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COMPARISON OF AGE AT NATURAL MENOPAUSE IN BRCA1/2 MUTATION CARRIERS TO A NON-CLINIC-BASED SAMPLE OF WOMEN IN NORTHERN CALIFORNIA

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Abstract

Objective—Germline mutations in *BRCA1/2* are related to increased lifetime risk of breast and ovarian cancer. While risk-reducing salpingo-oophorectomy reduces the risk for both cancers, loss of fertility is a major concern. A recent study suggested an association of *BRCA1* mutation with occult primary ovarian insufficiency. The aim of this study was to determine whether *BRCA1/2* mutation carriers have earlier onset of natural menopause than unaffected women.

Materials and Methods—Caucasian *BRCA1/2* carriers (n=382) were identified within the UCSF Breast Cancer Risk Program Registry and compared to non-clinic-based Caucasian women in Northern California (n=765). We compared the two groups regarding median age at natural menopause before and after adjustment for known risk factors, and examined the role of smoking within each group, using the Kaplan-Meier approach for unadjusted analyses and Cox proportional hazards regression analyses for adjusted analyses.

Results—The median age at natural menopause in *BRCA1/2* carriers was significantly earlier than the unaffected sample (50 vs 53years, p-value<0.001). The unadjusted hazard ratio for natural menopause comparing *BRCA1/2* carriers to unaffected women was 4.06(95% confidence interval 3.03-5.45), 3.98(2.87-5.53) after adjusting for smoking, parity, and oral contraceptive use. For

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BRCA1/2 carriers do undergo earlier natural menopause compared to the general population, and that current heavy smoking is associated with an even earlier age onset of menopause. This is the first study to explore the association between *BRCA1/2* and age at onset of menopause by comparing *BRCA1/2* carriers with women in the general population.

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BRCA1/2 carriers who were current heavy smokers (≥ 20 cigarettes/day), the median age at natural menopause was 46 vs. 49 years for non-smokers (p-value=0.027).

Conclusions—*BRCA1/2* mutation was associated with significantly earlier age at natural menopause, and heavy smoking compounded this risk. As the relationship between menopause and end of natural fertility is considered fixed, these findings suggest a risk of earlier infertility among *BRCA1/2* carriers.

Keywords

menopause; *BRCA1* gene; *BRCA2* gene; primary ovarian insufficiency; smoking; carcinogenesis

INTRODUCTION

In women, germ cells encounter one of several fates: they may remain quiescent, be recruited for further development and ovulation, or undergo apoptosis. Although somewhat controversial, over time and without regeneration, the population of oocytes (follicles) are depleted until <1000 remain, and menopause ensues [1] [2]. Natural menopause (the final menstrual period [FMP] followed by 12 months of amenorrhea [3]) occurs at a median age of 51.4 years with a distribution ranging between 40 and 60 years [4]. Before menopause, as the follicle count diminishes with age, the quality of oocytes also declines, resulting in infertility and increased miscarriage rates [5]. It has been proposed that the intervals between the decline in fertility, the age at last birth, and menopause are fixed at 10 years, with varying age for the onset of menopause [6].

Age at menopause is a complex outcome related to a number of factors [7] but is at least partially heritable, suggesting a genetic contribution. The heritability of menopausal age has been estimated to be between 30 and 85% [8] [9] [10], and a notable proportion (15–30%) of primary ovarian insufficiency cases is familial [11] [12] [13] [14]. Although multiple genes control some of the timing of natural menopause, very few genes have been identified that contain common genetic variants that are associated with age of menopause [15] [16].

Recent evidence has suggested that women with mutations in the DNA repair genes, *BRCA1* and *BRCA2* (*BRCA1/2*), have an increased risk of primary ovarian insufficiency [17]. The hypothesis asserts that because DNA repair mechanisms are impaired in patients with *BRCA1/2* mutations, oocytes are more prone to DNA damage and thereby experience accelerated follicular depletion [17]. If *BRCA1/2* carriers are more likely to experience occult ovarian insufficiency, this may lead to a higher incidence of infertility and early menopause. This circumstance may add to the significant psychosocial implications of being a *BRCA1/2* carrier and likely have an impact on reproductive decision-making. To test this hypothesis, we sought to determine if *BRCA1/2* carriers had an earlier age at menopause than non-carriers.

METHODS

Study population

The data for this study were collected from two populations: 1) The Cancer Risk Program (CRP) at the University of California, San Francisco (UCSF) and 2) the northern California (UC Davis/Kaiser) site of the Study of Women's Health Across the Nation (SWAN) [18]. Institutional review boards at all involved sites approved the study protocol, and all participants provided informed consent.

For all *BRCA1/2* carriers identified in the CRP registry, demographic, health behavior and clinical background information was obtained from a self-administered questionnaire completed at enrollment. This information included medical history, surgical history, detailed history and timing of cancer diagnosis and treatment, detailed menstrual history, parity, history of oral contraceptive (OCP) use, recent weight change, age and smoking history. It was then further cross-checked with the hospital charts and operations reports in electronic medical records to confirm its accuracy. Similar data were collected in SWAN, a multi-site, multi-race/ethnic study of health and menopause in midlife women. SWAN's first phase was a cross-sectional telephone survey of women aged 40-55 years, conducted at seven sites across the US.

Specifically, natural menopause was defined as at least 12 consecutive months of amenorrhea[3]. Similar questions were used in both CRP and SWAN cohorts to obtain menopause information. Women were asked if they had had a menstrual period in the past 12 months; if not, they were asked whether their periods stopped due to surgical intervention, medical treatment, pregnancy/breastfeeding, or severe weight loss or other reasons other than menopause. Women who answered no to all of these were asked what year their periods stopped. FMP age was calculated by subtracting the year of last menstruation from birth year. Analyses of the terminal digit for self-reported year in SWAN study had suggested self-reporting was accurate, as there was no digit preference [19].

Almost all *BRCA1/2* carriers within the CRP registry are Caucasian. Thus, to increase comparability of the *BRCA1/2* carriers and SWAN participants, we included only the Caucasians in the SWAN Northern California site to obtain an estimate of the age at the natural FMP for comparison. The present analyses included all Caucasian research participants with a positive *BRCA1/2* test between 1998 and 2006 in CRP program in UCSF. The participants included from the northern California SWAN sample were Caucasians aged 40-55 years who were selected at random from the northern California Kaiser Permanente membership in 1995 and interviewed for eligibility for the longitudinal SWAN cohort in 1996-7.

Statistical Analysis

Statistical analyses were performed with SAS version 9.2. Descriptive analyses were performed with independent t-tests to compare means for continuous data and with chi-square tests to compare frequencies for categorical data. Statistical significance was defined with $p\text{-value} < 0.05$.

To compare age at final menstrual period (FMP) in the two study samples (primary analysis), survival analyses were performed to allow for the inclusion of incomplete or censored observations. Age at FMP was censored for women who were still menstruating or whose FMP was not observable due to medical intervention, including exogenous hormone therapy, or to pregnancy/breastfeeding.

Unadjusted differences between *BRCA1/2* carriers and SWAN participants were estimated using Kaplan-Meier analyses with log-rank testing and Cox proportional hazards regression, and adjusted differences were estimated using Cox proportional hazards regression to control for potential confounding factors (parity, smoking, and oral contraceptive pill [OCP] use). These predictors were selected based on prior literature indicating their significant association with age at natural menopause [7]. We verified the validity of the proportional hazards assumption for each predictor by testing the significance of its interaction with age [20] and determining whether its inclusion improved model fit, as indicated by the AIC statistic [21].

Sensitivity analyses were run excluding *BRCA1/2* participants outside the 40-55 year age range of SWAN participants (172 *BRCA1/2* participants, 765 SWAN participants). Primary analyses included women whose FMP was not observable due to medical intervention (chemotherapy, radiation, and bilateral oophorectomy), consistent with previous studies [4]. Their inclusion reduces bias, as these women are not a random subsample of all participants, nor are they a small subset; moreover, they contribute information regarding age at FMP up to the age at which they are censored. To confirm that results were similar with and without these women, secondary analyses were run on 166 *BRCA1/2* carriers and 639 SWAN participants with a natural menopause or who were still menstruating, excluding patients who underwent hysterectomy or bilateral oophorectomy, or received chemotherapy or radiotherapy.

RESULTS

Within the UCSF CRP Registry, a total of 537 female *BRCA1/2* carriers were identified, and 382 (238 *BRCA1*, 144 *BRCA2*) were Caucasian. Among the SWAN participants, 765 were identified as living in northern California and Caucasian (figure 1). Comparison of health and behavior characteristics of *BRCA1/2* carriers to the northern California SWAN sample showed that *BRCA1/2* carriers had a higher rate of being nulliparous, a lower mean parity, a lower rate of OCP use, a higher rate of never smokers, but a lower rate of current smokers (table 1).

Overall, the median age at menopause for the *BRCA1/2* carriers in Northern California was 50 years, which was significantly earlier than the Caucasian population in Northern California at 53 years ($p < 0.001$) (Figure 2), while the median age at natural menopause for the entire SWAN sample was 51.4 years [4], consistent with prior studies [7].

Table 2 presents hazard ratios from the multivariate Cox proportional hazards model of the primary analysis, sensitivity analysis, and secondary analysis. Adjustment for parity, smoking, and OCP use had little impact on the difference in age at menopause between the *BRCA1/2* carriers and the northern California SWAN sample. In the subset of Caucasian women with complete covariate information ($n = 228$ in *BRCA* carriers, $n = 759$ in northern California SWAN), the unadjusted hazard ratio for time to menopause from Cox proportional hazards modeling was 4.06 (95% confidence interval 3.03 – 5.45, $p < 0.001$), and the corresponding adjusted hazard ratio was 3.98 (95% confidence interval 2.87 – 5.53, $p < 0.001$), both indicating a significantly earlier menopause in *BRCA1/2* carriers than in northern California Caucasians in SWAN. When restricting the age range for the *BRCA1/2* carriers to 40-55 years to be the same as the SWAN participants, the results were similar and remained statistically significant. The median age at menopause was 50 in the *BRCA1/2* carriers vs. 53 years in the SWAN participants ($p = 0.004$). In the subset aged 40-55 years with complete covariate information ($n = 103$ in *BRCA1/2* carriers, $n = 759$ in northern California SWAN), the unadjusted hazard ratio was 2.69 (95% confidence interval 1.57 – 4.62, $p = 0.0003$), and the corresponding adjusted hazard ratio was 2.82 (95% confidence interval 1.56 – 5.09, $p = 0.0006$).

Our secondary analysis excluded those who underwent surgical menopause or chemotherapy- or radiotherapy-related menopause. In this subset, the health and behavior characteristics of *BRCA1/2* carriers and the northern California Caucasian SWAN sample were similar to the primary analysis (Table 2). The median age at natural menopause among the *BRCA1/2* carriers ($n = 166$) remained significantly earlier than the SWAN sample ($n = 639$), specifically 49 vs. 53 years ($p < 0.001$) (Figure 3). In the subset with complete covariate information ($n = 146$ *BRCA1/2*, $n = 634$ SWAN), the unadjusted hazard ratio was 4.70 (95% confidence interval 3.50 – 6.30; $p < 0.001$). After adjusting for parity, smoking and

OCP use, the difference remained statistically significant; *BRCA1/2* carriers had a significantly earlier age onset of menopause with a hazard ratio of 4.33 (95% confidence interval 3.13 – 6.01; $p < 0.001$).

Interestingly, among *BRCA1/2* carriers, the Kaplan-Meier survival curves were very similar across the range of possible FMP ages, and no significant difference was observed in the median age at natural menopause between the 238 *BRCA1* carriers and the 144 *BRCA2* carriers in the primary analysis (50 vs. 49 years, $p = 0.231$) (Figure 4) or in the secondary analysis, excluding those who underwent surgical menopause or chemotherapy- or radiotherapy-related menopause ($N = 94$ vs. 72, 48 vs 49 years, $p = 0.759$).

BRCA1/2 carriers who were current smokers ($n = 25$) underwent earlier natural menopause than current smokers in SWAN ($n = 130$) (median age at natural menopause 48 vs. 51 years, $p = 0.0006$). Among *BRCA1/2* carriers, the median age at natural menopause was 49 years for both never- and former smokers (hazard ratio = 1.35, 95% CI 0.72 – 2.55, $p = 0.350$). Thus, subsequent analyses combined both subgroups as current non-smokers. Current heavy smokers (> 20 cigarettes/day) underwent even earlier menopause than non-smokers or current light or moderate smokers among *BRCA1/2* carriers (<10, 10-19 cigarettes/day) (median age was 46 years for heavy smokers, versus 49 years for non-smokers, 48.5 years for light smokers, and 50 years for moderate smokers; $p = 0.027$) (Figure 5). The patterns of smoking-related differences among northern California Caucasian SWAN participants were consistent. In SWAN samples, former and never smokers did not differ (hazard ratio = 1.08, 95% CI 0.69 – 1.68, $p = 0.733$), and the median age at natural menopause for never/past smokers ($n = 631$) was 54 years, vs. 52 years for current light smokers ($n = 31$), 49 years for current moderate smokers ($n = 46$), and 51 years for current heavy smokers ($n = 51$) (log-rank p -value = 0.0005).

Discussion

The novel results of this study showed that being a Caucasian *BRCA1/2* mutation carrier was associated with undergoing natural menopause a median of 3-4 years earlier than a community sample of midlife Caucasian women. In addition, when adjusted for variables known to affect age at menopause, *BRCA1/2* carriers had a significant four-fold increased hazard ratio, indicating a significantly earlier menopause than the community sample even after adjustment for such variables. Moreover, those carriers who were current heavy smokers underwent an even earlier age at menopause (median 46 years), an age at which only 7% of northern California Caucasian women had undergone natural menopause.

Data on the age at natural menopause among *BRCA1/2* carriers are limited as most women are recommended to undergo risk-reducing salpingo-oophorectomy on completion of childbearing, before menopause. One study reported *BRCA1* breast cancer patients to be at risk of premature ovarian failure compared to other breast cancer patients [22]. Although it is a study designed to investigate the association of *BRCA1* mutation and estrogenization of ovarian tissues with limited control groups, its menopause findings were consistent with our observation. Another study reported the median age at natural menopause among *BRCA1/2* carriers to be 49.1~49.8 years [23]. However, this study was designed to evaluate the associations between menopausal hormone therapy and the risk of ovarian cancer in *BRCA1/2* mutation carriers. Although no comparisons were made regarding the age at natural menopause in *BRCA1/2* carriers to the general population, the age at natural menopause for *BRCA1/2* carriers was consistent with our observations.

Our finding that *BRCA1/2* carriers who were current heavy smokers had an even earlier onset of menopause is consistent with the literature on the relation of smoking to earlier age

at menopause [24]. Cigarette smoking has also been demonstrated to alter menstrual cycles, estrogen status, spontaneous pregnancy rate, as well as assisted reproduction outcomes [25, 26] [27] [28] [29] [30]. The mechanism is thought to be due to, in part, an accumulation of toxic metabolites [31] [32], such as cadmium, nicotine, cotinine, and benzo[a]pyrene within the follicular fluid and ovarian stroma, which can have detrimental effects on ovarian function, potentially via oxidative damage [33, 34]. Some of these effects would seem to be reversible, as the literature and the present data largely indicates that women who stop smoking have a similar age at menopause to non-smokers [35]. This conclusion is not surprising given many other clinical observations, in addition to age onset of menopause, suggest smoking cessation resolves the additional risks present in current smokers (i.e. stroke) [36] [37].

The implications of *BRCA1/2* carriers having an earlier age at menopause may be a narrower reproductive window and possibly an increased risk of infertility. However, few studies have reported on fertility parameters for *BRCA1/2* carriers. Our results show that parity was different among carriers and non-carriers, which is in contrast to the previous published studies [38] [39]. However, parity in itself is an inadequate method for determining if the reproductive capacity is decreased, as it does not identify how many tried to conceive but were unable and does not take into account female age at the time of attempted conception. Interestingly, a recent study showed *BRCA1* carriers have poor ovarian stimulation responses, implying lower ovarian reserve for a given age than the general population [17]. These findings are of interest, given that lower ovarian reserve has been associated with infertility and earlier onset of menopause [40] [41]. Future studies are needed to clarify if fertility is compromised among *BRCA1/2* carriers.

Mechanistically, because DNA repair is deficient in patients with *BRCA1/2* mutations, oocytes may be more prone to DNA damage [42]. Prior basic research in non-reproductive cell types demonstrated that when DNA damage is severe and cannot be repaired, apoptotic pathways are activated, though there is no current evidence that this is in fact the case for the oocytes of women with *BRCA1/2*-mutations [43] [44] [45] [46]. It is possible based on the foregoing that oocytes with deficient *BRCA1/2* function may be prematurely eliminated by a similar mechanism, resulting in early depletion of egg reserve [17].

Nevertheless, *BRCA1/2* mutations are associated with a marked depletion of germ cells in adult female mice [39]. Mutations in *BRCA1/2* also have phenotypic presentations that range from female-to-male sex reversal at gametogenesis stage in zebrafish to impaired spermatogenesis in male mice [47] [48] [49]. In the extreme, in specific double mutants models, sterile males or females were observed, followed by neoplastic proliferation and elevated risk of testicular and ovarian tumors [47]. These observations, as well as the results of the present study, support the idea that the *BRCA1/2* genes may be important in reproductive function [17]. However, further basic research will be needed to understand the molecular mechanisms that lead to lower germ population both in animal models and women. This includes why in haplo-insufficient women oocyte yield in response to ovarian stimulation is low and menopause tends to occur earlier as reported here. Only studies that complement animal models with large prospective clinical studies will be able to answer these questions.

While this study has important practical implications it also has several limitations. First, the design was cross-sectional so that we cannot be positively certain of some temporal relations. Potential recall bias and not accounting for all possible confounders may have affected the effect estimates. However, women tend to have good recall about their age at natural FMP [50]. Additionally, while we may not have accounted for all possible confounders, we controlled for the factors that are known to be strongly associated with age

at natural FMP, including smoking and OCP use [7]. Further, we limited the analyses to Caucasians who lived within the same geographical area, which likely reduced some of the variability in estimating age at the natural FMP, but also have limited generalizability to other racial/ethnic groups. Another limitation of the present study was that the comparison SWAN sample of women was not tested for *BRCA1/2* and was not recruited in a similar fashion to the *BRCA1/2* carriers. The carrier rate in general population is 1-2%, so that we may have misclassified a very small proportion of the comparison SWAN sample in considering them to be non *BRCA1/2* carriers. However, this misclassification would potentially tend to lead to an earlier observed menopause in the controls and thus reduce the effect estimate of *BRCA1/2* carrier status on age at natural FMP such that our difference is a conservative estimate.

In conclusion, our study showed that both *BRCA1* and *BRCA2* carriers had an earlier age at natural menopause than a similar community-based sample of Caucasian women. Those carriers who were current heavy smokers had an even earlier menopause. These findings suggest that *BRCA1/2* carriers may have a narrower reproductive window than non-carriers. Therefore, in addition to discussing risk-reducing salpingo-oophorectomy, we would encourage the early initiation of fertility counseling for *BRCA1/2* carriers and consideration of earlier childbearing.

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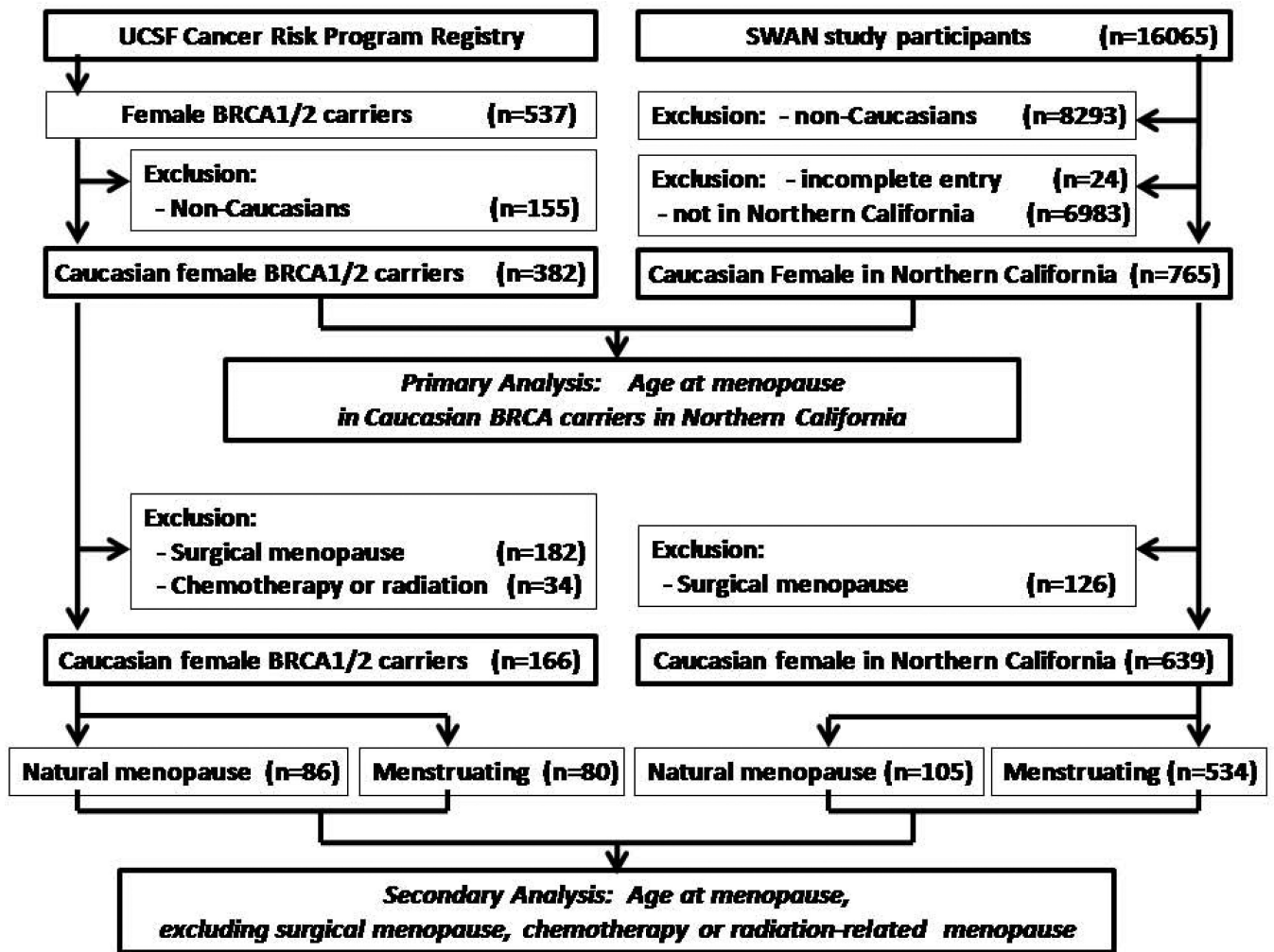


Figure 1. Study flow- Inclusion and exclusion criteria of the primary analysis and secondary analysis of *BRCA1/2* carriers from UCSF cancer risk program registry and SWAN study participants.

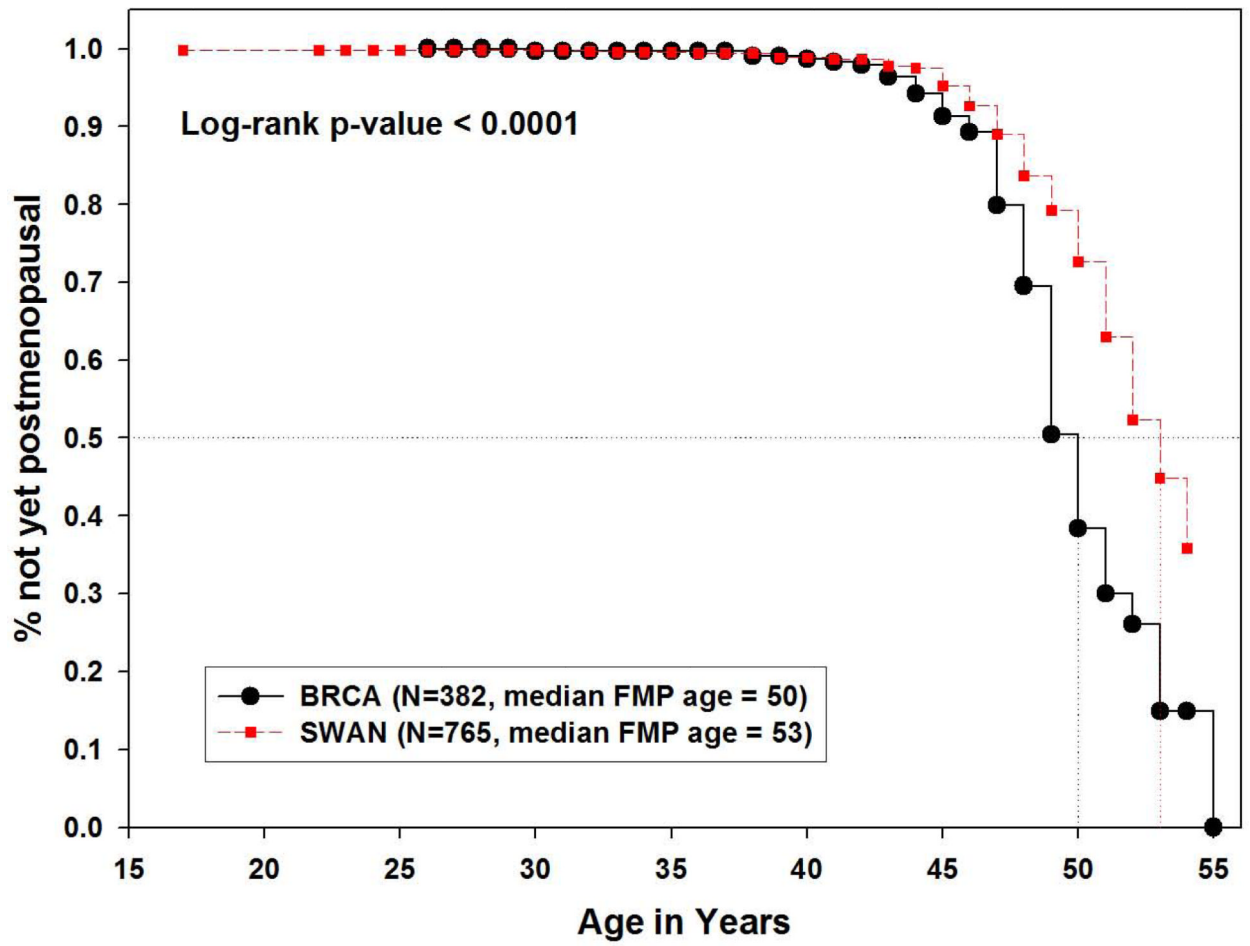


Figure 2. Kaplan-Meier estimates of age at natural menopause in Northern California Caucasians, comparing *BRCA1/2* carriers to SWAN cross-sectional screened sample. (Primary Analysis)

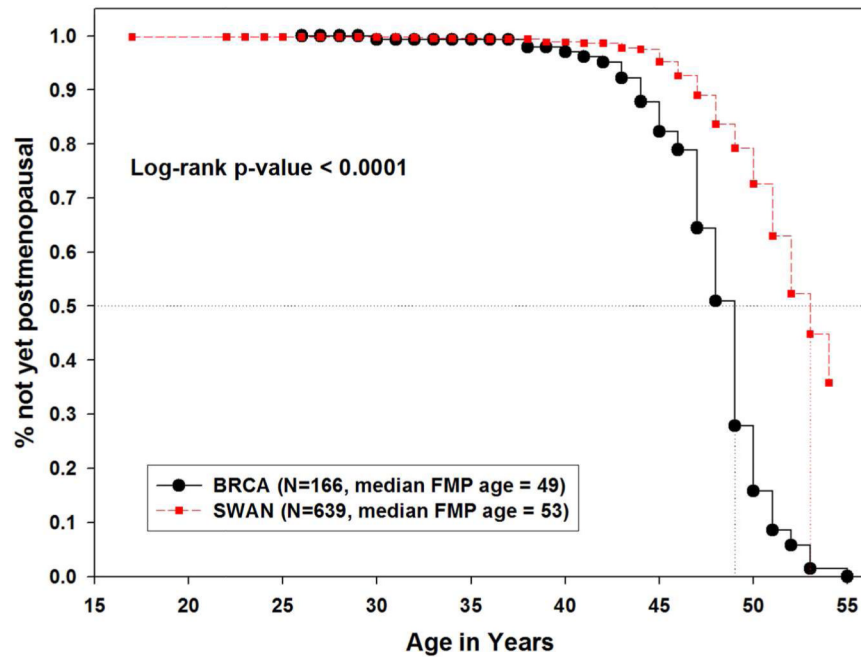


Figure 3. Kaplan-Meier estimates of age at natural menopause in Northern California Caucasians, comparing *BRCA1/2* carriers to SWAN cross-sectional screened sample, excluding medical and surgical menopause. (Secondary Analysis)

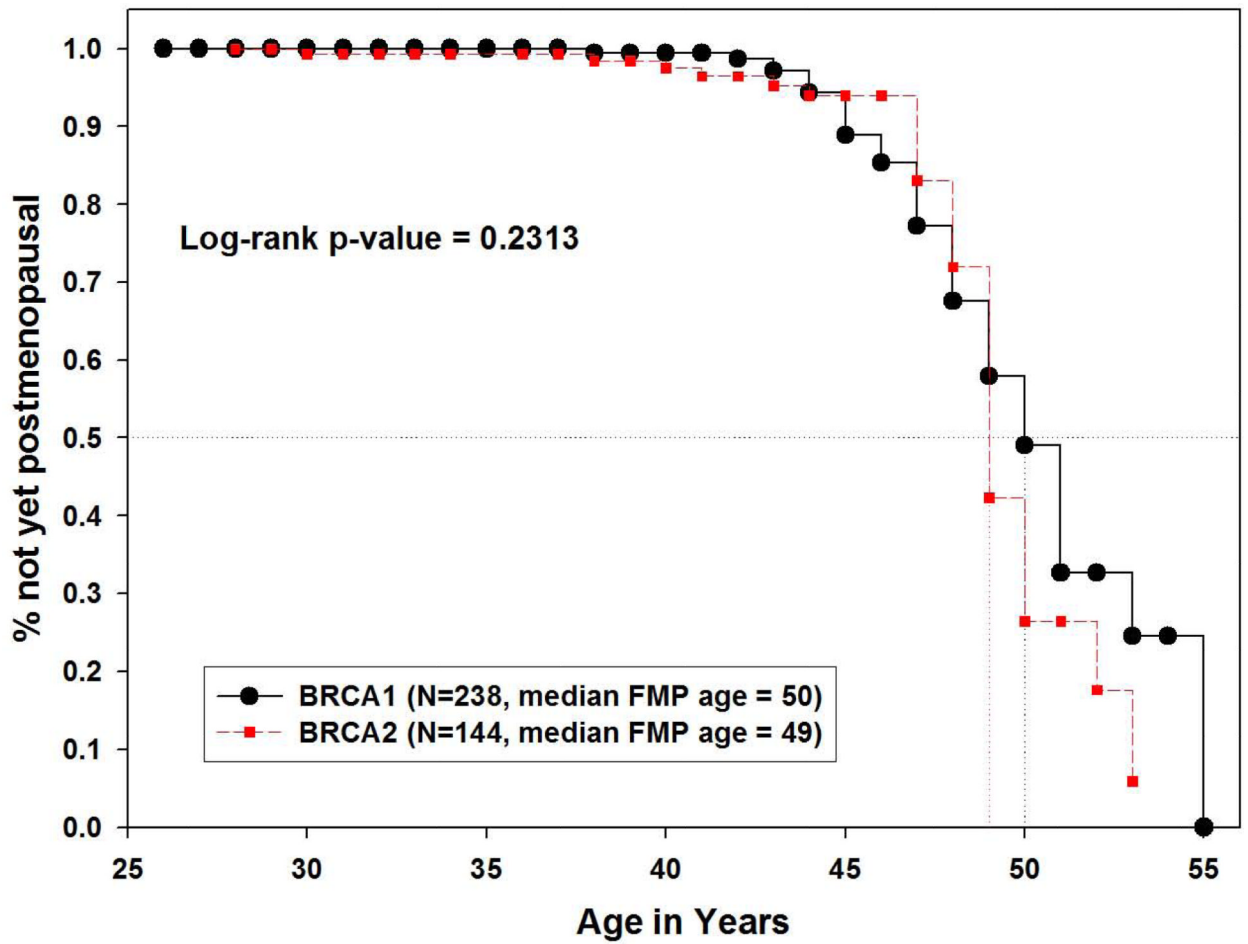


Figure 4. Kaplan-Meier estimates of age at natural menopause in Northern California Caucasians, comparing *BRCA1* to *BRCA2* carrier.

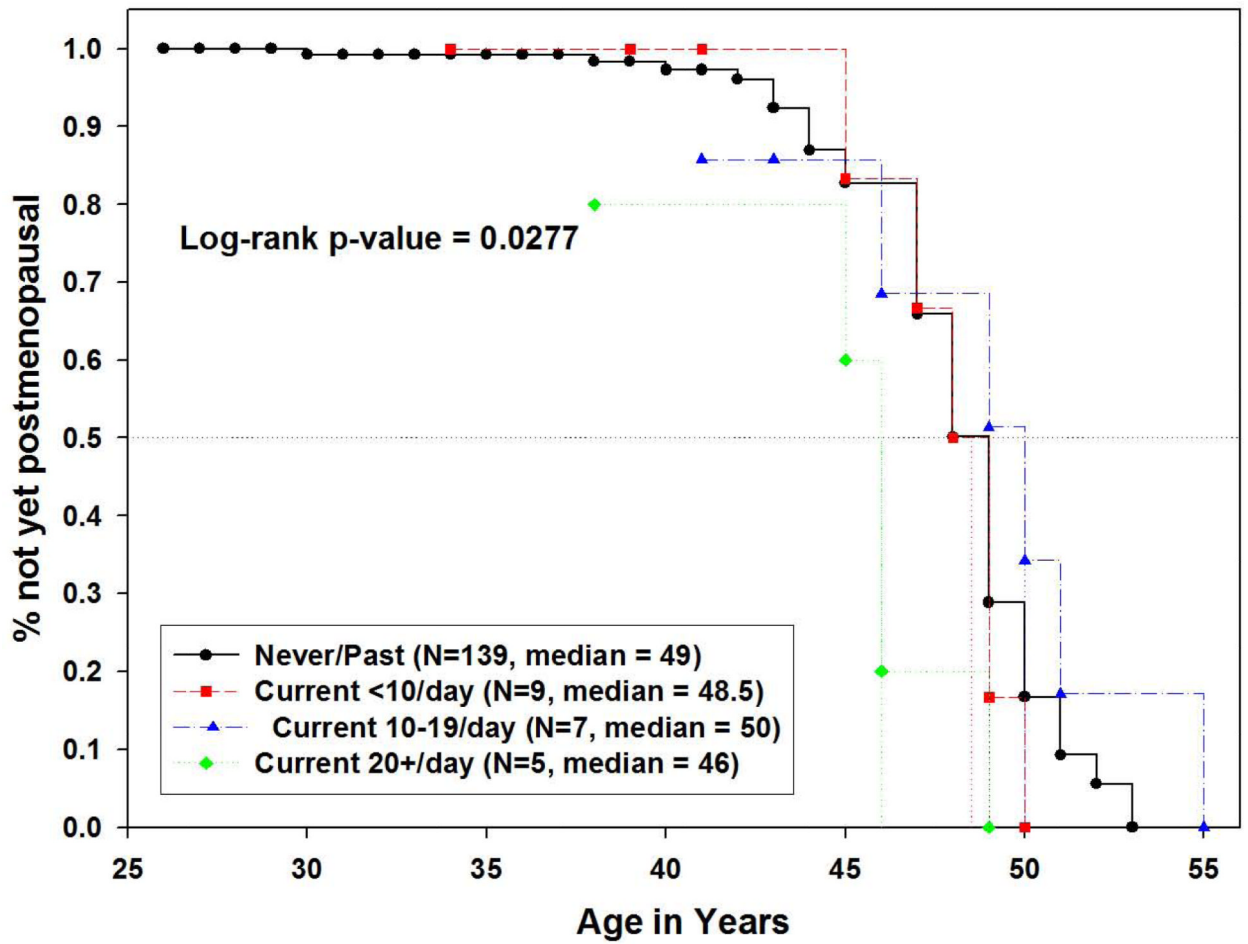


Figure 5. Kaplan-Meier estimates of age at natural menopause in Northern California Caucasian *BRCA1/2* carriers, comparing never/past smokers, current light, moderate, and heavy smokers.

Table 1

Characteristics of general population & BRCA carriers

Characteristic	% or Mean (SD)		p-value for difference
	SWAN* (N=765)	BRCA carriers** (N=382)	
Parity:			
0	25.5	39.4	<0.0001
1 +	74.5	60.6	
# children	1.5 (1.2)	1.3 (1.2)	0.0017
History of OCP use	88.2	48.0	<0.0001
History of smoking:			<0.0001
Never	49.3	83.7	
Past	33.6	6.4	
Current	17.1	9.9	

* The samples of general population were Caucasians extracted from the Study of Women's Health Across the Nation (SWAN) populations in Northern California.

** The BRCA carriers were obtained through The Cancer Risk Program (CRP) at the University of California, San Francisco (UCSF)

Table 2
Results of Cox proportional hazards modeling for age at final menstrual period

	Primary Analyses			Sensitivity Analyses (Ages 40 – 55 Only)			Secondary Analyses (Omit surgery, chemotherapy, radiotherapy)		
	HR (95% CI)	p-value	N	HR (95% CI)	p-value	N	HR (95% CI)	p-value	N
Model-BRCA vs. SWAN									
<i>Unadjusted (all)</i>			1147			937			805
BRCA	2.32 (1.74, 3.10)	<0.0001*		2.21 (1.30, 3.76)	0.0034*		4.38 (3.28, 5.85)	<0.0001*	
SWAN	Reference			Reference			Reference		
<i>Unadjusted (all with complete data)</i>			987			862			780
BRCA	4.06 (3.03, 5.45)	<0.0001*		2.69 (1.57, 4.62)	0.0003*		4.70 (3.50, 6.30)	<0.0001*	
SWAN	Reference			Reference			Reference		
<i>Adjusted:^(c)</i>			987			862			780
BRCA	3.98 (2.87, 5.53)	<0.0001*		2.82 (1.56, 5.09)	0.0006*		4.33 (3.13, 6.01)	<0.0001*	
SWAN	Reference			Reference			Reference		
Parity:		0.4958			0.8565			0.4746	
0	Reference			Reference			Reference		
1	1.25 (0.83, 1.89)			1.03 (0.59, 1.79)			0.95 (0.62, 1.47)		
2	1.21 (0.76, 1.93)			0.89 (0.56, 1.43)			0.80 (0.55, 1.17)		
3+	0.97 (0.65, 1.47)			1.11 (0.66, 1.85)			0.75 (0.49, 1.14)		
History of OCP use:		0.0659			0.0639			0.0631	
No	Reference			Reference			Reference		
Yes	0.72 (0.51, 1.02)			0.65 (0.41, 1.03)			0.72 (0.51, 1.02)		
History of smoking:		0.0013*			0.0275*			0.0085*	
Never	Reference			Reference			Reference		
Past	1.27 (0.88, 1.85)			1.14 (0.74, 1.76)			1.12 (0.77, 1.62)		
Current	2.01 (1.38, 2.93)			1.94 (1.19, 3.16)			1.79 (1.23, 2.61)		

* statistical significance; (a) HR=hazard ratio, CI=confidence interval; (b) Multivariate model including as predictors for all variables in the table