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Evaluation of chromosome 6p22 as a breast cancer risk modifier locus in a follow-up study of *BRCA2* mutation carriers

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Abstract

Several common germline variants identified through genome-wide association studies of breast cancer risk in the general population have recently been shown to be associated with breast cancer risk for *BRCA1* and/or *BRCA2* mutation carriers. When combined, these variants can identify marked differences in the absolute risk of developing breast cancer for mutation carriers, suggesting that additional modifier loci may further enhance individual risk assessment for *BRCA1* and *BRCA2* mutation carriers. Recently, a common variant on 6p22 (rs9393597) was found to be associated with increased breast cancer risk for *BRCA2* mutation carriers [Hazard ratio (HR)=1.55, 95% CI 1.25–1.92, $p=6.0 \times 10^{-5}$]. This observation was based on data from GWAS studies in which, despite statistical correction for multiple comparisons, the possibility of false discovery remains a concern. Here we report on an analysis of this variant in an additional 6,165 *BRCA1* and 3,900 *BRCA2* mutation carriers from the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA). In this replication analysis, rs9393597 was not associated with breast cancer risk for *BRCA2* mutation carriers [HR=1.09, 95% CI 0.96–1.24, $p=0.18$]. No association with ovarian cancer risk for *BRCA1* or *BRCA2* mutation carriers or with breast cancer risk for *BRCA1* mutation carriers was observed. This follow-up study suggests that, contrary to our initial report, this variant is not associated with breast cancer risk among individuals with germline *BRCA2* mutations.

Keywords

BRCA1; *BRCA2*; genetic modifier; association study

Introduction

Common genetic variants have been shown to be associated with breast cancer risk for *BRCA1* and *BRCA2* mutation carriers and can be potentially used for risk modeling and assessment [1–8]. These variants include susceptibility alleles that achieved genome-wide significant associations ($p < 5 \times 10^{-8}$) with breast cancer in *BRCA1* mutation carriers (19p13 rs8170, $P_{\text{trend}} = 2.3 \times 10^{-9}$) [1] and in *BRCA2* mutation carriers (*FGFR2* rs2981582, $P_{\text{trend}} = 1.7 \times 10^{-11}$) [2]. Variants in other known breast cancer risk loci such as *TNRC9/TOX3* (rs3803662), *MAP3K1* (rs889312), *LSP1* (rs3817198), 2q35 (rs13387042), *SLC4A7/NEK10* (rs4973768), 5p12 (rs10941679) and *ZNF365* (rs16917302) have also been found to display moderately significant associations with breast cancer in *BRCA2* mutation carriers [2, 4–6]. Similarly, variants in the *TNRC9/TOX3* 2q35, *ESR1*, and *PTHLH* loci have been moderately associated with risk for *BRCA1* mutation carriers [5, 7, 8]. These findings suggest that the common genetic factors involved in susceptibility to breast cancer in the general population may also influence risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers, although there appears to be little overlap in the breast cancer risk modifiers for women with *BRCA1* and *BRCA2* mutations. Furthermore, risk prediction models for *BRCA2* mutation carriers incorporating common risk modifier loci suggest that the combined effects of these SNPs can distinguish marked differences in the absolute risk of developing breast cancer [5].

Based on the observation that common breast cancer susceptibility loci influence risk for *BRCA1* and *BRCA2* mutation carriers, we recently reported a study evaluating SNPs identified from two breast cancer genome-wide association studies (GWAS) and risk of breast cancer in *BRCA1* (n=3,451) and *BRCA2* (n=2,006) mutation carriers in the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) [3]. We found an association between a locus at chromosome 6p22 (rs9393597) and risk of breast cancer in *BRCA2* mutation carriers (Hazard ratio (HR)=1.55; 95% Confidence interval (CI) 1.25–1.92; $p=6.0 \times 10^{-5}$). Since that time, women from an additional 19 CIMBA study centers have been genotyped for this variant for a total 6,165 *BRCA1* and 3,900 *BRCA2* mutation carriers. Here, we further evaluate this SNP in this larger cohort of CIMBA *BRCA1* and *BRCA2* mutation carriers to attempt to replicate our findings in an independent cohort of women.

Materials and Methods

Subjects

Female carriers of deleterious mutations in *BRCA1* and *BRCA2* were identified through CIMBA. A total of 28 studies from 19 countries provided clinical data, risk factor data, and rs9393597 genotypes from *BRCA1* and *BRCA2* mutation carriers (Table 1, Supplementary Table 1). Eligibility for CIMBA participation was restricted to women with deleterious *BRCA1* or *BRCA2* mutations over age 18 years at the time of ascertainment [3]. Information was collected on year of birth, age at last follow up or bilateral prophylactic oophorectomy and/or mastectomy, age at breast and/or ovarian cancer diagnosis, mutation description and country of residence. Related individuals were attributed common family identifiers. All contributing studies received approvals from institutional review committees at their host institutions.

SNP genotyping

DNA samples from 10 studies were genotyped for rs9393597 using the iPLEX Mass Array (Sequenom, San Diego, CA, USA). Another 21 studies genotyped samples using the 5' endonuclease assay (TaqMan) (Applied Biosystems, Carlsbad, CA, USA) using reagents

tested centrally. Genotyping centers included at least 2% duplicate samples and no template controls in every plate. Samples from affected and unaffected carriers were arranged randomly. All studies exhibited SNP call rates >95% and duplicate concordance >98%. To assess the accuracy of genotyping, a standard set of 95 Coriell DNA samples (HAPMAPP01) were genotyped by each genotyping center. All centers displayed >98% concordance for the Coriell samples. From the 12,128 *BRCA1* and *BRCA2* mutation carriers with genotyping data, 639 were excluded because of failure to meet eligibility criteria; missing follow-up information (n=15), overlapping samples between study groups (n=168), poor genotyping quality (n=421), discordant genotypes (n=13), mutation carrier in both *BRCA1* and *BRCA2* (n=22). A further 646 subjects were excluded due to non-European ethnicity. Further evaluation of the data identified one study with significant deviation from Hardy–Weinberg equilibrium (HWE) ($p=1\times 10^{-6}$) for rs9393597 in *BRCA1* carriers (n=879). All genotypes from this study were excluded, leaving 6,165 *BRCA1* and 3,900 *BRCA2* mutation carriers for analysis.

Statistical Analyses

Associations between rs9393597 and breast cancer risk for *BRCA1* and *BRCA2* mutation carriers were evaluated using a weighted Cox proportional hazards models [9]. For breast cancer analyses, each subject was followed from birth to the earliest event: breast cancer, bilateral mastectomy, ovarian cancer, last follow-up, or age 80 years. To adjust for the non-random sampling of mutation carriers with respect to disease phenotype, individuals were assigned weights based upon mutation status, breast cancer affected/unaffected status, and age at event or age at censoring [9], such that the weighted breast cancer incidence rates were consistent with established breast cancer risk estimates for *BRCA1* and *BRCA2* mutation carriers. For ovarian cancer analyses, separate disease-specific weights were applied and prophylactic oophorectomy replaced prophylactic mastectomy as a censoring variable [10]. All analyses were stratified by year of birth (based on quartiles of unaffected), ethnicity, and country of residence. A robust variance approach was used to estimate the standard errors of the parameters to allow for the dependence of individuals from the same family. Primary tests for association between rs9393597 and cancer risk were performed under a log-additive model. Hazard ratio estimates were obtained to summarize the relative risk of cancer for heterozygous and separately homozygous carriers of the minor allele of rs9393597. Heterogeneity among study sites was assessed by including a genotype by study interaction term in the model and testing for differences in the hazard ratios by study with a multiple degree-of-freedom test of significance. In sensitivity analyses, the data were analyzed while treating prophylactic oophorectomy as a time-dependent covariate and the influence of survival bias was explored by excluding subjects diagnosed with breast cancer more than five years prior to age at recruitment.

Results

In total, 6,165 *BRCA1* and 3,900 *BRCA2* mutation carriers were eligible for the pooled analysis of associations between rs9393597 and risk of breast and ovarian cancer. Characteristics of the eligible mutation carriers, after quality control exclusions, are summarized in Table 1. In breast cancer analyses, 3,120 *BRCA1* and 2,188 *BRCA2* mutation carriers were followed until the first breast cancer diagnosis and 3,045 *BRCA1* and 1,712 *BRCA2* mutation carriers were followed until bilateral prophylactic mastectomy, ovarian cancer or age at last follow up. Among the 3,900 women with *BRCA2* mutations, the minor (G) allele of rs9393597 was not significantly associated with breast cancer risk [HR=1.09, 95% CI 0.96–1.24, $p=0.18$] (Table 2). Consistent with our original report, there was no association between rs9393597 and risk of breast cancer among *BRCA1* mutation carriers [HR=0.98, 95% CI 0.90 – 1.07, $p=0.68$] (Supplementary Table 2).

To evaluate the possibility of survival bias, we excluded all cases diagnosed with breast cancer more than five years before recruitment in the overall dataset (Table 2). The association between rs9393597 and breast cancer risk remained non-significant [HR=1.03, 95% CI 0.88–1.19, $p=0.74$]. To assess the influence of risk-reducing oophorectomy (RRO), which is known to reduce the risk of breast cancer in mutation carriers by as much as 50% [11], RRO was next included as a time-dependent covariate in the analysis. We did not observe a substantial change in the estimate of significance of the rs9393597 association with breast cancer [HR=1.07, 95% CI 0.94–1.23, $p=0.27$] (Table 2). Similar analyses showed no significant associations in *BRCA1* mutation carriers (Supplementary Table 2). Since *BRCA1* and *BRCA2* mutations are also associated with increased risk of ovarian cancer, we next evaluated associations with ovarian cancer by following subjects until the first ovarian cancer diagnosis or until the earliest of bilateral prophylactic oophorectomy, breast cancer or age at last follow-up. No significant associations with ovarian cancer were observed for either *BRCA1* [HR=1.10, 95% CI 0.96–1.27, $p=0.18$] or *BRCA2* [HR=1.18, 95% CI 0.88–1.58, $p=0.28$] mutation carriers (Table 2, Supplementary Table 2).

Discussion

The SNP rs9393597 in the *LOC134997* locus on chromosome 6p22 has previously been shown to be associated with breast cancer risk for *BRCA2* mutation carriers [HR=1.55, 95% CI 1.25–1.92, $p=6\times 10^{-5}$] [3], although it has never been reported to be associated with breast cancer risk in the general population. Here we re-evaluated the association between rs9393597 and breast cancer in a total of 6,165 *BRCA1* and 3,900 *BRCA2* mutation carriers from 28 CIMBA study centers to replicate the association and to more accurately estimate the influence of the SNP on breast cancer risk. We found that the variant is not associated with breast cancer risk for either *BRCA2* mutation carriers [HR=1.09, $p=0.18$] or *BRCA1* mutation carriers [HR=0.98, $p=0.68$]. Additional analyses exploring potential sources of bias, such as the exclusion of prevalent cases and accounting for risk-reducing oophorectomies, did not substantially alter our results. Further, this locus was not associated with ovarian cancer risk for either *BRCA1* or *BRCA2* mutation carriers.

Compared to our original findings [3], we had a nearly two-fold increase in sample size for both women with *BRCA1* (6,165 compared to 3,451 carriers) and *BRCA2* (3,900 compared to 2,006 carriers) mutations. This suggests that the lack of association seen in the current study is unlikely to be due to inadequate power, even for modest rs9393597 effect sizes. This study also utilized subjects from the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) as in the original report, such that genotyping and analyses were performed using the same robust quality control methods.

Variation in eight loci has been associated with modification of breast cancer risk for *BRCA2* mutation carriers [22, 4–6], exhibiting effects ranging from HR=1.10 for *MAP3K1* to HR=1.30 for *FGFR2*. However, we show here that rs9393597 does not influence breast cancer risk for women with either *BRCA1* or *BRCA2* mutations, and should not be used in risk prediction models. CIMBA continues to enroll *BRCA1* and *BRCA2* mutation carriers, so that the association between rs9393597 and breast cancer risk may be re-evaluated in an even larger cohort of mutation carriers. However, given the current results we conclude that the 6p22 locus is not an important risk modifier for women with *BRCA1* or *BRCA2* mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1Summary characteristics for 10,065 eligible *BRCA1* and *BRCA2* carriers

Characteristic	<i>BRCA1</i>		<i>BRCA2</i>	
	Affected	Unaffected	Affected	Unaffected
Number	3120	3045	2188	1712
Person-years follow-up	127,298	131,164	95,616	75,949
Median age at censor	40.0 (9.0)	42.0 (12.4)	42.3 (9.9)	43.0 (13.7)
Age at censor, <i>N</i> (%)				
<30	277 (8.9%)	424 (13.9%)	127 (5.8%)	256 (14.9%)
30–39	1258 (40.3%)	830 (27.3%)	694 (31.7%)	427 (24.9%)
40–49	1107 (35.5%)	973 (31.9%)	814 (37.2%)	477 (27.9%)
50–59	371 (11.9%)	530 (17.4%)	388 (17.7%)	310 (18.1%)
60–69	87 (2.8%)	188 (6.2%)	135 (6.2%)	161 (9.4%)
70+	20 (0.6%)	100 (3.3%)	30 (1.4%)	81 (4.7%)
Year of birth, <i>N</i> (%)				
<1920	17 (0.5%)	15 (0.5%)	20 (0.9%)	15 (0.9%)
1920–1929	104 (3.3%)	86 (2.8%)	90 (4.1%)	58 (3.4%)
1930–1939	268 (8.6%)	223 (7.3%)	252 (11.5%)	126 (7.4%)
1940–1949	692 (22.2%)	467 (15.3%)	506 (23.1%)	241 (14.1%)
1950–1959	1058 (33.9%)	773 (25.4%)	725 (33.1%)	392 (22.9%)
1960+	981 (31.4%)	1481 (48.6%)	595 (27.2%)	880 (51.4%)
Mutation class, <i>N</i> (%)				
Class 1	2106 (67.5%)	2292 (75.3%)	2029 (92.9%)	1623 (94.9%)
Class 2	792 (25.4%)	599 (19.7%)	50 (2.3%)	29 (1.7%)
Other	220 (4.9%)	153 (5.0%)	105 (4.8%)	58 (3.4%)

Table 2Associations between rs9393957 and risk of cancer in *BRCA2* mutation carriers.

Analysis	Genotype	Unaffected	Affected	HR	95% C.I.	Ptrend
Breast cancer	A/A	1186	1514	1.00		
	A/G	487	600	1.02	0.88–1.18	
	G/G	39	74	1.52	1.01–2.29	
	per allele	1712	2188	1.09	0.96–1.24	0.18
Excluding prevalent cases	A/A	1186	788	1.00		
	A/G	487	298	0.91	0.76–1.09	
	G/G	39	47	1.15	0.92–1.38	
	per allele	1712	1133	1.03	0.88–1.19	0.74
Influence of oophorectomy	A/A	1194	1491	1.00		
	A/G	481	591	1.01	0.87–1.18	
	G/G	37	74	1.19	0.97–1.48	
	per allele	1712	2156	1.07	0.94–1.23	0.27
Ovarian cancer	A/A	2234	205	1.00		
	A/G	917	94	1.27	0.88–1.82	
	G/G	101	9	0.99	0.37–2.61	
	per allele	3252	308	1.18	0.88–1.58	0.28