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Common Genetic Variation at *BARD1* Is Not Associated with Breast Cancer Risk in *BRCA1* or *BRCA2* Mutation Carriers

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Abstract

Background: Inherited *BRCA1* and *BRCA2 (BRCA1/2)* mutations confer elevated breast cancer risk. Knowledge of factors that can improve breast cancer risk assessment in *BRCA1/2* mutation carriers may improve personalized cancer prevention strategies.

Methods: A cohort of 5,546 *BRCA1* and 2,865 *BRCA2* mutation carriers was used to evaluate risk of breast cancer associated with *BARD1* Cys557Ser. In a second nonindependent cohort of 1,537 of *BRCA1* and 839 *BRCA2* mutation carriers, *BARD1* haplotypes were also evaluated.

Results: The *BARD1* Cys557Ser variant was not significantly associated with risk of breast cancer from single SNP analysis, with a pooled effect estimate of 0.90 (95% CI: 0.71–1.15) in *BRCA1* carriers and 0.87 (95% CI: 0.59–1.29) in *BRCA2* carriers. Further analysis of haplotypes at *BARD1* also revealed no evidence that additional common genetic variation not captured by Cys557Ser was associated with breast cancer risk.

Conclusion: Evidence to date does not support a role for *BARD1* variation, including the Cy557Ser variant, as a modifier of risk in *BRCA1/2* mutation carriers.

Impact: Interactors of BRCA1/2 have been implicated as modifiers of *BRCA1/2*-associated cancer risk. Our finding that *BARD1* does not contribute to this risk modification may focus research on other genes that do modify *BRCA1/2*-associated cancer risk. *Cancer Epidemiol Biomarkers Prev; 20(5); 1032–38. ©2011 AACR.*

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Introduction

There is substantial interindividual variability in age at cancer diagnosis in *BRCA1* and *BRCA2* mutation carriers, which persists even among relatives that carry the same *BRCA1* and *BRCA2* mutation (1). Variation in genes that interact with *BRCA1* and *BRCA2* in the recognition and repair of DNA damage are strong candidates for study as genetic modifiers of *BRCA1* and *BRCA2* cancer risk. The *BRCA1–BARD1* heterodimer is known to be important for *BRCA1* function, with interaction mediated through the ring finger domains of the 2 proteins (2). In addition, although there is no evidence for a direct interaction between *BARD1* and *BRCA2*, they do operate in the same DNA repair processes, exemplified by the fact that the *BRCA2* partner RAD51, *BARD1*, and *BRCA1* all relocate to proliferating cell nuclear antigen structures after irradiation (3).

The *BARD1* Cys557Ser SNP (rs28997576) was first reported as a germ line alteration in a sporadic breast/uterine tumor (4). This variant lies between the ankyrin repeats and BRCT domains of *BARD1*, and the ectopically expressed Cys557 protein has growth suppression and proapoptotic effects relative to 557Ser (5). This SNP (minor allele frequency in Europeans: 0.025) has been reported to be associated with both breast cancer in the general population and familial breast cancer, but results have not shown consistent across all studies (6–12). Stacey and colleagues (6) initially reported that the Cys557Ser variant was associated with increased breast cancer risk in 756 Icelandic mutation carriers who carry *BRCA2* 999del5 founder mutation [OR = 3.1; 95% CI: 1.2–8.4]. However, subsequent studies reported no elevated risk in 228 Nordic *BRCA1* and *BRCA2* carriers (OR = 0.8, 95% CI: 0.3–2.0; ref. 8), or in 1,207 Polish *BRCA1* mutation carriers (OR = 0.9, 95% CI: 0.4–2.2; ref. 10). There have been no previous haplotype-based studies assessing the role of *BARD1* variation in breast cancer risk in *BRCA1* and *BRCA2* carriers specifically.

To resolve whether *BARD1* is a modifier of *BRCA1* and *BRCA2*-associated breast cancer risk, we undertook a large study to comprehensively assess the association of *BARD1* Cys557Ser as well as haplotypic variation with cancer risk in *BRCA1* and *BRCA2* carriers.

Materials and Methods

Study sample

The design for this study has been described in detail previously (13). Briefly, eligible participants included adult women with documented disease-associated inherited mutations in *BRCA1* or *BRCA2*. Mutations were included in the analysis if they were pathogenic according to generally recognized criteria (14, 15). Two overlapping cohorts of women with disease-associated *BRCA1* and *BRCA2* mutations were identitied (Table 1). First, a cohort of 5,546 *BRCA1* and 2,865 *BRCA2* mutation carriers from the multicenter CIMBA consortium (13) was used to evaluate risk of breast cancer associated with *BARD1* Cys557Ser. Second, a cohort of 1,537 of *BRCA1* and 839 *BRCA2* mutation carriers participating in the MAGIC consortium was used to further explore the relationship between *BARD1* haplotypes and breast cancer risk. Recruitment and genetic studies were approved by relevant ethics committees at all sites, and informed consent was obtained from each participant.

Laboratory methods

For analysis of the *BARD1* Cys557Ser SNP, existing genotype data from *BRCA1* and *BRCA2* mutation carriers was requested from members of the CIMBA consortium. The primary methods used for genotyping were Sequenom iPlex (EMBRACE, -HEBON, kConFab, SWE-BRCA, PISA, Penn, Austria, Mayo, FCCC, GEMO, Georgetown, HEBCS) and by Taqman assays (OUH, Baylor, Beth Israel, City of Hope, Creighton, Dana Farber, NorthShore, IHCC, UCLA, University of Chicago, University of Texas Health Science Center, University of Utah, and Women's College Hospital; ref. 16) Genotypes for the INHERIT samples were typed by direct sequencing using an ABI Prism 3730xl DNA Analyser automated sequencer, with version 3.1 of the Big Dye fluorescent method according to the manufacturer's instructions (Applied Biosystems). Sequence data were analyzed using the Staden preGap4 and Gap4 programs. Samples from IHCC were typed by PCR-RFLP (10). SNP quality control measures included more than 95% success rate, Hardy–Weinberg Equilibrium $P > 0.005$. In addition, concordance of more than 98% for duplicate samples was required for studies that had included 2% duplicated samples for quality control purposes (all studies undergoing Sequenom iplex for

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Table 1. Sample description and Cys557Ser association

Center	Data in Cys557Ser analysis		Data in haplotype analysis	
	BRCA1	*BRCA2*	*BRCA1*	*BRCA2*
Austria	285	122	196	60
Baylor			14	1
Beth Israel			7	12
City of Hope			68	42
Creighton			180	41
Dana Farber			90	38
EMBRACE	827	638		
NorthShore			31	20
Fox Chase	85	57	40	26
Georgetown	33	16	60	29
GEMO	1,140	559		
HEBON	777	294		
HEBCS	102	104		
IHCC	397			
INHERIT	73	82		
KConFab	506	400	379	302
MAGIC[a]	583	300		
Mayo	218	118	58	31
OUH	33	13		
Penn			202	98
Pisa (PBCS)	73	41		
Swe-BRCA	414	121		
UCLA			32	14
Univ of Chicago			27	11
UT Southwestern			28	19
Utah			99	78
Women's College			26	17
Total	5,546	2,865	1,537	839
Cys557Ser association	0.90 (0.71–1.15)	0.87 (0.59–1.29)	ND	ND

Abbreviation: ND, not done.
[a]Indicates MAGIC data included in single SNP analysis only.

BARD1 Cys557Ser, and all samples included in the haplotype substudy).

For studies of *BARD1* haplotypic variation, 11 haplotype tag SNPs were identified and assayed at the University of Pennsylvania as previously described (16). The rs IDs were as follows: rs6712055, rs16852689, rs280621, rs13021937, rs13423596, rs10190829, rs6751923, rs4234006, rs28997576, rs3768708, rs1374230.

Statistical methods

To assess the relationship between *BARD1* SNPs and breast cancer risk, proportional hazards models were used as previously described (16, 17). Briefly, participants were followed from the time of genetic testing or study ascertainment until the first diagnosis of breast cancer (the primary event in this analysis) or were censored at ovarian cancer. Participants who developed breast cancer were censored at bilateral prophylactic mastectomy if it occurred more than a year prior to the cancer diagnosis. This is to avoid censoring at bilateral mastectomies at which occult tumors were detected, but ages are rounded. The remaining participants were censored at the age at last observation. To address the problem of nonrandom sampling of mutation carriers with respect to the disease phenotype, analyses used the weighted Cox regression approach (17), where affected and unaffected individuals were differentially weighted such that observed breast cancer incidence rates in the study sample are consistent with established breast cancer risk estimates for *BRCA1* and *BRCA2* mutation carriers (18). Analyses assessing the association of the *BARD1* Cys557Ser SNP combined heterozygote and homozygote variant carriers under a dominant model because of the rare frequency of this variant. Analyses were assessed separately for *BRCA1* and *BRCA2* mutation carriers, adjusted for Study group, ethnicity (non-Jewish Caucasian, Jewish or other), and

Table 2. Analysis of *BARD1* haplotype data: failure time analyses stratified on mutation using the MAGIC consortium data[a]

Haplotypes detected in *BRCA1* carriers[b]

Ht no.	rs280621	rs28997576	rs1374230	rs10190829	rs4234006	rs16852689	rs3768708	rs6712055	rs13021937	Freq	HR	95% CI	
B1Ht1	T	**G**	C	T	G	C	A	T	T	0.268	1.00	Reference	
B1Ht2	C	**G**	T	A	G	C	A	T	T	0.011			
B1Ht3	T	**G**	T	A	G	C	G	T	T	0.008			
B1Ht4	T	**G**	C	A	G	C	A	C	T	0.009			
B1Ht5	T	**G**	T	A	A	T	A	T	T	0.047			
B1Ht6	T	**G**	T	T	A	C	G	T	C	0.009	1.07[b]	0.85	1.35
B1Ht7	C	**G**	T	A	A	C	A	T	C	0.038			
B1Ht8	C	**C**	T	A	G	C	A	T	T	0.025			
B1Ht9	T	**G**	C	A	G	C	A	T	T	0.005			
B1Ht10	C	**G**	T	T	A	C	G	T	C	0.008			
B1Ht11	T	**G**	C	T	G	C	A	C	C	0.019			
B1Ht12	T	**G**	T	A	G	C	A	C	T	0.056	1.15	0.82	1.62
B1Ht13	T	**G**	T	A	G	C	A	T	T	0.152	0.97	0.76	1.24
B1Ht14	T	**G**	T	A	G	C	G	C	C	0.203	1.00	0.80	1.26
B1Ht15	T	**G**	T	T	A	T	A	T	T	0.062	1.33	0.97	1.84
B1Ht16	T	**G**	T	T	G	C	A	T	T	0.052	0.99	0.66	1.48

Haplotypes detected in *BRCA2* carriers[b]

Ht#	rs280621	rs28997576	rs1374230.	rs6751923	rs16852689	rs13423596	rs3768708	rs6712055	rs13021937				
B2Ht1	T	**G**	C	T	C	G	A	T	T	0.270	1.00	Reference	
B2Ht2	T	**G**	T	C	C	G	A	T	T	0.016			
B2Ht3	C	**G**	T	T	C	G	G	T	C	0.012			
B2Ht4	C	**G**	T	C	C	G	A	T	C	0.043			
B2Ht5	T	**G**	T	C	C	G	A	T	C	0.004			
B2Ht6	T	**G**	T	C	C	G	A	C	T	0.049			
B2Ht7	T	**G**	C	C	C	G	A	C	T	0.020			
B2Ht8	C	**G**	T	C	C	G	A	T	T	0.008	0.88[b]	0.64	1.21
B2Ht9	T	**G**	C	T	C	G	A	C	C	0.020			
B2Ht10	C	**C**	T	T	C	A	A	T	T	0.019			
B2Ht11	T	**G**	T	T	C	G	G	T	T	0.007			
B2Ht12	T	**G**	T	T	C	G	G	C	T	0.004			
B2Ht13	T	**G**	T	T	C	G	G	T	C	0.018			
B2Ht14	T	**G**	T	T	C	G	A	T	T	0.172	0.82	0.54	1.25
B2Ht15	T	**G**	T	T	C	G	G	C	C	0.191	1.10	0.79	1.52
B2Ht16	T	**G**	T	T	T	A	A	T	T	0.062	0.62	0.37	1.06
B2Ht17	T	**G**	T	T	T	G	A	T	T	0.064	0.72	0.46	1.13

[a]The Cys557Ser variant is highlighted in bold.

[b]Haplotypes with frequency less than 5% were combined into a single "rare haplotype" analysis group.

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year of birth cohort (decade of birth, categorized as <1940, 1940–1949, 1950–1959, 1960–1969, 1970–1989). There were 3,047 breast cancer events of 5,546 total for BRCA1 (55%) and 1,578 breast cancer events of 2,865 total for BRCA2 (55%) for the Cys557Ser censored analysis datasets. The remainders were censored for analysis. Secondary analyses adjusted for prophylactic oophorectomy, or assessed risk for the subset of carriers with mutations determined to result in unstable transcripts/proteins (class 1 loss of function mutations). R version 2.7.0 was used for single SNP statistical analyses.

To investigate haplotype effects, the Estimation-maximization algorithm (19, 20) was used to estimate haplotype frequencies as implemented in R version 2.1.1 subroutine haplo.em (21) as previously described (16). In this analysis, we included 607 breast cancer cases and 863 censored observations for BRCA1, and 813 breast cancer cases and 423 controls for BRCA2.

Results and Discussion

The frequency of the Cys557Ser SNP in the combined dataset (Table 1) was similar to published reports, with 4.4% of individuals carrying at least 1 rare allele (4.5% in *BRCA1* carriers, 4.2% in *BRCA2* carriers). There were no significant associations of Cys557Ser and breast cancer risk for carriers of *BRCA1* mutations (HR = 0.90, 95% CI: 0.71–1.15) or *BRCA2* mutations (HR = 0.87, 95% CI: 0.59–1.29). There was no evidence for heterogeneity by center for either *BRCA1* or *BRCA2* analyses ($P > 0.5$). There was also no evidence for association with additional adjustment for prophylactic oophorectomy, or when analyses were restricted to Class 1 mutations. For example, the HR for the subset of 3,882 individuals with *BRCA1* Class 1 mutations was 0.84 (0.62–1.15), and for the 2,668 individuals with *BRCA2* class 1 mutations was 0.96 (0.64–1.45).

For the haplotype analysis (Table 2), we also observed no overall effect of variation at *BARD1* in either *BRCA1* false discovery rate (FDR-corrected value of $P = 0.152$) or *BRCA2* (FDR-corrected value of $P = 0.134$). No single *BARD1* haplotype was significantly associated with breast cancer risk. Cys557Ser is represented by SNP 16 in Table 2 (*BRCA1* haplotype 8 and *BRCA2* haplotype 10). Since this variant was relatively rare (approximately 2% in both *BRCA1* and *BRCA2* carriers), estimates of its effect were not made in our primary analysis. When we fit a model that allowed the estimation of effects for haplotypes with at least 1% frequency in controls, no single haplotype was significantly associated with risk. The haplotype that contained the 557Ser allele was also not significantly associated with risk in either *BRCA1* (HR = 0.91, 95% CI: 0.45–1.85) or *BRCA2* (HR = 0.69, 95% CI: 0.28–1.72). Indeed, neither of these estimates was associated with increased risk of breast cancer as previously reported.

The data presented here do not provide evidence that neither the *BARD1* Cys557Ser SNP nor additional haplotypic variability not captured by Cys557Ser is associated with breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. Our sample size had more than 99% power to detect the effect size reported by Stacey and colleagues (6) of OR = 3.1. The study had more than 80% power to detect risk ratios of 0.89 (or 1.13) for *BRCA1* carriers and 0.86 (or 1.17) for *BRCA2* carriers. The upper 95% confidence limits on the rate ratio in our analysis exclude any substantial risk.

Conclusion

Our study found no evidence to support substantial associations of *BARD1* variation with increased breast cancer risk in *BRCA1* and *BRCA2* carriers.

Disclosure of Potential Conflicts of Interest

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