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ARTICLE

Common Breast Cancer-Predisposition Alleles Are Associated with Breast Cancer Risk in *BRCA1* and *BRCA2* Mutation Carriers

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Germline mutations in *BRCA1* and *BRCA2* confer high risks of breast cancer. However, evidence suggests that these risks are modified by other genetic or environmental factors that cluster in families. A recent genome-wide association study has shown that common alleles at single nucleotide polymorphisms (SNPs) in *FGFR2* (rs2981582), *TNRC9* (rs3803662), and *MAP3K1* (rs889312) are associated with increased breast cancer risks in the general population. To investigate whether these loci are also associated with breast cancer risk in *BRCA1* and *BRCA2* mutation carriers, we genotyped these SNPs in a sample of 10,358 mutation carriers from 23 studies. The minor alleles of SNP rs2981582 and rs889312 were each associated with increased breast cancer risk in *BRCA2* mutation carriers (per-allele hazard ratio [HR] = 1.32, 95% CI: 1.20–1.45, $p_{trend} = 1.7 \times 10^{-8}$ and HR = 1.12, 95% CI: 1.02–1.24, $p_{trend} = 0.02$) but not in *BRCA1* carriers. rs3803662 was associated with increased breast cancer risk in both *BRCA1* and *BRCA2* mutation carriers (per-allele HR = 1.13, 95% CI: 1.06–1.20, $p_{trend} = 5 \times 10^{-5}$ in *BRCA1* and BRCA2 combined). These loci appear to interact multiplicatively on breast cancer risk in *BRCA2* mutation carriers. The differences in the effects of the *FGFR2* and *MAP3K1* SNPs between *BRCA1* and *BRCA2* carriers point to differences in the biology of *BRCA1* and *BRCA2* breast cancer tumors and confirm the distinct nature of breast cancer in *BRCA1* mutation carriers.

Introduction

BRCA1 (MIM 113705) and *BRCA2* (MIM 600185) mutations confer high risks of breast and other cancers. A meta-analysis of mutation-positive families identified through population-based studies of breast or ovarian cancer estimated the risk of breast cancer by age 70 years to be 65% and 45% for *BRCA1* and *BRCA2* mutation carriers, respectively.[1] Although the pattern of risk was similar, the absolute magnitude of risk in that study was lower than in previously published studies based on families with multiple affected individuals, in particular for *BRCA2* mutation carriers.[2] The breast cancer risks in *BRCA1* and *BRCA2* mutation carriers have also been found to vary by the age at diagnosis and the type of cancer (unilateral breast cancer, contralateral breast cancer, or ovarian cancer) in the index patient.[1,3,4] Such observations are consistent with the hypothesis that breast cancer risks in *BRCA1* and *BRCA2* mutation carriers are modified by other genetic or environmental factors that cluster in families.[1,3] Further evidence of genetic modifiers of risk comes from segregation-analysis models that have quantified the extent of variability in the risk of breast cancer in mutation carriers in terms of a polygenic-modifying variance[5,6]. In addition, Begg et al.[3] demonstrated significant between-family variation in risk.

A number of studies have evaluated associations between genetic variants and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers[7,8], but apart from a recent CIMBA (Consortium of Investigators of Modifiers of *BRCA1/2*) study that found evidence of association among *BRCA2* mutation carriers who are rare homozygotes for a single nucleotide polymorphism (SNP) in *RAD51*, no other such associations have been reliably identified.[9] A recent genome-wide association study in breast cancer identified five common susceptibility alleles that are associated with an increased risk of breast cancer in the general population.[10] To address whether these polymorphisms are also associated with the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers, we typed the three SNPs with the strongest evidence of association in *BRCA1* and *BRCA2* mutation carriers from the CIMBA study.[7]

Material and Methods

Study Sample

Eligibility was restricted to female carriers who had pathogenic mutations in *BRCA1* or *BRCA2* and were 18 years old or older. Twenty-three different studies submitted information on mutation carriers (Table 1). Information collected included the year of birth; mutation description, including nucleotide position and base change; age at last follow-up; ages at breast and ovarian cancer diagnosis; and age or date at bilateral prophylactic mastectomy. Information was also available on the country of residence, which was defined to be the country of the clinic at which the carriers were recruited (some studies included carriers from several countries). Related individuals were identified through a unique family identifier. Women were included in the analysis if they carried mutations that were pathogenic according to generally recognized criteria[9] (Breast Cancer Information Core, BIC). All carriers participated in clinical and research studies at the host institutions under IRB-approved protocols. Further details of the CIMBA initiative can be found elsewhere.[7]

Genotyping

All centers included at least 2% of the samples in duplicate, no template controls in every plate, and a random mixture of affected and unaffected carriers. Samples that failed in two or more of the SNPs genotyped were excluded from the analysis. A study was included in the analysis only if the call rate was over 95% after samples that failed at multiple SNPs had been excluded. The concordance between duplicates had to be at least 98%. To further validate the accuracy of genotyping across centers, we required all groups to genotype 95 DNA samples from a standard test plate for all three SNPs. If the genotyping was inconsistent for more than one sample in the test plate, the study was excluded from the analysis of that SNP. Based on these criteria, four studies were excluded from the analysis of rs2981582, and three studies were excluded from the analysis of rs3803662. As an extra genotyping quality-control check, we also evaluated deviation from Hardy-Weinberg equilibrium (HWE) among unrelated subjects separately for each SNP and study. Two studies gave HWE p values of 0.02 and 0.001. Examination of the cluster plots for these SNPs did not reveal any unusual patterns, and these studies were therefore included in the analysis. The genotype frequencies among unrelated individuals for all other studies and SNPs were consistent with HWE.

Statistical Analysis

After the above exclusions, a total of 10,358 unique *BRCA1* and *BRCA2* mutation carriers had an observed genotype for at least

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Table 1. Number of *BRCA1* and *BRCA2* Mutation Carriers by Study

Study	Country[a]	BRCA1	BRCA2	BRCA1 and BRCA2	Genotyping platform
EMBRACE	U.K. and Eire	658	471	3	iPLEX[b]
Spanish National Cancer Centre (CNIO)	Spain	167	205	0	Taqman
Deutsches Krebsforschungszentrum (DKFZ)	Germany	122	50	0	Taqman, MALDI-TOF MS, Biplex
Fox Chase Cancer Center (FCCC)	U.S.A.	50	41	1	iPLEX[b]
Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers (GEMO)	France	1102	554	0	Taqman
German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)	Germany	568	280	3	BIORAD iCycler
Hospital Clinico San Carlos (HCSC)	Spain	90	78	0	Taqman
Helsinki Breast Cancer Study (HBCS)	Finland	102	104	0	Taqman
*In*terdisciplinary *He*alth *R*esearch *I*nternational *T*eam *Br*east *Ca*ncer *S*usceptibility (INHERIT BRCAs)	Quebec- Canada	72	82	0	Taqman
kConFab	Australia	426	353	0	iPLEX[b]
Modifiers and Genetics in Cancer (MAGIC)	U.S.A.	683	378	1	Taqman
MAYO	U.S.A.	108	54	0	Taqman
Milan Breast Cancer Study Group (MBCSG)	Italy	251	135	0	Taqman
National Cancer Institute (NCI)	U.S.A.	147	50	0	Taqman
National Israeli Cancer Control Center (NICCC)	Israel	283	160	1	Taqman
Ontario Cancer Genetics Network (OCGN)	Canada	195	143	0	Taqman
Odense University Hospital(OUH)	Denmark	106	0	0	Taqman
Pisa Breast Cancer Study (PBCS)	Italy	54	30	0	iPLEX[b]
Sheeba Medical Centre (SMC)- Tel Hashomer	Israel	283	101	0	Taqman
SWE-BRCA	Sweden	426	127	0	iPLEX[b]
Mod-SQuaD	Czech Republic	138	37	0	
University of Pennsylvania (UPENN)	U.S.A.	271	124	1	iPLEX[b]
HEriditary Breast and Ovarian study Netherlands (DNA-HEBON)	The Netherlands	489	0	0	iPLEX[b]
Total		6791	3557	10	

[a] Coordinating center.
[b] Indicates that samples were genotyped at a central location (Queensland Institute of Medical Research).

one of the three polymorphisms (6,791 *BRCA1* carriers; 3,557 *BRCA2* carriers; and ten *BRCA1* and *BRCA2* carriers; Table 1). Individuals were classified according to their age at diagnosis of breast cancer or their age at last follow-up. For this purpose, individuals were censored at the age of the first breast cancer diagnosis (n = 5,489), ovarian cancer diagnosis (n = 975), or bilateral prophylactic mastectomy (n = 340) or the age at last observation (n = 3,554). Only individuals censored at breast cancer diagnosis were assumed to be affected (Table 2). Mutation carriers were censored at ovarian cancer diagnosis and were considered unaffected. We ignored data on breast cancer occurrence after an ovarian cancer because the risk of breast cancer may be affected by the treatment for ovarian cancer, and the recording of a second breast cancer may be inaccurate in a woman with advanced ovarian cancer.

We performed additional sensitivity analyses to investigate whether any bias could be introduced in our results as a result of our assumptions. If the SNPs under study were associated with disease survival in carriers, the estimated HRs might be affected by the inclusion of prevalent cases. We therefore performed

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Table 2. Patient Characteristics

Characteristic	Total	*BRCA1*[a]		*BRCA2*	
		Unaffected	Breast Cancer	Unaffected	Breast Cancer
Number	10,358	3300	3501	1574	1983
Person-years follow-up	440,252	140,541	14,2734	69,778	87,199
Median age at censure (IQR)	41 (34–49)	41 (33–50)	40 (34–46)	43 (34–52)	43 (37–50)
Age at Censure, N (%)					
<30	1222 (10.8)	499 (15.1)	320 (9.1)	196 (12.5)	107 (5.4)
30–39	3436 (33.2)	958 (29.0)	1416 (40.5)	443 (28.1)	619 (31.2)
40–49	3305 (31.9)	946 (28.7)	1200 (12.1)	428 (27.2)	731 (36.9)
50–59	1683 (16.3)	584 (17.7)	423 (12.1)	295 (18.7)	381 (19.2)
60–69	562 (5.4)	208 (6.3)	109 (3.1)	135 (8.6)	110 (5.5)
70+	250 (2.4)	105 (3.2)	33 (0.9)	77 (4.9)	35 (1.8)
Year of Birth, N (%)					
<1920	92 (0.9)	25 (0.8)	32 (0.9)	20 (1.3)	15 (0.8)
1920–1929	383 (3.7)	93 (2.8)	140 (4.0)	48 (3.0)	102 (5.1)
1930–1939	963 (9.3)	246 (7.4)	335 (9.6)	138 (8.8)	244 (12.3)
1940–1949	2066 (20.0)	511 (15.5)	836 (23.9)	228 (14.5)	491 (24.8)
1950–1959	2913 (28.1)	804 (24.4)	1,122 (32.0)	368 (23.4)	619 (31.2)
1960+	3741 (38.0)	1,621 (49.1)	1,036 (29.6)	772 (49.0)	512 (25.8)
Risk-Reducing Salpingo-Oophorectomy (RRSO)					
No RRSO	6613 (63.8)	2,032 (61.6)	2369 (67.7)	928 (59.0)	1284 (64.7)
RRSO	577 (5.6)	318 (9.6)	85 (2.4)	119 (7.6)	55 (2.8)
Missing	3168 (30.6)	950 (28.8)	1047 (29.9)	527 (33.4)	644 (32.5)

IQR: Interquartile range.
[a] Includes the ten females who have mutations in both BRCA1 and BRCA2.

analyses after excluding cases diagnosed more than 5 years prior to the age at last follow-up. Risk-reducing salpingo-oophorectomy (RRSO) reduces the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers.[11,12] To investigate whether allowance for RRSO alters our results in any way, we repeated the analysis after censoring the *BRCA1* and *BRCA2* mutation carriers at the time of surgery. In addition, because carriers diagnosed with ovarian cancer were treated as unaffected at the age of diagnosis, if any of these SNPs are associated with ovarian cancer risk, the hazard ratio (HR) estimates might be underestimated or overestimated depending on the direction of the association. Although there is no evidence of such an association between these SNPs and ovarian cancer in the general population (Song et al., American Society of Human Genetics meeting 2007, San Diego, USA, Abstract 428), we examined the sensitivity of our results to this assumption by excluding mutation carriers who were censored at a first ovarian cancer.

Our analyses are complicated by the fact that *BRCA1* and *BRCA2* mutation carriers are not randomly sampled with respect to their disease status. Many carriers are sampled through families seen in genetic clinics. The first tested individual in a family is usually someone diagnosed with cancer at a relatively young age. Such study designs therefore tend to lead to an oversampling of affected individuals, and standard analytical methods such as Cox regression might lead to biased estimates of the risk ratios.[13] For example, consider an individual affected at age *t*. In a standard analysis of a cohort study, the SNP genotype for the individual will be compared with those of all individuals at risk at age *t*. This analysis leads to consistent estimates of the HR estimates. However, in the present design, mutation carriers are already selected on the basis of disease status (where affected individuals are oversampled). If standard cohort analysis were applied to these data, it would cause affected individuals at age *t* to be compared to unaffected carriers selected on the basis of their future disease status. If the genotype is associated with the disease, the risk estimate will be biased to zero because too many affected individuals (in whom the at-risk genotype is overrepresented) are included in the comparison group. Simulation studies have shown that this effect can be quite marked.[13]

To correct for this potential bias, we analyzed the data within a survival analysis framework by modelling the retrospective likelihood of the observed genotypes conditional on the disease phenotypes. A detailed description of the retrospective-likelihood approach has been published.[9] The effect of each SNP was modeled either as a per-allele HR or as separate HRs for heterozygotes and homozygotes. The HRs were assumed to be independent of age (i.e., we used a Cox proportional-hazards model). We verified the assumption of proportional hazards by examining the Kaplan-Meier estimates of the survival functions by genotype and by subsequently adding a genotype × age interaction term to the model in order to fit models in which the HR changed with age. Analyses were carried out with the pedigree-analysis software MENDEL.[14] Under this approach, the baseline age-specific incidence rates in the Cox proportional-hazards model are chosen such that the overall breast cancer incidence rates, averaged over all genotypic categories, agree with external estimates of *BRCA1* and *BRCA2* incidence rates.[6] We examined between-study heterogeneity by comparing the models that allowed for study-specific log-hazard ratios against models in which the same log-hazard ratio was assumed to apply to all studies. All analyses were stratified by study group and country of residence (where numbers were sufficiently large) and used calendar-year- and cohort-specific

breast cancer incidence rates for *BRCA1* and *BRCA2*.[6] The risk of breast cancer in compound *BRCA1* and *BRCA2* mutation carriers was assumed to be that for *BRCA1* mutation carriers. We used a robust variance-estimation approach to allow for the nonindependence among related carriers.[15,16] To evaluate the combined effects of the significant SNPs on breast cancer risk, we fitted a multiplicative (log-additive) model that included a parameter for the per-allele log-hazard ratio for each of the SNPs and compared this to a fully saturated model in which a separate parameter was fitted for each multi-locus genotype. The proportions of the modifying variance explained by the *FGFR2*, *TNCR9*, and *MAP3K1* SNPs were estimated by $\ln(c)/\sigma^2$, where c is the estimated coefficient of variation in incidence rates due to each SNP[17,18] and σ^2 is the estimated modifying variance (1.32 and 1.73 for *BRCA1* and *BRCA2*, respectively[6]). We estimated the total proportion of the modifying variance due to all SNPs by adding the individual proportions, i.e., by assuming that the loci combined multiplicatively. In the text, the term "significant" is taken to mean a significance level of 5%.

Results

Results are shown in Table 3. SNP rs2981582 in *FGFR2* was associated with breast cancer risk in the combined sample of *BRCA1* and *BRCA2* mutation carriers ($p_{trend} = 0.0001$). However, when *BRCA1* and *BRCA2* carriers were analyzed separately, the association was restricted to *BRCA2* mutation carriers ($p_{trend} = 2 \times 10^{-8}$), and there was no evidence of an association among *BRCA1* carriers ($p_{trend} = 0.6$; $p = 1.3 \times 10^{-5}$ for the difference in the estimates between *BRCA1* and *BRCA2* carriers). The estimated effect among *BRCA2* mutation carriers was consistent with a multiplicative model in which each copy of the disease allele conferred a hazard ratio (HR) of 1.32 (95%CI: 1.20–1.45) (Figure 1). There was some suggestion that the HRs might differ between studies for *BRCA1* ($p = 0.03$), but there was no evidence of heterogeneity for *BRCA2* ($p = 0.11$).

TNRC9 SNP rs3803662 was associated with an increased risk of breast cancer in both *BRCA1* and *BRCA2* mutation carriers ($p_{trend} = 0.004$ and 0.009, respectively; joint $p_{trend} = 0.00005$). The per-allele HR was estimated to be 1.11 (95%CI: 1.03–1.19) for *BRCA1* carriers and 1.15 (95%CI: 1.03–1.27) for *BRCA2* carriers ($p = 0.6$ for the difference in the *BRCA1* and *BRCA2* per-allele HR estimates). There was no evidence of heterogeneity in the HRs among studies (*BRCA1:* $p = 0.67$; *BRCA2:* $p = 0.63$, Figure 2).

There was no evidence that SNP rs889312 in *MAP3K1* was associated with breast cancer risk in the combined sample of *BRCA1* and *BRCA2* mutation carriers or in *BRCA1* carriers alone ($p_{trend} = 0.29$ and 0.86, respectively). However, *BRCA2* mutation carriers who carried a copy of the minor allele of this SNP were at increased risk of breast cancer (per-allele HR = 1.12, 95% CI: 1.02–1.24, $p_{trend} = 0.02$). There was some evidence of heterogeneity in the HRs between studies for *BRCA2* ($p = 0.02$) but not for *BRCA1* ($p = 0.06$) mutation carriers (Figure 3). We also investigated whether the HRs change with age by including an age × genotype interaction term in the model. There was no significant evidence that HRs vary by age for any of the variants.

If these SNPs were associated with disease survival in carriers, the estimated HRs might be affected by the inclusion of prevalent cancer cases. We therefore repeated our analysis after excluding cancer cases diagnosed more than five years prior to their study recruitment. A total of 7,027 *BRCA1* and *BRCA2* mutation carriers were eligible for this analysis (2,523 affected; 4,504 unaffected). The estimated per-allele HRs among *BRCA2* mutation carriers were virtually unchanged for the *FGFR2* SNP rs2981582 (per-allele HR 1.37 (95%CI: 1.22–1.54; $p_{trend} = 2 \times 10^{-7}$) and the *MAP3K1* SNP rs889312 (HR: 1.11, 95%CI: 0.98–1.25, $p_{trend} = 0.11$), but slightly higher for the *TNRC9* SNP rs3803662 (*BRCA1*: 1.17 (95% CI:1.06-1.28, $p_{trend} = 0.001$; *BRCA2*: 1.24 (95%CI: 1.10–1.41, $p_{trend} = 0.0008$; *BRCA1*and *BRCA2* combined $p_{trend} = 9 \times 10^{-7}$).

Risk-reducing salpingo-oophorectomy (RRSO) reduces the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers.[11,12] To investigate whether allowance for RRSO alters our results in any way, we repeated the analysis after censoring the *BRCA1* and *BRCA2* mutation carriers at the time of surgery. Because information on RRSO was missing for approximately 30% of the carriers, we performed this analysis by first including all carriers in the analysis and assuming that carriers with no RRSO information did not have the surgery; we then repeated the analysis after including only carriers with data on RRSO as previously described.[9] When all *BRCA1* and *BRCA2* mutation carriers were included in this analysis, the HRs and significance test results were very similar to results of the analysis in which no censoring at RRSO took place (Table S1 in the Supplemental Data). When carriers with no information on RRSO were excluded, the sample size was reduced from 10,358 to 7,190. The estimated HRs remained virtually identical to those in the primary analysis, although the p values were increased, because of a reduced sample size (Table S1; rs2981582 in *BRCA2*: $p_{trend} = 6 \times 10^{-6}$; rs3803662 in *BRCA1*, *BRCA2* and combined: $p_{trend} = 0.03$, 0 .02, 0.001 respectively; rs889312 in *BRCA2*: $p_{trend} = 0.16$).

BRCA1 and *BRCA2* mutations are also associated with increased risks of ovarian cancer.[1] Carriers who had developed ovarian cancer were included in our analyses as unaffected. A possible bias could have been introduced if these SNPs were associated with ovarian cancer risk. Although there is no evidence of such an association in the general population (American Society of Human Genetics meeting 2007, San Diego, USA, Abstract 428), we repeated our analyses by excluding the 975 mutation carriers who were censored at an ovarian cancer diagnosis. The estimated HRs were unchanged (Table S2).

To evaluate the potential combined effects of the two most significant SNPs on breast cancer risk in *BRCA2* mutation carriers, we fitted a multiplicative model (log additive, 2 degrees of freedom [df]) for the effects of the *FGFR2* SNP rs2981582 and *TNRC9* SNP rs3803662 and compared this against a fully saturated model in which

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Table 3. Genotype Frequencies by Mutation and Disease Status and Hazard-Ratio Estimates

		Unaffected (%)	Affected (%)	HR[a]	95% CI	p Value
FGFR2 rs2981582						
BRCA1 and *BRCA2*	GG	1547 (36.0)	1647 (33.0)	1.00		
	GA	2051 (47.7)	2407 (48.2)	1.10	1.01–1.20	
	AA	703 (16.3)	936 (18.8)	1.24	1.11–1.38	
	2-df test					0.00045
	Per allele			1.11	1.05–1.17	0.000095
BRCA1	GG	1021 (35.5)	1114 (35.3)	1.00		
	GA	1376 (47.9)	1487 (47.2)	0.99	0.89–1.10	
	AA	477 (16.6)	553 (17.5)	1.05	0.92–1.20	
	2-df test					0.65
	Per allele			1.02	0.95–1.09	0.60
BRCA2	GG	526 (36.9)	533 (29.0)	1.00		
	GA	675 (47.3)	920 (50.1)	1.35	1.17–1.57	
	AA	226 (15.8)	383 (20.9)	1.72	1.41–2.09	
	2-df test					9.9×10^{-8}
	Per allele			1.32	1.20–1.45	1.7×10^{-8}
TNRC9 rs3803662						
BRCA1 and BRCA2	CC	2244 (50.3)	2422 (47.6)	1.00		
	CT	1831 (41.1)	2173 (42.7)	1.13	1.04–1.22	
	TT	382 (8.6)	497 (9.7)	1.28	1.11–1.46	
	2-df test					0.00027
	Per allele			1.13	1.06–1.20	5×10^{-5}
BRCA1	CC	1542 (50.9)	1571 (48.2)	1.00		
	CT	1238 (40.8)	1384 (42.4)	1.11	1.01–1.22	
	TT	251 (8.3)	308 (9.4)	1.24	1.04–1.46	
	2-df test					0.017
	Per allele			1.11	1.03–1.19	0.0043
BRCA2	CC	702 (49.2)	851 (46.5)	1.00		
	CT	593 (41.6)	789 (43.2)	1.15	1.00–1.32	
	TT	131 (9.2)	189 (10.3)	1.32	1.04-1.67	
	2-df test					0.033
	Per allele			1.15	1.03–1.27	0.009
MAP3K1 rs889312						
BRCA1 and *BRCA2*	AA	2440 (50.5)	2711 (49.9)	1.00		
	AC	1963 (40.7)	2195 (40.4)	1.02	0.94–1.10	
	CC	426 (8.8)	530 (9.8)	1.08	0.95–1.22	
	2-df test					0.53
	Per allele			1.03	0.97–1.09	0.29
BRCA1	AA	1637 (50.0)	1743 (50.2)	1.00		
	AC	1329 (40.6)	1394 (40.2)	1.00	0.91–1.09	
	CC	306 (9.4)	332 (9.6)	0.98	0.84–1.15	
	2-df test					0.98
	Per allele			0.99	0.93–1.06	0.86
BRCA2	AA	803 (51.6)	968 (49.2)	1.00		
	AC	634 (40.7)	801 (40.7)	1.08	0.94–1.24	
	CC	120 (7.7)	198 (10.1)	1.32	1.05–1.66	
	2-df test					0.049
	Per allele			1.12	1.02–1.24	0.020

[a] In all cases, where significant, the effect is consistent with a multiplicative model in which each copy of the disease allele confers the estimated, per-allele HR.

a separate parameter was fitted for each *FGFR2-TNRC9* combined genotype (8 df). The HR estimates for all nine genotypes under the multiplicative and fully saturated models are shown in Table 4. The HRs were remarkably similar under the two models, and there was no significant evidence that the fully saturated model fit better than the multiplicative model ($\chi^2 = 4.48$, df = 6, p-value:0.61). Under the multiplicative model, the highest HR was 2.26 for carriers who were homozygotes for the risk allele at both loci in comparison to *BRCA2* carriers who did not have any risk alleles. Based on the minor allele frequencies of the *FGFR2* and *TNRC9* SNPs in the general population,[10] approximately 36% of the BRCA2 mutation carriers will have HRs in excess of 1.5 in comparison to the 20% of

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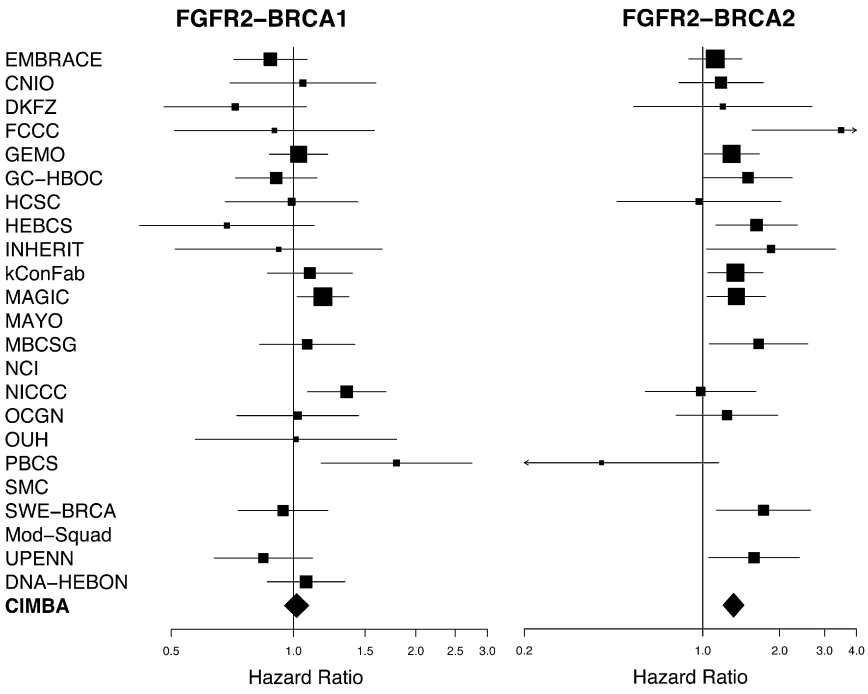

Figure 1. Study-Specific Estimates of the Per-Allele Hazard Ratio for SNP rs2981582 in *FGFR2*

The area of the square is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals.

carriers who will have no copies of the disease allele at either *FGFR2* or *TNRC9*.

Discussion

Our results provide strong evidence that SNP rs2981582 in *FGFR2* is associated with breast cancer risk in *BRCA2* mutation carriers and that SNP rs3803662 in *TNRC9* is associated with breast cancer risk in both *BRCA1* and *BRCA2* mutation carriers. With our sample size, we can rule out a comparable involvement of rs2981582 in the breast cancer risk for *BRCA1* mutation carriers. These results were unaltered when we accounted for survival bias and risk-reducing salpingo-oophorectomy or when we included ovarian cancer cases as unaffected in the analysis. There was no evidence of heterogeneity in the HRs between studies. The evidence of association with SNP rs889312 in *MAP3K1* was weaker and was restricted to *BRCA2* mutation carriers. For all three SNPs, the estimated HRs in *BRCA2* carriers were very similar to the corresponding estimated odds ratios (OR) for breast cancer derived from data from large population-based case-control studies[10] (per-allele ORs: 1.26, 1.20 and 1.13 for rs2981582 [*FGFR2*], rs3803662 [*TNRC9*], and rs889312 [*MAP3K1*], respectively). Based on the per-allele HR estimates, the frequencies of the risk alleles in the general population[10] and recent estimates of the genetic variance of the breast cancer risks in *BRCA1* and *BRCA2* mutation carriers ("modifying variance") derived from breast cancer segregation analyses[6], the *TNRC9* SNP is predicted to account for approximately 0.5% of the *BRCA1* modifying variance. The SNPs in *FGFR2*, *TNRC9*, and *MAP3K1* are estimated to account for 2.8% of the *BRCA2* modifying variance.

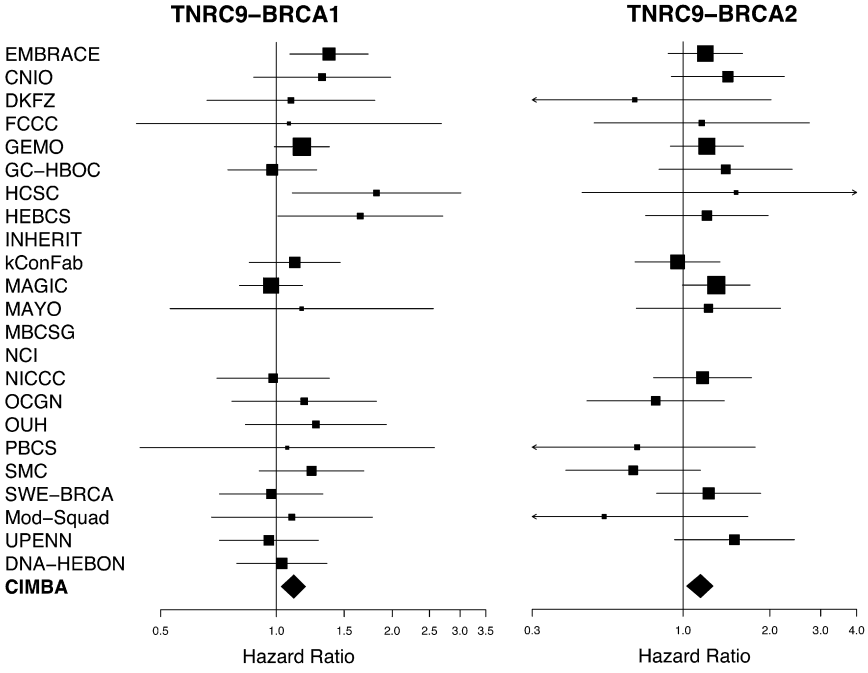

Figure 2. Study-Specific Estimates of the Per-Allele Hazard Ratio for SNP rs3803662 in *TNRC9*

The area of the square is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals.

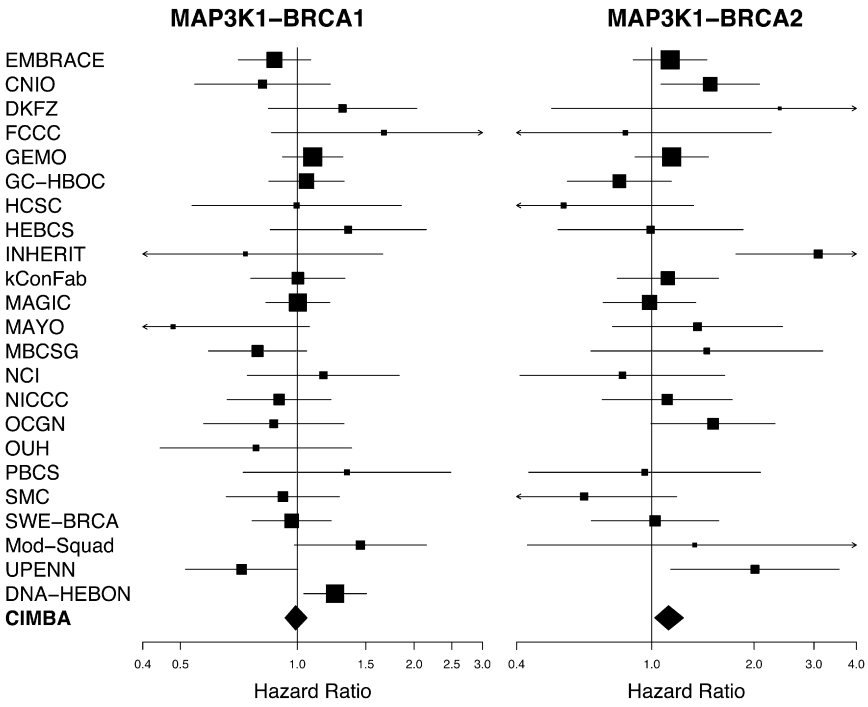

Figure 3. Study-Specific Estimates of the Per-Allele Hazard Ratio for SNP rs889312 in *MAP3K1*

The area of the square is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals.

It has been reported that more than 90% of *BRCA1* breast cancer tumors are estrogen receptor (ER) negative, whereas *BRCA2* breast cancer tumors have an ER distribution similar to that in the general population, in which the majority are ER positive.[19] A recent Breast Cancer Association Consortium study found that the *FGFR2* SNP rs2981582 was more strongly associated with ER-positive breast cancers than ER-negative tumors (OR: 1.31 versus 1.08, respectively).[20] The same study found that the *TNRC9* SNP rs3803662 was associated with the risk of both ER-positive and ER-negative breast cancers, which is again consistent with our results. Therefore, our results are consistent with the hypothesis that the SNPs modify the risk of breast cancer to a similar, relative extent in carriers for either *BRCA2* or (in the case of *TNRC9* rs3803662) *BRCA1* and noncarriers.

Table 4. HR Estimates for the Combined Genotypes of SNPs in *FGFR2* and *TNRC9* among *BRCA2* Carriers under a Multiplicative Model and under a Fully Saturated Model

FGFR2/TNRC9 Genotype	HR Multiplicative Model[a]	HR Fully Saturated Model	Predicted Genotype Distribution[b] (%)
GG/CC	1.00	1.00	20.4
GG/CT	1.16	1.05	14.3
GG/TT	1.35	1.23	2.5
GA/CC	1.29	1.25	26.1
GA/CT	1.50	1.44	18.3
GA/TT	1.75	1.72	3.2
AA/CC	1.67	1.41	8.3
AA/CT	1.94	2.08	5.9
AA/TT	2.26	2.08	1.0

[a] Multiplicative model, per-allele HRs. *FGFR2*: 1.29 (95% CI: 1.17–1.43); *TNRC9*: 1.16 (95% CI: 1.04–1.30).

[b] Assuming a minor allele frequency of 0.39 for *FGFR2* (rs rs2981582) and 0.26 for *TNRC9* (rs rs3803662).[10]

The weaker (or null) effect in *BRCA1* carriers for the *FGFR2* SNP rs2981582 is explicable by its weak effect on ER negative disease and is further confirmation of the distinct nature of breast cancer in *BRCA1* mutation carriers.

One potential limitation of this study is that it was not possible to take the precise family histories of carriers into account because CIMBA does not currently collect this information. Although this does not invalidate the statistical tests of association, we could not therefore assess directly how the breast cancer risk in carriers associated with these SNPs varies by the degree of family history. Such effects might be important in the context of genetic counseling. Another limitation is that we did not have detailed tumor characteristics such as ER status available for our carriers. For example, it might be that the *FGFR2* SNP is associated with the risk of ER-positive breast cancer in BRCA1 carriers, but this is not observable in our dataset because they only account for a small fraction of cases. In addition, information on whether any of the mutation carriers were on chemoprevention was also not available. However, chemoprevention is not expected to be a confounder in our analyses because its use is unlikely to be associated with the SNPs under investigation. A final uncertainty is that the SNPs we have tested are probably not the variants causally related to the disease, but are correlated with them. This does not invalidate the associations, but it might mean that the associations with the causal variants, when they are identified, will prove to be somewhat stronger.

Because *BRCA1* and *BRCA2* mutations confer high risks, the modest HRs associated with these SNPs translate into marked differences in absolute risk between extreme genotypes. For example, the absolute risk of breast cancer by age 70 among *BRCA2* mutation carriers is predicted to be 43% for common homozygotes at the *FGFR2* locus and 63% for rare homozygotes. The corresponding risks for *TNRC9* are 48% and 58% for common and rare homozygotes, respectively. However, when the combined effects of the two loci are considered, the absolute risk varies from 41% (for carriers with no risk alleles) to 70% (for carriers with four risk alleles; see Figure 4). Although only 1% of carriers are doubly homozygous, approximately 36% of carriers will have a HR of 1.5 or greater in comparison to the 20% of carriers with no risk alleles. This corresponds to an absolute

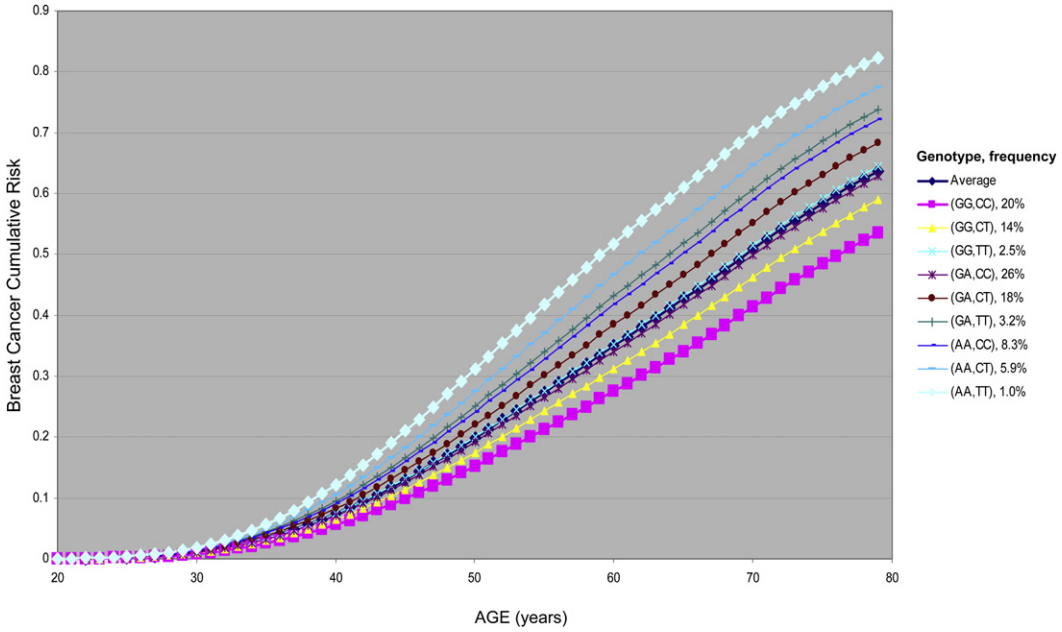

Figure 4. Cumulative Risk of Breast Cancer among *BRCA2* Mutation Carriers by Combined *FGFR2* and *TNRC9* Genotype under a Multiplicative Model for the Joint Effects of the Loci

The combined *FGFR2* and *TNRC9* genotypes are as follows: *FGFR2* = GG, GA, or AA; *TNRC9* = CC, CT, or TT. "Average" represents the cumulative breast cancer risk over all possible modifying effects among *BRCA2* mutation carriers born after 1950. The minor allele frequencies for the *FGFR2* and *TNRC9* SNPs were assumed to be 0.39 and 0.26, respectively.

risk of 55% or greater by age 70. If further such risk alleles are identified (for example, through additional genome scans), the proportion of carriers for whom the risk can be modified substantially will increase. These risks might also be affected by other factors, including family history, mutation type, and lifestyle risk factors, and future studies should aim to investigate these effects.

Supplemental Data

Two additional tables are available online at http://ajhg.org/.

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Web Resources

The URLs for data presented herein are as follows:

Breast Cancer Information Core (BIC), http://research.nhgri.nih.gov/projects/bic/

Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim

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