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Abstract

Recent genome-wide scans identified several novel breast cancer risk alleles, including variants of the *FGFR2*, *MAP3K1*, and *LSP1* genes, and a study of associations between these alleles and characteristics of breast cancer patients reported a borderline significant correlation between the number of *FGFR2* minor alleles and family history of breast/ovarian cancer. Given these results and similarities in the etiology of breast and ovarian cancer, we examined the association between seven novel breast cancer susceptibility alleles and epithelial ovarian cancer risk in two large study populations. Our analysis included 1,173 cases and 1,201 controls from a New England-based case-control study and 210 cases and 603 controls from the prospective Nurses' Health Study. We used logistic regression to estimate the odds ratio (OR) for individuals heterozygous or homozygous for the minor allele at each locus, compared to individuals with the wild-type genotype. We examined the associations separately in each population and, after testing for heterogeneity in the results, pooled the estimates using a random effects model. There was no clear association between these polymorphisms and ovarian cancer risk in either population. The pooled, per allele OR for *FGFR2* was 1.06 (95% confidence interval [CI]=0.95–1.18) for rs1219648 and 1.04 (95%CI=0.93–1.15) for rs2981582. We had over 80% power to detect a log-additive OR of 1.16–1.18 per allele at the alpha=0.05 level in the pooled analysis. Our results do not provide strong support for an association between these breast cancer susceptibility alleles and epithelial ovarian cancer risk.

INTRODUCTION

Women with inherited mutations in the *BRCA1* and *BRCA2* tumor suppressor genes have a greatly increased risk of both breast and ovarian cancer.1 Additionally, hormonal and reproductive exposures influence the risk of both cancers,2 suggesting that carcinogenesis of the breast and ovaries may involve some similar pathways. However, despite these parallels in the etiology of breast and ovarian cancer, no non-*BRCA* germline mutation has been clearly associated with risk of both cancers.

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Three recent genome-wide association studies identified several novel risk alleles for breast cancer.[3]–[5] Two of these studies reported strong positive associations with single nucleotide polymorphisms (SNPs) in intron 2 of *FGFR2*; the odds ratio (OR) for each minor allele was 1.26 (95% confidence interval [CI]=1.23–1.30) for rs2981582 in a study by Easton and colleagues and 1.32 (95% CI=1.17–1.49) for rs1219648 in a study by Hunter and colleagues. [3, 4] Easton et al. also reported slightly weaker but statistically significant positive associations with SNPs in the *MAP3K1* and *LSP1* genes, in a region near the *TNRC9* gene (LOC643714), and in a non-coding region on chromosome 8q.[3] Stacey et al. reported a positive association with the LOC643714 variant, as well as with a SNP in a non-coding region on chromosome 2q35.[5]

A subsequent study of clinical correlates of several of these breast cancer risk alleles reported a borderline significant correlation between the number of *FGFR2* minor alleles and family history of breast/ovarian cancer.[6] However, to our knowledge, no previous studies of the association between these variants and ovarian cancer risk have been published. We therefore analyzed the association between seven novel breast cancer susceptibility alleles and risk of epithelial ovarian cancer in two study populations with a total of 1,383 cases.

MATERIALS AND METHODS

New England Case-Control Study

The New England Case-Control Study (NECC) includes 1,231 epithelial ovarian cancer cases and 1,244 population-based controls from Massachusetts and New Hampshire. Participants were enrolled in the study in two phases, from May 1992 to March 1997 (563 cases, 523 controls) or from July 1998 to July 2003 (668 cases, 721 controls). Recruitment methods and eligibility criteria are described elsewhere.[7] Briefly, trained interviewers asked participants about exposures that occurred >1 year prior to the date of diagnosis for cases or >1 year prior to the interview date for controls. The institutional review boards of Brigham and Women's Hospital and Dartmouth Medical School approved both phases of the study, and all participants provided written informed consent.

Of 2,347 incident cases of ovarian cancer identified through hospital tumor boards and state cancer registries, 1,845 (79%) were eligible and 1,306 (71% of the eligible cases) were enrolled. Controls were identified using random digit dialing supplemented with town resident lists during phase 1 of enrollment, and drivers' license records and town resident lists during phase 2 of enrollment. Controls were frequency-matched to cases by age and state. During phase 1, 72% of the potentially eligible controls contacted by random digit dialing agreed to participate (n=421). Of 328 additional women identified using town resident lists, 21% could not be reached, 18% were ineligible, 30% declined to participate, and 31% enrolled in the study (n=102). Of 1,843 potential controls identified using drivers' license records and town resident lists during phase 2, 576 were ineligible, 546 declined to participate by phone or by mail via an "opt-out" postcard, and 721 were enrolled. Additional details of the control selection are published elsewhere.[7]

Over 95% of study participants provided a blood specimen at enrollment, and the heparinized samples were separated into plasma, red blood cell, and buffy coat components. DNA was extracted from the buffy coat using Qiagen DNA extraction (Qiagen Inc., Valencia, CA) and stored at −80°C.

Nurses' Health Study

In 1976, 121,701 female registered nurses aged 30–55 responded to a mailed questionnaire about known and suspected risk factors for disease, leading to the establishment of the

Nurses' Health Study (NHS). Study participants completed follow-up questionnaires every two years, providing information on new diagnoses of disease and updated information on risk factors. Participation in the study has remained high throughout follow-up; between 1976 and 2004 the percentage of follow-up information obtained (questionnaire responses plus deaths) was 98% for participants with a blood specimen and 99% for participants with a cheek cell specimen. The Institutional Review Board of Brigham and Women's Hospital, Boston, MA approved both the NHS and this analysis, and all participants provided implied consent by completing the baseline questionnaire.

In 1989–90, 32,826 participants submitted a blood sample; details of the collection are described elsewhere.8 In 2001–04, 33,040 additional women provided a buccal cell specimen using a mouthwash protocol. We extracted DNA from each white blood cell and buccal cell specimen within one week of receipt using Qiagen DNA extraction (Qiagen Inc., Valencia, CA), and stored the DNA at −80°C.

NHS nested case-control study

We collected information on new diagnoses of ovarian cancer and confirmed each diagnosis using methods described previously.9 For this analysis, we included all epithelial cases with a DNA specimen available from prior to diagnosis (incident cases), as well as cases who submitted a DNA specimen within four years after diagnosis (prevalent cases). The incident and prevalent cases were similar with respect to stage, histology, and survival time. All cases were diagnosed prior to June 1, 2004 and had no history of a prior cancer, other than non-melanoma skin cancer.

We randomly selected three controls per case from the study participants with DNA available, no prior bilateral oophorectomy, and no history of cancer, other than non-melanoma skin cancer, at the time of diagnosis of the matched case. We excluded 27 controls due to the unavailability of genotyping data (n=25) or because the participant was later diagnosed with ovarian cancer and was included in the analysis as a case (n=2). Cases and controls were matched on month and year of birth, DNA source, and menopausal status at diagnosis. Additional details of the matching criteria are published elsewhere.10

Genotyping methods

Genotyping was performed at the Dana Farber/Harvard Cancer Center High Throughput Genotyping Core. All samples were genotyped for seven SNPs: rs1219648 (*FGFR2*), rs2981582 (*FGFR2*), rs3803662 (*TNRC9*), rs889312 (*MAP3K1*), rs3817198 (*LSP1*), rs13281615 (chromosome 8q24), and rs13387042 (chromosome 2q35). Genotyping was performed on whole genome amplified DNA using the 5′ nuclease assay (Taqman) on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. Laboratory personnel were blinded to case-control status, and each plate included blinded replicate samples for quality control purposes. The replicate samples were 100% concordant for all genotypes except rs2981582 in the NECC, which was 98% concordant.

Statistical analysis

We used a chi-square test to examine whether each polymorphism was in Hardy-Weinberg equilibrium among the controls in each study population and to examine the distribution of each genotype by case-control status. We conducted all analyses separately in the NHS and NECC populations using consistent variable definitions, tested for heterogeneity in the results, and pooled the estimates using a random effects model.11 We used conditional (NHS) and unconditional (NECC) logistic regression to model the OR and 95% CI for the main effect of each variant genotype, compared to the wild-type genotype. We also

calculated the OR for a one-unit increase in the number of minor alleles (the log-additive model) by modeling a variable indicating the number of minor alleles, and we calculated the *p*-value for trend using the Wald test.

We adjusted all analyses for the relevant matching factors for each study population. Further, we examined several covariates as potential confounders, including menopausal status in the NECC analysis and family history of breast or ovarian cancer. There was no evidence of confounding by any covariate examined, so our final models are adjusted for the matching factors only.

In additional analyses, we stratified by menopausal status at diagnosis and known risk factors for ovarian cancer, to examine effect modification by these variables, and we calculated the *p*-value for interaction using the chi-square test for the difference between the log likelihoods for models with and without interaction terms between each variable and genotype. We also examined each two-way gene-gene interaction, assuming a log-additive genetic effect, using the methods described above. In addition to analyses of all epithelial ovarian cancers, we examined associations with the major histologic subtypes of epithelial ovarian cancer. We performed all analyses using SAS version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

Our study population included 1,173 cases and 1,201 frequency-matched controls from the NECC and 210 cases and 603 matched controls from the NHS, for a total of 1,383 cases and 1,804 controls. Of the NHS cases, 49 were prevalent (DNA collection ≤4 years after diagnosis) and 161 were incident with respect to the time of DNA collection. Characteristics of the NHS prevalent and incident cases were similar, although a higher percentage of the prevalent cancers had endometrioid histology (20% vs. 9%, *p*=0.04) and a nonsignificantly lower percentage were invasive (76% vs. 86%, *p*=0.09).

Among the controls in each population, the genotype frequencies were in Hardy-Weinberg equilibrium for all SNPs except the *MAP3K1* SNP in the NECC (*p*=0.04). The genotype frequency distributions were similar in the NECC and NHS populations (Table 1), and the minor allele frequencies were consistent with those reported in other populations.[3⁻5] Between 94% and 97% of the samples were successfully genotyped for each SNP in each study population. A small percentage of samples in each population had missing genotype data for multiple SNPs; 3% of the NECC samples and 2% of the NHS samples had missing data for three or more SNPs. However, in analyses excluding these samples the results were unchanged.

None of the genotypes examined were clearly associated with ovarian cancer risk in either population (Table 2). The results were generally consistent across the two study populations, and all *p*-values for the tests for heterogeneity comparing the NECC and NHS results were >0.05. Using the minor allele frequencies reported in the breast cancer genome-wide association studies,[3⁻5] we had >80% power to detect a log-additive OR of 1.16–1.18 per minor allele at the alpha=0.05 level in the pooled analysis. An association of this magnitude is within the range of the ORs reported for each *FGFR2*, *TNRC9*, and rs13387042 (chromosome 2q35) minor allele in the breast cancer genome-wide association studies.[3⁻5] In the NHS, women with two rs13387042 minor alleles had a significant decrease in ovarian cancer risk (OR=0.58, 95% CI=0.35–0.95), but there was no evidence of an association in the NECC or pooled analyses, suggesting that this result may have been due to chance.

Over 96% of the NECC participants and 98% of the NHS participants were of self-reported European ancestry. In analyses restricted to these participants, the results were similar to

those for the entire study population; we therefore included all participants in our analyses to maximize our sample size. The results were also similar when we restricted the NHS analyses to the incident cases only or the cases and controls with white blood cell DNA.

There was no clear association between any SNP examined and risk of the major histologic subtypes of ovarian cancer. In addition, we did not observe consistent evidence across both study populations of a gene-gene interaction between any two SNPs or gene-environment interactions between each SNP and menopausal status at diagnosis or known risk factors for ovarian cancer, including parity, tubal ligation history, and duration of oral contraceptive use (data not shown).

DISCUSSION

We did not observe a significant association between seven novel breast cancer susceptibility alleles and risk of epithelial ovarian cancer in this large combined study population. There was also no clear evidence of gene-gene or gene-environment interactions, or associations with the major histologic subtypes of ovarian cancer.

In genome-wide association studies by Hunter et al. and Easton et al., two SNPs in intron 2 of *FGFR2* were most strongly associated with breast cancer risk.[3, 4] The *FGFR2* gene encodes fibroblast growth factor receptor 2, a transmembrane tyrosine kinase involved in cell signaling and development of the embryonic mammary gland and other tissues.[12, 13] Although polymorphisms in the *FGFR2* gene have not been examined previously in relation to ovarian cancer risk, Huijts et al. reported a borderline significant association (p=0.05) between *FGFR2* SNP rs2981582 and the proportion of first- or second-degree female relatives with a history of breast or ovarian cancer.[6] In addition, Steele and colleagues reported that FGFR2 isoform IIIb was expressed in epithelial ovarian cancers but not in the normal ovarian surface epithelium and that ligands to FGFR2-IIIb promoted proliferation and prevented apoptosis of ovarian cancer cells.[14, 15] Although the *FGFR2* polymorphisms were not associated with ovarian cancer risk in our study, we cannot rule out the possibility that other polymorphisms in the *FGFR2* gene may influence ovarian cancer risk, or that FGFR2 may otherwise be involved in ovarian carcinogenesis.

With the exception of rs13281615 in chromosome 8q24, the other SNPs included in our analysis have not previously been examined in relation to risk of ovarian cancer. In the breast cancer genome-wide association study by Easton and colleagues, the estimated OR for each one-unit increase in the number of minor alleles was 1.13 for rs889312 (*MAP3K1*), 1.07 for rs3817198 (*LSP1*), 1.08 for rs13281615 (chromosome 8q24), and 1.20 for rs3803662 (*TNRC9*).[3] In the study by Stacey and colleagues, the corresponding ORs were 1.28 for rs3803662 (*TNRC9*) and 1.20 for rs13387042 (chromosome 2q35).[5] It is possible that one or more of these SNPs increases ovarian cancer risk but that our power was insufficient to detect the association. In a recent study by Ghoussaini et al., nine SNPs in 8q24 were associated with risk of one or more of the cancers examined (colorectal, ovarian, breast, or prostate), but none of the SNPs was associated with all four cancers. The SNP included in our analysis (rs13281615) was associated with breast cancer risk but not risk of colorectal, ovarian, or prostate cancer, while three other SNPs (rs10505477, rs10808556, and rs6983267) within a single haplotype block were associated with risk of colorectal, ovarian, and prostate cancer but not risk of breast cancer.[16] These results suggest that different risk alleles within the same gene or chromosomal region may be associated with different cancers and that, despite the lack of an association with the SNPs included in our analysis, other SNPs in these genes and chromosomal regions may be associated with ovarian cancer risk. Although there is little evidence suggesting a role of the *MAP3K1*, *LSP1*, and *TNRC9* genes or 2q35 in ovarian carcinogenesis, analyses of additional SNPs in

these regions, as well as analyses in larger study populations, would be helpful to further evaluate these associations.

Strengths of this study include the large number of cases in the combined study population, the examination of each association in two independent populations, and the availability of covariate data for analyzing gene-environment interactions. However, although our pooled analysis included almost 1,400 cases, we had insufficient power to detect very weak associations, gene-gene and gene-environment interactions, and associations with histologic subtypes of ovarian cancer. The participation rates in the NECC may have influenced our results if the cases or controls enrolled in the study differed from the population of eligible participants; however, the similar results in the NECC and the NHS, where the cases and matched controls were part of a large prospective cohort of women with excellent participation throughout the follow-up period, suggests that selection bias did not have a major impact on our results.

In conclusion, our results do not support modest or strong associations between seven novel breast cancer susceptibility alleles and risk of epithelial ovarian cancer. This suggests that the risk alleles identified in the breast cancer genome-wide scans to date may be specific to breast cancer and may not be associated more generally with risk of female reproductive cancers.

Acknowledgments

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Table 1

Genotype frequency distributions for 1,173 ovarian cancer cases and 1,201 controls in the New England Case-Control Study (NECC) and 210 ovarian cancer cases and 603 controls in the Nurses' Health Study (NHS)[*]

| SNP | Gene/location | Genotype | Study population | | | | Expected |
| | | | NECC | | NHS | | |
			Cases	Controls	Cases	Controls	
rs1219648	*FGFR2*	WT	363 (33%)	421 (36%)	77 (38%)	214 (37%)	
		Het	554 (51%)	545 (47%)	86 (42%)	262 (45%)	
		Hom	177 (16%)	188 (16%)	40 (20%)	104 (18%)	
		MAF[†]		0.40		0.41	0.40
rs2981582	*FGFR2*	WT	379 (35%)	427 (37%)	68 (35%)	211 (37%)	
		Het	550 (50%)	525 (46%)	89 (46%)	260 (46%)	
		Hom	166 (15%)	191 (17%)	35 (18%)	100 (18%)	
		MAF[†]		0.40		0.40	0.38
rs3803662	*TNRC9*	WT	568 (52%)	587 (51%)	110 (55%)	283 (50%)	
		Het	421 (38%)	454 (40%)	77 (39%)	227 (40%)	
		Hom	111 (10%)	107 (9%)	12 (6%)	54 (10%)	
		MAF[†]		0.29		0.30	0.25–0.27
rs889312	*MAP3K1*	WT	574 (52%)	610 (53%)	119 (59%)	318 (57%)	
		Het	447 (41%)	472 (41%)	68 (34%)	214 (38%)	
		Hom	73 (7%)	66 (6%)	14 (7%)	30 (5%)	
		MAF[†]		0.26		0.24	0.28
rs3817198	*LSP1*	WT	499 (45%)	504 (43%)	99 (49%)	291 (49%)	
		Het	480 (43%)	522 (45%)	86 (42%)	235 (40%)	
		Hom	132 (12%)	137 (12%)	19 (9%)	62 (11%)	
		MAF[†]		0.34		0.31	0.30
rs13281615	Chromosome 8q	WT	397 (36%)	423 (37%)	71 (35%)	199 (34%)	
		Het	514 (47%)	532 (46%)	101 (50%)	293 (50%)	
		Hom	194 (18%)	200 (17%)	32 (16%)	95 (16%)	
		MAF[†]		0.40		0.41	0.40

SNP	Gene/location	Genotype	Study population					Expected
			NECC		NHS			
			Cases	Controls	Cases	Controls		
rs13387042	Chromosome 2q35	WT	327 (29%)	336 (29%)	53 (27%)	143 (25%)		
		Het	525 (47%)	544 (47%)	109 (56%)	274 (48%)		
		Hom	263 (24%)	275 (24%)	33 (17%)	157 (27%)		
		MAF[†]	0.47		0.51			0.50

*
Frequencies do not add up to total N due to missing genotype data

[†]
Minor allele frequency among NECC and NHS controls; expected minor allele frequencies based on previous studies by Hunter et al., Easton et al., and Stacey et al.

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) for the association between breast cancer susceptibility alleles and ovarian cancer risk in the New England Case-Control Study (NECC) and the Nurses' Health Study (NHS)

SNP	Gene/location	Genotype	Study population		
			NECC*	NHS*	Pooled†
rs1219648	FGFR2	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	1.18 (0.98, 1.42)	0.95 (0.66, 1.37)	1.12 (0.94, 1.34)
		Hom	1.09 (0.85, 1.40)	1.08 (0.69, 1.69)	1.09 (0.88, 1.36)
		Per allele	1.07 (0.95, 1.21)	1.03 (0.82, 1.28)	1.06 (0.95, 1.18)
		P-trend	0.27	0.82	0.28
		Minor allele freq.‡	0.40	0.41	0.40
rs2981582	FGFR2	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	1.18 (0.98, 1.42)	1.12 (0.77, 1.63)	1.17 (0.99, 1.38)
		Hom	0.98 (0.77, 1.26)	1.13 (0.70, 1.81)	1.01 (0.81, 1.26)
		Per allele	1.03 (0.91, 1.16)	1.07 (0.85, 1.35)	1.04 (0.93, 1.15)
		P-trend	0.67	0.57	0.52
		Minor allele freq.‡	0.40	0.40	0.40
rs3803662	TNRC9	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	0.96 (0.81, 1.14)	0.89 (0.63, 1.26)	0.94 (0.81, 1.11)
		Hom	1.08 (0.81, 1.44)	0.59 (0.30, 1.15)	0.86 (0.48, 1.53)
		Per allele	1.01 (0.89, 1.14)	0.82 (0.63, 1.07)	0.94 (0.78, 1.14)
		P-trend	0.90	0.14	0.53
		Minor allele freq.‡	0.29	0.30	0.29
rs889312	MAP3K1	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	1.01 (0.85, 1.20)	0.89 (0.63, 1.25)	0.98 (0.84, 1.14)
		Hom	1.18 (0.83, 1.67)	1.21 (0.62, 2.37)	1.18 (0.87, 1.62)
		Per allele	1.04 (0.91, 1.20)	0.99 (0.76, 1.29)	1.03 (0.91, 1.16)
		P-trend	0.54	0.92	0.61
		Minor allele freq.‡	0.26	0.24	0.26
rs3817198	LSP1	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	0.93 (0.78, 1.11)	1.05 (0.75, 1.48)	0.95 (0.82, 1.11)

SNP	Gene/location	Genotype	Study population		
			NECC[*]	NHS[*]	Pooled[†]
		Hom	0.97 (0.74, 1.27)	0.89 (0.51, 1.55)	0.95 (0.75, 1.22)
		Per allele	0.97 (0.86, 1.09)	0.98 (0.77, 1.24)	0.97 (0.87, 1.08)
		P-trend	0.59	0.87	0.58
		Minor allele freq.[‡]	0.34	0.31	0.33
rs13281615	Chromosome 8q	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	1.03 (0.86, 1.24)	0.96 (0.68, 1.38)	1.02 (0.86, 1.20)
		Hom	1.04 (0.81, 1.32)	0.95 (0.57, 1.57)	1.02 (0.82, 1.27)
		Per allele	1.02 (0.91, 1.15)	0.97 (0.76, 1.24)	1.01 (0.91, 1.12)
		P-trend	0.74	0.81	0.84
		Minor allele freq.[‡]	0.40	0.41	0.41
rs13387042	Chromosome 2q35	WT	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
		Het	0.99 (0.82, 1.20)	1.06 (0.72, 1.57)	1.00 (0.84, 1.19)
		Hom	0.98 (0.78, 1.24)	0.58 (0.35, 0.95)	0.79 (0.47, 1.32)
		Per allele	0.99 (0.89, 1.11)	0.78 (0.62, 0.99)	0.89 (0.71, 1.12)
		P-trend	0.89	0.04	0.32
		Minor allele freq.[‡]	0.47	0.51	0.49

[*] NECC: unconditional logistic regression adjusted for age and study center; NHS: unadjusted conditional logistic regression matched on age, DNA source, and menopausal status at diagnosis

[†] *P*-values for tests for heterogeneity comparing the NECC and NHS results were all >0.05

[‡] Minor allele frequency among controls