ARTICLE

Interactions Between Genetic Variants and Breast Cancer Risk Factors in the Breast and Prostate Cancer Cohort Consortium

Daniele Campa, Rudolf Kaaks, Loïc Le Marchand, Christopher A. Haiman, Ruth C. Travis, Christine D. Berg, Julie E. Buring, Stephen J. Chanock, W.Ryan Diver, Lucie Dostal, Agnes Fournier, Susan E. Hankinson, Brian E. Henderson, Robert N. Hoover, Claudine Isaacs, Mattias Johansson, Laurence N. Kolonel, Peter Kraft, I-Min Lee, Catherine A. McCarty, Kim Overvad, Salvatore Panico, Petra H.M. Peeters, Elio Riboli, Maria José Sanchez, Fredrick R. Schumacher, Guri Skeie, Daniel O. Stram, Michael J. Thun, Dimitrios Trichopoulos, Shumin Zhang, Regina G. Ziegler, David J. Hunter, Sara Lindström, Federico Canzian

Manuscript received August 23, 2010; revised June 14, 2011; accepted June 21, 2011.

Correspondence to: Federico Canzian, PhD, Genomic Epidemiology Group, German Cancer Research Center (Deutsches Krebsforschungszentrum; DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany (e-mail: f.canzian@dkfz.de).

Background Recently, several genome-wide association studies have identified various genetic susceptibility loci for breast cancer. Relatively little is known about the possible interactions between these loci and the established risk factors for breast cancer.

Methods To assess interactions between single-nucleotide polymorphisms (SNPs) and established risk factors, we prospectively collected DNA samples and questionnaire data from 8576 breast cancer case subjects and 11892 control subjects nested within the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3). We genotyped 17 germline SNPs (*FGFR2*-rs2981582, *FGFR2*-rs3750817, *TNRC9*-rs3803662, 2q35-rs13387042, *MAP3K1*-rs889312, 8q24-rs13281615, *CASP8*-rs1045485, *LSP1*-rs3817198, *COL1A1*-rs2075555, *COX11*-rs6504950, *RNF146*-rs2180341, 6q25-rs2046210, *SLC4A7*-rs4973768, *NOTCH2*-rs11249433, 5p12-rs4415084, 5p12-rs10941679, *RAD51L1*-rs999737), and odds ratios were estimated by logistic regression to confirm previously reported associations with breast cancer risk. We performed likelihood ratio test to assess interactions between 17 SNPs and nine established risk factors (age at menarche, parity, age at menopause, use of hormone replacement therapy, family history, height, body mass index, smoking status, and alcohol consumption), and a correction for multiple testing of 153 tests (adjusted *P* value threshold = .05/153 = 3×10^{-4}) was done. Case–case comparisons were performed for possible differential associations of polymorphisms by subgroups of tumor stage, estrogen and progesterone receptor status, and age at diagnosis. All statistical tests were two-sided.

Results We confirmed the association of 14 SNPs with breast cancer risk (P_{trend} = 2.57×10^{-3} –3.96×10^{-19}). Three SNPs (*LSP1*-rs3817198, *COL1A1*-rs2075555, and *RNF146*-rs2180341) did not show association with breast cancer risk. After accounting for multiple testing, no statistically significant interactions were detected between the 17 SNPs and the nine risk factors. We also confirmed that SNPs in *FGFR2* and *TNRC9* were associated with greater risk of estrogen receptor–positive than estrogen receptor–negative breast cancer ($P_{heterogeneity}$ = .0016 for *FGFR2*-rs2981582 and $P_{heterogeneity}$ = .0053 for *TNRC9*-rs3803662). SNP 5p12-rs10941679 was statistically significantly associated with greater risk of progesterone receptor–positive than progesterone receptor–negative breast cancer ($P_{heterogeneity}$ = .0028).

Conclusion This study does not support the hypothesis that known common breast cancer susceptibility loci strongly modify the associations between established risk factors and breast cancer.

J Natl Cancer Inst 2011;103:1–12

Recently, multiple breast cancer susceptibility loci have been identified by several genome-wide association studies (GWAS) or studies of specific candidate single-nucleotide polymorphisms (SNPs) (1–10). Genetic variants that showed strong statistically significant associations with breast cancer risk (odds ratios [ORs] = 1.15–1.45, $P < 5 \times 10^{-7}$) were identified in fibroblast growth factor receptor 2 (*FGFR2*). The vicinity is referred to all genes, not only FGFR2. In other words, some of the SNPs mentioned in this paragraph are located directly within the mentioned genes, some others are near the genes. TOX high mobility group box family member 3 (*TOX3*; also known as *TNRC9*), mitogen-activated protein kinase kinase kinase 1 (*MAP3K1*), caspase 8 (*CASP8*), lymphocyte-specific protein 1 (*LSP1*), collagen type I alpha 1 (*COL1A1*), cytochrome c oxidase assembly homolog 11 (*COX11*),

CONTEXT AND CAVEATS

Prior knowledge

Genome-wide association studies have identified many single-nucleotide polymorphisms (SNPs) that are associated with breast cancer risk. Several established epidemiological factors are also associated with breast cancer risk. However, it is not well understood whether the interactions between the SNPs and established risk factors can modify breast cancer risk.

Study design

Prospective nested case–control study within the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3) to analyze the associations of 17 germline SNPs with breast cancer risk. Interactions between these 17 SNPs and nine established risk factors (age at menarche, parity, age at menopause, use of hormone replacement therapy, family history, height, body mass index, smoking status, and alcohol consumption) were tested.

Contribution

Of the 17 SNPs, 14 showed association with breast cancer risk. After correction for multiple testing, no statistically significant interactions between the 17 SNPs and the nine risk factors were detected.

Implication

The common polymorphisms associated with breast cancer risk tested in this study did not modify the association between established risk factors and breast cancer risk.

Limitation

Majority of the white subjects were of European descent, so analyses in other race or ethnicity were of limited statistical power.

From the Editors

ring finger protein 146 (*RNF146*), solute carrier family 4 member 7 (*SLC4A7*), neurogenic locus notch homolog protein 2 (*NOTCH2*), RAD51-like 1 (*RAD51L1*) genes, or in the vicinity of these genes, as well as in gene-poor regions on chromosomes 2q35, 8q24, 6q25, and 5p12 (1–10).

These SNPs are genetic markers and do not necessarily represent the functional variants responsible for the association with breast cancer risk. Relatively little is known about the possible interplay between established epidemiological and genetic risk factors for breast cancer risk (11–19). Previous reports suggest that specific SNPs in *FGFR2* (rs3750817 and rs1219648) modify the association between hormone replacement therapy (HRT) and breast cancer risk (13,14), although a large epidemiological study that included women from the Million Women Study did not confirm these findings (18).

Large-scale prospective data are needed to test reliably for interactions, defined here as departures from a multiplicative odds ratio model for the joint association of the SNPs and the established risk factors. In this study, we estimated interactions between 17 SNPs, previously reported to be associated with breast cancer risk and reaching genome-wide statistical significance in at least one previous study (*FGFR2*-rs2981582, *FGFR2*-rs3750817, *TNRC9*-rs3803662, 2q35-rs13387042, *MAP3K1*-rs889312, 8q24-rs13281615, *CASP8*-rs1045485, *LSP1*-rs3817198, *COL1A1*-rs2075555, *COX11*-rs6504950, *RNF146*-rs2180341, 6q25-rs2046210, *SLC4A7*-rs4973768, *NOTCH2*-rs11249433, 5p12-rs4415084, 5p12-rs10941679, *RAD51L1*-rs999737) (1–10), and nine established risk factors (age at menarche, parity, age at menopause, use of HRT, family history, height, body mass index (BMI), smoking status, and alcohol consumption), in the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3), a large consortium of prospective cohort studies from Europe and the United States.

Subjects and Methods

Study Population

The BPC3 has been described in detail elsewhere (20). Briefly, the consortium includes large well-established cohorts assembled in the United States and Europe, which have both DNA samples and extensive questionnaire information. These cohorts are the American Cancer Society Cancer Prevention Study II (CPS-II) (21), the European Prospective Investigation into Cancer and Nutrition (EPIC) (22), the Nurses Health Study (NHS) (23), the Women's Health Study (WHS) (24), the Prostate, Lung, Colorectal, Ovarian (PLCO) Cancer Screening Trial (25), and the Multiethnic Cohort (MEC) (26).

Case subjects were identified in each cohort by self-report with subsequent confirmation of the diagnosis from medical records or tumor registries and/or direct linkage with population-based tumor registries (method of breast cancer case confirmation varied by cohort). Control subjects were matched with case subjects by ethnicity and age and, in some cohorts, additional criteria, such as country of residence in EPIC. The requirement for each control subject was to be free of cancer up to the duration of follow-up of the matched case subject.

Most of the subjects were white and of European descent. One cohort (MEC) provided most of the DNA samples from nonwhite subjects. In total, we genotyped (described below) 8576 case subjects and 11 892 control subjects, of whom 7023 case subjects and 10 065 control subjects were white (of European descent), 389 case subjects and 423 control subjects were Latino, 430 case subjects and 471 control subjects were African American, 552 case subjects and 580 control subjects were Asian American (mostly of Japanese origin), and 148 case subjects and 297 control subjects were Native Hawaiian. Table 1 describes the study populations in detail.

Informed consent was obtained from all subjects, and the project was approved by the relevant institutional review boards for each cohort.

SNP Selection and Genotyping

We selected SNPs that were reported to be associated with breast cancer risk and reached a commonly accepted threshold for genome-wide statistical significance ($P < 5 \times 10^{-7}$) (27) in at least one previous study. For two loci, we genotyped either the SNP reported in the original study or a surrogate in a complete or nearly complete linkage disequilibrium, based on data from the International HapMap Project (28,29). Namely, we genotyped rs4415084 or surrogate rs920329 (correlation coefficient $r^2 = 0.981$ in HapMap Centre d'Etude du Polymorphisme Humain [CEPH] or CEU Utah residents with ancestry from Northern and Western Europe); likewise, we genotyped rs999737 or surrogate rs10483813 ($r^2 = 1$ in HapMap CEU). We selected two SNPs from the locus on chromosome 5p12. Both were reported to be strongly

Table 1. Summary characteristics of study population*

Characteristic	CPS-II		EPIC		MEC		NHS		PLCO		WHS		All	
	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects
No.	856	620	3275	2399	2252	1998	3839	2083	974	775	696	701	11892	8576
Age at diagnosis, y Mean(SD)	–	70.09 (6.51)	–	58.63 (7.80)	–	64.92 (8.48)	–	61.23 (10.39)	–	66.32 (5.69)	–	60.34 (7.59)	–	62.39 (9.08)
Stage of breast cancer, No.														
Localized	–	472	–	1154	–	1446	–	1356	–	434	–	378	–	5240
Advanced	–	124	–	497	–	529	–	514	–	231	–	274	–	2169
Unknown	–	24	–	748	–	23	–	213	–	110	–	49	–	1167
ER status, No.														
Positive	–	460	–	1047	–	1385	–	1500	–	520	–	563	–	5475
Negative	–	38	–	495	–	343	–	346	–	78	–	100	–	1400
Borderline or unknown	–	122	–	857	–	270	–	237	–	177	–	38	–	1701
PR status, No.														
Positive	–	370	–	563	–	1113	–	1258	–	438	–	504	–	4246
Negative	–	104	–	452	–	473	–	549	–	130	–	148	–	1856
Unknown	–	146	–	1384	–	412	–	276	–	207	–	49	–	2474
Ethnicity, No.														
Caucasian	854	618	3275	2399	570	524	3740	2040	961	772	665	670	10065	7023
Hispanic	0	0	0	0	400	380	10	3	9	2	4	4	423	389
African American	0	0	0	0	437	409	29	16	0	0	5	5	471	430
Asian	2	1	0	0	555	545	23	6	0	0	0	0	580	552
Hawaiian	0	0	0	0	290	140	0	1	0	0	7	7	297	148
Other or unknown	0	1	0	0	0	0	37	17	4	1	15	15	56	34
Height, m, Mean (SD)	1.64 (0.06)	1.64 (0.06)	1.62 (0.07)	1.63 (0.06)	1.61 (0.07)	1.61 (0.07)	1.64 (0.06)	1.65 (0.06)	1.64 (0.06)	1.64 (0.06)	1.64 (0.06)	1.64 (0.06)	1.63 (0.07)	1.63 (0.07)
BMI, kg/m^2, Mean (SD)														
Overall	25.42 (4.62)	25.56 (4.63)	25.54 (4.35)	25.51 (4.50)	26.76 (5.93)	26.65 (5.59)	25.84 (5.21)	25.98 (5.05)	27.08 (5.43)	26.84 (5.13)	25.95 (5.09)	25.49 (4.44)	26.01 (5.14)	26.01 (4.99)
Premenopausal	27.42 (7.05)	26.49 (4.97)	24.95 (4.28)	24.66 (4.30)	27.10 (6.47)	27.08 (5.96)	25.69 (5.59)	25.30 (5.00)	–	–	25.96 (4.92)	25.15 (4.61)	25.68 (5.35)	25.36 (4.96)
Postmenopausal	25.32 (4.46)	25.51 (4.63)	25.84 (4.38)	25.88 (4.55)	26.69 (5.81)	26.51 (5.47)	25.86 (5.01)	26.27 (4.98)	27.08 (5.44)	26.84 (5.12)	25.79 (4.76)	25.28 (4.06)	26.13 (5.07)	26.17 (4.96)
Age at menarche, No.														
Early (≤11 y)	169	121	667	277	183	170	876	491	194	161	160	173	2249	1393
Intermediate (12–13 y)	476	337	1390	1028	1789	1584	2192	1182	537	419	408	398	6792	4948
Late (≥14 y)	200	152	1080	956	261	212	759	395	240	195	109	110	2650	2020
Unknown	11	10	137	138	19	32	12	15	3	0	19	20	201	215
Ever full-term pregnancy, No.														
Yes	774	556	2737	1946	1974	1663	3259	1728	893	694	601	594	10238	7181
No	67	49	366	310	259	306	580	355	79	81	95	107	1446	1208
Unknown	15	15	172	143	19	29	0	0	2	0	0	0	208	187
Menopause, No.														
Premenopausal	35	25	816	523	338	236	1109	544	0	0	146	151	2444	1479
Postmenopausal	808	587	2049	1524	1825	1663	2609	1483	966	766	418	446	8675	6469

(Table continues)

Table 1 (Continued).

Characteristic	CPS-II		EPIC		MEC		NHS		PLCO		WHS		All	
	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects	Control subjects	Case subjects
Perimenopausal/ unknown	13	8	410	352	89	99	121	56	8	9	132	104	773	628
Age at menopause, No.														
Early (≤44 y)	200	122	249	156	633	502	358	174	252	185	92	72	1784	1211
Intermediate (45–49 y)	214	129	534	357	506	458	565	310	225	165	126	131	2170	1550
Late (≥50 y)	390	335	884	638	686	703	1151	682	489	416	176	218	3776	2992
Unknown	4	1	382	373	0	0	214	117	0	0	24	25	624	516
Ever use of HRT, No.														
Yes	504	386	771	691	1071	1081	1151	747	679	569	241	289	4417	3763
No	299	195	1173	797	692	533	1458	736	280	194	157	136	4059	2591
Unknown	5	6	105	36	62	49	0	0	7	3	20	21	199	115
Ever use of estrogen-only HRT, No.														
Yes	362	242	172	119	207	191	738	473	0	0	109	119	1588	1144
No	416	322	1215	797	249	254	1504	867	0	0	289	306	3673	2546
Unknown	30	23	662	608	1369	1218	367	143	966	766	20	21	3414	2779
Ever use of combined estrogen plus progestin HRT, No.														
Yes	146	157	296	337	131	198	504	390	0	0	132	170	1209	1252
No	632	407	1187	797	325	247	1738	950	0	0	266	255	4148	2656
Unknown	30	23	566	390	1369	1218	367	143	966	766	20	21	3318	2561
First-degree relatives with breast cancer, No.														
Yes	126	114	55	106	252	349	593	485	156	150	113	139	1295	1343
No	696	484	531	655	1900	1552	3246	1598	814	617	570	541	7757	5447
Unknown	34	22	2689	1638	100	97	0	0	4	8	13	21	2840	1786
Tobacco smoking†, No.														
Never smoker	505	310	1780	1280	1239	1050	2035	1031	578	405	346	348	6483	4424
Former smoker	297	268	831	560	669	630	1412	810	320	300	256	256	3785	2824
Current smoker	47	33	626	521	312	280	378	239	75	70	93	97	1531	1240
Unknown	7	9	38	38	32	38	14	3	1	0	1	0	93	88
Alcohol consumption‡, No.														
Non-drinker	455	319	1000	650	1574	1350	1669	843	482	343	369	351	5549	3856
Moderate	276	195	1568	1117	480	437	1573	806	268	226	278	282	4443	3063
Regular	76	76	704	632	198	211	347	269	127	149	49	68	1501	1405
Unknown	49	30	3	0	0	0	250	165	97	57	0	0	399	252

* Case subjects and matched control subjects were identified from the National Cancer Institute's Breast and Prostate Cancer Cohort Consortium (BPC3) that included the following studies: Cancer Prevention Study II (CPS-II), European Prospective Investigation into Cancer and Nutrition (EPIC), Multiethnic Cohort (MEC), Nurses' Health Study (NHS), Prostate, Lung, Colorectal, Ovarian (PLCO) Cancer Screening Trial, Women's Health Study (WHS). BMI = body mass index; ER = estrogen receptor; HRT = hormone replacement therapy; PR = progesterone receptor; - = not applicable.

† Never-smokers were defined as subjects who did not report any consumption of tobacco products up to the time of recruitment; former smokers were subjects who had given up tobacco smoking at the time of recruitment; current smokers were subjects who reported consumption of tobacco products at the time of recruitment.

‡ Non-drinker (<1 g alcohol per day); moderate drinker (<14 g alcohol per day); regular drinker (≥14 g alcohol per day).

associated with breast cancer risk (6), but there was only moderate linkage disequilibrium between the two SNPs (r^2 = 0.5 in the Icelandic population of the original study; r^2 = 0.51 in HapMap CEU subjects), and they could possibly represent two distinct susceptibility loci.

Genotyping assays were designed and performed using Taqman assays with reagents by Applied Biosystems (Foster City, CA). Details of primers and probes are available upon request. Genotyping of the breast cancer case subjects and control subjects was performed in four laboratories (located at the University of Southern California, the US National Cancer Institute, Harvard School of Public Health, and the German Cancer Research Center, DKFZ). Laboratory personnel were blinded to case–control status. Within each study, blinded duplicate samples (approximately 5%) were also included and concordance of these samples was greater than 99%.

The genotyping success rate was 94.14% overall (range 93.09%–95.62%). It was 97.11% (range 95.28%–98.86%) for white subjects of European descent.

Data Filtering and Statistical Analysis

DNA samples were excluded from further analysis if more than 25% of the SNPs in the samples failed genotyping. Genotype frequencies of each SNP were checked for deviation ($P < 10^{-3}$) from the expected Hardy–Weinberg proportions among control subjects of European ancestry within a cohort or overall.

We examined whether each of the 17 selected SNPs (1–10) was associated with risk of breast cancer by fitting for each SNP an unconditional logistic regression model involving the SNP and adjustment for age at baseline, study, ethnicity (within MEC), and country (for EPIC). An additional analysis was run with a model including also the established risk factors as adjustment variables. Genotypes were coded either as allele count (trend test) or as three categories: for major-allele homozygotes (reference category), for heterozygotes, and for minor-allele homozygotes (two *df* test). We calculated P_{trend} for each SNP as *P* value when coding minor alleles as trend variable. We performed these analyses in all subjects, separately for each ethnicity, and (for white subjects of European descent and African Americans) separately for each study within ethnicity.

We investigated the hypothesis that the odds ratios associated with nine established risk factors for breast cancer (age at menarche, parity, age at menopause, use of HRT, family history, height, BMI, smoking status, and alcohol consumption) could be modified by any one of 17 SNPs. Information on these established risk factors was recorded prospectively at the time the women joined the study or provided a blood sample (ie, before the diagnosis of breast cancer among the case subjects and at an equivalent time for the control subjects).

To test for interactions between SNPs and the established risk factors, we analyzed two models for each SNP—one with terms for the SNP and the covariate of interest, the other including additional interaction term(s) between the SNP and the covariate. SNPs were coded as counts of minor alleles (trend variable), and other risk factors were coded in categories. Both models were adjusted for age at baseline, study, ethnicity (within MEC), and country (for EPIC). We then computed the likelihood ratio test between the two models to test for a particular form of interaction, namely, departure from a multiplicative odds ratio model for the joint association of a genetic marker and an established risk factor. We did this for each SNP–covariate pair. The non-SNP variables were grouped as follows: age at menarche (early, ≤11 years; intermediate, 12–13 years; late, ≥14 years); age at menopause (early, ≤44 years; intermediate, 45–49 years; late, ≥50 years); BMI (BMI < 25 kg/m^2; 25 kg/m^2 ≤ BMI < 30 kg/m^2; BMI ≥ 30 kg/m^2, separately for pre- and postmenopausal women); alcohol intake (non-drinker, <1 g alcohol per day; moderate drinker, <14 g alcohol per day; regular drinker, 14 g alcohol per day); height (<1.63 m or ≥1.63 m); use of HRT (never or ever use of any type of HRT, never or ever use of estrogen-only HRT, never or ever use of combined estrogen plus progestin HRT); smoking status (never, former, or current smoker); family history (presence or absence of first-degree relatives diagnosed with breast cancer); and parity (nulliparous or parous). We also computed stratum-specific odds ratios and 95% confidence intervals (CIs) for each SNP. An additional analysis was performed with models including not only each covariate of interest but also all other established risk factors. To correct for multiple testing of the 153 (17 × 9 = 153) tests performed, we evaluated statistical significance at an adjusted *P* value threshold ($P = .05/153 = 3 \times 10^{-4}$).

We performed case-only analyses to test for differences in the associations of SNP with breast cancer risk with respect to different prognostic factors. Specifically, we compared estrogen receptor–negative (ER^-) case subjects with ER-positive (ER^+) case subjects as the reference, and in a similar fashion progesterone receptor–negative (PR^-) case subjects with PR-positive (PR^+) case subjects, advanced case subjects with nonadvanced case subjects (advanced disease was defined as having regional or distant metastasis), and case subjects diagnosed before the age of 55 with case subjects diagnosed after the age of 55 years. A statistically significant association between an SNP and breast cancer subgroup in this analysis was interpreted as a statistically significant heterogeneous association of the SNP on the different disease characteristics. We also performed case–control analyses by subgroups according to ER^+ or ER^- status, PR^+ or PR^- status, advanced or nonadvanced disease, and age at diagnosis.

For all subjects, analyses were performed after adjusting for cohort, age, country within EPIC, study phase in NHS, and ethnicity for MEC and PLCO.

We calculated the interaction odds ratios (ie, ORs of the interaction term between each SNP and each established risk factor) that we could detect in our study with 80% or greater power. The power calculation was performed assuming a multiplicative model of interaction and taking into account multiple testing. For these calculations, we considered only the SNPs that showed a statistically significant association with breast cancer risk.

Mathematical models for all analyses are reported in the Supplementary Methods (available online). All statistical tests were two-sided, and all statistical analyses were performed with SAS version 9.2 (SAS Institute Inc, Cary, NC).

Results

After exclusions, the analyses included 8576 breast cancer case subjects and 11 892 control subjects from the six cohorts. The

summary characteristics of the study population are shown in Table 1 and Supplementary Table 1 (available online). Case subjects and control subjects were predominantly white and were of European descent (7023 case subjects and 10 065 control subjects; overall 83%) and peri- or postmenopausal (7097 case subjects and 9448 control subjects; overall 81%) at the time of enrollment. The mean age at diagnosis was 62.39 (SD = 9.08 years). All established risk factors were associated with breast cancer risk, as shown in Supplementary Table 2 (available online). None of the SNPs were excluded from further analyses because of deviation from fitness for Hardy–Weinberg proportion ($P < 10^{-3}$).

For each SNP, the genotype frequencies in case subjects and control subjects and the association with breast cancer risk (P_{trend} = 2.57×10^{-3}–3.96×10^{-19}) are shown in Table 2. Associations between SNPs and breast cancer risk did not differ materially from those reported previously (1–10), except for three SNPs that did not show evidence of association with breast cancer risk (*LSP1*-rs3817198, P_{trend} = .89; *COL1A1*-rs2075555, P_{trend} = .42; and *RNF146*-rs2180341, P_{trend} = .11). Similar results were obtained when we corrected for multiple testing and adjusted for the established breast cancer risk factors; all SNPs remained statistically significantly associated with breast cancer risk (P_{trend} = .023–1.02×10^{-11}), except *LSP1*-rs3817198 (P_{trend} = .33), *COL1A1*-rs2075555 (P_{trend} = .74), and *RNF146*-rs2180341 (P_{trend} = .60), as shown in Supplementary Table 3 (available online). Tests of heterogeneity for the associations between SNPs and breast cancer risk of SNPs across cohorts and different ethnic groups (Supplementary Table 4, available online) were not statistically significant, with the exception of *TNRC9*-rs3803662 ($P_{heterogeneity} = 9.18 \times 10^{-5}$) that showed statistically significant association with breast cancer risk in the white ($P = 9.84 \times 10^{-6}$) and African American (P = .004) subjects, an association of borderline statistical significance among Hispanic subjects (P = .04), and a non-statistically significant association in Asian American (P = .53) and Native Hawaiian (P = .65) subjects.

Table 3 shows the results of interaction tests between each of the 17 SNPs and the established risk factors, and the P values of likelihood ratio tests comparing models with or without interaction term(s) between SNPs and covariates are presented. The detailed results of tests for associations between SNPs and breast cancer risk within each stratum of established risk factors are shown in Supplementary Table 5 (available online). After correction for multiple testing, we observed no statistically significant interactions in any of the 153 ($17 \times 9 = 153$) tests (adjusted P value threshold = $.05/153 = 3 \times 10^{-4}$). The strongest statistical significance was observed for interaction between 5p12-rs10941679 on chromosome 5 and use of estrogen-only HRT (P = .0072) (Table 3). This SNP was associated with increased breast cancer risk in users and nonusers of estrogen-only HRT but more strongly associated in the group of users, as measured by odds ratio of an increasing number of minor alleles (nonusers, OR_{allele} = 1.10, 95% CI = 1.02 to 1.19; users, OR_{allele} = 1.35, 95% CI = 1.19 to 1.53; $OR_{interaction}$ = 1.22, 95% CI = 1.06 to 1.41). We did not observe any interaction between 5p12-rs10941679 and use of HRT overall (P = .97), or with combined estrogen plus progestin HRT (P = .80). Analyses taking into account the duration of HRT use showed that *COX11*-rs6504950 was associated more strongly with breast cancer risk in women who used HRT for more than 5 years than in women who used HRT for less than 5 years, although the interaction did not reach statistical significance when corrected for multiple testing (P = .0035) (data not shown). When we adjusted the statistical models for all established breast cancer risk factors, we did not observe any statistically significant interaction with the same adjusted P value threshold (Supplementary Table 6, available online). It was previously suggested that *FGFR2*-rs3750817 shows an interaction with HRT (13); however, we did not find any clear evidence of interaction with the use of HRT. Indeed, point estimates of risks associated with this SNP in HRT users (OR = 0.87, 95% CI = 0.81 to 0.94) and nonusers (OR = 0.79, 95% CI = 0.73 to 0.85) differed only at borderline level of statistical significance (P = .05; Table 3 and Supplementary Tables 5 and 7, available online).

To investigate whether the SNPs were associated with particular forms of breast cancer, we analyzed the associations between 17 SNPs and breast cancer risk by subgroups of advanced or non-advanced disease, by ER or PR status, and by age at diagnosis (Table 4). We evaluated heterogeneity of associations between SNPs and breast cancer risk by case–case comparisons between case subjects grouped according to clinical variables. After correction for multiple testing for 17 SNPs (adjusted P value threshold = .05/17 = .0029), the results of the subgroup analyses showed that 5p12-rs10941679 was statistically significantly associated with greater risk of PR^+ breast cancer than PR^- breast cancer ($P_{heterogeneity}$ = .0028), and *FGFR2*-rs2981582 was statistically significantly associated with greater risk of ER^+ breast cancer than ER^- breast cancer ($P_{heterogeneity}$ = .0016). Additionally, if we considered associations showing P less than or equal to an arbitrary threshold of .01, another SNP on locus 5p12, rs4415084, also showed association with greater risk of PR^+ breast cancer than PR^- breast cancer ($P_{heterogeneity}$ = .010). *TNRC9*-rs3803662 and the second SNP in the *FGFR2* gene, rs3750817, were associated with greater risk of ER^+ breast cancer than ER^- breast cancer ($P_{heterogeneity}$ = .0053 and .0063, respectively) (Table 4). *FGFR2*-rs2981582 was also associated with a higher risk of being diagnosed with breast cancer at younger age ($P_{heterogeneity}$ = .0042), and similar was the observation for *COL1A1*-rs2075555 ($P_{heterogeneity}$ = .0098) (Table 4). However, *COL1A1*-rs2075555 did not show an association with breast cancer risk overall in this study (Table 2). The SNP 8q24-rs13281615 was previously reported to be associated with risk of ER^+ breast cancer, but not with risk of ER^- breast cancer (30). However, we did not observe any evidence of a differential association between this SNP and breast cancer risk depending on ER status ($P_{heterogeneity}$ = .13) (Table 4). The results of the subgroup analyses are shown in details in Supplementary Table 8 (available online).

Discussion

In this article, we report findings from a consortium of large prospective studies of possible interactions between 17 polymorphisms that have been associated with breast cancer and established risk factors for the disease. Data were examined using a nested case–control design within the National Cancer Institute's BPC3 and included 8576 case subjects with breast cancer and 11 892 control subjects without breast cancer.

Table 2. Associations between selected SNPs and breast cancer risk*

SNP	Gene	Chr	Location, bp†	Case subjects			Control subjects			OR_{het} (95% CI)§	OR_{hom} (95% CI)§	OR_{allele} (95% CI)§	P_{trend}‖	P_{2df}¶	Reference
				A/A‡	A/B‡	B/B‡	A/A‡	A/B‡	B/B‡						
rs11249433	*NOTCH2*	1	120,982,136	3356	3682	1323	4751	5058	1708	1.10 (1.03 to 1.18)	1.19 (1.09 to 1.30)	1.09 (1.05 to 1.14)	3.40×10^{-5}	1.76×10^{-4}	8
rs1045485	*CASP8*	2	201,857,834	6414	1539	110	8834	2345	197	0.91 (0.85 to 0.98)	0.79 (0.62 to 1.00)	0.91 (0.85 to 0.97)	2.57×10^{-3}	9.84×10^{-3}	5
rs13387042	Intergenic	2	217,614,077	2501	3698	2116	2900	5517	3176	0.78 (0.72 to 0.83)	0.73 (0.67 to 0.79)	0.85 (0.81 to 0.88)	4.06×10^{-15}	1.99×10^{-16}	3
rs4973768	*SLC4A7*	3	27,391,017	2388	4035	1915	3457	5615	2475	1.07 (1.00 to 1.14)	1.17 (1.08 to 1.27)	1.08 (1.04 to 1.13)	2.37×10^{-4}	1.06×10^{-3}	10
rs4415084#	Intergenic	5	44,698,272	2450	4113	1708	3820	5482	2239	1.14 (1.07 to 1.22)	1.14 (1.05 to 1.24)	1.08 (1.03 to 1.12)	4.49×10^{-4}	2.31×10^{-4}	6
rs10941679	Intergenic	5	44,742,255	4203	3422	782	6148	4383	900	1.13 (1.06 to 1.20)	1.25 (1.12 to 1.39)	1.12 (1.07 to 1.17)	5.14×10^{-7}	3.22×10^{-6}	6
rs889312	*MAP3K1*	5	56,067,641	3788	3509	985	5675	4793	1172	1.09 (1.03 to 1.16)	1.23 (1.11 to 1.36)	1.10 (1.05 to 1.15)	1.36×10^{-5}	6.83×10^{-5}	1
rs2180341	*RNF146*	6	127,642,323	4059	2428	393	5691	3584	587	0.95 (0.89 to 1.01)	0.94 (0.82 to 1.08)	0.96 (0.91 to 1.01)	1.14×10^{-1}	2.43×10^{-1}	7
rs2046210	Intergenic	6	151,990,059	3322	3796	1180	4904	5161	1478	1.11 (1.04 to 1.18)	1.20 (1.10 to 1.31)	1.10 (1.05 to 1.15)	1.41×10^{-5}	7.66×10^{-5}	9
rs13281615	Intergenic	8	128,424,801	2494	4044	1764	3813	5609	2193	1.09 (1.02 to 1.16)	1.19 (1.09 to 1.29)	1.09 (1.05 to 1.13)	3.31×10^{-5}	1.81×10^{-4}	1
rs3750817	*FGFR2*	10	123,322,567	2725	3115	930	3550	4755	1616	0.85 (0.79 to 0.91)	0.74 (0.67 to 0.81)	0.86 (0.82 to 0.90)	2.18×10^{-11}	1.66×10^{-10}	13
rs2981582	*FGFR2*	10	123,342,308	2794	3951	1568	4420	5456	1718	1.16 (1.09 to 1.24)	1.48 (1.36 to 1.61)	1.21 (1.16 to 1.26)	3.96×10^{-19}	1.30×10^{-18}	1,2
rs3817198	*LSP1*	11	1,865,583	4131	3382	779	5611	4875	1072	0.97 (0.91 to 1.03)	1.03 (0.93 to 1.14)	1.00 (0.95 to 1.04)	8.93×10^{-1}	3.73×10^{-1}	1,3
rs999737**	*RAD51L1*	14	68,104,435	4242	2356	332	5876	3474	599	0.93 (0.87 to 0.99)	0.77 (0.67 to 0.89)	0.91 (0.86 to 0.95)	2.41×10^{-4}	5.79×10^{-4}	8
rs3803662	*TNRC9*	16	51,143,843	3706	3528	1071	5721	4724	1150	1.14 (1.07 to 1.21)	1.38 (1.25 to 1.52)	1.16 (1.11 to 1.21)	9.04×10^{-12}	4.89×10^{-11}	1,3
rs2075555	*COL1A1*	17	45,629,290	5125	1646	139	7482	2320	210	1.04 (0.97 to 1.12)	0.96 (0.77 to 1.19)	1.02 (0.96 to 1.09)	4.18×10^{-1}	4.27×10^{-1}	4
rs6504950	*COX11*	17	50,411,470	4784	3066	537	6304	4467	860	0.92 (0.87 to 0.98)	0.85 (0.76 to 0.96)	0.92 (0.88 to 0.97)	5.83×10^{-4}	2.70×10^{-3}	10

* bp = base pair; Chr = Chromosome; CI = confidence interval; OR_{het} = odds ratio of heterozygotes vs homozygotes for the major allele; OR_{hom} = odds ratio of homozygotes for the minor allele vs homozygotes for the major allele; OR_{allele} = odds ratio of each increasing number of minor alleles; SNP = single-nucleotide polymorphism.

† National Center for Biotechnology Information genome, build 36 (http://genome.ucsc.edu/cgi-bin/hgGateway).

‡ A = major allele in HapMap CEU subjects; B = minor allele in HapMap CEU subjects (28,29).

§ Odds ratios were adjusted for age and subcohort (defined by country in the European Prospective Investigation into Cancer and Nutrition [EPIC]; ethnicity in the Multiethnic Cohort [MEC]; and Prostate, Lung, Colorectal, Ovarian [PLCO] Cancer Screening Trial and study phase in the Nurses' Health Study [NHS]; Cancer Prevention Study II [CPS-II]; and Women's Health Study [WHS] account each for a single subcohort).

‖ *P* values for trend (two-sided) were derived from Cochran–Armitage trend tests (*df* = 1).

¶ *P* values for the Cochran–Armitage trend test (two-sided; *df* = 2) were obtained by coding genotypes as three categories: for major-allele homozygotes (referent), heterozygotes, and for minor-allele homozygotes.

5p12-rs4415084 or surrogate 5p12-rs920329 (Pearson correlation coefficient $r^2 = 0.981$ in HapMap CEU).

** *RAD51L1*-rs999737 or surrogate *RAD51L1*-rs10483813 (Pearson correlation coefficient $r^2 = 1$ in HapMap CEU).

Table 3. Interactions between SNPs and established risk factors*

				Established risk factor, *P*†										
SNP	Gene	Chr	Location, bp‡	BMI§	Height‖	Age atmenarche¶	Full-termpregnancy#	Age atmenopause**	HRT use††	HRT-E use‡‡	HRT-C use§§	Familyhistory‖‖	Smoking¶¶	Alcohol##
rs11249433	*NOTCH2*	1	120,982,136	.86	.77	.55	.79	.59	.36	.18	.73	.49	.52	.97
rs1045485	*CASP8*	2	201,857,834	.74	.042	.99	.094	.10	.42	.76	.45	.61	.044	.23
rs13387042	Intergenic	2	217,614,077	.63	.76	.056	.53	.14	.78	.054	.26	.081	.53	.75
rs4973768	*SLC4A7*	3	27,391,017	.91	.047	.71	.92	.85	.84	.87	.39	.31	.088	.97
rs4415084***	Intergenic	5	44,698,272	.40	.41	.27	.084	.79	.62	.082	.97	.31	.61	.39
rs10941679	Intergenic	5	44,742,255	.64	.25	.41	.13	.46	.97	.0072	.80	.085	.089	.18
rs889312	*MAP3K1*	5	56,067,641	.42	.32	.52	.12	.12	.94	.76	.26	.50	.50	.14
rs2180341	*RNF146*	6	127,642,323	.92	.011	.15	.27	.54	.56	.89	.40	.23	.57	.97
rs2046210	Intergenic	6	151,990,059	.52	.22	.43	.34	.76	.48	.89	.53	.20	.26	.42
rs13281615	Intergenic	8	128,424,801	.60	.60	.018	.77	.15	.26	.43	.27	.57	.57	.075
rs2981582	*FGFR2*	10	123,342,308	.35	.40	.71	.10	.59	.27	.50	.53	.19	.83	.52
rs3750817	*FGFR2*	10	123,322,567	.58	.84	.60	.055	.21	.050	.10	.28	.16	.81	.036
rs3817198	*LSP1*	11	1,865,583	.87	.81	.73	.18	.047	.99	.52	.34	.50	.15	.94
rs999737†††	*RAD51L1*	14	68,104,435	.15	.80	.63	.46	.91	.19	.99	.16	.043	.27	.21
rs3803662	*TNRC9*	16	51,143,843	.079	.72	.44	.73	.41	.82	.49	.012	.42	.069	.75
rs2075555	*COL1A1*	17	45,629,290	.80	1.00	.84	.20	.80	.40	.92	.14	.70	.27	.42
rs6504950	*COX11*	17	50,411,470	.79	.10	.71	.062	.51	.52	.87	.67	.17	.22	.22

* Two models were analyzed for each SNP–covariate pair: one with the terms for the SNP and the covariate of interest, and one including the additional interaction term(s) between the SNP and the covariate. Both models were adjusted for age at baseline, study, ethnicity (within the Multiethnic Cohort [MEC]), and country (for the European Prospective Investigation into Cancer and Nutrition [EPIC]). BMI = body mass index; bp = base pair; Chr = chromosome; HRT = hormone replacement therapy; HRT-E = hormone replacement therapy-estrogen; HRT-C = hormone replacement therapy-combined; SNP = single-nucleotide polymorphisms.

† *P* values were calculated using two-sided likelihood ratio test.

‡ National Center for Biotechnology Information genome build 36 (http://genome.ucsc.edu/cgi-bin/hgGateway).

§ BMI (BMI < 25 kg/m^2; 25 kg/m^2 ≤ BMI < 30 kg/m^2; BMI ≥ 30 kg/m^2).

‖ Height (≤1.63 m; >1.63 m).

¶ Early menarche (age ≤ 11 years); intermediate menarche (age 12–13 years); late menarche (age ≥ 14 years).

Full-term pregnancy (ever or never).

** Early menopause (age ≤ 44 years); intermediate menopause (age 45–49 years); late menopause (age ≥ 50 years).

†† Use of HRT (ever or never).

‡‡ Use of estrogen-only HRT (ever or never).

§§ Use of combined (estrogen plus progestin) HRT (ever or never).

‖‖ First-degree relatives diagnosed with breast cancer (present or absent).

¶¶ Never-smokers were defined as subjects who did not report any consumption of tobacco products up to the time of recruitment; former smokers were subjects who had given up tobacco smoking at the time of recruitment; current smokers were subjects who reported consumption of tobacco products at the time of recruitment.

Non-drinker (<1 g alcohol per day); moderate drinker (<14 g alcohol per day); regular drinker (≥14 g alcohol per day).

*** 5p12-rs4415084 or surrogate 5p12-rs920329 (Pearson correlation coefficient r^2 = 0.981 in HapMap CEU).

††† *RAD51L1*-rs999737 or surrogate *RAD51L1*-rs10483813 (Pearson correlation coefficient r^2 = 1 in HapMap CEU).

Table 4. Heterogeneity of associations between SNPs and breast cancer risk by different prognostic factors*

SNP	Gene	Chr	Location, bp†	Stratification variable, *P*‡			
				Stage§	Age at diagnosis‖	ER¶	PR#
rs11249433	*NOTCH2*	1	120,982,136	.75	.83	.085	.79
rs1045485	*CASP8*	2	201,857,834	.23	.38	.96	.22
rs13387042	Intergenic	2	217,614,077	.73	.96	.12	.21
rs4973768	*SLC4A7*	3	27,391,017	.078	.47	.090	.13
rs4415084**	Intergenic	5	44,698,272	.34	.14	.21	.010
rs10941679	Intergenic	5	44,742,255	.23	.11	.10	.0028
rs889312	*MAP3K1*	5	56,067,641	.29	.17	.067	.48
rs2180341	*RNF146*	6	127,642,323	.86	.98	.80	.39
rs2046210	Intergenic	6	151,990,059	.51	.94	.46	.50
rs13281615	Intergenic	8	128,424,801	.89	.65	.13	.094
rs2981582	*FGFR2*	10	123,342,308	.24	.0042	.0016	.013
rs3750817	*FGFR2*	10	123,322,567	.73	.80	.0063	.51
rs3817198	*LSP1*	11	1,865,583	.35	.40	.58	.85
rs999737††	*RAD51L1*	14	68,104,435	.059	.15	.85	.48
rs3803662	*TNRC9*	16	51,143,843	.38	.59	.0053	.014
rs2075555	*COL1A1*	17	45,629,290	.77	.0098	.98	.55
rs6504950	*COX11*	17	50,411,470	.69	.077	.98	.078

* We performed case-only analysis to test for differences of the SNP association with breast cancer risk with respect to different prognostic factors. Specifically, we compared advanced case subjects with nonadvanced case subjects (advanced disease was defined as having regional or distant metastasis), case subjects diagnosed before the age of 55 years with case subjects diagnosed after 55 years, ER-negative (ER^-) case subjects with ER-positive (ER^+) case subjects (referent) and in a similar fashion PR-negative (PR^-) case subjects with PR-positive (PR^+) case subjects (referent). Analyses were performed for all subjects adjusting for cohort, age, country within the European Prospective Investigation into Cancer and Nutrition (EPIC), study phase in the Nurses' Health Study (NHS), and ethnicity for the Multiethnic Cohort (MEC) and the Prostate, Lung, Colorectal, Ovarian (PLCO) Cancer Screening Trial. bp = base pair; Chr = chromosome; ER = estrogen receptor; PR = progesterone receptor; SNP = single-nucleotide polymorphism.

† *P* values were calculated using two-sided Mantel–Haenszel test for heterogeneity.

‡ National Center for Biotechnology Information genome build 36 (http://genome.ucsc.edu/cgi-bin/hgGateway).

§ Advanced vs nonadvanced breast cancer case subjects (advanced disease was defined as having regional or distant metastasis).

‖ Case subjects diagnosed at age younger than 55 years vs diagnosed after 55 years of age.

¶ ER-positive breast cancer case subjects vs ER-negative breast cancer case subjects.

PR-positive breast cancer case subjects vs PR-negative breast cancer case subjects.

** 5p12-rs4415084 or surrogate 5p12-rs920329 (Pearson correlation coefficient $r^2 = 0.981$ in HapMap CEU).

†† *RAD51L1*-rs999737 or surrogate *RAD51L1*-rs10483813 (Pearson correlation coefficient $r^2 = 1$ in HapMap CEU).

We replicated all of the previously reported associations between SNPs and breast cancer risk, except for *LSP1*-rs3817198, *COL1A1*-rs2075555, and *RNF146*-rs2180341, which did not show association with breast cancer risk. It is worth noting that the association with *RNF146*-rs2180341 was reported only in a small study focusing on Ashkenazi Jews (7), which did not include replication in samples of other populations. Likewise, the association with *COL1A1*-rs2075555 was reported by a single study with only 58 cases of breast cancer, nested in the Framingham Heart Study (4). In light of the lack of association between these two SNPs and breast cancer risk in our study, we think that they most likely represent false positives or are relevant only to specific populations, such as women of Ashkenazi Jewish ancestry. The association between *LSP1*-rs3817198 and breast cancer risk was investigated in several studies: A statistically significant association at genome-wide level (albeit with a rather low $OR_{allele} = 1.07$) was reported by Easton et al. (1) but not confirmed in subsequent GWAS (8,9). Our results suggest that the association between this polymorphism and breast cancer risk is at best weak ($OR_{allele} = 1.00$; 95% CI = 0.95 to 1.04; $P_{trend} = .89$). For some of the other SNPs, whose associations with breast cancer risk are clearly replicated in our study, we found slightly lower odds ratios than reported in previous publications (1–10). However, the direction of associations was consistently the same, and our confidence intervals largely overlapped with those of the previous reports.

Previous studies have reported possible interactions between breast cancer susceptibility loci and established risk factors (13–17). These studies focused mainly on *FGFR2* and *MAP3K1* and hormonal and reproductive factors, particularly the use of HRT. A recent study within the Women's Health Initiative (13) showed a possible interaction between SNPs in *FGFR2* and HRT use. Another recent study (14) showed an interaction between SNP *FGFR2*-rs1219648 and use of combined HRT in women of European ancestry. These studies had smaller sample sizes than ours. *FGFR2*-rs1219648 is in strong linkage disequilibrium with *FGFR2*-rs2981582 (Pearson correlation coefficient $r^2 = 1$) (28,29), which did not show any evidence of interaction with HRT overall or with subtypes of HRT in this study (Table 3 and Supplementary Table 5, available online). As shown in the article by Prentice et al. (13), the *FGFR2* SNP showing the strongest interaction with HRT was rs3750817 ($P_{interaction} = .046$ for use of estrogen-only HRT, and $P_{interaction} = .033$ for use of estrogen–progestin HRT), which is only in modest linkage disequilibrium with rs2981582 (Pearson correlation coefficient $r^2 = 0.47$). We genotyped rs3750817 in all the case subjects and control subjects in our analyses but did not observe any clear evidence of interaction with use of HRT. There

was no evidence for interaction when we analyzed separately the use of estrogen-only HRT or combined estrogen plus progestin HRT.

A recent case–control study performed in a Japanese population (15) showed interactions between SNPs in *FGFR2* and family history of breast cancer, age at menarche, and parity. We did not observe any statistically significant interactions between SNPs *FGFR2*-rs2981582 or *FGFR2*-rs3750817 and any of these risk factors. The study by Kawase et al. (15) had a much smaller sample than ours (456 case subjects and 912 control subjects), and statistical significance of the interactions reported was modest (the strongest result was observed for interaction with family history of breast cancer $P_{interaction}$= .003); therefore, these could be chance findings.

Our results on interactions between SNPs and established risk factors are similar to those obtained in studies of comparable sample size, performed in the Million Women Study (18) and the Breast Cancer Association Consortium (19). Namely, no statistically significant interactions between SNPs and established breast cancer risk factors were detected in those studies, when multiple testing was taken into account (18,19).

Our study had greater than 80% power to detect interaction odds ratios (ie, ORs of the interaction term between each SNP and each established risk factor) ranging between 1.20 and 1.47 between the SNPs and the risk factors we considered. The power calculation was performed assuming a multiplicative model of interaction and taking into account multiple testing. Thus, we had a reasonably good chance to detect moderately large interactions between SNPs and established risk factors. This is also shown by the fact that 95% confidence intervals around interaction odds ratios were rather narrow for most SNP-established risk factor pairs (Supplementary Table 7, available online).

We cannot exclude the existence of real interactions of smaller magnitude (including interactions between SNPs and established risk factors that did not show a statistically significant association with breast cancer risk or had a relatively small association with breast cancer risk), which our study was not sufficiently powered to detect. If such interactions exist, they may shed light on poorly understood biological mechanisms, including the hitherto unknown function of most SNPs studied here. However, the relevance of such small interactions in terms of risk assessment and prevention would be limited.

Results from subgroup analyses on clinical characteristics of tumors were generally in agreement with previous reports (3,6,30–32), including a meta-analysis of all published data (32). Findings from previous studies suggested that several SNPs are predominantly associated with ER^+ breast cancer: *TNRC9*-rs3803662 (3,30–32), 5p12-rs4415084 (6), 5p12-rs10941679 (6), *FGFR2*-rs2981582 (6,30–32), 8q24-rs13281615 (30). In addition, *FGFR2*-rs2981582 was also reported to be more strongly associated with PR^+ cancers than with PR^- cancers (30). The SNP 2q35-rs13387042 was reported to be associated exclusively with ER^+ and PR^+ cancers (3), although later reports have shown that it is associated with both receptor-positive and receptor-negative cancers (31,32). In our data, SNPs on chromosome 5p12, *FGFR2* and *TNRC9* were preferentially associated with ER^+ and/or PR^+ breast cancer. In addition, SNP 2q35-rs13387042 showed a strongly statistically significant association with risk in ER^+ and PR^+ cases but not with ER^- and PR^- cases, although the heterogeneity was not statistically significant in our data, in agreement with previous studies (31,32). Because ER and PR status are the major markers of breast cancer subtypes, these observations suggest that inherited risk variants of these subtypes may vary. Contrary to one previous report (30) but consistent with results from a second study (32), we did not observe any evidence that SNP 8q24-rs13281615 had a stronger association with breast cancer risk depending on ER or PR status.

Our study has a few limitations. The vast majority of white subjects in the study are of European descent, and statistical power for analyses in other ethnicities is limited. In addition, many statistical tests were performed and, given that there were no a priori hypotheses about the possible interactions of SNPs and established risk factors, our findings should be taken with caution. Nevertheless, this is one of the largest cohort studies to systematically investigate possible interactions between major established risk factors for breast cancer and polymorphisms in the known susceptibility regions. It is very unlikely that we had nondifferential measurement error to the extent that could be a serious flaw in our study. Genotyping quality was monitored by a series of intra- and interlaboratory measures, including blind duplicated samples and measures of deviation from Hardy–Weinberg equilibrium. With respect to the established risk factors we included in our analyses, it is known that they are reliably measured in prospective cohorts, as documented by specific validation studies performed in some of the BPC3 cohorts (33–37).

Our study provides evidence against the hypothesis that common polymorphisms associated with breast cancer risk strongly modify the association of established factors with breast cancer risk. Our null findings are important given the size, prospective design, and the comprehensive approach of our study. However, our results do not rule out small departures from a multiplicative odds model for the joint association of pairs of individual SNPs and risk factors, nor does absence of departure from a multiplicative odds model necessarily imply that these genetic loci and risk factors do not interact in some causal mechanism. Moreover, absence of interaction as we have defined it here does not imply absence of a "public health interaction," where the benefit from reducing a risk factor in terms of absolute risk reduction differs across genotypes (38).

In conclusion, we studied almost 9000 women with breast cancer and 12 000 control subjects without breast cancer and showed that the 17 low-penetrance breast cancer susceptibility polymorphisms studied here do not strongly interact with established risk factors.

References

1. Easton DF, Pooley KA, Dunning AM, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature.* 2007; 447(7148):1087–1093.
2. Hunter DJ, Kraft P, Jacobs KB, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat Genet.* 2007;39(7):870–874.
3. Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet.* 2007;39(7):865–869.

4. Murabito JM, Rosenberg CL, Finger D, et al. A genome-wide association study of breast and prostate cancer in the NHLBI's Framingham Heart Study. *BMC Med Genet.* 2007;8(Suppl 1):S6.
5. Cox A, Dunning AM, Garcia-Closas M, et al. A common coding variant in CASP8 is associated with breast cancer risk. *Nat Genet.* 2007;39(3):352–358.
6. Stacey SN, Manolescu A, Sulem P, et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet.* 2008;40(6):703–706.
7. Gold B, Kirchhoff T, Stefanov S, et al. Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. *Proc Natl Acad Sci U S A.* 2008;105(11):4340–4345.
8. Thomas G, Jacobs KB, Kraft P, et al. A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet.* 2009;41(5):579–584.
9. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet.* 2009;41(3):324–328.
10. Ahmed S, Thomas G, Ghoussaini M, et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet.* 2009;41(5):585–590.
11. Huijts PE, Vreeswijk MP, Kroeze-Jansema KH, et al. Clinical correlates of low-risk variants in FGFR2, TNRC9, MAP3K1, LSP1 and 8q24 in a Dutch cohort of incident breast cancer cases. *Breast Cancer Res.* 2007;9(6):R78.
12. Liang J, Chen P, Hu Z, et al. Genetic variants in fibroblast growth factor receptor 2 (FGFR2) contribute to susceptibility of breast cancer in Chinese women. *Carcinogenesis.* 2008;29(12):2341–2346.
13. Prentice RL, Huang Y, Hinds DA, et al. Variation in the FGFR2 gene and the effects of postmenopausal hormone therapy on invasive breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2009;18(11):3079–3085.
14. Rebbeck TR, DeMichele A, Tran TV, et al. Hormone-dependent effects of FGFR2 and MAP3K1 in breast cancer susceptibility in a population-based sample of post-menopausal African-American and European-American women. *Carcinogenesis.* 2009;30(2):269–274.
15. Kawase T, Matsuo K, Suzuki T, et al. FGFR2 intronic polymorphisms interact with reproductive risk factors of breast cancer: results of a case control study in Japan. *Int J Cancer.* 2009;125(8):1946–1952.
16. Prentice RL, Huang Y, Hinds DA, et al. Variation in the FGFR2 gene and the effect of a low-fat dietary pattern on invasive breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2010;19(1):74–79.
17. Bhatti P, Doody MM, Rajaraman P, et al. Novel breast cancer risk alleles and interaction with ionizing radiation among U.S. radiologic technologists. *Radiat Res.* 2010;173(2):214–224.
18. Travis RC, Reeves GK, Green J, et al. Gene-environment interactions in 7610 women with breast cancer: prospective evidence from the Million Women Study. *Lancet.* 2010;375(9732):2143–2151.
19. Milne RL, Gaudet MM, Spurdle AB, et al. Assessing interactions between the associations of common genetic susceptibility variants, reproductive history and body mass index with breast cancer risk in the Breast Cancer Association Consortium: a combined case-control study. *Breast Cancer Res.* 2010;12(6):R110.
20. Hunter DJ, Riboli E, Haiman CA, et al. A candidate gene approach to searching for low-penetrance breast and prostate cancer genes. *Nat Rev Cancer.* 2005;5(12):977–985.
21. Calle EE, Rodriguez C, Jacobs EJ, et al. The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design and baseline characteristics. *Cancer.* 2002;94(9):2490–2501.
22. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr.* 2002;5(6B):1113–1124.
23. Colditz GA, Hankinson SE. The Nurses' Health Study: lifestyle and health among women. *Nat Rev Cancer.* 2005;5(5):388–396.
24. Rexrode K, Lee I, Cook N, et al. Baseline characteristics of participants in the Women's Health Study. *J Womens Health Gend Based Med.* 2000;9(1):19–27.
25. Hayes RB, Reding D, Kopp W, et al.; Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial Project Team. Etiologic and early marker studies in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Control Clin Trials.* 2000;21(6 Suppl):349S–355S.
26. Kolonel L, Henderson B, Hankin J, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am J Epidemiol.* 2000;151(4):346–357.
27. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007;447(7145):661–678.
28. International HapMap Consortium. The International HapMap Project. *Nature.* 2003;426(6968):789–796.
29. International HapMap Project. http://www.hapmap.org/.
30. Garcia-Closas M, Hall P, Nevanlinna H, et al. Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. *PLoS Genet.* 2008;4(4):e1000054.
31. Milne RL, Benítez J, Nevanlinna H, et al. Risk of estrogen receptor-positive and -negative breast cancer and single-nucleotide polymorphism 2q35-rs13387042. *J Natl Cancer Inst.* 2009;101(14):1012–1018.
32. Reeves GK, Travis RC, Green J, et al. Incidence of breast cancer and its subtypes in relation to individual and multiple low-penetrance genetic susceptibility loci. *JAMA.* 2010;304(4):426–434.
33. Spencer EA, Appleby PN, Davey GK, Key TJ. Validity of self-reported height and weight in 4808 EPIC-Oxford participants. *Public Health Nutr.* 2002;5(4):561–565.
34. Troy LM, Hunter DJ, Manson JE, Colditz GA, Stampfer MJ, Willett WC. The validity of recalled weight among younger women. *Int J Obes Relat Metab Disord.* 1995;19(8):570–572.
35. Colditz GA, Stampfer MJ, Willett WC, et al. Reproducibility and validity of self-reported menopausal status in a prospective cohort study. *Am J Epidemiol.* 1987;126(2):319–325.
36. Salvini S, Hunter DJ, Sampson L, et al. Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption. *Int J Epidemiol.* 1989;18(4):858–867.
37. Flagg EW, Coates RJ, Calle EE, Potischman N, Thun MJ. Validation of the American Cancer Society Cancer Prevention Study II Nutrition Survey Cohort Food Frequency Questionnaire. *Epidemiology.* 2000;11(4):462–468.
38. Siemiatycki J, Thomas DC. Biological models and statistical interactions: an example from multistage carcinogenesis. *Int J Epidemiol.* 1981;10(4):383–387.

Funding

US National Institutes of Health, National Cancer Institute (cooperative agreements U01-CA98233-07 to D.J.H.; U01-CA98710-06 to M.J.T.; U01-CA98216-06 to E.R. and R.K.; and U01-CA98758-07 to B.E.H.) and Intramural Research Program of National Institutes of Health and National Cancer Institute, Division of Cancer Epidemiology and Genetics.

Notes

The authors are solely responsible for the design of the study, the analysis and interpretation of the data, the writing of the article, and the decision to submit the article for publication. The writing team of the article included D. Campa, R. Kaaks, L. L. Marchand, C. A. Haiman, R. Travis, R. G. Ziegler, D. J. Hunter, S. Lindström, and F. Canzian.

Affiliations of authors: Genomic Epidemiology Group (DC, FC) and Division of Cancer Epidemiology (RK, LD), German Cancer Research Center (Deutsches Krebsforschungszentrum; DKFZ), Heidelberg, Germany; Cancer Research Center, University of Hawaii, Honolulu, HI (LLM, LNK); University of Southern California, Los Angeles, CA (CAH, BEH, FRS, DOS); Cancer Epidemiology Unit, University of Oxford, Oxford, United Kingdom (RCT); Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD (CDB, SJC, RNH, RGZ); Harvard School of Public Health, Boston, MA (JEB, PK, DT, DJH, SL); Brigham and Women's Hospital and Harvard Medical School, Boston, MA (JEB, SEH, I-ML, SZ); Epidemiology Research Program, American Cancer Society, Atlanta, GA (WRD, MJT);

Inserm, Centre for Research in Epidemiology and Population Health (Centre de recherche en Epidémiologie et Santé des Populations; CESP), U1018, Nutrition, Hormones and Women's Health Team, Villejuif, France (AF); Paris South University, UMRS 1018, Villejuif, France (AF); Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC (CI); International Agency for Research on Cancer (IARC), Lyon, France (MJ); Department of Surgical and Perioperative Sciences, Urology and Andrology, Umeå University, Umeå, Sweden (MJ); The Center for Human Genetics, Marshfield Clinic Research Foundation, Marshfield, WI (CAMC); Department of Clinical Epidemiology, Aarhus University Hospital, Aalborg, Denmark (KO); Department of Clinical and Experimental Medicine, Federico II University, Naples, Italy (SP); Julius Center, University Medical Center, Utrecht, the Netherlands (PHMP); Imperial College, London, UK (ER); Andalusian School of Public Health and Consorcio de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Granada, Spain (MJS); Department of Community Medicine, University of Tromsø, Tromsø, Norway (GS); Bureau of Epidemiologic Research, Academy of Athens, Athens, Greece (DT).