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Genetic variants of age at menopause are not related to timing of ovarian failure in breast cancer survivors

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Abstract

Objective—To determine if inter-individual genetic variation in single nucleotide polymorphisms related to age at natural menopause are associated with risk of ovarian failure in breast cancer survivors.

Methods—A prospective cohort of 169 premenopausal breast cancer survivors recruited at diagnosis with Stages 0 to III disease were followed longitudinally for menstrual pattern via self-reported daily menstrual diaries. Participants were genotyped for 13 single nucleotide polymorphisms (SNPs) previously found to be associated with age at natural menopause: *EXO1*, *TLK1*, *HELQ*, *UIMC1*, *PRIM1*, *POLG*, *TMEM224*, *BRSK1*, and *MCM8*. A risk variable summed the total number of risk alleles in each participant. The association between individual genotypes, as well as the risk variable, and time to ovarian failure (> 12 months of amenorrhea) was tested using time-to-event methods.

Results—Median age at enrollment was 40.5 years old (range 20.6–46.1). The majority of participants were white (69%) and underwent chemotherapy (76%). Thirty-eight participants (22%) experienced ovarian failure. None of the candidate SNPs or the summary risk variable were significantly associated with time to ovarian failure. Sensitivity analysis restricted to whites or

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Conflicts of Interest/Financial Disclosure

None

Disclaimers

None

only to participants receiving chemotherapy yielded similar findings. Older age, chemotherapy exposure and lower BMI were related to shorter time to ovarian failure.

Conclusions—Thirteen previously identified genetic variants associated with time to natural menopause were not related to timing of ovarian failure in breast cancer survivors.

Keywords

Breast Cancer; Genotype; Single Nucleotide Polymorphisms; Age at Menopause; Ovarian Failure

Introduction

Breast cancer is the most common type of cancer in premenopausal women.¹ In the United States, there are nearly 230,000 new breast cancer cases each year, with 11% of all new diagnoses in women younger than age 45.² Most young women with breast cancer will undergo gonadotoxic chemotherapy, which increases risks of primary ovarian insufficiency and early menopause.^{3,4} Several mechanisms have been proposed, including direct ovarian and vascular toxicity as well as cellular effects such as DNA damage and oxidative stress.⁵ The clinical impact of this shortened span of ovarian function includes lower birth rates,⁶ increased vasomotor and other estrogen deprivation symptoms,⁷ and rapid bone loss.⁶

It remains a challenge to counsel young breast cancer patients prior to cancer treatment on their future risk of ovarian failure. To date, known risk factors for increased risks of ovarian failure include older age,⁹ lower ovarian reserve^{7,8}, and undergoing chemotherapy.⁴ These predictors suggest that risk of ovarian failure is related to both innate factors, e.g. ovarian reserve, and exogenous exposures, e.g. cyclophosphamide-based chemotherapy.

There are a number of genetic variants that have been associated with age at menopause.⁹ In the general population, multiple genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with age at menopause.^{10–14} Following the initial GWAS, a meta-analysis examined the relationship of SNPs to age at natural menopause in a discovery cohort of 40,000 women and a replication cohort of 14,000 women.¹⁵ Together, these studies have identified 13 SNPs on 9 genes involved in DNA damage repair and replication (*EXO1*, *TLK1*, *HELQ*, *UIMC1*, *PRIM1* and *POLG*)^{16–18}; vesicle transport (*BRSK1*)^{19,20}; meiotic cell division and stability (*MCM8*)²¹; as well as one whose function is uncertain (*TMEM224*)²². Whether these genetic variants are related to the timing of ovarian failure in the context of gonadotoxic cancer treatment exposures is not known.

We have previously shown that SNPs in enzymes involved in cyclophosphamide metabolism are associated with time to ovarian failure in breast cancer survivors.²³ In a new, prospective cohort study of premenopausal breast cancer patients, we tested the hypothesis that SNPs associated with age at natural menopause would also be related to timing of ovarian failure after breast cancer diagnosis.

Materials and Methods

Study Population

Premenopausal breast cancer survivors (n=169) with Stages 0 to III disease as defined by the American Joint Committee on Cancer were enrolled at diagnosis at three academic breast centers (University of Pennsylvania, University of California, San Diego and University of Southern California) between 2008 and 2013 and followed prospectively. Participants were identified by systematic medical record screening of all new breast cancer patients at the three sites. Patients were eligible if they were between ages 18 and 45, had a new breast cancer diagnosis, had a uterus and at least one ovary, and reported at least 1 menses over the prior 12 months. Cancer treatments were determined by the primary oncologist and could include chemotherapy, radiation, endocrine therapy and/or surgery. Pregnancy, breastfeeding, use of psychotropic medications that interact with the hypothalamic-pituitary-ovarian axis, history of prior cancer and history of prior gonadotoxic therapy were exclusion criteria. For this secondary analysis, all participants were included, regardless of cancer treatment characteristics. Study approval was obtained from the three institutional review boards, and all participants provided written informed consent (NCT01197456).

Data Collection

At enrollment, participants provided peripheral whole blood specimens, from which the buffy coat and DNA were subsequently extracted and used for genotyping. After enrollment, participants were followed for up to 5 years. Participants prospectively recorded daily menstrual diaries and completed additional questionnaires on menstrual pattern every three to six months. Concordance between daily menstrual diaries and serial questionnaire data was high (data not shown). Primary medical records were reviewed to abstract cancer treatment and outcome data.

SNP Selection

Thirteen candidate SNPs were selected following a review of age at menopause GWAS and sample size considerations due to distribution of major and minor alleles. Participants were genotyped for SNPs in *EXO1* (rs1635501), *TLK1* (rs10183486), *HELQ* (rs4693089), *UIMC1* (rs365132), *PRIM1* (rs2277339), *POLG* (rs2307449), *TMEM224* (rs4806660), *BRSK1* (rs1172822, rs2384687, rs1551562, rs897798), *MCM8* (rs236114, rs16991615). Table 1 summarizes these candidate SNPs and their prior reported effect sizes on age at natural menopause.

Sample Preparation and Genotyping

Participant DNA was extracted from buffy coat specimens using the Qiagen QIAamp DNA Blood Kit (Qiagen, Valencia, CA) and quantitated using Invitrogen Pico Green dsDNA quantitation kit (Invitrogen, Paisley, U.K.). Participants were genotyped for the SNPs using the Taqman OpenArray System.

AMH and FSH measurement

At study enrollment, blood specimens were collected, processed for serum and frozen at -80°C . Due to urgency in starting chemotherapy, blood specimens were drawn across the menstrual cycle. Sera were assayed for AMH and FSH at the University of Southern California Reproductive Endocrine Research Laboratory. AMH was measured by the AMH enzyme-linked immunosorbent assay kits with limit of detectability of 0.17 ng/mL (Beckman Coulter, AMH Gen II assay, Brea, CA, USA). FSH was measured by a direct immunochemiluminometric assay using the automated Immulite system (Siemens Medical Solutions, Los Angeles, CA). E2 was measured by radioimmunoassay after an organic solvent extraction step, with limit of detectability of 3 pg/mL. Inter-assay coefficients of variation were $<10\%$ for all assays. Values below detection thresholds were given half of the threshold value in analyses.

Data analysis

Stata software (Release 12; Stata Corp., College Station, TX) was used for data analyses. The primary outcome was ovarian failure, defined as 12 months of amenorrhea following study enrollment. For participants who underwent chemotherapy, this outcome required 12 months of amenorrhea following the end of chemotherapy, as amenorrhea during the 3 to 6 months duration of chemotherapy would be attributable to acute loss of growing ovarian follicles and not to depletion of the entire follicle pool. Time to ovarian failure was calculated for each participant. Participants who underwent ovarian failure contributed the time from cancer diagnosis or end date of chemotherapy to the start date of amenorrhea. Participants who did not undergo ovarian failure contributed all of the time that they reported menstrual cycles until their last follow up or until the date when they were censored. Causes for censorship included starting GnRH agonist, bilateral oophorectomy, hysterectomy, withdrawal from the study, cancer metastasis, and death.

Descriptive statistics were performed for baseline characteristics. Genotypes were categorized as homozygous for the major allele versus any minor allele. In addition, in order to explore if there was an additive effect of having multiple risk alleles for earlier age at menopause, an omnibus variable summing the total number of risk alleles that were present in each participant was created for analysis. Kaplan-Meier survival curves for time to ovarian failure were generated. Cox proportional hazards models were used to assess the association between SNPs and time to ovarian failure, as well as between baseline characteristics and time to ovarian failure. For this exploratory analysis, a p-value of 0.05 was considered significant.

Results

In total, 169 breast cancer survivors met inclusion criteria. Baseline characteristics are depicted in Table 2. Median age at enrollment (range) was 40.5 (20.6–46.1) years. Sixty-nine percent of the population ($n=117$) was white, and 76% ($n=128$) underwent chemotherapy. The most common chemotherapy regimens included cyclophosphamide and doxorubicin, with or without a taxane ($n=86$), taxane with cyclophosphamide ($n=30$), and taxane and carboplatin ($n=11$). Fifty-four percent ($n=92$) underwent endocrine therapy with

tamoxifen. The median follow up time was 646 days (range 23 to 2119 days). During follow up, 22% of participants (n=38) met criteria for ovarian failure. Among these 38 participants, 27 underwent chemotherapy and never resumed menses following chemotherapy; 6 underwent chemotherapy and became amenorrheic at a median of 365 days (range 126 to 886 days) following end of chemotherapy; 5 did not undergo chemotherapy and became amenorrheic at a median of 262 days (range 57 to 805) from enrollment. Fifty participants were censored for start of GnRH agonist therapy (10.1%, n=17), oophorectomy (9.5%, n=16), breast cancer recurrence (6.0%, n=10), withdrew voluntarily (2.4%, n=4) and death (1.8%, n=3).

The associations of baseline characteristics with time to ovarian failure are depicted in Table 2. Cigarette smoking (6.5%, n=11) and alcohol use at least daily (4.1%, n=7) occurred infrequently in this population and were not related to ovarian failure. Older age (HR 1.15, 95% CI 1.05–1.26) and lower BMI (HR 0.92, 95% CI 0.85–0.99) were associated with shorter time to ovarian failure. Compared to no chemotherapy exposure, receiving cyclophosphamide-based chemotherapy was also associated with shorter time to ovarian failure (HR 3.31, 95% CI 1.17–9.40). Finally, lower baseline AMH (HR 0.67, 95% CI 0.45–0.98) and higher baseline FSH (HR 1.05, 95% CI 1.05–1.10) were related to shorter time to ovarian failure.

A summary of genotype frequencies is shown in Table 3. Overall, genotyping failure rates were low (<5%). Genotype frequencies were consistent with published allele frequencies for all genotypes. There were no statistically significant associations between any candidate genotype and time to ovarian failure in this cohort. In multivariable models adjusting for age, BMI and chemotherapy, there was also no statistically significant association between any candidate SNP and time to ovarian failure (all $p>0.40$). Restriction of analyses to white participants or to those who received chemotherapy also did not result in any significant associations with ovarian failure (data not shown).

To test the presence of potential dose-response relationships, we created a summary risk variable with a possible range of 0–13, with a point given for every risk allele for earlier time to menopause. The median score (range) was 7 (1–11). This summary risk variable was also not related to time to ovarian failure (HR 1.02, 95% CI 0.90–1.17).

Discussion

In a cohort of young breast cancer survivors, candidate SNPs associated with age at natural menopause were not related to timing of ovarian failure following breast cancer diagnosis. The lack of association did not change in sensitivity analyses restricted to those undergoing chemotherapy or by race. While exploratory, these analyses do not support using these candidate genetic variants to aid in predicting ovarian failure in premenopausal breast cancer patients.

The interest in these candidate genetic variants arose from the concept that ovarian function following breast cancer diagnosis is related to both innate ovarian reserve and exogenous exposures such as chemotherapy. Age and ovarian reserve markers at cancer diagnosis, both

measures of innate ovarian reserve, have been found to be related to long-term ovarian function in breast cancer survivors. As genetic variants associated with age at natural menopause may also be related to innate ovarian reserve, the study tested the hypothesis that these candidate variants would aid in predicting ovarian failure in breast cancer survivors.

The candidate genes participate in biological processes involved in ovarian aging, including DNA damage repair and replication (*EXO1*, *TLK1*, *HELQ*, *UIMC1*, *PRIM1* and *POLG*); vesicle transport (*BRSK1*); and meiotic germ cell division (*MCM8*). For example, *MCM8* encodes an oocyte protein involved in meiotic germ cell division, cell differentiation and stability.²¹ Moreover, several cohort studies have found associations of these genetic variants with other conditions of premature ovarian aging, including early menopause^{22,24} and Fragile X-associated primary ovarian insufficiency²⁵, as well as with age at menopause in Chinese, Iranian and in multi-ethnic cohorts.^{26–28} While these replicative studies are intriguing, the effect sizes of risk alleles are small, requiring larger sample sizes to detect difference by genotype. Hence, in the context of breast cancer treatment, we speculate that small effects may be dwarfed by exposure to ovarian toxic chemotherapy. Accordingly, we previously reported that SNPs in drug metabolizing enzymes involved in cyclophosphamide metabolism are significantly related to timing of ovarian failure after chemotherapy.²³ It is also possible that these SNPs do not impact ovarian aging in the breast cancer population, as the direction of effects were not clearly consistent with prior natural menopause studies. Should future studies to test the association between age at menopause SNPs and ovarian failure in breast cancer survivors be undertaken, this report provides data for sample size considerations.

Consistent with prior studies in breast cancer survivors, older age and cyclophosphamide-based chemotherapy were related to shorter time to ovarian failure in this study.²⁹ As well, ovarian reserve markers such as FSH and AMH measured prior to cancer treatment were significantly associated with the outcome. This was also in line with our findings among participants undergoing chemotherapy and in prior cohorts.^{8,30–33} These associations reinforce the concept that ovarian function following breast cancer diagnosis is correlated with innate ovarian reserve and exogenous exposures that are toxic to the finite oocyte pool. We also found a small but significant correlation between higher BMI and later time to ovarian failure. To our knowledge, this finding has not been reported in other cancer survivor cohorts, but the Study of Women's Health Across the Nation reported higher baseline weight to be associated with later age at the natural final menstrual period in a longitudinal study of 3,200 women in the U.S.^{34–36} As well, several smaller studies on menopause in non-cancer populations have reported this association. To date, the mechanism by which larger body size would result in later ovarian senescence remains unclear.

There are several strengths of this prospective cohort study. By prospectively collecting menstrual pattern outcomes, we limited recall bias. Recruitment of a relatively homogeneous population allowed us to examine the primary exposures of interest, menopause SNPs, given a small sample size. Careful collection of treatment exposures and outcomes allowed for accurate censoring and accounting for confounding. Sample size, censoring and lack of follow up of the entire cohort to age 51, the average age of menopause in the U.S., were significant limitations. Because the timing of ovarian recovery in breast cancer survivors

may be longer than 12 months, there is potential outcome misclassification, biasing our results toward the null.³⁷

Conclusion

In conclusion, in a prospective cohort study of menstrual pattern following breast cancer diagnosis in premenopausal women, we found no association between age at menopause SNPs and ovarian failure. Because ovarian function after breast cancer diagnosis is clinically important to survivors and their healthcare providers, there remains a need for future studies to identify predictors and refine prediction models of age at menopause in this population.

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Table 1

Effect of Minor Allele on Age of Natural Menopause.

Gene (SNP ID)	Minor Allele	Reported effect per minor allele (years) ^a	SE	P-value
<i>EXO1</i> (rs1635501)	C	-0.164	0.027	8.46×10^{-10}
<i>TLK1</i> (rs10183486)	T	-0.196	0.026	2.21×10^{-14}
<i>HELQ</i> (rs4693089)	G	0.228	0.025	2.38×10^{-19}
<i>UIMC1</i> (rs365132)	T	0.287	0.025	9.11×10^{-32}
<i>PRIM1</i> (rs2277339)	G	-0.380	0.042	2.47×10^{-19}
<i>POLG</i> (rs2307449)	G	-0.184	0.025	3.56×10^{-13}
<i>TMEM224</i> (rs4806660)	C	-0.406	0.03	8.88×10^{-16}
<i>BRSK1</i> (rs1172822)	T	-0.391	0.06	6.28×10^{-07}
<i>BRSK1</i> (rs2384687)	C	-0.381	0.059	1.39×10^{-10}
<i>BRSK1</i> (rs1551562)	G	-0.428	0.07	1.04×10^{-09}
<i>BRSK1</i> (rs897798)	G	-0.308	0.056	3.91×10^{-06}
<i>MCM8</i> (rs236114)	A	0.495	0.077	9.71×10^{-11}
<i>MCM8</i> (rs16991615)	A	0.948	0.052	1.42×10^{-73}

^aStolk, Lisette, et al. "Loci at chromosomes 13, 19 and 20 influence age at natural menopause." *Nature genetics* 41.6 (2009): 645–647. Stolk, Lisette, et al. "Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways." *Nature genetics* 44.3 (2012): 260–268. Murray, Anna, et al. "Common genetic variants are significant risk factors for early menopause: results from the Breakthrough Generations Study." *Human molecular genetics* 20.1 (2011): 186–192.

Table 2

Cohort characteristics and unadjusted hazard ratios (HR) for time to ovarian failure in 169 premenopausal breast cancer patients.

Characteristic	N (%) ^a	HR (95% CI)
Enrollment age, median (range)	40.5 (20.6–46.1)	1.15 (1.05–1.26)
Race		
White	117 (69)	Ref
African American	16 (9)	1.55 (0.61–4.07)
Other	35 (21)	1.23 (0.57–2.63)
Income		
< \$60,000	101 (62)	Ref
\$60,000	41 (25)	0.54 (0.22–1.30)
Education		
High school	22 (14)	Ref
College	91 (56)	0.69 (0.29–1.62)
Masters/doctorate	49 (30)	0.67 (0.26–1.73)
BMI, median (range)	23.9 (15.6–58.2)	0.92 (0.85–0.99)
0 (<24.9)	94 (56)	Ref
1 (<18.5)	5 (3)	2.61 (0.78–8.66)
2 (25–29.9)	43 (25)	0.57 (0.25–1.32)
3 (>30)	27 (16)	0.39 (0.12–1.28)
Number of menses in past year		
10–12	144 (89)	Ref
1–6	12 (7)	0.75 (0.18–3.12)
7–9	6 (4)	1.48 (0.35–6.19)
Median AMH (ng/ml) at enrollment, (range) [p25, 75]	0.68 (0.085–9.66) [0.21, 1.62]	0.67 (0.45–0.98)
Median FSH (IU/L) at enrollment, (range) [p25, 75]	5.29 (0.50–42.30) [3.27, 8.16]	1.05 (1.02–1.10)
Breast Cancer Type		
Ductal	152 (90)	Ref
Lobular	6 (4)	1.46 (0.35–6.11)
Mixed and other	9 (5)	1.53 (0.47–5.02)
Cancer stage		
0	14 (9)	Ref
1	46 (29)	1.99 (0.44–8.88)
2	73 (45)	1.59 (0.36–6.97)
3	28 (17)	3.21 (0.68–15.02)

Characteristic	N (%) ^a	HR (95% CI)
ER+ or PR+	120 (71)	0.88 (0.43–1.80)
Her2-neu +	41 (25)	1.03 (0.50–2.14)
Tamoxifen	92 (54s)	1.45 (0.75–2.83)
Radiation therapy	104 (65)	1.37 (0.69–2.72)
Chemotherapy		
None	40 (24)	Ref
Cyclophosphamide-based	116 (69)	3.31 (1.17–9.40)
Carboplatin-based	12 (7)	1.73 (0.32–9.43)

^aNot all variables sum to 169 participants due to missing data.

Table 3

Genotype frequencies and unadjusted hazard ratios (HR) for time to ovarian failure in 169 young breast cancer patients.

Gene (SNP ID)	Genotypes	Genotype frequencies n (%) ^a	Unadjusted HR (95% CI)
<i>EXO1</i> (rs1635501)	TT TC/CC	52 (31) 109 (64)	ref 1.32 (0.64–2.72)
<i>TLK1</i> (rs10183486)	CC CT/TT	55 (32.5) 113 (66.9)	ref 1.33 (0.66–2.68)
<i>HELQ</i> (rs4693089)	GG GA/AA	59 (34.9) 108 (63.9)	ref 0.68 (0.36–1.27)
<i>UIMC1</i> (rs365132)	TT TG/GG	33 (19.5) 135 (79.9)	ref 1.09 (0.48–2.48)
<i>PRIM1</i> (rs2277339)	TT TG/GG	130 (76.9) 39 (23.1)	ref 0.72 (0.32–1.64)
<i>POLG</i> (rs2307449)	TT TG/GG	64 (37.9) 105 (62.1)	ref 0.92 (0.48–1.76)
<i>TMEM224</i> (rs4806660)	TT TC/CC	76 (45.0) 93 (55.0)	ref 0.95 (0.51–1.79)
<i>BRSK1</i> (rs1172822)	CC CT/TT	80 (47.3) 89 (52.7)	ref 0.85 (0.45–1.59)
<i>BRSK1</i> (rs2384687)	AA AG/GG	70 (41.4) 98(58.0)	ref 0.83 (0.44–1.55)
<i>BRSK1</i> (rs1551562)	AA AG/GG	104 (61.5) 64 (37.9)	ref 1.15 (0.61–2.17)
<i>BRSK1</i> (rs897798)	AA AG/GG	63 (37.3) 106 (67.7)	ref 0.95 (0.50–1.81)
<i>MCM8</i> (rs236114)	CC CT/TT	117 (69.2) 52 (30.8)	ref 0.70 (0.34–1.45)
<i>MCM8</i> (rs16991615)	GG GA/AA	149 (88.2) 20 (11.8)	ref 0.81 (0.29–2.28)

^aNot all variables sum to 169 participants due to missing data.