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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Variants in 6q25.1 Are Associated with Mammographic Density in Malaysian Chinese Women

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Abstract

Background: Mammographic density is an established risk factor for breast cancer and has a strong heritable component. Genome-wide association studies (GWAS) for mammographic density conducted in women of European descent have identified several genetic associations, but none of the studies have been tested in Asians. We sought to investigate whether these genetic loci, and loci associated with breast cancer risk and breast size, are associated with mammographic density in an Asian cohort.

Methods: We conducted genotyping by mass spectrometry in 1,189 women (865 Chinese, 187 Indian, and 137 Malay). Quantitative measurements of mammographic density were performed using ImageJ, a fully automated thresholding technique. The associations of SNPs to densities were analyzed using linear regression models.

Results: We successfully evaluated the associations of 36 SNPs with mammographic densities. After adjusting for age, body mass index, parity, and menopausal status, we found that in our cohort of 865 Malaysian Chinese, three SNPs in the 6q25.1 region near *ESR1* (rs2046210, rs12173570, and rs10484919) that were associated with mammographic density, breast cancer risk, or breast size in previous GWAS were significantly associated with both percentage density and absolute dense area. We could not replicate the most significant association found previously in European women (rs10995190, *ZNF365* gene) because the minor allele was absent for Asian women.

Conclusion: We found that the directions of genetic associations were similar to those reported in Caucasian women.

Impact: Our results show that even in Asian women with lower population risk to breast cancer, there is shared heritability between mammographic density and breast cancer risk. *Cancer Epidemiol Biomarkers Prev; 25(2); 327–33. ©2015 AACR.*

Introduction

Mammographic density, which reflects the amount of radiodense epithelial and stromal tissues in the breast, has a large heritable component. Twin studies have reported heritability estimates ranging from 0.60 to 0.75 (1). Given the associations of percentage density, dense area, and nondense area with risk of breast cancer (2–4), identifying genetic modifiers of this trait may be important in understanding breast cancer etiology and pathogenesis. Although candidate gene and linkage analyses have been mostly unsuccessful in identifying genetic loci associated with mammographic density, genome-wide association studies (GWAS) have uncovered a number of candidate loci. These include known breast cancer susceptibility loci in *ZNF365*, near *ESR1*, *LSP1*, *RAD51L1*, 8q24, and *TOX3* (5–9). In addition, recent studies show that genetic loci associated with breast size (measured using adjusted bra cup size), such as the 4q13.3 region where the *AREG* gene lies, are also associated with radiologically dense area (10, 11). Bra cup size has been shown to have moderate heritability (12), and common genetic factors may be associated with mammographic density and bra size.

To the best of our knowledge, none of the GWAS of mammographic density have thus far included Asian women. Given the diversity in genetic variants and the variability in mammographic density and breast cancer incidence rates across different ethnicities, it is unclear whether results from GWAS conducted in European women can be extrapolated to non-European populations. In this study, we sought to assess if SNPs previously known to be associated with mammographic density, breast size, and/or breast cancer risk are associated with mammographic density phenotypes in our cohort of Asian women from Malaysia.

Materials and Methods

Study population

Participants of this study comprised of women recruited from October 2011 to October 2013 ($N = 1,646$) through the Malaysian Mammogram (MyMammo) study, one of the several subsidized opportunistic mammogram screening programs in the country. The study design, recruitment method, and study

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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population are as described previously (13). The MyMammo study was approved by the Ramsay Sime Darby Healthcare Independent Ethics Committee (Ethics Committee/IRB Reference Number: 201109.4), and all women provided written informed consent.

Mammograms were available for 1,603 women. Women were excluded from this study if they were of mixed ethnicities or ethnicities other than Malay, Indian, and Chinese ($n = 84$). Among women who were known to be related, we selected the oldest in the family and excluded 178 women from subsequent analysis. Of the 1,341 unrelated participants, we excluded women who were known to have had silicone breast injections ($n = 3$), were symptomatic ($n = 91$) or diagnosed with cancer ($n = 8$), had no body mass index (BMI) data ($n = 16$), or never had menstrual periods ($n = 1$). A total of 1,225 women were eligible for genotyping.

Selection of SNPs

We conducted a PubMed online literature search dated November 2013 to identify SNPs of interest for genotyping. We included SNPs associated with mammographic density in other populations, SNPs associated with breast cancer risk in Asian women, and SNPs associated with breast size. The following key words or their combinations were used to select for SNPs associated with mammographic density: mammographic, density, GWAS, SNP*, variant*, polymorphism*, and Asia*. We identified 76 publications from this search. Publications that were not within the scope of this study were excluded. Nineteen full-text articles were retrieved and reviewed. We selected five SNPs from three studies that showed genome-wide significant associations with mammographic density (6, 7, 14). We also selected two SNPs significantly associated with mammographic density in two Asian studies (15, 16), which looked at candidate regions in *PPARγ* and *TGFβ1* for genotyping. Apart from SNPs identified through this PubMed search, we included eight SNPs shown to have genome-wide significant associations with density in a study, now published (10), presented at the American Society of Human Genetics 2013 Annual Meeting, Boston (17).

To select SNPs associated with breast cancer risk in Asian women, we conducted a literature search using the following key words: Asia*, breast, cancer, and GWAS. A total of 56 publications were identified, and full versions of 4 publications were retrieved, from which we selected 20 genome-wide significant SNPs from four publications (18–21) for genotyping. Four SNPs identified as breast cancer susceptibility loci in a then unpublished GWAS from the Asian Breast Cancer Consortium were also included for genotyping (22).

We identified only two publications from our PubMed search for SNPs associated with breast size using the key words: breast, size, cancer, density, and GWAS. We selected seven SNPs from one study in European women (11), two of which are also associated with mammographic density and breast cancer risk and therefore already selected for genotyping from the previous two searches.

DNA extraction and genotyping

Genomic DNA was extracted from white blood cells of peripheral blood. Genotyping was performed by MALDI-TOF MS using the Sequenom MassARRAY. We included one negative control and at least three replication controls in each of the 96-well plates for genotyping. Samples with assay call rates of <95% were removed from all analyses. SNPs with genotyping call rates <95% were excluded from all analyses. SNPs were also excluded if minor allele frequencies (MAF) were <5% or the *P* value for Hardy–Weinberg equilibrium (HWE) was <0.01.

Mammographic density assessment

Two-view mammograms were performed using the Hologic Selenia full-field digital mammography system. To measure mammographic density, we used a fully automated thresholding method using the ImageJ software, as previously described (23). Validations for measurements obtained using this method are as described (13). We used the conversion factor available from the images to convert measurements in pixels into area measurements. Nondense areas were calculated by subtracting dense area from total breast area. The craniocaudal (CC) and mediolateral oblique (MLO) view density measurements were strongly correlated, but the means were significantly different. There was no statistically significant difference between measurements from the left and right mammograms. We used randomly selected left or right CC view mammograms for this current analysis.

Statistical analysis

We stratified our cohort by ethnicity for all analyses. Three mammographic density phenotypes, namely percent mammographic density, dense area, and nondense area, were considered. Total breast area was used as a surrogate for breast size. Using linear regression analysis, we assessed the relationship of known determinants of mammographic density and breast cancer risk with mammographic density phenotypes and total breast area. Variables with significant associations after accounting for all other potential confounding variables were adjusted for in subsequent association analyses. The additive effect of each SNP was assessed using the linear regression model, adjusted for age, BMI, menopausal status, and parity for the three mammographic density measures, and adjusted for age, BMI, and parity for total breast area. Bonferroni adjustments for multiple testing corrections were performed. *P* values less than 0.05 divided by the number of independent SNPs were considered statistically significant. We used the Broad Institute's SNAP pairwise linkage disequilibrium function to calculate linkage disequilibrium between SNPs of interest. Regression models were checked for normality by inspecting the normal probability plots of the regression-standardized residuals. No major deviations from normality were observed. Linearity, homoscedasticity, and independence of residuals for continuous independent variables were checked from the residuals scatter plots. Outliers on dependent variables were also identified from the scatter plots. Correlation coefficients between each variable, the variance inflation factor, and tolerance values were inspected to check for multicollinearity among the independent variables. Statistical analyses were performed using SPSS Version 16.0 and R Version 2.12.1.

Results

Genotyping data were available for 1,189 unrelated, asymptomatic Chinese, Malay, and Indian women, after excluding samples with genotype call rates of <95% ($n = 36$). The majority of the women were Chinese (72.8%) while 15.7% and 11.5% were Indians and Malays, respectively. Given the small sample size of Indians ($n = 187$) and Malays ($n = 137$), only Chinese women ($n = 865$) were included in the main analyses. We

explored the direction of association of each phenotype in Indian and Malay women for a selected number of SNPs separately. For Chinese women in this study, the mean age at mammography was 50.8 years old, and mean BMI was 23.7 kg/m^2. Eighty-four percent have had at least one full-term pregnancy, and 48.4% were postmenopausal (Table 1). As reported previously (13), Indian and Malay women in our cohort had higher BMI, were more likely to have had at least one or more full-term pregnancies, and had larger total breast areas compared with Chinese women (Supplementary Table S1).

For this study, we included 44 SNPs that were shown previously to be associated with either one or more of the following variables in European or Asian women: mammographic density, breast size, and breast cancer risk (Supplementary Table S2). Two SNPs (rs10483813 and rs12371778) failed in the assay design phase, and two SNPs (rs3817198 and rs3803662) were excluded because of call rate <95%. The mean genotype call rate of all samples was 99.8% (median, 100%). The concordance across replicates was 100%. Two SNPs (rs1292011 and rs2241716) that were not in HWE ($P < 0.01$) in Chinese women and two rare SNPs (rs10995190 and rs6472903) with MAF of <5% were also excluded from subsequent analyses (Supplementary Table S2).

Linear regression models were used to assess the association of the remaining 36 SNPs with three mammographic density phenotypes: percent mammographic density, dense area, and nondense area. Based on data from the Asian populations in the 1000 Genomes Project, two of these SNPs, namely rs2046210 and rs12173570, located near the *ESR1* gene were in linkage disequilibrium with each other ($r^2 = 0.8$; $D' = 1.0$). We used a Bonferroni-corrected P value threshold of 1.43e–03, considering 35 independent loci.

After adjusting for age, BMI, parity, and menopausal status, three SNPs, rs2046210, rs12173570, and rs10484919, which were previously reported to be associated with mammographic density in women with European ancestry, were found to also have significant associations with percent density and absolute dense area in the Malaysian Chinese women in our study. All three SNPs were located near the *ESR1* gene. Two of these SNPs (rs2046210 and rs12173570) were in strong linkage disequilibrium with each other. The T-allele for the SNP rs2046210 was also previously found to be associated with breast cancer risk in European and Chinese women, and was the only breast cancer risk SNP that was significantly associated with mammographic density in our cohort. The SNP rs12173570 is associated with breast size in European women and was the only breast size SNP of the six analyzed that was associated with mammographic density in our cohort. We investigated the association of each SNP with total breast area and found that the three SNPs associated with density in our cohort had significance values that were below 0.05. None of these SNPs however were associated with breast area after the Bonferroni correction. We also found weak associations ($P < 0.05$) with both percent density and dense area for SNPs located in *INHBB* (rs4849887), *AREG* (rs10034692 and rs62314947), and *SGSM3* (rs17001868; Table 2 and Fig. 1).

For each phenotype, we selected SNPs with P values that were below 0.05 in either Chinese, Indian, or Malay women and investigated their associations across the three ethnic groups (Supplementary Fig. S1). The directions of associations of the three *ESR1* SNPs (rs10484919, rs2046210, and rs12173570) that were significantly associated with percent density and dense area in Chinese women were similarly associated with percent density and dense area in Malay women in our study. For Indian women, the associations of all three SNPs with dense area were in the same direction as that for Chinese and Malay women, whereas for percent density, this association was found for all but one SNP, rs2046210. Due to the limited number of samples for Indian and Malay women, there was insufficient power to find any associations that were significant after correcting for multiple tests.

Given that menopause affects mammographic density (24), we sought to determine whether there were differences between the associations of SNPs in premenopausal and postmenopausal women. In the stratified cohort (among Chinese women only), the means for percent density, dense area, and nondense area were 35.8%, 30.2 cm^2, and 57.3 cm^2, respectively, for premenopausal women and 25.8%, 22.9 cm^2, and 72.5 cm^2 for postmenopausal women. The most significant SNPs for both premenopausal and postmenopausal women were similar to those reported in the combined analysis. The effect sizes of the associations were however smaller in postmenopausal women (Supplementary Table S3).

Table 1. Characteristics of subjects

	$N = 865$		
Characteristic	**Range**	**Mean (SD)**	**%**
Age (years)	40–73	50.83 (7.30)	
Height (m)	1.38–1.76	1.58 (0.05)	
Weight (kg)	38.00–118.00	58.87 (9.57)	
BMI (kg/m^2)	16.23–43.34	23.72 (3.66)	
Parous			83.9
Number of FTP	0–7	2.15 (1.32)	
Menopausal status (post)			48.4
Oral contraceptives use (ever), $n = 860$			25.8
Hormone therapy use (ever), $n = 859$			8.7
Percent mammographic density (%)	2.24–70.40	30.92 (13.82)	
Dense area (cm^2)	1.66–78.71	26.61 (12.82)	
Nondense area (cm^2)	2.33–212.94	64.64 (30.92)	
Total breast area (cm^2)	27.35–234.83	91.26 (31.29)	

Abbreviation: FTP, full-term pregnancies.

Discussion

We assessed the association of mammographic densities and genetic loci that have been known to be associated with one or more of these variables: mammographic density or breast size or both, in women of European ancestry and breast cancer risk in Asian women (6, 11, 17, 18, 20–22, 25). We also investigated the association of one SNP in *PPARγ* that was found to be associated with mammographic density in Singaporean Chinese women (15).

We found that the direction of association of all SNPs previously reported to be associated with mammographic density was similarly associated with both percent density and dense area in our study with the exception of two SNPs: rs7289126 in *TMEM184B* and rs7816345 in *ZNF703*. Lindstrom and colleagues (17) reported that the A allele for rs7289126 was associated with a decrease in percent density and dense area, and the T allele in rs7816345 was associated with increased percent density. In our study however, we observed that the effects of these alleles were in the opposite direction from those reported previously, although neither of these were statistically significant. Notably, the

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Table 2. Analysis of SNPs associated with percent mammographic density, mammographic dense area, mammographic nondense area, and total breast area in Malaysian Chinese women ($N = 865$)

		Gene/	Alleles			Percent density[b]		Dense area[b]		Nondense area[b]		Total breast area[c]	
SNP ID	Chr	nearest gene	major/minor	MAF	HWE[a]	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value	β (SE)	*P* value
rs616488	1	*PEX14*	T/C	0.327	0.928	0.70 (0.56)	0.210	0.45 (0.62)	0.476	−1.78 (1.21)	0.144	−1.34 (1.35)	0.320
rs4951011	1	*ZC3H11A/ZBED6*	A/G	0.331	0.746	−0.35 (0.55)	0.529	−0.90 (0.62)	0.145	−1.63 (1.20)	0.176	−2.52 (1.33)	0.059
rs17625845	2	*INHBB*	T/C	0.075	0.557	0.36 (0.99)	0.714	1.31 (1.10)	0.237	2.85 (2.15)	0.185	4.16 (2.38)	0.081
rs4849887	2	*IHNBB*	C/T	0.269	0.415	1.26 (0.58)	0.031	1.31 (0.65)	0.045	−0.22 (1.27)	0.865	1.12 (1.41)	0.428
rs16857609	2	*DIRC3*	T/C	0.380	0.540	−0.05 (0.54)	0.930	−0.12 (0.60)	0.841	1.22 (1.17)	0.296	1.10 (1.29)	0.393
rs880663	3	*PPARγ*	T/C	0.375	0.345	−0.64 (0.55)	0.240	−0.27 (0.62)	0.667	1.36 (1.19)	0.255	1.08 (1.33)	0.415
rs4973768	3	*SLC4A7*	C/T	0.196	0.093	0.75 (0.64)	0.242	0.41 (0.72)	0.565	−0.93 (1.40)	0.506	−0.51 (1.55)	0.742
rs10034692	4	*AREG*	A/G	0.308	0.774	−1.45 (0.56)	0.010	−1.37 (0.63)	0.030	1.16 (1.22)	0.341	−0.19 (1.36)	0.889
rs62314947	4	*AREG*	C/T	0.306	0.694	−1.44 (0.56)	0.011	−1.47 (0.63)	0.020	0.97 (1.23)	0.433	−0.48 (1.37)	0.727
rs7697216	4	*ADAM29*	C/T	0.281	0.494	−0.04 (0.59)	0.951	0.63 (0.66)	0.344	0.33 (1.28)	0.798	0.96 (1.42)	0.498
rs10941679	5	*MRPS30*	G/A	0.468	0.768	−0.38 (0.53)	0.474	−0.85 (0.59)	0.150	−1.35 (1.15)	0.239	−2.17 (1.27)	0.088
rs10474352	5	*ARRDC3*	C/T	0.468	0.528	−0.10 (0.53)	0.854	−0.43 (0.59)	0.466	−0.28 (1.15)	0.808	−0.73 (1.28)	0.570
rs186749	5	*PRDM6*	T/C	0.495	0.038	−0.37 (0.54)	0.501	−0.56 (0.61)	0.353	0.64 (1.18)	0.587	0.09 (1.31)	0.946
rs1432679	5	*EBF1*	C/T	0.389	0.471	−1.21 (0.54)	0.026	−1.09 (0.61)	0.075	1.39 (1.19)	0.244	0.31 (1.32)	0.814
rs9485372	6	*TAB2*	G/A	0.416	0.615	−0.74 (0.53)	0.163	−0.38 (0.59)	0.524	0.49 (1.15)	0.672	0.12 (1.27)	0.923
rs2046210	6	*ESR1*	C/T	0.381	0.941	2.45 (0.53)	4.9E−6[d]	3.29 (0.59)	3.8E−8[d]	−0.23 (1.17)	0.842	3.05 (1.30)	0.019
rs12173570	6	*ESR1*	C/T	0.350	0.359	2.51 (0.55)	5.9E−6[d]	3.36 (0.61)	5.8E−8[d]	−0.14 (1.21)	0.905	3.20 (1.34)	0.017
rs10484919	6	*ESR1*	C/T	0.322	0.467	2.32 (0.56)	3.9E−5[d]	3.36 (0.63)	1.1E−7[d]	0.35 (1.24)	0.776	3.70 (1.37)	0.007
rs9693444	8	*RPL17P33*	C/A	0.283	0.514	0.55 (0.58)	0.340	0.59 (0.65)	0.358	−1.19 (1.25)	0.343	−0.60 (1.39)	0.666
rs7816345	8	*ZNF703*	C/T	0.336	0.499	−0.19 (0.55)	0.728	−0.11 (0.61)	0.860	−0.74 (1.19)	0.537	−0.86 (1.32)	0.518
rs7089814	10	*ZNF365*	T/C	0.308	0.609	−0.89 (0.56)	0.111	−0.21 (0.63)	0.744	1.27 (1.22)	0.300	1.07 (1.36)	0.431
rs10822013	10	*ZNF365*	T/C	0.498	0.071	−0.30 (0.54)	0.579	−0.14 (0.60)	0.817	0.51 (1.18)	0.663	0.38 (1.30)	0.770
rs1219648	10	*FGFR2*	A/G	0.370	0.825	0.10 (0.54)	0.849	−0.60 (0.61)	0.328	−1.46 (1.19)	0.218	−2.06 (1.31)	0.117
rs909116	11	*TNNT3*	C/T	0.328	0.888	0.21 (0.56)	0.703	0.35 (0.63)	0.577	−0.22 (1.22)	0.854	0.11 (1.35)	0.937
rs7107217	11	*BARX2*	A/C	0.297	0.264	0.85 (0.56)	0.129	1.45 (0.63)	0.021	−0.02 (1.20)	0.984	1.41 (1.34)	0.293
rs10771399	12	*PTHLH*	A/G	0.186	0.365	−0.55 (0.66)	0.406	−0.88 (0.74)	0.235	−1.48 (1.44)	0.304	−2.37 (1.60)	0.137
rs17356907	12	*NTN4*	A/G	0.257	0.596	−0.23 (0.60)	0.700	0.49 (0.68)	0.473	0.53 (1.32)	0.685	1.05 (1.46)	0.470
rs703556	12	-	T/C	0.131	0.974	−0.93 (0.77)	0.229	−1.34 (0.87)	0.122	0.09 (1.69)	0.956	−1.27 (1.87)	0.498
rs1265507	12	*TBX5* and *TBX3*	T/C	0.194	0.801	0.12 (0.67)	0.861	0.40 (0.75)	0.592	0.32 (1.45)	0.826	0.71 (1.61)	0.660
rs2236007	14	*PAX9*	G/A	0.286	0.855	0.02 (0.58)	0.972	−0.04 (0.65)	0.957	1.34 (1.26)	0.289	1.30 (1.40)	0.353
rs2290203	15	*PRC1*	C/T	0.492	0.913	0.33 (0.52)	0.526	0.42 (0.59)	0.476	−0.86 (1.14)	0.453	−0.44 (1.27)	0.726
rs4784227	16	*TOX3*	C/T	0.262	0.199	−0.87 (0.61)	0.153	−0.26 (0.68)	0.701	2.31 (1.33)	0.082	2.04 (1.47)	0.165
rs17817449	16	*FTO*	T/G	0.128	0.060	0.02 (0.81)	0.980	−0.70 (0.91)	0.439	−2.21 (1.76)	0.209	−2.91 (1.95)	0.137
rs11082321	18	*CABLES1*	G/A	0.195	0.228	0.77 (0.68)	0.253	0.27 (0.76)	0.720	−1.55 (1.47)	0.295	−1.27 (1.64)	0.437
rs7289126	22	*TMEM184B*	C/A	0.457	0.745	0.89 (0.53)	0.091	1.04 (0.59)	0.078	0.05 (1.15)	0.966	1.10 (1.28)	0.390
rs17001868	22	*SGSM3*	A/C	0.152	0.427	−1.60 (0.72)	0.026	−2.41 (0.80)	0.003	−0.64 (1.56)	0.684	−3.06 (1.73)	0.078

Abbreviation: Chr, chromosome.

[a]*P* value of the χ^2 test for HWE.

[b]Adjusted for age, BMI, menopausal status, and parity.

[c]Adjusted for age, BMI, and parity.

[d]Significant after Bonferroni correction considering 35 SNPs.

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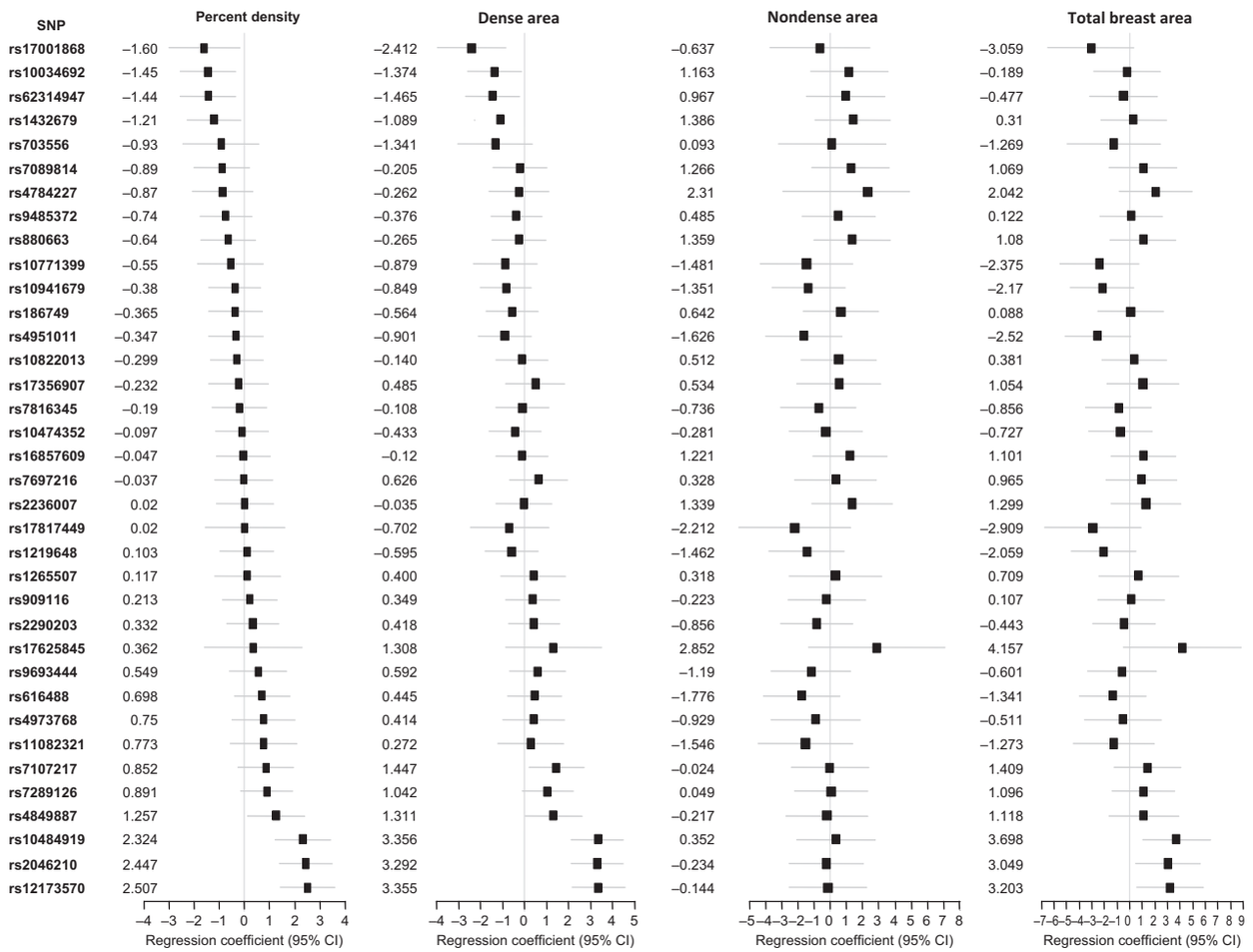

Figure 1.
Analysis of SNPs associated with percent mammographic density, mammographic dense area, mammographic nondense area, and total breast area in Malaysian Chinese women ($N = 865$).

associations for both rs7289126 with percent density and dense area and rs7816345 with percent density could not be replicated in a recent large-scale genetic association study including about 88,400 Swedish women (26).

The SNP rs4849887 in *INHBB* was the only SNP included in our study that was reported to be associated with nondense area at genome-wide significance level previously (10, 26). We observed a similar association with nondense area in the Chinese women.

Results from a recent association study of common breast cancer susceptibility variants with percent density, dense area, and nondense area involving over 10,700 women suggest that 18% of the variants associated with breast cancer risk are also associated with mammographic density (27). We found that the association of SNPs associated with breast cancer susceptibility was in the expected direction as that for breast cancer risk for all except four SNPs for percent density (rs4951011, rs2290203, rs4784227, and rs17817449) and seven SNPs for dense area (rs4951011, rs7697216, rs1219648, rs17356907, rs2236007, rs2290203, and rs4784227). None of the associations for the eight abovementioned SNPs were statistically significant, even before correcting for multiple testing ($P > 0.05$). Two explanations are possible. First, the effect of these loci on breast cancer risk may not be mediated by mammographic densities. Notably, previous GWAS have not reported any associations with mammographic densities in these regions. Second, effects of these breast cancer risk SNPs on percent density and dense area, if present, are likely to be very small and need further evaluation in a larger cohort.

Our results showed that all effect alleles of SNPs associated with breast size measured by bra cup size in a previous GWAS (11) were associated with total breast area, which we used in this study as a surrogate for breast size, in the same direction.

Overall, we found that the SNPs located in the *ESR1* region were most significantly associated with mammographic density. The 6q25.1 region was first identified as a breast cancer susceptibility locus in East Asians (25) and subsequently validated in women of European ancestry (18). Three variants in 6q25.1 had the strongest associations with mammographic density in our cohort. The SNPs, rs2046210 and rs10484919, previously shown to be associated with density and breast cancer risk in other populations (6, 7, 25), are also significantly associated with both percent density

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($P = 4.9e{-}06$; $P = 3.9e{-}05$) and dense area ($P = 3.8e{-}08$; $P = 1.1e{-}07$) in our study, in the expected direction as that for risk to disease. A two-stage fine mapping study of the 6q25 region in the Chinese population (28) revealed that rs10484919 is a candidate SNP for breast cancer risk where the risk allele is more common in Asian (35.4% in HapMap CHB) compared with European women (5.8% in HapMap CEU). The rs2046210 SNP is in strong linkage disequilibrium with rs60705924 ($r^2 = 0.93$, 1000 Genomes Asian populations), a variant in *CCDC170/ESR1* that was identified to be associated with volumetric and area-based mammographic density in Swedish women (26), but this was not evaluated in our study. Brand and colleagues (26) also reported a genome-wide significant association with density for another SNP, rs9485370, in the 6q25.1 locus mapping to the *TAB2* gene. In our study, we assessed the association of rs9485372, an Asian breast cancer risk SNP (20, 21), which is in strong linkage disequilibrium with rs9485370 ($r^2 = 0.97$, 1000 Genomes Asian populations) but did not find a significant association with density. Another SNP near *ESR1*, shown previously to be associated with breast size in European women, rs12173570 (11), was also found to be significantly associated with percent density ($P = 5.9e{-}06$) and dense area ($P = 5.8e{-}08$) in our study. This SNP is in high linkage disequilibrium with rs12665607 ($r^2 = 0.79$, 1000 Genomes Asian populations), which was reported to be associated with dense area previously (10). The rs12173570SNP is also in linkage disequilibrium with rs2046210 and another breast cancer susceptibility variant, rs9397435.

Taken together, our findings show that there is a shared genetic basis for mammographic density, breast cancer risk, and breast size in the 6q25.1 region in Chinese women. It is hypothesized that the estrogen receptor gene *ESR1* may play an essential role in estrogen-dependent mammary epithelial proliferation, which is associated with increased mammographic density and subsequently risk to breast cancer.

In addition to the *ESR1* locus, our study found four SNPs (rs4849887, rs10034692, rs62314947, and rs17001868) to be weakly associated with both percent density and dense area ($P < 0.05$). These results should be validated in a larger cohort of Asian women. Two of these SNPs (rs10034692 and rs62314947) are located in *AREG*. AREG is a member of the EGF family and promotes growth of normal epithelial cells by interacting with EGF and TGFα. The rs4849887 SNP is located in *INHBB*, which has been shown to be upregulated by estrogen (29). Interestingly, a previous study found that the C allele of rs17001868 was associated with mammographically dense area and breast cancer risk in opposing directions of association. The direction of association of rs17001868, in the *SGSM3* gene which may be involved in NF2-mediated growth suppression of cells (30), to dense area in this study was similar to that reported previously.

A meta-analysis of five GWAS in women of European ancestry showed that rs10995190 in *ZNF365* had the most significant association with mammographic density (6). The G allele in this SNP was found to be associated with a decrease in both density and breast cancer risk. We however could not evaluate this SNP in our study because the A allele was absent. Asian populations in the International HapMap Project have also reported low minor allele frequencies for this SNP. The minor allele frequencies for HapMap's Han Chinese in Beijing, Japanese in Tokyo, and Gujarati Indians in Houston were 0, 0.05, and 0.12, respectively. Identifying the causal locus will be useful to enable further investigation of this region in Asian women.

We did not observe a significant association of the variant in *PPARγ* that was associated with mammographic density in a study of more than 2,000 Singaporean Chinese women (15) with density in the Chinese women in this study. The directions of association and minor allele frequencies for this SNP however were similar in both studies (36% and 37.5%). We could not evaluate the association of another SNP, rs2241716 in *TGFβ1*, reported to be associated with density in East Asians (16) because this SNP was not in HWE in our cohort.

One of the strengths of this study is that the mammograms were obtained from the same mammography system for all women, and a fully automated thresholding method was used to measure mammographic density. Thus, variability due to differences in image acquisition parameters and inconsistencies in user-dependent thresholding methods is avoided.

The minor allele frequencies and directions of associations of several SNPs included in this study varied across the three ethnic groups. We therefore performed all analyses after stratifying by ethnic group. We however lacked statistical power to investigate the effect of the SNPs we selected in Malay and Indian women, and these need further investigation in a larger study. In addition, replication in an independent cohort of Asian women is warranted.

To the best of our knowledge, our study is the first study to evaluate the association of genome-wide significant mammographic density loci in Asian women. We conclude that although results from GWAS from other populations may be extrapolated to the Asian population, there are differences in risk allele frequencies and effect sizes of these variants across different populations. Various studies have reported ethnic differences in mammographic densities that correspond to variation in risk to disease across ethnic groups (13, 31–33). Genetic factors identified to date explain only a small fraction (<2%) of the variation in density. A GWAS in Asian women would enable identification of additional mammographic density–associated genetic regions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Mariapun, S.H. Teo
Development of methodology: S. Mariapun
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Mariapun, P.C.E. Kang, J. Li, C.H. Yip
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. Mariapun, W.K. Ho, J. Li, S. Lindström
Writing, review, and/or revision of the manuscript: S. Mariapun, W.K. Ho, P.C.E. Kang, J. Li, S. Lindström, S.H. Teo
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. Mariapun, P.C.E. Kang, J. Li
Study supervision: S.H. Teo

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