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Ages at menarche- and menopause-related genetic variants in relation to terminal duct lobular unit involution in normal breast tissue

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Abstract

Purpose—Reduced levels of terminal duct lobular unit (TDLU) involution, as reflected by higher numbers of TDLUs and acini per TDLU, have been associated with higher breast cancer risk.

Younger age at menarche and older age at menopause have been previously related to lower levels of TDLU involution. To determine a possible genetic link, we examined whether single nucleotide

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polymorphisms (SNPs) previously established in genome-wide association studies (GWAS) for ages at menarche and menopause are associated with TDLU involution.

Methods—We conducted a pooled analysis of 862 women from two studies. H&E tissue sections were assessed for numbers of TDLUs and acini/TDLU. Poisson regression models were used to estimate associations of 36 menarche- and 21 menopause-SNPs with TDLU counts, acini counts/TDLU, and the product of these two measures, adjusting for age and study site.

Results—Fourteen percent of evaluated SNPs (8 SNPs) were associated with TDLU counts at $p < 0.05$, suggesting an enrichment of associations with TDLU counts. However, only menopause-SNPs had $>50\%$ that were either significantly or nonsignificantly associated with TDLU measures in the directions consistent with their relationships shown in GWAS. Among 10 SNPs that were statistically significantly associated with at least one TDLU involution measure ($p < 0.05$), seven SNPs (rs466639: *RXRG*; rs2243803: *SLC14A2*; rs2292573: *GAB2*; rs6438424: *3q13.32*; rs7606918: *METAP1D*; rs11668344: *TMEM150B*; rs1635501: *EXO1*) were associated in the consistent directions.

Conclusions—Our data suggest that the loci associated with ages at menarche and menopause may influence TDLU involution, suggesting some shared genetic mechanisms. However, larger studies are needed to confirm the results.

Keywords

menarche; menopause; lobular involution; TDLU; SNP; breast histology

INTRODUCTION

Terminal duct lobular units (TDLUs) are the milk-producing structures of the breast and the predominant anatomical structures from which breast cancers originate [1]. As women age, the total number of TDLUs and acini (epithelial substructures) decrease, a process called age-related TDLU involution [2]. Studies have shown that less advanced TDLU involution, indicated by higher numbers of TDLUs and acini measured at a single time point, is a significant risk factor for breast cancer [3–6]. Recent longitudinal data also support that women whose TDLUs fail to involute are at higher subsequent breast cancer risk as compared with those who do not [7]. Further, measures of TDLU involution likely reflect a global process occurring throughout the breast as data show TDLU measures to be largely correlated within and between breasts in a woman [8–10]. Therefore, identification of factors that are associated with TDLU involution may reveal underlying biological pathways related to breast cancer risk.

Menarche indicates the onset of female sexual maturation and attainment of reproductive capacity that involves important physiologic changes of breast development [11,12]. Before or around the time at menarche, ovaries start producing estrogens and progesterone, which stimulate the branching of the duct system, appearance of TDLUs, and accumulation of fat in the connective tissue of breasts. During puberty, breasts continue to mature, growing in size and number of glands and lobules, to support lactation at a future birth. In contrast, menopause is marked by significant atrophy in the breast compared with premenopausal women. In response to the reduction in estrogen levels at menopause, glandular tissue of the

breast shrinks, which is then replaced by adipose tissue, and connective tissue becomes inelastic [11,12].

Younger age at menarche and older age at menopause are well-established risk factors for breast cancer [13], although the exact biologic mechanisms by which they are associated with risk have not been identified. Younger age at menarche and older age at menopause are thought to indicate high cumulative exposure to sex hormones [14] that stimulate cell proliferation [15] and breast development [16,11,12]. Previously, we have observed that women with higher estrogens and prolactin levels have higher TDLU counts [17,18]. We have also shown younger age at menarche and fewer years since menopause to be associated with higher TDLU counts [19], suggesting that increasing epithelial cell content, which indicates delayed involution, in part may mediate their associations with breast cancer risk. However, we have limited knowledge of the biological mechanisms that may influence TDLU involution.

Recently, genome-wide association studies (GWAS) have identified single nucleotide polymorphism (SNP) markers that are associated with ages at menarche [20] and menopause [21], which should further enhance our understanding of the biology of menarche and menopause as well as normal breast development. Findings from these studies suggest that ages at menarche and menopause are complex traits that involve various markers and pathways, some of which are related to obesity, hormonal regulation, coenzyme A and fatty acid biosynthesis, and DNA repair [20,21]. Given that menarche and menopause are critical periods when the breast undergoes large changes, many of the mechanisms related to menarche and menopause may also influence TDLU involution and thus breast cancer risk. In a pooled analysis of two studies, the Susan G. Komen Tissue Bank (KTB) at the Indiana University Simon Cancer Center [22] and the NCI Breast Radiology Evaluation and Study of Tissues (BREAST) Stamp Project [23], we examined whether SNPs related to age at menarche (36 SNPs) and age at menopause (21 SNPs) are associated with standardized measures of TDLU involution in order to provide some insights into the influence of potentially biologically relevant loci on the histology of the breast.

MATERIALS AND METHODS

Study population

The KTB is an annotated specimen bank which has recruited healthy volunteer women, aged 18–91 years, since 2007. The current analysis targeted a subset of 2,321 KTB participants who were recruited from January 10, 2009 to September 14, 2012. Participants provided demographic, lifestyle, and cancer-related information via self-administered questionnaire. Details of the KTB have been described elsewhere (<http://komentissuebank.iu.edu>) [22,19].

The NCI BREAST Stamp Project is a cross-sectional study of mammographic density conducted among 465 women, aged 40–65 years, who were referred for diagnostic image-guided breast biopsy from 2007 through 2010 at the University of Vermont College of Medicine and University of Vermont Medical Center. Women with a prior history of breast cancer or receipt of non-surgical treatment for any cancer, had undergone breast surgery within one year, had breast implants, or were taking breast cancer chemoprevention were

ineligible. A self-administered questionnaire and a supplementary telephone interview assessed health and breast cancer risk factor information. Details of the study have been described elsewhere [23].

Women were excluded if they had a diagnosis of *in situ* or invasive carcinoma at biopsy (n=177 KTB, n=78 BREAST) and if they were of non-white, non-European descent (n=555 KTB, n=23 BREAST), pregnant at biopsy (n=21 KTB), currently using hormone therapy (n=95 KTB, n=23 BREAST), and missing genotype data (n=796 KTB, n=17 BREAST) or TDLU measurements (n=1 KTB, n=14 BREAST). Repeated data from the previous donors (n=124 KTB) were also excluded. A total of 862 women (n=552 KTB, n=310 BREAST) were included in the final analytic population.

Participants provided written informed consent and the studies were approved by the Institutional Review Board at the Indiana University and the NIH Office of Human Subjects Research for the KTB and by the Institutional Boards at the University of Vermont and the National Cancer Institute for the BREAST Stamp Project.

KTB sample collection and DNA extraction

Whole blood samples were collected using Vacuette® EDTA tubes. DNA was extracted from blood cells at the Indiana CTSI Specimen Storage Facility (ICTSI-SSF) lab using an AutogenFlex Star (SN 401033) instrument and the Flexigene AGF3000 blood kit for DNA extractions from whole blood specimens following manufacturer's specifications. For this study, a 50 µL aliquot of sample was stored using Biomatrix® DNASTable® Handbook. Samples were reconstituted at the Cancer Genomics Research laboratory (CGR) (Frederick, MD, USA) for genotyping.

BREAST Stamp sample collection and DNA extraction

Whole blood samples were collected using standard techniques and allowed to clot for 30 minutes. Mouthwash samples were collected as previously described [24]. Blood and mouthwash samples were processed at the University of Vermont General Clinical Research Center. Frozen samples were shipped to SeraCare Life Sciences (Gaithersburg, MD), where they were stored in liquid nitrogen. At SeraCare, leukocyte DNA was isolated from blood clots using phenol chloroform extraction methods, and DNA was isolated from buccal cells using Puregene methods (Gentra Puregene Buccal Cell Kits, Qiagen). DNA was quantified at the CGR with the QuantiFluor® dsDNA System (Promega) according to the manufacturer's instructions.

Genotyping assay

Genotyping was performed at the CGR using Taqman nuclease assay (Taqman®), with reagents designed by Applied Biosystems (<http://www.appliedbiosystems.com/>) as Assays-by-Design™ and using the ABI PRISM 7900HT, 7700 or 7500 Sequence Detection Systems or Fluidigm format. Duplicate concordance was 100% for all SNPs.

SNPs were selected *a priori* from previous GWAS meta-analyses that examined associations with ages at menarche [20] and menopause [21,25] as continuous traits in years. We

confirmed that the allele frequencies and the directions of associations between the selected SNPs and ages at menarche and menopause estimated in our data were largely consistent with those previously reported in the GWAS, although we generally had limited power to detect the modest associations (Supplementary Table 1, methods in Appendix).

TDLU measurements

In the KTB, tissue cores were obtained from the upper outer quadrant of the breast using a 10-gauge needle. The tissue samples were then fixed in formalin to construct formalin-fixed paraffin embedded (FFPE) blocks. In the BREAST Stamp Project, FFPE blocks of benign breast tissue were collected from diagnostic ultrasound-guided core needle (14-gauge) or stereotactic-guided vacuum-assisted (9-gauge) breast biopsies. For both studies, each prepared FFPE tissue block was sectioned at 5 μ m and stained with hematoxylin and eosin (H&E). All TDLU measures in the H&E slides were centrally annotated at the NCI by a single study pathologist (MES) as previously described [19,9]. The H&E slides were scanned as digital images suitable for web-based viewing, electronic marking of regions of interest, and image analysis on Digital Image Hub software (SlidePath/Leica, Dublin, Ireland). TDLU analyzer software [18,26] was used to quantify the number of acini per TDLU (“acini count/TDLU”) for up to 10 TDLUs per woman and the median value was selected as a summary measure for each woman. A previous study [19] demonstrated high intra-observer agreement (Spearman $r > 0.90$) for the study pathologist for the TDLU measures. The TDLU measures were also inversely correlated with the subjective, qualitative evaluation of TDLU involution (none, partial, complete involution) that had been previously linked to breast cancer risk [3]. To estimate the cumulative epithelial content, a product of the TDLU counts and the median acini counts/TDLU was calculated for each woman.

Statistical analysis

All analyses were conducted using the pooled data of the two studies. For each outcome, Poisson regression models, with a robust variance estimator, were performed to examine the relationships with candidate SNPs. To confirm the robustness of results, linear regression models were additionally performed as secondary analyses. Because different needle sizes were used for the breast biopsies, we standardized TDLU counts by total tissue area on the H&E slides by using an offset in the Poisson models and by including total tissue area within the denominator of the outcome in the linear models. For linear models, TDLU measures were log-transformed to improve normality of the data. To avoid log-transformation of zeros, we added one to each TDLU measure before the transformation. Per-allele risk estimates were presented adjusted for age (10-year intervals) and study (KTB, BREAST), where coding of each variable with values of 0, 1, and 2 was based on the number of risk alleles (i.e., alleles associated with younger age at menarche or older age at menopause implicating increased breast cancer risk) that each woman carried. Additional adjustment for menopausal status, percent fat on the H&E slides, body mass index, parity, and ages at menopause and menarche did not materially change the results, and thus were not included in the final models. P-values for trend were estimated using the Wald test. In secondary analyses, we stratified by study, menopausal status, and parity to assess the variation in associations by these variables; we tested for heterogeneity by these variables

using likelihood ratio tests for interaction terms in the pooled model. For SNPs showing heterogeneous associations by study, we further stratified by low to high-risk benign lesion type (n=128 non-proliferative, 152 proliferative without atypia, 28 atypical hyperplasia) within the BREAST Stamp Project to examine whether the associations varied by biopsy diagnosis. In separate secondary analyses, we also created a composite polygenic risk score to assess the polygenic contribution of loci associated with ages at menarche and menopause by summing the number of risk alleles multiplied by the corresponding beta estimates from the previously published GWAS. Associations with TDLU involution measures were estimated for quintile categories of the polygenic risk scores.

All statistical tests were two-sided with 5% type I error. Because we used established SNPs identified from previous GWAS [20,21], we used the threshold of $p < 0.05$ to identify significant associations. We tested for enrichment of associations using binomial tests comparing the proportion of SNPs with significant associations to 5% which was expected by chance alone at 5% type I error. We also adjusted for multiple testing using Bonferroni correction (corrected $\alpha = 0.0003$ for 171 tests). To account for directions of associations, we compared the proportion of evaluated SNPs associated (regardless of significance) in the directions consistent with our expectation ($RR > 1.0$) based on their relationships with ages of menarche or menopause as shown in GWAS to 50% which would have been expected by chance.

Analyses were conducted with SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Participant characteristics

Of the 862 women evaluated in the two studies, the majority was premenopausal (72% in the KTB, 63% in the BREAST) (Table 1). Forty seven percent of KTB and 39% of BREAST participants had menarche at age 12 years. The mean age of the women was 43.1 years and, among postmenopausal women, the mean age at natural menopause was 49.8 years (49.4 years in the KTB, 50.3 years in the BREAST). Women from the KTB and BREAST studies were enriched for family history of breast cancer in a first degree relative compared with the general population at a similar frequency (23% in the KTB and 25% in the BREAST vs. 11% in the general U.S. population [27,28]). The median numbers of TDLUs per 100 mm² tissue area and acini per TDLU were 21.7 and 13.0 in the KTB and 18.8 and 11.3 in the BREAST.

TDLU counts

Out of 36 menarche SNPs and 21 menopause SNPs evaluated, eight SNPs (5 menarche and 3 menopause SNPs) were significantly associated with TDLU counts ($p < 0.05$) (Table 2, results for all 57 SNPs in Supplementary Table 2). The number of significant associations was significantly higher than that would have been expected by chance alone (14% vs. 5%; $p = 0.007$), suggesting an enrichment of association with TDLU counts. Among these, the risk alleles of rs466639 (*RXRG*), rs2243803 (*SLC14A2*), and rs2292573 (*GAB2*), which have been associated with younger age at menarche, were also associated with higher TDLU

counts. Rs7606918 (*METAP1D*), rs11668344 (*TMEM150B*), and rs1635501 (*EXO1*), which have been associated with older age at menopause, were associated with higher TDLU counts. Regardless of statistical significance, 50% of menarche SNPs and 69% of menopause SNPs were associated with TDLU counts in the consistent directions based on their associations with ages at menarche or menopause shown in GWAS.

Acini counts per TDLU

One menarche SNP and none of menopause SNPs were significantly associated with acini counts/TDLU ($p < 0.05$) (Table 3, results for all 57 SNPs in Supplementary Table 3), which does not demonstrate a significant enrichment of associations (1.8% vs. 5%; $p = 0.37$). Rs6438424 (3q13.32) was associated with both younger age at menarche and higher acini counts/TDLU. Regardless of statistical significance, 37% of menarche SNPs and 67% of menopause SNPs were associated with acini counts/TDLU in the consistent directions based on their associations with ages at menarche or menopause shown in GWAS.

The product of TDLU counts and acini counts per TDLU

Five menarche SNPs and one menopause SNP were significantly associated with the product of TDLU counts and acini counts/TDLU ($p < 0.05$) (Table 4, results for all 57 SNPs in Supplementary Table 4); however, significant enrichment of associations was not found (11% vs. 5%; $p = 0.16$). The risk alleles of rs2243803 (*SLC14A2*) and rs6438424 (3q13.32) have been associated with younger at age menarche and were related to higher product of TDLU counts and acini counts/TDLU. The risk allele of rs7606918 (*METAP1D*) has been associated with older age at menopause and was related to higher product of TDLU counts and acini counts/TDLU in this study. Regardless of statistical significance, 45% of menarche SNPs and 71% of menopause SNPs were associated with the product in the consistent directions.

Secondary analyses

Similar results were observed with linear models (Supplementary Tables 2–4). However, none of the associations remained statistically significant after Bonferroni adjustment.

Although the directions of associations did not vary by study, menopausal status, and parity, we observed statistically significant heterogeneity in the strength of associations by study and menopausal status (p -heterogeneity < 0.05) (data not shown). Specifically, a positive association of rs2243803 with acini counts/TDLU and the inverse associations of rs1398217 with product of TDLU counts and acini counts/TDLU were stronger in premenopausal women. Positive associations of rs6438424 with TDLU counts, acini counts/TDLU, and product of the two measures were stronger in the KTB. Associations of rs466639 and rs1635501 with higher TDLU counts were stronger in the BREAST. Within the BREAST, positive associations of rs466639 and rs1635501 with TDLU counts were suggestively stronger among women with proliferative vs. non-proliferative lesions (rs466639: RR=1.38 non-proliferative vs. 1.76 proliferative without atypia vs. 2.17 atypical hyperplasia; rs1635501: RR=1.25 vs. 1.41 vs. 1.31), although the differences were not statistically significant (p -heterogeneity=0.80 for rs466639, 0.86 for rs1635501).

No significant associations were observed with polygenic risk scores for ages at menarche and menopause (data not shown).

DISCUSSION

In our pooled analysis of *a priori* selected SNPs and standardized TDLU involution measures, we observed that a subset of SNPs previously found to be related to ages at menarche or menopause was also associated with standardized measures of TDLU involution. Specifically, out of 36 menarche SNPs and 21 menopause SNPs tested, 14% of evaluated SNPs were found to be significantly associated with TDLU counts, whereas only 5% would have been expected to be found by chance alone; these findings suggest an enrichment of associations with TDLU counts and are consistent with our previous data showing that self-reported early age at menarche is associated with higher TDLU counts [19]. Further, a total of 10 SNPs were associated with at least one TDLU involution measure; seven (4 menarche SNPs and 3 menopause SNPs) of these SNPs were associated in the directions consistent with our expectation based on their associations with ages at menarche or menopause as shown in GWAS. Our data suggest that some menarche- and menopause-SNPs may be related to variation in normal breast histology, specifically with regard to the number of TDLUs. In two cohorts of women with benign breast disease, women with lower levels of TDLU involution were at higher risk of developing breast cancer [29,3]. Menarche and menopause are critical periods of marked changes within the breast and both are related to hormonal fluctuations and cellular proliferation [11,16,12], which are likely to be reflected in the process of TDLU involution. However, when taking into account the direction of associations regardless of statistical significance, only menopause SNPs, but not menarche SNPs, had more than the expected number of SNPs (>50%) that were associated in the consistent directions based on their relationships with ages at menarche or menopause, suggesting that the loci associated with delayed menopause may be more closely related to the biological mechanisms underlying delayed lobular involution. Further, our data suggest that the biological mechanisms related to these loci may also influence TDLU involution, independent of ages at menarche and menopause, as their associations with TDLU and acini/TDLU counts persisted after adjustment for these variables in the models.

As expected based on their associations with ages at menarche or menopause reported in GWAS, the risk alleles for four menarche SNPs (rs466639: *RXRG*; rs2243803: *SLC14A2*; rs2292573: *GAB2*; rs6438424: 3q13.32) and three menopause SNPs (rs7606918: *METAP1D*; rs11668344: *TMEM150B*; rs1635501: *EXO1*) were significantly associated with higher TDLU counts, higher acini counts/TDLU, or higher product of these two measures. Although there is limited knowledge about the causal variants in each locus or the biological functions of the involved genes, some of these SNPs were located near or in the genes that are implicated in hormonal regulation (rs466639: *RXRG*), cell proliferation (rs2292573: *GAB2*), and DNA repair (rs1635501: *EXO1*), suggesting some plausible biologic mechanisms that may be involved in TDLU involution and breast tissue aging.

In contrast, two alleles related to younger age at menarche showed significant associations with lower TDLU counts. The findings could be due to chance or suggest that the alleles related to age at menarche are not related to normal breast microanatomy.

There was no overlap between the SNPs that were significantly associated with TDLU counts and the SNP that was associated with acini counts/TDLU. Although little is known about how TDLU involution occurs in the breast, TDLU counts and acini counts/TDLU may indicate different biologic processes of involution, as suggested by a modest correlation between these two measures [19,9]. TDLU involution may occur in women by either reducing the number of TDLUs or reducing the number of acini within the TDLUs, both resulting in decreased overall epithelial content in the breast. Our data suggest that genetic variants may differentially influence each of these processes.

Although the directions of associations were consistent, the magnitude of associations for three SNPs (rs6438424, rs466639, rs1635501) varied by study, possibly due to differences in population characteristics and biopsy methods. As women in the BREAST underwent diagnostic breast biopsies, they tended to be older and at higher risk of breast cancer. TDLU measures in the BREAST were also estimated using tissues obtained by targeted image-guided biopsies, which inherently captured more epithelial-rich tissue area than in the KTB. Our findings of two SNPs (rs466639: *RXRG*; rs1635501: *EXO1*) that were more strongly associated with TDLU counts in the BREAST suggest that these SNPs may be related to mechanisms that are more strongly associated with TDLU involution among the higher risk group by acting later in the natural history of breast cancer (i.e., closer to the disease state in the risk spectrum). This hypothesis was further supported in our secondary analysis that showed these SNPs were also suggestively more strongly associated with TDLU counts among women with proliferative (vs. non-proliferative) lesions, as women with proliferative lesions (with and without atypia) have higher breast cancer risk as compared to women with non-proliferative lesions [30].

Strengths of this study are the use of standardized, reproducible measures of TDLU involution, the use of validated genetic markers associated with ages at menarche and menopause, and the use of two study populations including a unique resource of normal breast tissues (the KTB). Limitations of the study are the relatively small sample size, despite the pooling of two study populations; hence, we had limited statistical power to detect modest associations and none of the associations remained significant after Bonferroni adjustment for multiple testing. Additional studies with larger samples sizes are needed to replicate these findings. Furthermore, the results may not be generalizable to other populations since our study populations were enriched for family history of breast cancer. However, it is also important to understand and identify biomarkers among women at elevated breast cancer risk.

In conclusion, our data suggest that the processes of TDLU involution may be influenced by the genetic loci associated with ages at menarche and menopause may influence TDLU involution, suggesting some shared genetic mechanisms underlying these factors. However, given the limited sample size of this study, larger studies are needed to confirm the genetic correlations between TDLU involution and reproductive risk factors for breast cancer. Our

findings also provide the basis to pursue a large-scale GWAS to identify susceptibility loci related to TDLU involution and provide further support for the evaluation of normal breast tissues, specifically TDLUs, in potentially clarifying etiologic pathways to breast cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of participants by study

	KTb (n=552)	BREAST (n=310)
	N (%)	
Age		
<30 years	180 (32.6)	0
30–39 years	105 (19.0)	0
40–49 years	127 (23.0)	162 (52.3)
50–59 years	85 (15.4)	112 (36.1)
60 years	55 (10.0)	36 (11.6)
Age at menarche		
12 years	260 (47.1)	120 (39.2)
13 years	169 (30.6)	113 (36.9)
14 years	123 (22.3)	73 (23.9)
Postmenopausal	157 (28.4)	113 (36.5)
Surgical menopause (among postmenopausal women)	18 (15.0)	7 (8.3)
Age at natural menopause		
<45 years	9 (11.8)	5 (8.1)
45–49 years	22 (29.0)	13 (21.0)
50–54 years	34 (44.7)	41 (66.1)
55 years	11 (14.5)	3 (4.8)
Body mass index		
<25 kg/m ²	222 (40.6)	140 (45.2)
25–29.9 kg/m ²	160 (29.3)	86 (27.7)
30 kg/m ²	165 (30.2)	84 (27.1)
Parity		
Nulliparous	267 (48.4)	79 (25.5)
1	64 (11.6)	39 (12.6)
2	140 (25.4)	125 (40.3)
3	81 (14.7)	67 (21.6)
First-degree family history of breast cancer	127 (23.0)	76 (24.9)
TDLU involution measures		
TDLU observed	382 (69.2)	226 (72.9)
	Median (IQR)	
Among women with at least one TDLU:		
TDLU counts per 100 mm ² area	21.7 (41.5)	18.8 (32.4)
Median number of acini per TDLU	13.0 (13.5)	11.3 (10.0)
Product of TDLU counts per 100 mm ² area and median number of acini per TDLU	284 (728)	212 (440)

Abbreviations: KTB=Susan G. Komen Tissue Bank, BREAST= NCI Breast Radiology Evaluation and Study of Tissues Stamp Project, TDLU=terminal duct lobular unit, IQR=interquartile range

Table 2
Top associations (p<0.05) of ages at menarche- and menopause-SNPs with TDLU counts in the pooled analysis

	Genomic region	Nearest gene(s)	Risk/non-risk allele ^b	AF ^c	N	Poisson model ^{1a}	
						RR (95% CI) per risk allele	p-trend
Menarche SNPs							
rs466639 *	1q23.3	RXRG	A/G	0.14	843	1.26 (1.05, 1.51)	0.01
rs1079866	7p14.1	INHBA	C/G	0.85	814	0.82 (0.69, 0.97)	0.02
rs2243803 *	18q12.3	SLC14A2	T/A	0.59	814	1.16 (1.02, 1.32)	0.02
rs9389666	6q16.2	PRDM13, MCHR2	C/G	0.40	803	0.87 (0.77, 0.98)	0.02
rs2292573 *	11q14.1	GAB2	T/C	0.84	804	1.23 (1.02, 1.48)	0.03
Menopause SNPs							
rs7606918 *		METAP1D	A/G	0.85	806	1.31 (1.07, 1.60)	0.01
rs11668344 *	19q13.42	TMEM150B	T/C	0.65	821	1.17 (1.01, 1.36)	0.04
rs1635501 *	1q43	EXO1	T/C	0.53	798	1.14 (1.00, 1.30)	0.04

^a Adjusted for study (KTB, BREAST) and age (<30, 30–39, 40–49, 50–59, 60 years).

^b Risk allele indicates an allele associated with younger age at menarche or older age at menopause. Based on their associations with ages at menarche and menopause in GWAS, we expect to observe RR>1 for their associations with TDLU counts.

^c Allele frequency of the risk allele

* Alleles with expected directions of associations based on their associations with ages at menarche or menopause as shown in GWAS.

Abbreviations: TDLU=terminal duct lobular unit, AF=allele frequency, RR=relative risk, CI=confidence interval, SNP= single nucleotide polymorphism

Table 3

Top associations (p<0.05) of ages at menarche- and menopause-SNPs with acini counts per TDLU in the pooled analysis

	Genomic region	Nearest gene(s)	Risk/non-risk allele ^b	AFC	N	Poisson model ^a	
						RR (95% CI) per risk allele	p-trend
Menarche SNPs							
rs6438424 *	3q13.32	3q13.32	T/G	0.52	840	1.15 (1.05, 1.28)	0.005

^a Adjusted for study (KTB, BREAST) and age (<30, 30–39, 40–49, 50–59, 60 years).

^b Risk allele indicates an allele associated with younger age at menarche. Based on their associations with age at menarche in GWAS, we expect to observe RR>1 for their associations with acini counts per TDLU.

^c Allele frequency of the risk allele.

^{*} Alleles with expected directions of associations based on their associations with age at menarche as shown in GWAS

Note: None of the 21 menopause-SNPs was significantly associated with acini counts per TDLU (p 0.05).

Abbreviations: TDLU=terminal duct lobular unit, AF=allele frequency, RR=relative risk, CI=confidence interval, SNP= single nucleotide polymorphism

Table 4

Top associations ($p < 0.05$) of ages at menarche- and menopause-SNPs with the product of TDLU counts and acini counts per TDLU in the pooled analysis

	Genomic region	Nearest gene(s)	Risk/non-risk allele ^b	AF ^c	N	Poisson model ^a	
						RR (95% CI) per risk allele	p-trend
Menarche SNPs							
rs1079866	7p14.1	INHBA	C/G	0.85	802	0.73 (0.59, 0.90)	0.003
rs9389666	6q16.2	PRDM13/MCHR2	C/G	0.40	791	0.82 (0.70, 0.95)	0.01
rs1398217	18q21.1	FUSSELL8	C/G	0.41	835	0.81 (0.68, 0.96)	0.01
rs2243803 [*]	18q12.3	SLC14A2	T/A	0.59	802	1.19 (1.02, 1.39)	0.03
rs6438424 [*]	3q13.32	3q13.32	T/G	0.52	833	1.22 (1.02, 1.45)	0.03
Menopause SNPs							
rs7606918 [*]		METAP1D	A/G	0.85	793	1.51 (1.16, 1.95)	0.002

^a Adjusted for study (KTB, BREAST) and age (<30, 30–39, 40–49, 50–59, 60 years).

^b Risk allele indicates an allele associated with younger age at menarche or older age at menopause. Based on their associations with ages at menarche and menopause in GWAS, we expect to observe $RR > 1$ and $\beta > 0$ for their associations with the product of TDLU counts and acini counts.

^c Allele frequency of the risk allele

* Alleles with expected directions of associations based on their associations with ages at menarche or menopause as shown in GWAS.

Abbreviations: TDLU=terminal duct lobular unit, AF=allele frequency, RR=relative risk, CI=confidence interval, SNP= single nucleotide polymorphism