2)	1104 (15.2)	234 (15.8)	210 (16.1)
3-6	1126 (18.4)	1283 (17.6)	282 (19.1)	254 (19.4)
7	1242 (20.3)	1388 (19.1)	317 (21.4)	276 (21.1)
Menopausal status				
Premenopausal	2385 (38.9)	2801 (38.5)	565 (38.1)	470 (36.0)
Postmenopausal	3355 (54.7)	4029 (55.4)	822 (55.4)	742 (56.8)

Characteristic	All participants		Participants with a DNA sample	
	Cases N (%) (N = 6131)	Controls N (%) (N = 7274)	Cases N (%) (N = 1484)	Controls N (%) (N = 1307)
Age at menopause (years)				
<45	481 (14.3)	767 (19.0)	123 (15.0)	130 (17.5)
45-49	700 (20.9)	871 (21.6)	171 (20.8)	173 (23.3)
50-54	1085 (32.3)	1216 (30.2)	255 (31.0)	220 (29.7)
55	396 (11.8)	388 (9.6)	91 (11.1)	59 (8.0)
Age at natural menopause (years)				
<45	247 (10.8)	372 (14.0)	64 (11.7)	69 (14.1)
45-49	514 (22.5)	660 (24.8)	127 (23.2)	133 (27.2)
50-54	986 (43.2)	1104 (41.4)	232 (42.3)	205 (41.9)
55	375 (16.4)	369 (13.8)	85 (15.5)	55 (11.3)
Postmenopausal hormone therapy				
Never	1534 (45.7)	2046 (50.8)	333 (40.5)	336 (45.3)
Estrogen-only	767 (22.9)	1000 (24.8)	191 (23.2)	192 (25.9)
Estrogen+ progestogen	945 (28.2)	867 (21.5)	265 (32.2)	192 (25.9)
Other combination	109 (3.2)	116 (2.9)	33 (4.0)	22 (2.9)
Body mass index ^b (kg/m ²)				
<25 (average)	2945 (48.0)	3477 (47.8)	708 (47.7)	629 (48.1)
25-29 (overweight)	1906 (31.1)	2321 (31.9)	453 (30.5)	418 (32.0)
30 (obese)	1237 (20.2)	1410 (19.4)	314 (21.2)	248 (19.0)
Recent alcohol use (drinks/week)				
0	1056 (17.2)	1279 (17.6)	232 (15.6)	198 (15.2)
1-6	2170 (35.4)	2736 (37.6)	546 (36.8)	536 (41.0)
7	1995 (32.5)	2334 (32.1)	499 (33.6)	420 (32.1)
Education				
Less than high school	365 (6.0)	500 (6.9)	84 (5.7)	82 (6.3)
High school	2418 (39.4)	2886 (39.7)	565 (38.1)	515 (39.4)
Some college	1600 (26.1)	1961 (27.0)	396 (26.7)	341 (26.1)
College degree	1747 (28.5)	1924 (26.5)	439 (29.6)	369 (28.2)
Histologic subtype				
Ductal	4791 (78.1)		1317 (79.2)	
Lobular	575 (9.4)		154 (9.3)	
Mixed ductal-lobular	302 (4.9)		85 (5.1)	
Other	463 (7.6)		106 (6.4)	

Certain subgroups do not add to total due to missing data.

^aParous women only.

^bBody mass index levels were as defined by the World Health Organization.

Table 2

Odds ratios and 95% confidence intervals for the association between interval between age at menarche and age at first birth (quintiles) and invasive breast cancer.

Quintile (years)	Cases	Controls	Odds ratio (95% CI) ^a	Odds ratio (95% CI) ^b
1 st <7.3	932	1316	1 (ref.)	1 (ref.)
2 nd 7.3-9.4	970	1317	1.05 (0.93-1.18)	1.02 (0.91-1.15)
3 rd 9.5-11.6	1040	1321	1.13 (1.01-1.28)	1.10 (0.98-1.25)
4 th 11.7-15.2	1193	1316	1.32 (1.18-1.48)	1.31 (1.16-1.47)
5 th 15.3	1356	1319	1.52 (1.36-1.71)	1.52 (1.34-1.71)
per-quintile estimate				1.09 (1.07-1.12)
P-trend				<0.0001

Participants with a DNA sample

Quintile (years)	Cases	Controls	Odds ratio (95% CI) ^a	Odds ratio (95% CI) ^b
1 st <7.3	217	237	1 (ref.)	1 (ref.)
2 nd 7.3-9.4	252	235	1.17 (0.90-1.51)	1.14 (0.88-1.49)
3 rd 9.5-11.6	246	207	1.32 (1.02-1.72)	1.28 (0.98-1.68)
4 th 11.7-15.2	295	243	1.36 (1.05-1.75)	1.35 (1.04-1.77)
5 th 15.3	325	253	1.49 (1.16-1.92)	1.49 (1.14-1.95)
per-quintile estimate				1.07 (1.00-1.12)
P-trend				0.02

Analyses exclude time for oral contraceptive use before first pregnancy. CI indicates confidence interval; ref, reference category.

^aOdds ratios are adjusted for age and state.

^bOdds ratios further adjusted for family history of breast cancer, recent alcohol consumption, body mass index, education, age at menopause and postmenopausal hormone therapy use.

Table 3

Odds ratios and 95% confidence intervals for the association between age at menarche and age at natural menopause (quintiles) and invasive breast cancer, by menopausal type.

Quintile(years)	Natural menopause				All-cause menopause		
	Cases	Controls	OR (95% CI) ^a	OR (95% CI) ^b	Cases	Controls	OR (95% CI) ^a
1 st <26.0	349	491	1 (ref.)	1 (ref.)	564	868	1 (ref.)
2 nd 26.1-31.1	387	492	1.12 (0.93-1.36)	1.14 (0.94-1.39)	517	643	1.25 (1.07-1.47)
3 rd 31.2-34.3	399	498	1.15 (0.95-1.36)	1.20 (0.99-1.46)	486	597	1.28 (1.09-1.50)
4 th 34.4-37.3	408	486	1.20 (0.99-1.45)	1.23 (1.01-1.49)	453	539	1.31 (1.11-1.54)
5 th 37.4	549	492	1.62 (1.35-1.95)	1.65 (1.37-1.99)	597	533	1.77 (1.51-2.08)
per-quintile estimate				1.03 (1.01-1.06)			1.06 (1.03-1.08)
P-trend				<0.0001			<0.0001

Participants with a DNA sample

Quintile(years)	Natural menopause				All-cause menopause		
	Cases	Controls	OR (95% CI) ^a	OR (95% CI) ^b	Cases	Controls	OR (95% CI) ^a
1 st <26.0	91	109	1 (ref.)	1 (ref.)	150	168	1 (ref.)
2 nd 26.1-31.1	91	89	1.21 (0.81-1.82)	1.30 (0.86-1.96)	118	116	1.13 (0.80-1.59)
3 rd 31.2-34.3	97	84	1.40 (0.93-2.11)	1.50 (1.00-2.27)	118	96	1.40 (0.99-1.99)
4 th 34.4-37.3	94	97	1.19 (0.79-1.77)	1.22 (0.81-1.84)	105	109	1.10 (0.77-1.56)
5 th 37.4	124	76	2.01 (1.34-3.02)	2.12 (1.40-3.19)	136	82	1.92 (1.34-2.76)
per-quintile estimate				1.08 (1.02-1.13)			1.07 (1.02-1.12)
P-trend				0.05			0.05

Reproductive lifespan analyses completed only among postmenopausal women. Analyses exclude time for oral contraceptive use, lactation, and pregnancy. OR indicates odds ratio; CI, confidence interval; ref, reference category.

^a Odds ratios are adjusted for age and state.

^b Odds ratios further adjusted for family history of breast cancer, recent alcohol consumption, body mass index, education and postmenopausal hormone therapy use.

Table 4

Per-allele odds ratios^a for the associations between breast cancer susceptibility loci and breast cancer risk.

Chromosomal location/Gene	SNP	Major/Minor Allele	MAF	OR (95% CI)	P _{trend}
10q26/ <i>FGFR2</i>	rs2981582	C/T	0.40	1.22 (1.08-1.38)	0.001
16q12/ <i>TOX3</i>	rs3803662	C/T	0.28	1.22 (1.07-1.39)	0.003
5p12	rs10941679	A/G	0.27	1.20 (1.05-1.37)	0.01
3p24/ <i>SLC4A7</i>	rs4973768	C/T	0.50	1.19 (1.06-1.34)	0.004
11p15/ <i>LSP1</i>	rs3817198	T/C	0.32	1.15 (1.02-1.31)	0.02
8q24	rs13281615	A/G	0.42	1.07 (0.95-1.20)	0.23
1p11	rs11249433	T/C	0.43	1.06 (0.95-1.19)	0.31
5q11/ <i>MAP3K1</i>	rs889312	A/C	0.29	1.06 (0.93-1.20)	0.43
6q25	rs2046210	C/T	0.37	1.00 (0.91-1.13)	0.80
2q33/ <i>ALS2CR12/CASP8</i>	rs17468277	G/A	0.12	1.00 (0.84-1.19)	0.99
14q24/ <i>RAD51B</i>	rs10483813	T/A	0.24	0.98 (0.85-1.12)	0.73
2q35	rs13387042	T/C	0.46	0.89 (0.79-1.00)	0.04
17q23/ <i>STXBP4</i>	rs6504950	G/A	0.27	0.83 (0.73-0.95)	0.01

^aOdds ratios are adjusted for age and state of residence. MAF indicates minor allele frequency; OR, odds ratio; CI, confidence interval. P-trend calculated by inclusion of an ordinal term to represent the number of increasing minor alleles.

Table 5

Odds ratios^a and 95% confidence intervals for the association between the interval from age at menarche to age at first birth and breast cancer risk, stratified by rs10483813 and rs10941679.

Quintiles (years)	OR (95%CI)	OR (95%CI)	P _{Interaction}
rs10483813	TT	AT/AA	
1 st <7.3	1 (ref.)	1 (ref.)	
2 nd 7.3-9.4	1.46 (1.01-2.11)	1.08 (0.69-1.70)	
3 rd 9.5-11.6	1.41 (0.96-2.06)	1.08 (0.59-1.70)	
4 th 11.7-15.2	1.48 (1.03-2.13)	1.16 (0.75-1.80)	
5 th 15.3	2.12 (1.47-3.05)	0.99 (0.65-1.53)	0.02
per-quintile estimate	1.16 (1.07-1.26)	1.00 (0.91-1.10)	
rs10941679	AA	AG/GG	
1 st <7.3	1 (ref.)	1 (ref.)	
2 nd 7.3-9.4	1.13 (0.76-1.69)	1.66 (1.09-2.53)	
3 rd 9.5-11.6	1.08 (0.71-1.62)	1.66 (1.09-2.52)	
4 th 11.7-15.2	1.00 (0.68-1.46)	2.08 (1.37-3.17)	
5 th 15.3	1.23 (0.84-1.80)	1.96 (1.30-2.95)	0.04
per-quintile estimate	1.03 (0.95-1.12)	1.16 (1.06-1.28)	

OR indicates odds ratio; CI, confidence interval; ref., reference.

^a Odds ratios are adjusted for age and state. Analyses exclude time for oral contraceptive use before first pregnancy.