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Association of *BRCA1* Mutations With Occult Primary Ovarian Insufficiency: A Possible Explanation for the Link Between Infertility and Breast/Ovarian Cancer Risks

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ABSTRACT

Purpose
Germline mutations in *BRCA* genes are associated with breast and ovarian cancer susceptibility. Because infertility is associated with breast and ovarian cancer risks, we hypothesized that the mutations in the *BRCA* gene may be associated with low response to fertility treatments.

Methods
We performed ovarian stimulation in 126 women with breast cancer by using letrozole and gonadotropins for the purpose of fertility preservation by embryo or oocyte cryopreservation. As surrogates of ovarian reserve, the oocyte yield and the incidence of low response were compared with ovarian stimulation according to *BRCA* mutation status.

Results
Of the 82 women who met the inclusion criteria, 47 women (57%) had undergone *BRCA* testing, and 14 had a mutation in *BRCA* genes, of which two were of clinically undetermined significance. In *BRCA* mutation–positive patients, low ovarian response rate was significantly higher compared with *BRCA* mutation–negative patients (33.3 *v* 3.3%; *P* = .014) and with *BRCA*-untested women (2.9%; *P* = .012). All *BRCA* mutation–positive low responders had *BRCA1* mutations, but low response was not encountered in women who were only *BRCA2* mutation positive. Compared with controls, *BRCA1* mutation– but not *BRCA2* mutation–positive women produced lower numbers of eggs (7.4 [95% CI, 3.1 to 17.7] *v* 12.4 [95% CI, 10.8 to 14.2]; *P* = .025) and had as many as 38.3 times the odds ratio of low response (95% CI, 4.1 to 353.4; *P* = .001).

Conclusion
BRCA1 mutations are associated with occult primary ovarian insufficiency. This finding may, at least in part, explain the link between infertility and breast/ovarian cancer risks.

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INTRODUCTION

Through recombination with undamaged, homologous DNA strands, *BRCA* genes play an essential role in double-strand DNA break (DSB) repair.[1] Mutations in either gene (ie, *BRCA1* or *BRCA2*) are associated with breast and ovarian cancer susceptibility, and these mutations are inherited in an autosomal dominant fashion.[2] We have recently developed a method of ovarian stimulation that uses the aromatase inhibitor letrozole (ie, controlled ovarian stimulation treatment with letrozole supplementation study; COST-LESS) for women with breast cancer who wish to preserve their fertility by oocyte or embryo cryopreservation before undergoing chemotherapy. In this protocol, administration of letrozole concurrently with gonadotropins reduces estrogen exposure significantly compared with standard ovarian stimulation regimens.[3-5] During

the aforementioned study we repeatedly encountered young patients with breast cancer who have no history of infertility and who unexpectedly have had low response to ovarian stimulation. Because low response to ovarian stimulation is associated with diminished oocyte reserve, occult primary ovarian insufficiency, and—although unconfirmed—increased DNA errors in oocytes, we hypothesized that the mutations in the *BRCA* gene may also be associated with primary occult ovarian insufficiency and infertility, as determined by low ovarian response to in vitro fertilization treatments.[6,7]

METHODS

Data for this study were generated from a secondary analysis of the prospective, controlled COST-LESS study,

which involved women with breast cancer who underwent oocyte or embryo cryopreservation for fertility preservation. Details of the COST-LESS protocol have been published.[3,5] Briefly, all women received letrozole 5 mg/d, starting on menstrual cycle day 2. Follicle-stimulating hormone (FSH) was added 2 days later at 150 to 300 IU/d on the basis of age and body mass index (BMI). Because patients were referred shortly after diagnosis of breast cancer and because BRCA testing was ordered by the referring oncologists, BRCA status was known in only one patient at the time of ovarian stimulation. The investigators, thus, were blinded to BRCA status of all but one patient. Because an association between BRCA mutations and ovarian response was not known during the COST-LESS study, BRCA status did not play a role in ovarian stimulation decisions in that patient.

A widely accepted definition of low ovarian response, retrieval of four or fewer oocytes in women younger than 38 years, was utilized in this study.[8,9] Women older than 38 years and women with prior ovarian surgery or with infertility treatments were excluded from consideration. BRCA testing was performed at commercial clinical laboratories, and the decision to perform such testing was made by the patient's oncologist, who was not involved with ovarian stimulation.

Statistical Analysis

Statistical analysis was performed with the SPSS 15.0 for Windows package (SPSS, Chicago, IL). Continuous data were analyzed with an independent t test if a normal distribution was likely and with log-converted data if the distribution was skewed. Log-converted data are presented as the harmonic mean and 95% CIs of the mean. Normally distributed data is presented as means ± standard deviations. To examine the association of BRCA mutation and low ovarian response, cross tabulations and Pearson's χ^2 test were used. We performed linear and logistic regression analyses to adjust for age. Various models also were employed to take into account total FSH dose and BMI. Statistical significance was reached at $P < .05$. The fit of logistic models was assessed with the Hosmer-Lemeshow test. Differences in continuous data were presented as mean differences and 95% CIs. Differences in categoric data (2 × 2) were expressed in terms of odds ratios (ORs) and 95% CIs.

RESULTS

One hundred twenty-six patients underwent ovarian stimulation for fertility preservation via embryo or oocyte cryopreservation according to the COST-LESS protocol. Of those, 82 patients met the study criteria. Of the excluded, 12 (27%) of 44 patients were tested for

BRCA, and only one was BRCA mutation positive. She was excluded because of a history of prior chemotherapy.

Of the 82 women who met inclusion criteria, 47 (57%) had undergone BRCA testing. Of those, 14 (30%) had a mutation in BRCA genes, and 33 (70%) did not. Of those 14 women, nine had a mutation in the BRCA1 gene, four had a mutation in the BRCA2 gene, and one had mutations in both genes (Table 1). Of the 14 BRCA mutations, two were of clinically undetermined significance; thus, the primary analysis was performed with 12 BRCA mutation–positive patients. Mean ages of untested, BRCA mutation–negative and –positive women were similar (Table 2). Low ovarian response rate was significantly higher in BRCA mutation–positive patients (four [33.3%] of 12) compared with BRCA mutation–negative patients (one [3.3%] of 33; $P = .014$) and BRCA-untested women (one [2.9%] of 35; $P = .012$). All BRCA mutation–positive low responders had BRCA1 mutations, and one patient also had a mutation in BRCA2. Low response was not encountered in women who were only BRCA2 mutation positive. When analysis was controlled for age, a BRCA mutation of known significance was associated with 28.7 times the normal OR of low response (95% CI, 1.8 to 447; $P = .016$) compared with BRCA mutation–negative women. When compared with the combined group of BRCA mutation–negative and -untested women, the OR was 24.7 (95% CI, 1.9 to 208; $P = .003$).

Mean oocyte numbers were significantly lower in BRCA mutation–positive women than in BRCA mutation–negative women (7.9 [95% CI, 4.6 to 13.8] v 11.3 [95% CI, 9.1 to 14.1]; $P = .025$). On analysis of variance, BRCA-negative and -untested groups were similar and, thus, merged to increase statistical power (Table 2). When deleterious BRCA1 mutation–positive women were compared with the combined group of BRCA mutation–negative and -untested women, mean oocyte numbers were significantly lower in BRCA1 mutation–positive women (7.4 [95% CI, 3.1 to 17.7] v 12.4 [95% CI, 10.8 to 14.2]; $P = .03$; (Table 2).

Analysis of BRCA subtypes revealed that BRCA1, but not BRCA2, mutations were associated with low response with an OR of 38.3 (95% CI, 4.1 to 353.4; $P = .001$). Inclusion of two nondeleterious BRCA1 mutations in this analysis reduced the OR but tightened the 95% CI (OR, 25.5; 95% CI, 3.2 to 204.2; $P = .002$). In BRCA1 mutation–positive patients, no specific deletion appeared to be associated with low response, but the sample size was too small to reach statistically sound conclusions. (Fig 1, OR data).

DISCUSSION

On the basis of previous studies and large clinical experience with in vitro fertilization, low response to ovarian stimulation is one of the strongest indications of diminished ovarian reserve and infertility.[10] By utilizing the largest and only prospective database available for women with breast cancer undergoing ovarian stimulation, we described here for the first time, to our knowledge, that mutations in the BRCA1 gene are associated with low response to ovarian stimulation and, by inference, infertility risks. We therefore hypothesize that, because DNA repair is deficient in patients with BRCA mutations, oocytes may be more prone to DNA damage. Follicles can reside in the ovary for decades, their oocytes potentially accumulating lethal DNA damage.[6] Research in nonreproductive cell types demonstrate that

Table 1. Mutation Types and Number of Oocytes Retrieved in BRCA Mutation–Positive Women Ordered by Age

Patient No.	Age (years)	No. of Total Oocytes	BRCA	Mutation
1	28	15	2	9637del4
2	30	27	1	187delAG
3	30	5	1	k1109n 3446A>C*
4	31	17	1	W1815X 5563G->A
5	32	8	1	187delAG
6	32	30	1	187delAG
7	32	6	2	IVS22-1del3insAA
8	33	3	1	187delAG
9	34	10	2	6174delT
10	35	34	1	M1083VG 3366A>G*
11	35	3	1 and 2	1(185delAG), 2(6174delT)
12	36	3	1	3889delAG
13	37	3	1	187delAG
14	37	8	2	6174delT

*Variant of unknown significance.

**Table 2.** Age, FSH, and Oocyte Number Comparisons Among *BRCA* Mutation–Negative, –Positive, and –Untested Women

| Variable | *BRCA* Mutation Status | | | | | P |
	All Positive (n = 12)	All Negative (n = 33)*	Untested (n = 35)	*BRCA1* Positive (n = 8)†	All Negative and Untested (n = 68)‡	
Age, years						NS
Mean	33.1	32.8	33.0	33.9	32.9	
SD	2.8	2.9	2.9	2.7	2.9	
Day-2 FSH, mU/mL						NS
	5.7	7.1	6.4	6.2	6.7	
	3.0	2.7	2.3	3.4	2.5	
Oocytes						
Mean	7.9	11.3	13.5	7.4	12.4	
95% CI§	4.6 to 13.8	9.1 to 14.1	11.4 to 16.0	3.1 to 17.7	10.8 to 14.2	

Abbreviations: FSH, follicle-stimulating hormone; NS, not significant; SD, standard deviation.
*P positive v negative = .025.
†P *BRCA1* mutation–positive v –negative and untested combined = .03.
‡P positive v negative and untested combined = .003.
§Analysis was performed after log conversion because of non-normal distribution. Thus, 95% CIs were used instead of SDs.

when DNA damage is severe and cannot be repaired, apoptotic pathways are activated.[11-14] Thus oocytes with deficient *BRCA* function may be prematurely eliminated by a similar mechanism, resulting in early depletion of egg reserve and, as a consequence, primary ovarian insufficiency.

Primary ovarian insufficiency is a continuum that ranges from occult insufficiency that is only detectable by laboratory markers to low response to fertility drugs to its overt form, which presents with clinical symptoms.[15-17] We demonstrated that *BRCA* mutations are associated with occult primary ovarian insufficiency.

As in the case of occult primary ovarian insufficiency, although mutations in *BRCA1* and *BRCA2* are associated with ovarian cancer risk, the risk is considerably higher for *BRCA1* mutations. The latter observation, thus, may suggest a common pathway in the development of diminished ovarian reserve and ovarian cancer in *BRCA1* mutation–positive women. Because occult primary ovarian insufficiency is associated with female infertility, *BRCA* mutations may contribute to the long-known association between breast as well as ovarian cancer risks.[18,19]
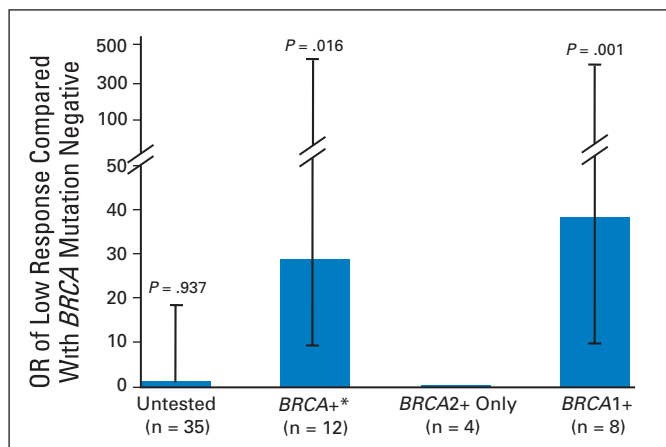
Fig 1. Odds ratios (ORs) of low ovarian response in women with BRCA mutations. (*) Only mutations with proven clinical significance for breast and ovarian cancer risk are included.

It is estimated that, in the general population, one in every 1,000 women is *BRCA* mutation positive, and this incidence is as high as 2.5% in certain ethnic groups, such as people with Jewish-Ashkenazi origin.[2,20] Regardless of underlying mechanisms of occult primary ovarian insufficiency in *BRCA1* mutation–positive women, our findings may have profound implications for the future fertility of a large number of women in the general population. In this study, we did not measure serum ovarian reserve markers, such as antimullerian hormone, but we used response to ovarian stimulation as a surrogate of ovarian reserve. The presumed lower ovarian reserve, which was based on the low oocyte yield, may place *BRCA1* mutation–positive women with breast cancer also at higher risk for chemotherapy-induced ovarian failure. This underscores the importance of fertility preservation in *BRCA* mutation–positive women with cancer.[21] We do not know to what extent *BRCA* mutations affect the fertility of women who have not developed cancer; thus, widespread fertility preservation of *BRCA* mutation–positive women cannot be recommended at this time. Similarly, we do not yet know to what extent variations in the sequence of the *BRCA* gene contribute to ovarian dysfunction in the general population.

Nevertheless, considering that 1% of females suffer from primary ovarian insufficiency, that the underlying mechanism is unknown in 90%, and that an even a larger fraction of infertile women may suffer from occult primary ovarian insufficiency, discovery of susceptibility genes for premature ovarian insufficiency, such as *BRCA1*, will have positive implications for understanding the link between infertility and breast/ovarian cancer risks.[17,22]

An association between *BRCA* mutations and diminished oocyte reserve or occult primary ovarian insufficiency is biologically plausible on the basis of the previous laboratory and clinical data. *BRCA1* expression is reduced in oocytes of aging mice, and RNAi-mediated reduction of *BRCA1* in oocytes from young female mice perturbs oocyte spindle formation, which indicates that intact *BRCA1* function is essential for oocyte survival.[23] It has been demonstrated recently that *BRCA1* localizes to unsynapsed chromosomes at the pachytene stage in human oocytes, so it may play a similar role in humans. The DSB repair pathway not only involves *BRCA* genes but also coordinates activity of at least six other genes, all linked to Fanconi anemia

(FA).[11,24] In FA, another disease caused by mutations in DSB repair genes, women experience early menopause; Fanconi gene–mutated mice display premature reproductive aging, and their ovarian primordial follicle reserves are severely reduced.[14,25] Interestingly, the activated version of *FANCD2*, a gene involved in FA, targets *BRCA1*, and disruption of *BRCA1* results in the disruption of DNA damage–inducible *FANCD2*-containing subnuclear foci. Although homozygous *BRCA* knockout mice are generally not viable, spermatogenesis in *BRCA1* mutant mice was impaired, but ovarian follicle numbers or function was not quantified.[26]

We could not detect an association between *BRCA2* mutations and the probability of low ovarian response. However, because of the smaller number of patients with those mutations, an impact of *BRCA2* mutations on ovarian function cannot be ruled out. In fact, in mice with a truncating *BRCA2* mutation, both testis and ovaries were devoid of germ cells and were hypoplastic, which resulted in infertility.[27,28] Also, *BRCA1* mutations did not always result in clinically overt low response, which suggests that *BRCA1* may be, as is the case in breast and ovarian cancer, only a susceptibility gene for oocyte death. Accumulation of additional environmental factors with age may determine whether germline mutations in *BRCA* will result in occult primary ovarian insufficiency. In fact, in this study, all *BRCA* mutation–positive low responders were 33 years of age or older, which indicated that the degree of occult primary ovarian insufficiency may only become clinically significant in older women. This, coupled with the fact that many of the women in their late thirties are undergoing risk-reducing oophorectomies, may explain why the association of occult ovarian insufficiency and BRCA mutation status was not discovered previously. In addition, before our introduction of ovarian stimulation with aromatase inhibitors in women with breast cancer, most women would not have been given the option of ovarian stimulation for embryo or oocyte freezing; thus, an association between breast cancer and occult primary ovarian insufficiency could not have been recognized.

The proportion of women with *BRCA* mutations in this study may be perceived as higher than expected. Besides the young age of patients in our study, this can be additionally explained by the fact that a large population of people in our geographic area are of Ashkenazi-Jewish origin.[20] It is also possible that *BRCA* mutation–positive women are more likely to remain childless because of the effects of these mutations on fertility; therefore, they will be more likely to need fertility preservation when faced with the prospects of chemotherapy-induced ovarian failure.

More speculatively, in majority of oligo-azospermic men, no underlying cause can be identified currently.[29] Given that sperm production is altered in both FA and *BRCA* mutant rodent models), it is conceivable that *BRCA* mutations may be responsible for male-factor infertility in some of these men.

In conclusion, we showed a novel association between low response to ovarian stimulation and *BRCA1* mutations, which suggests a possible link between DSB repair gene function, infertility, and breast/ovarian cancer risks. The analysis of the *BRCA* gene in women with infertility and low response to ovarian stimulation may be worthwhile, especially when there is family history of breast and/or ovarian cancer. Larger studies are warranted to investigate the impact of *BRCA* mutations on fertility in general population.

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