No evidence for association of inherited variation in genes involved in mitosis and percent mammographic density

Celine M Vachon1*, Jingmei Li3,4, Christopher G Scott1, Per Hall3, Kamila Czene3, Xianshu Wang2, Jianjun Liu4, Zachary S Fredericksen1, David N Rider1, Fang-Fang Wu1, Janet E Olson1, Julie M Cunningham2, Kristen N Stevens1, Thomas A Sellers5, Shane V Pankratz1 and Fergus J Couch2

Abstract

Introduction: Increased mammographic breast density is one of the strongest risk factors for breast cancer. While two-thirds of the variation in mammographic density appears to be genetically influenced, few variants have been identified. We examined the association of inherited variation in genes from pathways that mediate cell division with percent mammographic density (PMD) adjusted for age, body mass index (BMI) and postmenopausal hormones, in two studies of healthy postmenopausal women.

Methods: We investigated 2,058 single nucleotide polymorphisms (SNPs) in 378 genes involved in regulation of mitosis for associations with adjusted PMD among 484 unaffected postmenopausal controls (without breast cancer) from the Mayo Clinic Breast Cancer Study (MCBCS) and replicated the findings in postmenopausal controls (n = 726) from the Singapore and Sweden Breast Cancer Study (SASBAC) study. PMD was assessed in both studies by a computer-thresholding method (Cumulus) and linear regression approaches were used to assess the association of SNPs and PMD, adjusted for age, BMI and postmenopausal hormones. A P-value threshold of 4.2 × 10^-5 based on a Bonferroni correction of effective number of independent tests was used for statistical significance. Further, a pathway-level analysis was conducted of all 378 genes using the self-contained gene-set analysis method GLOSSI.

Results: A variant in PRPF4, rs10733604, was significantly associated with adjusted PMD in the MCBCS (P = 2.7 × 10^-7), otherwise, no single SNP was associated with PMD. Additionally, the pathway analysis provided no evidence of enrichment in the number of associations observed between SNPs in the mitotic genes and PMD (P = 0.60). We evaluated rs10733604 (PRPF4), and 73 other SNPs at P < 0.05 from 51 genes in the SASBAC study. There was no evidence of an association of rs10733604 (PRPF4) with adjusted PMD in SASBAC (P = 0.23). There were, however, consistent associations (P < 0.05) of variants at the putative locus, LOC375190, Aurora B kinase (AURKB), and Minichromosome maintenance complex component 3 (MCM3) with adjusted PMD, although these were not statistically significant.

Conclusions: Our findings do not support a role of inherited variation in genes involved in regulation of cell division and adjusted percent mammographic density in postmenopausal women.

* Correspondence: vachon.celine@mayo.edu
1Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, MN 55905, USA
Full list of author information is available at the end of the article

© 2012 Vachon et al; licensee BioMed Central Ltd. This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Introduction

Mammographic density is a trait that represents the proportion of stromal and epithelial tissues in a radiographic image of the breast. Women with >50% dense tissue are at an estimated four- to six-fold increased risk of breast cancer relative to those with <10% [1,2].

Little is known about the biology of mammographic density or the mechanisms underlying the association between density and breast cancer. However, there is mounting evidence that genetic influences account for a large proportion of variation in mammographic density [3-5]. Indeed, it has been estimated that 61% to 67% of the variation in percent mammographic density adjusted for age and covariates, may be attributable to genetics [3]. To date, few loci have been shown associated with the mammographic density measures that predict breast cancer [6]. A recent study by Odefrey and colleagues [7] confirmed the association of a variant (rs3817198) in lymphocyte-specific protein 1, LSP1, with mammographic density. Importantly, this variant was initially identified as a breast cancer susceptibility locus [8]. Also, the first meta-analysis of genome-wide association studies of adjusted percent mammographic density identified an association with rs10995190 in ZNF365 [9], which has also been shown to be a risk factor for breast cancer [10]. Although these loci are promising, they explain little variation in the density measures and suggest other genetic variation for mammographic density remains to be determined.

Mammographic density has been hypothesized to reflect the cumulative exposure of breast stroma and epithelium to hormones and growth factors that can stimulate cell division and proliferation [11]. Evidence for this can be seen in the multiple studies showing positive associations of mammographic density with use of postmenopausal hormone therapy (PMH), especially estrogen and progesterone therapy [12-19], as well as with circulating IGF-1 (in premenopausal women) [20-23], both of which have been shown to exert proliferative effects on the breast. In one study of PMH, density, and tissue characteristics, PMH was associated with increased density, greater fibrous stroma, and less complex lobule type (lobule type 1), independent of estrogen and progesterone receptor up-regulation [24]. Increased density was also associated with Ki67 activity in the ducts and lobules [24], although this has not been confirmed in the majority of other studies [25-28]. These findings are consistent with the evidence that fibrous stroma differentiates dense and non-dense breast tissue [28-32]. Recent histologic studies that have compared targeted regions of dense and non-dense tissue in healthy patients suggest the proportion of connective tissue and relative cellularity of stromal cells is higher in dense vs. non-dense areas of the breast; this was not consistently seen or was seen to a lesser extent for the epithelial tissues [28,33]. We have also shown increased aromatase expression in stromal cells from dense vs. non-dense areas of the breast, which could result in increased production of estrogens, and consequent stimulation of cellular proliferation [34]. Furthermore, stromal cells can produce growth factors such as IGF-1 that may also stimulate proliferation through paracrine mechanisms [24,35].

Genes involved in regulation of cell division or mitosis could mediate the influence of these endogenous and exogenous exposures on breast tissue, reflected in variations in mammographic density. For instance, virgin Sprague-Dawley rats treated with the placental hormone human chorionic gonadotropin (hCG) to mimic pregnancy show unique genomic signatures, including expression of genes involved in cell division control, which were not seen in rats receiving 17beta-estradiol and progesterone [36]. In addition, non-epithelial nuclear area, which may represent increased nuclear size due to failed or delayed cell division, has been associated with mammographic breast density in women over age 50 [37]. Here we present a comprehensive analysis of the association between variation in 378 genes involved in regulation of mitosis and mammographic density in postmenopausal women.

Materials and methods

Study population

The Mayo Clinic Breast Cancer Study (MCBCS) is an on-going clinic-based case-control study initiated in February 2001 at Mayo Clinic, Rochester, MN. Details of the study design and data collection procedures have been previously described [38]. Briefly, cases were women over age 20 years with histologically confirmed primary invasive breast carcinoma enrolled within six months of the date of diagnosis. Controls without prior history of cancer (other than non-melanoma skin cancer) were matched on age (± 5 years) and region of residence to cases. Controls were selected from the outpatient clinic in the Department of Internal Medicine at Mayo Clinic where they were seen for general medical examinations. A self-administered risk factor questionnaire, blood sample, permission to obtain mammograms and written informed consent were obtained from all participants. Case participation was 69% (n = 798 cases) and control participation was 71% (n = 843 controls). All subjects provided written, informed consent and the protocol was reviewed by the Mayo Clinic Institutional Review Board.

Mammographic density measurement

Mammograms were only ascertained on controls for this analysis, as the focus of the study was to understand the genetics of mammographic density among healthy
women. The closest screening mammogram to enrollment date (median 0 days, 59% were same day, 82% within 1 year) was obtained and digitized on a Kodak Lumiscan 75 scanner (LS 75) (Lumisys/Eastman Kodak Co, Rochester, New York, USA) with 12-bit grayscale pixel depth for 686 of the 843 (81%) control women; analyses focused on the 484 mammograms (of 579 postmenopausal and 84%) from postmenopausal Caucasian women due to the composition of the SASBAC replication study. We estimated mammographic density using the cranial-caudal (CC) or top-down view from the left breast using a validated computer-assisted thresholding program (Cumulus [39] University of Toronto, Toronto, Ontario, Canada) that we have used in previous reports [40-42] and has been shown by our group to predict breast cancer [43]. We assessed percent mammographic density defined as the absolute area of dense tissue on the mammogram divided by the total area multiplied by 100. All images were read by one trained technician who consistently maintained high reliability ($r > 0.90$) while reading duplicate images across varying time frames [41,43].

**Gene and SNP selection**

We identified genes encoding proteins involved in regulation of all aspects of cell division, identified through the literature and known pathways. Specifically, we chose genes implicated in mitotic entry, mitotic progression, the mitotic checkpoint, cytokinesis and mitotic exit. In addition, we included genes implicated in mitotic function through functional screens [44,45] and genes involved in the structure and function of centrosomes [46], which are directly involved in chromosome segregation. SNPs representing common genetic variation within these 378 genes were identified and examined with percent mammographic density. SNP selection has been described in detail elsewhere [38,46]. Briefly, we first selected tagSNPs ($r^2 > 0.80$) from SNPs with MAF ≥ 0.05 located within 5 kb of the largest cDNA isoform (genome build 35) to represent a reduced set of SNPs in each gene [47]. We prioritized putative functional SNPs (within 1 kb upstream, 5' UTR, 3' UTR or non-synonymous) with MAF ≥ 0.05 identified in Ensembl version 34. A total of 2,058 SNPs in 378 genes were identified.

As detailed elsewhere [38], samples from both cases and controls, (including 5% duplicate samples), were assayed at Illumina Corporation (San Diego, CA, USA) on an Illumina BeadLab using the Illumina GoldenGate Assay™. DNA activation, incubation with assay oligonucleotides, PCR amplification and analysis using the BeadStudio software for automated genotype clustering and calling was performed according to a standard protocol [48-50]. Successful genotyping was achieved for 99.9% of DNA samples (seven case DNAs failed).

Analyses of SNPs with mammographic density were limited to postmenopausal controls. We assessed departures from Hardy-Weinberg equilibrium (HWE) ($P < 0.001$) in these 484 postmenopausal control subjects using a Pearson goodness-of-fit test. Of the 2,058 SNPs genotyped, 2,048 (99.5%) were in HWE, and SNP call rates were > 99% in 2,041 SNPs (2,053 SNPs > 98%). Also, only 24 (5%) of the 484 had sample call rates below 98%, but these were all above 95%.

**SASBAC study**

The Singapore and Sweden Breast Cancer Study (SASBAC) is a population-based case-control study of postmenopausal breast cancer in women aged 50 to 74 years born in Sweden. Details on data collection and subjects have been described previously [51]. Controls were white Europeans randomly selected from the Swedish population and frequency matched to the expected age distribution of cases and on geographical area. They served as the replication sample for this study. The final study group with both mammographic density and genotype data included 726 controls of 764 eligible (95%). Approval of the study was given by the ethical review board at the Karolinska Institute (Stockholm, Sweden) and six other ethical review boards in the respective regions in which the subjects were based.

Screening film mammograms corresponding to the enrollment date were obtained. The medio-lateral oblique (MLO) view was digitized using an Array 2905HD Laser Film Digitizer (Array Corporation, Roden, The Netherlands, which covers a range of 0 to 4.7 optical density. Similar to the MCBCS, the Cumulus software was used for determination of percent mammographic density on a randomly selected side. A random 10% of the images were included as replicates to assess the intra-observer reliability, which was high with a Spearman rank correlation coefficient of 0.95.

Associations of any SNP with $P < 0.05$ in MCBCS by the log-additive test were attempted for in silico replication within SASBAC using available genotype information from a genome wide association study (GWAS) of breast cancer [52]. Briefly, 764 controls were genotyped on the HumanHap550 BeadChip; of these, 726 (95%) controls had films available. When the exact SNP was genotyped and available as part of the SASBAC GWAS (which occurred 56% of the time), the corresponding $P$-value for that SNP was used. If the exact SNP was not available, we examined the association with available SNPs in high linkage disequilibrium (LD), defined as $r^2 > 0.70$ with the SNP of interest, using HapMap CEU.

**Mammographic density comparison studies**

We compared the similarity of percent mammographic density (PMD) assessment between the readers from
MCBCS and SASBAC using a standard set of 20 film mammogram images across varying densities. The intra-reader reliability assessed as the intraclass correlation or ICC for PMD between readers was high (ICC = 0.99; Figure 1). We also assessed the intraclass correlations between our readers with Dr. Norman Boyd, an expert in the estimation of density and found strong agreement (ICC = 0.98 and 0.99 for MCBCS and SASBAC, respectively, Figure 1).

Further, since MCBCS and SASBAC ascertained and estimated PMD from different mammogram views (CC vs. MLO, respectively), we were interested in the differences in PMD between the two mammogram views. A previous study of 30 women found strong correlations of CC and MLO views (between 0.86 and 0.96), suggesting representative information is provided in a single view [53]. We conducted a larger study of 700 controls with both right and left CC and MLO views [43]. We examined the differences in mean PMD as well as Pearson correlations (r) assessed from the CC and MLO views from the same breast. We found the average absolute difference in PMD between CC and MLO views to be 2.0% (SD = 6.5) for the left and 2.2% (SD = 6.4%) for the right breast. The Pearson correlations between the PMD from CC and MLO were also very high, with r = 0.90 for both left and right breasts (Figure 2).

**Statistical analysis**

Primary analyses focused on the 484 postmenopausal controls in the MCBCS study, since SASBAC was
comprised only of postmenopausal women. Initially, we examined the distribution of risk factors and mammographic density among postmenopausal controls from MCBCS and SASBAC. Genotypes from controls were used to estimate allele frequencies within each study set.

Individual SNP associations with PMD were assessed using linear regression. No transformation of PMD was made in MCBCS since residuals were approximately normal. Tests for associations were carried out assuming an ordinal (log-additive or additive) genotypic relationship using simple tests for trend within the linear regression models. All analyses were adjusted for age, body mass index (BMI) and current post-menopausal hormone (PMH) use. Examination of transformations of BMI and age did not result in substantial improvement in model fit when compared to models that were based on the original scale of these covariates. Because of this, and because linear regression assumptions were met in the analysis models, the original scale for these variables was used in all analyses on MCBCS data.

Similar analyses were performed for the replication of SNPs in the SASBAC sample with PMD, although a square root transformation of PMD was required to meet linear regression assumptions. Analyses were adjusted as above. Mean PMD from SASBAC was back-transformed for each genotype within the context of the general model in order to more directly compare results to those from MCBCS.

A pathway-level analysis was conducted using the self-contained gene-set analysis method, GLOSSI [54]. This algorithm, based on Fisher’s combined probability test, is designed to determine if the distribution of $P$-values in a set of genes deviates from what is expected based on the null hypothesis of no association. GLOSSI was implemented using 2,028 SNPs from the 378 genes among the 484 postmenopausal breast cancer-free controls in the MCBCS study to test for an association with this pathway and PMD adjusted for age, BMI and PMH use as above. A pathway-level $P$-value was obtained based on 500 permutations.

To assess heterogeneity of associations by study, a meta-analysis was performed on the ordinal parameter estimates and the Q-test was calculated [55]. In order to make the ordinal estimates comparable between studies for the meta-analysis, ordinal estimates for the MCBCS sample were estimated using square root transformed percent density within this sample. These parameter estimates were used to calculate the Q-test statistic and resulting heterogeneity $P$-value.

In order to set a threshold for statistical significance that appropriately reflects the number of SNPs tested, recognizing that SNPs within genes may not be independent, we calculated an effective number of

![Figure 2 Comparison of percent mammographic density estimation on MLO vs. CC views from 700 controls](http://breast-cancer-research.com/content/14/1/R7)
independent tests within each gene using an eigenvalue based measure as proposed by Galwey [56]. We summed the effective number of independent tests per gene across all genes in the study to estimate the effective number of independent tests \( (n = 1,178 \text{ for stage I and } n = 64 \text{ for replication}) \). We use this result to set our threshold for significance via a Bonferroni correction for the number of independent tests \( (0.05/1,178 = 4.2 \times 10^{-5} \text{ for stage I and } 0.05/64 = 7.8 \times 10^{-4} \text{ for replication}) \). Analyses were implemented using SAS (SAS Institute, Cary, NC, USA, Version 8, 1999), S-Plus (Insightful Corp, Seattle, WA, USA, Version 7.05, 2005) and R software systems.

**Results**

The 2,058 SNPs from 378 genes were examined for associations with PMD among 484 postmenopausal women within MCBCS. Characteristics of the MCBCS postmenopausal controls are described in Table 1. One variant, rs10733604 in PRPF4, was associated with adjusted PMD \( (P = 2.7 \times 10^{-7}) \). A second, albeit not statistically significant, association was seen with rs12563929 in PRKACB \( (P = 2.2 \times 10^{-4}) \) (Additional file 1). In total, we found 88 SNPs in 58 genes associated at \( P < 0.05 \) with percent density. These SNPs were selected for examination within the SASBAC study (Additional file 1). The pathway analysis incorporating all 378 genes showed no evidence of enrichment in the number of associations observed between SNPs in the mitotic genes and PMD \( (P = 0.60) \).

The 726 controls from SASBAC were slightly younger (mean age 62.8 ± 6.2 vs. 63.6 ± 9.2), less likely to use PMH (38.7% vs. 65.3%) and had lower average BMI (25.7 vs. 26.9) and PMD (14.4% vs. 18.6%) than the MCBCS controls (Table 1). Both studies showed inverse associations of age and BMI with PMD and positive associations with current PMH use (Table 2).

Within SASBAC, genotype information was available for 73 of the 88 SNPs (located in 51 genes) associated with PMD at \( P < 0.05 \) in MCBCS. Of these, 56% were the exact SNP but the remainder were SNPs in moderate to high linkage disequilibrium \( (LD > 0.70) \) with the SNP of interest within MCBCS. Both of the SNPs rs10733604 in PRPF4 and rs12563929 in PRKACB were available in SASBAC (noted in Additional file 1). There were no associations of rs10733604 \( (P = 0.23) \) or rs12563929 \( (P = 0.93) \) with percent density in the SASBAC study. Eight of the 73 (10.9%) candidate SNPs, located in seven genes, displayed associations also at \( P < 0.05 \) with PMD in the SASBAC study (Table 3), although none reached the statistical threshold. Only variants in LOC375190 (rs2080727) AURKB (rs4792590 and rs3027260, \( LD \) of \( r^2 = 0.71 \)) and MCM3 (rs3765447), showed consistent direction of effect in both the MCBCS and SASBAC studies, which was also reflected in the tests of heterogeneity (Table 3).

**Discussion**

Overall, we found no statistically significant associations between SNPs involved in mitosis with percent density.

---

**Table 1 Characteristics of two postmenopausal control populations (MCBCS\(^a\), 2001-2005 and SASBAC\(^b\), 1993-1995).**

| Characteristic                  | Level               | MCBCS\(^a\) \(n = 484\) | SASBAC\(^b\) \(n = 726\) |
|--------------------------------|---------------------|--------------------------|---------------------------|
| Age, years \(40 \text{ to } 49\) | 26 \(5.4\)          | 1 \(0.1\)                |
| Age, years \(50 \text{ to } 59\) | 157 \(32.4\)        | 237 \(32.6\)             |
| Age, years \(60 \text{ to } 69\) | 176 \(36.4\)        | 363 \(50.0\)             |
| Age, years \(70+\)             | 125 \(25.8\)        | 125 \(17.2\)             |
| Body mass index, kg/m\(^2\)    | Mean (SD) \(464 \text{ (5.3)}\) | 718 \(25.7 \text{ (4.1)}\) |
| Postmenopausal hormone use      | Current \(153 \text{ (3.6)}\) | 95 \(13.1\)             |
| Postmenopausal hormone use      | Former \(163 \text{ (3.7)}\) | 186 \(25.6\)             |
| Postmenopausal hormone use      | Never \(136 \text{ (2.8)}\) | 362 \(49.9\)             |
| Postmenopausal hormone use      | Unknown \(32 \text{ (6.6)}\) | 83 \(11.4\)             |
| Percent Mammographic Density, (%)| Mean (SD) \(484 \text{ (13.9)}\) | 726 \(14.4 \text{ (13.9)}\) |

\(^a\)Mayo Clinic Breast Cancer Study. \(^b\)Singapore and Sweden Breast Cancer Study.

---

http://breast-cancer-research.com/content/14/1/R7
mammographic density. There were consistent associations (albeit only at a significance of $P < 0.05$) among four SNPs in three genes involved in either cell division ($AURKB$ and $LOC375190$) or cellular proliferation ($MCM3$) and adjusted percent density among two studies of postmenopausal women. These associations warrant further investigation.

Strengths of this study include examination of a novel pathway with adjusted percent mammographic density, SNP associations examined in two independent populations, similar quantitative measures of density used in both studies, adjustment for potential confounding factors, and focus on the homogenous subgroup of postmenopausal, healthy women. These associations warrant further investigation.

Strengths of this study include examination of a novel pathway with adjusted percent mammographic density, SNP associations examined in two independent populations, similar quantitative measures of density used in both studies, adjustment for potential confounding factors, and focus on the homogenous subgroup of postmenopausal, healthy women. These associations warrant further investigation.

Table 2 Adjusted mean percent mammographic density by age, BMI, postmenopausal hormone use (MCBCS and SASBAC controls)$^a$.

| Characteristic                  | Level       | MCBCS Controls $(n = 484)$ | SASBAC Controls $(n = 726)$ |
|--------------------------------|-------------|----------------------------|----------------------------|
|                                |             | Mean (95% CI)              | Mean (95% CI)              |
| Age, years                     | 40 to 49    | 23.6 (18.3 to 28.9)        | 8.2 (14.9 to 18.6)         |
|                                | 50 to 59    | 20.1 (17.9 to 22.2)        | 16.7 (12.4 to 15.1)        |
|                                | 60 to 69    | 18.2 (16.1 to 20.2)        | 13.7 (12.0 to 14.5)        |
|                                | 70+         | 16.4 (14.0 to 18.8)        | 9.5 (9.5 to 14.5)          |
| Body mass index, kg/m$^2$      | < 25        | 24.8 (24.0 to 27.6)        | 19.2 (17.5 to 20.8)        |
|                                | 25 to 30    | 16.0 (14.2 to 17.9)        | 11.2 (10.0 to 12.5)        |
|                                | > 30        | 10.6 (8.2 to 13.0)         | 6.8 (5.1 to 8.5)           |
| Postmenopausal hormone use$^c$ | Current     | 21.9 (19.8 to 24.0)        | 19.6 (16.4 to 22.7)        |
|                                | Former      | 18.1 (16.1 to 20.2)        | 17.0 (14.9 to 19.1)        |
|                                | Never       | 15.6 (13.4 to 17.9)        | 11.7 (10.6 to 12.8)        |
|                                | Unknown     | 15.9 (10.2 to 21.5)        | 10.5 (3.9 to 17.2)         |

*Mayo Clinic Breast Cancer Study; Singapore and Sweden Breast Cancer Study.

$^a$ Adjusted for age. $^b$ Adjusted for age and BMI.

Interestingly, two of the genetic loci ($AURKB$, $LOC375190$) containing SNPs that displayed consistent, albeit nonsignificant, associations with adjusted percent mammographic density in the MCBCS and SASBAC studies, have been implicated in the regulation of the metaphase to anaphase transition during chromosome segregation. Although little is known about the protein encoded by $LOC375190$, a functional siRNA based screen has shown that reduced levels of $LOC375190$ causes severe spindle defects and mitotic arrest and subsequent formation of polyploid cells due to premature mitotic exit [44]. In contrast, much is known about the role of the $AURKB$-encoded Aurora B protein in mitotic regulation. While Aurora B has been implicated in mitotic entry and also in cytokinesis and mitotic exit, the primary role for this kinase is in the assembly of factors involved in spindle attachment and tension and regulation of the mitotic checkpoint. Loss or gain of Aurora B results in defects in the metaphase to anaphase transition and subsequent aneuploidy due to chromosome segregation defects or polyploidy due to premature mitotic exit. Interestingly, Aurora B is localized at centrosomes during mitosis and may influence spindle growth from the centrosome to the kinetochore during mitosis, similar to $LOC375190$, resulting in defects in chromosome segregation and/or premature mitotic exit [57]. Given these common functions, it is tempting to speculate that common genetic variation in these two loci may result in defects in chromosome segregation,
premature mitotic exit and an increase in the number of cells with multiple nuclei.

Since non-epithelial nuclear area, which may represent increased nuclear size due to failed or delayed cell division, has been associated with mammographic breast density in women over age 50 [37], studies of cell division in dense and non-dense mammary tissues may provide further insight into the associations reported here. However, since these proteins are multifunctional and may influence other cellular processes, including growth factors [58], alternative explanations for the associations with density must also be considered.

The third locus of interest, a variant in minichromosome maintenance complex component 3 or MCM3, however, plays a role in cellular proliferation. The protein encoded by MCM3 is one of the highly conserved minichromosome maintenance proteins (MCM) that are involved in the initiation of genome replication.

| Chr | Gene Name   | Effect | SNP   | Position (bp) | N     | PMD Est (SE)/adj mean | P-value | N     | Sqrt PMD Est (SE)/adj mean | P-value | P-Het |
|-----|-------------|--------|-------|--------------|-------|-----------------------|---------|-------|----------------------------|---------|-------|
| 1   | GALNT2      | Ordinal| rs1043908| 22843917   | 484   | 2.88 (1.28)            | 0.025   | 725   | -0.314 (0.135)             | 0.02    | < 0.001 |
|     | A/A         |        | 373    | 17.9        | 555    | 19.0                  |         |       |                            |         |       |
|     | A/G         |        | 102    | 20.8        | 158    | 16.3                  |         |       |                            |         |       |
|     | G/G         |        | 9      | 23.6        | 12     | 13.9                  |         |       |                            |         |       |
| 2   | LOC375190   | Ordinal| rs2080727| 24204411   | 484   | 1.69 (0.85)            | 0.048   | 725   | 0.185 (0.095)             | 0.05    | 0.54   |
|     | A/A         |        | 195    | 17.2        | 305    | 17.1                  |         |       |                            |         |       |
|     | A/G         |        | 215    | 19.4        | 332    | 18.7                  |         |       |                            |         |       |
|     | G/G         |        | 74     | 20.2        | 88     | 20.3                  |         |       |                            |         |       |
| 6   | MCM3        | Ordinal| rs3765447| 52249471   | 483   | 3.62 (1.76)            | 0.040   | 725   | 0.45 (0.188)              | 0.017   | 0.96   |
|     | A/A         |        | 422    | 18.2        | 644    | 17.7                  |         |       |                            |         |       |
|     | A/G         |        | 60     | 22.3        | 76     | 21.7                  |         |       |                            |         |       |
|     | G/G         |        | 1      | 12.7        | 5      | 26.1                  |         |       |                            |         |       |
| 8   | TNKS        | Ordinal| rs12549064| 9479437   | 484   | -2.15 (1.09)           | 0.048   | 725   | 0.321 (0.116)             | 0.006   | 0.001 |
|     | A/A         |        | 303    | 19.5        | 496    | 17.8                  |         |       |                            |         |       |
|     | A/C         |        | 166    | 17.4        | 205    | 20.6                  |         |       |                            |         |       |
|     | C/C         |        | 15     | 15.1        | 24     | 23.6                  |         |       |                            |         |       |
| 10  | KIF11       | Ordinal| rs2275220| 94362686   | 484   | -4.23 (2.12)           | 0.046   | 726   | 0.45 (0.172)              | 0.009   | 0.005 |
|     | A/A         |        | 448    | 19.0        | 613    | 17.9                  |         |       |                            |         |       |
|     | A/G         |        | 34     | 13.5        | 111    | 22.0                  |         |       |                            |         |       |
|     | G/G         |        | 2      | 20.4        | 2      | 26.4                  |         |       |                            |         |       |
| 11  | STIM1       | Ordinal| rs3794050| 4068476   | 484   | 2.85 (1.38)            | 0.039   | 726   | -0.383 (0.142)            | 0.007   | 0.003 |
|     | G/G         |        | 389    | 17.8        | 547    | 19.4                  |         |       |                            |         |       |
|     | G/A         |        | 89     | 23.0        | 173    | 16.2                  |         |       |                            |         |       |
|     | A/A         |        | 6      | 8.6         | 6      | 13.3                  |         |       |                            |         |       |
| 17  | AURKB       | Ordinal| rs3027260| 8053911   | 484   | -3.33 (1.52)           | 0.029   | 726   | -0.404 (0.177)            | 0.022   | 0.73   |
|     | G/G         |        | 404    | 19.2        | 632    | 18.7                  |         |       |                            |         |       |
|     | G/A         |        | 77     | 15.8        | 91     | 15.4                  |         |       |                            |         |       |
|     | A/A         |        | 3      | 12.8        | 3      | 12.4                  |         |       |                            |         |       |
|     | Ordinal     |        | rs4792590| 8057840   | 484   | -3.14 (1.34)           | 0.020   | 726   | -0.39 (0.152)             | 0.010   | 0.89   |
|     | G/G         |        | 377    | 19.3        | 597    | 18.6                  |         |       |                            |         |       |
|     | G/A         |        | 102    | 16.6        | 121    | 15.4                  |         |       |                            |         |       |
|     | A/A         |        | 5      | 9.5         | 8      | 12.5                  |         |       |                            |         |       |

*P ≤ 0.05 in both studies. **Mayo Clinic Breast Cancer Study; Singapore and Sweden Breast Cancer Study.

1PMD Est(SE): ordinal parameter estimate and standard error reflecting the estimated change in percent density per each additional copy of the minor allele carried. Adjusted mean from general model: least squares estimate of mean percent density for each genotype, adjusted for age, BMI, and PMH. 2P-value from analyses adjusted for age, BMI, and PMH. 3Sqrt PMD Est(SE): ordinal parameter estimate and standard error reflecting the estimated change in percent density error per each additional copy of the minor allele carried. Adjusted mean from general model: least squares estimate of mean percent density for each genotype, adjusted for age, BMI, and PMH. Least squares means back transformed from square root to original scale. 4P-value from test for heterogeneity from meta-analysis performed on the ordinal parameter estimates (ordinal estimates for the MCBCS sample were estimated using square root transformed PMD).
acetylation of this protein inhibits the initiation of DNA replication and cell cycle progression. Proliferation of stroma and/or epithelium has been hypothesized to underlie increased mammographic density [11], although few studies have shown positive associations between proliferation markers and PMD [24,26,27,59] or tissue from dense areas of the breast [28].

Conclusion
In summary, we present the first report of variation in genes involved in regulation of cell division and mammographic density. We find no strong evidence of association between variants in genes involved in mitosis and adjusted percent mammographic density; however, further investigation of variants in AURKB, LOC375190 and MCM3 is warranted.

Additional material

Additional file 1: Associations between variants in genes in the mitotic pathway and percent mammographic density (PMD) in two studies of postmenopausal women. 484 Caucasian subjects from the Mayo Clinic Breast Cancer Study (MCBCS, 2001 to 2005) and 726 Caucasian subjects (controls) from Singapore and Sweden Breast Cancer Study (SASBC, 1993-1995).

Abbreviations
AURKB: Aurora B Kinase; BMI: Body Mass Index; CC: cranio-caudal; CELU: One of 11 populations in the Hapmap (Northern and Western European Ancestry); GWAS: genome wide association study; hCG: human chorionic gonadotropin; HWE: Hardy-Weinberg equilibrium; LD: linkage disequilibrium; MCBCS: Mayo Clinic Breast Cancer Study; MCM3: mini-chromosome maintenance complex component 3; MLO: medio-lateral oblique; PMH: postmenopausal hormones; PMD: Percent Mammographic Density; SASBC: Sweden and Singapore Breast Cancer Study; SNP: single nucleotide polymorphism

Acknowledgements
This work was supported by grants from the National Institutes of Health (R01 CA128931; P50 CA116201; R01 CA122340; R01 CAS8427); Märit and Sweden Breast Cancer Research Initiative Against Breast Cancer; W81XWH-05-1-0314

Authors’ contributions
CMV, FJC, JEO and TAS contributed to all aspects of the study, including study design, data collection, analyses, interpretation and preparation of the manuscript. XX, JMC and DNR were responsible for samples and genotyping for MCBCS. JLI, PH, KC and JLI contributed to the SASBC replication results, interpretation and publication. VSP, ZSF, CGS and JLI conducted statistical analyses and provided results. KNS performed the pathway analyses and provided interpretation of results and scientific review of the manuscript. FFW was responsible for all aspects of mammogram retrieval and mammographic density estimation. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 15 February 2011 Revised: 1 December 2011 Accepted: 7 January 2012 Published: 7 January 2012

References
1. Cummings SR, Tice JA, Bauer S, Browner WS, Cuzick J, Ziv E, Vogel V, Shepherd J, Vachon C, Smith-Blundman R, Kerlikowske K: Prevention of breast cancer in postmenopausal women: approaches to estimating and reducing risk. J Natl Cancer Inst 2009, 101:384-398.
2. McCormack VA, dos Santos Silva I: Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2006, 15:1159-1169.
3. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tischkier D, Chiarelli A, Yaffe MJ, Hopper JL: Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002, 347:886-894.
4. Vachon CM, Sellers TA, Carlson EE, Cunningham JH, Hilkier CA, Smalley R, Schaid DJ, Kelemen LE, Couch FJ, Pankratz VS: Strong evidence of a genetic determinant for mammographic density, a major risk factor for breast cancer. Cancer Res 2007, 67:9412-9418.
5. Pankow JS, Vachon CM, Kuni CC, King RA, Amett DK, Grabrick DM, Rich SS, Anderson VE, Sellers TA: Genetic analysis of mammographic breast density in adult women: evidence of a gene effect. J Natl Cancer Inst 1997, 89:549-556.
6. Kelemen LE, Sellers TA, Vachon CM: Can genes for mammographic density inform cancer aetiology? Nat Rev Cancer 2008, 8:812-823.
7. Odefrey F, Stone J, Gunnin LC, Byrnes G, Apicella C, Dite GS, Cawson JN, Giles GG, Treloar SA, English DR, Hopper JL, Southey MC, Australian Twins and Sisters Mammographic Density Study: Common genetic variants associated with breast cancer and mammographic density measures that predict disease outcome. Cancer Res 2010, 70:1449-1458.
8. Easton DF, Pooley KA, Dunning AM, Pharoah PO, Thompson D, Ballinger DG, Struweiping JP, Morrison J, Field H, Luken R, Warren J, Ahmed S, Healey CS, Bowman R, SEARCH collaborators, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brenner P, Sangrajrang S, Gaboreau I, Vodrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Petco J, et al. Genomewide association study identifies novel breast cancer susceptibility loci. Nature 2007, 447:1087-1093.
9. Lindstrom S, Vachon CM, Liu J, Varghese J, Thompson D, Warren R, Brown J, Leyland J, Aylott T, Wareham NJ, Loos RJ, Paterson AD, Rommens J, Waggott D, Martin LJ, Scott CG, Kankvatz V, Hankinson SE, Hazra A, Hunter DJ, Hopper JL, Southey MC, Chanoon SJ, Silva ID, Liu J, Eriksson L, Couch FJ, Stone J, Apicella C, Czene K, et al. Common variants in ZNF365 are associated with both mammographic density and breast cancer risk. Nat Genet 2011, 43:185-187.
10. Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranan M, Seal S, Ghousainni M, Hines S, Healey CS, Hughes D, Warren-Perry M, Tapper W, Eccles D, Evans DG, Breast Cancer Susceptibility Collaboration (UK), Hoening M, Schutte M, van den Ouweland A, Houlston R, Ross G, Langford C, Pharoah PD, Stratton MR, Dunning AA, Rahman N, Easton DF. Genomewide association study identifies five new breast cancer susceptibility loci. Nat Genet 2010, 42:504-507.
11. Boyd NF, Martin LJ, Bronskill M, Yaffe MJ, Duric N, Minkin S: Breast tissue composition and susceptibility to breast cancer. J Natl Cancer Inst 2010, 102:1224-1237.
12. Boyd NF, Melnickouk O, Martin LJ, Hilsop G, Chiarelli AM, Yaffe MJ, Minkin S: Mammographic density, response to hormones, and breast cancer risk. J Clin Oncol 2011, 29:2985-2992.
13. Greendale GA, Rebourssin BA, Se A, Singh HR, Olson LK, Gaterwood O, Bassett LW, Waislauskaus C, Bush T, Barrett-Conner E: Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Postmenopausal Estrogen/Progestin Interventions (PEPI) Investigators. Men Int Med 1999, 130:262-269.
14. Laya MB, Gallagher JC, Schreiman JS, Larson BB, Watson P, Weinstein L: Effect of postmenopausal hormonal replacement therapy on...
mammographic density and parenchymal pattern. Radiology 1995, 196:433-437.
15. Stomper PC, Van Voorhis BJ, Ravnikar VA, Meyer JE: Mammographic changes associated with postmenopausal hormone replacement therapy: a longitudinal study. Radiology 1990, 174:471-490.
16. Marug NC, van der Moorren MJ, Hendriks JH, Rolland R, Ruijs SH: Mammographic changes in postmenopausal women on hormonal replacement therapy. Eur Radiol 1997, 7:749-755.
17. Rutter CM, Mandelson MT, Laya MB, Seger DJ, Taplin S: Changes in breast density associated with initiation, discontinuation, and continuing use of hormone replacement therapy. JAMA 2001, 285:171-176.
18. McTiernan A, Chlebowski RT, Martin C, Peck JD, Aragaki A, Pisano ED, Wang CY, Johnson KC, Manson JE, Wallace RB, Vitolins MZ, Heiss G: Conjugated equine estrogen influence on mammographic density in postmenopausal women in a substudy of the women's health initiative randomized trial. J Clin Oncol 2007, 25:1613-1614.
19. Verheus M, Maskarinec G, Erber E, Steude JS, Killeen J, Hernandez BY, Harvey JA, Santen RJ, Petroni GR, Bovbjerg VE, Smolkin ME, Sheriff FS, Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Byrne C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Premenopausal insulin-like growth factor-I (IGF-I), IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
20. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
21. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
22. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
23. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
24. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
25. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
26. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
27. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
28. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
29. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
30. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
31. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
32. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
33. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
34. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
35. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
36. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
37. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
38. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
39. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
40. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
41. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
42. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
43. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
44. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
45. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
46. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
47. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
48. Dierio C, Pollak M, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, Cote G, Berube S, Morin C, Brison J: Insulin-like growth factor-I, IGF-binding protein-3, and mammographic breast density. Cancer Epidemiol Biomarkers Prev 2005, 14:1065-1073.
49. Boyd NF, Stone J, Martin LJ, Jong R, Fishell E, Yaffe M, Hammond G, Minkin S: The association of breast mitogens with mammographic densities. Br J Cancer 2002, 87:867-882.
50. Verheus M, Peeters PH, Kaaks R, van Noorden PA, Grobbee DE, van Gils CH: Premenopausal insulin-like growth factor-I serum levels and changes in breast density over menopause. Cancer Epidemiol Biomarkers Prev 2007, 16:451-7.
51. Orem C, Colditz GA, Willett WC, Speizer FE, Pollak M, Hankenson SE: Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000, 60:3474-3478.
53. Byng JW, Boyd NF, Little L, Lockwood G, Fishell E, Jong RA, Yaffe MJ: Symmetry of projection in the quantitative analysis of mammographic images. *Eur J Cancer Prev* 1996, 5:319-327.
54. Chai HS, Sicotte H, Bailey KR, Turner ST, Asmann YW, Kocher JP: GLOSSI: a method to assess the association of genetic loci-sets with complex diseases. *BMC Bioinformatics* 2009, 10:102.
55. Cochran WG: The combination of estimates from different experiments. *Biometrics* 1954, 10:101-129.
56. Galwey NW: A new measure of the effective number of tests, a practical tool for comparing families of non-independent significance tests. *Genet Epidemiol* 2009, 33:559-568.
57. Ganem NJ, Godinho SA, Pellman D: A mechanism linking extra centrosomes to chromosomal instability. *Nature* 2009, 460:278-282.
58. Montoya-Durango DE, Velu CS, Kazanjian A, Rojas ME, Jay CM, Longmore GD, Grimes HL: Ajuba functions as a histone deacetylase-dependent co-repressor for autoregulation of the growth factor-independent-1 transcription factor. *J Biol Chem* 2008, 283:32056-32065.
59. Hawes D, Downey S, Pearce CL, Bartow S, Wan P, Pike MC, Wu AH: Dense breast stromal tissue shows greatly increased concentration of breast epithelium but no increase in its proliferative activity. *Breast Cancer Res* 2006, 8:R24.

doi:10.1186/bcr3088
Cite this article as: Vachon et al.: No evidence for association of inherited variation in genes involved in mitosis and percent mammographic density. *Breast Cancer Research* 2012 14:R7.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit