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EPIDEMIOLOGY

Evaluation of established breast cancer risk factors as modifiers of *BRCA1* or *BRCA2*: a multi-center case-only analysis

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Abstract The incomplete penetrance of mutations in *BRCA1* and *BRCA2* suggests that some combination of environmental and genetic factors modifies the risk of breast cancer in mutation carriers. This study sought to identify possible interactions between established breast cancer risk factors and *BRCA1* or *BRCA2* mutations using a case-only study design. Breast cancer cases that had been tested for *BRCA1* and *BRCA2* mutations were identified from 11 collaborating centers. Comparisons of reproductive and lifestyle risk factors were made between women with breast cancer who were positive for *BRCA1* mutations ($n = 283$), *BRCA2* mutations ($n = 204$), or negative for both *BRCA1* and *BRCA2* mutations ($n = 894$). Interaction risk ratios (IRRs) were calculated using multinominal logistic regression models. Compared with non-carriers, statistically significant IRRs were observed for later age at menarche among *BRCA2* mutation carriers, for a greater number of pregnancies among both *BRCA1* and *BRCA2* mutation carriers, and for alcohol use among *BRCA1* mutation carriers. Our data suggest that the risk for breast

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cancer among *BRCA1* or *BRCA2* carriers may be modified by reproductive characteristics and alcohol use. However, our results should be interpreted cautiously given the overall inconsistency in the epidemiologic literature on modifiers of *BRCA1* and *BRCA2*.

Keywords Breast cancer · *BRCA1* · *BRCA2* · Mutations · Modifiers

Introduction

Carriers of mutated forms of the *BRCA1* and *BRCA2* genes are at markedly higher risk for developing breast and ovarian cancer. Estimates of the lifetime risk for developing breast cancer for *BRCA1* and *BRCA2* mutation carriers range from approximately 50 to 80% [1]. Despite the high cancer risk associated with these mutations, their incomplete penetrance suggests that other factors, likely a combination of genetic variants and environmental exposures, influence the risk of a mutation carrier developing the disease.

Comparisons of cancers among *BRCA1*- and *BRCA2*-mutation carriers and non-mutation carriers show differences in clinical characteristics suggesting that they may arise from distinct etiologic pathways [2, 3]. Breast cancers diagnosed in individuals with *BRCA1* mutations tend to have a higher prevalence of the triple negative (negative for estrogen receptor (ER), progesterone receptor (PR), and *HER2*) subtype of breast cancer [3, 4]. Although triple-negative breast cancer is generally associated with poorer prognosis [4], most studies show survival is not significantly worse for *BRCA1* carriers than those without mutations [5], providing further evidence that *BRCA1* mutation-related cancers are distinct from both sporadic cancers and *BRCA2*-related cancers.

Differences in epidemiologic risk factors for *BRCA1*- and *BRCA2*-associated cancers as compared to risk factors for breast cancer in non-carriers may provide insight into factors that modify the penetrance of these genes. A number of investigations using a variety of study designs have been conducted in an attempt to identify modifiers of *BRCA1* or *BRCA2*. The most common approach has been to compare established breast cancer risk factors between women with and without breast cancer in study populations that comprised *BRCA1* and/or *BRCA2* mutation carriers [6–19]. Other studies compared affected *BRCA1* or *BRCA2* mutation carriers to unaffected women without mutations [20–22], or compared breast cancer cases with and without mutations [20, 21, 23]. The extent to which breast cancer risk factors among *BRCA1* or *BRCA2* carriers differ from risk factors for sporadic disease may be an indicator of factors that may modify the penetrance of mutated *BRCA1* or *BRCA2*. To date, most reports have focused on reproductive and hormonal risk factors such as parity, breast-feeding, and oral contraceptive use. No clear picture has yet emerged on whether the associations with these risk factors among *BRCA1* or *BRCA2* mutation carriers are similar or different to the established associations in sporadic breast cancer.

Although the majority of studies to date have examined breast cancer risk factors among affected and unaffected mutation carriers, this approach does not directly evaluate gene–environment interaction because the study population comprised entirely mutation carriers. A more direct approach to evaluating potential modifiers of *BRCA1* and *BRCA2* mutations is to compare the epidemiologic characteristics of women with *BRCA1* or *BRCA2* mutations who develop breast cancer to non-mutation carriers with breast cancer—a case-only design. In this report, we used a case-only analysis to evaluate differences in clinical characteristics and risk factors between breast cancers diagnosed in women with *BRCA1* mutations, *BRCA2* mutations, and no known mutation. The objective of these analyses was to obtain a better understanding of differences in risk factors across genotypes, which ultimately could lead to prevention strategies that are tailored specifically to *BRCA1* or *BRCA2* mutation carriers.

Methods

Study design

The Genetic and Environmental Modifiers of *BRCA1*/*BRCA2* Study (GEMS) is a multi-center study coordinated at Duke University Medical Center that was designed to

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investigate genetic variants and epidemiologic risk factors that may affect the penetrance of *BRCA1* and *BRCA2* mutations. The study involved 11 centers in the United States and Australia that contributed data and biological samples for analysis. Cases included in the study were females aged 20 years or older who had been diagnosed with breast cancer and had been tested for *BRCA1* and/or *BRCA2* mutations. We included only women with known deleterious mutations as mutation-positive cases. Women who had variants of unknown significance but no known deleterious mutation were excluded from the analysis. This study was designed such that each case with a *BRCA* mutation would be frequency matched by center and age (±4 years) to two cases who tested negative; however, in some instances there was only one negative for each positive case. To minimize survival bias, eligible cases had to have been tested for *BRCA1* and/or *BRCA2* mutations and have had epidemiologic data collected no later than 3 years after diagnosis. This analysis involved a total of 1,381 cases of whom 283 are *BRCA1* mutation positive (*BRCA1*+), 204 are *BRCA2* mutation positive (*BRCA2*+), and 894 are negative for both *BRCA1* and *BRCA2* mutations (*BRCA*−). Four women who had both *BRCA1* and *BRCA2* mutations were excluded from the analyses. Mutation status was based on testing for *BRCA1* and *BRCA2* performed prior to enrollment in GEMS that was done in various laboratories using methods established at each center. Seventy-five percent of subjects had full sequencing, 19% had partial sequencing (Jewish panel or family mutations), and 6% had various other tests such as protein truncation tests, single-strand conformation polymorphisms (SSCP), or denaturing high performance liquid chromatography (DHPLC). Among the women that had full sequencing, 65% of them had large deletion or duplication testing such as multiplex ligation-dependent probe analysis (MLPA). Participating centers and the number of cases contributed by each site are listed in Table 1.

All centers sent DNA or whole blood/cell lines from which DNA could be extracted to Duke University Medical Center. They also sent questionnaire data, pathology data from the breast cancer diagnosis, and mutation testing results. The study protocol was approved by the Institutional Review Boards at Duke University Medical Center and all participating centers.

Cases were identified both prospectively (newly diagnosed and tested) and retrospectively (previously diagnosed and tested); thus, there was some variation in the questionnaire data that were obtained. Centers that prospectively enrolled cases for the GEMS study and were not administering a questionnaire to patients at their hereditary cancer clinic used a questionnaire developed for the GEMS study. Centers that prospectively enrolled cases but were already administering a questionnaire to their clinic patients added a supplement to their survey to obtain additional risk factor information. Centers that contributed retrospectively identified cases for this analysis sent data from a previously administered risk factor questionnaire. Data from centers that did not use the GEMS questionnaire were mapped to the questions on the GEMS survey. Missing data in the analyses are due in most cases to questions not being asked on surveys administered to retrospectively identified cases.

Table 1 Number of *BRCA1* mutation positive, *BRCA2* mutation positive, and *BRCA* negative cases by participating site

Center	*BRCA1*+	*BRCA2*+	*BRCA*−	Total
Duke University Medical Center	28	21	70	119
Dana Farber Cancer Institute	22	13	70	105
Georgetown University	25	14	79	118
Johns Hopkins University	13	8	42	63
kConFab (Australia)	43	49	133	225
University of Southern California	57	30	161	248
University of Pennsylvania	28	25	113	166
Moffitt Cancer Center	6	4	23	33
University of North Carolina	28	20	93	141
University of Michigan	4	3	11	18
M.D. Anderson Cancer Center	29	17	99	145
Totals	283	204	894	1381

Data analysis

The analysis took into account the different centers from which cases were recruited and the age matching within each center. All models treated *BRCA1* or *BRCA2* genotype as the response variable and included a random effect for center. We fit these unconditional logistic link multinomial response models using the GLIMMIX procedure in SAS (version 9.2, SAS Institute, Cary, NC). The models used for characterizing relationships between demographic and clinical characteristics and *BRCA1* or *BRCA2* genotype were unadjusted (Table 2), while those used to evaluate epidemiologic factors as potential modifiers of *BRCA1* or *BRCA2* (Table 3) were age-adjusted. The latter class of models was used to estimate the interaction risk ratios (IRRs) and 95% confidence intervals (CIs) for each parameter, where $IRR = [P(D = 1|BRCA+, E+)P(D = 1|BRCA-, E-)]/[P(D = 1|BRCA+, E-)P(D = 1|BRCA-, E+)]$, with D = disease (breast cancer) and E = environmental exposure. The IRR is a ratio of risk ratios and can be expressed as the RR of disease given E among carriers to the RR of disease given E among non-carriers [24, 25]. In these analyses, the IRRs are case-only comparisons and reflect heterogeneity in risk factors between case groups.

Table 2 Demographic and clinical characteristics of breast cancer cases by *BRCA1* or *BRCA2* mutation status

	BRCA1+ ($n = 283$) *N* (%)	*BRCA2*+ ($n = 204$) *N* (%)	*BRCA*− ($n = 894$) *N* (%)	*P* value	
				BRCA1+ vs. *BRCA*−	*BRCA2*+ vs. *BRCA*−
Age at diagnosis (years)					
22–29	18 (6.4)	4 (2.0)	37 (4.1)	0.0004	0.09
30–39	111 (39.2)	66 (32.4)	248 (27.7)		
40–49	116 (41.0)	89 (43.6)	409 (45.8)		
50–59	27 (9.5)	39 (19.1)	142 (15.9)		
60+	11 (3.9)	6 (2.9)	58 (6.5)		
Race					
White	252 (89.1)	184 (90.2)	823 (92.1)	0.4	0.5
African-American	11 (3.9)	7 (3.4)	28 (3.1)		
Hispanic	13 (4.6)	6 (2.9)	25 (2.8)		
Asian or Pacific Islander	3 (1.1)	6 (2.9)	12 (1.3)		
Other	4 (1.4)	1 (0.5)	6 (0.7)		
Jewish					
Yes	63 (23.3)	44 (22.0)	142 (16.2)	0.01	0.02
No	207 (76.7)	156 (78.0)	737 (83.9)		
Missing	13	4	15		
Behavior					
Invasive	266 (94.0)	185 (90.7)	791 (88.5)	0.01	0.3
In situ	17 (6.0)	19 (9.3)	103 (11.5)		
Stage					
1	171 (70.1)	120 (71.4)	452 (64.3)	0.2	0.4
2	60 (24.6)	34 (20.2)	188 (26.7)		
3	10 (4.1)	11 (6.6)	54 (7.7)		
4	3 (1.2)	3 (1.8)	9 (1.3)		
Missing	39	36	191		
Histologic grade					
Well differentiated	44 (19.3)	27 (17.9)	145 (23.5)	<0.0001	0.2
Moderately differentiated	30 (13.2)	51 (33.8)	211 (34.1)		
Poorly differentiated	154 (67.5)	73 (48.3)	262 (42.4)		
Missing	55	53	276		
Estrogen receptor (ER) status					
Positive	49 (20.6)	125 (78.6)	498 (72.8)	<0.0001	0.3
Borderline	8 (3.4)	3 (1.9)	18 (2.6)		
Negative	181 (76.1)	31 (19.5)	168 (24.6)		
Missing	45	45	210		
Progesterone receptor (PR) status					
Positive	52 (22.0)	97 (63.8)	432 (64.6)	<0.0001	0.7
Borderline	12 (5.1)	7 (4.6)	22 (3.3)		
Negative	172 (72.9)	48 (31.6)	215 (32.1)		
Missing	47	52	225		
HER2 status					
Positive	15 (10.3)	16 (16.8)	96 (22.6)	0.01	0.3
Borderline	6 (4.1)	5 (5.3)	14 (3.3)		
Negative	125 (85.6)	74 (77.9)	315 (74.1)		
Missing	137	109	469		

Table 2 continued

	BRCA1+ ($n = 283$) *N* (%)	*BRCA2*+ ($n = 204$) *N* (%)	*BRCA*− ($n = 894$) *N* (%)	*P* value	
				BRCA1+ vs. *BRCA*−	*BRCA2*+ vs. *BRCA*−
Triple negative status (ER−/PR−/*HER2*−)					
Yes	86 (59.3)	17 (18.9)	56 (13.6)	<0.0001	0.2
No	59 (40.7)	144 (81.1)	634 (86.4)		
Missing	138	43	204		
BRCAPRO probability of being a *BRCA1* or *BRCA2* mutation carrier					
<25%	124 (44.6)	120 (60.0)	709 (81.1)	<0.0001	<0.0001
25% to <50%	45 (16.2)	24 (12.0)	69 (7.9)		
50% to <75%	29 (10.4)	24 (12.0)	48 (5.5)		
75% to <90%	24 (8.6)	12 (6.0)	27 (3.1)		
>90%	56 (20.1)	20 (10.0)	21 (2.4)		
Missing	5	4	20		

Table 3 Case-only interaction risk ratios (IRRs) and 95% confidence intervals (CI) comparing *BRCA1*+ or *BRCA2*+ breast cancer cases to *BRCA*− breast cancer cases

	BRCA1+ *N* (%)	*BRCA2*+ *N* (%)	*BRCA*− *N* (%)	*BRCA1*+ vs. *BRCA*− IRR* (95% CI)	*BRCA2*+ vs. *BRCA*− IRR* (95% CI)
Age at menarche (years)					
<11	57 (20.7)	35 (17.6)	182 (20.6)	1.00 Reference	1.00 Reference
12–13	156 (56.7)	98 (49.2)	497 (56.2)	0.98 (0.69–1.39)	1.02 (0.67–1.56)
≥14	62 (22.6)	66 (33.2)	205 (23.2)	0.95 (0.63–1.44)	1.65 (1.04–2.61)
Missing	8	5	10		
Full-term pregnancies					
None	81 (28.8)	48 (23.5)	281 (31.6)	1.00 Reference	1.00 Reference
1	41 (14.6)	35 (17.2)	133 (15.0)	1.09 (0.71–1.68)	1.55 (0.95–2.52)
2	96 (34.2)	78 (38.2)	299 (33.7)	1.28 (0.91–1.81)	1.54 (1.03–2.32)
≥3	63 (22.4)	43 (21.1)	175 (19.7)	1.54 (1.04–2.28)	1.46 (0.90–2.35)
Missing	2	0	6		
Age at first full-term pregnancy					
<20 years	19 (9.6)	15 (9.8)	50 (8.3)	1.00 Reference	1.00 Reference
20–24 years	62 (31.5)	42 (27.4)	164 (27.3)	1.03 (0.56–1.90)	0.86 (0.44–1.67)
25–29 years	63 (32.0)	41 (26.8)	185 (30.7)	0.83 (0.45–1.53)	0.73 (0.37–1.43)
≥30 years	53 (26.9)	55 (36.0)	203 (33.7)	0.63 (0.34–1.17)	0.90 (0.46–1.74)
Missing	3	3	5		
Oral contraceptive use					
Never	47 (17.7)	24 (12.2)	120 (14.4)	1.00 Reference	1.00 Reference
Ever	219 (82.3)	173 (87.8)	716 (85.7)	0.70 (0.48–1.02)	1.19 (0.74–1.91)
Missing	17	7	58		
Oral contraceptive duration					
<12 months	66 (26.4)	40 (21.4)	187 (23.9)	1.00 Reference	1.00 Reference
12–35 months	26 (10.4)	29 (15.5)	102 (13.0)	0.69 (0.41–1.15)	1.30 (0.76–2.22)
36–59 months	27 (10.8)	10 (5.4)	75 (9.6)	0.95 (0.56–1.61)	0.60 (0.28–1.27)
≥60 months	131 (52.4)	108 (57.8)	418 (53.5)	0.81 (0.57–1.15)	1.13 (0.74–1.71)
Missing duration (among users)	16	10	54		

Table 3 continued

	BRCA1+ *N* (%)	*BRCA2*+ *N* (%)	*BRCA*− *N* (%)	*BRCA1*+ vs. *BRCA*− IRR* (95% CI)	*BRCA2*+ vs. *BRCA*− IRR* (95% CI)
Breastfeeding					
Never	32 (20.0)	30 (22.3)	97 (19.4)	1.00 Reference	1.00 Reference
Ever	128 (80.0)	101 (77.7)	402 (80.6)	0.88 (0.56–1.39)	0.76 (0.47–1.23)
Missing	40	25	108		
BMI (kg/m^2) 1 year before diagnosis					
<25	98 (54.1)	72 (53.0)	309 (55.8)	1.00 Reference	1.00 Reference
≥25 to <30	42 (23.2)	38 (27.9)	147 (26.5)	0.99 (0.65–1.51)	1.09 (0.70–1.70)
≥30	41 (22.7)	26 (19.1)	98 (17.7)	1.38 (0.89–2.13)	1.15 (0.69–1.90)
Missing	102	68	340		
BMI (kg/m^2) at age 18					
<25	138 (72.2)	109 (75.7)	413 (70.2)	1.00 Reference	1.00 Reference
≥25 to <30	29 (15.2)	25 (17.4)	120 (20.4)	0.76 (0.48–1.20)	0.81 (0.50–1.31)
≥30	24 (12.6)	10 (6.9)	55 (9.4)	1.15 (0.68–1.94)	0.68 (0.33–1.38)
Missing	92	60	306		
Alcohol use					
No	74 (28.0)	49 (25.1)	173 (20.9)	1.00 Reference	1.00 Reference
Yes	190	146 (74.9)	656 (79.1)	0.65 (0.48–0.90)	0.80 (0.55–1.16)
Missing	19 (72.0)	9	65		
Smoking (ever)					
No	175 (62.5)	111 (55.0)	514 (58.3)	1.00 Reference	1.00 Reference
Yes	105 (37.5)	91 (45.0)	367 (41.7)	0.87 (0.66–1.15)	1.14 (0.84–1.56)
Missing	3	2	13		

*Adjusted for age and center

Because these analyses are case-only comparisons, the IRRs are estimates of the interaction between the genetic factor (*BRCA* mutation) and the environmental factor provided that the two factors are independent in the population giving rise to the data (women with a family history of breast and/or ovarian cancer) and conditional on the factors adjusted for in the model (i.e., age). Since there is no traditional 'control group' of unaffected subjects, we are not able to assess main effects. For example, an IRR of 2.0 generated from this analysis for environmental factor E comparing *BRCA1*+ cases to *BRCA*− cases indicates that the relative risk of disease given E is twice as high among *BRCA1*+ individuals as it is among *BRCA*− individuals, but the strength of the association in *BRCA*− individuals (the main effect) is unknown. Similarly, an IRR of 1.0 for environmental factor E comparing *BRCA1*+ cases to *BRCA*− cases indicates that there is no interaction between *BRCA1* mutation status and factor E, i.e., the RR of disease given E is the same for both *BRCA1*+ and *BRCA*− individuals. This could indicate that either the factor is unassociated with disease in both genotype groups or has a similar association (main effect) in both genotype groups.

Genetic testing for *BRCA1* and *BRCA2* mutations is specific, but its sensitivity is less than one with rates that vary by the method employed, gene, and mutation type. As a result, we expect that test positives are truly positive for a deleterious mutation and that a fraction of the test negatives are also positive. This has the potential to result in biased estimates of effect. To assess the sensitivity of our results to misclassification of *BRCA1* or *BRCA2* genotype, we conducted a sensitivity analysis in which we repeated the above analyses after removing test negative subjects with pre-test probabilities of carrying a deleterious *BRCA1* or *BRCA2* mutation ranging from greater than 90% to greater than 25%. We used BRCAPRO as implemented in BayesMendel V2.0-2 to calculate each subject's BRCAPRO probability assuming the penetrance functions found in Chen et al. [26] and Tai et al. [27] and implemented in the structure BRCApenet.metaDSL.2008. In calculating these probabilities, we conditioned on each subject's first- and second-degree family history of breast and ovarian cancer as well as oophorectomy status, when known.

A factor, E, that is a modifier of penetrance may either result in a different risk of disease by genotype or shift the

age at diagnosis distribution as a function of genotype, or both. Therefore, we also looked at whether the environmental factors modified the penetrance of *BRCA1* and *BRCA2* through a shift in the age at onset distributions by constructing multiple linear regression models with age at diagnosis as the response (dependent) variable and the independent variables gene mutation status (*BRCA1*+, *BRCA2*+, *BRCA*−), the environmental factor, and a cross-product gene by environmental factor interaction term. Statistically significant differences in the mean age at diagnosis by genetic and environmental factors provide evidence that the environmental factor acts as a genetic modifier. The analysis of mean age differences does not require the G by E independence assumption as stated for the case-only interaction analyses described above.

Results

Table 2 shows the demographic and clinical characteristics of the cases by *BRCA* mutation status. The *BRCA1*+ cases were slightly younger than the other case groups (mean age 41.2 years vs. 44.0 for *BRCA2*+ cases and 44.1 for *BRCA*− cases). Only about 10% of the cases in each group were non-white, which is similar to the race/ethnic distribution of tested women reported by Myriad Genetics Laboratories [28]. The proportion of Jewish women was higher among the *BRCA1*+ and *BRCA2*+ cases than controls ($P = 0.01$ and 0.02, respectively), reflecting the high prevalence of known founder mutations among women with Ashkenazi ancestry.

The *BRCA1*+ cases had clinical characteristics that were distinct from the *BRCA2*+ and the *BRCA*− cases. As previously reported, the stage distribution of cancers among *BRCA1* mutation carriers was not statistically significantly different from the other two case groups, but they were more likely to have higher grade (poorly differentiated) and to be ER negative, PR negative, and *HER2* negative. Among women with information on all three markers (ER, PR, and *HER2*), 59.3% of the *BRCA1*+ cases were negative for all the three, compared to a triple negative proportion of 18.9% for *BRCA2*+ and 13.6% for *BRCA*− cases. *BRCA1*+ cases had higher prior probabilities of being a mutation carrier than either *BRCA2*+ or *BRCA*− cases.

In Table 3, we present the case-only comparisons of the epidemiologic risk factors. Age at menarche showed a statistically significant difference in its association with *BRCA2*+ cancer as compared to *BRCA*− cases. A greater proportion of *BRCA2*+ cases had an age at menarche ≥14 years. The IRR of 1.65 (95% CI 1.04–2.61) comparing *BRCA2*+ to *BRCA*− cases indicates that the protective effect of late age at menarche typically observed for sporadic breast cancer was reduced among the *BRCA2*+ cases.

IRRs for number of full-term pregnancies were greater than one for both *BRCA1*+ and *BRCA2*+ cases, suggesting that the inverse relationship with pregnancy usually noted for breast cancer is attenuated or absent for *BRCA1*+ and *BRCA2*+ cases. There were no statistically significant differences between the case groups for breastfeeding, although the IRRs of 0.88 for *BRCA1*+ and 0.76 for *BRCA2*+ cases as compared to *BRCA*− cases suggest that breastfeeding has a protective effect regardless of *BRCA* mutation status. The IRRs for oral contraceptive use were not statistically significant and showed no clear trends with duration of use.

Among the other characteristics examined, the only statistically significant IRR observed was alcohol use among *BRCA1*+ cases (IRR = 0.65, 95% CI 0.48–0.90), suggesting that the effect of alcohol on *BRCA1*+ cases as compared to *BRCA*− cases is weaker or absent. No clear interactions with BMI or smoking were observed.

We also performed multivariable analyses, with terms for age, age at menarche, number of pregnancies, oral contraceptive use, breastfeeding, alcohol use, and smoking included in the model. Overall, results were not substantively different than the age-adjusted analyses.

In the sensitivity analysis, we repeated the modeling excluding test negative cases that had pre-test probabilities of being a mutation carrier based on BRCAPRO scores ranging from >90% (21 cases excluded) to >25% (165 cases excluded). No substantive differences in any of the IRRs were observed when excluding test negative cases that had a high pre-test probability of being a mutation carrier (data not shown). For example, the statistically significant IRR for late age at menarche that we observed for *BRCA2* carriers (1.65, 95% CI 1.04–2.61) changed only slightly when we excluded cases that had high pre-test probabilities (IRRs ranged from 1.60 to 1.69). Therefore, we conclude that misclassification of mutation status among test negative cases had minimal effect on our findings.

Results of the analyses that modeled the age at diagnosis by genetic and environmental factors are presented in Table 4. For each factor, we assessed whether each breast cancer risk factor modified the age at diagnosis according to the *BRCA* mutation status. For all but one of the factors examined, the *P* values for gene–environment interaction were not statistically significant implying that the relationship between the risk factors and age at diagnosis did not vary according to *BRCA* mutation status. The only statistically significant interaction observed was for alcohol use. Among *BRCA1*+ and *BRCA2*+ cases, the age at diagnosis was younger for women who consumed alcohol whereas the age at diagnosis was slightly older for women

Table 4 Modeled age at breast cancer diagnosis by gene and risk factor status and *P* values for gene effects (G), environmental factor effects (E), and gene by environment (G × E) interactions

Risk factor	Categories	*BRCA1+*	*BRCA2+*	*BRCA−*	*P* values
No. of full-term pregnancies	0	38.2	41.9	41.3	G: <0.0001
	1	39.1	40.5	42.0	E: <0.0001
	2	41.8	44.5	44.3	G × E: 0.91
	≥3	42.8	44.7	46.1	
Age at last pregnancy	<20 years	41.2	43.6	45.2	G: 0.0004
	20–24 years	41.7	46.6	45.5	E: 0.0087
	25–29 years	41.8	40.8	43.3	G × E: 0.37
	≥30 years	40.6	42.7	43.4	
Age at menarche	<12 years	41.4	43.9	43.5	G: 0.0001
	12–14 years	40.2	42.3	43.2	E: 0.34
	>14 years	40.0	43.4	42.9	G × E: 0.86
Oral contraceptive duration of use	<12 months	43.8	45.2	44.8	G: < 0.0001
	12–35 months	38.8	41.6	44.4	E: < 0.0001
	36–59 months	38.3	44.6	43.5	G × E: 0.20
	>60 months	39.6	41.9	42.2	
Breastfed	Never	42.1	45.3	46.7	G: 0.0016
	Ever	41.3	42.4	43.8	E: 0.014
					G × E: 0.60
BMI kg/m^2 (1 year before diagnosis)	<25	40.3	43.1	42.9	G: 0.0004
	25 to <30	41.6	42.6	43.4	E: 0.13
	≥30	41.3	45.7	45.9	G × E: 0.14
BMI kg/m^2 (at age 18)	Underweight	40.9	44.2	45.5	G: 0.0048
	Normal	37.6	41.5	39.2	E: 0.0076
	Overweight	40.9	42.9	43.2	G × E: 0.65
Alcohol Use	Yes	39.8	42.6	43.1	G: 0.015
	No	42.7	44.3	42.8	E: 0.031
					G × E: 0.047
Smoking status	Never	40.7	42.8	42.7	G: < 0.0001
	Ever	40.3	43.2	44.0	E: 0.54
					G × E: 0.34

who consumed alcohol among the *BRCA−* cases. Although the interaction *P* value for mutation status by alcohol consumption was nominally statistically significant, we made no adjustment for multiple comparisons so these results should be interpreted cautiously.

Discussion

In the classic situation of evaluating gene–environment interaction, the study population (case–control or cohort) involves eight groups of subjects: cases (1) and controls (2) that have neither the genetic nor environmental factor; cases (3) and controls (4) with the genetic factor but not the environmental factor; cases (5) and controls (6) with the environmental factor but not the genetic factors; and cases (7) and controls (8) with both the genetic factor and the environmental factor. Such a design allows the estimation of the effect of the gene, the effect of the environmental factor, and the effect of both factors combined, allowing a determination of any interaction effects. In the published studies that have evaluated potential modifying factors of *BRCA1* or *BRCA2*, none has included subjects with all eight of the possible combinations of disease status, gene, and environmental exposure.

The design that has been used most frequently in the reports published to date, e.g. [6, 8, 9, 29] is a case–control study among carriers only, which is depicted in part A of Fig. 1. Risk factors are compared between mutation carriers affected with breast cancer and unaffected carriers. The

Fig. 1 Study designs for evaluating modifiers of *BRCA1*/*BRCA2*

A Case-Control Study among carriers: All study subjects have BRCA1 (or BRCA2) mutation

		Breast Cancer	
		Yes	No
Risk Factor	Yes		
	No		

B Case-only Design. All study subjects have breast cancer

		BRCA1 (or BRCA2) Mutation	
		Yes	No
Risk Factor	Yes		
	No		

logORs generated in these studies describe the association with a given risk factor within a population of mutation carriers; hence they correspond to the sum of the population logOR of D given E and the population interaction logOR of D given E and G in the classical case–control or cohort study. If the associations are similar to established risk factors for sporadic breast cancer, the data may be interpreted as no evidence of gene by environment interaction. If the estimated associations are different, it is suggestive of interaction between the genetic and environmental factor.

The case-only design used in this study, which is depicted in part B of Fig. 1, compares risk factors between breast cancer cases who have a *BRCA* mutation and those who do not. The case-only odds of environmental exposure given genotype is mathematically equivalent to the IRR for disease given genotype and environmental exposures when those variables are independent in the population [24, 25]. When this assumption is met, the case-only design has two distinct advantages over a full case–control study for estimating gene by environment interaction: it provides direct estimates of the IRR and is more efficient, requiring fewer subjects.

Some investigations have combined aspects of both designs in case–control studies with multiple case groups, in which both case–control comparisons and case-only comparisons were made [20, 21]. In these studies, case groups were defined by *BRCA1* and/or *BRCA2* genotype and the controls were all *BRCA*− (either based on testing or presumed to be negative if untested) [20, 21]. Lee [20] estimated odds of disease given a variety of environmental exposures for each case group and tested for heterogeneity in effects (which would suggest gene–environment interaction/modification). In addition, they carried out case-only analyses to formally test for modifiers. The report by Tryggvadottir et al. [21], which focused on the 999del5 Icelandic *BRCA2* founder mutation, took a similar analytic tack in terms of utilizing case-only analysis to test for modifiers.

The different parameters estimated with the case–control design and the case-only design must be kept in mind when interpreting results from this study and comparing these results to other published studies. Only when (1) G and E are independent in the population conditional on the variables adjusted for in the modeling (age) and (2) the disease is rare for all combinations of the covariates can the case-only IRR be compared to an interaction OR generated by a classical case–control or cohort study. Because assumption 2 is clearly not met for *BRCA1* or *BRCA2* carriers, the IRRs we estimate are not directly comparable to interaction ORs but remain interpretable as RRs [25].

Our data on pregnancy characteristics suggest that there is a weaker protective effect of pregnancy among mutation carriers than non-carriers. There was no evidence of significant risk modification with age at first pregnancy and *BRCA* mutation status. Several publications have evaluated the effect of pregnancy characteristics on breast cancer risk in mutation carriers. A recent article describing the association between parity and breast cancer in *BRCA1* and *BRCA2* mutation carriers summarized the published literature [29]. When considering parity, results ranged from statistically significant inverse relationships among *BRCA1* carriers [9] or pooled *BRCA1* and *BRCA2* carriers [30], to non-statistically significant inverse relationships among *BRCA1* or *BRCA2* carriers [8, 9, 20, 29] to statistically significant positive relationships among *BRCA1* [19] carriers. Similar inconsistencies in results were observed for age at first birth. Inverse associations with age at first birth were observed for *BRCA2*+ breast cancer in two studies [9] whereas no association was reported in other studies [20, 21, 29]. For *BRCA1*+ breast cancer, one study reported a statistically significant positive association with age at first birth [9] and two others reported no association [9, 29]. There is no clear reason for the disparate findings between studies.

Other characteristics that we identified within our study as being potential modifiers are age at menarche in *BRCA2* carriers and alcohol consumption in *BRCA1* carriers. Our finding of a statistically significant IRR for *BRCA2*+ cancers (IRR = 1.65, 95% CI 1.04–2.61) suggests that later age at menarche is not as protective for *BRCA2*+ cancers as for *BRCA*− cancers. Several other published studies have reported on age at menarche in relation to breast cancer given *BRCA* mutation status [10, 16, 19–21]. In one case–control study of carriers only, age at menarche was not statistically significantly associated with breast cancer risk in either *BRCA1*+ or *BRCA2*+ carriers [10] whereas two other studies reported inverse associations

with age at menarche for *BRCA1*+ cancers [16, 19]. The studies by Lee et al. [20] and Tryggvadottir et al. [21] each conducted case-only analyses. Lee et al. [20] reported a significant IRR for age at menarche ≥14 years, when comparing *BRCA1/2*+ cases to *BRCA*− cases, but a non-significant *P* value for trend. They observed no protective effect of later age at menarche among *BRCA2* carriers, albeit with a small sample size, which is consistent with results from this study. Tryggvadottir et al. reported that the interaction between age at menarche and *BRCA2* mutation status was not statistically significant [21].

To our knowledge, only one previous report, a case–control study among carriers, has described a relation between alcohol and *BRCA1*+ and *BRCA2*+ breast cancer [12], and their results were not consistent with our findings. McGuire et al. [12] reported that ever use of alcohol was inversely associated with *BRCA2*+ cancer compared to unaffected carriers, but there was no trend between amount of alcohol and breast cancer risk. No significant associations were found with *BRCA1*+ cancer.

To date, the results of studies that have attempted to identify modifiers of *BRCA1* or *BRCA2* do not present a consistent picture of any factor that appears to interact with these genes. There are a number of possible reasons that various studies have reached different conclusions. Some of the inconsistency may be related to small sample sizes [22, 23]. There is also variability in the characteristics of the cases included in the studies. Some early reports that included both *BRCA1* and *BRCA2* mutation carriers did not perform separate analyses for *BRCA1* and *BRCA2*. However, the evidence is quite compelling that *BRCA1*+ cancers have distinct clinical characteristics. Presuming that they arise from distinct biological pathways, differences in risk factors would be obscured when *BRCA1*+ and *BRCA2*+ cancers are evaluated as a single entity.

Other studies have been conducted within select populations, for example Tryggadottir et al. from Iceland [21] or Gronwald et al. from Poland [19] in which there is a preponderance of certain founder mutations that are distinct from those in other populations. Among studies that are multi-center collaborations with cases derived from a variety of populations, including this study, there is a much greater spectrum of distinct mutations within the genes. If different factors act as modifiers of different mutations, it will be extremely challenging to detect such modifiers.

Our data provide evidence that the distributions of age at menarche, number of pregnancies, and alcohol consumption vary between breast cancer-affected carriers of a *BRCA1* or *BRCA2* mutation, suggesting these factors are potential modifiers of *BRCA1* and/or *BRCA2* mutations. However, the limitations of this study should be considered when interpreting the results of the analyses. Evaluations of potential modifiers of *BRCA1* and *BRCA2* rely on multi-center collaborations to achieve reasonable sample sizes, but the use of breast cancer cases from multiple sites also introduces variability that may not be completely controlled for in statistical analyses, including differences in testing methods, criteria for testing individuals, and the type of epidemiologic data collected.

The women included in this study were tested for *BRCA1* or *BRCA2* mutations over a broad time span using a variety of methods, including full sequencing with or without the use of large deletion/duplication testing, the Jewish panel only or family mutations only. It is possible that there were mutations among the test-negative cases that were not detected because of the testing methods used. The sensitivity analyses we performed in which we excluded test-negative cases with a high BRCAPRO probability of being mutation positive did not suggest our results were strongly influenced by false negative results; however, the possibility of misclassification of mutation status remains a limitation.

It is also possible that the criteria for testing breast cancer cases for *BRCA1* or *BRCA2* mutations varied across the centers. All sites except the University of Southern California recruited women from high-risk clinics, but there may have been subtle differences in the characteristics of women tested across sites. Further, the recruitment from high-risk clinics means that the non-mutation carriers are a select sub-group of sporadic breast cancers, and they could have a distinct set of risk factors.

The epidemiologic data collected across the sites also varied such that we were limited in exploring the effects of certain environmental factors. For example, differences across sites in how information on alcohol consumption or smoking was collected precluded us from doing more detailed analyses of the duration or timing of these exposures.

Finally, although the number of subjects needed to detect statistically significant interactions is smaller with a case-only design, our analyses were limited by sample size considerations. With only 283 *BRCA1*+ and 204 *BRCA2*+ cases, it is possible that we had inadequate power to detect modifying factors that had modest effects.

Although we found evidence of potential modification of *BRCA1* and *BRCA2* by environmental factors, these results have to be interpreted in light of the overall lack of consistency in the literature regarding *BRCA1* and *BRCA2* modifiers. Much of this inconsistency is due, as we have shown, to use of different study designs and effect measures. The case-only design used in this study allows us to estimate IRRs, which cannot be directly compared to interaction ORs estimated from a traditional case–control study of breast cancer given exposure and *BRCA1* or *BRCA2* mutations status. While there are compelling reasons to suspect that there are environmental modifiers of

BRCA1 and *BRCA2*, the results from this study combined with the overall body of literature does not provide consistent evidence in result or form for any factor. Future work on the identification of modifiers of *BRCA1* and *BRCA2* will need to rely on collaborative efforts involving thousands of affected women.

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