Variants of the Adiponectin and Adiponectin Receptor 1 Genes and Breast Cancer Risk

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Abstract

Breast cancer risk is higher among obese women and women with diabetes. Adiponectin is a protein exclusively secreted by adipose tissue, circulating levels of which have been associated with breast cancer risk. Whether genetic variants within the adiponectin pathway are associated with breast cancer risk is unknown. To explore the association of genetic variants of the adiponectin (ADIPOQ) and adiponectin receptor 1 (ADIPOR1) genes with breast cancer risk, we conducted a case control study of female patients with breast cancer and healthy female controls from New York City recruited between 1999 and 2004. We genotyped 733 hospital-based breast cancer cases and 839 controls for 10 haplotype-tagging single nucleotide polymorphisms (SNP) of ADIPOQ and ADIPOR1. Two ADIPOQ SNPs (rs2241766 and rs1501299), which have been associated with circulating levels of adiponectin, were associated with breast cancer risk [rs1501299*GG: odd ratios (OR), 1.80; 95% confidence interval (95% CI), 1.14–2.85; rs2241766*TG: OR, 0.61; 95% CI, 0.46–0.80]. One ADIPOR1 SNP (rs7539542), which modulates expression of adiponectin receptor 1 mRNA, was also associated with breast cancer risk (OR, 0.51; 95% CI, 0.28–0.92). Based on the known function of these adipokines, adiponectin is associated with breast cancer risk (6). Adiponectin, a protein secreted by the adipose tissue, has been found to be an endogenous insulin sensitizer, the circulating levels of which are decreased in obese and diabetic subjects. Moreover, adiponectin has the potential of regulating the secretion of estrogens, tumor necrosis factor (TNF) (12, 13), and IGF (14).

Several proteins produced by adipose tissue have been studied in relation to breast cancer risk. There is emerging evidence that one of these adipokines, adiponectin, is associated with breast cancer risk (6). Adiponectin, a protein secreted by the adipose tissue, has been found to be an endogenous insulin sensitizer, the circulating levels of which are decreased in obese and diabetic subjects. Moreover, adiponectin has the potential of regulating the secretion of estrogens, tumor necrosis factor (TNF) (12, 13), and IGF (14).

Recently, circulating levels of adiponectin have been found to correlate with breast cancer risk (15–17). Because its levels are inversely correlated with adiposity (13), it has been suggested that decreased levels of adiponectin may explain the increased risk of breast cancer in obesity (13, 18). In fact, several groups have shown that after adjustment for body mass index (BMI), women with higher adiponectin levels had a 65% reduced risk for breast cancer (15, 16, 19). Furthermore, the breast cancer cell lines MCF-7, MDB-MB-231, and T47D were found to express both adiponectin receptors ADIPOR1/R2 (15, 20), and exposure of T47D cells to adiponectin significantly inhibited their proliferation (15).

Several adiponectin polymorphisms have been shown to affect adiponectin levels, and polymorphisms of both the ligand and its type I receptor (ADIPOR1) have been associated with risk for insulin resistance, cardiovascular disease, and DM (21–28). However, to date, the association of these polymorphisms with breast cancer risk has not been studied. In this study of breast cancer cases and controls, we systematically evaluated selected haplotype-tagging single nucleotide polymorphisms (SNP) in genes encoding adiponectin and its type I receptor in relation to breast cancer risk.

Materials and Methods

Study participants. As part of institutional review board–approved protocols, we collected blood samples from 733 consecutive female patients admitted to Memorial Sloan-Kettering Cancer with a diagnosis of breast cancer. Recruitment of cases occurred in two phases, the first one between January 1, 1998 and December 31, 1999, the second one occurred between...
December 1, 2002 and January 31, 2004. All breast cancer cases were histologically confirmed at Memorial Sloan-Kettering Cancer Center. Information regarding sex, age, age at breast cancer diagnosis, and ethnic status was recorded. In a subset of 152 patients, information on estrogen receptor (ER) and progesterone receptor (PR) status as assessed by immunohistochemistry was collected at the time of case collection. A sample of 839 healthy female volunteers from New York City ages 20 to 87 years was recruited between January 1, 2003 and December 31, 2004. The last date of follow-up is September 30, 2006. None of the controls had any personal history of cancer at the time of blood donation. This was ascertained by a questionnaire completed by each healthy volunteer. The controls were matched to cases on gender and geographic region and, thus, are representative of the source population where the cases come from. Although age categories were obtained for all participants, exact age information was not available for some controls. However, in that subgroup of individuals, which represents 7.9% of controls, the age ranged from 20 to 40 years (Table 1). None of the controls had any personal history of cancer as ascertained by a questionnaire administered at the time of enrollment. Ethnic status for cases and controls was self-defined. All cases and controls signed an informed consent. All personal identifiers from both cases and controls were permanently removed. The study was approved by the Institutional Review Board.

**Table 1. Characteristics of breast cancer cases and controls**

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–40</td>
<td>114 (16.2)</td>
<td>27 (7.9)</td>
</tr>
<tr>
<td>41–50</td>
<td>186 (26.4)</td>
<td>80 (23.5)</td>
</tr>
<tr>
<td>51–60</td>
<td>185 (26.3)</td>
<td>117 (34.3)</td>
</tr>
<tr>
<td>61–70</td>
<td>130 (18.5)</td>
<td>73 (21.4)</td>
</tr>
<tr>
<td>71+</td>
<td>89 (12.6)</td>
<td>44 (12.9)</td>
</tr>
</tbody>
</table>

**Race**

<table>
<thead>
<tr>
<th></th>
<th>Cases, n (%)</th>
<th>Controls, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>563 (76.8)</td>
<td>801 (95.5)</td>
</tr>
<tr>
<td>Black</td>
<td>68 (9.3)</td>
<td>19 (2.3)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>33 (4.5)</td>
<td>10 (1.2)</td>
</tr>
<tr>
<td>Asian</td>
<td>22 (3.0)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>47 (6.4)</td>
<td>7 (0.8)</td>
</tr>
</tbody>
</table>

**Selection of SNPs.** Ten haplotype-tagging SNPs were selected for genotyping (Table 2). The adiponectin gene has >10 SNPs (22, 27) and two common SNPs for genotyping (Table 2). The adiponectin gene has >10 SNPs (22, 27) and two common SNPs for genotyping (Table 2). For block 1, we selected rs1501299 (+276) GG is associated with decreased levels of serum adiponectin (29, 24). Rs2241766 (+45) TT has also been associated with decreased levels of serum adiponectin (29, 30). We therefore classified individuals who had 276*GG/45*TT, 276*GT/45*TT, and 276*GG/45*GT as low signalers; individuals with 276*TT/45*TT, 276*GT/45*GT, and 276*GG/45*GG as intermediate signalers; and individuals with 276*GT/45*GG, 276*TT/45*GG, and 276*TT/45*GT genotypes as high signalers (Fig. 1C). For ER and PR studies, genotype distributions of SNPs were analyzed based on comparison of ER and PR status within breast cancer cases. Statistical analysis of the data were performed with SAS 9.1 (SAS Institute). Bonferroni adjustment for multiple comparisons was also performed. We present both unadjusted and Bonferroni adjusted P values in the Tables.

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Results

Haplotypes of ADIPOQ and breast cancer risk. Of the five haplotype-tagging SNPs selected for the analysis two of them (rs2241766 and rs1501299), both tagging block 2 of ADIPOQ were found to be significantly associated with breast cancer risk. Rs2241766 and rs1501299 have been found to alter adiponectin levels (21, 23, 25, 27) and are also associated with obesity (31), risk of insulin resistance, hypertension, and cardiovascular disease (21, 22, 24, 25). The high-expressing rs2241766 G allele (GG and GT genotypes) was associated with decreased breast cancer risk [OR, 0.64; 95% confidence interval (95% CI), 0.49–0.83; Table 4]. Breast cancer population attributable risk (PAR) for the low-expressing rs2241766 TT genotype is 70 per 1,000 individuals. The low expressing rs1501299 G allele was associated with increased breast cancer risk (TG: OR, 1.59; 95% CI, 1.03–2.48; GG: OR, 1.80; 95%, CI, 1.14–2.85; Table 3). Breast cancer PARs for the rs1501299 TG and GG genotypes are 76 and 75 per 1,000 individuals, respectively.

Haplotypes of ADIPOR1 and breast cancer risk. Of the five haplotype SNPs genotyped, we found that rs2232853 CT genotype (OR, 1.67; 95% CI, 1.23–2.26) and the combination of rs7539542 GC (OR, 0.59; 95% CI, 0.36–0.98) and CC genotypes (OR, 0.57; 95% CI, 0.35–0.94) were significantly associated with breast cancer risk...
(Tables 3 and 4). Studies on ADIPOR1 show that haplotype-tagging SNPs of the second block and especially rs7539542, are mostly associated with CAD and DM (26, 32). Moreover, rs7539542 GC and CC have been associated with higher mRNA levels compared with GG (26). Breast cancer PAR for individuals that harbor the rs7539542 GG genotype is 8 per 1,000 individuals.

Analysis according to age and ER and PR status. We analyzed our results according to ER and PR status among cases for which data were available. One genotype (rs1342387*CC) was significantly associated with ER positivity (OR, 4.09, 1.39–12.02; data not shown). However, the confidence intervals are wide given the small number of cases available for this analysis. The lack of clear associations may be a surprise because at present, there is no clearly shown association between adiponectin levels and ER status (4, 16, 19). Another explanation is the limited power of our analysis. There was also no association with age (data not shown).

Table 3. Crude and adjusted ORs (95% CI) of breast cancer by ADIPOQ and ADIPOR1 SNP genotypes

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Crude OR</th>
<th>Adjusting for age*, SNPs †, and race</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs266729</td>
<td>GG</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>GC</td>
<td>1.15 (0.76–1.74)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1.00 (0.67–1.50)</td>
</tr>
<tr>
<td>rs22395</td>
<td>GG</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>1.55 (0.85–2.81)</td>
</tr>
<tr>
<td>rs2241766</td>
<td>TT</td>
<td>1.00</td>
</tr>
<tr>
<td>rs1501299</td>
<td>TT</td>
<td>1.00</td>
</tr>
<tr>
<td>rs2232853</td>
<td>TT</td>
<td>1.00</td>
</tr>
<tr>
<td>rs12733285</td>
<td>TC</td>
<td>1.18 (0.87–1.60)</td>
</tr>
<tr>
<td>rs1342387</td>
<td>CC</td>
<td>1.11 (0.82–1.50)</td>
</tr>
<tr>
<td>rs7539542</td>
<td>GC</td>
<td>1.00</td>
</tr>
<tr>
<td>rs10920531</td>
<td>AA</td>
<td>0.89 (0.72–1.09)</td>
</tr>
</tbody>
</table>

*Age adjustment was done by using categorical age groups (age ≥50 y and age <50 y).
† Adjustment for SNPs was only done with SNPs from the same gene.
‡ P < 0.001 without Bonferroni adjustment; P < 0.05 with Bonferroni adjustment.
§ P < 0.05 without Bonferroni adjustment; 0.05 < P < 0.10 with Bonferroni adjustment.

Discussion

In this study, we found that two functionally relevant adiponectin polymorphisms, +45 T→G (rs2241766) and +276 G→T (rs 1501299), were significantly associated with breast cancer risk. Furthermore, a polymorphism of ADIPOR1, +10225 C→G (rs7539542), which has been shown to alter mRNA levels of the receptor (with the GG and CG polymorphisms increasing mRNA levels; ref. 26), was also significantly associated with breast cancer risk. This study shows that adiponectin signaling, as assessed by a combination of functionally relevant SNPs, may predict breast cancer risk.
High BMI has been associated with postmenopausal breast cancer (33, 34). The prevailing theory behind the association between obesity and breast cancer is based on the increased levels of estrogens and/or insulin resistance observed in obese women (35, 36). The established association between breast cancer and insulin resistance and obesity has drawn interest for adipokines, a group of proteins synthesized in the adipose tissue (4, 13, 37).

Adiponectin is secreted by adipose tissue, and its levels are inversely correlated to BMI. Several studies have shown that low levels of adiponectin are directly associated with increased breast cancer risk (11, 13, 15, 16, 18, 19). Adiponectin can act as a growth inhibitor for breast cancer cell lines. More specifically, adiponectin has been shown to inhibit the growth of the breast cancer cell lines MDA-MB-231 (38) and T47D (15). Furthermore, it was shown that in MCF-7 cells, growth stimulation with estradiol was suppressed by the presence of adiponectin (20). Several theories exist as to the link between adiponectin and breast carcinogenesis. Adiponectin is inversely correlated with insulin levels and is also associated with IGF-binding proteins, which have been associated with breast cancer (39, 40). Adiponectin has also been shown to inhibit the production of TNF-α in macrophages and its actions in endothelial cells (41), and has been found to bind several growth factors, such as fibroblast growth factor and platelet-derived growth factor–β polypeptide, which can induce cell proliferation. Adiponectin also inhibits the activation of nuclear factor-κB, which is involved in breast cancer development (42). One of the downstream elements in the adiponectin pathway is 5'-AMP–activated protein kinase (AMPK; refs. 43, 44). Once activated, AMPK targets mammalian target of rapamycin, and protein kinase B (43), and the c-Jun-NH2-kinase, and signal transducers and activators of transcription 3 pathways that are involved in breast carcinogenesis (45).

Table 5. Adjusted OR for adiponectin signaling status

<table>
<thead>
<tr>
<th>Genotype combinations</th>
<th>n (cases/controls)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI) adjusted for age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low signalers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>GG/45</em>TT</td>
<td>581/598</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>276<em>GT/45</em>TT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>GG/45</em>GT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>TT/45</em>GT</td>
<td>124/191</td>
<td>0.67 (0.52–0.86)</td>
<td>0.64 (0.49–0.83)</td>
</tr>
<tr>
<td>Intermediate signalers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>TT/45</em>GT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>GT/45</em>GT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>GG/45</em>GG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>TT/45</em>GG</td>
<td>1/8</td>
<td>0.13 (0.02–1.03)</td>
<td>0.15 (0.02–1.28)</td>
</tr>
<tr>
<td>High signalers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>GT/45</em>GG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>TT/45</em>GG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>276<em>TT/45</em>GT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P trend</td>
<td>0.001</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

Given the previously shown functional significance of two of the SNPs tested (rs1501299 and rs2241766), we elected to divide patients into high, intermediate, and low signalers. Our hypothesis was that high signalers, i.e., individuals with higher predicted levels of adiponectin and/or expression of its receptor, would have a lower risk of breast cancer compared with low signalers. The findings provide support for our hypothesis in that individuals with intermediate and high adiponectin signaling had significantly lower breast cancer risk (OR, 0.64 and 0.15, respectively).

Haplotypetagging SNPs for ADIPOR1 have also been shown to alter mRNA levels. More specifically, rs7539542 GG is associated with 30% to 40% lower ADIPOR1 mRNA levels than heterozygotes or CC homozygotes (26). This SNP has also been associated with risk for DM and CAD (26, 32). In our analysis, we found that rs7539542 CC and CG, which are associated with higher mRNA levels, i.e., more adiponectin receptor 1, are associated with a 43% lower risk for breast cancer.

A limitation in our study is the lack of exact age at the time of blood draw for a small portion of our controls as only age range was available. To address this, we did analyses controlling for age. We also conducted sensitivity analyses using hypothetical models to show that the effect of lack of detailed age information in a portion of our samples was negligible. It is possible that age differences in cases and controls affected the allele frequencies observed. Nonetheless, this would be expected to create a bias toward the null hypothesis because it would overestimate the deleterious allele frequency in controls given that a fraction of younger women who would have developed breast cancer were not present.
removed from the control group. Thus, the younger mean age of controls could have resulted in a bias toward the null hypothesis, resulting in a weaker association. Furthermore, inaccurate information with respect to any variable classification would result in “random misclassification,” which would also be expected to result in suppression of effect estimates with a trend toward the null hypothesis. Other limitations of this study are the lack of information on confounding factors such as BMI and family history of breast cancer. However, it has been shown that the association of adiponectin with breast cancer is independent of BMI (15, 16, 19), and the presence of a strong family history of the disease would only weaken the power of our study. Our study also has several strengths. It includes a relatively large number of cases and controls from the same geographic location. The selection of our SNPs was based on previous studies, and the SNPs significantly associated with breast cancer are functionally significant. Although exposure was assessed in the context of a case control study, it is impossible that breast cancer would have changed SNP classification, which is already determined at birth. Thus, this study does fulfill the “time sequence” criterion for causality. This in association with existing epidemiologic evidence and biological plausibility support a causal association between these SNPs and risk for breast cancer.

There is currently only limited information on the allelic frequency of some of the SNPs studied in various ethnic groups. For example, the allelic frequency of rs241766 has only been studied in 752 anonymous unrelated Japanese volunteers. There is no additional information on the allelic frequency in other populations besides the previously cited reports. With respect to rs2232853, the C/T allelic frequency differs between Caucasians (0.675:0.325), Asians (0.956:0.044), and sub-Saharan Africans (0.950:0.050). Finally, for rs7539542, the C/G allelic frequency also differs between Caucasians (0.712:0.288) and Asians (0.310:0.690), but there is no information on its frequency in other ethnic groups.

To our knowledge, this is the first study reporting an association of polymorphisms of adiponectin and its type I receptor with breast cancer risk. If confirmed in subsequent studies, our findings suggest that genetic variants of the adiponectin and adiponectin receptor 1 alter breast cancer risk. Our findings confirm the important role of adiponectin in breast cancer. In the future, we may be able to identify a population of breast cancer patients with low adiponectin levels, either due to genetic predisposition and/or environmental factors, who may benefit from adiponectin therapy. If these exciting results can be confirmed in other studies, the adiponectin axis may emerge as an important modifier of breast cancer risk.

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