Genetic Variation in Adipokine Genes and Associations with Adiponectin and Leptin Concentrations in Plasma and Breast Tissue

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Abstract

Background: Circulating adipokines may be associated with breast cancer risk. Genetic variants governing adipokines and adipokine receptors may also predict risk, but their effect on breast adipokine concentrations is unknown.

Methods: We conducted a cross-sectional analysis of functional SNPs in 5 adipokine genes [adiponectin, leptin (LEP), and their receptors] among 85 cancer-free women who were undergoing reduction mammoplasty.

Results: In multivariable-adjusted regression models, compared with the common GG genotype, the AA genotype of the LEP A19G SNP was associated with 27% lower plasma adiponectin [ratio, 0.73; 95% confidence interval (CI), 0.54–0.98] and leptin (ratio, 0.73; 95% CI, 0.55–0.96). Women with the AG genotype of LEP A19G had 39% lower breast leptin (ratio, 0.61; 95% CI, 0.39–0.97) compared with those with the GG genotype. No associations were observed for SNPs in the remaining genes.

Conclusions: Genetic variation in LEP may alter endogenous adipokine concentrations in circulation and in breast tissues.

Impact: These preliminary findings may support the hypothesis that genetic variation in adipokine genes modifies circulating adipokine concentrations and possibly leptin concentrations in local breast tissues, which may be associated with breast cancer risk. Cancer Epidemiol Biomarkers Prev; 23(8); 1559–68. ©2014 AACR.

Introduction

Although there is consistent evidence that obesity is associated with increased breast cancer risk among postmenopausal women, and with poorer prognosis following diagnosis irrespective of menopausal status (1, 2), the molecular mechanisms by which obesity influences breast cancer remain unclear. These mechanisms likely involve exposure of breast epithelial tissues to bioactive molecules, including the adipokines, adiponectin, and leptin, which are produced by adipocytes in the breast and other tissues.

The biologic activities of adiponectin and leptin tend to be opposing. For example, adiponectin inhibits cell proliferation and promotes apoptosis in vitro (3–5), whereas leptin induces cell proliferation and angiogenesis (6–9). In several epidemiologic studies, there is evidence that plasma adiponectin (10–18) and the adiponectin-to-leptin (A/L) ratio (10, 19) are inversely associated with breast cancer risk. There is a less consistent positive association between plasma leptin and breast cancer. Some studies reported a positive association (10, 17, 19–23), some reported an inverse association, particularly among premenopausal women (24, 25), whereas others have reported no association (26–28). Many of the studies showing associations of plasma adipokine with breast cancer were limited by their lack of prediagnostic assessment of these biomarkers. Some of the prospective studies have not confirmed an inverse association for adiponectin (18, 19, 28) or a positive association for leptin (24, 26, 29).

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In all of the studies to date, there is an assumption that blood adipokine concentrations reflect tissue levels and exposures to breast epithelial cells. Previously, we reported only modest correlations between plasma and breast tissue adipokines (38). A few studies (39–41) have compared adipokine concentrations in breast tumors and nontumor tissues. One study (40) reported higher leptin concentrations in breast tumor tissues compared with adjacent, nontumor breast tissues. Adiponectin concentrations were higher in breast tumors compared with nontumor tissues in one study (39), but not another (41). In the latter study, there was a significant inverse association between adiponectin concentrations within breast tumors and tumor stage (41). Given the difficulty of measuring breast tissue adipokines for breast cancer risk in the general population, we sought to determine if assessing genetic polymorphisms in adipokine and adipokine receptor genes, which may affect adipokine levels via a negative feedback loop, would influence adipokine concentrations within the breast. Examining genetic variation may provide additional information about breast tissue adipokine concentrations and contribute to our understanding of these biomarkers in breast carcinogenesis.

Findings from the few studies examining associations of genetic variation in genes for adiponectin, leptin, and their receptors and blood hormone concentrations have not been consistent and few studies have examined the same variants (36, 42–63). To our knowledge, no study has previously described (38). For the breast tissue measurements, frozen tissues (0.80 g starting weight) were homogenized in 5 mL ice-cold extraction solution containing TPER buffer (contains a proprietary detergent in 25 mmol/L Tris HCl, 150 mmol/L sodium chloride; pH 7.6) and protease inhibitors (Pierce). Each sample was homogenized in Lysing Matrix D tubes using the FastPrep-24 Instrument (MP Biomedicals) at 6.0 m/s for 6 cycles of 30 seconds. Homogenates were centrifuged at 1,600 × g for 10 minutes, and supernatants were collected, aliquoted, and stored at −80°C. Total protein concentration was quantified using the Coomassie Plus-The Better Bradford Assay Kit (Pierce). Breast hormone concentrations were normalized by total protein concentrations and reported in nanograms of hormone per milligram of total protein (ng/mg). Each plasma and breast homogenate sample was assayed in duplicate and in random order. Every batch included replicates, commercial controls, and plasma or breast homogenate controls to assess laboratory variation. The coefficients of variation (CV) for the plasma assays were 5.92% and 4.48% for leptin and adiponectin, respectively. CVs for the breast tissue assays were 9.24% and 9.33% for leptin and adiponectin, respectively.

Materials and Methods

Study sample and biospecimen collection

The details of the study design have been described previously (38). Briefly, healthy women undergoing elective reduction mammoplasty surgery, age >16 years with no prior history of cancer, were recruited between 1997 and 2009 at Georgetown University Medical Center, the University of Maryland, the Washington Hospital Center and the Center for Plastic Surgery (Buffalo, NY). Within 24 hours before surgery, participants completed interviewer-administered questionnaires, which ascertained personal and family medical history, diet and alcohol, smoking history, and reproductive history. All participants provided written, informed consent (for patients ages 16 to 18 years a parent/guardian provided consent), and the study was approved by the Institutional Review Boards of all participating institutions.

Both blood and breast tissue specimens were collected from the study participants. Heparinized plasma samples were collected within 24 hours before surgery, processed, aliquoted, and stored at −80°C until used for the adipokine analyses. Plasma adipokine concentrations and adipokine SNP genotype data were assessed in 155 study participants; among these, sufficient breast tissues for adipokine analyses were available from 94 participants. Breast tissues were inspected and determined to be free from gross abnormalities by board-certified pathologists. Women found to have benign breast disease (n = 9) on microscopic examination were excluded; thus, a subset of the total study sample (n = 85) was included in the present analyses. Epithelial/stromal tissues were dissected (to remove adipose tissues) and snap-frozen in liquid nitrogen within 1 hour of surgery. Aliquots of breast tissues were stored at −80°C until used for adipokine analyses.

Plasma and breast adipokine measurements

Plasma and breast adiponectin and leptin concentrations were measured by ELISA kits [Human Adiponectin/Acrp30 Quantikine ELISA and Human Leptin Quantikine ELISA, respectively (R&D Systems)], as previously described (38). For the breast tissue measurements, frozen tissues (0.80 g starting weight) were homogenized in 5 mL ice-cold extraction solution containing TPER buffer (contains a proprietary detergent in 25 mmol/L bicine, 150 mmol/L sodium chloride; pH 7.6) and protease inhibitors (Pierce). Each sample was homogenized in Lysing Matrix D tubes using the FastPrep-24 Instrument (MP Biomedicals) at 6.0 m/s for 6 cycles of 30 seconds. Homogenates were centrifuged at 1,600 × g for 10 minutes, and supernatants were collected, aliquoted, and stored at −80°C. Total protein concentration was quantified using the Coomassie Plus-The Better Bradford Assay Kit (Pierce). Breast hormone concentrations were normalized by total protein concentrations and reported in nanograms of hormone per milligram of total protein (ng/mg). Each plasma and breast homogenate sample was assayed in duplicate and in random order. Every batch included replicates, commercial controls, and plasma or breast homogenate controls to assess laboratory variation. The coefficients of variation (CV) for the plasma assays were 5.92% and 4.48% for leptin and adiponectin, respectively. CVs for the breast tissue assays were 9.24% and 9.33% for leptin and adiponectin, respectively.

Genotypic analysis of adipokine and adipokine receptor genes

DNA was extracted from dissected breast tissues using the Puregene Tissue Kit for DNA purification by Gentra Systems (Qiagen Inc.), and SNP genotyping was conducted in the Genomics and Epigenomics Shared
Resource at the Lombardi Comprehensive Cancer Center (Georgetown University) using the Illumina BeadXpress platform according to the manufacturer’s instructions (29). The leptin (LEP A19G; rs2167270), leptin receptor (LEPR Q223R; rs1137101), adiponectin (ADIPOQ G276T; rs1501299), adiponectin receptor 1 (ADIPOR1 Intron 1; rs2275737), and adiponectin receptor 2 (ADIPOR2 C33447T; rs1044471) polymorphisms were included (on the basis of their association with circulating adipokines concentrations) in our custom, 96-plex Illumina BeadXpress Assay, which included several other variants in various other pathways. Briefly, 500 ng genomic DNA at 50 ng/µL was subjected to several steps of activation and ligation, followed by PCR and hybridization to the custom-made genotyping beads, specific for each SNP in a 96-well plate format. Plates were then washed and scanned in the BeadXpress instrument, and genotypes were called using the BeadStudio Software (Illuma Inc.).

Statistical analysis

Descriptive statistics were used to examine the distributions of demographic characteristics and plasma and breast adipokine concentrations. Mean and 95% confidence intervals (CI) of adipokine concentrations were calculated for each SNP genotype. Differences in least-squares mean concentration of plasma and breast adipokines, adjusted for age, race, and BMI (kg/m²), were compared using an F test. Associations between genotypes and plasma and breast adipokines were estimated using multivariable regression models (codominant), in which log-transformed adipokine concentrations among those carrying variant genotypes were considered the dependent variables. Results for each genotype are given as eβ, representing the ratio of adipokine concentrations among those carrying variant genotypes relative to those carrying both common-type alleles, and eβ±1.96SEβ, the corresponding 95% CI. We also examined these associations by race and examined race by genotype interactions with adipokine concentrations. All regression models were adjusted for age, race, and BMI. P values for trend (P trend) were calculated by treating ordered genotype data as continuous in multivariable regression models. All P values were two-sided, and P < 0.05 was considered statistically significant. All statistical analyses were performed using SAS v9.2.

Results

Baseline characteristics of the study sample are shown in Table 1. The mean age of women included in the present analyses was 37.6 ± 12.5 years and approximately 57% were white. Although there was a range of body mass among the study participants (18.0–52.0 kg/m²), on average, participants were obese (mean ± SD BMI, 31.0 ± 7.2 kg/m²). Mean concentrations of plasma adiponectin, leptin, and the A/L ratio were 8.5 ± 5.1 µg/mL, 27.6 ± 18.5 ng/mL, and 0.6 ± 0.9, respectively, whereas mean breast concentrations were 688.0 ± 538.1 ng/mg, 3.4 ± 3.7 ng/mg, and 557.1 ± 1,410.1, respectively.

Overall, genotypes of LEP A19G, ADIPOQ G276T, and ADIPOR2 C33447T in this study sample were in Hardy–Weinberg equilibrium (HWE) and were similar to those reported in the NCBI dbSNP database; however, for genotypes of LEPR Q223R and ADIPO1 Intron 1, there were significant deviations from HWE (P values = 0.004 and 0.01, respectively) in the overall study sample. For LEPR Q223R, HWE was achieved among whites (P = 0.06), but not among blacks (P < 0.0001), whereas for ADIPO1 Intron 1, the race-specific genotype frequencies were in HWE (whites, P = 0.93; blacks, P = 0.55). Age-, race-, and BMI-adjusted distributions of plasma adiponectin, leptin, and the A/L ratio by adipokine genotype are shown in Table 2. Compared with those with the common GG genotype, carriers of the LEP A19G homozygous variant genotype (AA) had 27% lower concentrations of plasma adiponectin (ratio, 0.73; 95% CI, 0.54–0.98) and leptin (ratio, 0.73; 95% CI, 0.55–0.96). No significant differences were observed in plasma adipokine concentrations for SNPs in ADIPOQ, ADIPO1, ADIPO2, or LEPR. In log-additive models of plasma adiponectin and leptin concentrations, for each additional variant A allele of LEP A19G, there were 14% reductions in plasma adiponectin (ratio, 0.86; 95% CI, 0.75–0.98; P trend = 0.03) and leptin (ratio, 0.86; 95% CI, 0.76–0.97; P trend = 0.02). The association between LEP A19G and plasma leptin was stronger among blacks than whites (Table 3). Among whites, the ratio comparing plasma leptin in AA genotype carriers with GG genotype carriers was 0.92 (95% CI, 0.64–1.31), whereas among blacks the ratio was 0.54 (95% CI, 0.35–0.82); however, the race by genotype interaction did not attain statistical significance.
not achieve statistical significance (P interaction = 0.08). In addition, some marginally significant racial differences in plasma adiponectin were observed by LEPR Q223R and ADIPOR2 C3347T genotypes. In adjusted analyses, white women with the AA variant genotype of LEPR Q223R had lower plasma adiponectin than blacks carrying the same genotype (whites: ratio, 0.87; 95% CI, 0.61–1.24 vs. blacks: ratio, 1.42; 95% CI, 0.99–2.02; P interaction = 0.06). Conversely, black women who were carriers of the ADIPOR2 C3347T homozygous variant genotype had lower plasma adiponectin than whites carrying the same genotype (whites: ratio, 1.02; 95% CI, 0.74–1.41 vs. blacks: ratio, 0.35; 95% CI, 0.19–0.64; P interaction = 0.07).

Age-, race-, and BMI-adjusted distributions of concentrations of breast tissue adiponectin, leptin, and the A/L ratio by SNP genotype are shown in Table 4. Compared with carriers of the common GG genotype, those with the AG genotype of LEPR A19G had 39% lower breast leptin concentrations (ratio, 0.61; 95% CI, 0.39–0.97), whereas no statistically significant association was observed among AA genotype carriers (ratio, 1.67; 95% CI, 0.86–3.26). There were no significant differences in breast adipokine concentrations by genotype of ADIPOQ, ADIPOR1, ADIPOR2, or LEPR. Although there were no significant differences in the associations between LEPR A19G genotype and breast adipokine, leptin, or the A/L ratio by race (Table 5), the interaction of LEPR A19G genotype and race on breast adipokine concentrations was marginally significant (P interaction = 0.07); white women who were carriers of the AA genotype had lower breast adiponectin, whereas black women with that genotype had higher concentrations (whites: ratio, 0.69; 95% CI, 0.33–1.45 vs. blacks: ratio, 1.73; 95% CI, 0.82–3.71). Similarly, white women who were carriers of the LEPR Q223R AA genotype had lower breast tissue A/L concentrations than black women carrying the same genotype (whites: ratio, 0.38; 95% CI, 0.16–0.88 vs. blacks: ratio, 1.20; 95% CI, 0.60–2.41; P interaction = 0.05). However, for these tests of interaction, the numbers in each cell were small and the CIs were wide.
Leptin is thought to be a growth factor in breast cancer; however, no other study has investigated the association between genetic variation and leptin concentrations within the breast. *LEP* A19G, or other polymorphisms that are in linkage disequilibrium with this variant, may be low-penetrant risk factors for breast cancer. There is very little study of the association between *LEP* A19G and breast cancer. In one study, *LEP* A19G was not significantly associated with breast cancer risk (or plasma leptin concentrations) among premenopausal women (43); however, it is not clear what the association is among postmenopausal women. The current literature is lacking data about the association between *LEP* A19G and breast cancer risk, which may differ by race/ethnicity (as observed for other adipokine-related polymorphisms; refs. 71, 72) and possibly other patient characteristics, thus warranting further study. Given the conflicting data about the association between plasma leptin and breast cancer risk, as well as our recent finding of only modest correlations between plasma and breast leptin concentrations (38), a deeper understanding of the association between breast tissue leptin concentrations and its influence on breast cancer risk would be essential as findings from our studies (38, 73), as well as from others (74), indicate that circulating hormone concentrations are weak surrogates for levels in local tissues.

Several studies have examined associations between SNPs in *LEP* and *LEPR* and circulating leptin and have provided support for a genetic basis of variation in leptin concentrations (36, 42–53). We found that carriers of the variant A allele of *LEP* A19G had significantly lower plasma leptin concentrations compared with the GG genotype (51, 53, 64); results from those studies were not consistent with ours; they found higher leptin among carriers of the AA genotype. One explanation for the discrepancy between these studies and the current one could be differences in study samples, including gender.

Table 3. Association between *LEP* A19G SNP genotype and plasma adipokine concentrations, by race

<table>
<thead>
<tr>
<th>Race</th>
<th>n (%)</th>
<th>Plasma adiponectin ratio (95% CI)</th>
<th>Plasma leptin ratio (95% CI)</th>
<th>Plasma A/L ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Plasma adiponectin ratio (95% CI)</strong></td>
<td><strong>Plasma leptin ratio (95% CI)</strong></td>
<td><strong>Plasma A/L ratio (95% CI)</strong></td>
</tr>
<tr>
<td>White</td>
<td></td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>GG</td>
<td>36 (40.0)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AG</td>
<td>43 (47.8)</td>
<td>0.85 (0.68–1.07)</td>
<td>0.89 (0.70–1.13)</td>
<td>0.95 (0.66–1.36)</td>
</tr>
<tr>
<td>AA</td>
<td>11 (12.2)</td>
<td>0.73 (0.52–0.93)</td>
<td>0.92 (0.64–1.31)</td>
<td>0.79 (0.45–1.36)</td>
</tr>
<tr>
<td>Black</td>
<td></td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>GG</td>
<td>23 (36.5)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AG</td>
<td>33 (52.4)</td>
<td>0.83 (0.59–1.15)</td>
<td>0.89 (0.70–1.15)</td>
<td>0.92 (0.61–1.39)</td>
</tr>
<tr>
<td>AA</td>
<td>7 (11.1)</td>
<td>0.64 (0.36–1.11)</td>
<td>0.54 (0.35–0.82)</td>
<td>1.18 (0.60–2.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P</em> interaction = 0.94</td>
<td><em>P</em> interaction = 0.08</td>
<td><em>P</em> interaction = 0.23</td>
</tr>
</tbody>
</table>

NOTE: All analyses were adjusted for age and BMI.


Table 4. Associations between ADIPOQ, ADIPOR1, ADIPOR2, LEP, and LEPR SNP genotype and breast tissue adipokine concentrations

<table>
<thead>
<tr>
<th></th>
<th>Breast adiponectin (µg/mL)</th>
<th>Breast leptin (ng/mL)</th>
<th>Breast A/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (95% CI)</td>
<td>Ratio (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Ratio (95% CI)</td>
</tr>
<tr>
<td>ADIPOQ G276T (rs1501299)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC 585.87 (452.23–759.00)</td>
<td>1.00 (referent)</td>
<td>2.29 (1.62–3.25)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AC 503.21 (390.84–647.49)</td>
<td>0.86 (0.61–1.22)</td>
<td>2.04 (1.45–2.86)</td>
<td>0.89 (0.56–1.42)</td>
</tr>
<tr>
<td>AA 395.05 (229.50–679.39)</td>
<td>0.67 (0.38–1.19)</td>
<td>1.45 (0.70–2.99)</td>
<td>0.63 (0.29–1.35)</td>
</tr>
<tr>
<td><strong>P trend = 0.16</strong></td>
<td><strong>P trend = 0.27</strong></td>
<td><strong>P trend = 0.27</strong></td>
<td><strong>P trend = 0.95</strong></td>
</tr>
<tr>
<td>ADIPOR1 Intron 1 (rs2275737)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC 436.16 (324.31–587.10)</td>
<td>1.00 (referent)</td>
<td>1.85 (1.25–2.73)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AC 579.40 (456.51–735.83)</td>
<td>1.33 (0.93–1.90)</td>
<td>2.35 (1.72–3.21)</td>
<td>1.27 (0.79–2.03)</td>
</tr>
<tr>
<td>AA 531.13 (331.06–852.78)</td>
<td>1.22 (0.72–2.05)</td>
<td>1.22 (0.66–2.27)</td>
<td>0.66 (0.33–1.31)</td>
</tr>
<tr>
<td><strong>P trend = 0.25</strong></td>
<td><strong>P trend = 0.56</strong></td>
<td><strong>P trend = 0.56</strong></td>
<td><strong>P trend = 0.11</strong></td>
</tr>
<tr>
<td>ADIPOR2 C33447T (rs1044471)</td>
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<tr>
<td>GG 520.09 (394.30–686.22)</td>
<td>1.00 (referent)</td>
<td>1.93 (1.34–2.78)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AG 538.08 (416.51–695.83)</td>
<td>1.03 (0.72–1.49)</td>
<td>1.92 (1.37–2.69)</td>
<td>0.99 (0.62–1.60)</td>
</tr>
<tr>
<td>AA 530.91 (338.53–832.64)</td>
<td>1.02 (0.61–1.70)</td>
<td>3.28 (1.82–5.93)</td>
<td>1.70 (0.87–3.32)</td>
</tr>
<tr>
<td><strong>P trend = 0.90</strong></td>
<td><strong>P trend = 0.21</strong></td>
<td><strong>P trend = 0.21</strong></td>
<td><strong>P trend = 0.20</strong></td>
</tr>
<tr>
<td>LEP A19G (rs2162770)</td>
<td></td>
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<tr>
<td>GG 598.24 (458.38–781.33)</td>
<td>1.00 (referent)</td>
<td>2.44 (1.73–3.44)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AG 440.54 (337.55–575.42)</td>
<td>0.74 (0.52–1.05)</td>
<td>1.50 (1.06–2.11)</td>
<td>0.61 (0.39–0.97)</td>
</tr>
<tr>
<td>AA 625.78 (389.79–1,004.25)</td>
<td>1.04 (0.62–1.76)</td>
<td>4.08 (2.22–7.50)</td>
<td>1.67 (0.86–3.26)</td>
</tr>
<tr>
<td><strong>P trend = 0.59</strong></td>
<td><strong>P trend = 0.75</strong></td>
<td><strong>P trend = 0.75</strong></td>
<td><strong>P trend = 0.42</strong></td>
</tr>
<tr>
<td>LEPR Q232R (rs11760956)</td>
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<td></td>
</tr>
<tr>
<td>GG 529.96 (377.47–744.12)</td>
<td>1.00 (referent)</td>
<td>1.89 (1.21–2.97)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>AG 518.01 (395.64–674.32)</td>
<td>0.97 (0.64–1.48)</td>
<td>1.98 (1.39–2.82)</td>
<td>1.05 (0.60–1.82)</td>
</tr>
<tr>
<td>AA 531.66 (385.45–733.63)</td>
<td>1.00 (0.64–1.57)</td>
<td>2.28 (1.49–3.49)</td>
<td>1.20 (0.66–2.18)</td>
</tr>
<tr>
<td><strong>P trend = 0.98</strong></td>
<td><strong>P trend = 0.53</strong></td>
<td><strong>P trend = 0.53</strong></td>
<td><strong>P trend = 0.51</strong></td>
</tr>
</tbody>
</table>

NOTE: All analyses were adjusted for age, race, and BMI.

*P < 0.05, comparing mean breast leptin concentration among AG genotype carriers with GG genotype carriers.

**P < 0.05, ratio comparing AG genotype carriers with GG genotype carriers.

There were many strengths of this study. The cross-sectional examination of plasma and breast adipokine concentrations in relation to common genetic variation in adipokine genes, in healthy women without breast cancer as well as without benign breast disease who underwent reduction mammoplasty, afforded us the
opportunity to study these relationships with sufficient quantities of breast tissues that would not be feasible in any other setting for women without cancer. Our use of highly reproducible immunoassays and genomics techniques also strengthened the study. In addition, as previously described (38), our examination of tissue heterogeneity (i.e., amounts of adipose and epithelial/stromal tissues) and our finding that tissue heterogeneity had no significant effect on breast adipokine concentrations were also important strengths of this study.

There were also limitations that should be considered when interpreting our results. We are aware that our fairly small sample size could have limited our power to detect meaningful associations; however, the preliminary findings reported herein are useful for generating hypotheses that can be investigated in larger studies. Relatedly, the sample size of this study limited our ability to determine the relative contributions of rare genetic variants and nongenetic factors, which have been suggested to have considerable importance in variation of circulating adipokine concentrations, and possibly local breast tissue concentrations as well. Some of our previous analyses (38) demonstrated that overall, BMI and race explained most of the variation in breast adiponectin, while oral contraceptive use and smoking status explained most of the variation in breast adiponectin. Inclusion of the SNPs examined herein in the linear models of plasma and breast adiponectin concentrations did not yield statistically significant genotype associations, so the clinical significance of these SNPs (taking into account other patient characteristics) is unclear. Furthermore, we cannot exclude the possibility that other variants unexamined in this study, particularly low frequency adipokine and/or adipokine receptor variants, contribute to adipokine concentrations as well. Thus, examination of the contribution of these and other genetic factors to variation in adipokine concentrations is a priority for our future research. The candidate gene approach, studying only a few, rare SNPs, in association with our outcomes of interest was also a limitation. There are additional variants in adipokine and adipokine receptor genes that may be associated with plasma and breast adipokine concentrations. Identifying these variants would be useful in drawing inferences about adipokines and their association with breast cancer, as well as in identifying low-penetrant genes that may influence a woman’s susceptibility to the disease. Also, our observation that the frequencies of LEP Q223R and ADIPOR1 Intron 1 SNPs in the overall study sample did not reach statistical significance for HWE was a limitation. Therefore, our lack of significant associations between these variants and plasma and breast tissue adipokine concentrations should be considered with caution until confirmed by other larger studies. And finally, women who undergo reduction mammoplasty may not be representative of all women, given that they have larger breasts and generally higher BMI and socioeconomic status, and may have increased breast cancer risk due to large breast size, all factors which may limit the generalizability of our findings. However, our analyses included a sample of women with a wide range of BMI and adipokine concentrations, thereby strengthening the validity of our findings.

In summary, we observed significant associations between LEP A19G genotype and plasma adiponectin and leptin, and breast leptin concentrations in this study of cancer-free women undergoing elective reduction mammoplasty. These findings provide evidence to support a role of genetic variation in adipokine genes in variation of endogenous adipokine concentrations in blood and breast tissues but also underscore the differences in determinants of adipokines in blood and breast tissues. Our findings add to a growing literature on the biologic mechanisms linking the adipokines, adiponectin and leptin, and breast cancer susceptibilities.

### Table 5. Association between LEP A19G SNP genotype and breast adipokine concentrations, by race

<table>
<thead>
<tr>
<th>Race</th>
<th>n (%)</th>
<th>Breast adiponectin Ratio (95% CI)</th>
<th>Breast leptin Ratio (95% CI)</th>
<th>Breast A/L Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>White</td>
<td>GG</td>
<td>36 (40.0)</td>
<td>0.67 (0.41–1.09)</td>
<td>0.65 (0.34–1.26)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>43 (47.8)</td>
<td>0.69 (0.33–1.45)</td>
<td>1.25 (0.46–3.42)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>11 (12.2)</td>
<td></td>
<td>0.55 (0.21–1.42)</td>
</tr>
<tr>
<td>Black</td>
<td>GG</td>
<td>23 (36.5)</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>33 (52.4)</td>
<td>0.90 (0.52–1.55)</td>
<td>0.79 (0.42–1.48)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>7 (11.1)</td>
<td>1.73 (0.82–3.71)</td>
<td>3.16 (1.34–7.39)</td>
</tr>
</tbody>
</table>

NOTE: All analyses were adjusted for age and BMI. Minor allele frequencies of LEP A19G among whites and blacks were 0.375 and 0.326, respectively.
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A.A.M. Llanos, C. Marian, R.G. Dumitrescu, P.G. Shields.
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.A.M. Llanos, J. Mathew, C. Marian, R.G. Dumitrescu, P.G. Shields.
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.A.M. Llanos, T.M. Brasky, K.H. Makambi, R.G. Dumitrescu, J.L. Freudenheim, P.G. Shields.
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References

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