A meta-analysis of genome-wide association studies of breast cancer identifies two novel susceptibility loci at 6q14 and 20q11

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Genome-wide association studies (GWAS) of breast cancer defined by hormone receptor status have revealed loci contributing to susceptibility of estrogen receptor (ER)-negative subtypes. To identify additional genetic variants for ER-negative breast cancer, we conducted the largest meta-analysis of ER-negative disease to date, comprising 4754 ER-negative cases and 31 663 controls from three GWAS: NCI Breast and Prostate Cancer Cohort Consortium (BPC3) (2188 ER-negative cases; 25 519 controls of European ancestry), Triple Negative Breast Cancer Consortium (TNBCC) (1562 triple negative cases; 3399 controls of European ancestry) and African American Breast Cancer Consortium (AABC) (1004 ER-negative cases; 2745 controls). We performed *in silico* replication of 86 SNPs at $P \leq 1 \times 10^{-5}$ in an additional 11 209 breast cancer cases (946 with ER-negative disease) and 16 057 controls of Japanese, Latino and European ancestry. We identified two novel loci for breast cancer at 20q11 and 6q14. SNP rs2284378 at 20q11 was associated with ER-negative breast cancer (combined two-stage OR = 1.16; $P = 1.1 \times 10^{-8}$) but showed a weaker association with overall breast cancer (OR = 1.08, $P = 1.3 \times 10^{-6}$) based on 17 869 cases and 43 745 controls and no association with ER-positive disease (OR = 1.01, $P = 0.67$) based on 9965 cases and 22 902 controls. Similarly, rs17530068 at 6q14 was associated with breast cancer (OR = 1.12; $P = 1.1 \times 10^{-9}$), and with both ER-positive (OR = 1.09; $P = 1.5 \times 10^{-5}$) and ER-negative (OR = 1.16, $P = 2.5 \times 10^{-7}$) disease. We also confirmed three known loci associated with ER-negative (19p13) and both ER-negative and ER-positive breast cancer (6q25 and 12p11). Our results highlight the value of large-scale collaborative studies to identify novel breast cancer risk loci.

INTRODUCTION

Breast cancer is a heterogeneous disease and has multiple histological and molecular subtypes, likely with distinct etiologies. Tumors that lack expression of the estrogen receptor (ER) tend to have more aggressive disease, higher histological grade and lower survival rates (1). ER-negative breast cancer is more common in women of African ancestry, accounting for as much as 40% of cases in African American women compared with 15–20% in women of European ancestry. The etiologic heterogeneity between breast cancer subtypes is supported by different associations with ER-positive versus ER-negative disease for many of the known breast cancer risk factors (such as reproductive factors and BMI) (2). Tumors in women with *BRCA1* mutations are predominantly ER-negative, while tumors in *BRCA2* mutation carriers are predominantly ER-positive (3). Furthermore, GWAS have identified multiple common genetic variants more strongly associated with ER-positive than ER-negative breast cancer (4). Through collaborative efforts, we recently identified risk loci on 5p15 and 19p13 that are associated specifically with ER-negative and triple negative (TN) [ER-negative, progesterone (PR)-negative and HER2-negative] breast cancer (5–7).

In order to identify genetic loci associated with the risk of ER-negative breast cancer, we conducted a meta-analysis of three GWAS of ER-negative breast cancer, comprising 4754 cases and 31 663 controls with further replication in an additional 11 209 cases (946 with ER-negative disease) and 16 057 controls.

RESULTS

The meta-analysis included genome-wide association studies (GWAS) of ER-negative breast cancer (4754 ER-negative cases and 31 663 controls) from the NCI Breast and Prostate Cancer Cohort Consortium (BPC3) (2188 ER-negative cases and 25 519 controls of European ancestry), the Triple Negative Breast Cancer Consortium (TNBCC) (1562 TN cases and 3399 controls of European ancestry) and the African American Breast Cancer Consortium (AABC) (1004 ER-negative cases and 2745 controls). (Fig. 1, Supplementary Material, Table S1). We observed little evidence of over-inflation in the test statistics ($\lambda \leq 1.04$ for each study; $\lambda = 1.04$ for meta-analysis) (Supplementary Material, Fig. S1). A total of 86 SNPs were associated with ER-negative breast cancer at $P \leq 10^{-5}$ (Supplementary Material, Table S2). An *in silico* replication of the 86 SNPs was conducted using GWAS of European (BCAC combined), Latino (MEC-LAT, SFBCS/ NC-BCFR) and Japanese (MEC-JPT) ancestry populations, totaling 11 209 breast cancer cases (946 with ER-negative disease) and 8404 controls (Stage 2) (Supplementary Material, Table S1).

Combining results for ER-negative breast cancer from Stages 1 and 2, variants in three regions showed genome-wide significance [20q11-rs2284378, T allele: odds ratio, OR = 1.16, $P = 1.1 \times 10^{-8}$ (Table 1); 19p13-rs8100241, G allele: OR = 1.14, $P = 3.5 \times 10^{-8}$; 6q25-rs9383938, T allele: OR = 1.28, $P = 2.37 \times 10^{-10}$]. Variants at 6q25 have previously been associated with breast cancer risk (8), and variants at the 19p13 locus have been associated with ER-negative and

TN breast cancer risk (5,7). The rs2284378 variant at 20q11 is located in a region containing *RALY* (RNA binding protein, autoantigenic), *EIF2S2* (eukaryotic translation initiation factor 2, subunit 2 beta) and ∼100 kb upstream of *ASIP* (agouti signaling protein), and is in high linkage disequilibrium (LD) ($r^2 = 0.96$ and $D' = 1$) with rs4911414, which has been associated with melanoma and basal cell carcinoma (9) (Supplementary Material, Fig. S2). The T allele at rs2284378 was associated with an increased ER-negative breast cancer risk (OR>1) in all racial/ethnic populations, except Japanese (OR = 0.99) (Table 1). However, this group had the smallest sample size. Furthermore, no significant evidence of heterogeneity was observed by race ($P = 0.28$) or study ($P = 0.54$) (Table 1, Supplementary Material, Table S3). When the study was extended to include all available breast cancer cases (ER-positive and ER-negative) and controls from the participating GWAS, rs2284378 showed a weaker association with overall breast cancer (OR = 1.08, $P = 1.3 \times 10^{-6}$ based on 17 868 cases and 43 744 controls; Table 1) and no evidence for association with ER-positive disease [OR = 1.01, $P = 0.67$ based on 9965 cases and 22 902 controls (Supplementary Material, Table S5)]. A case-only analysis of ER-negative versus ER-positive breast cancer indicated a highly significant difference in ORs by ER status ($P = 1.3 \times 10^{-4}$, Supplementary Material, Table S5). Furthermore, rs2284378 appeared more strongly associated with TN breast cancer (OR = 1.16; $P = 6.4 \times 10^{-3}$), than ER-negative, PR-negative and HER2-positive breast cancer (OR = 1.07, $P = 0.41$), although these differences were not statistically significant (case-only $P = 0.44$) (Supplementary Material, Table S5).

Next, we examined the associations between all candidate loci from Stage 1 ($n = 86$ SNPs) and overall breast cancer risk using all available breast cancer cases and controls from the studies in Stages 1 and 2 (Fig. 1). We identified genome-wide statistically significant associations with variants at 6q25 (rs9383938, T allele: OR = 1.20; $P = 8.7 \times 10^{-14}$), and a recently reported risk locus near the *PTHLH* gene at 12p11 (rs1975930, T allele: OR = 1.22; $P = 1.4 \times 10^{-13}$) (10). In addition, we observed genome-wide significant associations with multiple variants in a gene-desert located at 6q14. Allele C of rs17530068 at 6q14 was associated with increased risk for overall breast cancer risk (OR = 1.12; $P = 1.1 \times 10^{-9}$) (Table 2, Supplementary Material, Fig. S3 and Table S4) and both ER-positive (OR = 1.09; $P = 1.5 \times 10^{-5}$) (Supplementary Material, Table S6) and ER-negative (OR = 1.16, $P = 2.5 \times 10^{-7}$) (Table 2) breast cancer. We observed no evidence of risk heterogeneity for rs17530068 by ER status (case-only analysis $P = 0.53$) (Supplementary Material, Table S6); study ($P_{het} = 0.16$); or race/ethnicity ($P_{het} = 0.30$) (Table 2). Furthermore, rs17530068 appeared more strongly associated with ER-negative, PR-negative and HER2-positive breast cancer (OR = 1.26, $P = 8.0 \times 10^{-3}$) than TN breast cancer (OR = 1.12, $P = 0.07$), although these differences were not statistically significant (case-only $P = 0.17$) (Supplementary Material, Table S6).

We also evaluated associations for 25 known breast cancer risk markers in European-ancestry women from our study (Supplementary Material, Table S7 and Fig. S4). In our samples 8 of the 13 markers previously associated with both
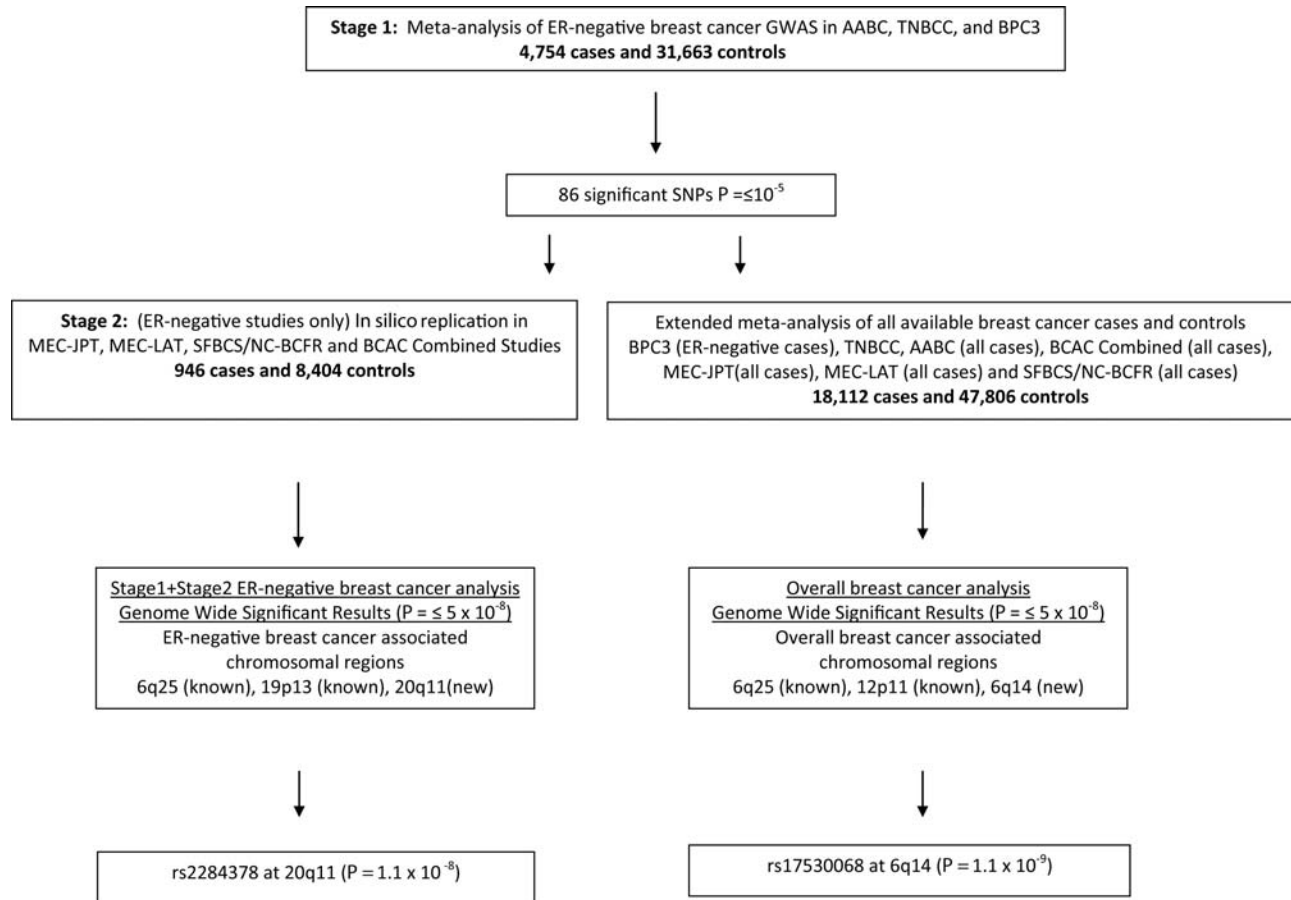
Figure 1. Multi-stage study design.

ER-negative and ER-positive disease or with ER-negative disease only (TERT and 19p13.1) were nominally significantly associated ($P < 0.05$) with ER-negative disease. In contrast, none of the 10 markers previously associated with ER-positive disease only was associated with ER-negative disease. A risk score formed by summing the risk alleles at all 25 previously identified loci was significantly associated with ER-negative disease in our study [OR = 1.06 (1.04–1.07); $P = 2.9 \times 10^{-14}$]. Risk scores for subsets of markers associated with ER-negative disease only (2 markers) or both ER-negative and ER-positive disease (11 markers) were also significantly associated with ER-negative disease [OR = 1.22 (1.14–1.31), $P = 1.0 \times 10^{-8}$ and OR = 1.08 (1.05–1.10), $P = 9.5 \times 10^{-12}$, respectively]. A risk score for the subset of loci previously associated with ER-positive disease only (10 markers) was not associated with the risk of ER-negative disease [OR = 1.02 (1.00–1.04), $P = 0.08$]. These score results provide some confirmation of earlier results and an estimate of the effects of previously identified breast cancer risk markers on the risk of ER-negative disease.

DISCUSSION

We present results from the largest meta-analysis to date to specifically focus on ER-negative disease. We identify two novel loci for breast cancer: 20q11 associated with ER-negative and

TN, but not ER-positive breast cancer, and 6q14 associated with both ER-positive and ER-negative breast cancer. In addition, we confirm three known regions previously associated with ER-negative (19p13) or ER-negative and ER-positive breast cancer (6q25 and 12p11). Correction for genomic control results in similar but attenuated findings for 20q11-rs2284378 ($P_{GC} = 2.4 \times 10^{-8}$) and 6q14-rs17530068 ($P_{GC} = 3.2 \times 10^{-9}$).

The novel association at 20q11 with ER-negative breast cancer spans the *ASIP, RALY* and *EIF2S2* genes. Agouti signaling protein (product of the *ASIP* gene) was first described to inhibit melanogenesis in human melanocytes in 1997 (11). ASIP is a melanocortin 1 receptor (MC1R) ligand that antagonises the function of the transmembrane receptor (12). The variants we identified at 20q11 for breast cancer are highly correlated with variants previously associated with pigmentation traits as well as the risk of both cutaneous melanoma and basal cell carcinoma (9), suggesting a possible biological link between these cancers. Further studies have confirmed the importance of the genetic variation spanning the *ASIP* locus, where a variant at 20q11 showed the strongest association with pigmentation and was implicated in a probable LD with variants within an *ASIP* regulatory region (13). *EIF2S2* encodes eukaryotic translation initiation factor 2, subunit 2 beta, which is involved in early steps of protein synthesis by forming a ternary complex with GTP and initiator tRNA. The deletion of *Eif2s2* has been

Table 1. Association of SNP rs2284378 (T/C) at chromosome 20q11 and breast cancer risk by study and race/ethnicity

Consortium/study	Race/ethnicity	Case/control[a]	RAF (T allele)[b]	OR (95% CI)[c]	P-value[d]	$P_{\text{Het-study}}/P_{\text{Het-race}}^{e}$
Stage 1: ER-negative cases versus controls						
BPC3	European	2188/25 519	0.31	1.14 (1.05–1.24)	0.0028	
TNBCC	European	1478/3345	0.33	1.18 (1.07–1.30)	0.0010	
AABC	African	1004/2744	0.16	1.19 (1.03–1.37)	0.020	
Stage 1		4670/31 608		1.16 (1.09–1.23)	6.5×10^{-7}	0.85/0.76
Stage 2: ER-negative cases versus controls						
BCAC combined GWAS	European	562/6410	0.35	1.10 (0.96–1.25)	0.17	
MEC-JPT	Japanese	84/830	0.26	0.99 (0.68–1.44)	0.95	
MEC-LAT	Latino	112/553	0.29	1.27 (0.94–1.71)	0.13	
SFBCS/NC-BCFR	Latino	188/611	0.29	1.45 (1.13–1.87)	0.004	
Stage 2 (ER-negative)		946/8404		1.16 (1.04–1.29)	0.0048	0.98/0.12
Stage 1 + 2 (ER-negative)		5616/40 012		1.16 (1.10–1.22)	1.1×10^{-8}	0.54/0.28
All breast cancer cases versus controls						
AABC	African	3016/2745	0.16	1.06 (0.95–1.17)	0.30	
BCAC combined GWAS	European	8785/10 142	0.35	1.04 (0.99–1.09)	0.11	
MEC-JPT	Japanese	886/830	0.26	1.08 (0.91–1.24)	0.46	
MEC-LAT	Latino	546/553	0.29	1.24 (1.03–1.48)	0.021	
SFBCS/NC-BCFR	Latino	970/611	0.29	1.23 (1.05–1.44)	0.011	
Stage 2 (all cases)		14 202/14 880		1.06 (1.02–1.10)	0.0025	0.14/0.073
Stage 1 + 2 (all cases)		17 869/43 745		1.08 (1.05–1.12)	1.3×10^{-6}	0.056/0.19

[a]Number of cases and controls with genotype data for rs2284378.
[b]Risk allele frequency (RAF) in controls.
[c]Adjusted for age, study and principal components in AABC. Adjusted for age and country in TNBCC. Adjusted for age categories and top six eigenvectors in BPC3. Adjusted for age and top 10 eigenvectors in MEC-JPT, MEC-LAT and SFBCS/NC-BCFR studies. Combined analysis (Stage 1, Stage 2 and Stage 1 + 2) are from the meta-analysis.
[d]P for trend (1-d.f.).
[e]P for heterogeneity by study and race/ethnicity, respectively.

Table 2. Association of SNP rs17530068 (C/T) at chromosome 6q14 and breast cancer risk by study and race/ethnicity

Consortium/study	Race/ethnicity	Case/control[a]	RAF (C allele)[b]	OR (95% CI)[c]	P-value[d]	$P_{\text{Het-study}}/P_{\text{Het-race}}^{e}$
Stage 1: ER-negative cases versus controls						
BPC3	European	2188/25 519	0.24	1.23 (1.12–1.35)	2.23×10^{-5}	
TNBCC	European	1478/3345	0.24	1.13 (1.02–1.26)	0.023	
AABC	African	1004/2745	0.07	1.07 (0.86–1.34)	0.54	
Stage 1		4670/31 609		1.17 (1.09–1.26)	3.5×10^{-6}	0.37/0.41
Stage 2: ER-negative cases versus controls						
BCAC combined GWAS	European	562/6410	0.22	1.09 (0.95–1.25)	0.24	
MEC-JPT	Japanese	84/830	0.19	1.16 (0.79–1.71)	0.45	
MEC-LAT	Latino	112/553	0.23	1.06 (0.75–1.50)	0.73	
SFBCS/NC-BCFR	Latino	188/611	0.22	1.40 (1.07–1.84)	0.014	
Stage 2 (ER-negative)		946/8404		1.14 (1.02–1.28)	0.022	0.41/0.52
Stage 1 + 2 (ER-negative)		5616/40 013		1.16 (1.10–1.23)	2.5×10^{-7}	0.54/0.78
All breast cancer cases versus controls						
AABC	African	3016/2745	0.07	1.04 (0.89–1.21)	0.63	
BCAC combined GWAS	European	8785/10 142	0.22	1.08 (1.02–1.14)	0.0021	
MEC-JPT	Japanese	886/830	0.19	1.13 (0.96–1.34)	0.14	
MEC-LAT	Latino	546/553	0.23	1.21 (0.99–1.47)	0.056	
SFBCS/NC-BCFR	Latino	970/611	0.22	1.27 (1.07–1.51)	0.006	
Stage 2 (all cases)		14 203/14 881		1.10 (1.05–1.15)	1.8×10^{-5}	0.31/0.20
Stage 1 + 2 (all cases)		17 869/43 745		1.12 (1.08–1.16)	1.1×10^{-9}	0.16/0.30

[a]Number of cases and controls with genotype data for rs17530068.
[b]Risk allele frequency (RAF) in controls.
[c]Adjusted for age, study and principal components in AABC. Adjusted for age and country in TNBCC. Adjusted for age categories and top six eigenvectors in BPC3. Adjusted for age and top 10 eigenvectors in MEC-JPT, MEC-LAT and SFBCS/NC-BCFR studies. Combined analysis (Stage 1, Stage 2 and Stage 1 + 2) are from the meta-analysis.
[d]P for trend.
[e]P for heterogeneity by study and race/ethnicity, respectively.

associated with suppression of testicular germ cell tumor incidence and recessive lethality in mice (14). The agouti-yellow (*AV*) deletion is a genetic modifier known to suppress testicular germ cell tumor susceptibility in mice and humans. The *AV* mutation deletes both *RALY* and *Eif2s2,* and induces the ectopic expression of *agouti,* all of which are potential testicular germ cell tumor-modifying variations (14). Both *RALY* and *EIF2S2* are expressed in many tissues, including mammary gland (15). The SNP rs2284378 was not consistently associated with expression of *EIF2S2*, *RALY* or *ASIP* in lymphocytes (11), adipocytes or skin cells(16), although there was marginal evidence for association between rs2284378 and *EIF2S2* expression in one study (16) (Supplementary Material, Table S8). However, several SNPs in high LD with SNP rs2284378 ($r^2 > 0.8$) within a 1 Mb region were significantly associated with expression of nearby genes *EIF2S2* and *RALY*. Rs4911379 ($r^2 = 0.96$) is statistically significantly associated with *EIF2S2* expression in fibroblasts ($P = 3.6 \times 10^{-4}$) (17) and SNPs rs761238 and rs761236 ($r^2 = 0.85$) are associated with *RALY* expression in lymphocytes ($P = 8.3 \times 10^{-4}$) (16). An additional 13 SNPs ($r^2 > 0.85$) have been associated with expression of *RALY*, *GGTL3*, *DYNLRB1* and *AK054906* in liver cells, monocytes and lymphoblastoid cell lines (Supplementary Material, Table S9). In addition to expression, several enhancer as well as promoter regions defined by overlapping chromatin marks in human mammary epithelial cells (HMEC) were found at 20q11 (Supplementary Material, Fig. S5). SNPs in high LD with rs2284378 ($r^2 > 0.7$), such as rs4911395, rs4911396 and rs1007090, are located in the promoter region of *RALY*. SNPs rs6142101, rs6087557 and rs4911408 ($r^2 > 0.7$) are present in the promoter region of *EIF2S*2, and rs1054534, rs1555075, rs2268086, rs2268088, rs4911401, rs2284388, rs2284389 and rs932388 are located in predicted enhancer regions in introns of *RALY*. Thus, variants at 20q11 may influence expression of multiple genes in mammary epithelial cells, as has been seen in prostate cancer (18).

In contrast, rs17530068 at 6q14 is located in a gene desert with no evidence of an open/active regulatory region in HMEC (Supplementary Material, Fig. S6). The closest gene (~262 kb), family with sequence similarity 46, member A (*FAM46A/C6orf37*), encodes a protein of unknown function. Five SNPs in this region in low LD with SNP rs17530068 ($r^2 < 0.02$) were associated with expression of *IBTK* in lymphoblastoid cell lines (Supplementary Material, Table S10). Additional studies of both of these novel regions will be necessary to identify the underlying biologically relevant variant/s.

The SNP rs17530068 at chromosome 6q14 was associated with overall breast cancer risk and showed no differential association depending on ER status. The association of SNP rs2284378 at 20q11, however, was stronger for ER-negative than ER-positive breast cancer. This finding underscores the importance of investigating genetic variants for specific subtypes of breast cancer, as this locus had not been previously identified in the many GWAS of breast cancer to date that did not focus on this specific breast cancer subtype. The etiology of ER-negative disease is largely unknown. Identifying new loci associated with ER-negative and TN breast cancer will continue to provide insight into the biological

mechanisms underlying this more aggressive form of breast cancer, and could result in improvements in risk prediction and treatment.

MATERIALS AND METHODS

Study populations

Stage 1 included the studies of the NCI BPC3, TNBCC and AABC. The BPC3 study includes 2188 ER-negative cases and 25 519 controls, AABC includes 3153 cases (1004 ER-negative) and 2745 controls from 9 studies and TNBCC includes 1562 cases and 3399 controls from 15 studies (Supplementary Material, Table S1). Replication studies include 886 cases (84 ER-negative) and 830 controls from a GWAS of breast cancer in Japanese (MEC-JPT) women and 546 cases (112 ER-negative) and 558 controls from a GWAS of breast cancer in Latino (MEC-LAT) women in the Multiethnic Cohort (MEC), 992 cases (188 ER-negative) and 640 controls from the San Francisco Bay Area Breast Cancer Study (SFBCS) and the Northern California Breast Cancer Family Registry (NC-BCFR), and 8785 cases (562 ER-negative) and 14 029 controls from 8 combined GWAS of breast cancer from BCAC. All participants in these studies have provided written consent for the research and approval for the study was obtained from the ethical review board from all local institutions. A description of each participating study has been provided in Supplementary Material.

Stage 1: genotyping and quality control

Genotyping in AABC was conducted using the Illumina Human1M-Duo BeadChip. Of the 5984 samples in the AABC Consortium (3153 cases and 2831 controls), we attempted genotyping of 5932, removing samples ($n = 52$) with DNA concentrations <20 ng/µl. Following genotyping, we removed samples based on the following exclusion criteria: (i) unknown replicates (≥98.9% genetically identical) that we were able to confirm, ($n = 15$); (ii) unknown replicates pair or triplicate removed, ($n = 14$); (iii) samples with call rates <95% after a second attempt ($n = 100$); (iv) samples with ≤5% African ancestry ($n = 36$) (discussed below); and (v) samples with <15% mean heterozygosity of SNPs in the X chromosome and/or similar mean allele intensities of SNPs on the X and Y chromosomes ($n = 6$). In the analysis, we removed SNPs with <95% call rates ($n = 21 732$) or minor allele frequencies (MAFs) <1% ($n = 80 193$). The concordance rate for blinded duplicates was 99.95%. We also eliminated SNPs with genotyping concordance rates <98% based on the replicates ($n = 11 701$). The final analysis data set included 1 043 036 SNPs genotyped on 3016 cases (988 ER-negative, 1520 ER-positive and the remaining 508 cases with unknown ER status) and 2745 controls, with an average SNP call rate of 99.7% and average sample call rate of 99.8%.

Genotyping for the TNBCC GWAS was conducted on 1718 cases from 10 studies (ABCTB, BBCC, DFCI, FCCC, GENICA, MARIE, MCBCS, MCCS, POSH, SBCS) using the Illumina 660-Quad SNP array. In addition, a subset of MARIE cases ($n = 52$) was genotyped using the Illumina CNV370 SNP array. HEBCS cases ($n = 85$) were genotyped

using the Illumina 550 SNP array and population allele and genotype frequencies on healthy population controls ($n = 222$) were genotyped on Illumina 370 SNP array, and obtained from the NordicDB, a Nordic pool and portal for genome-wide control data (19) from the Finnish Genome Center. GWAS data for public controls ($n = 3448$) were generated using the following arrays: Illumina 660-Quad SNP array (QIMR), Illumina 550 SNP array (CGEMS), Illumina 550 SNP array (KORA) and Illumina 1.2M (WTCCC). These GWAS data were independently evaluated by an iterative QC process with the following exclusion criteria: MAF <0.01, call rate $<95\%$, Hardy–Weinberg equilibrium (HWE) P-value $<1 \times 10^{-7}$ among controls and sample call rate $<98\%$. In total, we excluded previously unknown replicates ($n = 2$) and samples with call rates $<98\%$ ($n = 83$), samples that failed sex check ($n = 10$), cases identified as non-TN breast cancer ($n = 20$) and related samples ($n = 27$). We removed SNPs with $<95\%$ call rates or MAF $<5\%$. Because a number of our samples were genotyped at different locations, we removed SNPs if there was a difference >0.10 between the study allele frequency and the median frequency across all studies. The Eigensoft software which uses principle component analysis (PCA) was used to evaluate confounding due to population stratification. We removed 101 subjects that did not cluster with the CEU HapMap Phase 2 samples, and a further 179 controls were removed which overlapped with CGEMS/NHS controls in BPC3, resulting in 1562 cases and 3399 controls in the GWAS analyses.

BPC3 GWAS genotyping was conducted at three genotyping centers (NCI Core Genotyping Facility, USA; University of Southern California, USA; and Imperial College London, UK). Subjects from CPSII, EPIC, MEC, PLCO and PBCS were genotyped using the Illumina Human 660k-Quad SNP array (Illumina, Inc.), NHSI/NHSII and part of the PLCO study were genotyped previously using the Illumina Human 550 SNP array (Illumina, Inc.) (20). SNPs were filtered and removed based on deviations from Hardy–Weinberg proportions in control subjects ($P < 10^{-5}$), autosomal SNPs with MAF of $<5\%$ and completion rate $<95\%$. Samples were excluded based on genotyping call rates $<95\%$ ($n = 195$), samples with extreme heterozygosity were excluded from the analysis ($n = 35$), sex discordance ($n = 3$) and unexpected duplicates and relatedness ($n = 6$). Subjects with evidence of significant non-European ancestry and population structure were also excluded. Non-European ancestry was assessed utilizing a subset of unlinked, population informative SNPs (21). Individuals determined to have $<80\%$ European ancestry were excluded from future analyses ($n = 16$). The average concordance rate of blinded duplicates was 99.95%. In order to resolve a more detailed population substructure, PCA was conducted using *struct.pca* module of GLU (http://code. google.com/p/glu-genetics/). PCA was only performed in subjects with over 80% European ancestry. Furthermore, 958 controls from NHS (CGEMS) were removed from BPC3 analyses due to overlap between TNBCC and BPC3 studies. The overall number of cases and controls after all exclusions which contributed to the Stage 1 analysis were 1998 cases and 2305 controls.

The WHS cohort subjects in BPC3 were previously genotyped using the Human-Hap300 Duo-plus BeadChip (22).

Among the final 23 294 individuals of verified European ancestry, genotypes for a total of 2 608 509 SNPs were imputed from the experimental genotypes and LD relationships implicit in the HapMap r. 22 CEU samples. WHS contributed 190 cases and 23 214 control subjects to Stage 1. WHS was meta-analyzed with the remaining BPC3 studies contributing a total of 2188 cases and 25 519 control subjects to Stage 1 analysis.

SNPs rs2284378 and rs17530068 were genotyped in all Stage 1 studies.

Stage 2: genotyping and quality control

The SFBCS (23) and the NC-BCFR (24) study samples were genotyped with the Affymetrix 6.0 array according to the manufacturer's instructions (https://www.affymetrix.com) in the laboratory of Esteban Gonzalez Burchard at UCSF. A total of 15 cases and 30 controls were excluded from the SFBCS and NC-BCFR sample set that had a genotyping call rate $<95\%$ or showed either known or cryptic relatedness. The final sample included in the analysis was 992 cases (188 ER-negative cases) and 640 controls. Imputation was conducted with the program BEAGLE, with all unrelated HapMap Phase II samples included as references (http:// hapmap.ncbi.nlm.nih.gov).

GWAS of breast cancer in Latino (MEC-LAT) and Japanese (MEC-JPT) samples from the MEC were genotyped with the Illumina 660W array at USC. For MEC-LAT, we excluded 48 samples from the MEC that had a genotyping call rate of $<95\%$ and 34 that showed either known or cryptic relatedness. The final MEC-LAT sample included 546 (112 ER-negative) and 558 controls. With similar exclusions, the final MEC-JPT sample included 886 (84 ER-negative) and 830 controls.

The BCAC combined GWAS includes primary genotype data from eight breast cancer GWAS in populations of European ancestry (ABCFS, BBCS, GC-HBOC, MARIE, HEBCS, SASBAC, UK2, DFBBCS). All studies were genotyped with various versions of Illumina arrays, except GC-HBOC which was performed with the Affymetrix 5.0 (cases) and 6.0 (controls) arrays. Standard QC was performed on all scans. Specifically, all individuals with low call rate ($<95\%$), extreme high or low heterozygosity ($P<10^{-5}$), and all individuals evaluated to be of non-European ancestry ($>15\%$ non-European component, by multidimensional scaling using the three Hapmap2 populations as a reference) were excluded. SNPs with call rate $<95\%$; call rate $<99\%$ and MAF$<5\%$, all SNPs with MAF$<1\%$, and SNPs with genotype frequencies departing from HWE at $P<10^{-6}$ in controls or $P < 10^{-12}$ in cases were also excluded. Data were imputed for ~ 2.6 M SNPs for all scans using Mach v1.0 with HapMap version 2 CEU as a reference. BBCS and UK2 used the same control data (WTCCC2). These studies were imputed separately. For the combined analysis, the control set was divided randomly between the two studies, in proportion to the size of case series, to provide disjoint strata. Estimated per-allele ORs and standard errors were generated from the imputed genotypes using Probabel (25).

SNPs rs2284378 and rs17530068 were genotyped in all Stage 2 studies except SFBCS and NC-BCFR where they

were imputed. Both SNPs were genotyped by TaqMan in 483 samples from these studies and genotype concordance versus imputed genotypes was 93.3% for rs2284378 and 94.9% for rs17530068.

TaqMan genotyping in BPC3 for SNP rs2284378 and SNP rs17530068

In BPC3, genotyping of SNP rs2284378 and rs17530068 was performed for all available breast cancer cases and controls by TaqMan in four laboratories (CPS-II and MEC at the University of Southern California; NHS and WHS at Harvard University; EPIC at the German Cancer Research Center in Heidelberg; and PLCO at the NCI/Core Genotyping Facility). All studies typed SNP rs17530068; however for SNP rs2284378, PLCO and CPS-II typed a proxy SNP rs6059651 ($r^2 = 1$, $D' = 1$). The concordance for the TaqMan genotyping data with that generated from Illumina for Stage 1 ER-negative cases and controls was 0.997 for rs17530068 and 0.986 for rs2284378 for CPS2, MEC, NHS, EPIC and PLCO. The genotype concordance versus imputed for WHS was 95% for rs2284378 and 97% for rs17530068.

Statistical analysis

In AABC, we tested for gene dosage effects in models adjusted for age, study and eigenvectors 1–10. OR and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression. In TNBCC, unconditional logistic regression was used to assess each SNP association analyses also assuming a log-additive model, adjusting for country and the first two principal components. In BPC3, unconditional logistic regression model was used to assess single SNP associations adjusting for age categories and the top six eigenvectors.

In both AABC and TNBCC, phased haplotype data from the founders of the CEU and YRI HapMap Phase 2 samples (build 21) were used to infer LD patterns in order to impute untyped markers. For BPC3, Hapmap Phase 2 (release 21) and Hapmap Phase 3 were used to impute untyped markers. For all studies, genome-wide imputation was carried out using the software MACH. Filtered from the analysis were SNPs with Rsq < 0.3 and MAF <1%.

We conducted a fixed effect meta-analysis of AABC, TNBCC and BPC3 using the inverse variance weighted method. The number of SNPs available for meta-analysis from AABC, TNBCC and BPC3 in Stage 1 were 3 055 415, 2 134 490 and 245 3207, respectively. The union of these three data sets was meta-analyzed using the program METAL. We conducted *in silico* replication of 86 SNPs with P-values $\leq 10^{-5}$ in Stage 1 in the Stage 2 studies, and a meta-analysis of these SNPs from Stages 1 and 2 for both ER- negative and overall breast cancer. P-values from our top two loci were corrected for genomic inflation (P_{GC}) using the lambda value from the overall meta-analysis. Testing for heterogeneity by study was evaluated using the Q-statistic. Case-only analyses were performed to test for differences in the association by tumor subtypes, study and race/ethnicity.

The association between risk scores of 25 previously identified breast cancer risk alleles and risk of breast cancer in our samples was calculated using meta-regression, assuming the per-allele odds ratio was constant across the markers analyzed. This is equivalent to combining the summary log odds ratio estimates at independent loci using inverse-variance weighted meta-analysis. The overlap between subjects contributing to this study and those contributing to previous studies varied from marker to marker [e.g. the TNBCC contributed to the initial report on rs8170 (5) and the BPC3 and TNBCC contributed to the initial report on the *TERT* locus (6)].Thus, the results could be overestimates since some of the studies here contributed to the discovery of these 25 loci.

Functional analysis

Expression quantitative trait loci (eQTL) were assessed for all SNPs in the chromosome 6 and 20 loci using the GTEX database (http://www.ncbi.nlm.nih.gov/gtex/GTEX2/gtex.cgi), University of Chicago eQTL Browser (http://eqtl.uchicago.edu) and Genevar (http://www.sanger.ac.uk/resources/software/genevar/) (26).

In an attempt to identify functionality at the two novel breast cancer risk loci, we used the open-source R/Bioconductor package FunciSNP version 0.99 (27), which systematically integrates the 1000 Genomes Project SNP data (April 2012 data release) with chromatin features of interest. For each of the two novel breast cancer markers, we analyzed all SNPs with an r^2-value > 0.5 with each index SNP in the 1000 Genomes Project EUR populations in a 1 Mb window around each index variant. We assessed whether these SNPs were co-located with 12 different chromatin features generated by next-generation sequencing technologies, which capture open chromatin regions, promoters and enhancers genome-wide in HMEC as well as known DNaseI hypersensitive locations, FAIRE-seq peaks and CTCF-binding sites from >100 different cell types, which were collected in ENCODE data (28). We utilized the UCSC Genome Browser (http://genome.ucsc.edu/) to illustrate the correlated SNPs, which overlap chromatin features as well as chromatin feature tracks (Supplementary Material, Figs S5 and S6).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

REFERENCES

1. Parl, F.F., Schmidt, B.P., Dupont, W.D. and Wagner, R.K. (1984) Prognostic significance of estrogen receptor status in breast cancer in relation to tumor stage, axillary node metastasis, and histopathologic grading. *Cancer*, **54**, 2237–2242.
2. Yang, X.R., Chang-Claude, J., Goode, E.L., Couch, F.J., Nevanlinna, H., Milne, R.L., Gaudet, M., Schmidt, M.K., Broeks, A., Cox, A. *et al.* (2011) Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. *J. Natl Cancer Inst.*, **103**, 250–263.
3. Milne, R.L. and Antoniou, A.C. (2011) Genetic modifiers of cancer risk for BRCA1 and BRCA2 mutation carriers. *Ann. Oncol.*, **22**(Suppl. 1), i11– i 17.
4. Broeks, A., Schmidt, M.K., Sherman, M.E., Couch, F.J., Hopper, J.L., Dite, G.S., Apicella, C., Smith, L.D., Hammet, F., Southey, M.C. *et al.* (2011) Low penetrance breast cancer susceptibility loci are associated with specific breast tumor subtypes: findings from the Breast Cancer Association Consortium. *Hum. Mol. Genet.*, **20**, 3289–3303.
5. Antoniou, A.C., Wang, X., Fredericksen, Z.S., McGuffog, L., Tarrell, R., Sinilnikova, O.M., Healey, S., Morrison, J., Kartsonaki, C., Lesnick, T. *et al.* (2010) A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. *Nat. Genet.*, **42**, 885–892.
6. Haiman, C.A., Chen, G.K., Vachon, C.M., Canzian, F., Dunning, A., Millikan, R.C., Wang, X., Ademuyiwa, F., Ahmed, S., Ambrosone, C.B. *et al.* (2011) A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. *Nat. Genet.*, **43**, 1210–1214.
7. Stevens, K.N., Vachon, C.M., Lee, A.M., Slager, S., Lesnick, T., Olswold, C., Fasching, P.A., Miron, P., Eccles, D., Carpenter, J.E. *et al.* (2011) Common breast cancer susceptibility loci are associated with triple-negative breast cancer. *Cancer Res.*, **71**, 6240–6249.
8. Zheng, W., Long, J., Gao, Y.T., Li, C., Zheng, Y., Xiang, Y.B., Wen, W., Levy, S., Deming, S.L., Haines, J.L. *et al.* (2009) Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat. Genet.*, **41**, 324–328.
9. Gudbjartsson, D.F., Sulem, P., Stacey, S.N., Goldstein, A.M., Rafnar, T., Sigurgeirsson, B., Benediktsdottir, K.R., Thorisdottir, K., Ragnarsson, R., Sveinsdottir, S.G. *et al.* (2008) ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. *Nat. Genet.*, **40**, 886–891.
10. Schadt, E.E., Molony, C., Chudin, E., Hao, K., Yang, X., Lum, P.Y., Kasarskis, A., Zhang, B., Wang, S., Suver, C. *et al.* (2008) Mapping the genetic architecture of gene expression in human liver. *PLoS Biol.*, **6**, e107.
11. Stranger, B.E., Montgomery, S.B., Dimas, A.S., Parts, L., Stegle, O., Ingle, C.E., Sekowska, M., Smith, G.D., Evans, D., Gutierrez-Arcelus, M. *et al.* (2012) Patterns of cis regulatory variation in diverse human populations. *PLoS Genet.*, **8**, e1002639.
12. Scherer, D. and Kumar, R. (2010) Genetics of pigmentation in skin cancer—a review. *Mutat. Res.*, **705**, 141–153.
13. Barrett, J.H., Iles, M.M., Harland, M., Taylor, J.C., Aitken, J.F., Andresen, P.A., Akslen, L.A., Armstrong, B.K., Avril, M.F., Azizi, E. *et al.* (2011) Genome-wide association study identifies three new melanoma susceptibility loci. *Nat. Genet.*, **43**, 1108–1113.
14. Heaney, J.D., Michelson, M.V., Youngren, K.K., Lam, M.Y. and Nadeau, J.H. (2009) Deletion of eIF2beta suppresses testicular cancer incidence and causes recessive lethality in agouti-yellow mice. *Hum. Mol. Genet.*, **18**, 1395–1404.
15. Mosca, E., Alfieri, R., Merelli, I., Viti, F., Calabria, A. and Milanesi, L. (2010) A multilevel data integration resource for breast cancer study. *BMC Syst. Biol.*, **4**, 76.
16. Nica, A.C., Parts, L., Glass, D., Nisbet, J., Barrett, A., Sekowska, M., Travers, M., Potter, S., Grundberg, E., Small, K. *et al.* (2011) The architecture of gene regulatory variation across multiple human tissues: the MuTHER study. *PLoS Genet.*, **7**, e1002003.
17. Dimas, A.S., Deutsch, S., Stranger, B.E., Montgomery, S.B., Borel, C., Attar-Cohen, H., Ingle, C., Beazley, C., Gutierrez Arcelus, M., Sekowska, M. *et al.* (2009) Common regulatory variation impacts gene expression in a cell type-dependent manner. *Science*, **325**, 1246–1250.

18. Pomerantz, M.M., Shrestha, Y., Flavin, R.J., Regan, M.M., Penney, K.L., Mucci, L.A., Stampfer, M.J., Hunter, D.J., Chanock, S.J., Schafer, E.J. *et al.* (2010) Analysis of the 10q11 cancer risk locus implicates MSMB and NCOA4 in human prostate tumorigenesis. *PLoS Genet.*, **6**, e1001204.

19. Leu, M., Humphreys, K., Surakka, I., Rehnberg, E., Muilu, J., Rosenstrom, P., Almgren, P., Jaaskelainen, J., Lifton, R.P., Kyvik, K.O. *et al.* (2010) NordicDB: a Nordic pool and portal for genome-wide control data. *Eur. J. Hum. Genet.*, **18**, 1322–1326.

20. Hunter, D.J., Kraft, P., Jacobs, K.B., Cox, D.G., Yeager, M., Hankinson, S.E., Wacholder, S., Wang, Z., Welch, R., Hutchinson, A. *et al.* (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat. Genet.*, **39**, 870–874.

21. Yu, K., Wang, Z., Li, Q., Wacholder, S., Hunter, D.J., Hoover, R.N., Chanock, S. and Thomas, G. (2008) Population substructure and control selection in genome-wide association studies. *PLoS ONE*, **3**, e2551.

22. Ridker, P.M., Chasman, D.I., Zee, R.Y., Parker, A., Rose, L., Cook, N.R. and Buring, J.E. (2008) Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. *Clin. Chem.*, **54**, 249–255.

23. John, E.M., Schwartz, G.G., Koo, J., Wang, W. and Ingles, S.A. (2007) Sun exposure, vitamin D receptor gene polymorphisms, and breast cancer risk in a multiethnic population. *Am. J. Epidemiol.*, **166**, 1409–1419.

24. John, E.M., Miron, A., Gong, G., Phipps, A.I., Felberg, A., Li, F.P., West, D.W. and Whittemore, A.S. (2007) Prevalence of pathogenic BRCA1 mutation carriers in 5 US racial/ethnic groups. *JAMA*, **298**, 2869–2876.

25. Aulchenko, Y.S., Struchalin, M.V. and van Duijn, C.M. (2010) ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*, **11**, 134.

26. Yang, T.P., Beazley, C., Montgomery, S.B., Dimas, A.S., Gutierrez-Arcelus, M., Stranger, B.E., Deloukas, P. and Dermitzakis, E.T. (2010) Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. *Bioinformatics*, **26**, 2474–2476.

27. Coetzee, S.G., Rhie, S.K., Berman, B.P., Coetzee, G.A. and Noushmehr, H. (2012) FunciSNP: an R/bioconductor tool integrating functional non-coding data sets with genetic association studies to identify candidate regulatory SNPs. *Nucleic Acids Res.*, [Epub ahead of print, 22 June].

28. Ernst, J., Kheradpour, P., Mikkelsen, T.S., Shoresh, N., Ward, L.D., Epstein, C.B., Zhang, X., Wang, L., Issner, R., Coyne, M. *et al.* (2011) Mapping and analysis of chromatin state dynamics in nine human cell types. *Nature*, **473**, 43–49.