Hormone Metabolism Genes and Mammographic Density in Singapore Chinese Women

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Abstract

Background: Female steroid hormone levels and exogenous hormone use influence breast cancer risk. We investigated the association between genetic variation in the hormone metabolism and signaling pathway and mammographic density, a strong predictor of breast cancer risk.

Methods: We genotyped 161 SNPs in 15 hormone metabolism pathway gene regions and evaluated mammographic density in 2,038 Singapore Chinese women. Linear regression analysis was used to investigate single-nucleotide polymorphism (SNP) and mammographic density association. An overall pathway summary was obtained using the adaptive ranked truncated product test.

Results: We did not find any of the individually tested SNPs to be associated with mammographic density after a multiple testing correction. There was no evidence of an overall effect on mammographic density of genetic variation in the hormone metabolism pathway.

Conclusions: In this cross-sectional study, genetic variation in hormone metabolism pathway was not associated with mammographic density in Singapore Chinese women.

Impact: Consistent with existing data from Caucasian populations, polymorphisms in hormone pathway genes are not likely to be strong predictors of mammographic density in Asian women. Cancer Epidemiol Biomarkers Prev; 22(5): 984–6. ©2013 AACR.

Introduction

Mammographic density is a relative measure of the amount of epithelium and stroma in the breast, and one of the strongest known predictors of breast cancer risk (1). Endogenous sex steroid hormone levels and exogenous hormone therapy use have been associated with increased breast cancer risk (2). To date, 18 studies, conducted mainly among Caucasian populations, have described the association between single-nucleotide polymorphisms (SNP) in one or more hormone pathway genes (reviewed in ref. 3) and mammographic density and generally reported no associations. In the current study, we investigated the associations between SNPs in hormone metabolism pathway genes and mammographic density among participants of the Singapore Chinese Health Study (SCHS), a population-based prospective study initiated in 1993 in Singapore (4).

Materials and Methods

Study participants

Details of the Mammography Subcohort, who were women enrolled in both the SCHS and the Singapore Breast Screening Project (SBSP), have been described (4). In brief, 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%). Of these, DNA samples were collected from 2,164 women (1,848 blood, 316 buccal; ref 4). Mammographic density was assessed by one of the authors (G. Ursin, Cancer Registry of Norway, Oslo, Norway; ref. 4). The Institutional Review Boards at the National University of Singapore, the National Cancer Center Singapore (Singapore), the University of Southern California (Los Angeles, CA), the University of Minnesota (Minneapolis, MN) and the University of Pittsburgh (Pittsburgh, PA) had approved this study.

We selected tagging SNPs in the hormone metabolism pathway genes (see Table 1), from 20 kb upstream of 5' untranslated region (UTR) to 10 kb downstream of 3' UTR of each gene. We tagged all common SNPs [minor allele frequency (MAF) ≥5%] found among non-Hispanic White or Chinese populations, with r² ≥ 0.80. This selection was done using the Snagger software and a custom
database of the Hapmap CEU data (release 24) merged with unique SNPs in the Affymetrix 500 K panel as well as the Hapmap CHB data release 24 (4).

Genotyping of 2,164 samples was conducted using the Illumina Golden Gate Assay (Illumina Inc.) at the University of Southern California Epigenome Core Facility. We excluded 126 samples whose genotyping success rates were less than 85%. Genotyping concordance based on 42 random duplicate samples was more than 99.9%. We excluded SNPs with MAF less than 0.001 or which depart significantly from Hardy–Weinberg equilibrium ($P < 0.01$).

**Statistical analysis**

We used linear regression to examine the association between SNPs and mammographic density, adjusting for age at mammogram, body mass index (BMI; kg/m²) at mammogram, and dialect group (Cantonese, Hokkien). Additional adjustment for other breast cancer risk factors, including parity, menopausal status, and hormone therapy use, did not materially change the results; these risk factors were not included in the final model. The linear regression models were based on additive genetic models, in which the regression coefficients are estimates of the difference in mammographic density per copy of the minor allele of a given polymorphism. We tested whether the association between SNPs and mammographic density is modified by established determinants of mammographic density by introducing product terms and conducting Wald tests, adjusting for age, BMI, and dialect. Analyses were conducted using SAS 9.2 (SAS Inc.). All $P$ values are 2 sided.

To summarize the overall statistical significance of the entire set of SNPs under investigation, we applied the adaptive ranked truncated product (ARTP) test of Yu and colleagues (5).

**Results**

We genotyped 161 SNPs in 15 hormone metabolism pathway gene regions (Table 1). We did not observe a

### Table 1. Number of genotyped SNPs in each hormone pathway gene, and the gene-level, and pathway-level summary $P$ values

<table>
<thead>
<tr>
<th>Pathway-level summary $P$ value</th>
<th>Number of genotyped SNPs</th>
<th>Tagging SNPs selected</th>
<th>Tagging coverage (%) when using tagging approach; list of selected SNPs when not using tagging approach</th>
<th>Gene-level/pathway-level summary $P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR1C4</td>
<td>1</td>
<td>No</td>
<td>rs17134592 (Leu311Val)</td>
<td>0.79</td>
</tr>
<tr>
<td>AR</td>
<td>2</td>
<td>No</td>
<td>rs5918757, rs1204038</td>
<td>0.82</td>
</tr>
<tr>
<td>COMT</td>
<td>29</td>
<td>Yes</td>
<td>75%</td>
<td>0.14</td>
</tr>
<tr>
<td>CYP1A1/CYP1A2</td>
<td>13</td>
<td>Yes</td>
<td>81%</td>
<td>0.18</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>16</td>
<td>Yes</td>
<td>78%</td>
<td>0.69</td>
</tr>
<tr>
<td>CYP17A1</td>
<td>1</td>
<td>No</td>
<td>rs743572</td>
<td>0.72</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>2</td>
<td>No</td>
<td>rs724797, rs749202</td>
<td>0.54</td>
</tr>
<tr>
<td>ESR2</td>
<td>11</td>
<td>No</td>
<td>rs2077647, rs2295190, rs9479130, rs728524, rs2250122, rs12681, rs3798577, rs1801132, rs9340799, rs1062577, rs3798758</td>
<td>0.49</td>
</tr>
<tr>
<td>ESR2</td>
<td>28</td>
<td>Yes</td>
<td>90%</td>
<td>0.18</td>
</tr>
<tr>
<td>HSD3B1/HSD3B2</td>
<td>4</td>
<td>No</td>
<td>rs6428830, rs6428828, rs6686779, rs10802107</td>
<td>0.12</td>
</tr>
<tr>
<td>HSD1B1</td>
<td>5</td>
<td>Yes</td>
<td>57%</td>
<td>0.37</td>
</tr>
<tr>
<td>PGR</td>
<td>32</td>
<td>Yes</td>
<td>86%</td>
<td>0.19</td>
</tr>
<tr>
<td>SHBG</td>
<td>10</td>
<td>No</td>
<td>rs6259, rs2955617, rs858518, rs858521, rs858524, rs1624085, rs9898876, rs9913778, rs1642796, rs1619016</td>
<td>0.36</td>
</tr>
<tr>
<td>SPTD5A2</td>
<td>1</td>
<td>No</td>
<td>rs523349</td>
<td>0.58</td>
</tr>
<tr>
<td>SULT1A1/SULT1A2</td>
<td>6</td>
<td>Yes</td>
<td>68%</td>
<td>0.40</td>
</tr>
</tbody>
</table>

$a$Tagging SNPs were selected using Snagger (see ref. 4 for further description and citation of original article).

$^b$Hapmap SNPs (Chinese population; release 27) in each gene region captured by the genotyped tag SNPs with minimum pairwise $r^2 \geq 0.80$. Each gene region covers from 20 kb upstream of the start of each gene to 10 kb downstream of the end of each gene. Full list of tagging SNPs are available upon request.
significant association between SNPs in the hormone metabolism genes and mammographic density in Singapore Chinese women. The overall ARTP test for the set of SNPs investigated was not significant ($P = 0.70$; Table 1). For single SNP analyses, only rs4680 (COMT) had a $P$ value less than 0.01, but this association was not statistically significant after Bonferroni adjustment. There was no evidence of effect modification by parity, menopausal status, or BMI ($<25$ kg/m$^2$, $\geq 25$ kg/m$^2$); however, the sample size for nulliparous ($n = 133$) or premenopausal women ($n = 216$) was limited.

**Discussion**

Consistent with published data (3), our results do not support hormone pathway SNPs as genetic determinants of mammographic density in Chinese women. To our knowledge, this is the first study to investigate hormone metabolism pathway genes in association with mammographic density in a large population-based study of Asians.

For SNPs with MAF of 0.2 (average MAF of all tested SNPs), we had 80% power to detect a 3.2% difference in mammographic density per minor allele, with a Bonferroni-corrected type I error rate of 5%. One limitation of this study is that we were not able to evaluate effect modification by hormone therapy (6) due to the very low prevalence of hormone therapy use in this population. Another limitation is the incomplete tagging of the selected genes. Nonetheless, the list of hormone pathway genes and the number of investigated SNPs in the current study is by far the largest among all published studies in Asian population, which examined 2 to 25 SNPs (3, 7).

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

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

Development of methodology: M.C. Yu

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

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): E. Lee, Y.-C. Su, J.P. Lewinger, C. Hsu, G. Ursin, D.O. Stram, M.C. Yu, A.H. Wu

Writing, review, and/or revision of the manuscript: E. Lee, Y.-C. Su, J.P. Lewinger, G. Ursin, W.-P. Koh, J.-M. Yuan, D.O. Stram, M.C. Yu, A.H. Wu

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y.-C. Su, D.V.D. Berg, J.-M. Yuan, A.H. Wu

Study supervision: A.H. Wu

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