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Breast cancer susceptibility loci in association with age at menarche, age at natural menopause and the reproductive lifespan

Shaneda Warren Andersen[a,b,*], **Amy Trentham-Dietz**[a,b], **Ronald E. Gangnon**[b], **John M. Hampton**[a], **Halcyon G. Skinner**[b], **Corinne D. Engelman**[b], **Barbara E. Klein**[c], **Linda J. Titus**[d], **Kathleen M. Egan**[e], and **Polly A. Newcomb**[a,f]

Shaneda Warren Andersen: snandersen@uwalumni.com

[a]University of Wisconsin, Carbone Cancer Center, Madison, WI 53726, USA

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

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

[d]Norris Cotton Cancer Center, Dartmouth Medical School, Lebanon, NH 03756, USA

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

[f]Cancer Prevention Program, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Abstract

Background—Genome-wide association studies have identified single nucleotide polymorphisms (SNPs) associated with breast cancer risk. Some of these loci have unknown functional significance and may mediate the effects of hormonal exposures on breast cancer risk. We examined relationships between breast cancer susceptibility variants and menstrual/reproductive factors using data from two population-based studies.

Methods—The first analysis was based on a sample of 1328 women age 20–74 who participated as controls in a case-control study of breast cancer conducted in three U.S. states. We evaluated the associations between age at menarche, age at natural menopause and the reproductive-lifespan with 13 previously identified breast cancer variants. Associations were also examined with a genetic score created as the sum of at-risk alleles across the 13 variants. For validation, significant results were evaluated in a second dataset comprised 1353 women age 43–86 recruited as part of a cohort study in Wisconsin.

*Corresponding author at: University of Wisconsin, Carbone Cancer Center, 610 Walnut Street, WARF Room 307, Madison, WI 53726, USA. Tel.: +1 615 875 4193; fax: +1 615 343 0719.

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Results—Neither the genetic score nor any of the 13 variants considered individually were associated with age at menarche or reproductive lifespan. Two SNPs were associated with age at natural menopause; every increase in the minor allele (A) of rs17468277 (CASP8) was associated with a 1.12 year decrease in menopause age (p = 0.02). The minor allele (G) of rs10941679 (5p12) was associated with a 1.01 year increase in age at natural menopause (p = 0.01). The results were not replicated in the validation cohort (B = −0.61, p = 0.14 and B = −0.01, p = .0.98, respectively).

Conclusions—The evaluated variants and reproductive experiences may work through separate pathways to influence breast cancer risk.

Keywords

Epidemiology; Breast cancer; Breast cancer susceptibility loci; Menstrual factors

1. Introduction

Genome-wide association studies (GWAS) and their subsequent consortia projects have identified at least 64 distinct loci associated with breast cancer risk [1]. Previous epidemiologic studies have demonstrated that although many of the breast cancer GWAS-identified variants have unknown functional significance they may act through underlying hormonal pathways [2,3]. Age at menarche, age at natural menopause and the period which encompasses the time between age at menarche and age at natural menopause, termed the total “reproductive lifespan” are breast cancer risk factors also hypothesized to work through hormonal pathways to influence breast cancer risk [4,5]. Individual variation in the timing of these menstrual factors is known to be influenced by genetic variation [6]. We used a sequential study design to explore associations between 13 breast cancer risk variants with age at menarche, age at natural menopause and the reproductive lifespan.

2. Materials and methods

2.1. Study population

Data from two population-based studies were used in this analysis. The first study, the Three State Study, is a previously described population-based case–control study focused on breast cancer etiology [7,8]. The present analyses were restricted to control participants in the study. Telephone interviews were used to obtain information on known and suspected risk factors for breast cancer.

Participants were asked to donate a buccal cell sample for genetic studies using an oral rinse protocol (*N* = 1 545; 61% of eligible). To reduce the potential for population stratification in the data all analyses were limited to persons self-identified as White/Caucasian in race (97% of participants). Samples were sent through the mail to a National Cancer Institute-affiliated laboratory for processing. DNA collection, isolation and storage were conducted according to previously described protocols [9]. DNA was quantitated from frozen aliquots and plated for the genotyping assays. Genome-wide association studies were used to identify variants for inclusion in this analysis. In total 13 SNPs were genotyped: rs4973768, rs10941679, rs2981582, rs3817198, rs3803662, rs13281615, rs11249433, rs889312, rs2046210, rs17468277, rs10483813, rs13387042, and rs6504950. Genotyping for the Three State Study

was conducted using Taqman nuclease assay (Taqman®) with reagents designed by Applied Biosystems (http://www.appliedbiosystems.com/) as Assays-by-Design™ and genotyping performed using the ABI PRISM 7900HT, 7700 or 7500 Sequence Detection Systems according to the manufacturer's instructions.

Quality control measures were taken to remove poor quality genotype data. SNPs missing >20% of values or individual participants with a call rate <80% for genotypic data were excluded from the analysis. All SNPs passed quality control measures. 174 participants failed genetic quality control measures and were removed from genetic analyses leaving a total of 1 328 participants for analysis.

2.2. Validation dataset: Beaver Dam Eye Study

To increase power and reduce the number of tests, significant associations (p-value ≤0.05) from the Three State Study dataset were subsequently evaluated in the Beaver Dam Eye Study (BDS). The BDS is a cohort study focused on eye conditions and other processes of aging, among 2 762 (83% of eligible) female residents ages 43–86 in Beaver Dam, Wisconsin initially identified through a private census conducted in 1987 [10]. The cohort has been re-contacted and information updated every five years since baseline. BDS participants were considered ineligible for the current analysis if they had any cancer diagnosis prior to or within one year of baseline (N = 382), survived less than one year after the initial interview (N = 22) or had been diagnosed with breast cancer since baseline (N = 133) leaving 2 225 eligible participants.

In-person visits with BDS women included blood sampling and an interview covering health histories. DNA was extracted from whole blood cells or buffy coat separation following standard procedures [11]. DNA was stored at −80° Celsius until genotyped. The variants used in this project were genotyped as part of an Illumina iSelect 4608 Custom Genotyped Array at a Case Westernaffiliated laboratory. In the BDS sample rs999737 was genotyped instead of rs10483813. These SNPs are highly correlated in the HapMap CEU population (r^2 = 1.0) and are located 3 398 basepairs apart. The SNP rs2046210 could not be genotyped in BDS cohort due to infeasibility on the customized array. Samples which failed Illumina quality control recommendations or labeled as genetic outliers based upon principal components analysis were excluded (N = 872). All remaining BDS participants had an overall genotype array call rate >85% leaving 1 353 cohort participants.

2.3. Statistical analyses

Descriptive analyses were completed separately for each study population. Hardy–Weinberg equilibrium was tested by using chi-squared tests to compare the observed to expected genotype frequencies in participants. We used linear regression to assess the associations between each SNP and the following menstrual factor exposures: age at menarche, age at natural menopause and reproductive lifespan. The number of minor alleles present for each SNP (0,1,2) was represented in the regression model by an ordinal term. Standard errors (SEs) were also calculated for each point estimate. Subjects with missing data were excluded from analyses which included the missing variable. All statistical models included a term for age and Three State Study models also included a term for state of residence.

2.4. Genetic risk score

A composite genetic risk score was created to assess the polygenic contribution of the evaluated breast cancer risk variants on menstrual exposures. All SNPs were coded according to the direction of association from low (0) to high (2) risk of breast cancer with the number of risk alleles (0–2) then summed across all 13 SNPs and the score regressed onto the menstrual factor of interest.

2.5. Outcome variable definitions

Age at menarche, age at menopause and the reproductive lifespan were coded as continuous variables. Three State Study participants were considered postmenopausal if they reported their menstrual cycles had stopped for at least six months prior to a reference date, defined as approximately one year before interview. BDS participants were considered postmenopausal if their cycles had stopped at the date of the baseline interview. The postmenopausal participants were categorized into two groups: participants with natural menopause or a second group defined as menopause due to other causes. In the Three State Study reproductive lifespan was defined as the interval between age at menarche and age at natural menopause, which excluded the following phases: pregnancy, lactation and oral contraceptive use. In the BDS, information on pregnancy and lactation was not collected. Statistical analysis was conducted using SAS (Cary, NC 9.1). Participants in both studies provided informed consent during study enrollment. The current study was conducted under the approval of the University of Wisconsin Health Sciences Institutional Review Board.

3. Results

Three State Study and BDS participants had similar ages at menarche and natural menopause (Table 1). BDS participants were somewhat older than Three State Study participants. In both studies women experienced menarche on average around age of 13 and natural menopause near age 50.

There was no evidence for departure from Hardy-Weinberg Equilibrium for any of the study SNPs (p-values >0.05). The minor allele (A) of rs17468277 (*CASP8*) was associated with a 1.12 ($p = 0.02$) per year decrease in age at natural menopause per increase in the minor alleles (Table 2). Women homozygous for the major allele had mean age at natural menopause of 49.4 years whereas the mean age at natural menopause for heterozygous women was 47.9 years. In the second stage of the study, rs17468277 was not associated with age at natural menopause ($B = -0.609$, $p = 0.14$). In the Three State Study the rs10941679 risk allele (G) increased age at natural menopause by 1.01 year ($p = 0.01$). The result was not replicated in the BDS population ($B = -0.009$, $p = 0.98$). None of the remaining SNPs or the genetic score were associated with age at natural menopause in the Three State Study (Table 2). There was no evidence to suggest that any of the individual SNPs or the genetic score were associated with reproductive lifespan or age at menarche at p-value ≤ 0.05 in the Three State Study (Table 2).

4. Discussion

In the initial study stage we found evidence of association between rs10941679 and an one year increase in age at natural menopause per increase in minor allele. The direction of the association was as expected by the SNP's association with breast cancer risk [12]. Moreover, in both the Three State Study and the BDS the direction of the association between a second variant, rs17468277, and age at natural menopause was as predicted by its association with breast cancer risk. However, the associations between rs10941679 and rs17468277 with age at natural menopause were not significantly replicated in the BDS and in light of the number of tests conducted our findings could have occurred by chance.

We did not find evidence of associations between 13 GWAS-identified breast cancer susceptibility loci or a genetic score with age at menarche or reproductive lifespan. It is possible that a lack of power contributed to our null results. However, our results are in line with one previous study. A study using Million Women Study data found no evidence of associations between 12 breast cancer susceptibility loci and age at menarche or age at menopause [13]; the Million Women Study included five of the variants evaluated in the present analysis. The current study had 80% power to detect a B estimate for age at menarche as small as 0.17 years per risk allele increase for a variant with minor allele frequency greater than 0.45. Reproductive lifespan analyses were restricted to postmenopausal women and had a reduced sample size. For the same minor allele frequency comparison we had 80% power to detect a 1.3 year or greater change in reproductive lifespan per risk allele increase. For rarer variants, such as rs17468277 (*CASP8*), we had 80% power to detect a B estimate of 0.26 for age at menarche and 2.0 for reproductive lifespan. We were able to exclude the possibility that there are large effects attributed to the associations between the examined breast cancer susceptibility loci and three menstrual factors, however, are unable to rule out the possibility that we may have missed more subtle associations between these factors. Of interest, none of the genetic variants linked to breast cancer have been implicated in the age at onset of puberty or age at natural menopause in GWAS [6,15,16], further supporting the conclusion that common breast cancer variants operate independently of the timing of these events in a woman's reproductive life.

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References

1. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013 Apr; 45(4): 353–61. [PubMed: 23535729]

2. Garcia-Closas M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA, et al. Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLoS Genet. 2008 Apr.4(4):e1000054. [PubMed: 18437204]
3. Garcia-Closas M, Chanock S. Genetic susceptibility loci for breast cancer by estrogen receptor status. Clin Cancer Res. 2008 Dec; 14(24):8000–9. [PubMed: 19088016]
4. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev. 1993; 15(1):36–47. [PubMed: 8405211]
5. Clavel-Chapelon F. E3N Group. Cumulative number of menstrual cycles and breast cancer risk: results from the E3N cohort study of French women. Cancer Causes Control. 2002 Nov; 13(9):831–8. [PubMed: 12462548]
6. He C, Kraft P, Chen C, Buring JE, Paré G, Hankinson SE, et al. Genome-wide association studies identify loci associated with age at menarche and age at natural menopause. Nat Genet. 2009 Jun; 41(6):724–8. [PubMed: 19448621]
7. García-Closas M, Egan KM, Newcomb PA, Brinton LA, Titus-Ernstoff L, Chanock S, et al. Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. Hum Genet. 2006 May; 119(4):376–88. [PubMed: 16485136]
8. Sprague BL, Trentham-Dietz A, Garcia-Closas M, Newcomb PA, Titus-Ernstoff L, Hampton JM, et al. Genetic variation in TP53 and risk of breast cancer in a population-based case control study. Carcinogenesis. 2007 Aug; 28(8):1680–6. [PubMed: 17449902]
9. García-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev. 2001 Jun; 10(6):687–96. [PubMed: 11401920]
10. Klein R, Klein BE, Linton KL, De Mets DL. The Beaver Dam Eye Study: visual acuity. Ophthalmology. 1991 Aug; 98(8):1310–5. [PubMed: 1923372]
11. Duggal P, Klein AP, Lee KE, Klein R, Klein BEK, Bailey-Wilson JE. Identification of novel genetic loci for intraocular pressure: a genomewide scan of the Beaver Dam Eye Study. Arch Ophthalmol. 2007 Jan; 125(1):74–9. [PubMed: 17210855]
12. Nickels S, Truong T, Hein R, Stevens K, Buck K, Behrens S, et al. Evidence of gene-environment interactions between common breast cancer susceptibility loci and established environmental risk factors. PLoS Genet. 2013; 9(3):e1003284. [PubMed: 23544014]
13. Travis RC, Reeves GK, Green J, Bull D, Tipper SJ, Baker K, et al. Gene-environment interactions in 7610 women with breast cancer: prospective evidence from the Million Women Study. Lancet. 2010 Jun; 375(9732):2143–51. [PubMed: 20605201]
15. Elks CE, Perry JRB, Sulem P, Chasman DI, Franceschini N, He C, et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet. 2010 Dec; 42(12):85–1077.
16. Stolk L, Perry JRB, Chasman DI, He C, Mangino M, Sulem P, et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nat Genet. 2012 Mar; 44(3):260–8. [PubMed: 22267201]

Table 1

Descriptive characteristics of menstrual traits by study stage.

Characteristic (years)	Stage I: Three State Study				Stage II: Beaver Dam Study			
	N	Median	SD	Range	*N*	Median	SD	Range
Age	1328	54.8	9.6	26–74	1353	61.0	11.0	43–86
Age at menarche	1319	13.0	1.6	8–22	1341	13.0	1.6	8–21
Age at menopause[a]	462	50.0	5.2	15–59	688	50.0	5.1	24–70
Reproductive lifespan[b]	455	32.3	7.2	2.0–45.1	683	37.0	5.3	10–58

[a] Analyses restricted to women who underwent natural menopause.

[b] The reproductive lifespan is defined by the interval between age at menarche and age at natural menopause. Analyses in the Three State Study account for pregnancy related events. SD indicates standard deviation.

Table 2

Estimates[a] for the association between putative breast cancer susceptibility loci and menstrual traits.

Chromosomal location/gene	SNP	Allele	MAF	Age at natural menopause			Reproductive lifespan			Age at menarche		
				B **(years)**	**SE**	***p*-Value**	***B*** **(years)**	**SE**	***p*-Value**	***B*** **(years)**	**SE**	***p*-Value**
2q33/*CASP8*	rs17468277	G/A	0.12	−1.116	0.49	0.02	−0.533	0.74	0.47	−0.035	0.11	0.75
Validation result				−0.609	0.41	0.14						
5p12	rs10941679	A/G	0.27	1.009	0.39	0.01	0.931	0.60	0.12	0.054	0.08	0.52
Validation result				−0.009	0.30	0.98						
1p11	rs11249433	T/C	0.43	0.079	0.32	0.80	−0.465	0.48	0.33	0.113	0.07	0.11
2q35	rs13387042	T/C	0.46	−0.250	0.34	0.47	−0.240	0.52	0.64	−0.001	0.07	0.98
3p24/*SLC4A7*	rs4973768	C/T	0.50	0.174	0.34	0.61	0.874	0.52	0.09	−0.088	0.07	0.23
5q11/*MAP3K1*	rs889312	A/C	0.29	−0.533	0.36	0.14	−0.802	0.54	0.14	−0.015	0.08	0.85
6q25	rs2046210	C/T	0.37	0.317	0.35	0.36	0.283	0.49	0.56	−0.033	0.07	0.64
8q24	rs13281615	A/G	0.42	−0.258	0.35	0.46	−0.490	0.52	0.35	0.051	0.07	0.48
10q26/*FGFR2*	rs2981582	C/T	0.40	0.092	0.35	0.80	−0.390	0.54	0.47	−0.046	0.08	0.54
11p15/*LSP1*	rs3817198	T/C	0.32	0.110	0.37	0.76	−0.651	0.56	0.24	0.020	0.08	0.79
14q24/*RAD51B*	rs10483813	T/A	0.24	−0.049	0.39	0.90	0.456	0.59	0.44	0.046	0.08	0.58
16q12/*TOX3*	rs3803662	C/T	0.28	0.153	0.40	0.70	0.433	0.60	0.47	0.009	0.08	0.91
17q23/*STXBP4*	rs6504950	G/A	0.27	0.250	0.37	0.50	−0.122	0.56	0.83	0.025	0.08	0.75
Genetic risk score				−0.136	0.16	0.39	0.009	0.10	0.93	−0.007	0.02	0.77

[a]Estimates are adjusted for age. Three State Study estimates are also adjusted for state of residence. Alleles are displayed major/minor allele. B estimate is for the effect of the minor allele on the menstrual trait. MAF indicates minor allele frequency; SE, standard error.