Genome- and transcriptome-wide association studies of 386,000 Asian and European-ancestry women provide new insights into breast cancer genetics

Authors

Guochong Jia, Jie Ping, Xiang Shu, ..., Xiao-ou Shu, Jirong Long, Wei Zheng

Correspondence

wei.zheng@vanderbilt.edu

Using data from 386,000 Asian- and European-ancestry women, we conducted extensive genome- and transcriptome-wide association studies that identified 222 risk loci and 137 genes in association with breast cancer risk. These studies, along with pathway analyses, provide a comprehensive understanding of and new biological insights into the genetics of breast cancer.

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Genome- and transcriptome-wide association studies of 386,000 Asian and European-ancestry women provide new insights into breast cancer genetics

Guochong Jia,[1,56] Jie Ping,[1,56] Xiang Shu,[2] Yaohua Yang,[1] Qiuyin Cai,[1] Sun-Seog Kweon,[3,4] Ji-Yeob Choi,[5,6,7] Michiaki Kubo,[8] Sue K. Park,[5,6,7] Manjeet K. Bolla,[9] Joe Dennis,[9] Qin Wang,[9] Xingyi Guo,[1] Bingshan Li,[10] Ran Tao,[11,12] Kristan J. Aronson,[13] Tsun L. Chan,[14,15] Yu-Tang Gao,[16] Mikael Hartman,[17,18,19] Weang Kee Ho,[20] Hidemi Ito,[21,22] Motoki Iwasaki,[23] Hiroji Iwata,[24]

(Author list continued on next page)

Summary

By combining data from 160,500 individuals with breast cancer and 226,196 controls of Asian and European ancestry, we conducted genome- and transcriptome-wide association studies of breast cancer. We identified 222 genetic risk loci and 137 genes that were associated with breast cancer risk at a $p < 5.0 \times 10^{-8}$ and a Bonferroni-corrected $p < 4.6 \times 10^{-6}$, respectively. Of them, 32 loci and 15 genes showed a significantly different association between ER-positive and ER-negative breast cancer after Bonferroni correction. Significant ancestral differences in risk variant allele frequencies and their association strengths with breast cancer risk were identified. Of the significant associations identified in this study, 17 loci and 14 genes are located 1Mb away from any of the previously reported breast cancer risk variants. Pathways analyses including 221 putative risk genes identified multiple signaling pathways that may play a significant role in the development of breast cancer. Our study provides a comprehensive understanding of and new biological insights into the genetics of this common malignancy.

Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide, with an estimated 2.3 million new cases in 2020.[1] Genetic factors play a critical role in the etiology of both familial and sporadic breast cancers. In addition to breast cancer predisposition genes, such as *BRCA1* and *BRCA2*,[2–4] common genetic variants in approximately 200 loci have been identified in genome-wide association studies (GWASs).[5–7] However, most GWASs of breast cancer have been conducted among women of European ancestry,[8] and GWASs conducted among women of Asian ancestry have had relatively smaller sample sizes.[9,10] Although most susceptibility loci have been shown to be shared across European and Asian populations, the lead variants at some susceptibility loci can be different between these two populations given their differences in genetic architecture.[11,12] To identify additional genetic risk

[1]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 2525 West End Avenue, Suite 800, Nashville, TN, USA; [2]Department of Epidemiology & Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [3]Department of Preventive Medicine, Chonnam National University Medical School, Hwasun, Korea; [4]Jeonnam Regional Cancer Center, Chonnam National University Hwasun Hospital, Hwasun, Korea; [5]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea; [6]Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea; [7]Cancer Research Institute, Seoul National University College of Medicine, Seoul, Korea; [8]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan; [9]Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK; [10]Department of Molecular Physiology & Biophysics, Vanderbilt Genetics Institute, Vanderbilt University, Nashville, TN, USA; [11]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, USA; [12]Vanderbilt Genetics Institute, Vanderbilt University Medical Center, Nashville, TN, USA; [13]Department of Public Health Sciences and Queen's Cancer Research Institute, Queen's University, Kingston, ON, Canada; [14]Hong Kong Hereditary Breast Cancer Family Registry, Hong Kong SAR, China; [15]Department of Molecular Pathology, Hong Kong Sanatorium & Hospital, Hong Kong SAR, China; [16]State Key Laboratory of Oncogene and Related Genes & Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; [17]Department of Surgery, National University Hospital, Singapore, Singapore; [18]Saw Swee Hock School of Public Health, National University of Singapore, Singapore, Singapore; [19]Department of Surgery, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore; [20]Department of Applied Mathematics, Faculty of Engineering, University of Nottingham Malaysia Campus, Semenyih, Selangor, Malaysia; [21]Division of Cancer Information and Control, Aichi Cancer Center Research Institute, Nagoya, Japan; [22]Department of Descriptive Cancer Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [23]Division of Epidemiology, National Cancer Center Institute for Cancer Control, Tokyo, Japan; [24]Department of Breast Oncology, Aichi Cancer Center, Nagoya, Aichi, Japan; [25]Departments of Epidemiology, Cancer Prevention Institute of California, Fremont, CA, USA; [26]Departments of Health Research and Policy, School of Medicine, Stanford University, Stanford, CA, USA; [27]Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA; [28]Department of Surgery, Nagano Matsushiro General Hospital, Nagano, Japan; [29]Division of Cancer Epidemiology and Management, National Cancer Center, Goyang, Korea; [30]Department of Surgery, University of Hong Kong, Hong Kong SAR, China; [31]Department of Surgery, Hong Kong Sanatorium & Hospital, Hong Kong SAR, China; [32]Human Genetics, Genome Institute of Singapore, Singapore, Singapore; [33]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden; [34]Division of Health Sciences, Warwick Medical School, Warwick University, Coventry, UK; [35]Institute of Population Health, University of Manchester, Manchester, UK; [36]Cancer Research Malaysia, Subang Jaya, Selangor, Malaysia; [37]Laboratory of Clinical Genome Sequencing, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan; [38]Division of

(Affiliations continued on next page)

Esther M. John,[25,26,27] Yoshio Kasuga,[28] Mi-Kyung Kim,[29] Allison W. Kurian,[26] Ava Kwong,[14,30,31] Jingmei Li,[19,32,33] Artitaya Lophatananon,[34,35] Siew-Kee Low,[8] Shivaani Mariapun,[36] Koichi Matsuda,[37] Keitaro Matsuo,[38,39] Kenneth Muir,[34,35] Dong-Young Noh,[7,40] Boyoung Park,[41] Min-Ho Park,[42] Chen-Yang Shen,[43,44] Min-Ho Shin,[3] John J. Spinelli,[45,46] Atsushi Takahashi,[8,47] Chiuchen Tseng,[48] Shoichiro Tsugane,[49] Anna H. Wu,[48] Taiki Yamaji,[23] Ying Zheng,[50] Alison M. Dunning,[51] Paul D.P. Pharoah,[9,51] Soo-Hwang Teo,[36,52] Daehee Kang,[6,7,53,54] Douglas F. Easton,[9,51] Jacques Simard,[55] Xiao-ou Shu,[1] Jirong Long,[1] and Wei Zheng[1,*]

loci and provide a more comprehensive understanding of breast cancer genetics, we conducted cross-ancestry meta-analyses of data from the Asia Breast Cancer Consortium (ABCC) and the Breast Cancer Association Consortium (BCAC), including 386,696 women (139,523 of Asian ancestry and 247,173 of European ancestry). Furthermore, we performed a transcriptome-wide association study (TWAS) to uncover putative breast cancer susceptibility genes and gain biological insights into the genetics of this common malignancy.

Subjects and methods

Study population

In this study, we conducted a cross-ancestry meta-analysis using data from two large breast cancer genetic research consortia: ABCC and BCAC. All studies were approved by relevant institutional ethical committees. The detailed descriptions of participating studies are described in the supplemental information. In brief, the 133,384 individuals with breast cancer and 113,789 controls of European ancestry included in this analysis were from BCAC, which consisted of three datasets: iCOGS (38,349 individuals with breast cancer and 37,818 controls), OncoArray (80,125 individuals with breast cancer and 58,383 controls), and other GWASs (14,910 individuals with breast cancer and 17,588 controls).[6] For European-ancestry participants, we used summary statistics data generated in BCAC, following the data use agreements. Individuals of Asian ancestry included in this analysis were 27,116 individuals with breast cancer and 112,407 controls recruited by studies in AABC and BCAC (Table S1). Proper informed consent was obtained from all study participants.

Genotyping and quality control

Genotyping and quality control procedures for the contributing studies have been described previously.[5–7,9–11,13–19] After quality control, we imputed all datasets using the 1000 Genomes Project Phase 3 and excluded variants with an imputation quality score (R^2) <0.3. Variants with a minor allele frequency (MAF) of >0.01 in Asian-ancestry datasets or >0.005 in European-ancestry datasets were included for association analyses.

Statistical meta-analyses

Analyses using logistic regression models were performed within each of the ABCC studies, except Biobank Japan project (BBJ2), to estimate the per-allele odds ratio (OR) for each variant using PLINK 2.0.[20] Age and the top two principal components (PCs) were adjusted as covariates. The number of PCs included in the regression was determined by evaluating the Scree plot. Summary statistics were acquired for BBJ2 and BCAC-European dataset. Age and top five PCs were adjusted in BBJ as covariates.[13] The country of contributing studies and the first ten PCs were adjusted in the BCAC-European dataset.[6] A fixed-effects model was used for ancestry-specific meta-analyses and cross-ancestry meta-analyses for risk of overall breast cancer and estrogen receptor (ER) subtypes using METAL.[21] The heterogeneity of risk estimates was evaluated using Cochran's Q statistic and I^2. We estimated the statistical power of our cross-ancestry meta-analyses with α at 5×10^{-8} (Figure S1). We had 80% power to detect a minimum per-allele OR of 1.07, 1.05, 1.04, and 1.03 for variants with a MAF of 0.05, 0.15, 0.20, and 0.30, respectively. In order to take into account of the population heterogeneity, we also used the meta-regression approach implemented in MR-MEGA[22] in cross-ancestry meta-analyses for overall breast cancer. At each risk locus, we performed fine-mapping analysis using SuSiE[23] and constructed a 95% credible set for the lead variant at the locus (detailed methods in supplemental information). We investigated the ancestral heterogeneity of the lead variants and all variants in the credible sets.

Novel risk loci were defined as loci with the sentinel variants located at least 1 Mb away from any of the risk variants identified by previous GWASs included in the NHGRI-EBI GWAS Catalog.[24] For each novel locus, we conducted conditional analyses to identify additional independent signals located flanking ± 500 kb from the lead variant. The GCTA-COJO was used for the conditional analyses. In each iteration of the stepwise conditional analysis, we conducted ancestry-specific conditional analyses and combined the results by a fixed-effects model using METAL. Asian samples (N = 20,554) genotyped by Multi-Ethnic Genotyping

Cancer Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan; [39]Division of Cancer Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [40]Department of Surgery, Seoul National University Hospital, Seoul, South Korea; [41]Department of Medicine, Hanyang University College of Medicine, Seoul, Korea; [42]Department of Surgery, Chonnam National University Medical School, Gwangju, Korea; [43]College of Public Health, China Medical University, Taichong, Taiwan; [44]Taiwan Biobank, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan; [45]Department of Cancer Control Research, British Columbia Cancer Agency, Vancouver, BC, Canada; [46]School of Population and Public Health, University of British Columbia, Vancouver, BC, Canada; [47]Department of Genomic Medicine, Research Institute, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan; [48]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [49]National Institute of Health and Nutrition, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo, Japan; [50]Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China; [51]Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, UK; [52]Department of Surgery, Faculty of Medicine, University Malaya, Kuala Lumpar, Malaysia; [53]Department of Biomedical Sciences, Seoul National University Graduate School, Seoul, Korea; [54]Institute of Environmental Medicine, Seoul National University Medical Research Center, Seoul, Korea; [55]Genomics Center, Centre Hospitalier Universitaire de Québec - Université Laval, Research Center, Québec City, QC, Canada
[56]These authors contributed equally
*Correspondence: wei.zheng@vanderbilt.edu
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Array (MEGA) chips were used as a reference panel for linkage disequilibrium (LD) estimation among women of Asian ancestry. For women of European ancestry, we used 5,000 samples from the Vanderbilt University Medical Center biobank (BioVU) genotyped by MEGA as a reference panel for LD estimation.[25,26] Since the conditional analyses were restricted to local regions of the novel loci identified at genome-wide significance, we used 1×10^{-4} as significance level (adjusting for ~500 comparisons in each locus). If the variant with the lowest conditional p was lower than 1×10^{-4}, it was considered an independent signal at that locus, and it was subsequently adjusted, along with the lead variant, from cross-ancestry meta-analyses in later iterations. This process was repeated until there were no variants with a cross-ancestry conditional $p < 1 \times 10^{-4}$.

Genetic variance explained by novel risk variants

We estimated the genetic variance explained by novel risk variants identified in this study using a log-additive model:

$$\sum_{i}^{n} 2p_i(1 - p_i)(\beta_i^2 - \tau_i^2)$$

where n is the total number of novel risk variants, p_i is the MAF of the ith variant, β_i is the log-OR for the ith variant and τ_i is the standard error of β_i. The explained genetic variance was estimated for overall breast cancer and by ER subtypes for Asian- and European-ancestry populations, respectively.

Transcriptome-wide association analysis

We used RNA sequencing data from 115 samples collected from European-ancestry women from the Genotype-Tissue Expression Project (GTEx, version 8) to build prediction models for each gene expressed in normal breast tissue. Germline genotyping data were obtained using whole-genome sequencing (WGS) of genomic DNA extracted from blood samples. The details of data processing are described in the supplemental information. We used a cross-tissue approach, joint-tissue imputation (JTI), to build prediction models for gene-expression levels in normal breast tissue.[27] Besides breast tissue, data from all 31 other tissues were borrowed in the JTI approach to leverage shared genetic regulation and improve prediction performance in a tissue-dependent manner (Table S10). Prediction models were built using genetic variants within flanking +/− 500 kb from the respective gene boundaries. Five-fold cross-validation was conducted to validate the models internally. Genes with a model prediction $R > 0.1$ were included for association analyses.

To evaluate the performance of prediction models, we performed an external validation using 86 tumor-adjacent normal breast tissue samples from European-ancestry females with breast cancer in The Cancer Genome Atlas (TCGA). We calculated the Spearman's correlation between the prediction performance (R^2) in GTEx and TCGA.

We conducted association analyses of predicted gene expression with breast cancer risk with S-PrediXcan tool,[28] using the summary statistics from our ancestry-specific and cross-ancestry meta-analyses of GWASs for breast cancer. For genes identified at Bonferroni correction in the association analyses, we also conducted TWAS fine-mapping analyses and colocalization analyses. Pathway analyses were conducted for protein-coding genes. The details of statistical analyses were described in supplemental information.

Results

By cross-ancestry meta-analyzing GWAS data from 160,500 individuals with breast cancer and 226,196 controls of Asian and European ancestry using fixed-effects models, we identified 23,461 variants in 184 regions that were associated with overall breast cancer risk at genome-wide significance level ($p < 5.00 \times 10^{-8}$; Table S2). Twenty-seven additional risk loci were uncovered in population-specific analyses, including 25 loci identified in European-specific GWASs and two in Asian-specific GWASs. In total, we identified 211 loci showing a significant association with risk of overall breast cancer. Of them, 16 loci are novel, with the sentinel variants located at least 1 Mb away from any of the risk variants identified by previous GWASs (Table 1).

Analyses by ER status identified 13,392 variants in 100 loci and 2,425 variants in 34 loci that were associated with ER-positive and ER-negative breast cancer, respectively, at the genome-wide significance level (Tables S3 and S4). Two loci for ER-positive and nine loci for ER-negative breast cancer did not overlap with any of the loci identified for overall breast cancer. Of them, 17p13.2, associated with ER-negative breast cancer risk, has not yet been reported in previous GWASs (Table 1).

Of the 222 lead risk variants identified in our study that were associated with the risk of either overall breast cancer (n = 211) or exclusively ER-positive (n = 2) or ER-negative (n = 9) breast cancer, 68 variants showed a significantly different association by ER status at a false discovery rate (FDR) <0.05 in heterogeneity tests (Table S7). Among them, eight risk loci were not reported previously. Except for rs12335941 at 9p21.3, all other seven variants had a stronger association with ER-positive than ER-negative breast cancer. Of the 32 variants showing a different association at a Bonferroni-corrected $p < 2.25 \times 10^{-4}$ (0.05/222, Table 2), five lead variants showed an opposite direction of the association by ER status.

Of the 211 lead risk variants for overall breast cancer, 166 variants had a >25% difference in the effect allele frequency between Asian-ancestry and European-ancestry women (Figure S2). Seventeen lead variants, all identified from ancestry-specific GWASs, are rare (a MAF of <0.01) in one population but common in the other population. For nine of these lead variants, all variants included in their 95% credible sets were rare in one population but common in the other population (Table S2). Of the 194 common risk variants in both populations, 36 showed a significant difference in risk estimates between Asian- and European-ancestry populations at $p < 0.05$, including 31 lead variants with the entire credible sets showing ancestral heterogeneity in risk estimates ($p < 0.05$). Three variants showed ancestral heterogeneity with a $p < 2.58 \times 10^{-4}$, the significance level after adjusting for multiple comparisons (0.05/194) (Table S2). In particular, variant rs59957907 showed a highly significant ancestral difference in risk estimate with a p for heterogeneity of

Table 1. Results for the lead risk variants at 17 novel loci identified in cross-ancestry meta-analyses of GWAS data

Variants	Loci	Nearest gene	Gene region	Alleles[a]	EAF[b]	OR (95% CI)	p[c]	I^2, %	p_het
Overall									
rs727477	2p22.1	*SLC8A1*	Intron	G/T	0.36	0.97 (0.96, 0.98)	2.85×10^{-8}	52.1	0.03
rs3010266	5q13.2	*LINC02056*	8.5 kb from 5′	A/G	0.24	0.96 (0.95, 0.98)	3.56×10^{-8}	0	0.83
rs6890591[d]	5q35.2	*CPEB4*	3.3 kb from 3′	A/T	0.38	0.97 (0.96, 0.98)	3.25×10^{-8}	50.5	0.04
rs3829964	6p21.2	*CDKN1A*	Intron	T/C	0.47	0.97 (0.96, 0.98)	4.61×10^{-9}	0	0.46
rs74392007	6q22.31	*HSF2*	5.4 kb from 5′	T/C	0.12	1.05 (1.03, 1.07)	1.55×10^{-8}	0	0.93
rs3778663	6q27	*AFDN*	Intron	A/G	0.13	1.06 (1.04, 1.07)	8.51×10^{-9}	0	0.69
rs17167576	7p21.2	*AC005019.3*[e]	5.5 kb from 3′	A/T	0.37	1.03 (1.02, 1.04)	6.93×10^{-9}	47.2	0.05
rs3988353	8p22	*PCM1*	Intron	CT/C	0.42	1.03 (1.02, 1.04)	4.32×10^{-8}	0	0.81
rs1937680	10q21.1	*PRKG1*	Intron	C/A	0.36	1.03 (1.02, 1.04)	8.18×10^{-9}	1.3	0.42
rs11354045	11q23.1	*ALG9*	Intron	CT/C	0.35	1.03 (1.02, 1.04)	2.68×10^{-8}	22.3	0.25
rs36028244	11q23.3	*PCSK7*	Intron	C/CTTA	0.07	1.06 (1.04, 1.08)	1.77×10^{-8}	0	1.00
rs3809114	12q13.3	*INHBE*	5′ UTR[f]	G/A	0.47	0.97 (0.96, 0.98)	2.33×10^{-8}	37.8	0.12
rs956006	15q22.2	*TLN2*	Intron	T/C	0.32	1.03 (1.02, 1.05)	3.54×10^{-8}	1.7	0.42
rs4797754	18p11.21	*LDLRAD4*	Intron	G/C	0.31	1.03 (1.02, 1.05)	2.08×10^{-8}	0	0.50
rs112208395	20q11.23	*PHF20*	Intron	C/CT	0.14	1.05 (1.03, 1.07)	4.11×10^{-8}	0	0.96
rs74157632[g]	10q26.11	*DENND10*	Missense	G/A	0.05	0.86 (0.81, 0.90)	1.41×10^{-8}	0	1.00
ER-negative									
rs2123844	17p13.2	*ZZEF1*	Intron	A/C	0.07	1.13 (1.09, 1.18)	2.81×10^{-10}	37.4	0.16

[a]Effect allele/reference allele.
[b]Effect allele frequency.
[c]Unless otherwise specified, p derived from meta-analyses using fixed-effects model.
[d]Identified using cross-ancestry meta-regression (Table S6). The p derived from cross-ancestry fixed-effects model is 1.16×10^{-7} (Table S2).
[e]*AC005019.3* (ENSG00000224330) does not have a gene symbol in HUGO yet.
[f]UTR, untranslated region.
[g]Identified in Asian-specific GWASs. The p for cross-ancestry fixed-effects model is 1.74×10^{-7} (Table S2).

1.27×10^{-104}. Overall, risks estimated in European-ancestry populations are larger than those estimated in Asian-ancestry populations with a regression beta coefficient of 0.579 derived from linear regression (Figure 1, Table S2). The ancestral difference observed in our study could be underestimated, as variants with similar risk estimates were more likely to be identified by cross-ancestry meta-analyses.

Twenty-three previously reported index variants are not located at the regions identified at genome-wide significance in our meta-analyses. However, 16 of them were associated with breast cancer risk at $p < 2.04 \times 10^{-4}$, a significant level with Bonferroni correction for comparisons of 245 index variants. Of the remaining seven index risk variants, four were previously identified in a GWAS by breast cancer intrinsic subtypes[6] (Table S8). Two index variants showed a nominally significant association with breast cancer in cross-ancestry and European-ancestry meta-analyses ($p < 0.05$). Only variant rs9348512 showed a null association with overall breast cancer risk ($p = 0.505$). The association with this variant was originally reported in a GWAS conducted among individuals with *BRCA2* mutation[29] but was not replicated in subsequent studies.[5,6]

The sentinel variants at all 17 newly identified risk loci showed the same association direction in both Asian- and European-ancestry populations (Tables S2 and S4). Except for the Asian-specific risk variant rs74157632, all other lead variants are common, with a MAF >0.01 in both populations. Significant ancestral heterogeneity was observed for rs6890591 (identified by meta-regression) and rs74157632 (identified as Asian specific). The estimated ORs for these 17 lead variants in the BCAC and AABC studies are shown in Table S5. The proportion of variance explained by the 17 novel loci identified in our study was 1.15% for overall breast cancer, 1.07% for ER-positive breast cancer, and 1.03% for ER-negative breast cancer in Asian-ancestry populations. The corresponding numbers are 0.74%, 0.61%, and 1.03% for European-ancestry populations. The higher percentage of genetic variation explained by these new loci in Asian- compared to European-ancestry populations was because of the population differences in the risk estimates at the new loci. Of the 17 novel loci, one locus was specific to the Asian populations. For the remaining 16 loci, the effect size, as measured using OR, was larger in Asian- than in European-ancestry populations for nine loci, including two

Table 2. Results for breast cancer risk loci showing different associations by estrogen receptor status

				ER-Positive		ER-Negative		
Variants	**Loci**	**Allele[a]**	**EAF[b]**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**p for ER heterogeneity**
rs2506885	1p36.22	T/A	0.34	0.95 (0.94, 0.97)	5.91×10^{-10}	0.88 (0.86, 0.90)	3.68×10^{-27}	2.63×10^{-8}
rs11249433	1p11.2	G/A	0.39	1.13 (1.11, 1.15)	3.45×10^{-59}	1.01 (0.99, 1.04)	0.29	1.01×10^{-15}
rs12129456	1q32.1	G/T	0.38	1.02 (1.00, 1.03)	0.03	0.92 (0.90, 0.94)	1.52×10^{-13}	2.00×10^{-13}
rs2169137	1q32.1	G/C	0.25	1.00 (0.98, 1.02)	0.9	1.13 (1.11, 1.16)	4.03×10^{-24}	2.30×10^{-17}
rs56158184	2p23.2	C/T	0.09	1.03 (1.00, 1.05)	0.02	0.89 (0.86, 0.92)	1.01×10^{-9}	1.60×10^{-10}
rs2016394	2q31.1	A/G	0.44	0.94 (0.93, 0.96)	1.05×10^{-16}	1.00 (0.98, 1.02)	0.91	2.51×10^{-6}
rs4442975	2q35	G/T	0.46	1.15 (1.14, 1.17)	1.42×10^{-92}	1.05 (1.03, 1.07)	1.12×10^{-5}	3.72×10^{-14}
rs552647	3p24.1	A/C	0.48	1.12 (1.10, 1.14)	6.35×10^{-60}	1.05 (1.03, 1.07)	4.89×10^{-6}	1.06×10^{-7}
rs7697216	4q34.1	T/C	0.15	0.89 (0.87, 0.91)	1.17×10^{-30}	0.98 (0.96, 1.01)	0.24	1.49×10^{-8}
rs2853669	5p15.33	G/A	0.31	0.96 (0.95, 0.97)	3.29×10^{-8}	0.89 (0.87, 0.91)	3.03×10^{-24}	4.32×10^{-8}
rs7710996	5p12	A/G	0.25	1.00 (0.98, 1.02)	0.97	1.07 (1.04, 1.09)	1.50×10^{-8}	3.84×10^{-6}
rs10941679	5p12	G/A	0.31	1.16 (1.14, 1.18)	5.38×10^{-86}	1.02 (1.00, 1.05)	0.04	1.45×10^{-20}
rs59957907	5q11.2	G/A	0.22	1.19 (1.17, 1.21)	2.95×10^{-90}	1.06 (1.04, 1.09)	2.09×10^{-6}	2.46×10^{-13}
rs60954078	6q25.1	G/A	0.17	1.16 (1.14, 1.19)	1.75×10^{-41}	1.33 (1.29, 1.37)	6.92×10^{-76}	2.18×10^{-12}
rs910416	6q25.1	C/T	0.46	0.95 (0.94, 0.96)	3.23×10^{-13}	0.91 (0.89, 0.93)	1.08×10^{-21}	1.02×10^{-4}
rs116426014	8p23.3	G/A	0.26	1.03 (1.01, 1.04)	0.01	1.09 (1.06, 1.12)	1.83×10^{-10}	1.68×10^{-4}
rs60037937	9q31.2	T/TAA	0.26	1.10 (1.08, 1.11)	7.92×10^{-28}	1.03 (1.00, 1.05)	0.04	1.57×10^{-5}
rs7862747	9q31.2	C/A	0.36	0.88 (0.87, 0.90)	1.89×10^{-58}	0.98 (0.96, 1.00)	0.05	4.49×10^{-13}
rs7098100	10p12.31	A/G	0.34	1.07 (1.06, 1.09)	9.46×10^{-21}	0.97 (0.95, 1.00)	0.02	1.42×10^{-12}
rs9420318	10q26.12	A/G	0.33	0.94 (0.93, 0.95)	2.55×10^{-17}	1.00 (0.98, 1.02)	0.74	6.53×10^{-6}
rs2981579	10q26.13	A/G	0.41	1.32 (1.31, 1.34)	3.72×10^{-359}	1.06 (1.04, 1.08)	4.23×10^{-8}	5.37×10^{-74}
rs78540526	11q13.3	T/C	0.07	1.39 (1.35, 1.42)	3.11×10^{-137}	1.01 (0.97, 1.05)	0.73	1.67×10^{-36}
rs199504893	11q22.3	CA/C	0.41	1.02 (1.00, 1.03)	0.01	0.94 (0.92, 0.96)	3.31×10^{-9}	1.56×10^{-10}
rs1292011	12q24.21	G/A	0.39	0.90 (0.89, 0.92)	3.34×10^{-47}	0.97 (0.95, 0.99)	0	1.05×10^{-7}
rs1744947	14q24.1	T/C	0.15	1.08 (1.06, 1.10)	8.58×10^{-14}	1.00 (0.97, 1.03)	0.82	2.26×10^{-5}
rs4784227	16q12.1	T/C	0.24	1.26 (1.25, 1.28)	1.03×10^{-202}	1.15 (1.13, 1.18)	3.57×10^{-36}	3.21×10^{-11}
rs2123844	17p13.2	A/C	0.07	1.03 (1.00, 1.06)	0.03	1.13 (1.09, 1.18)	2.81×10^{-10}	6.69×10^{-5}
rs745983748	18q11.2	A/AAGTGTT	0.32	0.93 (0.91, 0.94)	6.12×10^{-24}	1.01 (0.99, 1.03)	0.44	3.07×10^{-10}
rs4609972	19p13.11	C/G	0.48	1.00 (0.98, 1.01)	0.80	0.88 (0.86, 0.90)	6.13×10^{-35}	6.60×10^{-24}
rs34753522	20q12	C/T	0.35	0.96 (0.94, 0.97)	3.21×10^{-8}	1.02 (1.00, 1.04)	0.1	8.07×10^{-6}
rs2403907	21q21.1	A/C	0.29	0.91 (0.90, 0.93)	1.09×10^{-32}	0.97 (0.95, 1.00)	0.02	3.14×10^{-6}
rs4822992	22q12.1	A/G	0.02	1.25 (1.19, 1.31)	7.16×10^{-19}	1.00 (0.93, 1.09)	0.91	6.23×10^{-6}

[a]Effect allele/reference allele.
[b]Effect allele frequency.

loci showing a significant difference (p for heterogeneity <0.05). In only two loci, the OR for the lead variant was larger in European- than in Asian-ancestry populations, but no significant heterogeneity was found in either locus. The Asian-specific lead variant rs74157632 (GenBank: NM_207009.4; c.658A>G; p.Asn220Asp) is a missense variant of protein-coding gene *DENND10*, which has been shown to regulate the progression of epidermal growth factor receptor (EGFR) trafficking.[30] Eleven lead variants are located in the intronic regions of genes. Some of these genes have been reported to be involved in breast cancer cell migration and invasion (*SLC8A1*,[31] *CDKN1A*,[32] *AFDN*,[33] *TLN2*[34]), resistance to radiotherapy (*ALG9*[35]), and TGF-β (*LDLRAD4*[36]) or p53 (*PHF20*[37]) signaling pathways.

For each of the novel loci identified in this study, we performed conditional analyses for variants located within 500 kb of the lead variant, adjusted for the lead variant

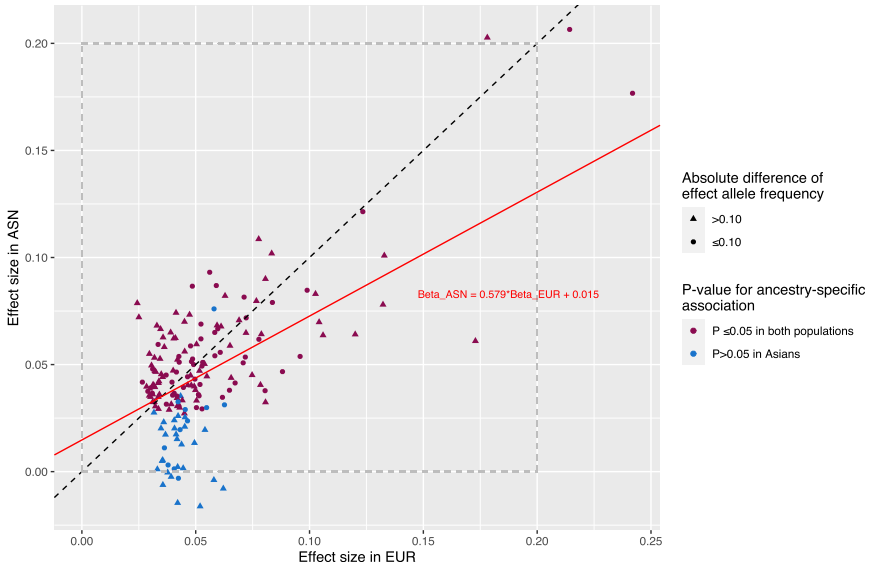

Figure 1. Comparison of risk estimates for lead risk variants between Asian- and European-ancestry women

The red regression line shows the trend of risk estimates in both ancestry groups. To be conservative, the regression was performed excluding four variants with risk estimates >0.15 in European-ancestry women, which could be outliers or with a high leverage. The black dashed diagonal line shows where risk estimates are the same in both ancestries.

separately for Asian and European descendants, to identify potential secondary association signals. These results were then combined by meta-analyses. We found eight independent association signals (conditional $p < 1.0 \times 10^{-4}$) at six loci: 2p22.1, 6q22.31, 6q27, 8p22, 15q22.2, and 18p11.21 (Table S9). There were two additional independent association signals found at loci 8p22 and 18p11.21.

To identify putative breast cancer susceptibility genes, we conducted a transcriptome-wide association analysis (TWAS). We used whole-genome sequencing data generated in genomic DNA samples and RNA sequencing data generated in normal tissues obtained from 115 individuals included in the GTEx project (version 8) to build genetic models to predict gene expression across the transcriptome (Material and methods, Table S10). Of the 30,362 genes evaluated, models were successfully built for 17,127 genes, in which 10,820 genes could be predicted with $R > 0.1$. The performance of the models was evaluated using the adjacent normal breast tissue samples from TCGA. Overall, genes that were predicted with $R > 0.1$ in GTEx data were also predicted well in TCGA tumor-adjacent normal tissue data (correlation coefficient of 0.69; Figure S3).

Of the 10,820 genes evaluated using GWAS data from 160,500 individuals with breast cancer and 226,196 controls, we identified 137 genes in association with risk of breast cancer at the Bonferroni-corrected threshold of $p < 4.62 \times 10^{-6}$, including 76 protein-coding genes (Tables S11 and S18). Of them, 14 genes at 13 loci are located at least 1 Mb away from any of the previous GWAS-identified risk variants for breast cancer (Table 3), including 11 genes associated with overall breast cancer risk and three additional genes associated with ER-positive breast cancer. *CPNE1* is located at a novel risk locus identified in our cross-ancestry meta-analyses. *CPNE1* has been reported to be overexpressed in triple-negative breast cancer and promotes tumorigenesis and radio-resistance by the AKT signaling pathway.[38] In addition, we also identified 87 genes (including 39 protein-coding genes) that are located in known risk loci but have not yet been reported in previous TWASs[39,40–42] (Table S11).

Of the 137 genes identified by TWAS, 15 genes showed different associations with ER-positive and ER-negative breast cancer, with a p for heterogeneity $<3.65 \times 10^{-4}$ (0.05/137; Tables 4 and S12). Of them, protein-coding genes *ABHD8* and *ANKLE1* at 19p13.11 showed an exclusive association with ER-negative breast cancer, and similar heterogeneity also was found for the lead variant rs4808616 at this risk locus. These findings were supported by a previous study, which identified *ABHD8* and *ANKLE1* as potential target genes at the risk locus 19p13.11.[43]

In addition, 16 genes showed a significantly different association between Asian- and European-ancestry women at the Bonferroni-corrected threshold p for heterogeneity $<3.65 \times 10^{-4}$, including seven protein-coding genes (Table S13). Of them, *CASP8* and *ALS2CR12* at 2q33.1 and *HLA-F* at 6p22.1 showed a stronger association with breast cancer risk in Asian-ancestry women than in European-ancestry women. The *CASP8* gene plays a central role in extrinsic apoptosis[44] and has been reported to be associated with breast cancer risk in previous TWASs among European-ancestry women.[39,40–42]

To identify the most likely target genes in the locus in which multiple genes were found to be associated with breast cancer risk in TWASs, we performed fine-mapping analyses using FOCUS.[45] In total, we identified 69 genes showing significant posterior inclusion probability and thus included them in the credible target gene sets (Table S14). In addition, we identified 50 genes that were colocalized with both GWASs and eQTL signals from colocalization analyses using COLOC[46] (Table S15), including 28 genes included in the credible target gene sets from TWAS fine-mapping analyses.

We performed pathway analyses to identify biological pathways that may play a role in breast cancer etiology. Of the 137 genes identified in our TWASs in association with breast cancer risk, 76 located in 53 genomic regions are protein-coding genes. In 47 regions, we were able to identify 53 genes as putative target genes with supporting

Table 3. Genes identified in TWASs in novel loci in association with breast cancer risk

Loci[a]	Gene	Gene type	*Z* score	p	R^{2}[b]
Overall					
1p11.2	*NBPF8*	Pseudogene	7.05	1.76×10^{-12}	0.23
1p11.2	*PFN1P2*	Pseudogene	9.22	2.87×10^{-20}	0.22
3p21.31	*RNF123*	Protein coding	4.63	3.62×10^{-6}	0.26
5p15.31	*NSUN2*	Protein coding	−4.89	1.01×10^{-6}	0.37
10q26.13	*EEF1AKMT2*	Protein coding	−4.70	2.63×10^{-6}	0.34
15q15.1	*SRP14-DT*	LincRNA	−4.80	1.55×10^{-6}	0.29
15q15.3	*STRCP1*	Pseudogene	−4.66	3.18×10^{-6}	0.12
17p12	*MAP2K4*	Protein coding	4.99	6.06×10^{-7}	0.02
19q13.12	*ZNF793-AS1*	Antisense RNA	−4.94	7.64×10^{-7}	0.10
20q11.22	*CPNE1*	Protein coding	−4.68	2.88×10^{-6}	0.38
20q13.33[c]	*RGS19*	Protein coding	4.64	3.47×10^{-6}	0.07
ER-positive					
6p22.1	*H4C12*	Protein coding	5.01	5.54×10^{-7}	0.07
11q13.2	*RHOD*	Protein coding	4.78	1.73×10^{-6}	0.19
5q13.2[c]	*GUSBP14*	Pseudogene	5.08	3.73×10^{-7}	0.08

[a]Unless otherwise specified, results are based on TWAS analyses using cross-ancestry GWAS data.
[b]Prediction performance derived using GTEx data.
[c]Genes identified from association analysis using European-ancestry GWAS data.

evidence from either fine-mapping analyses (n = 25), co-localization analyses (n = 10), or both (n = 18). Additionally, for the remaining 152 loci, in which no target genes were identified in TWASs, we selected 89 protein-coding genes previously reported as putative target genes[47] and 79 protein-coding genes located nearby the lead variants identified in our GWAS. In total, 221 putative risk genes for breast cancer were included in our pathway analysis (supplemental methods and Table S16). We identified multiple signaling pathways that were significantly associated with breast cancer risk at FDR <0.05, including p53, cGMP-PKG, TNF, and MAPK signaling pathways, as well as pathways of DNA-binding transcription activator activity and cell cycle phase transition[48–50] (Table S17).

Discussion

We conducted a large GWAS and TWAS of breast cancer, including 386,696 women of Asian and European ancestry. In total, 222 genetic risk loci and 137 genes were identified by GWAS and TWAS, respectively, in association with breast cancer risk after adjusting for multiple comparisons.

Our pathway analyses identified multiple biological pathways that have been implicated in the development of breast and other cancers. For example, *CACNA1A*, *DUSP4*, *FGFR2*, *MAP2K4*, *MAP3K1*, *MYC*, *NF1*, *PLA2G6*, *TAB2*, *TGFBR2*, and *TP53* are involved in mitogen-activated protein kinase (MAPK) signaling pathway.[48,51] *ATG10*, *CDKAL1*, *KLF4*, *MAF8*, and *MAP3K1* are regulated by the activation of *KRAS*.[51] *KRAS* is a proto-oncogene from the *RAS* family and a part of the RAS/MAPK pathway. Although the RAS signaling pathway is commonly activated in breast cancer, somatic mutations of *RAS* are not common in individuals with breast cancer.[52] Our findings indicate that the germline alternation of genes involved in the RAS signaling pathway could play a role in the development and progression of breast cancer.

Although the p53 pathway is often altered in breast cancer tissues, particularly those from ER-negative and triple-negative cancer, germline mutations of *TP53* are detected only in less than 1% of individuals with breast cancer.[53] In this study, we found that 15 genes (*CASP8*, *CCND1*, *CCNE1*, *CDKN1A*, *CHEK2*, *MDM4*, *INHBB*, *KLF4*, *MXD1*, *PHLDA3*, *PIDD1*, *TNNI1*, *TP53*, *ZFP36L1*, *ZNF365*) are involved in the p53 signaling pathway,[48,51] providing support that germline alterations of this pathway could play a more significant etiologic role than what is appreciated based on analyzing *TP53* alone. Intriguingly, the *MDM4* and *CCNE1* are located at risk loci with a stronger association with ER-negative than ER-positive breast cancer. Our TWAS also found that the expression of *MDM4* was exclusively associated with an increased risk of ER-negative breast cancer. These findings suggest that the p53 signaling pathway plays an important role in the risk of breast cancer, especially ER-negative breast cancer.

By increasing the sample size and incorporating transcriptome data, we were able to identify 30 novel associations in loci and genes that are located >1 Mb away from any of the previously reported breast cancer risk variants.

Table 4. TWAS-identified breast cancer risk genes showing a significantly different association by estrogen receptor status

			ER-Positive		ER-Negative		
Loci	**Gene**	**Gene type**	***Z* score**	**P**	***Z* score**	**p**	**p for ER heterogeneity**
1p11.2	*SRGAP2C*	Protein coding	−9.45	$3.32'10^{-21}$	−1.47	0.14	$6.99'10^{-5}$
1p11.2	*H3P4*	Pseudogene	8.89	$6.05'10^{-19}$	1.10	0.27	$1.72'10^{-4}$
1p11.2	*RP11-343N15.2*[a]	LincRNA	−8.74	$2.27'10^{-18}$	−1.00	0.32	$3.35'10^{-5}$
1p11.2	*EMBP1*	Pseudogene	−8.38	$5.23'10^{-17}$	−0.27	0.78	$9.32'10^{-6}$
1p36.13	*KLHDC7A*	Protein coding	−7.10	$1.27'10^{-12}$	0.10	0.92	$5.79'10^{-6}$
1p36.22	*DFFA*	Protein coding	4.37	$1.26'10^{-5}$	7.60	$2.96'10^{-14}$	$9.54'10^{-5}$
1q22	*GBAP1*	Pseudogene	−6.66	$2.73'10^{-11}$	0.59	0.56	$2.54'10^{-5}$
1q22	*THBS3*	Protein coding	5.72	$1.07'10^{-8}$	−0.89	0.38	$8.72'10^{-5}$
1q32.1	*PTPRVP*	Pseudogene	−1.50	0.14	6.67	$2.52'10^{-11}$	$1.36'10^{-10}$
2q35	*TNP1*	Protein coding	5.85	$5.04'10^{-9}$	−0.37	0.71	$5.44'10^{-5}$
5p12	*MRPS30-DT*	Antisense RNA	16.38	$2.48'10^{-60}$	−0.15	0.88	$4.20'10^{-21}$
5q11.2	*CTD-2310F14.1*[a]	Antisense RNA	14.50	$1.17'10^{-47}$	3.73	$1.90'10^{-4}$	$4.24'10^{-7}$
8p23.3	*SEPT14P8*	Pseudogene	−2.29	0.02	−6.00	$1.98'10^{-9}$	$2.53'10^{-4}$
19p13.11	*ABHD8*	Protein coding	−0.51	0.61	9.64	$5.25'10^{-22}$	$2.39'10^{-15}$
19p13.11	*ANKLE1*	Protein coding	−0.24	0.81	6.74	$1.62'10^{-11}$	$8.17'10^{-9}$

[a]*RP11-343N15.2* (ENSG00000231429) and *CTD-2310F14.1* (ENSG00000271828) do not have gene symbols in HUGO yet.

The discovery of these novel associations further expanded our understanding of the genetic and biological mechanism of breast cancer development. For example, the lead variant at the novel risk locus 6p21.2 is located at the intronic region of *CDKN1A*. *CDKN1A* regulates cell-cycle progression as a cyclin-dependent kinase inhibitor[32] and plays an important role in both PI3K/AKT signaling pathway and p53 pathway.[51]

MAP2K4 at 17p12 is a novel target gene identified by our TWAS. This gene encodes a member of the mitogen-activated protein kinase and it is involved in multiple signaling pathways, including MAPK pathway, EGF pathway, FAS signaling pathway,[51] and PI3K/AKT signaling pathway.[54] In addition, our TWAS identified 39 protein-coding genes that are located in known risk loci but have not yet been reported in previous TWAS. Of them, *MDM4*, *PLA2G6*, and *RIT1* are involved in the p53 pathway, RAS/MAPK pathway, and PI3K/AKT pathway, respectively. These newly identified putative breast cancer risk genes could be potential targets for therapies.

Given the much larger sample size for GWASs conducted in European descendants compared to those conducted in East Asians, many of the associations were driven by data from European-ancestry GWASs. Increasing the sample size for GWASs of non-European populations will be valuable to fully uncover the genetic basis for breast cancer. In our TWAS, we built gene prediction models using European-ancestry samples from GTEx. Given the difference in genetic architectures between Asian and European descendants, some of these models may not perform well in TWASs in Asian populations, affecting the detection of significant association signals, particularly in regions where significant ancestral differences exist. Using Asian-specific gene prediction models in future studies should help to identify additional genes associated with breast cancer risk.

In summary, in this large GWAS and TWAS for breast cancer, we uncovered a large number of genetic variants associated with breast cancer risk and identified potential target genes for this common cancer. We discovered significant differences for many of these variants and genes in association with breast cancer risk by ER status and ancestry. We identified multiple signaling pathways that play an etiologic role in breast cancer risk and propose that germline alterations in *TP53, RAS*, and *MAPK* pathways may play a more significant role in the etiology of breast cancer than what is currently appreciated. Our study provides substantial insights into the genetics and biology of breast cancer.

Data and code availability

Access to the ABCC data can be requested by submission of an inquiry to Dr. Wei Zheng (wei.zheng@vanderbilt.edu). Request for access to the BCAC data can be submitted directly to BCAC (http://bcac.ccge.medschl.cam.ac.uk/). All GTEx data are publicly available through dbGaP: phs000424.v8.p2. TCGA data are publicly available through National Cancer Institute's Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov/). Access to the custom code: https://github.com/pingjie/EURASN_GWAS/.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2022.10.011.

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Declaration of interests

The authors declare no competing interests.

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References

1. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J. Clin. *71*, 209–249. https://doi.org/10.3322/caac.21660.
2. Hu, C., Hart, S.N., Gnanaolivu, R., Huang, H., Lee, K.Y., Na, J., Gao, C., Lilyquist, J., Yadav, S., Boddicker, N.J., et al. (2021). A population-based study of genes previously implicated in breast cancer. N. Engl. J. Med. *384*, 440–451. https://doi.org/10.1056/NEJMoa2005936.
3. Breast Cancer Association Consortium, Dorling, L., Carvalho, S., Allen, J., González-Neira, A., Luccarini, C., Wahlström, C., Pooley, K.A., Parsons, M.T., Fortuno, C., et al. (2021). Breast cancer risk genes - association analysis in more than 113, 000 women. N. Engl. J. Med. *384*, 428–439. https://doi.org/10.1056/NEJMoa1913948.
4. Narod, S.A. (2021). Which genes for hereditary breast cancer? N. Engl. J. Med. *384*, 471–473. https://doi.org/10.1056/NEJMe2035083.
5. Michailidou, K., Lindström, S., Dennis, J., Beesley, J., Hui, S., Kar, S., Lemaçon, A., Soucy, P., Glubb, D., Rostamianfar, A., et al. (2017). Association analysis identifies 65 new breast cancer risk loci. Nature *551*, 92–94. https://doi.org/10.1038/nature24284.
6. Zhang, H., Ahearn, T.U., Lecarpentier, J., Barnes, D., Beesley, J., Qi, G., Jiang, X., O'Mara, T.A., Zhao, N., Bolla, M.K., et al. (2020). Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. Nat. Genet. *52*, 572–581. https://doi.org/10.1038/s41588-020-0609-2.
7. Shu, X., Long, J., Cai, Q., Kweon, S.-S., Choi, J.-Y., Kubo, M., Park, S.K., Bolla, M.K., Dennis, J., Wang, Q., et al. (2020). Identification of novel breast cancer susceptibility loci in meta-analyses conducted among Asian and European descendants. Nat. Commun. *11*, 1217. https://doi.org/10.1038/s41467-020-15046-w.
8. Martin, A.R., Kanai, M., Kamatani, Y., Okada, Y., Neale, B.M., and Daly, M.J. (2019). Clinical use of current polygenic risk scores may exacerbate health disparities. Nat. Genet. *51*, 584–591. https://doi.org/10.1038/s41588-019-0379-x.
9. 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. https://doi.org/10.1038/ng.318.
10. Cai, Q., Zhang, B., Sung, H., Low, S.-K., Kweon, S.-S., Lu, W., Shi, J., Long, J., Wen, W., Choi, J.-Y., et al. (2014). Genome-wide association analysis in East Asians identifies breast cancer susceptibility loci at 1q32.1, 5q14.3 and 15q26.1. Nat. Genet. *46*, 886–890. https://doi.org/10.1038/ng.3041.
11. Zheng, W., Zhang, B., Cai, Q., Sung, H., Michailidou, K., Shi, J., Choi, J.-Y., Long, J., Dennis, J., Humphreys, M.K., et al. (2013). Common genetic determinants of breast-cancer risk in East Asian women: a collaborative study of 23 637 breast cancer cases and 25 579 controls. Hum. Mol. Genet. *22*, 2539–2550. https://doi.org/10.1093/hmg/ddt089.
12. Yang, Y., Tao, R., Shu, X., Cai, Q., Wen, W., Gu, K., Gao, Y.-T., Zheng, Y., Kweon, S.-S., Shin, M.-H., et al. (2022). Incorporating polygenic risk scores and nongenetic risk factors for breast cancer risk prediction among asian women. JAMA Netw. Open *5*. e2149030. https://doi.org/10.1001/jamanetworkopen.2021.49030.
13. Ishigaki, K., Akiyama, M., Kanai, M., Takahashi, A., Kawakami, E., Sugishita, H., Sakaue, S., Matoba, N., Low, S.-K., Okada, Y., et al. (2020). Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. Nat. Genet. *52*, 669–679. https://doi.org/10.1038/s41588-020-0640-3.
14. Michailidou, K., Beesley, J., Lindstrom, S., Canisius, S., Dennis, J., Lush, M.J., Maranian, M.J., Bolla, M.K., Wang, Q., Shah, M., et al. (2015). Genome-wide association analysis of more than 120, 000 individuals identifies 15 new susceptibility loci for breast cancer. Nat. Genet. *47*, 373–380. https://doi.org/10.1038/ng.3242.
15. Cai, Q., Long, J., Lu, W., Qu, S., Wen, W., Kang, D., Lee, J.-Y., Chen, K., Shen, H., Shen, C.-Y., et al. (2011). Genome-wide association study identifies breast cancer risk variant at 10q21.2: results from the Asia Breast Cancer Consortium. Hum. Mol. Genet. *20*, 4991–4999. https://doi.org/10.1093/hmg/ddr405.
16. Long, J., Cai, Q., Sung, H., Shi, J., Zhang, B., Choi, J.-Y., Wen, W., Delahanty, R.J., Lu, W., Gao, Y.-T., et al. (2012). Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. PLoS Genet. *8*. e1002532. https://doi.org/10.1371/journal.pgen.1002532.
17. Han, M.-R., Long, J., Choi, J.-Y., Low, S.-K., Kweon, S.-S., Zheng, Y., Cai, Q., Shi, J., Guo, X., Matsuo, K., et al. (2016). Genome-wide association study in East Asians identifies two novel breast cancer susceptibility loci. Hum. Mol. Genet. *25*, 3361–3371. https://doi.org/10.1093/hmg/ddw164.
18. Zhang, Y., Long, J., Lu, W., Shu, X.-O., Cai, Q., Zheng, Y., Li, C., Li, B., Gao, Y.-T., and Zheng, W. (2014). Rare coding variants and breast cancer risk: evaluation of susceptibility Loci

identified in genome-wide association studies. Cancer Epidemiol. Biomarkers Prev. *23*, 622–628. https://doi.org/10.1158/1055-9965.EPI-13-1043.

19. Kim, H.c., Lee, J.-Y., Sung, H., Choi, J.-Y., Park, S.K., Lee, K.-M., Kim, Y.J., Go, M.J., Li, L., Cho, Y.S., et al. (2012). A genome-wide association study identifies a breast cancer risk variant in ERBB4 at 2q34: results from the Seoul breast cancer study. Breast Cancer Res. *14*, R56. https://doi.org/10.1186/bcr3158.
20. Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., and Lee, J.J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience *4*, 7. https://doi.org/10.1186/s13742-015-0047-8.
21. Willer, C.J., Li, Y., and Abecasis, G.R. (2010). METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics *26*, 2190–2191. https://doi.org/10.1093/bioinformatics/btq340.
22. Mägi, R., Horikoshi, M., Sofer, T., Mahajan, A., Kitajima, H., Franceschini, N., McCarthy, M.I., COGENT-Kidney Consortium T2D-GENES Consortium, Morris, A.P., and Morris, A.P. (2017). Trans-ethnic meta-regression of genome-wide association studies accounting for ancestry increases power for discovery and improves fine-mapping resolution. Hum. Mol. Genet. *26*, 3639–3650. https://doi.org/10.1093/hmg/ddx280.
23. Wang, G., Sarkar, A., Carbonetto, P., and Stephens, M. (2020). A simple new approach to variable selection in regression, with application to genetic fine mapping. J. Roy. Stat. Soc. B *82*, 1273–1300. https://doi.org/10.1111/rssb.12388.
24. Buniello, A., MacArthur, J.A.L., Cerezo, M., Harris, L.W., Hayhurst, J., Malangone, C., McMahon, A., Morales, J., Mountjoy, E., Sollis, E., et al. (2019). The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res. *47*, D1005–D1012. https://doi.org/10.1093/nar/gky1120.
25. Roden, D.M., Pulley, J.M., Basford, M.A., Bernard, G.R., Clayton, E.W., Balser, J.R., and Masys, D.R. (2008). Development of a large-scale De-identified DNA biobank to enable personalized medicine. Clin. Pharmacol. Ther. *84*, 362–369. https://doi.org/10.1038/clpt.2008.89.
26. Kasimatis, K.R., Abraham, A., Ralph, P.L., Kern, A.D., Capra, J.A., and Phillips, P.C. (2021). Evaluating human autosomal loci for sexually antagonistic viability selection in two large biobanks. Genetics *217*, 1–10. https://doi.org/10.1093/genetics/iyaa015.
27. Zhou, D., Jiang, Y., Zhong, X., Cox, N.J., Liu, C., and Gamazon, E.R. (2020). A unified framework for joint-tissue transcriptome-wide association and Mendelian randomization analysis. Nat. Genet. *52*, 1239–1246. https://doi.org/10.1038/s41588-020-0706-2.
28. Barbeira, A.N., Dickinson, S.P., Bonazzola, R., Zheng, J., Wheeler, H.E., Torres, J.M., Torstenson, E.S., Shah, K.P., Garcia, T., Edwards, T.L., et al. (2018). Exploring the phenotypic consequences of tissue specific gene expression variation inferred from GWAS summary statistics. Nat. Commun. *9*, 1825. https://doi.org/10.1038/s41467-018-03621-1.
29. Gaudet, M.M., Kuchenbaecker, K.B., Vijai, J., Klein, R.J., Kirchhoff, T., McGuffog, L., Barrowdale, D., Dunning, A.M., Lee, A., Dennis, J., et al. (2013). Identification of a BRCA2-specific modifier locus at 6p24 related to breast cancer risk. PLoS Genet. *9*. e1003173. https://doi.org/10.1371/journal.pgen.1003173.
30. Zhang, J., Zhang, K., Qi, L., Hu, Q., Shen, Z., Liu, B., Deng, J., Zhang, C., and Zhang, Y. (2019). DENN domain-containing protein FAM45A regulates the homeostasis of late/multivesicular endosomes. Biochim. Biophys. Acta Mol. Cell Res. *1866*, 916–929. https://doi.org/10.1016/j.bbamcr.2019.02.006.
31. Zhu, Q., Zhang, X., Zai, H.-Y., Jiang, W., Zhang, K.-J., He, Y.-Q., and Hu, Y. (2021). circSLC8A1 sponges miR-671 to regulate breast cancer tumorigenesis via PTEN/PI3k/Akt pathway. Genomics *113*, 398–410. https://doi.org/10.1016/j.ygeno.2020.12.006.
32. Zaremba-Czogalla, M., Hryniewicz-Jankowska, A., Tabola, R., Nienartowicz, M., Stach, K., Wierzbicki, J., Cirocchi, R., Ziolkowski, P., Tabaczar, S., and Augoff, K. (2018). A novel regulatory function of CDKN1A/p21 in TNFα-induced matrix metalloproteinase 9-dependent migration and invasion of triple-negative breast cancer cells. Cell. Signal. *47*, 27–36. https://doi.org/10.1016/j.cellsig.2018.03.010.
33. Fournier, G., Cabaud, O., Josselin, E., Chaix, A., Adélaïde, J., Isnardon, D., Restouin, A., Castellano, R., Dubreuil, P., Chaffanet, M., et al. (2011). Loss of AF6/afadin, a marker of poor outcome in breast cancer, induces cell migration, invasiveness and tumor growth. Oncogene *30*, 3862–3874. https://doi.org/10.1038/onc.2011.106.
34. Li, L., Li, X., Qi, L., Rychahou, P., Jafari, N., and Huang, C. (2017). The role of talin2 in breast cancer tumorigenesis and metastasis. Oncotarget *8*, 106876–106887. https://doi.org/10.18632/oncotarget.22449.
35. Sun, X., He, Z., Guo, L., Wang, C., Lin, C., Ye, L., Wang, X., Li, Y., Yang, M., Liu, S., et al. (2021). ALG3 contributes to stemness and radioresistance through regulating glycosylation of TGF-β receptor II in breast cancer. J. Exp. Clin. Cancer Res. *40*, 149. https://doi.org/10.1186/s13046-021-01932-8.
36. Nakano, N., Maeyama, K., Sakata, N., Itoh, F., Akatsu, R., Nakata, M., Katsu, Y., Ikeno, S., Togawa, Y., Vo Nguyen, T.T., et al. (2014). C18 ORF1, a novel negative regulator of transforming growth factor-β signaling. J. Biol. Chem. *289*, 12680–12692. https://doi.org/10.1074/jbc.M114.558981.
37. Cui, G., Park, S., Badeaux, A.I., Kim, D., Lee, J., Thompson, J.R., Yan, F., Kaneko, S., Yuan, Z., Botuyan, M.V., et al. (2012). PHF20 is an effector protein of p53 double lysine methylation that stabilizes and activates p53. Nat. Struct. Mol. Biol. *19*, 916–924. https://doi.org/10.1038/nsmb.2353.
38. Shao, Z., Ma, X., Zhang, Y., Sun, Y., Lv, W., He, K., Xia, R., Wang, P., and Gao, X. (2020). CPNE1 predicts poor prognosis and promotes tumorigenesis and radioresistance via the AKT singling pathway in triple-negative breast cancer. Mol. Carcinog. *59*, 533–544. https://doi.org/10.1002/mc.23177.
39. Wu, L., Shi, W., Long, J., Guo, X., Michailidou, K., Beesley, J., Bolla, M.K., Shu, X.-O., Lu, Y., Cai, Q., et al. (2018). A transcriptome-wide association study of 229, 000 women identifies new candidate susceptibility genes for breast cancer. Nat. Genet. *50*, 968–978. https://doi.org/10.1038/s41588-018-0132-x.
40. Ferreira, M.A., Gamazon, E.R., Al-Ejeh, F., Aittomäki, K., Andrulis, I.L., Anton-Culver, H., Arason, A., Arndt, V., Aronson, K.J., Arun, B.K., et al. (2019). Genome-wide association and transcriptome studies identify target genes and risk loci for breast cancer. Nat. Commun. *10*, 1741. https://doi.org/10.1038/s41467-018-08053-5.
41. Lawrenson, K., Kar, S., McCue, K., Kuchenbaeker, K., Michailidou, K., Tyrer, J., Beesley, J., Ramus, S.J., Li, Q., Delgado, M.K., et al. (2016). Functional mechanisms underlying pleiotropic risk alleles at the 19p13.1 breast-ovarian cancer susceptibility

locus. Nat. Commun. *7*, 12675. https://doi.org/10.1038/ncomms12675.

42. Fritsch, M., Günther, S.D., Schwarzer, R., Albert, M.-C., Schorn, F., Werthenbach, J.P., Schiffmann, L.M., Stair, N., Stocks, H., Seeger, J.M., et al. (2019). Caspase-8 is the molecular switch for apoptosis, necroptosis and pyroptosis. Nature *575*, 683–687. https://doi.org/10.1038/s41586-019-1770-6.
43. Wen, W., Chen, Z., Bao, J., Long, Q., Shu, X.-O., Zheng, W., and Guo, X. (2021). Genetic variations of DNA bindings of FOXA1 and co-factors in breast cancer susceptibility. Nat. Commun. *12*, 5318. https://doi.org/10.1038/s41467-021-25670-9.
44. Feng, H., Gusev, A., Pasaniuc, B., Wu, L., Long, J., Abu-Full, Z., Aittomäki, K., Andrulis, I.L., Anton-Culver, H., Antoniou, A.C., et al. (2020). Transcriptome-wide association study of breast cancer risk by estrogen-receptor status. Genet. Epidemiol. *44*, 442–468. https://doi.org/10.1002/gepi.22288.
45. Mancuso, N., Freund, M.K., Johnson, R., Shi, H., Kichaev, G., Gusev, A., and Pasaniuc, B. (2019). Probabilistic fine-mapping of transcriptome-wide association studies. Nat. Genet. *51*, 675–682. https://doi.org/10.1038/s41588-019-0367-1.
46. Giambartolomei, C., Vukcevic, D., Schadt, E.E., Franke, L., Hingorani, A.D., Wallace, C., and Plagnol, V. (2014). Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. PLoS Genet. *10*. e1004383. https://doi.org/10.1371/journal.pgen.1004383.
47. Fachal, L., Aschard, H., Beesley, J., Barnes, D.R., Allen, J., Kar, S., Pooley, K.A., Dennis, J., Michailidou, K., Turman, C., et al. (2020). Fine-mapping of 150 breast cancer risk regions identifies 191 likely target genes. Nat. Genet. *52*, 56–73. https://doi.org/10.1038/s41588-019-0537-1.
48. Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. *28*, 27–30. https://doi.org/10.1093/nar/28.1.27.
49. Gene Ontology Consortium (2021). The Gene ontology resource: enriching a GOld mine. Nucleic Acids Res. *49*, D325–D334. https://doi.org/10.1093/nar/gkaa1113.
50. Ashburner, M., Ball, C.A., Blake, J.A., Botstein, D., Butler, H., Cherry, J.M., Davis, A.P., Dolinski, K., Dwight, S.S., Eppig, J.T., et al. (2000). Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat. Genet. *25*, 25–29. https://doi.org/10.1038/75556.
51. Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J.P., and Tamayo, P. (2015). The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. *1*, 417–425. https://doi.org/10.1016/j.cels.2015.12.004.
52. Galiè, M. (2019). RAS as supporting actor in breast cancer. Front. Oncol. *9*, 1199.
53. Schon, K., and Tischkowitz, M. (2018). Clinical implications of germline mutations in breast cancer: TP53. Breast Cancer Res. Treat. *167*, 417–423. https://doi.org/10.1007/s10549-017-4531-y.
54. Liu, S., Huang, J., Zhang, Y., Liu, Y., Zuo, S., and Li, R. (2019). MAP2K4 interacts with Vimentin to activate the PI3K/AKT pathway and promotes breast cancer pathogenesis. Aging (Albany NY) *11*, 10697–10710. https://doi.org/10.18632/aging.102485.

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Supplemental information

Genome- and transcriptome-wide association studies of 386,000 Asian and European-ancestry women provide new insights into breast cancer genetics

Guochong Jia, Jie Ping, Xiang Shu, Yaohua Yang, Qiuyin Cai, Sun-Seog Kweon, Ji-Yeob Choi, Michiaki Kubo, Sue K. Park, Manjeet K. Bolla, Joe Dennis, Qin Wang, Xingyi Guo, Bingshan Li, Ran Tao, Kristan J. Aronson, Tsun L. Chan, Yu-Tang Gao, Mikael Hartman, Weang Kee Ho, Hidemi Ito, Motoki Iwasaki, Hiroji Iwata, Esther M. John, Yoshio Kasuga, Mi-Kyung Kim, Allison W. Kurian, Ava Kwong, Jingmei Li, Artitaya Lophatananon, Siew-Kee Low, Shivaani Mariapun, Koichi Matsuda, Keitaro Matsuo, Kenneth Muir, Dong-Young Noh, Boyoung Park, Min-Ho Park, Chen-Yang Shen, Min-Ho Shin, John J. Spinelli, Atsushi Takahashi, Chiuchen Tseng, Shoichiro Tsugane, Anna H. Wu, Taiki Yamaji, Ying Zheng, Alison M. Dunning, Paul D.P. Pharoah, Soo-Hwang Teo, Daehee Kang, Douglas F. Easton, Jacques Simard, Xiao-ou Shu, Jirong Long, and Wei Zheng

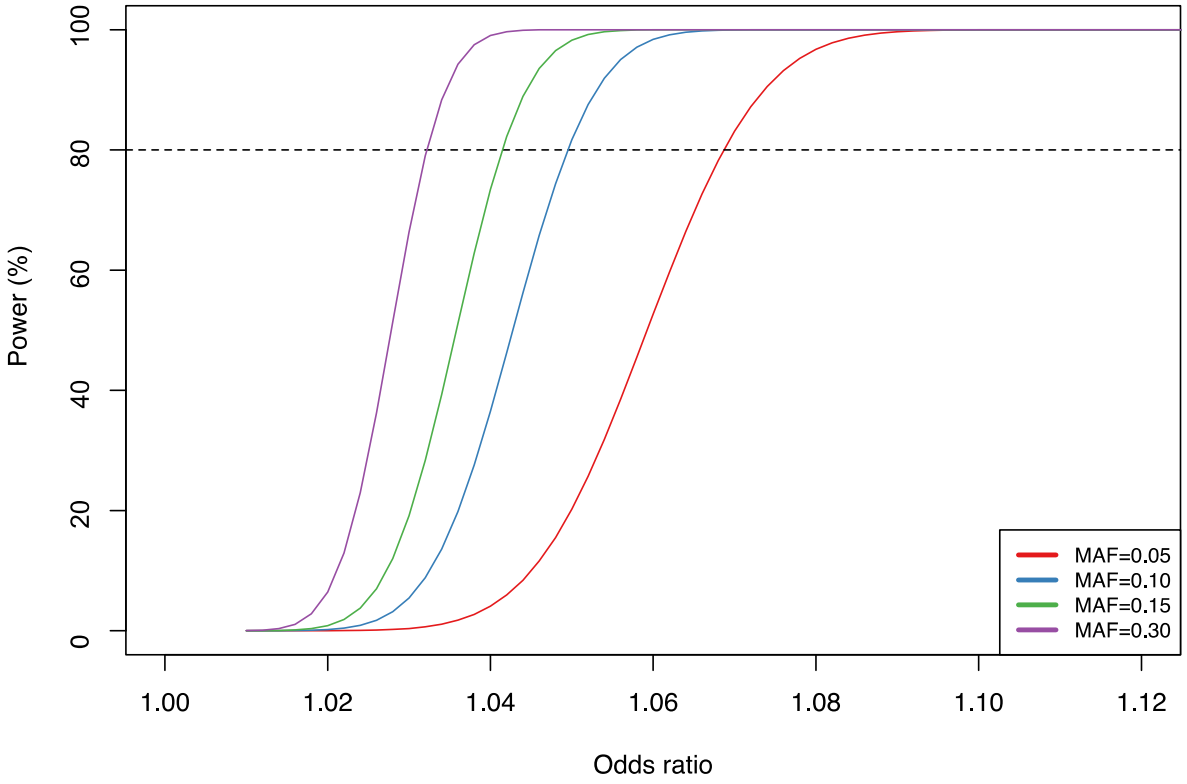

Figure S1. Estimated power of cross-ancestry meta-analysis using samples from ABCC and BCAC.

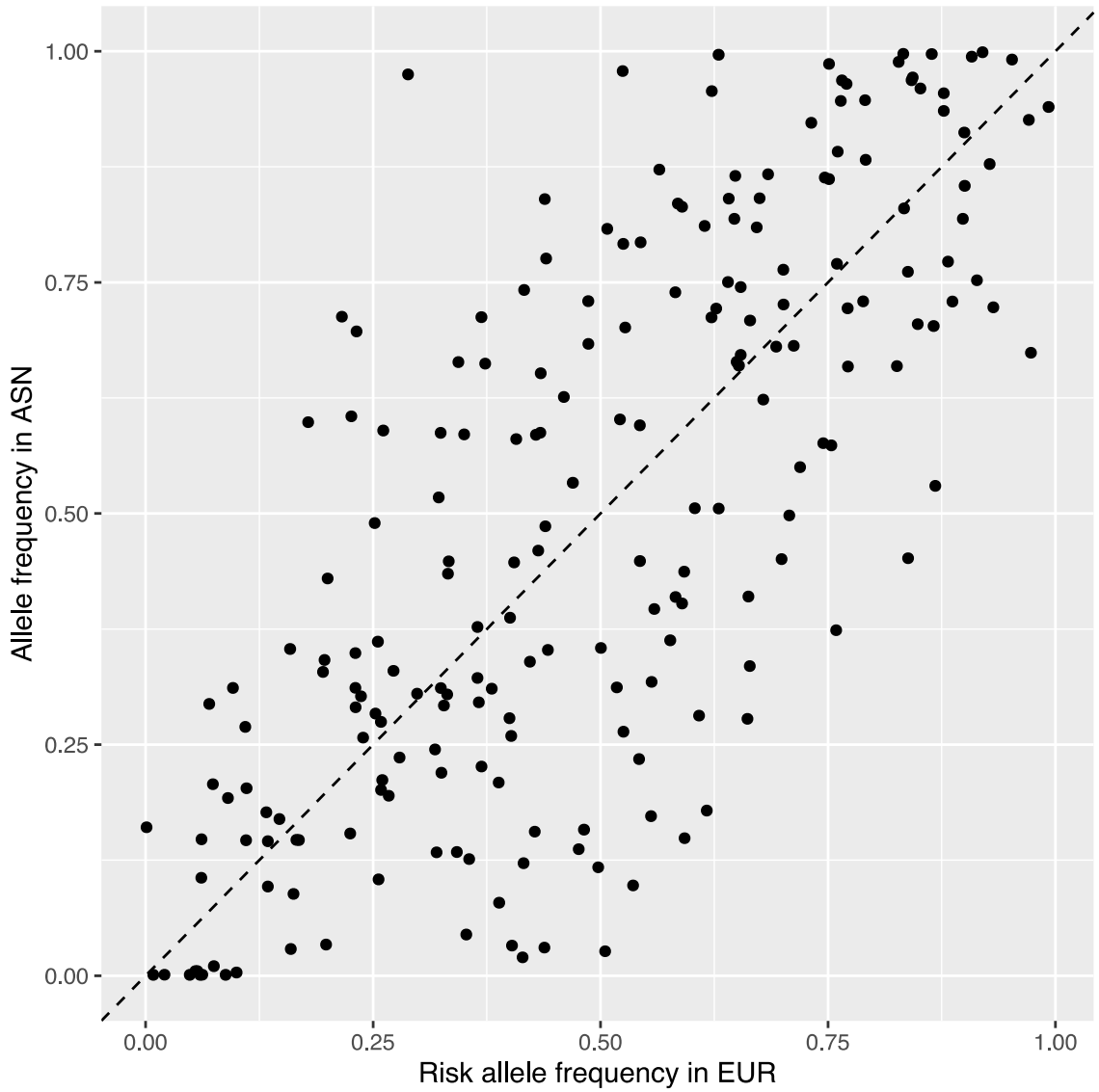

Figure S2. Comparison of allele frequency in Asian- and European-ancestry women for lead variants at risk loci identified by cross-ancestry meta-analysis. The counted allele was the allele in association with an increased risk of breast cancer in European-ancestry women. The black dashed line is the diagonal line.

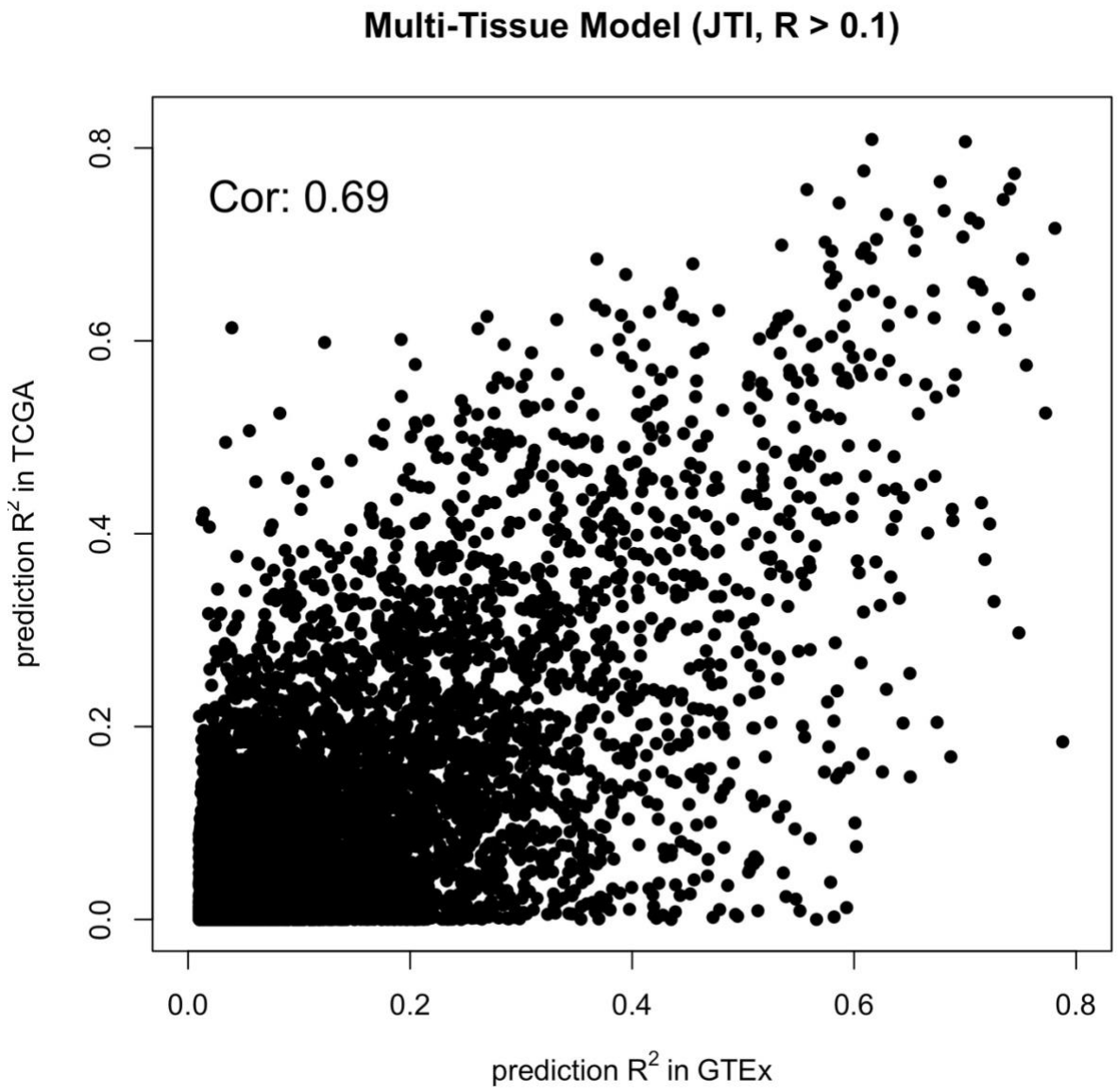

Figure S3. Performance of expression prediction model in GTEx and TCGA data for genes with over 10% correlation in GTEx data. The x axis represents the prediction performance (R^2) in the GTEx dataset (n = 115) and the y axis represents the prediction performance in the TCGA dataset (n = 86). Each dot represents the expression prediction model for one gene. There is a trend that genes with high prediction performance in the GTEx data also have high prediction performance in the TCGA (Pearson's correlation coefficient: 0.69).

Legends for Supplemental Tables

Table S1. Studies included in the cross-ancestry meta-analysis.

Table S2. Lead variants at risk loci for risk of overall breast cancer identified by meta-analyses.

Table S3. Lead variants at risk loci for risk of ER-positive breast cancer identified by meta-analyses.

Table S4. Lead variants at risk loci for risk of ER-negative breast cancer identified by meta-analyses.

Table S5. Results for the association of breast cancer risk with 17 novel risk loci in women from ABCC and BCAC

Table S6. Associations of novel risk variants for overall breast cancer risk from analyses using meta-regression.

Table S7. Associations by ER status for lead variants at risk loci identified by cross-ancestry meta-analyses.

Table S8. Associations with breast cancer risk for previously reported index SNPs not located at loci identified by our cross-ancestry meta-analysis.

Table S9. Independent association signals at novel breast cancer risk loci identified by conditional analysis in women of Asian and European ancestry.

Table S10. Samples by tissue type used in cross-tissue model building.

Table S11. Genes associated with breast cancer risk at the Bonferroni-corrected signficance level.

Table S12. Associations with breast cancer by ER status for genes identified at the Bonferroni-corrected significant level.

Table S13. Ancestry-specific associations with breast cancer risk for genes identified at the Bonferroni-corrected significant level.

Table S14. TWAS fine-mapping results for significant genes.

Table S15. Colocalization analysis results for TWAS significant genes from COLOC.

Table S16. Putative target protein-coding genes at risk loci for breast cancer risk.

Table S17. Pathway analyses for protein-coding genes associated with breast cancer.

Table S18. Summary of findings from genome- and transcriptome-wide association analyses with overall breast cancer and ER subtypes.

Supplemental Methods

I. Description of Study Populations

1. Description of Studies of the Asia Breast Cancer Consortium (ABCC)

1.1 Shanghai Breast Cancer Genetics Study (SBCGS)

The Chinese participants were drawn from Shanghai Breast Cancer Genetics Study (SBCGS), which consists of the Shanghai Breast Cancer Study (SBCS), Shanghai Breast Cancer Survival Study (SBCSS), Shanghai Endometrial Cancer Study (SECS, contributed control data only), and the Shanghai Women's Health Study (SWHS), four large population-based studies in urban Shanghai. All participants provided written informed consent prior to interview, and institutional review boards of all institutes in both China and the United States approved the study.

The SBCGS contributed samples to both ABCC and the BCAC Asian samples. Samples overlapped between ABCC and BCAC were only kept in the ABCC.

1.1.1 Shanghai Breast Cancer Study (SBCS)

The SBCS is a two-phase (SBCS-I and SBCS-II) population-based case-control study that recruited incident patients with breast cancer and controls in urban Shanghai, China.[1,2] The first phase (SBCS-I) recruited 1,602 eligible breast cancer cases and 1,724 eligible controls, from August 1996 to March 1998. Cases were recruited by a rapid case-ascertainment system and the population-based Shanghai Cancer Registry, and controls were randomly selected from the general population using the Shanghai Resident Registry. There were 1,459 cases (91.1%) and 1,556 controls (90.3%) who completed in-person interviews. Blood samples (10 ml from each woman) were obtained who completed the in-person interview (1,193 (82%) cases and 1,310 (84%) controls). A sample of exfoliated buccal cells was obtained using cotton swabs from virtually all study participants who did not provide a blood sample. The second phase (SBCS-II) recruited subjects between April 2002 and February 2005 using a protocol similar to the one used in the initial phase. Similar to the SBCS-I subjects, the majority of newly-recruited cases (n=1,932, 97.1%) and controls (n=1,857, 93.4%) provided a blood sample or an exfoliated buccal cell sample to the study. The modified mouthwash method initially reported by Lum A *et al*. was used.[3] Eligibility criteria for study participation were identical for SBCS-I and SBCS-II except age. The age ranged from 25 to 65 years for SBCS-I, and from 25 to 70 years in SBCS-II.

1.1.2 Shanghai Breast Cancer Survival Study (SBCSS)

The SBCSS included 6,303 breast cancer cases ascertained via the population-based Shanghai Cancer Registry between April 2002 and December 2006.[1] Information on known breast cancer risk factors as well as anthropometrics was collected by in-person interviews using a protocol and questionnaire similar to that used in the SBCS. Buccal cell samples were collected from 96% of study participants using the modified mouthwash method. There were 1,469 breast cancer patients participated in both SBCS-II and SBCSS due to the time overlap in the participant recruitment period.

1.1.3 Shanghai Endometrial Cancer Study (SECS)

The SECS is a population-based, case-control study of endometrial cancer conducted between January 1997 and December 2003 using a protocol similar to the SBCS, and only the community controls from the SECS were included in the present study.[1] Eligible cases were identified through the population-based Shanghai Cancer Registry and controls were randomly selected from the general population of Shanghai using the Shanghai Resident Registry and were age frequency matched to cases. Detailed information was collected by in-person interviews and anthropometrics measurements were taken. A total of 1,039 controls provided a blood sample or buccal cell sample using the mouthwash method, and these women were included in SBCGS.

1.1.4 Shanghai Women's Health Study (SWHS)

The SWHS is a population-based cohort study which recruited approximately 75,000 adult women from urban Shanghai between 1997 and 2000.[4] A total of 56,831 subjects, 75.8% of those who completed baseline survey through an in-person interview, donated a blood sample. An exfoliated buccal cell sample was collected from an additional 8,934 (49.3%) of the 18,111 subjects who did not provide a blood sample at baseline. Genomic DNA was available for about 88% of cohort members. Cancer cases were identified via record linkage with the population-based cancer registry and data collected at the Vital Statistic Unit, followed by home visits or telephone calls if necessary to confirm the diagnoses. Cancer diagnoses were verified by a review of medical records obtained from the diagnosing hospital.

Participants in SBCGS have been genotyped by Affymetrix Genome-Wide Human SNP Array 6.0, the Asian ExomeChip, and the Multi-Ethnic Global Array (MEGA). Similar genotyping and QC procedures have been described previously.[1,5] After imputation with the 1000 Genomes Project Phase 3 and QC exclusions, the final dataset included 2,511 cases and 2,135 controls for 11.1 million markers for the Affy6 dataset, 1,563 cases and 2,396 controls for 2.95 million markers for the ExomeChip dataset, and 1,794 cases and 2,059 controls for 14.1 million markers for the MEGA dataset.

1.2 Hwasun Cancer Epidemiology Study-Breast (HCES-Br)

The Hwasun Cancer Epidemiology Study (HCES-Br) is a hospital-based case-control study to identify factors of the cancer development and clinical progression in a Korean population.[6,7] The study included 3,387 female breast cancer cases diagnosed between April 2004 and February 2013 at Chonnam National University Hwasun Hospital, a cancer specified hospital in Jeollanam-do province, South Korea. Patients with secondary or recurrent tumor were excluded. Controls were 3,186 women who were randomly selected from among women with no previous cancer diagnosis at enrollment in the Namwon Study and the Dong-gu study, ongoing community-based cohort studies in South Korea.[8] Genomic DNA was extracted from their peripheral blood. Demographics data and conventional factors of breast cancer were collected by structured questionnaire and review of medical records. All cases and control subjects provided the informed consent to participate in the study and Institutional Review Board of Chonnam National University Hwasun Hospital approved this study. In the HCES-Br, there were 274 cases and 273 controls genotyped by MEGA and imputed with the 1000 Genomes Project Phase 3 data as reference.

1.3 Korea Precision Oncology Program (KPOP) - Breast Cancer

The KPOP – Breast Cancer study is a study to investigate genetic mutation/variants distribution of hereditary breast/ovarian cancer and risk stratification for women with or without family history of breast cancer. In addition, the risk factors of breast cancer were studied in women, stratified by family history of breast cancer. All cases had a histologically confirmed diagnosis of invasive breast cancer or ductal carcinoma in situ. The breast cancer cases were recruited from breast cancer center and genetic counseling clinic, National Cancer Center in Korea between 2013 and 2018. The controls were recruited from health screening examinees from National Cancer Center between 2013 and 2016 and they were women free of any cancer. After obtaining informed consent, cases and controls were asked to complete questionnaire on reproductive factors, lifestyle factors, and family history of cancer and provided blood samples. After separating plasma, serum, and whole blood, samples were stored at -70°C until assayed. Overall, 1904 breast cancer cases and 1195 controls were recruited. In KPOP, there were 963 cases and 921 controls were successfully genotyped by MEGA and imputed with the 1000 Genomes Project Phase 3 data as reference.

1.4 The Biobank Japan Project (BBJ2)

The BioBank Japan Project recruited around 200,000 patients with 47 diseases in Japan and collaboratively collected DNA and serum samples (https://biobankjp.org/english/index.html).[9,10] There were a total of 5,552 breast cancer patients and 89,731 female controls registered in Biobank Japan. Control samples were from population-based prospective cohorts and samples without related diagnoses. Samples were genotyped using the Illumina HumanOmniExpressExome BeadChip or a combination of the Illumina HumanOmniExpress and HumanExome BeadChips, and imputed with the 1000 Genomes Project Phase 3 data as reference.[11]

1.5 Seoul Breast Cancer Study (SeBCS):

The SeBCS is a hospital-based case-control study conducted in two teaching hospitals in Seoul.[12,13] Between 2001 and 2007, there were 2,342 patients with primary breast cancer recruited in the study. Information on known breast cancer risk factors and anthropometrics were collected by in-person interviews using a protocol and questionnaire. Medical charts were reviewed to verify clinical information. Eligible controls were derived from a large urban cohort included in the Korea Genome Epidemiology Study (KoGES), which was an ongoing cohort study that has sought to understand the causes and risk factors of disease in South Korea. A total of 2,052 controls were recruited between May 2006 and December 2007. They were frequency-matched to cases on the case's age at diagnosis in five-year intervals. Using a structured questionnaire and a protocol similar to the SeBCS, trained interviewers collected the demographic characteristics of the controls, their family histories with regard to breast cancer in first-degree relatives, reproductive and menstrual factors, and life-style habits. Samples were genotyped using Affymetrix 6.0 array. After quality control and imputation by the 1000 Genomes Project Phase 3, the final data set included 2,246 cases and 2,052 controls.[14]

In addition to AABC, the SeBCS also contributed samples to BCAC Asian dataset.

2. BCAC Asian samples

The studies included in the BCAC that contributed individual-level data to the Asian-specific meta-analysis were listed as Study, Location and BCAC project(s): ACP, Thailand, Oncoarray and iCOGS; CBCS, Canada, Oncoarray; HERPACC, Japan, Oncoarray and iCOGS; HKHBCFR, Hong Kong, Oncoarray; KOHBRA, Korea, Oncoarray; LAABC, USA, iCOGS; MYBRCA, Malaysia, Oncoarray and iCOGS; NC-BCFR, USA, Oncoarray; NGOBCS, Japan, Oncoarray; SBCGS, China, Oncoarray and iCOGS; SeBCS, Korea, Oncoarray and iCOGS; SGBCC, Singapore, Oncoarray and iCOGS; TWBCS, Taiwan, Oncoarray and iCOGS.

2.1 Asia Cancer Program (ACP):
The ACP is a hospital-based case-control study conducted in Thailand. Breast cancer cases were recruited between 1999-2000, and 2008-present at The National Cancer Institute (Central region), The Prince Songkla University Research Centre (South region), The HRH Princess Maha Chakri Sirindhorn Medical Centre (MSMC)-Srinakarinviroj University (Eastern region), Khon-Kaen University Cancer Centre (North-Eastern region). Women who were less than 71 years of age and underwent biopsy were eligible to participate in the study. All cases were pathologically diagnosed with breast cancer. Women resided in the same geographic area, younger than 71 years old, and reported no prior history of cancer were recruited as controls. In total, 944 invasive cases and 1,382 controls were included in the BCAC Asian dataset.

2.2 Canadian Breast Cancer Study (CBCS)
The CBCS is a population-based case-control study conducted in Canada.[15–18] Incident cases diagnosed between 2005 and 2009 were recruited from two areas, Vancouver, British Columbia and Kingston, Ontario. The cases were ascertained either from the population cancer registry (Vancouver, British Columbia) or participants of the Hotel Dieu Breast Assessment Program (Kingston, Ontario). Cancer-free controls were recruited through the Screening Mammography Program of British Columbia or the Hotel Dieu Breast Assessment Program in Kingston, Ontario. Controls were frequency matched by 5-year age groups.

2.3 Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC)
The participants were recruited from a hospital-based case-control study conducted in Aichi, Japan.[19] All incident breast cancer cases were newly diagnosed within 1 year from the first visit to the Aichi Cancer Center between 2001 and 2013. Controls were selected from pool of non-cancer patients who firstly visited Aichi Cancer Center between 2001 and 2011. Subjects with previous cancer history were excluded.

2.4 Hong Kong Hereditary Breast Cancer (HKHBCFR)
Genetic screening of high-risk breast cancer patients was approached for the study enrollment from all hospitals in Hong Kong, China between 2006 and 2014.[20–22] Controls were selected from pool of non-cancer patients who visited hospitals in Hong Kong during the same period of recruitment as cases.

2.5 Korean Hereditary Breast Cancer (KOHBRA)

The KOHBRA study is an ongoing cohort study since 2007 to examine high risk groups for hereditary breast cancer such as female breast cancer patients with a family history, ovarian cancer, or other coincidental cancers, male breast cancer patients, and family members of breast cancer patients with *BRCA1/2* mutation. Final dataset included selected 1,397 female cancer patients without *BRCA1/2* mutation among KOHBRA subjects recruited in 2007-2009.[23]

2.6 Los Angeles County Asian-American Breast Cancer Case-Control Study (LAABC)

The LAABC is a population-based case-control study of incident breast cancer among Asian American women in Los Angeles County. Breast cancer cases were ascertained through the Los Angeles Cancer Surveillance Program. The included women were identified as Chinese, Japanese or Filipino women (aged 25-74 years) with a histologically confirmed primary breast cancer diagnosed between 1996 and 2006.[24–26] Controls were recruited from the same neighborhood as where cancer cases resided at the time of diagnosis. Cases and controls were frequency-matched on specific Asian ethnicities and 5-year age groups.

2.7 Malaysian Breast Cancer Genetic Study (MYBRCA)

Prevalent or incident breast cancer cases identified at the Breast Cancer Clinic in University Malaya Medical Centre from January 2003 to July 2014 and Subang Jaya Medical Centre from September 2012 to September 2014.[27] Controls are cancer-free individuals (37-74 years) selected from women attending mammographic screening at the same hospitals.

2.8 Northern California Breast Cancer Family Registry (NC-BCFR)

Incident breast cancer cases included women aged <65 years diagnosed from 1995-2009, identified through the SEER cancer registry of the Greater San Francisco Bay Area. All cases with indicators of increased genetic risk were eligible to enroll (diagnosed at age <35 years, personal history of ovarian or childhood cancer, bilateral breast cancer with 1st diagnosis at age <50, family history of breast or ovarian cancer in first-degree relatives).[28,29] Cases not meeting these criteria were randomly sampled (2.5% of non-Hispanic whites, 32% of other race/ethnicities). Incident cases also included men aged <80 years diagnosed from 1995-1998. Controls were those unaffected family members enrolled from 1995-2011 or unaffected unrelated subjects identified through random digit dialing conducted from 1999-2000 in the San Francisco Bay Area. Controls were frequency matched to cases diagnosed from 1995-1998 on 5-year age group and race/ethnicity, at a ratio of 1 control per 2 cases. Only women were included in the current analysis.

2.9 Nagano Breast Cancer Study (NGOBCS)

The Nagano Breast Cancer Study is a multicenter, hospital-based case-control study which was conducted from May 2001 to September 2005 at four hospitals in Nagano Prefecture, Japan.[30,31] Cases were admitted to the four hospitals during the survey period, and were a consecutive series of women aged 20-74 years with newly diagnosed, histologically confirmed invasive breast cancer. Among the 412 eligible patients, 405 (98%) agreed to participate. Controls were selected from medical checkup examinees in two of the hospitals who were confirmed having no cancer, with one control matched for each case by age (within three years) and residential area during the study period. Only one declined to participate among potential control subjects. Written informed consent was obtained from 405 matched pairs. Since two controls refused to provide blood samples, the analysis was restricted to 403 matched pairs. Participants completed a self-

administered questionnaire, which included questions on demographic characteristics, anthropometric factors, smoking habits, family history of cancer, physical activity, medical history, and menstrual and reproductive history. Dietary habits were investigated using a 136-item semi-quantitative food-frequency questionnaire, which was developed and validated in the Japanese population. The ER status of the patient's breast cancer tissue was obtained from medical records. Hormone receptor positivity values were determined either as specified by the laboratory that performed the assay, in accordance with the laboratory's written interpretation thereof, or both. The study protocol was approved by the Institutional Review Board of the National Cancer Center (Tokyo, Japan).

2.10 Singapore Breast Cancer Cohort (SGBCC)

The SGBCC is an open cohort with a recruitment target of 16,000 patients diagnosed with either breast carcinoma in situ or invasive breast cancer. Details of the study design has been published elsewere.[32] Briefly, recruitment started in 2010. All breast cancer patients who are at least 21 years of age at diagnosis, who are citizens or permanent residents of Singapore and who are attending any of the seven tertiary hospitals are invited to participate in SGBCC. Cases are a mixture of prevalent and incident cases. Three main ethnic groups are represented, namely, Chinese, Malays and Indians. Controls matched by age and ethnicity were selected from the Multi-ethnic Cohort (Phase 2, part of the Singapore Population Health Studies (SPHS).[33] Exclusion criteria for controls included a medical history of cancer, acute myocardial infarction or stroke, or major psychiatric morbidity including schizophrenia, psychotic depression, and advanced Alzheimer's disease.

2.11 Taiwanese Breast Cancer Study (TWBCS)

The study is a part of an ongoing collaborative study with a focus on understanding the cause of breast cancer among Taiwanese.[34,35] Breast cancer patients were recruited from those who were diagnosed and treated at the Tri-Service General Hospital or the Changhua Christian Hospital between March 2002 and August 2005. The controls were randomly selected from women who attended the same hospitals for a comprehensive health examination during the same period. If any evidence of breast cancer, precancerous lesions of breast or other cancers was found, the subject was excluded from the control group. Epidemiologic data were collected from the participants via a structured questionnaire by research nurses. Blood biospecimen was also collected. All the participants provided their informed consent before the data and sample collection.

3. BCAC European samples

Summary statistics data of European descendants from studies involved in the BCAC OncoArray, iCOGS, and GWAS projects were obtained and utilized in the cross-ancestry meta-analysis. Among 82 studies from the BCAC, the OncoArray dataset included 80,125 female cases with breast cancer and 58,383 female controls of European ancestry, and the Collaborative Oncological Gene-environment Study (iCOGS) included 38,349 breast cancer cases and 37,818 controls.[36] In addition, summary statistics from 11 other breast cancer genome-wide association

studies were also used in the meta-analysis with a combined sample of 14,910 cases and 17,588 controls. The genotyping data were imputed by IMPUTE version 2[37] with the 1000 Genomes Project Phase 3 as the reference panel.

II. Supplemental Statistical Analyses

Fine-mapping. We investigated the ancestral heterogeneity of the lead variants at risk loci. However, lead variants are not necessarily the causal variants, and the observed heterogeneity may be related to the different linkage disequilibrium (LD) pattern across populations. Therefore, we performed fine-mapping analyses to construct the 95% credible sets for the lead variants, and further investigated the ancestral heterogeneity of all variants in the credible sets. Fine-mapping analysis was performed using SuSiE[38]. Samples from 1000 Genome Project Phase 3 (EAS and EUR) were used as LD reference. An ancestry-specific LD matrix was used for risk loci identified by ancestry-specific analyses. For risk loci identified by cross-ancestry analyses, a cross-ancestry LD matrix was constructed by combining ancestry-specific LD matrices using weights of population sample sizes.

Gene prediction model building. We used whole genome sequencing (WGS) data in blood samples and RNA sequencing (RNA-seq) data from the Genotype-Tissue Expression Project (GTEx, version 8) to build prediction models for genes expressed in normal breast tissue. All genotyping and expression data were downloaded from dbGap (Accession Number: phs000424.v8.p2).

We kept samples from European-ancestry women with both expression and genotyping data (N =115). The following genetic variants were used to build genetic prediction models: 1) MAF ≥0.05, and 2) Hardy-Weinberg equilibrium $P \geq 10^{-4}$, and 3) call rate ≥ 95%, and 4) non A/T, C/G bi-allelic, and 5) available in BCAC. Finally, a total of 4,853,854 variants were kept for gene expression prediction model building.

There were 32 tissues with both RNA-Seq and WGS data available with sample size >50, and these 32 tissues were kept for model building. Detailed sample sizes by each tissue type were shown in Supplementary Table 10. Within each tissue type, we kept genes with a median expression level (transcript per million, TPM) >0 across samples for each tissue, and the expression level was log2 transformed. Then we performed quantile normalization to bring the expression profile of each sample to the same scale and performed inverse quantile normalization for each gene to the same scale. Then the expression levels were adjusted for age, the top three principal components (PCs) and the top probabilistic estimation of expression residuals (PEER) factors[39] to correct for batch effects and experimental confounders. After adjusting all these covariates, we performed another inverse quantile normalization for the residuals after PEER adjustment of each gene.

We built genetic models to predict gene expression levels in normal breast tissue using the joint-tissue imputation (JTI) approach, which borrows information across transcriptomes of different tissues to improve prediction performance.[40] Besides breast tissue, data from all 31 other tissues were borrowed in the JTI approach to leverage shared genetic regulation and improve prediction performance in a tissue-dependent manner. Gene expression levels were predicted using genetic variants within a flanking +/- 500kb from the respective gene boundaries. Five-fold cross-validation was used to validate the models internally. Genes with a model prediction R >0.1 (≥10% correlation between predicted and observed gene expression) were included for association analyses.

To evaluate the performance of prediction models, we further performed an external validation using 86 tumor-adjacent normal breast tissue samples from European-ancestry female breast cancer patients in the Cancer Genome Atlas (TCGA). Expression data were processed and normalized in similar approach for GTEx data as described above. We calculated the Spearman's correlation between the prediction performance (R^2) in GTEx and TCGA.

Association analyses of predicted gene expression with breast cancer risk. Based on the weight matrix from the prediction models and the summary statistics from meta-analysis of GWAS, we evaluated the association between genetically predicted gene expression and breast cancer risk using the method from the S-PrediXcan tool[41]. The details of the formula used in this method are

$$Z_g \approx \sum_{l \in Model_g} w_{lg} \frac{\widehat{\sigma_l}}{\widehat{\sigma_g}} \frac{\widehat{\beta_l}}{se(\widehat{\beta_l})}$$

In brief, the Z-score was used to estimate the association between predicted gene expression and breast cancer risk. In this formula, w_{lg} is the weight of variant l for predicting the expression of gene g. $\hat{\beta}_l$and $se(\hat{\beta}_l)$ are the association regression coefficient and its standard error for variant l in GWAS, and $\hat{\sigma}_l$ and $\hat{\sigma}_g$ are the estimated variances of variant l and the predicted expression of gene g, respectively. For this study, we estimated the correlations between variants included in the prediction models.

TWAS fine-mapping analyses. We performed TWAS fine-mapping for all genomic regions that contain one or more TWAS-identified risk genes using FOCUS (Fine-mapping Of CaUsal

gene Sets, v0.6.10)[42]. Regions were defined using the correlation matrix of predicted effects on gene expression around TWAS-identified genes. A posterior inclusion probability (PIP) was assigned to each gene for being possibly causal in each TWAS uncovered association signal. Based on the PIP of each gene and a null model, whereby no gene in the region is causal for the TWAS signal, a gene set for each region in which the sum of PIPs for all the genes was greater than or equal to 90% probability ($\sum_{i=1}^{k} nPIP \geq 90\%$) was defined as a credible gene set.

Colocalization analyses. COLOC were conducted to assess the probability that molecular traits as estimated by eQTL and physiological traits as estimated by GWAS share the same causal variant[43]. For each TWAS-identified risk gene, we only estimated variants with both gene-variant paired eQTL results from GTEx and GWAS association statistics (effect size estimate, standard error, and *P* value) and reached association p value less than 0.5. We obtained reference information such as MAF, sample size, and case-to-control proportions (in case of binary traits) for each variant. We defined a gene as having evidence of co-localization when gene-based posterior probability of co-localization PP[4] > 0.5.

Pathway analyses. Protein-coding genes identified by our TWAS were located at 46 GWAS-identified risk loci and seven novel risk loci. If there were multiple TWAS-identified genes at the same locus, genes which were included in the fine-mapping credible set or supported by colocalization analyses were selected for pathway analyses. At 150 additional GWAS-identified loci without protein-coding genes identified by our TWAS, previously reported putative target genes[44] or nearby protein-coding genes were selected for pathway analyses. A total of 221 putative genes for breast cancer were included for pathway analyses (Table S16). The WEB-

based Gene Set Analysis Toolkit (WebGestalt) was used to perform for KEGG pathways and gene ontology terms enrichment analyses[45,46].

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[1] Breast Department, KK Women's and Children's Hospital, Singapore
[2] SingHealth Duke-NUS Breast Centre, Singapore
[3] Department of General Surgery, Tan Tock Seng Hospital, Singapore
[4] Division of Surgical Oncology, National Cancer Centre Singapore, Singapore
[5] Department of General Surgery, Singapore General Hospital, Singapore
[6] Division of Breast Surgery, Department of General Surgery, Changi General Hospital, Singapore
[7] National University Health System, Department of Surgery, Singapore, Singapore
[8] Division of Radiation Oncology, National Cancer Centre Singapore, Singapore
[9] Division of Medical Oncology, National Cancer Centre Singapore, Singapore
[10] Cancer Genetics Service, National Cancer Centre Singapore, Singapore
[11] Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Singapore
[12] Yong Loo Lin School of Medicine, National University of Singapore and National University Health System, Singapore
[13] Genome Institute of Singapore, Human Genetics, Singapore, Singapore

Supplemental references:

1. Zheng W, Long J, Gao YT, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet*. 2009;41(3):324-328.

2. Gao YT, Shu XO, Dai Q, et al. Association of menstrual and reproductive factors with breast cancer risk: Results from the Shanghai breast cancer study. *International Journal of Cancer*. 2000;87(2):295-300.

3. Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. *Cancer Epidemiol Biomarkers Prev*. 1998;7(8):719-724.

4. Zheng W, Chow WH, Yang G, et al. The Shanghai Women's Health Study: rationale, study design, and baseline characteristics. *Am J Epidemiol*. 2005;162(11):1123-1131.

5. Zhang Y, Long J, Lu W, et al. Rare coding variants and breast cancer risk: evaluation of susceptibility Loci identified in genome-wide association studies. *Cancer Epidemiol Biomarkers Prev*. 2014;23(4):622-628.

6. Song HR, Shin MH, Kim HN, et al. Sex-specific differences in the association between ABO genotype and gastric cancer risk in a Korean population. *Gastric Cancer*. 2013;16(2):254-260.

7. Shim HJ, Lee R, Shin MH, Kim HN, Kweon SS. Association between the TCF7L2 polymorphism and colorectal cancer does not differ by diabetes and obesity statuses. *Cancer Epidemiol*. 2016;45:108-111.

8. Kweon SS, Shin MH, Jeong SK, et al. Cohort Profile: The Namwon Study and the Dong-gu Study. *Int J Epidemiol*. 2014;43(2):558-567.

9. Hirata M, Kamatani Y, Nagai A, et al. Cross-sectional analysis of BioBank Japan clinical data: A large cohort of 200,000 patients with 47 common diseases. *J Epidemiol*. 2017;27(3S):S9-S21.

10. Nagai A, Hirata M, Kamatani Y, et al. Overview of the BioBank Japan Project: Study design and profile. *J Epidemiol*. 2017;27(3S):S2-S8.

11. Ishigaki K, Akiyama M, Kanai M, et al. Large-scale genome-wide association study in a Japanese population identifies novel susceptibility loci across different diseases. *Nat Genet*. 2020;52(7):669-679.

12. Han S, Lee KM, Choi JY, et al. CASP8 polymorphisms, estrogen and progesterone receptor status, and breast cancer risk. *Breast Cancer Res Treat*. 2008;110(2):387-393.

13. Cho YS, Go MJ, Kim YJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. *Nat Genet*. 2009;41(5):527-534.

14. Han MR, Long J, Choi JY, et al. Genome-wide association study in East Asians identifies two novel breast cancer susceptibility loci. *Hum Mol Genet*. 2016;25(15):3361-3371.

15. Grundy A, Schuetz JM, Lai AS, et al. Shift work, circadian gene variants and risk of breast cancer. *Cancer Epidemiol*. 2013;37(5):606-612.

16. Kobayashi LC, Janssen I, Richardson H, Lai AS, Spinelli JJ, Aronson KJ. Moderate-to-vigorous intensity physical activity across the life course and risk of pre- and post-menopausal breast cancer. *Breast Cancer Res Treat*. 2013;139(3):851-861.

17. Grundy A, Richardson H, Burstyn I, et al. Increased risk of breast cancer associated with long-term shift work in Canada. *Occup Environ Med*. 2013;70(12):831-838.

18. Kobayashi LC, Janssen I, Richardson H, Lai AS, Spinelli JJ, Aronson KJ. A case-control study of lifetime light intensity physical activity and breast cancer risk. *Cancer Causes Control*. 2014;25(1):133-140.

19. Kawase T, Matsuo K, Suzuki T, et al. FGFR2 intronic polymorphisms interact with reproductive risk factors of breast cancer: results of a case control study in Japan. *Int J Cancer*. 2009;125(8):1946-1952.

20. Kwong A, Ng EKO, Law FBF, et al. Novel BRCA1 and BRCA2 genomic rearrangements in Southern Chinese breast/ovarian cancer patients. *Breast Cancer Res Treat*. 2012;136(3):931-933.

21. Kwong A, Ng EKO, Wong CLP, et al. Identification of BRCA1/2 founder mutations in Southern Chinese breast cancer patients using gene sequencing and high resolution DNA melting analysis. *PLoS One*. 2012;7(9):e43994.

22. Kwong A, Shin VY, Au CH, et al. Detection of Germline Mutation in Hereditary Breast and/or Ovarian Cancers by Next-Generation Sequencing on a Four-Gene Panel. *J Mol Diagn*. 2016;18(4):580-594.

23. Han SA, Park SK, Ahn SH, et al. The Korean Hereditary Breast Cancer (KOHBRA) study: protocols and interim report. *Clin Oncol (R Coll Radiol)*. 2011;23(7):434-441.

24. Wu AH, Yu MC, Tseng CC, Stanczyk FZ, Pike MC. Dietary patterns and breast cancer risk in Asian American women. *Am J Clin Nutr*. 2009;89(4):1145-1154.

25. Wu AH, McKean-Cowdin R, Tseng CC. Birth weight and other prenatal factors and risk of breast cancer in Asian-Americans. *Breast Cancer Res Treat*. 2011;130(3):917-925.

26. Wu AH, Vigen C, Butler LM, Tseng CC. Metabolic conditions and breast cancer risk among Los Angeles County Filipina Americans compared with Chinese and Japanese Americans. *Int J Cancer*. 2017;141(12):2450-2461.

27. Phuah SY, Looi LM, Hassan N, et al. Triple-negative breast cancer and PTEN (phosphatase and tensin homologue) loss are predictors of BRCA1 germline mutations in women with

early-onset and familial breast cancer, but not in women with isolated late-onset breast cancer. *Breast Cancer Res*. 2012;14(6):R142.

28. John EM, Hopper JL, Beck JC, et al. The Breast Cancer Family Registry: an infrastructure for cooperative multinational, interdisciplinary and translational studies of the genetic epidemiology of breast cancer. *Breast Cancer Res*. 2004;6(4):R375-389.

29. Terry MB, Phillips KA, Daly MB, et al. Cohort Profile: The Breast Cancer Prospective Family Study Cohort (ProF-SC). *Int J Epidemiol*. 2016;45(3):683-692.

30. Itoh H, Iwasaki M, Hanaoka T, et al. Serum organochlorines and breast cancer risk in Japanese women: a case-control study. *Cancer Causes Control*. 2009;20(5):567-580.

31. Shimada N, Iwasaki M, Kasuga Y, et al. Genetic polymorphisms in estrogen metabolism and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. *J Hum Genet*. 2009;54(4):209-215.

32. Ho PJ, Yeoh YS, Miao H, et al. Cohort profile: The Singapore Breast Cancer Cohort (SGBCC), a multi-center breast cancer cohort for evaluation of phenotypic risk factors and genetic markers. *PLOS ONE*. 2021;16(4):e0250102.

33. Tan KHX, Tan LWL, Sim X, et al. Cohort Profile: The Singapore Multi-Ethnic Cohort (MEC) study. *Int J Epidemiol*. 2018;47(3):699-699j.

34. Hsu HM, Wang HC, Chen ST, Hsu GC, Shen CY, Yu JC. Breast cancer risk is associated with the genes encoding the DNA double-strand break repair Mre11/Rad50/Nbs1 complex. *Cancer Epidemiol Biomarkers Prev*. 2007;16(10):2024-2032.

35. Ding SL, Yu JC, Chen ST, et al. Genetic variants of BLM interact with RAD51 to increase breast cancer susceptibility. *Carcinogenesis*. 2009;30(1):43-49.

36. Zhang H, Ahearn TU, Lecarpentier J, et al. Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. *Nat Genet*. 2020;52(6):572-581.

37. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet*. 2009;5(6):e1000529.

38. Wang G, Sarkar A, Carbonetto P, Stephens M. A simple new approach to variable selection in regression, with application to genetic fine mapping. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*. 2020;82(5):1273-1300.

39. Stegle O, Parts L, Durbin R, Winn J. A Bayesian Framework to Account for Complex Non-Genetic Factors in Gene Expression Levels Greatly Increases Power in eQTL Studies. *PLOS Computational Biology*. 2010;6(5):e1000770.

40. Zhou D, Jiang Y, Zhong X, Cox NJ, Liu C, Gamazon ER. A unified framework for joint-tissue transcriptome-wide association and Mendelian randomization analysis. *Nat Genet.* 2020;52(11):1239-1246.

41. Barbeira AN, Dickinson SP, Bonazzola R, et al. Exploring the phenotypic consequences of tissue specific gene expression variation inferred from GWAS summary statistics. *Nat Commun*. 2018;9(1):1825.

42. Mancuso N, Freund MK, Johnson R, et al. Probabilistic fine-mapping of transcriptome-wide association studies. *Nat Genet.* 2019;51(4):675-682.

43. Giambartolomei C, Vukcevic D, Schadt EE, et al. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet.* 2014;10(5):e1004383.

44. Fachal L, Aschard H, Beesley J, et al. Fine-mapping of 150 breast cancer risk regions identifies 191 likely target genes. *Nature Genetics*. 2020;52(1):56-73.

45. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res*. 2019;47(W1):W199-W205.

46. Zhang B, Kirov S, Snoddy J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Research*. 2005;33(suppl_2):W741-W748.