Risk of Estrogen Receptor–Positive and –Negative Breast Cancer and Single–Nucleotide Polymorphism 2q35-rs13387042

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Background A recent genome-wide association study identified single-nucleotide polymorphism (SNP) 2q35-rs13387042 as a marker of susceptibility to estrogen receptor (ER)–positive breast cancer. We attempted to confirm this association using the Breast Cancer Association Consortium.

Methods 2q35-rs13387042 SNP was genotyped for 31 510 women with invasive breast cancer, 1101 women with ductal carcinoma in situ, and 35 969 female control subjects from 25 studies. Odds ratios (ORs) were estimated by logistic regression, adjusted for study. Heterogeneity in odds ratios by each of age, ethnicity, and study was assessed by fitting interaction terms. Heterogeneity by each of invasiveness, family history, bilaterality, and hormone receptor status was assessed by subclassifying case patients and applying polytomous logistic regression. All statistical tests were two-sided.

Results We found strong evidence of association between rs13387042 and breast cancer in white women of European origin (per-allele OR = 1.12, 95% confidence interval [CI] = 1.09 to 1.15; $P_{trend} = 1.0 \times 10^{-19}$). The odds ratio was lower than that previously reported ($P = .02$) and did not vary by age or ethnicity (all $P \geq .2$). However, it was higher when the analysis was restricted to case patients who were selected for a strong family history ($P = .02$). An association was observed for both ER-positive (OR = 1.14, 95% CI = 1.10 to 1.17; $P = 10^{-15}$) and ER-negative disease (OR = 1.10, 95% CI = 1.04 to 1.15; $P = .0003$) and both progesterone receptor (PR)–positive (OR = 1.15, 95% CI = 1.11 to 1.19; $P = 5 \times 10^{-14}$) and PR-negative disease (OR = 1.10, 95% CI = 1.06 to 1.15; $P = .00002$).

Conclusion The rs13387042 is associated with both ER-positive and ER-negative breast cancer in European women.

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Genome-wide association studies (GWASs) have proved effective in identifying low-penetrance genetic loci associated with many complex diseases, including breast cancer. However, the relative risks associated with these common susceptibility alleles are generally very low (<1.3) and can only be estimated reliably by using very large samples. We previously identified five breast cancer susceptibility loci through a GWAS: *FGFR2*-rs2981582, *TOX3*-rs3803662, *LSP1*-rs3817198, *MAP3K1*-rs889312, and 8q-rs13281615 (1). The *FGFR2* and *TOX3* loci were also identified by other groups using similar approaches (2,3). In our study, confirmation of these associations at a genome-wide level of statistical significance ($P<10^{-7}$) was provided by replication through the Breast Cancer Association Consortium (BCAC). The BCAC is an international consortium of case–control studies formed to provide a resource for the reliable assessment of the breast cancer risks associated with such variants.

A GWAS of 1600 Icelandic breast cancer case patients and 11 563 control subjects, with replication in 2954 case patients and 6014 control subjects, found that an additional single-nucleotide polymorphism (SNP) on 2q35, rs13387042, was associated with an increased risk of breast tumors that were positive for the estrogen receptor (ER) (2).

The aim of this study was to define more precisely the breast cancer risk associated with 2q35-rs13387042 using more than 30 000 case patients and 30 000 control subjects from the BCAC.

Subjects and Methods

A total of 25 collaborating BCAC studies contributed genotypes for 2q35-rs13387042 to this study. Of these studies, 18 (Bavarian

CONTEXT AND CAVEATS

Prior knowledge

2q35-rs13387042 has been identified as a marker of susceptibility to estrogen receptor (ER)–positive breast cancer.

Study design

Genetic association study of breast cancer risk and rs13387042 using data from 25 case–control studies in the Breast Cancer Association Consortium.

Contribution

Having the rs13387042 genotype was associated with increased risk of breast cancer among white women of European origin, but the magnitude of the association was lower than that found previously. The association did not vary by age or ethnicity but was stronger among patients with a family history and was observed in ER-positive and -negative and progesterone receptor–positive and –negative disease.

Implications

The single-nucleotide polymorphism 2q35-rs13387042 is associated with increased risk of ER-positive and -negative breast cancer among white European women in this study.

Limitations

Hormone receptor status was not available for all patients and available data were taken from medical records rather than being confirmed by pathology review; thus, the statistical power was lower in the analyses for hormone receptor status.

From the Editors

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Breast Cancer Case Patients and Controls [BBCC], British Breast Cancer Study [BBCS], Copenhagen General Population Study [CGPS], Spanish National Cancer Research Centre Breast Cancer Study [CNIO-BCS], Familial Breast Cancer Study [FBCS], German Consortium for Hereditary Breast and Ovarian Cancer [GC-HBOC], Gene Environment Interaction and Breast Cancer in Germany [GENICA], Genetic Epidemiology Study of Breast Cancer [GESBC], Hannover Breast Cancer Study [HABCS], Helsinki Breast Cancer Study [HEBCS], Hannover-Minsk Breast Cancer Study [HMBCS], Hannover-Ufa Breast Cancer Study [HUBCS], Kuopio Breast Cancer Project [KBCP], Leiden University Medical Centre Breast Cancer Study [ORIGO], Polish Breast Cancer Study [PBCS], Singapore and Swedish Breast Cancer Study [SASBAC], Sheffield Breast Cancer Study [SBCS], and Studies of Epidemiology and Risk Factors in Cancer Heredity [SEARCH]) were conducted in Europe, three (Australian Breast Cancer Family Study [ABCFS], Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer and Australian Ovarian Cancer Study [kConFab/AOCS], and Melbourne Collaborative Cohort Study [MCCS]) in Australia, two (Mayo Clinic Breast Cancer Study [MCBCS] and University of California Irvine Breast Cancer Study [UCIBCS]) in the United States, and two (Seoul Breast Cancer Study [SEBCS] and Taiwanese Breast Cancer Study [TWBCS]) in Southeast Asia. Four studies (kConFab/AOCS, FBCS, GC-HBOC, and BBCS) selected case patients with a strong family history of breast cancer (typically two or more affected relatives) and/or bilateral disease, and a further two studies (CNIO-BCS and HEBCS) sampled a subset of case patients in the same way. Details of each study along with the numbers of samples and genotyping technology used in each are provided in Supplementary Table 1 (available online). HMBCS and HUBCS had not participated in previous BCAC studies.

All 25 studies provided information on disease status, ethnic group (white European, Asian, or other), and first-degree family history of breast cancer. All but three studies (FBCS, HUBCS, and UCIBCS) provided information on age at diagnosis for case patients and age at enrollment for control subjects, and all but GESBC, HUBCS, and SEBCS collected information on the bilaterality of breast cancer at diagnosis. Eighteen studies (BBCC, CGPS, CNIO-BCS, GENICA, GESBC, HABCS, HEBCS, KBCP, kConFab/AOCS, MCBCS, MCCS, ORIGO, PBCS, SASBAC, SBCS, SEARCH, TWBCS, and UCIBCS) provided information on the ER status and progesterone receptor (PR) status of a subset of case patient tumors (4). For the vast majority of case patients, this information was abstracted from medical reports.

Subjects who reported having ethnicity other than white European were excluded, with the exception of those from the Seoul and Taiwanese Breast Cancer Studies (SEBCS and TWBCS), for which only subjects of Asian origin were included. This gave a total of 32 591 case patients with invasive breast cancer, 1163 case patients with ductal carcinoma in situ (DCIS), and 36 995 control subjects. All women gave written informed consent, and each study was approved by the relevant local institutional review boards.

For five studies (ABCFS, GENICA, HEBCS, kConFab/AOCS, and SASBAC), genotyping was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry for the determination of allele-specific primer extension products using Sequenom's MassARRAY system and iPLEX technology (Sequenom, Inc, San Diego, CA). Oligonucleotides were designed according to the guidelines of Sequenom and performed using MassARRAY Assay Design software (version 3.1, Sequenom, Inc). Genotyping was carried out for the remaining studies by nuclease assay (Taqman; Applied Biosystems, Foster City, CA). Taqman genotyping reagents were designed by Applied Biosystems (http://www.appliedbiosystems.com/) as Assays-by-Design. Genotyping was performed using the ABI PRISM 7900HT, 7700, or 7500 Sequence Detection Systems according to the manufacturer's instructions.

All studies complied with BCAC genotyping quality control standards by including at least two blank wells (containing no DNA template) per 384-well assay plate, at least 2% of samples in duplicate, and a common set of 93 samples from the Centre d'Etude Polymorphisme Humain (CEPH) used by the HapMap Consortium (HAPMAPPT01, Coriell Institute for Medical Research, Cambden, NJ). Genotyping call rates and duplicate concordance rates were calculated after excluding samples that had previously repeatedly failed to genotype successfully and those that failed for more than 20% of the SNPs attempted in the current genotyping round.

Statistical Analysis

Departure from Hardy–Weinberg equilibrium was tested in control subjects from each center using Pearson's χ^2 test with 1 *df*. The association of 2q35-rs13387042 with breast cancer risk was assessed using multivariable logistic regression, adjusted for study, and therefore, implicitly, ethnicity (white European or Asian). We performed one analysis estimating separate odds ratios (ORs) and 95% confidence intervals (CIs) for each of heterozygotes (AG) and A-allele homozygotes relative to G-allele homozygotes and a second analysis estimating the odds ratio per A-allele assuming a log-additive model. Between-study heterogeneity in odds ratios was tested for using a likelihood ratio test, comparing the model with interaction terms for the per-allele log–odds ratio by study with the model with no interaction terms. Differences in odds ratios by each of ethnicity (white European or Asian) and age (<40, 40–49, 50–59, and 60 years and older) were also evaluated using a similar likelihood ratio test. A linear trend in the per-allele log-odds ratio with age was tested for by creating a dummy variable with values 1, 2, 3 and 4 corresponding to the same categories of age (<40, 40–49, 50–59, and ≥60, respectively), and comparing models with and without an interaction term for the per-allele log-odds ratio multiplied by the dummy variable. Differences in odds ratios between case patient groups defined by ER status, PR status, family history, bilaterality, selection for strong family history or bilaterality, and invasive breast cancer vs DCIS were tested for using a likelihood ratio test comparing polytomous logistic regression models with and without the per-allele odds ratio constrained to be equal for the two corresponding case groups (1 *df*). All statistical tests were two-sided. The term statistically significant implies *P* less than .05. However, in the context of GWASs, much more stringent levels of statistical significance are appropriate and statistical significance at a "genome-wide" level was defined as *P* less than 10^{-7}. All analyses were carried out using Stata: Release 10 (5).

Results

Genotyping results for rs13387042 complied with the following quality control criteria at all 25 study centers: at least 98% genotype concordance between duplicated samples and 95% concordance for the CEPH samples. A total of 1864 samples (951 invasive breast cancer case patients, 57 DCIS case patients, and 856 control subjects) were excluded because of repeated failures in genotyping. The call rate for rs13387042 was greater than or equal to 97.9% for the remaining case patients and control subjects at each center, with 305 failing (130 invasive breast cancer case patients, five DCIS case patients, and 170 control subjects), leaving 31 510 case patients with invasive breast cancer, 1101 case patients with DCIS, and 35 969 control subjects for analysis.

The call rate, *P* for compliance with Hardy–Weinberg equilibrium, and estimated frequency of the previously reported risk (A) allele (2) for each study were determined (Supplementary Table 2, available online). No statistically significant evidence of departure from Hardy–Weinberg equilibrium was observed for any control group (all $P \geq .07$). The A-allele frequency was substantially lower for Asian women than for white European women (10% vs 51%, respectively), with estimates ranging between 45% and 57% across the white European control groups.

We estimated odds ratios based on data from all studies combined, for invasive breast cancer and DCIS, and by ethnicity (Table 1). For invasive breast cancer, the estimated odds ratio per A-allele under a log-additive model was 1.13 (95% CI = 1.11 to 1.16; $P = 2 \times 10^{-27}$). There was no evidence of heterogeneity in odds ratios across studies ($P = .2$; Figure 1) and no difference in the odds ratio by ethnicity ($P_{interaction} = .6$).

Genotype-specific odds ratios by study are provided in Supplementary Table 2 (available online). There was evidence of departure from log-additivity for invasive disease ($P = .02$), with the odds ratio for AA homozygotes (1.28) higher than the square of that for heterozygotes ($1.19 = 1.09^2$). The estimated per-A-allele odds ratio for DCIS (OR = 1.12, 95% CI = 1.03 to 1.23; $P = .01$) was similar to that for invasive disease ($P = .7$ for difference).

We examined whether the observed association was specific to ER-positive or PR-positive breast cancer, as suggested by Stacey et al. (2), by stratified analyses of data from the 18 studies that recorded the hormone receptor status of case patient tumors (Table 2). There was evidence that the A-allele of rs13387042 was associated with increased risk, regardless of ER and PR status (all $P \leq .0005$). The odds ratio estimate was higher for ER-positive (ER+) disease than for ER-negative (ER−) disease and for PR-positive (PR+) disease than for PR-negative (PR−) disease, but the evidence that these differences are real was weak ($P = .11$ and $P = .08$, respectively). Additional polytomous regression analysis suggested no statistically significant difference in odds ratios by ER and PR status in combination. Per-A-allele–associated odds ratio estimates (and their corresponding 95% confidence intervals) were 1.09 (1.04 to 1.15) for ER−/PR− disease, 1.11 (1.04 to 1.18) for ER+/PR− disease, 1.11 (10.8 to 1.16) for ER−/PR+ disease, and 1.15 (1.11 to 1.19) for ER+/PR+ disease ($P = .3$ for heterogeneity).

There was no evidence of differences in the odds ratio by age (estimated per-A-allele ORs were 1.10 [95% CI = 1.02 to 1.19], 1.20 [95% CI = 1.13 to 1.27], 1.11 [95% CI = 1.06 to 1.16], and 1.14 [95% CI = 1.09 to 1.19] for age categories <40, 40–49, 50–59, and ≥60, respectively; $P = .2$ for heterogeneity, $P = .6$ for log-linear trend with age). There was also no evidence of a difference in the per-allele odds ratio between case groups defined by bilaterality ($P = .7$) or first-degree family history of breast cancer ($P = .2$), although the estimate was slightly higher when considering case patients with a first-degree family history (OR = 1.16, 95% CI = 1.11 to 1.20) than when considering case patients with no first-degree family history (OR = 1.12, 95% CI = 1.10 to 1.15). However, the per-allele odds ratio was statistically significantly higher when considering those studies (and subsets of studies) in which case patients were selected for a strong family history of breast cancer or bilaterality (kConFab/AOCS, FBCS, GC-HBOC, BBCS, CNIO-BCS, and HEBCS; OR = 1.20, 95% CI = 1.14 to 1.26) than when considering the remaining unselected case patients (OR = 1.12, 95% CI = 1.09 to 1.15; $P = .02$ for difference).

To generate odds ratio estimates for white Europeans that were not biased by selection for strong family history or bilaterality, we excluded all subjects from the kConFab/AOCS, FBCS, GC-HBOC, and BBCS and selected case patients from the CNIO-BCS and HEBCS (this entailed excluding 4667 [15%] case patients with invasive breast cancer and 4835 [13%] control subjects). This exclusion had no substantial effect on the estimates obtained. The per-A-allele odds ratio estimate for white Europeans was 1.12 (95% CI = 1.09 to 1.15, $P = 10^{-19}$), and the genotype-specific odds ratios relative to GG were 1.07 (95% CI = 1.02 to 1.11) and 1.25 (95% CI = 1.19 to 1.32) for AG and AA, respectively, with evidence of departure from log-additivity ($P = .009$). The estimated per-allele odds ratio was 1.14 (95% CI = 1.10 to 1.17, $P = 10^{-15}$) for ER-positive disease and 1.10 (95% CI = 1.04 to 1.15, $P = .0003$) for

Table 1. Estimated odds ratios and 95% confidence intervals according to 2q 35-rs13387042 genotype for invasive breast cancer and ductal carcinoma in situ in white Europeans and Asians*

Genotype by invasiveness and ethnic group	Control subjects, No. (%)	Case patients, No. (%)	OR† (95% CI)
Invasive disease			
All subjects			
GG	9843 (27)	8263 (26)	1.00 (Referent)
AG	17 198 (48)	14 413 (46)	1.09 (1.05 to 1.13)
AA	8928 (25)	8834 (28)	1.28 (1.22 to 1.34)
White Europeans			
GG	7999 (24)	6016 (21)	1.00 (Referent)
AG	16 803 (50)	13 900 (48)	1.09 (1.05 to 1.14)
AA	8906 (26)	8797 (31)	1.28 (1.22 to 1.34)
Asians			
GG	1844 (82)	2247 (80)	1.00 (Referent)
AG	395 (17)	513 (18)	1.07 (0.93 to 1.24)
AA	22 (1.0)	37 (1.3)	1.37 (0.81 to 2.34)
DCIS			
All subjects‡			
GG	4525 (26)	250 (23)	1.00 (Referent)
AG	8455 (48)	527 (48)	1.09 (0.92 to 1.27)
AA	4453 (26)	324 (29)	1.25 (1.05 to 1.50)

* DCIS = ductal carcinoma in situ.

† Adjusted for study.

‡ Data included only from studies with DCIS case patients.

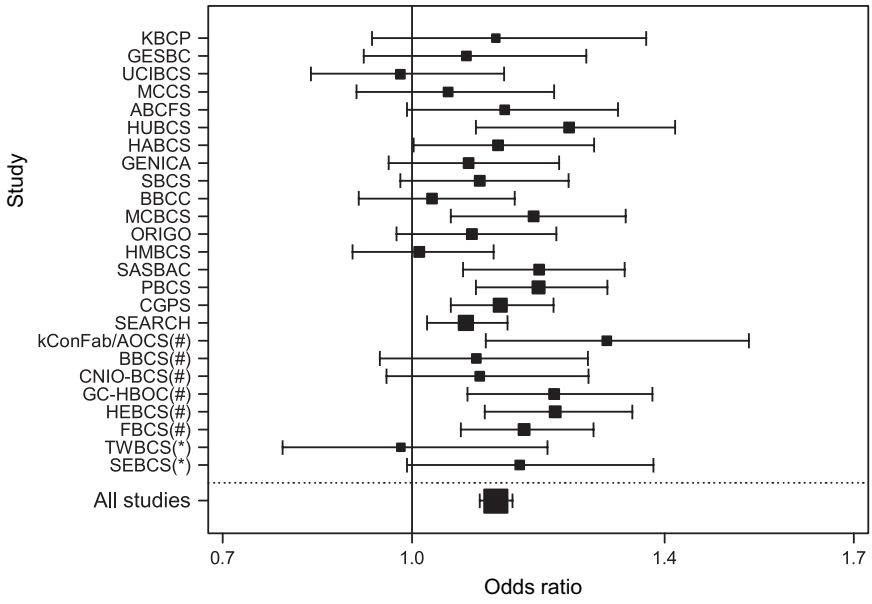

Figure 1. The per-A-allele odds ratio estimate for 2q-rs13387042, by study. The size of the box is inversely proportional to the standard error of the log-odds ratio estimate. Study acronyms are defined in Supplementary Table 1 (available online) (#, studies that selected case patients with a strong family history and/or bilateral disease; *, studies of Asian women).

ER-negative disease (P = .2 for difference). The corresponding estimates for PR-positive and PR-negative disease were 1.15 (95% CI = 1.11 to 1.19, $P = 5 \times 10^{-14}$) and 1.10 (95% CI = 1.06 to 1.15, P = .00002), respectively (P = .3 for difference).

Discussion

In this study of more than 30 000 case patients and 30 000 control subjects, we have provided strong confirmation of the association between 2q35-rs13387042 and breast cancer, previously reported by Stacey et al. (2). We observed no evidence of heterogeneity in the odds ratios across studies or age categories. Stacey et al. (2) reported a per-A-allele odds ratio of 1.20 (95% CI = 1.14 to 1.26) based on a pooled analysis of 4533 case patients and 17 513 control subjects. In the current much larger study, the estimated odds ratio in white Europeans, from 12 countries, was lower (per-A-allele, OR = 1.12, 95% CI = 1.09 to 1.15; P = .02 for difference).

This association was not identified in a GWAS we conducted previously (1). This is not surprising, given the estimated odds ratio. According to HapMap data, rs13387042 was perfectly tagged

Table 2. Estimated odds ratios and 95% confidence intervals for 2q35-rs13387042, by tumor subtypes defined by estrogen receptor and progesterone receptor status

Genotype by hormone receptor status	Control subjects*, No. (%)	Case patients, No. (%)	OR† (95% CI)	P‡
Estrogen receptor positive				
GG	7050 (25)	3077 (23)	1.00 (Referent)	
AG	13643 (49)	6388 (47)	1.08 (1.03 to 1.14)	
AA	7105 (26)	4038 (30)	1.29 (1.22 to 1.37)	
Per-A-allele			1.14 (1.11 to 1.17)	3×10^{-17}
Estrogen receptor negative				
GG	7050 (25)	1021 (25)	1.00 (Referent)	
AG	13643 (49)	1898 (47)	1.05 (0.96 to 1.15)	
AA	7105 (26)	1130 (28)	1.18 (1.07 to 1.31)	
Per-A-allele			1.09 (1.04 to 1.14)	.0005
Progesterone receptor positive				
GG	7050 (25)	2286 (23)	1.00 (Referent)	
AG	13643 (49)	4671 (47)	1.09 (1.02 to 1.16)	
AA	7105 (26)	3007 (30)	1.31 (1.23 to 1.41)	
Per-A-allele			1.15 (1.11 to 1.19)	2×10^{-15}
Progesterone receptor negative				
GG	7050 (25)	1304 (24)	1.00 (Referent)	
AG	13643 (49)	2553 (48)	1.08 (1.00 to 1.17)	
AA	7105 (26)	1517 (28)	1.20 (1.10 to 1.31)	
Per-A-allele			1.10 (1.05 to 1.15)	.00003

* Data included only from the 18 studies that collected hormone receptor status. OR = odds ratio; CI = confidence interval.

† Adjusted for study.

‡ Two-sided P values were calculated using a likelihood ratio test.

by a SNP (rs4442975) in stage 1 of our GWAS, but this SNP did not reach the *P* value cutoff ($P < .05$) to be selected for stage 2. Nevertheless, the odds ratio estimate in stage 1 (OR = 1.11, 95% CI = 0.89 to 1.37) was not inconsistent with the estimate from the current study. Based on the odds ratio estimate in this study, the statistical power to have reached $P < .05$ in stage 1 was relatively low (~57%), even after allowing for the greater power provided by familial case patients (6).

We observed evidence of departure from log-additivity in genotype-specific risks, with the odds ratio for carriers of two A-alleles being substantially greater than the square of the odds ratio for carriers of just one. A similar departure from log-additivity was also observed by Stacey et al. (2). Consistent with HapMap data, the A-allele was much more frequent in Europeans than in Southeast Asians. Despite this difference, the odds ratio estimates were very similar between these two ethnic groups. However, the genotype frequencies were not statistically significantly different between Southeast Asian case patients and control subjects, and larger studies in Asian populations are needed to estimate the risk more precisely for this group. Both the per-allele and genotype-specific odds ratio estimates were very similar for invasive and in situ disease. Based on our odds ratio estimates and the allele frequencies in Europeans, the 2q35-rs13387042 SNP would account for 0.6% of the excess familial risk of breast cancer (ie, 0.6% of the twofold increased risk to first-degree relatives of affected women in the general population).

We observed a higher odds ratio associated with the A-allele of 2q35-rs13387042 when considering case patients selected for strong family history and/or bilaterality. The kConFab/AOCS, FBCS, GC-HBOC, CNIO-BCS, and HEBCS all selected case patients from multiple case families that were considered likely to carry mutations in *BRCA1* and *BRCA2*, but which tested negative for such mutations, whereas the BBCS selected case patients with two primary breast cancers. The observed higher odds ratio is broadly consistent with the effect one would expect under a polygenic model of susceptibility in which large numbers of susceptibility polymorphisms act multiplicatively on risk (6). It also corroborates the theoretical argument that such selection can increase the power to detect associations with common, low-penetrance variants (6).

The original publication on 2q35-rs13387042 (2) reported that the associated risk was confined to ER-positive breast cancer. We found that the association with rs13387042 was apparent for both ER-positive and ER-negative disease. However, the association appeared to be slightly stronger for ER-positive disease. This tendency to be more strongly associated with the risk of ER-positive breast cancer has been observed for other clearly established susceptibility SNPs, notably *FGFR2*-rs2981582, 8q-rs13281615, and 5p-rs10941679 (2,4,7), perhaps reflecting the fact that they were initially identified by GWASs for which most of the case patients in the hypothesis-generating phases had ER-positive disease. It will be of interest to see if this phenomenon is reversed if and when loci are identified through GWASs enriched for ER-negative disease.

A limitation of our study was that information on ER and PR status was not available for all case patients, and where it was, the data were predominantly abstracted from medical records rather than being obtained through a standardized pathology review. A study comparing ER status abstracted from medical records with ER status determined on the same specimens by immunohistochemical assay at a central laboratory reported very good agreement between the two (8). Nevertheless, some misclassification may have been present in our study. Although this, as well as missing data, is unlikely to be related to the genotype at rs13387042, it may have led to a loss of power in differentiating the effects of the 2q35 SNP by hormone receptor status. Future studies based on standardized measures of hormone status and indeed other markers of tumor subtypes may help elucidate phenotype-specific effects for susceptibility alleles.

SNP 2q35-rs13387042 lies in a 90-kb region of high linkage disequilibrium that contains neither known genes nor noncoding RNAs (2). The causal variant (or variants) in this region has not been determined, and it is possible that it may confer a higher risk than rs13387042. Elucidating the causal mechanism may improve our understanding of the etiology of breast cancer. In the meantime, our results emphasize the need for large collaborative studies to evaluate with precision the strength of the risks associated with susceptibility variants.

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