

## Genetic Variation in Transforming Growth Factor Beta 1 and Mammographic Density in Singapore Chinese Women

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### Abstract

TGF- $\beta$  plays a critical role in normal mammary development and morphogenesis. Decreased TGF- $\beta$  signaling has been associated with increased mammographic density. Percent mammographic density (PMD) adjusted for age and body mass index (BMI) is a strong risk factor and predictor of breast cancer risk. PMD is highly heritable, but few genetic determinants have been identified. We investigated the association between genetic variation in *TGFBI* and PMD using a cross-sectional study of 2,038 women who were members of the population-based Singapore Chinese Health Study cohort. We assessed PMD using a computer-assisted method. We used linear regression to examine the association between nine tagging single-nucleotide polymorphisms (SNP) of *TGFBI* and PMD and their interaction with parity, adjusting for age, BMI, and dialect group. We calculated *P* values adjusted for correlated tests ( $P_{ACT}$ ) to account for multiple testing. The strongest association was observed for rs2241716. Adjusted PMD was higher by 1.5% per minor allele ( $P_{ACT} = 0.04$ ). When stratifying by parity, this association was limited to nulliparous women. For nulliparous women, adjusted PMD was higher by 8.6% per minor allele ( $P_{ACT} = 0.003$ ; *P* for interaction with parity = 0.002). Three additional *TGFBI* tagging SNPs, which were in linkage disequilibrium with rs2241716, were statistically significantly associated with adjusted PMD ( $P_{ACT} < 0.05$ ) for nulliparous women. However, none of these three SNPs showed statistically significant association after adjusting for rs2241716. Our data support that *TGFBI* genetic variation may be an important genetic determinant of mammographic density measure that predicts breast cancer risk, particularly in nulliparous women. *Cancer Res*; 73(6): 1876–82. ©2012 AACR.

### Introduction

Percent mammographic density (PMD), the percentage of white-appearing tissue in the breast in radiographic films, is a measure of the amount of epithelium and stroma relative to the amount of fat tissue in the breast. PMD, adjusted for age and body mass index (BMI), is one of the strongest known predictors of breast cancer risk (1). Women with greater than 75% PMD have a 4 to 5 times higher risk of breast cancer than women of the same age and BMI with little or no density (<5%;

ref. 1). Data from twin and family studies suggest that genetic factors explain 30% to 60% of the variation in PMD, when adjusted for age, BMI, and other covariates (2–4). However, few genetic determinants of PMD have been identified, and the few existing studies were mainly conducted in European whites. A recent genome-wide association study (GWAS) of age and BMI-adjusted PMD among 4,877 women of European descent identified one locus (*ZNF365*—rs10995190) that was significantly associated with PMD, but this association explained only 0.5% of the variance in PMD (5). Results from a study of twins and their sisters identified several breast cancer susceptibility loci, including *LSPI*, that are associated with adjusted PMD (6). Subsequently, a large international collaboration (DENSEP consortium) of PMD in 16,895 Caucasian women found that 2 breast cancer susceptibility variants in the genes *LSPI* and *RAD51L1* are associated with age- and BMI-adjusted PMD (7), but these 2 single-nucleotide polymorphisms (SNP) after adjustment explained only 2% of interindividual variation in PMD (7).

It is well-accepted that TGF- $\beta$  functions as a tumor suppressor during early tumor development, but at later stages, it promotes tumor progression and metastasis (8, 9). TGF- $\beta$  also plays a critical role in normal mammary development and morphogenesis with growth-inhibitory properties (10, 11). Consistent with the antiproliferative role of TGF- $\beta$  in normal mammary tissue, decreased TGF- $\beta$  signaling was shown to

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be associated with increased breast density in a microarray-based analysis of normal breast tissue of patients with breast cancer (12).

Among the three isoforms of TGF- $\beta$  (TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3), TGF- $\beta$ 1 is most frequently unregulated in tumor cells (13). Two potentially functional SNPs of the gene encoding TGF- $\beta$ 1 (*TGFB1*) have been described and extensively studied in relation to breast cancer risk: a nonsynonymous polymorphism L10P (Leu10Pro substitution; rs1800470, formerly known as rs1982073) and a promoter polymorphism C-509T (rs1800469). These 2 SNPs are in strong linkage disequilibrium (LD) in European whites (14) and in Chinese (15). Minor allele of rs1800470 (L10P) was shown to increase TGF- $\beta$ 1 secretion *in vitro* (14). In human association studies, minor alleles of L10P and C-509T were associated with higher serum concentration of TGF- $\beta$ 1 (16, 17).

Data from the Breast Cancer Association Consortium (BCAC) have shown that P-allele of rs1800470 (L10P) was statistically significantly associated with a 4% increased breast cancer risk (18). When stratifying by tumor stage, this association was observed for advanced (stage II or above) breast cancer but not for stage I breast cancer (18). In an earlier report from the BCAC, where a limited number of ductal carcinoma *in situ* (DCIS) cases ( $n = 328$ ) were included, there was some indication that P-allele is inversely associated with risk of DCIS tumors (19). The OR associated with L10P among Asians was similar to that among Caucasians (18, 20). *TGFB1* C-509T (rs1800469) was not significantly associated with breast cancer risk in a 2010 meta-analysis including 10,197 cases and 13,382 controls (21), but there was a significant between-study heterogeneity. GWAS of breast cancer have genotyped several (i.e., 3–6) tagging SNPs in the *TGFB1* region, with about 30% to 50% tagging coverage (22–28). *TGFB1* SNPs were not among the top-hits in these GWAS, including one breast cancer GWAS conducted in an Asian population (24). However, GWAS is thought to have missed true susceptibility loci due to limited statistical power in the initial scan (28, 29).

Few studies have investigated the association between *TGFB1* SNPs and PMD. The DENSNP consortium analysis of Caucasian women investigated the association between *TGFB1* L10P and adjusted PMD but did not find a significant association (7). In the GWAS of PMD, which was a meta-analysis of 5 unpublished GWAS of PMD conducted in European whites, *TGFB1* SNPs were not reported to be associated with adjusted PMD (5); tagging coverage of *TGFB1* region was lower than 40% for the majority of participating studies (5), whereas the coverage was more than 80% in our study (see below). In the current study, we investigated the association between genetic variation in *TGFB1* and age- and BMI-adjusted PMD in healthy Singapore Chinese women. Parity, in particular, induces substantial proliferation, differentiation, and subsequent involution of breast tissue cells (30). As the effect of a genetic factor on PMD may differ between nulliparous and parous women, we hypothesized *a priori* to test interaction by parity. To our knowledge, *TGFB1* genetic variation has not been investigated in relation to PMD in Asians.

## Materials and Methods

### Study subjects

Details of the Mammography Subcohort who were women enrolled in both the Singapore Chinese Health Study (SCHS) and the Singapore Breast Screening Project (SBSP) have been described previously (31, 32). In brief, the SCHS includes 35,298 Chinese women and 27,959 men, ages 45 to 74 years, who were enrolled during 1993 to 1998. Subjects were residents of government housing estates; during the enrollment period, 86% of the Singapore population resided in such housing facilities. During 1994 to 1997, Singaporean women ages 50 to 64 years were invited for a screening mammography as part of the SBSP (33). We identified 3,777 women common to the SBSP and SCHS databases through a computer linkage and successfully retrieved mammograms of 3,702 women (98%). After excluding 6 women who had missing information on key variables and 1 woman who was later found not to be a Singapore resident, 3,695 women were included in the cross-sectional analysis of the Mammography Subcohort (31, 34, 35).

### Data collection

At entry to the SCHS, a trained interviewer conducted a face-to-face interview in the subject's home using a structured questionnaire that asked about demographic and anthropometric factors, menstrual and reproductive history (women only), medical history, family history of cancer, use of tobacco, and other dietary and lifestyle factors. We included only women without any history of cancer, as lifestyle habits may change as a result of cancer diagnosis, and the presence of tumor and/or cancer treatment may additionally influence PMD.

As part of the SBSP, participants completed a brief questionnaire that asked about demographic and body size characteristics and reproductive history. As factors such as age, BMI, parity, and menopausal status are established to be strongly associated with PMD, we used the data collected at the same time the mammogram was conducted, as part of the SBSP, for these analyses (31, 32).

PMD was assessed by one of the authors (G. Ursin) as previously described (31) using a highly reproducible computer-assisted method (31, 35–37). In brief, subjects' mammograms were scanned using a Cobrascan 812T scanner (Radiographic Digital Imaging Inc.) and Adobe Photoshop software with the plug-in program Scanwizard 3.0.9. Two research assistants trained by G. Ursin outlined the entire breast using an outlining tool on the computer screen and the computer counted the total number of pixels, which represents the total breast area. The average of the 2 assistants' readings was used. The density readings were done by G. Ursin: The reader draws a region of interest that excludes the pectoralis muscle and other artifacts. The software counts the number of pixels within the region of interest, which represents the area with absolute density. G. Ursin read the mammograms by random batches of 50. Each batch included subjects of all age groups. The PMD, or the mammographic percent density, is the absolute density (dense area) divided by the total breast area. Nondense area was calculated by subtracting dense area from

total breast area. The reproducibility on PMD assessment based on 237 pairs of duplicated mammograms was high ( $r = 0.97$ ,  $P < 0.0001$  for percent density;  $r = 0.97$ ,  $P < 0.0001$  for absolute density,  $r = 0.99$ ,  $P < 0.0001$  for total breast area; ref. 32).

The Institutional Review Boards at the National University of Singapore (Singapore, Singapore), the National Cancer Center Singapore (Singapore, Singapore), the University of Southern California (Los Angeles, California), the University of Minnesota (Minneapolis, Minnesota), and the University of Pittsburgh (Pittsburgh, Pennsylvania) had approved this study.

### Blood specimen collection and DNA extraction

In April 1994, we began collecting blood specimens from all consenting subjects from a 3% random sample of SCHS enrollees. Red cells, plasma, serum, and buffy coat were extracted from blood. Subjects who refused to donate blood were asked to donate buccal cells using a mouthwash protocol. Eligibility for this biospecimen subcohort was extended to all surviving cohort participants starting in January 2000. By April 2005, samples were obtained from 32,535 subjects (~60% participation). Of the 3,695 women evaluated for PMD, DNA samples were available on 2,164 women (1,848 blood, 316 buccal). Compared with the excluded members of the subcohort (i.e., those without DNA samples), study subjects were more educated and more likely to be Cantonese but otherwise similar to other subcohort members in factors such as age, BMI, parity, menopausal status, soy intake, and total caloric intake (32). All extracted components from blood/mouthwash specimens were stored in  $-80^{\circ}\text{C}$  freezers until analysis.

### Tagging SNP selection and genotyping

We selected tagging SNPs in the *TGFBI*, from 20 kb upstream of 5'-untranslated region (UTR) to 10 kb downstream of 3'-UTR. We tagged all common SNPs [minor allele frequency (MAF)  $\geq 5\%$ ] found among non-Hispanic white or Chinese populations, with  $r^2 \geq 0.80$ . This selection was done using the Snagger (38) software and a custom database of the Hapmap CEU data (release 24; ref. 39) merged with unique SNPs in the Affymetrix 500K panel as well as the Hapmap CHB data release 24.

Genotyping of 2,164 samples was conducted using the Illumina Golden Gate Assay (Illumina, Inc.) at the University of Southern California Epigenome Core Facility, as a part of 384 SNPs in multiple candidate gene pathways. We excluded 126 samples with a genotyping success rate (call rate) less than 85%; the remaining 2,038 samples were included in the current analyses. Genotyping concordance based on the 42 random duplicate samples was more than 99.9%. In the *TGFBI* region, we genotyped 11 SNPs. Of these, we excluded 2 SNPs that had  $\text{MAF} < 0.001$ . All remaining 9 SNPs did not depart significantly from Hardy-Weinberg equilibrium (HWE;  $P \geq 0.01$ ).

### Statistical analysis

We used linear regression to examine the association between *TGFBI* genotype and PMD, adjusting for age at mammogram, BMI ( $\text{kg}/\text{m}^2$ ) at mammogram, and dialect group

(Cantonese, Hokkien). Additional adjustment for other breast cancer risk factors including parity (nulliparous, 1–2, 3–4, 5+ deliveries), menopausal status (pre- and postmenopausal), and hormone therapy use (yes, no) did not change the results; these risk factors were not included in the final model. PMD and absolute density (i.e., dense area) had a skewed distribution. When we square root-transformed these variables, the results were identical with regard to the magnitude and statistical significance of the regression coefficients. We therefore present the results from the untransformed density variables. The linear regression models were based on additive genetic models, where the regression coefficients are estimates of the difference in PMD per copy of the minor allele of a given polymorphism.

We tested whether the association between *TGFBI* SNPs and PMD is modified by established determinants of PMD, such as parity, BMI, and menopausal status. Parity was defined as a dichotomized variable (nulliparous and parous). Additional analyses were conducted subdividing parous women by number of deliveries (1–2 deliveries, 3 or more). We evaluated the interaction between BMI ( $<24$ ,  $\geq 24$   $\text{kg}/\text{m}^2$ ), parity (nulliparous and parous; nulliparous, 1–2 deliveries, 3 or more deliveries), menopausal status (premenopausal, postmenopausal), and *TGFBI* genotype by introducing product terms and conducting Wald tests, adjusting for age, BMI (continuous), and dialect. We calculated "P values adjusted for correlated tests" ( $P_{\text{ACT}}$ ), which is a less conservative method to adjust statistical significance of correlated tests for multiple testing (40). Analyses were conducted using SAS 9.2 (SAS Inc.). All  $P$  values are 2-sided.

### Results

The LD structure of *TGFBI* was assessed using the Hapmap dataset of Chinese population (Hapmap CHB data; release 27; Supplementary Fig. S1; ref. 39). The 9 SNPs in the *TGFBI* that were analyzed in the current study tagged 82% of SNPs ( $\text{MAF} > 0.01$ ) in the Hapmap CHB population with pairwise  $r^2 > 0.80$ .

One (rs2241716) of 9 *TGFBI* SNPs showed a statistically significant association with PMD after multiple testing correction ( $P_{\text{ACT}} = 0.04$ ; Table 1) in a model adjusted for age, BMI, and dialect group. The estimated difference in adjusted PMD per minor allele of rs2241716 was 1.52%. The adjusted means (adjusted for age, BMI, and dialect) of PMD for women with rs224176 genotype of C/C, C/T, and T/T was 21.0% (SE, 0.5), 23.0% (SE, 0.6), and 23.5% (SE, 1.3), respectively (data not shown). When we conducted subgroup analyses stratified by parity, the association with rs2241716 was limited to nulliparous women (Table 2). The estimated PMD was higher by 8.55% per copy of minor allele (T) (uncorrected  $P = 0.0005$ ;  $P_{\text{ACT}} = 0.003$ ; Table 2) for nulliparous women. The adjusted means of PMD for nulliparous women with rs224176 genotype of C/C ( $n = 71$ ), C/T ( $n = 52$ ), and T/T ( $n = 10$ ) was 30.3% (SE, 2.0), 43.7% (SE, 2.3), and 38.9% (SE, 5.5), respectively (Supplementary Fig. S2). As the sample size for T/T genotype was limited, we repeated the analyses combining the C/T ( $n = 52$ ) and T/T ( $n = 10$ ) genotypes. Adjusted PMD was higher by 12.7% in women with C/T or T/T genotype than in women with C/C genotype (data not shown). For parous women, adjusted PMD

**Table 1.** Association between *TGFB1* SNPs and percent mammographic density, adjusted for age, BMI, and dialect group, in the Mammography Subcohort of the Singapore Chinese Health Study

SNP	Chromosome 19 position	Major/minor allele	MAF	N (WW/WV/VV)	$\beta$	95% CI	P	$P_{ACT}$
rs2241718	46521446	G/A	0.30	1000/841/188	0.10	-0.99 to 1.20	0.85	0.98
rs12981053	46524296	C/T	0.30	1001/838/189	0.13	-0.97 to 1.22	0.82	0.97
rs10417924	46525007	C/T	0.10	1652/356/21	-0.04	-1.73 to 1.65	0.96	>0.99
rs12983047	46526339	A/G	0.36	829/933/259	0.14	-0.91 to 1.20	0.79	0.99
rs8110090	46537712	A/G	0.004	2017/17/0	-1.02	-8.86 to 6.82	0.80	0.99
rs4803455	46543349	C/A	0.34	867/920/237	1.15	0.09 to 2.22	0.034	0.144
rs2241716	46545926	C/T	0.28	1054/806/171	1.52	0.42 to 2.63	0.007	0.041
rs1982072	46556349	T/A	0.42	663/1015/354	1.32	0.28 to 2.35	0.012	0.063
rs2241713	46561308	G/C	0.42	666/1015/344	1.24	0.20 to 2.28	0.019	0.094

NOTE: From linear regression models adjusting for age at mammogram (continuous), BMI at mammogram (continuous), and dialect group (Cantonese, Hokkien). Additive genetic model was used. Abbreviation:  $P_{ACT}$ , P values adjusted for correlated tests.

was higher by 1.13% per copy of T allele; however, this association did not remain statistically significant after multiple testing correction. P value for interaction between rs2241716 and parity was 0.002. When we further divided the parous group by number of births, the estimated regression coefficient (i.e., adjusted PMD difference per T allele) was 1.85 (P = 0.14) for women with 1 to 2 births and 0.82 (P = 0.18) for those with 3 or more births (P for interaction = 0.004 with 3 ordinal categories of parity; Supplementary Table S4).

For nulliparous women, 3 other *TGFB1* SNPs (rs4803455, rs1982072, rs2241713) showed statistically significant association: adjusted PMD was higher by 6.6% to 7.7% per minor allele of each SNP ( $P_{ACT}$  ranging from 0.006–0.018; Table 2). Minor alleles of these 3 *TGFB1* SNPs were positively correlated with minor allele of rs2241716, with a pairwise correlation coefficient ranging from 0.71 to 0.84 (Supplementary Table S1).

After adjusting for rs2241716, none of these 3 SNPs showed statistically significant association with adjusted PMD (P > 0.05; regression coefficient ranging from 1.5 to 2.1 per minor allele of each SNP). For parous women, none of these 3 *TGFB1* SNPs was associated with adjusted PMD. Compared with parous women, nulliparous women were significantly more likely to have lower BMI, earlier age at menarche, and receive formal and higher education (Supplementary Table S2). Additional adjustment for age at menarche and education did not change the results.

We evaluated the association of *TGFB1* SNPs with absolute density, total breast area, and nondense area for nulliparous women, adjusting for age, BMI, and dialect group (Table 3). Per minor allele of rs2241716, absolute density was higher by 11.3 cm<sup>2</sup> (P = 0.0007). *TGFB1* SNPs were not associated with total breast area and nondense area. For parous women, *TGFB1*

**Table 2.** Association between rs2241716 and other *TGFB1* SNPs and percent mammographic density, adjusted for age, BMI, and dialect group, by parity in the Mammography Subcohort of the Singapore Chinese Health Study

SNP	Major/minor allele	MAF	Nulliparous (n = 133)				Parous (n = 1,905)				
			n (WW/WV/VV)	$\beta$ (95% CI)	P	$P_{ACT}$	n (WW/WV/VV)	$\beta$ (95% CI)	P	$P_{ACT}$	$P_{interaction}$
rs2241718	G/A	0.30	63/54/15	1.08 (-3.49 to 5.64)	0.64	0.87	937/787/173	-0.05 (-1.17 to 1.06)	0.92	0.99	0.57
rs12981053	C/T	0.30	64/53/15	1.34 (-3.22 to 5.9)	0.56	0.87	937/785/174	-0.03 (-1.14 to 1.08)	0.95	0.95	0.50
rs10417924	C/T	0.10	107/24/1	-4.69 (-12.2 to 2.82)	0.22	0.57	1545/332/20	0.27 (-1.44 to 1.97)	0.76	0.99	0.19
rs12983047	A/G	0.36	54/60/18	1.18 (-3.31 to 5.67)	0.61	0.90	775/873/241	0.05 (-1.02 to 1.11)	0.93	0.99	0.57
rs8110090	A/G	0.004	132/1/0	—	—	—	1885/16/0	-0.71 (-8.59 to 7.18)	0.86	1.00	0.88
rs4803455	C/A	0.34	60/60/12	7.71 (3.07 to 12.3)	0.001	0.006	807/860/225	0.87 (-0.21 to 1.94)	0.11	0.40	0.004
rs2241716	C/T	0.28	71/52/10	8.55 (3.83 to 13.3)	0.0005	0.003	983/754/161	1.13 (0.01 to 2.24)	0.048	0.24	0.002
rs1982072	T/A	0.42	46/65/21	6.85 (2.45 to 11.3)	0.003	0.015	617/950/333	1.02 (-0.02 to 2.06)	0.055	0.25	0.007
rs2241713	G/C	0.42	47/65/20	6.58 (2.14 to 11.0)	0.004	0.018	619/950/324	0.97 (-0.08 to 2.02)	0.069	0.30	0.011

NOTE: The results are from linear regression models adjusting for age at mammogram (continuous), BMI at mammogram (continuous), and dialect group (Cantonese, Hokkien). Additive genetic model was used. Abbreviation:  $P_{ACT}$ , P values adjusted for correlated tests.

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**Table 3.** Association of *TGFB1* SNPs with absolute mammographic density, total breast area, and nondense breast area for nulliparous women ( $n = 133$ ), adjusted for age, BMI, and dialect group

SNP	Major/ minor allele	MAF	Mammographic density features					
			Absolute density		Total breast area		Nondense area	
			$\beta$ (95% CI)	<i>P</i>	$\beta$ (95% CI)	<i>P</i>	$\beta$ (95% CI)	<i>P</i>
rs2241718	G/A	0.30	6.34 (0.15 to 12.5)	0.045	7.22 (-1.23 to 15.7)	0.093	0.88 (-6.16 to 7.92)	0.80
rs12981053	C/T	0.30	6.56 (0.38 to 12.7)	0.038	7.13 (-1.31 to 15.6)	0.097	0.57 (-6.46 to 7.6)	0.87
rs10417924	C/T	0.10	-7.78 (-18.0 to 2.45)	0.13	-5.09 (-18.9 to 8.7)	0.47	2.69 (-8.81 to 14.2)	0.64
rs12983047	A/G	0.36	6.25 (0.16 to 12.3)	0.044	6.77 (-1.55 to 15.1)	0.11	0.52 (-6.41 to 7.45)	0.88
rs8110090	A/G	0.004	—	—	—	—	—	—
rs4803455	C/A	0.34	9.30 (2.85 to 15.8)	0.005	2.80 (-6.18 to 11.8)	0.54	-6.50 (-13.9 to 0.87)	0.08
rs2241716	C/T	0.28	11.3 (4.82 to 17.7)	0.0007	5.76 (-3.28 to 14.8)	0.21	-5.51 (-13.0 to 1.97)	0.15
rs1982072	T/A	0.42	9.71 (3.73 to 15.7)	0.002	4.88 (-3.47 to 13.2)	0.25	-4.84 (-11.7 to 2.07)	0.17
rs2241713	G/C	0.42	9.51 (3.47 to 15.6)	0.002	5.01 (-3.42 to 13.4)	0.24	-4.50 (-11.5 to 2.47)	0.20

NOTE: From linear regression models adjusting for age at mammogram (continuous), BMI at mammogram (continuous), and dialect group (Cantonese, Hokkien). Additive genetic model was used.

SNPs were not associated with any of the density measures (Supplementary Table S3). In our study, PMD was positively correlated with absolute density (Pearson correlation coefficient,  $r = 0.85$ ), and inversely correlated with nondense area ( $r = -0.66$ ) and total breast area ( $r = -0.27$ ).

BMI or menopausal status did not significantly modify the association between *TGFB1* SNPs and adjusted PMD (data not shown).

## Discussion

In this study of Singapore Chinese women, genetic variation in *TGFB1* was associated with age- and BMI-adjusted PMD; this association was statistically significant only in nulliparous women. To our knowledge, this is the first study to investigate an association between *TGFB1* genetic variation and PMD in Asians.

Our finding that genetic variation in *TGFB1* (rs2241716 or a causal variant tagged by rs2241716) is associated with adjusted PMD in Singapore Chinese women is consistent with the known biologic roles of TGF- $\beta$  signaling on mammary tissue. TGF- $\beta$  signaling has been shown to be critical for normal mammary development and morphogenesis. Experimental and animal studies have shown that TGF- $\beta$  signaling inhibits proliferation of human mammary epithelial cells and controls mammary gland morphogenesis (10, 11). Consistent with the antiproliferative role of TGF- $\beta$  in mammary tissue, normal breast tissue of patients with breast cancer who had high breast density showed decreased TGF- $\beta$  signaling compared with normal breast tissue of the patients who had low breast density (12). Our observation of effect modification by parity may be related to the fact that breast tissue undergoes substantial proliferation, differentiation, and involution through pregnancy and lactation (30). The association between *TGFB1* SNPs and PMD could have been weakened through this process.

Our findings, however, differ from the null findings in the 2 published studies on *TGFB1* SNPs and age- and BMI-adjusted PMD in European whites, although subgroup-specific results in nulliparous women were not presented in these studies (5, 7). Notably, rs2241716 was monomorphic in European whites (39), suggesting that previous studies (in European whites) were not likely to capture the causal variant linked to rs2241716. In addition, recent data from Norwegian women suggest that known determinants of adjusted PMD such as menopausal hormone use modify the association between genetic polymorphisms and adjusted PMD (41). Chinese women in Singapore rarely consume alcohol or use menopausal hormones and have smaller breast size and relatively low BMI than Western populations (31). Mean BMI of nulliparous Singapore Chinese women was low (23.0 kg/m<sup>2</sup>) in our study (Supplementary Table S2). These factors may explain the different findings between our study and the previous studies (5, 7).

TGF- $\beta$  signaling also has been implicated in breast carcinogenesis, and *TGFB1* genetic variation has been extensively studied in relation to breast cancer risk. Two recent meta-analyses of rs1800470 (L10P) suggest that the P allele is associated with an about 5% elevated risk of breast cancer (20, 42); the relative risk estimate in Asians was slightly stronger (OR, 1.11) than the estimate in whites (20). *In vitro* experiments have shown that cells overexpressing the P allele form of TGF- $\beta$ 1 secreted more than twice the amount of TGF- $\beta$ 1 than cells overexpressing the L allele form of TGF- $\beta$ 1 (14). The P allele also was found to be associated with increased serum concentration of TGF- $\beta$ 1 in Japanese (16). A promoter polymorphism rs1800469 (C-509T), which is in strong LD with rs1800470 in Whites (14) and in Chinese (15), was associated with increased serum TGF- $\beta$ 1 in whites (17). The totality of evidence, therefore, supports the notion of TGF- $\beta$ 1 duality in carcinogenesis (20), namely, that TGF- $\beta$ 1 promotes tumor progression in later stages of carcinogenesis (8, 9, 13).

Consistent with this view, data from BCAC suggest that P allele of rs1800470 is positively associated with stage II and III/IV breast cancer but not with stage I breast cancer (18) and that P allele may be inversely associated with DCIS (19). The OR (per P allele) for stage I, II, III/IV breast cancer was 1.02 [95% confidence interval (CI), 0.99–1.06], 1.06 (95% CI, 1.02–1.10), 1.11 (95% CI, 1.01–1.22), respectively (18); the OR (for LP vs. LL) for DCIS was 0.89 (95% CI, 0.70–1.13; ref. 19). Similar risk patterns were observed in one study of Chinese women, where P allele of rs1800470 was significantly associated with decreased risk of earlier stage breast cancer (*in situ* or stage I) but was nonsignificantly associated with increased risk of more advanced stage breast cancer (stage III or IV; ref. 15).

In the current study, the strongest association was observed for rs2241716. This SNP is located in the intron region of *TGFB1*. The 3 SNPs that are in LD with rs2241716 and showed significant associations are located in the intron region (rs4803455) or in 5' upstream of *TGFB1* (rs1982072 and rs2241713). Functional significance of these SNP as well as other genotyped SNPs in our study is not known. Nonetheless, rs2241716 is in moderate LD with L10P ( $r^2 = 0.46$ ) and C-509T ( $r^2 = 0.45$ ) in Asians in 1000 Genome dataset (43). Minor allele of rs2241716 (C/T) was linked to major alleles of L10P and C-509T, which have been shown to be associated with decreased serum level of TGF- $\beta$ 1 (14, 16, 17). *In vitro* data show that cells transfected with the L allele form of *TGFB1* exhibited reduced secretion of TGFB1 compared with cells transfected with P allele form of *TGFB1* (14). Decreased expression of TGF- $\beta$  signaling pathway genes has been associated with higher breast density in a microarray-based expression profiling study (12). Therefore, our observation that rs2241716 T allele is associated with increased PMD is consistent with existing evidence. The potential impact of our finding on breast cancer risk may have practical implications. The estimated 8% elevation in adjusted PMD per rs2241716 T allele for nulliparous women is comparable in magnitude with the 3% to 6% increase in adjusted PMD in women receiving 1 year treatment of estrogen–progestin combined hormone therapy (36, 44), an established risk factor of breast cancer (45, 46). However, given the likely dual roles of TGF- $\beta$ 1 signaling in breast carcinogenesis and the seemingly differential associations between *TGFB1* genetic variation and breast cancer risk by tumor staging (15, 18), the clinical significance of our findings is unclear at this time. *TGFB1* genetic variation may put some women at higher risk of developing breast cancer, but their tumors may be less likely to progress and metastasize. Further research is needed to replicate our findings and determine the relevance to breast cancer risk.

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To our knowledge, this study represents one of the first studies to investigate genetic determinants of adjusted PMD using a large, well-characterized population-based study of Asians. Recently, we reported a significant association between adjusted PMD and genetic variation in *PPAR $\gamma$* , a gene implicated in lipid and glucose metabolism in the same study population of Singapore Chinese women (32). Our current results suggest an association between *TGFB1* and adjusted PMD which appeared to be modified by parity. One limitation of our study is that 2 well-studied, potentially functional SNPs (namely, L10P and C-509T) were not included in our tagging SNP selection, thus precluding direct comparison of our findings with those from other epidemiologic studies of *TGFB1* on breast cancer risk. However, these 2 SNPs were in moderate LD with one tagging SNP that was significantly associated with adjusted PMD in our study. In conclusion, our data from a Singapore Chinese population support that *TGFB1* genetic variation may be important in determining age- and BMI-adjusted PMD, particularly in nulliparous women.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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**Conception and design:** G. Ursin, M.C. Yu, A.H. Wu

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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** D. Van Den Berg, W.-P. Koh, J.-M. Yuan, M.C. Yu, A.H. Wu

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