Common Breast Cancer Susceptibility Loci Are Associated with Triple-Negative Breast Cancer


Abstract

Triple-negative breast cancers are an aggressive subtype of breast cancer with poor survival, but there remains little known about the etiologic factors that promote its initiation and development. Commonly inherited breast cancer risk factors identified through genome-wide association studies display heterogeneity of effect among breast cancer subtypes as defined by the status of estrogen and progesterone receptors. In the Triple Negative Breast Cancer Consortium (TNBCC), 22 common breast cancer susceptibility variants were investigated in 2,980 Caucasian women with triple-negative breast cancer and 4,978 healthy controls. We identified six single-nucleotide polymorphisms, including rs2046210 (ESR1), rs12662670 (ESR1), rs3803662 (TOX3), rs999737 (RAD51L1), rs8170 (19p13.1), and rs8100241 (19p13.1), significantly associated with the risk of triple-negative breast cancer. Together, our results provide convincing evidence of genetic susceptibility for triple-negative breast cancer. Cancer Res; 71(19); 6240–9. © 2011 AACR.

Introduction

Triple-negative breast cancers are a biologically and clinically distinct subtype of breast cancer, defined as tumors that exhibit no or low expression of estrogen receptor (ER), progesterone receptor (PR), and HER2 (1). Women with triple-negative disease account for approximately 15% of all invasive breast cancers and are more likely to be younger, African American, have an earlier age at menarche, higher body mass index during premenopausal years, higher parity, and a lower lifetime duration of breastfeeding (2–4). In addition, triple-negative tumors are typically of higher histologic grade and are associated with more aggressive disease and poorer survival (1, 5, 6). These differences in tumor pathology, nongenetic risk factors, and survival among women with triple-negative disease suggest that the etiology of these tumors may differ from other breast cancer subtypes.
Genome-wide association studies (GWAS) have recently identified common, low-penetrance susceptibility variants that are associated with risk of breast cancer (7–16). Growing evidence suggests substantial heterogeneity by tumor subtype, defined by hormone receptor status, for associations with these single-nucleotide polymorphisms (SNP). In particular, variants in 5p12, FGFR2, 8q24, 1p11.2, 9p21.3, 10q22.1, and 11q13 are associated with the risk of developing ER-positive tumors (9–12, 14, 17, 18) but not ER-negative tumors, whereas variants in 2q35, TOX3, LSP1, MAP3K1, TGFBI, and RAD51LI are associated with both ER-positive and ER-negative diseases (19). To date, no variants have been specifically associated with ER-negative or triple-negative disease. However, variants at TOX3, 2q35, and 2 distinct signals at 19p13.1 have been associated with breast cancer risk in BRCA1 mutation carriers, who predominantly develop tumors displaying an ER-negative and triple-negative phenotype (15, 20, 21). Thus, additional studies specifically investigating ER-negative and triple-negative disease are necessary to understand genetic susceptibility to these breast cancer subtypes.

Here, we report on the first Triple Negative Breast Cancer Consortium (TNBCC) study of genetic susceptibility to triple-negative breast cancer in which associations between 22 common breast cancer susceptibility loci and risk among 2,980 cases and 4,978 controls were evaluated. This comprehensive study included 21 common variants from all known susceptibility loci identified through currently published breast cancer GWAS (1p11.2, 2q35, 3p24/NEK10, 5p12/MAP3K1, MAP3K1, LSP1, 8q24, 9q31.2, 10p15.1, 10q21.3/ZNF365, 10q22.3/TOX3, FGFR2, LSP1, 11q13, RAD51LI, TOX3, 17q23/COX11, and 19p13.1) and a SNP from CASP8 identified in a candidate-gene study are strongly associated with risk of triple-negative breast cancer.

Materials and Methods

Ethics statement

Study subjects were recruited on protocols approved by the Institutional Review Boards at each participating institution, and all subjects provided written informed consent.

Study populations

Samples from several triple-negative breast cancer case–control series, including 2,778 triple-negative breast cancer cases and 1,406 unaffected controls, were genotyped on the iPLEX platform. These subjects were ascertained by 22 studies in 9 different countries as follows: United States, Australia, Great Britain, Finland, Germany, Netherlands, Greece, Ireland, and Sweden. These included cases from the KBKP and POSH cohort studies, cases and controls from the MCCS cohort study, and cases and controls from established population-based breast cancer case–control studies (BBCC, GENICA, MARIE, and SEARCH), hospital or clinic-based case–control studies (ABCS, BIGGS, LMBC, MCBCS, OBCS, SBCS, and RPCI), case-only studies with geographically matched controls (BBCC, KARBAC, SKDKFZS, and FCCC), and unselected cases identified in tumor collections (DFCI, ABCB, and DEMOKRITOS). Data from an ongoing GWAS of triple-negative breast cancer, including cases and controls from several of the studies described earlier, and the triple-negative cases from the HEBCS GWAS along with population control data (n = 273) were also included (24). In addition, data from 4 publicly available control GWAS data sets [Wellcome Trust Case Control Consortium UK 1958 birth cohort (WTCCC), National Cancer Institute’s Cancer Genetic Markers of Susceptibility (CGEMS) project, Cooperative Health Research in

Hospital, Amsterdam, the Netherlands; \(^{16}\)Department of Oncology, Institute for Cancer Studies, and \(^{17}\)Academic Unit of Pathology, Department of Neurosurgery, Faculty of Medicine, Dentistry & Health, University of Sheffield, Sheffield; \(^{18}\)National Institute for Health Research Comprehensive Biomedical Research Centre, Guy’s & St. Thomas’ NHS Foundation Trust; \(^{19}\)Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine; \(^{20}\)Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom; \(^{21}\)Institute of Pathology, \(^{22}\)Institute of Diagnostic Radiology, \(^{23}\)Institute of Human Genetics, and \(^{24}\)Department of Gynecology and Obstetrics, Breast Cancer Centre Franconia, Friedrich-Alexander University Erlangen-Nuremberg, University Hospital Erlangen, Erlangen; \(^{25}\)Division of Cancer Epidemiology, German Cancer Research Center; \(^{26}\)Department of Pathology, University Hospital Heidelberg; \(^{27}\)Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum, Heidelberg, Germany; \(^{28}\)Institute for Medical Biometrics and Epidemiology, University Clinic Hamburg-Eppendorf, Hamburg, Germany; \(^{29}\)Molecular Diagnostics Laboratory IRRP, National Centre for Scientific Research “Demokritos”; \(^{30}\)Department of Internal Medicine, Oncology Section, Hippokration Hospital; \(^{31}\)Department of Clinical Therapeutics, “Alexandra” Hospital, University of Athens School of Medicine, Athens; \(^{32}\)Department of Medical Oncology, Aristotle University of Thessaloniki, Papageorgiou Hospital, Thessaloniki, Greece; \(^{33}\)Department of Translational Oncology, Westmead Hospital, Western Sydney Local Health Network, Westmead, New South Wales, Australia; Departments of \(^{34}\)Medical Oncology and \(^{35}\)Biostatistics, Fox Chase Cancer Center, Philadelphia, Pennsylvania; \(^{36}\)Institute of Pathology, Stadtklinikum Karlsruhe, Karlsruhe, Germany; \(^{37}\)Division of Molecular and Deutsches Krebsforschungszentrum, Heidelberg, Germany, and Center for Primary Health Care Research, University of Lund, Malmo, Sweden; \(^{38}\)Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany; \(^{39}\)Multidisciplinary Breast Center, University Hospital Gasthuisberg; \(^{40}\)Sawas Research Centre, VIB; \(^{41}\)Sawas Research Centre, University of Leuven, Leuven, Belgium; \(^{42}\)Cancer Epidemiology Centre, The Cancer Council Victoria, and Centre for Molecular, Environmental, Genetic, and Analytic Epidemiology, The University of Melbourne, Melbourne, Australia; \(^{43}\)Wellcome Trust Centre for Human Genetics and Oxford Comprehensive Biomedical Research Centre, University of Oxford, Oxford, United Kingdom; \(^{44}\)Department of Pathology, Institute of Clinical Medicine, University of Eastern Finland and Kuopio University Hospital, Biocenter Kuopio, Kuopio, Finland; \(^{45}\)Genetics and Population Health Division, and \(^{46}\)QIMR GWAS Collective, Queensland Institute of Medical Research, Brisbane, Australia; Departments of \(^{47}\)Obstetrics and Gynecology and \(^{48}\)Oncology, Helsinki University Central Hospital, Helsinki, Finland; \(^{49}\)Human Genetics Division, Genome Institute of Singapore, Singapore; \(^{50}\)Department of Oncology and Department of Public Health and Primary Care, University of Cambridge; \(^{51}\)Department of Genetic Epidemiology, Cancer Research UK Genetic Epidemiology Unit, Strangeways Research Laboratory, Cambridge, United Kingdom; and \(^{52}\)Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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the Region of Augsburg (KORA) study, and the Australian Twin Cohort study from the Queensland Institute of Medical Research (QIMR); \( n = 3,593 \) were used. Age distributions and years of diagnosis for individual study sites are provided in Supplementary Table S1, and these studies are described in more detail in Supplementary Material.

Pathology and tumor markers
A triple-negative breast cancer case was defined as an individual with an ER-negative, PR-negative, and HER2-negative [0 or 1 by immunohistochemical staining (IHC)] breast cancer diagnosed after age 18. Criteria used for defining ER, PR, and HER2 status varied by study. These are described in detail in Supplementary Table S2. IHC data for cytokeratin 5/6 and epidermal growth factor receptor for identification of basal tumors were not available.

Genotyping
The following 22 SNPs were genotyped on the iPLEX platform: rs11249433 (1p11.2), rs13387042 (2q35), rs4973768 (3p24), rs10941679 (5p12), rs889312 (6q24), rs2046210 (ESR1), rs12662670 (ESR1, surrogate for rs9397435), rs13281615 (8q24), rs1011970 (9p21.3), rs865686 (9q31.2), rs2380205 (10p1.5), rs10509168 (10q21.2, surrogate for rs10995190), rs704010 (10q22.3), rs2981582 (FGFR2), rs3817198 (LSP1), rs614367 (11q13), rs999737 (RAD51L1), rs3803662 (TOX3), rs6504950 (17q23), rs81870 (19p13.1), rs8100241 (19p13.1), and rs17468277 (tagSNP for rs6504950 (17q23), and only partial data were available for 5 other SNPs (17q23), and only partial data were available for 5 other SNPs because of the absence of these SNPs from some or all of the iPLEX genotyping platforms (Table 1). As a further measure of genotype quality, genotype concordance was evaluated for the

### Table 1. Subjects by country and genotyping platform (iPLEX, GWAS)

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of studies</th>
<th>Age, range (mean)</th>
<th>Years of diagnosis</th>
<th>iPLEX</th>
<th>GWAS</th>
<th>Combined</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Total</td>
</tr>
<tr>
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<td>5</td>
<td>25–92 (52) 24–92 (62)</td>
<td>1990–2010</td>
<td>711</td>
<td>1,159</td>
<td>35</td>
</tr>
<tr>
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<td>22–93 (45) 42–81 (53)</td>
<td>1971–2010</td>
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<td>111</td>
<td>684</td>
</tr>
<tr>
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<td>3</td>
<td>27–90 (55) 18–80 (57)</td>
<td>1990–2004</td>
<td>101</td>
<td>88</td>
<td>189</td>
</tr>
<tr>
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<td>21–79 (53) 34–82 (50)</td>
<td>1997–2010</td>
<td>273</td>
<td>85</td>
<td>358</td>
</tr>
<tr>
<td>Netherlands</td>
<td>1</td>
<td>26–62 (39) NA</td>
<td>1995–2007</td>
<td>60</td>
<td>0</td>
<td>67</td>
</tr>
</tbody>
</table>

*Study-specific distributions are shown in Supplementary Table S1.*
1,402 samples included in both the iPlex and GWAS. Eighteen of 19 SNPs had concordance rates of more than 98% and rs8100241 showed concordance of 96.3%.

Statistical methods

Allele frequencies for each of the 22 SNPs included in these analyses were estimated using the iPlex and GWAS genotype data and the combined GWAS and iPlex data for cases, controls, and all subjects (Supplementary Table S3). Associations for triple-negative breast cancer were estimated using unconditional logistic regression adjusted for country of residence. The sites were categorized by country of origin (American, Australian, British, Finnish, German, Greek, Irish, and Swedish; Table 1). SNPs were coded for a gene–dose effect by assigning a 3-level (0, 1, and 2) variable to each genotype (log-additive model). We calculated P values, ORs, and 95% CIs from these logistic regressions. Pairwise interactions were tested by including multiplicative interaction terms in logistic regression models. Homogeneity of ORs by country was tested using the Q-statistic (26), and the extent of heterogeneity was estimated by the F statistic (27). All analyses were conducted using SAS version 9.2, R version 2.11.0, or Plink version 1.07.

Results

We evaluated 22 breast cancer susceptibility SNPs identified in breast cancer GWAS for associations with triple-negative disease using genotype data from an iPlex study of the 22 SNPs supplemented with data from a different GWAS. The combined data resulted in a case–control study of 2,980 cases and 4,978 controls from 25 studies in 8 countries (Table 1). All 22 SNPs were in HWE among controls at P > 0.01. Only rs17468277, rs13387042, rs10941679, and rs614367 showed evidence of heterogeneity by country (rs17468277: P = 0.065, F = 47.4%; rs13387042: P = 0.037, F = 53.1%; rs10941679: P = 0.063, F = 47.8%; rs614367: 0.054, F = 49.4%). Of the 22 SNPs from 20 loci, 7 were significantly associated with risk of triple-negative breast cancer (P < 0.05; Table 2). Six SNPs from 4 loci, rs2046210 (P = 4.38 × 10⁻⁷), rs12662670 (P = 1.13 × 10⁻⁴), rs999737 (P = 2.96 × 10⁻⁴), rs3803662 (P = 3.66 × 10⁻³), rs8170 (P = 2.25 × 10⁻⁶), and rs8100241 (P = 8.66 × 10⁻⁷), remained significant after correction for multiple testing (P < 2.27 × 10⁻⁵). Adjustment for age did not change the magnitude or significance of our results. In addition, we did not find evidence of significant interactions with age for any of the 22 SNPs.

rs2046210, located upstream of ESR1 on chromosome 6q25.1, exhibited a strong association with triple-negative disease (OR = 1.29, 95% CI = 1.17–1.42; P = 4.38 × 10⁻⁷; Fig. 1A), whereas rs12662670, located further upstream of ESR1, displayed a similar effect but slightly less significant association with triple-negative disease (OR = 1.33, 95% CI = 1.15–1.53; P = 1.13 × 10⁻⁴; Fig. 1B). To assess the independence of these 2 ESR1 SNPs, which are not correlated in HapMap subjects of European ancestry (r² = 0.09), we included both SNPs in a multivariate model. rs2046210 was more strongly associated with triple-negative risk than rs12662670 (rs2046210: OR = 1.24, 95% CI = 1.12–1.38; P = 5.64 × 10⁻⁵; rs12662670: OR = 1.20, 95% CI = 1.00–1.44; P = 0.053) in this model, suggesting that rs2046210 may account in part for these 2 associations. In addition, 2 SNPs at 19p13.1, shown to have genome-wide significant associations with breast cancer in BRCA1 mutation carriers, were highly significantly associated with triple-negative breast cancer (rs8170: OR = 1.27, 95% CI = 1.17–1.38; P = 2.25 × 10⁻⁸; rs8100241: OR = 0.84, 95% CI = 0.78–0.90; P = 8.66 × 10⁻⁷; Fig. 1C and D). Multivariate modeling of these 2 SNPs, which are moderately correlated in HapMap subjects of European ancestry (r² = 0.74), showed that rs8170 is more strongly associated with triple-negative breast cancer risk (rs8170: OR = 1.22, 95% CI = 1.10–1.34; P = 7.56 × 10⁻⁵; rs8100241: OR = 0.90, 95% CI = 0.83–0.98; P = 0.014), although both variants are retained in the model. In addition, rs3803662 (TOX3), which has been strongly associated with risk of ER-negative breast cancer (OR = 1.15, P = 2.1 × 10⁻¹⁰; ref. 19), was associated with a 1.17-fold increase in risk of triple-negative disease (OR = 1.17, 95% CI = 1.09–1.26; P = 3.66 × 10⁻⁵; Fig. 1E). Likewise, the rs999737 (RAD51L1) SNP was significantly associated with risk of triple-negative breast cancer (rs999737: OR = 0.86, 95% CI = 0.80–0.93; P = 2.96 × 10⁻⁴; Fig. 1F). In contrast, rs17468277 (ALS2CR12/CASP8; P = 0.005) was not significantly associated with triple-negative breast cancer risk after correction for multiple testing, suggesting that this result should be interpreted with caution. None of these 6 SNPs showed evidence of heterogeneity by country (Fig. 1). To further understand the influence of variants in the 6q25.1 and 19p13.1 loci on triple-negative risk, we looked for statistical interactions between the SNPs in these regions. Although there was no evidence for a statistical interaction between rs2046210 and rs1266270 (P = 0.820) at 6q25.1, we found strong evidence of an interaction (P = 0.004) between rs8170 and rs8100241 from 19p13.1 in a multiplicative model.

Next, we conducted a subset analysis using the iPlex data alone (2,707 cases and 1,385 controls) for the 19 SNPs with both iPlex and GWAS genotypes to assess the consistency of our results. Analysis of associations with triple-negative disease in the iPlex-only data set showed that ORs for the 19 SNPs were consistent in both direction and magnitude of effect compared with the analysis using all available genotype data, although some variation in the significance of the associations was observed (Table 2). Four of the SNPs significantly associated with triple-negative breast cancer in the overall analysis retained statistical significance in the iPlex-only analysis (rs12662670: P = 5.32 × 10⁻⁴; rs3803662: P = 8.25 × 10⁻⁵; rs8170: P = 7.30 × 10⁻⁵; rs8100241: P = 1.81 × 10⁻⁴) after correction for multiple testing. Results were unchanged for rs2046210 from the ESR1 locus, because the overall analysis was restricted to iPlex data as a result of missing GWAS data for this variant. Finally, although the rs999737 (RAD51L1) SNP was only marginally associated with triple-negative breast cancer risk in the iPlex-only analysis (rs999737: P = 0.053), the estimate of effect for this SNP was consistent with the effect observed in the overall analysis. Importantly, genotype data from a subset of these cases and controls have previously been used in association studies involving a number of these SNPs by the Breast Cancer
<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene/locus</th>
<th>Chromosome</th>
<th>Tested allele</th>
<th>Overall</th>
<th>iPLEX</th>
<th>Published OR (95% CI)</th>
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<td>2.705</td>
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<td>1.385</td>
</tr>
</tbody>
</table>

*These SNPs showed evidence of country-based heterogeneity.

No additional samples included in overall analysis compared with iPLEX-only.

¢Estimated ORs in Europeans.
Association Consortium (BCAC). To avoid duplication and to assess the degree to which these BCAC samples influenced our results, we also conducted a subset analysis in which we excluded all cases and controls used in the BCAC studies ($n = 1,819$ cases and $n = 4,038$ controls; Supplementary Table S4). The effect estimates and significance of associations with triple-negative disease in either the iPLEX or combined analyses were not substantially modified following the removal of these cases and controls (Supplementary Table S5).

### Discussion

Here, we report on the first study by the TNBC and the largest study to date of genetic susceptibility to triple-negative breast cancer, which is composed of 2,980 cases and 4,978 controls from 25 studies in 9 countries. We show that a subset of breast cancer susceptibility SNPs identified through GWAS is also associated with risk of triple-negative breast cancer. Specifically, we determined that 6 breast cancer susceptibility SNPs from 4 loci, rs2046210 ($ESR1$), rs12662670 ($ESR1$), rs999377 ($RAD51L1$), rs3803662 ($TOX3$), rs8170 (19p13.1), and rs800241 (19p13.1), are associated with risk of triple-negative breast cancer. Of these, rs8170 (19p13.1) achieved genome-wide significance ($P = 2.25 \times 10^{-8}$). Overall, these findings provide strong evidence of genetic susceptibility to triple-negative breast cancer.

We identified highly significant associations between SNPs at 6q25.1, including rs12662670 ($P = 1.13 \times 10^{-4}$) and rs2046210, which reached near genome-wide significance ($P = 4.38 \times 10^{-7}$), and risk of triple-negative breast cancer. These variants are located approximately 30 and 60 kb upstream of the first untranslated exon and 180 and 210 kb upstream of the first coding exon of $ESR1$, which encodes the ER$\alpha$ protein.

The rs2046210 SNP was originally reported in a breast cancer GWAS in Chinese women (13), where a stronger association was observed among ER-negative than among ER-positive breast cancer cases. Importantly, the magnitude of effect in this triple-negative study ($OR = 1.29, 95\% CI = 1.17–1.42$) was identical to that reported for ER-negative breast cancer in the Chinese study ($OR = 1.29, 95\% CI = 1.21–1.37$). In contrast, a study of women of European ancestry
did not observe an association with breast cancer, although analyses were not stratified by ER status (28). When combined with our results, the suggestion is that this SNP may be specifically associated with triple- or ER-negative disease. The second variant in the ESR1 locus rs12662670 was originally associated with breast cancer in the same study of women of European ancestry (OR = 1.12, 95% CI = 1.03–1.21) and was used as a surrogate for rs9397435, which is associated with breast cancer risk (OR = 1.15, 95% CI = 1.06–1.25) independently of rs2046210 (28). Here, rs12662670 showed a strong influence on triple-negative breast cancer risk (OR = 1.33, 95% CI = 1.15–1.53), again suggesting that variation in the ESR1 locus is specifically associated with risk of ER-negative and/or triple-negative breast cancer. It remains to be determined whether a single locus represented by rs2046210 or 2 loci accounted for by rs2046210 and rs9397435 are associated with ER-negative and triple-negative breast cancer at chromosome 6q25.

Because triple-negative breast cancer is defined in part by the absence of expression of ERs, we can speculate that inherited variation may downregulate ESR1 expression and promote formation of ERα-negative tumors. However, recent studies in mice have shown that the mammary stem cell compartment can be regulated by 17β-estradiol and progesterone through a paracrine-signaling mechanism from steroid receptor–positive luminal cells to steroid receptor–negative stem cells (29, 30). Thus, SNPs in the ESR1 locus may promote expansion of receptor-negative precursors and subsequent development of triple-negative tumors. Interestingly, variation in the 5′ region of ESR1 has been associated with an increased risk of breast cancer relapse in a British prospective cohort study (31), which was accounted for by including tumor grade and nodal status in multivariate models. Thus, the causal SNPs in this area may be associated with a more aggressive tumor phenotype.

The SNPs rs8170 (P = 2.25 × 10^{-5}) and rs8100241 (P = 8.66 × 10^{-5}) located at 19p13.1 were first identified both as modifiers of breast cancer risk in BRCA1 carriers (15) and as risk factors for ovarian cancer (32), as well as shown to be significantly associated with ER-negative breast cancer (15). In this study, we showed that rs8170 displayed a genome-wide significant association with triple-negative breast cancer, suggesting that we can now identify variation in the 19p13.1 locus as a risk factor for triple-negative disease. Interestingly, rs8170 attenuated the significance of rs8100241 when the SNPs were included in a multivariate regression model for breast cancer whereas both these SNPs retained significance in multivariate models evaluating effects on BRCA1-associated breast cancer and ER-negative breast cancer (15). In addition, our data suggest that these SNPs have a multiplicative effect on triple-negative breast cancer risk. Further studies are required to determine whether these SNPs represent independent signals in the 19p13.1 locus. Additional studies are also needed to identify the underlying causative genetic events in this locus and to determine whether the causative events for BRCA1, ER-negative, and triple-negative breast cancer as well as ovarian cancer are common.

These 19p13.1 variants are located in a cluster of genes including C19orf62, ANKLE1, and ABHD8. ABHD8 encodes the hydrolase domain containing 8 protein, which is a gene of uncharacterized function, and is located about 13 kb downstream of both rs8170 and rs8100241. The SNP rs8170 is located within C19orf62, which encodes the MERIT40 protein, whereas rs8100241 is located within ANKLE1, a protein of unknown function that encodes ankyrin repeat and LEM domains. MERIT40 is the most plausible candidate in this region for breast cancer susceptibility because it is a component of the BRCA1-A complex and is required to ensure the integrity and localization of this complex during the repair of DNA double-strand breaks, specifically through the recruitment and retention of the BRCA1–BARD1 ubiquitin ligase and the BRCC36 deubiquitination enzyme (33–35). However, it remains to be determined whether the causal variants at 19p13.1 alter MERIT40 expression or function or influence other genes in the region such as ANKLE1 or ABHD8.

We also found that variants in RAD51L1 (rs999737: P = 2.96 × 10^{-4}) and TOX3 (rs3803662: P = 3.66 × 10^{-5}) were strongly associated with risk of triple-negative breast cancer. rs999737 (RAD51L1) was originally identified in a recent breast cancer GWAS of women of European ancestry (12). Detailed studies of breast tumors have suggested that rs999737 is associated with both ER-positive and ER-negative breast cancers, which is consistent with our findings. RAD51L1 is a member of the Rad51-like family and functions in the double-strand break repair and homologous recombination pathway (36). When coupled with the association of the 19p13.1/MERIT40 locus with triple-negative risk, the suggestion is that modification of DNA repair genes is an important mechanism involved in predisposition to triple-negative breast cancer. The SNP rs3803662, located telomeric to the gene TOX3, was also strongly associated with triple-negative breast cancer in our study (P = 3.66 × 10^{-5}). This SNP was originally identified in 2 GWASs of breast cancer (7, 9) and has been associated with risk of developing both ER-positive and ER-negative tumors (9). The SNP is also associated with the risk of BRCA1-related breast cancers (15), which are primarily ER-negative or triple-negative. TOX3 encodes a protein containing an HMG-box that is speculated to be involved in the modification of DNA and chromatin structure (37).

Only a subset of the 22 susceptibility loci was associated with triple-negative disease in this study. This suggests that there may be heterogeneity in the predisposition loci associated with different breast tumor subtypes. However, it is important to consider whether limited statistical power may have influenced our results. Among the 16 SNPs that did not reach statistical significance in this study, the effect estimates for variants at 1p11.2, 2q35, 8q24, 9q31.2, 10p15.1, 10p21.2/ZNF365, 10q22.3/ZMIZ1, and FGFR2 either showed no evidence for association or were in the opposite direction compared with the original GWAS findings. Interestingly, 2q35 has been associated with both ER-negative (19) and BRCA1-related breast cancers (21) and was marginally significant in a smaller set of triple-negative breast cancer (19). However, we found no evidence for the association at 2q35 among...
Our results are also consistent with a recent study reporting the most stringent criteria for defining triple-negative cases. This analysis (Fig. 1) would suggest that our findings are lack of heterogeneity in effect estimates across study sites in ER-negative disease. However, we did successfully identify 6 with susceptibility loci that are specific to triple-negative or ER-negative breast cancer (19). These results suggest that we may have had insufficient power to detect significant associations for these SNPs among triple-negative breast cancers.

Several limitations should be considered when interpreting these results. First, different ascertainment criteria were used among the contributing breast cancer studies, with cases being ascertainment from population-based or hospital-based case–control studies. Importantly, genetic main effects models in other large breast cancer consortia such as BCAC have provided stable risk estimates for SNPs across a wide range of study designs. This would suggest that in the case of these genetic variants, ascertainment and study design issues had limited influence on the results of genetic association studies for breast cancer. The consistency in effect estimates among BRCA1-related breast cancers, ER-negative breast cancer, and now triple-negative breast cancer for variants at 19p13.1, 6q25, and TOX3 provides additional evidence that these estimates are robust to variability in study design. Furthermore, our evaluation of interactions with age was underpowered, and unavailability of family history on most studies precluded investigations of interactions by family history. There is also variability in the criteria used to define the status of ER, PR, and HER2 of cases between studies (Supplementary Table S2). For HER2, cases with scores of 0 or 1 by IHC were defined as HER2 negative. Cases with IHC of 2+ were not included to minimize erroneous inclusion of HER2-positive cases. In general, cases were considered ER- or PR-negative on the basis of IHC of tumors using thresholds of less than 1% of cells stained, less than 10% of cells stained, or an Alfred score of 0 to 2, which incorporates both intensity and percentage of staining in tumor cells. In addition to variability in thresholds for positivity, factors such as tissue fixation, antibody choice, and interpretation of positive immunostaining may also affect the definition or the status of ER or PR across study sites (38, 39). The resulting heterogeneity in the definition of triple-negative breast cancer may influence our ability to detect associations with susceptibility loci that are specific to triple-negative or ER-negative disease. However, we did successfully identify 6 genetic loci associated with triple-negative disease, and the lack of heterogeneity in effect estimates across study sites in this analysis (Fig. 1) would suggest that our findings are generally robust to the differences noted earlier. In addition, in a sensitivity analysis including only cases from studies with the most stringent criteria for defining triple-negative cases (<1% of cells stained positive for ER and PR, HER2 0 or 1+ on IHC), the effect estimates were very similar to those from the complete analysis for the 6 SNPs in ESRI, 19p13.1, TOX3, and RAD51L1, with some attenuation of significance. Finally, it is important to note that the results of this study are specific to Caucasian women. Although greater proportions of African Americans and Latinas than do Caucasians develop triple-negative breast cancer, it is not known whether similar associations with the SNPs described here exist in these populations. Further studies are needed to address this question.

In conclusion, our study provides convincing evidence for genetic susceptibility to triple-negative breast cancer and suggests that susceptibility loci may differ by histologic breast tumor subtype, defined by the status of ER, PR, and HER2. These findings add to the evidence suggesting that these subtypes likely arise through distinct etiologic pathways. Additional studies, such as those from the BCAC, will be important for determining whether these SNPs are exclusively associated with ER-negative, triple-negative disease, or even basal breast cancer, a more refined subgroup of triple-negative tumors. Fine mapping and functional analyses of these susceptibility loci are needed to identify the causative variants and mechanisms underlying the associations with triple-negative breast cancer risk.

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No potential conflicts of interest were disclosed.

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