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BRCA1 Germline Mutations May Be Associated With Reduced Ovarian Reserve

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Abstract

Objective—To determine whether *BRCA* carriers have a decreased ovarian reserve compared to women without *BRCA* mutations, as *BRCA* mutations may lead to accelerated oocyte apoptosis due to accumulation of damaged DNA.

Design—Cross-sectional study

Setting—Academic tertiary care center

Patients—143 women, ages 18–45, who underwent clinical genetic testing for *BRCA* deleterious mutations due to a family history of cancer, were included. The cohort was classified into three groups: *BRCA1* carriers, *BRCA2* carriers, and women without *BRCA* mutations (controls). None had a personal history of breast or ovarian cancer.

Intervention—none

Main Outcome Measures—The main outcome was serum anti-Mullerian hormone (AMH) level. Linear and logistic regression models adjusting for age and body mass index (BMI) were performed to determine the association between *BRCA* mutations and AMH.

Results—*BRCA1* mutation carriers had a significant decrease in AMH levels compared to controls after adjusting for age and BMI (0.53 ng/mL 95% CI 0.33–0.77 vs. 1.05 ng/mL 95% CI 0.76–1.40). Logistic regression confirmed that *BRCA1* carriers had a 4-fold higher odds of having

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AMH <1 ng/mL compared to controls (OR=4.22, 95% CI 1.48–12.0). There was no difference in AMH levels between *BRCA2* carriers and controls.

Conclusions—*BRCA1* carriers have lower age- and BMI-adjusted serum AMH levels compared to women without *BRCA* mutations. Our results contribute to the current body of literature regarding *BRCA* carriers and their reproductive outcomes. Larger prospective studies with clinical outcomes such as infertility and age at menopause in this population are needed to further substantiate our findings.

Keywords

anti-Mullerian hormone; *BRCA* mutations; ovarian reserve

Introduction

BRCA1 and *BRCA2* germline mutations are associated with an inherited predisposition to breast and ovarian cancers (1, 2). Women with *BRCA* germline mutations face significant reproductive pressure given the guidelines for prophylactic surgery in the form of risk-reducing bilateral salpingo-oophorectomy (3).

In addition, recent literature also suggests that *BRCA* mutations adversely affect ovarian function by decreasing ovarian reserve, which is defined as oocyte quantity, oocyte quality, and reproductive potential (4). Since *BRCA* genes play critical roles in the repair of double-stranded DNA breaks (5, 6), it is biologically plausible that germline mutations in these genes lead to accelerated oocyte apoptosis and depletion. In a small case-control study of women with breast cancer undergoing fertility preservation, *BRCA1* carriers produced fewer oocytes than control women after ovarian stimulation (7). Three epidemiological studies concluded an earlier age of natural menopause in *BRCA* carriers, which supports the concept of a decreased follicular pool (8–10). However, studies have demonstrated conflicting results regarding markers of ovarian reserve in *BRCA* carriers compared to women without *BRCA* mutations (11, 12).

In recent years, serum anti-Mullerian hormone (AMH), a member of the transforming growth factor (TGF)- β family, has come to the forefront as a validated biomarker of ovarian reserve. AMH decreases with age (13), predicts age of menopause (14, 15), and correlates with the number of oocytes retrieved following ovarian stimulation for fertility treatment (16, 17), all of which speak to the validity of AMH as a biomarker for ovarian reserve. To test the hypothesis that *BRCA* carriers have a decreased ovarian reserve compared to women without *BRCA* mutations, we measured serum AMH in a cohort of 143 reproductive-aged women who underwent *BRCA* testing for family history of cancer and compared AMH levels in those with and without deleterious *BRCA* mutations. These results may provide additional information when counseling *BRCA* carriers regarding fertility and reproductive health.

Methods

This was an IRB-approved cross-sectional study of participants in the Gilda Radner Hereditary Cancer Program at Cedars Sinai Medical Center (18). Stored serum samples from women, who previously underwent genetic counseling and testing for *BRCA* mutations due to family histories of cancer, were obtained from the Women's Cancer Program biorepository. Serum samples were collected between 1991 and 2008 and stored at -80°C . All participants were between 18–45 years, had intact ovaries at the time of sample collection, and consented to biobanking and future studies. Participants were classified into three groups: *BRCA1* carriers, *BRCA2* carriers, and controls, defined as women who tested negative for both *BRCA1* and *BRCA2* mutations. Given the low expected frequency in our cohort ($<1\%$), participants who were positive for both *BRCA1* and *BRCA2* mutations were excluded. Participants who had a medical history of breast or ovarian cancer prior to sample collection were excluded. Clinical data abstraction was performed retrospectively to obtain additional information pertinent to the assessment of ovarian reserve, including sociodemographic information, reproductive history, oral contraceptive use, tobacco history, and body mass index (BMI) (19, 20).

The AMH Gen II ELISA assays (Beckman, Coulter), with an intra-and inter-assay coefficient of variation of 5.4 and 5.6% respectively, were performed using standard methods in the Cedars-Sinai Molecular Phenotyping and Biochemistry Laboratory (21, 22). Samples were stored on average 13.8 years prior to processing. All but one of the serum aliquots used for this study were thawed for the first time since storage. Upon reviewing the AMH assay results, two *BRCA1* mutation carriers were excluded as their AMH results were noted to be extreme outliers, 9.06 ng/mL and 8.49 ng/mL, suggestive of alternate etiologies of ovarian dysfunction such as polycystic ovary syndrome (23, 24).

Descriptive statistics, including analysis of variance (for parametric data), Chi-squared test (for count data), or the Fisher's exact test (for sparse count data) were used to compare data across the three groups: *BRCA1* carriers, *BRCA2* carriers, and controls. Serum AMH was transformed by a square root function to achieve a normal distribution prior to linear regression analysis after failing the Kolmogorov-Smirnov test and based on the results of the Box-Cox procedure (25). Inter-correlations between variables were investigated by Pearson's correlation coefficient. Linear regression analyses were used to test for differences in AMH levels as a continuous measure across groups with post-hoc pairwise multiple group comparisons adjusted via the Tukey-Kramer test. Logistic regression analyses were performed to determine the association between *BRCA* status and serum AMH as a dichotomized variable, $<1\text{ ng/mL}$ or $\geq 1\text{ ng/mL}$. Although the literature suggests multiple thresholds of AMH in predicting ovarian response and pregnancy outcomes for *in vitro* fertilization cycles, a cut-off of 1 ng/mL or 0.5 ng/mL is a frequent choice (16, 26–28). We chose the cut-off of 1 ng/mL *a priori* based on the literature and the fact that it is the median AMH level in this study. Regression analyses were adjusted for significant clinical covariates (age and BMI) and regression diagnostics were used to confirm the overall fit of final modeling. There were no overly influential outliers to skew the results. For all analyses, the level of statistical significance was set at $P < 0.05$.

Results

Between 1991–2008, 3623 women consented to the Gilda Radner Hereditary Cancer Program and the Women's Cancer Program biorepository. Seven hundred eighty two women underwent *BRCA* testing. Women were sequentially excluded based on eligibility criteria – 421 women were excluded due to a prior history of breast or ovarian cancer, 122 women were then excluded for age >45 years. Samples were identified from 205 eligible women; however our analysis was restricted to the 143 cases with nearly complete clinical data. Overall, we found no difference in the AMH levels between cases included and excluded (AMH 1.13 ± 1.05 vs. 1.24 ± 1.18 ng/mL, $P=0.496$).

Of the 143 women included in this analysis, 62 were *BRCA1* carriers, 27 were *BRCA2* carriers, and 54 were controls. *BRCA1* and *BRCA2* carriers (35.5 ± 5.2 years and 35.6 ± 6.2 years, respectively) were significantly younger than controls (39.3 ± 3.7 years) (Table 1). Over 98% of the cohort self-identified as Caucasian, with 70% reporting Ashkenazi Jewish background. This was reflected in the *BRCA* mutations detected, with the three known founder mutations (185delAG, 5385insC, 6174delT) being the most prevalent (Table 2). The three groups were similar in terms of BMI, gravidity, current oral contraception use, and smoking history based on available data. Although 41% (59/143) were missing data on oral contraceptive use and 26% (37/143) were missing data on smoking, both variables were represented in Table 1 because of its known effects on serum AMH and ovarian reserve (20, 29, 30). The mean serum AMH levels were not significantly different among *BRCA1* carriers (1.07 ± 1.02 ng/mL), *BRCA2* carriers (1.33 ± 1.11 ng/mL), and controls (1.11 ± 1.05 ng/mL) ($P=0.32$).

AMH was found to have a significant negative correlation with age ($r=-0.38$, $P<0.001$) and BMI ($r=-0.19$, $P=0.033$). AMH was not correlated with the length of time in which samples were stored ($r=-0.07$, $P=0.421$). There were no differences in AMH levels by use of oral contraceptives ($P=0.333$), gravida ($P=0.145$), or smoking status ($P=0.272$), which may have been due to our sample size.

In the final linear regression modeling of AMH levels, after adjusting for age ($P<0.001$) and BMI ($P=0.011$) (Table 3), AMH levels were found to be significantly different by *BRCA* status ($P=0.034$). There were significant differences between *BRCA1* carriers and controls ($P=0.026$), but not between *BRCA2* carriers and controls ($P=0.470$) nor between *BRCA1* and *BRCA2* carriers ($P=0.634$) (Table 4). *BRCA1* carriers had a 0.52 ng/mL decrease in AMH levels compared to controls after adjusting for age and BMI (Table 4). Analyses stratified by age groups results in a loss of statistical significance due to multiple pairwise comparisons within a modest sample size; however, the trend was in the same direction with *BRCA1* carriers having lower serum AMH levels compared to controls across all age groups (data not shown). Logistic regression analysis (Table 3) confirmed that *BRCA1* carriers had a 4-fold higher odds of having AMH <1 ng/mL compared to controls (OR=4.22, 95% CI 1.48–12.0, $P=0.012$) after adjusting for age and BMI, but not for *BRCA2* carriers compared to controls (OR=1.38, 95% CI 0.39–4.80, $P=0.499$).

Discussion

In this cohort of healthy reproductive-age women, *BRCA1* carriers had a 0.52 ng/mL decrease in age- and BMI- adjusted serum AMH levels compared to women without *BRCA* mutations. *BRCA1* carriers were noted to have a 4-fold increased odds of having AMH levels <1ng/mL. It is important to note that although the unadjusted AMH levels were comparable across all 3 groups (Table 1), the estimated mean AMH levels from the regression model (Table 4) demonstrated a significant decrease in age- and BMI- adjusted AMH levels in *BRCA1* carriers. This was due to the fact that age is negatively correlated with AMH, and the controls were on average 4 years older than the *BRCA* carriers. Our data suggest that *BRCA1* carriers have a lower ovarian reserve, and thus, contribute to the current body of literature on *BRCA* carriers and their reproductive outcomes. However, larger prospective studies with clinical outcomes such as infertility and age at menopause are needed to further substantiate our findings.

Because *BRCA* genes play critical roles in the repair of double-stranded DNA breaks (5, 6), germline mutations in these genes may lead to accelerated oocyte apoptosis and depletion. The mechanism of the decline in ovarian reserve in *BRCA1* carriers was elucidated in an important recent study by *Titus et. al.* In this landmark paper, the authors demonstrated using RNA interference in mouse oocytes that the inhibition of *BRCA1* increased DNA double stranded breaks and reduced survival (11, 29). *Titus et. al.* also determined that the primordial follicle pool was reduced in *BRCA1* heterozygous mutant mice, but not in *BRCA2* heterozygous mutant mice, which supports our findings.

Based on our findings, we were likely underpowered to detect a significant difference in serum AMH levels in the *BRCA2* carrier group. However, it is intriguing to consider the possibility that *BRCA1* and *BRCA2* have a differential effect on ovarian reserve, as the epidemiology of ovarian cancer differs between the two mutations as well. *BRCA1* mutations carry a higher lifetime risk of epithelial ovarian cancer compared to *BRCA2* mutations (40–65% vs. 20%), and *BRCA1*-associated ovarian cancers occur over a decade earlier than *BRCA2*-associated ovarian cancers (2, 30). This difference in age penetrance is mirrored by *in vitro* studies where significant age-related decline in *BRCA1* gene expression was observed in human oocytes from reproductive-age women; however, this was not noted for *BRCA2* gene expression suggesting that the decline may occur in later years (11).

Furthermore, *BRCA1* and *BRCA2* have distinct molecular functions in the DNA repair pathway. As a multifunctional protein, *BRCA1* interacts with other DNA repair proteins to detect damaged DNA, participates in homologous recombination-mediated repair of double-strand breaks, and activates checkpoints that delay the cell cycle to ensure that genetic errors are not transmitted to the next generation (31, 32). On the other hand, *BRCA2* plays a more limited role in homologous recombination repair by recruiting recombinase RAD51 to double strand breaks (32, 33). Taken together, this evidence supports a more critical role of *BRCA1* in preventing oocyte depletion in reproductive-age women.

Our results of lower AMH levels in *BRCA1* carriers may be suggestive of a shorter reproductive lifespan in these women, as AMH has been shown to be predictive of age of

menopause (14, 15). This is a difficult question to investigate as *BRCA* carriers often opt to undergo risk-reducing bilateral salpingo-oophorectomies resulting in premature surgical menopause. Based on limited follow-up, 45 *BRCA1* carriers and 17 *BRCA2* carriers in this cohort subsequently underwent prophylactic bilateral salpingo-oophorectomies. Results from epidemiologic studies on *BRCA* carriers and age of natural menopause have been mixed. One small study suggested that affected and unaffected *BRCA1* mutation carriers had an comparable self-reported age of menopause, but that this was earlier than the age of menopause in women without *BRCA* mutations (10). Lin et. al. compared over 382 *BRCA* carriers with 765 women in a population-based cohort, and found that *BRCA* carriers underwent natural menopause three years earlier than the control group; however, this study did not differentiate between *BRCA1* and *BRCA2* carriers (8). Another large case-control study concluded that although *BRCA1* and *BRCA2* carriers experienced menopause 1–2 years earlier than controls, there was no clinical difference in parity and infertility (9). More recently, a familial study did not demonstrate an earlier age of menopause in *BRCA1* or *BRCA2* carriers compared to their non-mutation carrier relatives; however, only 19% of the cohort reached natural menopause (34).

There is conflicting data in the literature on the association between ovarian reserve, as determined by AMH, and BMI. A large population-based Dutch cohort of over 2000 women did not find an association between AMH and BMI (35); however, other studies have demonstrated that accounting for BMI improves the prediction of age of menopause based on AMH (19) and that obese women in their fifth decade of life have lower AMH levels than normal weight women (36). In our analyses, BMI had a significant impact on AMH and was noted to be negatively correlated. Increased levels of inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin-6 (IL-6) have been detected in the follicular fluid of obese women undergoing assisted reproductive technology (37) providing a possible mechanism for decreased ovarian reserve in obese women. This is an intriguing question as an increasing number of studies emphasize the role of inflammatory markers, particularly IL-6, in the development of epithelial ovarian cancer (38). Interestingly, women with polycystic ovary syndrome, a reproductive phenotype characterized by irregular menstrual cycles, androgen excess, and obesity, are known to have elevated AMH levels (23, 24), further complicating the association between AMH and BMI.

To our knowledge, there are two relevant studies in the literature comparing AMH levels in *BRCA* carriers and controls. One study evaluated 15 *BRCA1* carriers and 60 controls (11), and although this study reported similar results to ours, it is unclear whether their *BRCA1* carriers were affected by breast or ovarian cancers, which would represent a selection bias for *BRCA1* carriers of a more severe phenotype. Our study included unaffected *BRCA* carriers only, eliminating the possibility of such a selection bias. A more recent study evaluated healthy unaffected carriers with at least one of the three Ashkenazi Jewish founder mutations and found contradictory results suggesting that *BRCA* carriers do not have lower serum AMH levels compared to controls (12). However, this study was limited by its small sample size and did analyze *BRCA1* and *BRCA2* carriers separately, which may explain the difference in our findings.

Our study has several important limitations. First, it is important to note that our findings are based on a specific subset of women- reproductive-age women with intact ovaries and a family history, but not a personal history, of breast or ovarian cancer. The findings may not be generalizable to the larger population. Second, although emerging data suggests that serum AMH levels may be affected by oral contraception use (20, 35, 39) and cigarette smoking (35, 40, 41), our analyses did not account for these two factors in the regression model due to missing data. Further studies are needed to determine whether these two factors confound the association between *BRCA1* carriers and AMH levels we observed. Third, the sample size of this study remains modest and limited particularly by the smaller sample of *BRCA2* carriers compared to *BRCA1* carriers, which likely contributes to the lack of a significant finding in the *BRCA2* group. Based on our preliminary results, a power analysis indicates that a future study would require approximately 100 *BRCA2* carriers and 100 controls to determine a significant difference in AMH levels, while covarying for age and BMI in a linear regression model. Lastly, as previously stated, we do not have longitudinal clinical data in this cohort to determine whether the difference in serum AMH levels predicted reproductive outcomes such as fertility or age of menopause. This limits the ability to apply our findings to affect clinical care and counseling of *BRCA* carriers. Despite these drawbacks, we present the largest study thus far of unaffected *BRCA* carriers and our findings contribute to the limited literature that exists on the effect of *BRCA* germline mutations and ovarian reserve.

In conclusion, *BRCA1* carriers may have a decreased ovarian reserve, as evidenced by lower serum AMH levels, compared to women without *BRCA* mutations. Our study broadens the scope of research on *BRCA* carriers beyond malignancies, a necessary step as surveillance and surgical interventions have provided enhanced opportunities for cancer-free living.

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Table 1

Baseline Characteristics

	<i>BRCA1</i> carrier N=62	<i>BRCA2</i> carrier N=27	Controls N=54	P-value
Age, y ^d	35.5 ± 5.2	35.6 ± 6.2	39.3 ± 3.7	<0.001 ^a
<35 years	26	11	5	
35–37 years	15	5	16	
38–40 years	12	4	13	
41 years	9	7	20	
Caucasian, n (%) ^f	61/62 (98%)	27/27 (100%)	52/54 (96%)	0.515 ^b
BMI, kg/m ^d	23.6 ± 5.0	22.0 ± 3.2	24.5 ± 6.3	0.202 ^a
Gravida, n (%) ^f				0.512 ^b
0	13/59 (22%)	10/27 (37%)	11/44 (25%)	
1–2	26/59 (44%)	7/27 (26%)	17/44 (39%)	
3	20/59 (34%)	10/27 (37%)	16/44 (35%)	
Current OCP use, n(%) ^e	9/47 (19%)	9/24 (38%)	4/13 (18%)	0.231 ^b
Former or current smoking, n(%) ^e	6/48 (12%)	3/23 (13%)	5/35 (14%)	0.972 ^b
Serum AMH, ng/mL ^d	1.07 ± 1.02	1.33 ± 1.11	1.11 ± 1.05	0.679 ^a
Serum AMH <1ng/mL, n(%) ^e	36/62 (58%)	11/27 (41%)	29/54 (54%)	0.320 ^c

P-values computed by

^a ANOVA,^b Fisher's exact test, or^c Chi-square test^d Continuous variables represented as means ± standard deviations^e Categorical variables represented as counts (percentages)

Table 2*BRCA* Deleterious Mutations Represented in the Study Cohort

<i>BRCA1</i> Deleterious Mutations		<i>BRCA2</i> Deleterious Mutations	
187delAG	36	6174delT	16
5385insC	9	8525delC	2
1623del15	1	6056delC	1
1675delA	1	R2336P (7235G>C)	1
2953delGTainsC	1	276del3ins10	1
3875del4	1	Q742X	1
4184del4	1	Q1994X	1
4873delCA	1	5579insA	1
5612insTT	1	W3127X	1
A1708E	1	Y1655X	1
IVS2+1G>A	1		
Q1200X	1		
Q494X	1		

Table 3

Results of Linear and Logistic Regression Modeling of AMH Levels

Linear Regression Modeling of AMH Levels ^a			Logistic Regression Modeling of AMH <1 ng/mL			
	Coefficient	SE	P-value	Coefficient	SE	P-value
Intercept	3.631	0.484	<0.001	-12.2	2.7	<0.001
<i>BRCA1</i> carriers	-0.298	0.113	0.010	0.854	0.341	0.012
<i>BRCA2</i> carriers	-0.169	0.112	0.242	-0.268	0.397	0.499
Controls	(ref)			(ref)		
BMI	-0.024	0.009	0.011	0.135	0.055	<0.001
Age	-0.054	0.010	<0.001	0.244	0.056	0.014

^aRegression of square-root transformed AMH levels

Table 4

Estimated least-square mean of AMH levels after adjusting for age and BMI.

	Mean adjusted AMH, ng/mL	(Adjusted 95% CI)	P-value ^a
<i>BRCA1</i> carrier	0.53	(0.33, 0.77)	0.026
<i>BRCA2</i> carrier	0.73	(0.39, 1.19)	0.470
Controls	1.05	(0.76, 1.40)	

^aP-value of linear regression modeling compared to controls and adjusted for multiple comparisons