Energy homeostasis genes modify the association between serum concentrations of IGF-1 and IGFBP-3 and breast cancer risk

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Breast cancer is a multifactorial disease in which the interplay among multiple risk factors remains unclear. Energy homeostasis genes play an important role in carcinogenesis and their interactions with the serum concentrations of IGF-1 and IGFBP-3 on the risk of breast cancer have not yet been investigated. The aim of this study was to assess the modifying effect of the genetic variation in some energy homeostasis genes on the association of serum concentrations of IGF-1 and IGFBP-3 with breast cancer risk. We analyzed 78 SNPs from 10 energy homeostasis genes in premenopausal women from the 4-Corner’s Breast Cancer Study (61 cases and 155 controls) and the Mexico Breast Cancer Study (204 cases and 282 controls). After data harmonization, 71 SNPs in HWE were included for interaction analysis. Two SNPs in two genes (MBOAT rs13272159 and NPY rs16131) showed an effect modification on the association between IGF-1 serum concentration and breast cancer risk ($P_{\text{interaction}} < 0.05$, adjusted $P_{\text{interaction}} < 0.20$). In addition, five SNPs in three genes (ADIPOQ rs182052, rs822391 and rs7649121, CARTPT rs3846659, and LEPR rs12059300) had an effect modification on the association between IGFBP-3 serum concentration and breast cancer risk ($P_{\text{interaction}} < 0.05$, adjusted $P_{\text{interaction}} < 0.20$). Our findings showed that variants of energy homeostasis genes modified the association between the IGF-1 or IGFBP-3 serum concentration and breast cancer risk in premenopausal women. These findings contribute to a better understanding of this multifactorial pathology.

Breast cancer is a multifactorial disease in which the interplay among multiple risk factors remains unclear. Some central and peripheral regulators of food intake and energy expenditure (energy homeostasis) foster cancer development. Dysregulated energetic metabolism has been strongly associated with breast cancer (BC) risk. In addition to their role in food intake or fuel metabolism, energy homeostasis factors regulate tumorigenic processes. Central regulators such as the hypothalamic orexigenic neuropeptide Y (NPY) and anorexigenic cocaine and amphetamine regulated transcript (CART) peptide have been involved in different kinds of cancers. NPY promotes cellular proliferation, invasion, metastasis and angiogenesis through its different receptors, and the CART peptide is expressed in primary and metastatic BC cells. Among peripheral regulators, leptin (LEP) signaling stimulates proliferation, survival, migration and cell invasion in BC, and adiponectin (ADIPOQ) inhibits proliferation and metastasis of BC cells. The ghrelin (GHRL) peptide has been associated with increased proliferation of BC cellular lines. The enzyme ghrelin-O-acyltransferase (GOAT or MBOAT4), implicated in the activation of GHRL, is overexpressed in BC tissue samples. Although cholecystokinin (CCK) has not been
associated with the risk of BC, it has been reported that it affects the proliferation of pancreatic cancer cells. Recently, in the Breast Cancer Health Disparities Study, we found associations between genetic variants of some of these genes and the risk of BC.

Another key factor associated with BC development is insulin-like growth factor-1 (IGF-1) signaling. IGF-1 regulates fuel metabolism and is an important regulator of cell growth, proliferation, survival, differentiation and cellular transformation in many types of cancer, including BC. Insulin-like growth factor binding protein-3 (IGFBP-3) is the main protein that binds to IGF-1. IGFBP-3, by binding to IGF-1, increases the half-life of circulating IGF-1, modulating its availability and its mitogenic and anti-apoptotic effects. In an independent manner, IGFBP-3 is able to regulate the survival and proliferative activity of healthy and cancer cells. Several studies have shown that IGF-1 serum concentration is positively associated with BC risk. The association to < 0.10 for three SNPs in three genes, rs11585329, rs182052, and rs7649121; and LEPR, reported positive associations, while others have shown inverse relationships. At a genetic level, variants between the serum concentration of IGFBP-3 and the risk of BC has been inconsistent. Some studies have shown an increased risk of BC (OR = 2.79; 95% CI 1.88, 4.13) (Table 2).

For this study, 78 SNPs from 10 energy homeostasis genes were included (Supplementary Table S1); all of them had a minor allele frequency higher than 1%. After data from the 4-CBCS and MBCS populations were harmonized, HWE was tested in controls from both studies. Three SNPs (LEPR rs6673324, and rs1137101; POMC rs6713532) in women from the 4-CBCS (Supplementary Table S2) and four SNPs (CARTPT rs2239670; LEPR rs9436739, and rs1938484; NPY rs2023890) from the MBCS were not in HWE (Supplementary Table S3). These SNPs were not included in the analysis. The comparison of genotype frequencies between cases and controls in the 4-CBCS showed statistically significant differences (P < 0.05) in two genes: ADIPOQ (rs3821799 and rs1063537) and LEPR (rs3828942) (Supplementary Table S2). In the MBCS, differences were observed in LEPR (rs12145690) (Supplementary Table S3). The genotype frequency comparison between studies showed significant differences for SNP rs3774261 in the ADIPOQ gene (Supplementary Table S3).

**Energy homeostasis genes modified the association of serum concentrations of IGF-1 and IGFBP-3 with BC risk**

**IGF-1.** The unadjusted analysis of the association between IGF-1 serum concentration and the risk of BC showed that compared to women in the lowest tertile, those in the highest tertile had a statistically significant increased risk of BC (OR = 2.79; 95% CI 1.88, 4.13) (Table 2).

We assessed the interaction between IGF-1 and the 71 SNPs and found an effect modification with a P_interaction < 0.05 for four SNPs in four genes (GHRL, LEPR, MBOAT, and NPY) and with a P_interaction from 0.05 to < 0.10 for three SNPs in three genes (ADIPOQ, LEPR, and POMC) in co-dominant models (Table 2). When comparing the effect of the highest IGF-1 tertile vs. the lowest, for most SNPs, there was a protective effect in heterogeneous women; these effects were statistically significant for ADIPOQ rs17366568 (OR = 0.09; 95% CI 0.01, 0.91; P = 0.04), and POMC rs7565427 (OR = 0.05; 95% CI 0.01, 0.51; P = 0.01) (Table 2). Of particular interest, the SNP rs13272159 of MBOAT4 showed an increased risk when comparing the effect of the highest IGF-1 tertile vs. the lowest (OR = 7.16; 95% CI 1.64, 31.25; P = 0.009) in women with AA; the SNP rs7565427 of POMC also showed an increased risk (OR = 1.89; 95% CI 1.07, 3.35; P = 0.03) in women with GG. After adjusting for multiple comparisons (FDR correction), only interactions for three SNPs in three genes (LEPR rs12059300, MBOAT rs13272159 and NPY rs16131) were statistically significant with an adjusted P_interaction < 0.20.

After assessing the association between the Table 2 SNPs and IGF-1 serum concentration, we found that for the LEPR rs970468 SNP, control women who were carriers of the GG variant had higher IGF-1 serum concentration (1.23 ng/mL, P = 0.010) than TT homozygotes. However, after adjusting for multiple comparisons, this association was not statistically significant (adjusted P > 0.05) (data not shown).

**IGFBP-3.** The unadjusted analysis of the association between IGFBP-3 serum concentration and the risk of BC showed that compared to women in the lowest tertile, those in the highest tertile had a statistically significant increased risk of BC (OR = 5.55, 95% CI 3.55, 8.68) (Table 3).

For IGFBP-3, we also assessed the interaction with the 71 SNPs and found an effect modification with a P_interaction < 0.05 for five SNPs in three genes (ADIPOQ, CARTPT and LEPR) and with a P_interaction from 0.05 to < 0.10 for five SNPs in three (ADIPOQ, LEPR, and NPY) out of the 10 genes included in the analysis (Table 3). In the codominant models, all SNPs except three (ADIPOQ rs182052, and rs7649121; and LEPR rs11585329) showed the same pattern. For seven SNPs (ADIPOQ rs822391, rs3821799, and rs3774261; CARTPT rs3846659;
LEPR rs12059300; NPY rs16141 and rs16129), when comparing the effect of the highest IGFBP-3 serum concentration tertile vs. the lowest, there was a statistically significant increased BC risk in those who were homozygous for the major allele. This effect was lower in heterozygous, and the effect was not observed in women who were homozygous for the minor allele. For example, for ADIPOQ rs3774261 GG genotype, we observed an OR = 10.80 (95% CI 3.74, 31.19; \( P < 0.001 \)); for the GA genotype, an OR = 3.81 (95% CI 1.75, 8.27; \( P = 0.001 \)); and for the AA genotype, an OR = 2.24 (95% CI 0.57, 8.88; \( P = 0.25 \)) (Table 3). All interactions except (LEPR rs11585329) remained statistically significant with an adjusted \( P_{\text{interaction}} < 0.20 \) after adjusting for multiple comparisons (FDR correction).

After assessing the association between the Table 3 SNPs and IGFBP-3 serum concentration, we found that in control women, there was an association between 4 ADIPOQ SNPs (rs7649121, rs182052, rs3774261, rs3821799) and the IGFBP-3 serum concentration \( (P < 0.05) \). However, after adjusting for multiple comparisons, only the association for rs7649121 remained statistically significant. Compared to AA homozygotes TT carriers had a lower IGFBP-3 serum concentration (-710.1 ng/mL) \( (P < 0.001) \) (data not shown).

**Discussion**

In this study, we showed that variants of some energy homeostasis genes modified the association between IGF-1 or IGFBP-3 serum concentration and the risk of BC in premenopausal women. Several epidemiological studies have highlighted the existing association between IGF-1 and IGFBP-3 serum concentration and the risk of BC. IGF-1 and IGFBP-3 regulate multiple biological mechanisms, including fuel metabolism and cell growth, proliferation, migration, metastasis and angiogenesis. IGF-1 activates downstream pathways involved in carcinogenic processes such as the PI3K/AKT, RAS/RAF/MAPK, and STAT cascades. IGFBP-3, in addition to modulating IGF-1 activity, may also trigger IGF-independent activation of various signaling pathways.

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Table 1. Subject characteristics by study (premenopausal women), the Breast Cancer Health Disparities Study.

| Characteristics            | 4-Corner's Breast Cancer Study (4-CBCS) | Mexico Breast Cancer Study (MBCS) |
|---------------------------|----------------------------------------|----------------------------------|
|                           | Control (n = 155)                      | Case (n = 61)                    | Control (n = 282) | Case (n = 204) |
| Age (years)\(^a\)          | 42.5 (6.5)                             | 44.3 (5.0)                      | 42.2 (4.5)       | 42.9 (5.1)     |
| Age at menarche (years)\(^a\) | 12.8 (1.6)                             | 13.1 (1.7)                      | 12.7 (1.7)       | 12.7 (1.5)     |
| Genetic ancestry (% Indigenous)\(^a\) | 19.7 (22.6)                           | 16.3 (22.9)                     | 71.1 (17.4)      | 69.1 (18.0)    |
| BMI (Kg/m2)\(^a\)         | 28.4 (6.7)                             | 26.4 (6.4)                      | 30.0 (5.4)       | 28.3 (4.9)     |
| Height (cm)\(^a\)         | 161.9 (7.3)                            | 161.7 (7.5)                     | 153.4 (5.5)      | 154.5 (5.5)    |
| Energy intake (Kcal/day)\(^a\) | 2322.5 (994.7)                      | 2500.4 (1092.9)                | 2023.4 (729.6)   | 2232.7 (755.0) |
| IGF-1 serum concentration (ng/mL)\(^a\) | 145.9 (50.0)                          | 152.3 (42.9)                   | 171.2 (98.7)     | 224.7 (101.5)  |
| IGFBP-3 serum concentration (ng/mL)\(^a\) | 4241.4 (761.6)                      | 4455.0 (611.2)                 | 3158.3 (995.9)   | 4160.5 (1153.4) |
| Ever use Oral Contraceptives (yes)\(^b\) | 69.7                                  | 78.0                           | 53.9             | 57.4           |
| Ever alcohol consumption (yes)\(^b\) | 41.8                                  | 47.5                           | 1.8              | 3.9            |
| Ever cigarette smoking (yes)\(^b\) | 28.4                                  | 31.2                           | 22.7             | 27.9           |
| First-degree family history of breast cancer (yes)\(^b\) | 16.1                                  | 18.3                           | 4.3              | 5.4            |
| **Parity**\(^b\)          |                                       |                                 |                  |               |
| Nulliparous                | 18.1                                  | 16.4                           | 7.1              | 5.9            |
| 1 or 2                     | 43.9                                  | 47.5                           | 40.1             | 45.1           |
| 3 or more                  | 38.1                                  | 36.1                           | 52.8             | 49.0           |
| **Education**\(^b\)       |                                       |                                 |                  |               |
| Low                        | 7.1                                   | 4.9                            | 85.5             | 76.5           |
| Middle                     | 17.4                                  | 18.0                           | 8.2              | 13.2           |
| High                       | 75.5                                  | 77.1                           | 6.4              | 10.3           |
| **IGF-1 serum concentration (ng/mL)**\(^c\) |             |                                 |                  |               |
| Tertile 1                  | 33.6                                  | 27.9                           | 33.3             | 17.2           |
| Tertile 2                  | 33.6                                  | 31.2                           | 33.7             | 25.0           |
| Tertile 3                  | 32.9                                  | 41.0                           | 33.0             | 57.8           |
| **IGFBP-3 serum concentration (ng/mL)**\(^d\) |             |                                 |                  |               |
| Tertile 1                  | 33.6                                  | 21.3                           | 33.3             | 8.8            |
| Tertile 2                  | 33.6                                  | 34.4                           | 33.3             | 29.4           |
| Tertile 3                  | 32.9                                  | 44.3                           | 33.3             | 70.6           |

\(^a\) Mean and standard deviation (SD). \(^b\) Percentage. \(^c\) Cut points of tertiles of serum IGF-1 in 4-Corner’s Breast Cancer Study were (122.0, 161.0); in Mexico Breast Cancer Study (123.6, 200.7). \(^d\) Cut points of tertiles of serum IGFBP-3 1 in 4-Corner’s Breast Cancer Study were (3916, 4490); in Mexico Breast Cancer Study (2744, 3535).
including MAPK, AKT, Smad and STAT15,25. Recently, we reported an association between some SNPs of energy homeostasis genes and the risk11 or mortality26 of BC. Other authors have also reported associations between variants of these genes and BC risk27–29. As we described below, evidence shows that some energy homeostasis genes may also regulate signaling pathways involved in cancer development, supporting their potential to modify the association between IGF-1 and IGFBP-3 and the risk of BC.

In our study, seven SNPs in five genes (MBOAT4 rs13272159, ADIPOQ rs16131, LEPR rs12059300) showed a significant modifying effect (Pinteraction < 0.05; adjusted Pinteraction < 0.20), with at least one tertile of IGF-1 or IGFBP-3 associated with the risk of BC (Pvalue < 0.05).

We found that one SNP of MBOAT4 significantly modified the association of IGF-1 serum concentration and the risk of BC in premenopausal women. For the intronic (3'UTR) SNP of MBOAT4, rs13272159, the positive effect of the highest tertile of IGF-1 on the risk of BC was observed in women with the AA genotype but not in

| Table 2. Association between serum concentration of IGF-1 and breast cancer by SNPs of genes regulating energy homeostasis, the Breast Cancer Health Disparities Study. The model at the top of the table was not adjusted for potential confounders. The rest of the models were adjusted for IGFBP-3 serum concentration (ng/mL), age (years), genetic ancestry (% Indigenous), BMI (Kg/m2), energy intake (Kcal/day), height (cm), age at menarche (years), ever use oral contraceptives (no,yes), parity (nulliparous, 1 or 2, 3 or more), study (4-CBCS, MBCS), first-degree family history of breast cancer (no, yes), ever alcohol consumption (no, yes) and ever cigarette smoking (no, yes). aBenjamini-Hochberg adjusted P values (correction for the number of SNPs tested per gene); a threshold of 0.2 was considered significant. |

| SNP | Variables | OR 95% CI | P value |
|-----|-----------|------------|---------|
| IGF-1 T1 | 1.00 | |
| IGF-1 T2 | 1.34 | 0.87, 2.04 | 0.18 |
| IGF-1 T3 | 2.79 | 1.88, 4.13 | < 0.0001 |

Interaction Co-dominant models

| SNP | Variables | OR 95% CI | P value |
|-----|-----------|------------|---------|
| ADIPOQ | 0 = GG; Cases = 223; Controls = 338 | 1 = GA; Cases = 26; Controls = 49 | 2 = AA; Cases = 0; Controls = 2 |
| rs17366568 | IGF-1 T2 | 0.96 | 0.57, 1.63 | 0.89 | 0.14 | 0.01, 1.54 | 0.11 |
| n = 561 | IGF-1 T3 | 1.62 | 0.93, 2.82 | 0.09 | 0.09 | 0.01, 0.91 | 0.04 |
| GHRL | 0 = TT; Cases = 155; Controls = 234 | 1 = TC; Cases = 83; Controls = 126 | 2 = CC; Cases = 11; Controls = 29 |
| rs27647 | IGF-1 T1 | 1.00 | | |
| n = 389 | IGF-1 T3 | 1.75 | 0.90, 3.42 | 0.10 | 1.13 | 0.45, 2.86 | 0.79 |
| LEPR | 0 = TT; Cases = 96; Controls = 148 | 1 = TG; Cases = 119; Controls = 193 | 2 = GG; Cases = 40; Controls = 56 |
| rs970468 | IGF-1 T1 | 1.00 | | |
| n = 389 | IGF-1 T3 | 1.52 | 0.61, 3.79 | 0.37 | 1.42 | 0.67, 3.02 | 0.36 |
| MBOAT4 | 0 = AA; Cases = 53; Controls = 99 | 1 = AG; Cases = 120; Controls = 176 | 2 = GG; Cases = 76; Controls = 114 |
| rs13272159 | IGF-1 T1 | 1.00 | | |
| n = 504 | IGF-1 T3 | 3.92 | 0.91, 16.97 | 0.07 | 0.60 | 0.27, 1.32 | 0.60 |
| NPY | 0 = AA; Cases = 214; Controls = 332 | 1 = AG; Cases = 32; Controls = 53 | 2 = GG; Cases = 3; Controls = 4 |
| rs16131 | IGF-1 T1 | 1.00 | | |
| n = 546 | IGF-1 T3 | 1.14 | 0.66, 2.00 | 0.64 | 0.13 | 0.03, 0.71 | 0.02 |
| POMC | 0 = GG; Cases = 209; Controls = 340 | 1 = GA; Cases = 39; Controls = 48 | 2 = AA; Cases = 1; Controls = 1 |
| rs7565427 | IGF-1 T1 | 1.00 | | |
| n = 549 | IGF-1 T3 | 1.20 | 0.70, 2.06 | 0.52 | 0.05 | 0.01, 0.41 | 0.006 |

Including MAPK, AKT, Smad and STAT15,25. Recently, we reported an association between some SNPs of energy homeostasis genes and the risk11 or mortality26 of BC. Other authors have also reported associations between variants of these genes and BC risk27–29. As we described below, evidence shows that some energy homeostasis genes may also regulate signaling pathways involved in cancer development, supporting their potential to modify the association between IGF-1 and IGFBP-3 and the risk of BC.

In our study, seven SNPs in five genes (MBOAT4 rs13272159, ADIPOQ rs182052, rs822391 and rs7649121, CARTPT rs3846659, and LEPR rs12059300) showed a significant modifying effect (Pinteraction < 0.05; adjusted Pinteraction < 0.20), with at least one tertile of IGF-1 or IGFBP-3 associated with the risk of BC (Pvalue < 0.05).

We found that one SNP of MBOAT4 significantly modified the association of IGF-1 serum concentration and the risk of BC in premenopausal women. For the intronic (3'UTR) SNP of MBOAT4, rs13272159, the positive effect of the highest tertile of IGF-1 on the risk of BC was observed in women with the AA genotype but not in
Table 3. Association between serum concentration of IGFBP-3 and breast cancer by SNPs of genes regulating energy homeostasis, the Breast Cancer Health Disparities Study. The model at the top of the table was not adjusted for potential confounders. The rest of the models were adjusted for IGF-1 serum concentration (ng/mL), age (years), genetic ancestry (% Indigenous), BMI (Kg/m²), energy intake (Kcal/day), height (cm), age at menarche (years), ever use oral contraceptives (no,yes), parity (nulliparous, 1 or 2, 3 or more), study (4-CBCS, MBCS), first-degree family history of breast cancer (no, yes), ever alcohol consumption (no, yes) and ever cigarette smoking (no, yes). *Benjamini-Hochberg adjusted P value (correction for the number of SNPs tested per gene); a threshold of 0.2 was considered significant.
the presence of the G allele. There is evidence that supports the possible role of MBOAT4 in BC. The MBOAT4 gene encodes GHRL O-acyltransferase (GOAT), an enzyme that catalyzes GHRL octanoylation regulating GHRL activity\(^9\). The regulation of growth hormone (GH) and IGF-1 release is under the influence of GHRL\(^7\,\,9\). In our study, we did not find an association between the MBOAT4 rs13272159 SNP and the serum concentration of IGF-1 (data not shown). The peptide hormone GHRL, in addition to having a role in the regulation of feeding and energy balance, also regulates processes associated with cancer, such as cell proliferation, apoptosis, cell migration, cell invasion, angiogenesis and inflammation. The GHRL proliferation effect could be mediated through the activation of key signaling pathways, such as MAPK/ERK and/or PI3K/AKT/mTOR\(^8\,\,9\,\,30\). Although a precise role for GHRL in BC has not yet been established, it has been suggested that an imbalance in the expression of the GHRL system in mammary tissue could be implicated in breast tumor pathogenesis\(^8\). MBOAT4 mRNA expression seems to be upregulated in BC tumors\(^8\). Our group and other authors have reported an association between GHRL SNPs and BC risk\(^11\,\,28\,\,31\) or BC-specific mortality\(^26\). In addition, in a recent cohort study, GHRL expression was associated with increased survival in node-negative patients\(^32\).

For the intronic SNP of NPY, rs16131, the highest tertile of IGF-1 concentration was borderline associated with an increased risk of BC in the presence of AA but not in G carriers. The central orexigenic regulator NPY and its receptors have been implicated as growth-promoting factors in various cancer types. It has been shown that NPY regulates proliferation, migration, and vascular endothelial growth factor (VEGF) release to promote angiogenesis in BC cells. The mitogenic effects of NPY seem to be mediated by the p44/42 MAPK pathway in some malignancies\(^3\). It has been suggested that NPY may influence GH release (by modulating growth hormone-releasing hormone (GHRH) secretion)\(^3\), thus modulating IGF-1 release. There was no association between the NPY rs16131 SNP and the serum concentration of IGF-1 (data not shown). NPY receptor type 1 (NPY1R) has been proposed as a novel peripheral blood marker predictive of metastasis and prognosis in patients with BC\(^34\). In a previous work, we found that another NPY SNP (rs16129), was significantly associated with BC-specific mortality; however, no effect was observed for the rs16131 SNP\(^26\).

Our results showed that the association between IGFBP-3 serum concentration and BC risk differed by ADIPOQ SNPs (rs822391, rs7649121 and rs182052). In the case of ADIPOQ SNP rs822391, an increase in BC risk was observed for the highest tertile of serum concentration of IGFBP-3 in TT carriers and was lost in the presence of the minor allele C. Interestingly, other authors have reported that rs822391 (minor allele C) decreases the risk of overall prostate cancer\(^35\). Regarding the ADIPOQ rs7649121 SNP, compared to women in the lowest IGFBP-3 serum concentration tertile, women in the highest tertile showed higher BC risk. This association was higher in carriers of the T allele, than in AA homozygotes. To our knowledge, no association between this SNP and the risk of any cancer has been previously reported. For ADIPOQ SNP rs182052, our data showed that the positive effect of the highest tertile of IGFBP-3 concentration on the risk of BC was higher in the presence of the minor allele A. These results are consistent with other studies that have found that rs182052 (minor allele A) increased the risk of other types of cancer\(^5\,\,36\). Furthermore, these studies observed an association between high LEP plasma concentrations and BC risk\(^5\,\,7\). High LEP serum concentration tertile on the BC risk observed in women with the GG decreased in the presence of the minor allele A. Interestingly, it has been reported that rs12059300 interacts with methylation sites in the 5´UTR of LEPR\(^9\). There was no association between the IGFBP-3 highest serum concentration tertile and BC risk by ADIPOQ SNP rs12059300. Our results show that the positive effect of the IGFBP-3 highest serum concentration tertile on the BC risk observed in women with the GG decreased in the presence of the minor allele A. Interestingly, it has been reported that rs12059300 interacts with methylation sites in the 5´UTR of LEPR, influencing LEP serum levels (LEP levels decrease for AA compared to GG genotype)\(^37\). Some studies have observed an association between high LEP plasma concentrations and BC risk\(^37\). High LEP serum concentrations and LEPR overexpression have been positively correlated with the reduction in overall survival rates in patients with BC\(^6\). In a previous work, using the samples of the present study plus samples from other centers, we found that some LEPR SNPs were associated with BC risk\(^31\) and with BC mortality\(^26\) within specific ancestry strata; however, there was no association between rs12059300 and BC risk or mortality\(^41\,\,28\). Experimental data obtained with LEPR knockout mice suggested that LEP signaling could induce GH release\(^42\), thus regulating not only IGF-1 but also IGFBP-3 release. We did not find an association between the LEPR rs12059300 SNP and the IGFBP-3 serum concentration (data not shown). In addition to its neuroendocrine role, LEP and LEPR regulate a wide range of biological processes, including mammary tumorigenesis\(^5\,\,7\). LEP signaling promotes cell proliferation, differentiation, differentiation, survival, migration, and invasion via the activation of downstream pathways, including the Jak2/Stat3, MAPK and PI3K/Akt pathways\(^5\,\,7\). LEP crosstalk has also been reported with estrogen receptor alpha (ERa) via ERa transactivation\(^3\) and the IGF-1 pathway through mTORC1 and Stat5\(^43\). Based on these studies, our results suggest that the LEPR rs12059300 minor allele A could contribute to the reduction in BC risk.
Notably, in addition to its modifying effect on the association between IGFBP-3 and BC risk, rs12059300 also showed an effect modification on the association between IGF-1 and the risk of BC ($P_{\text{Interaction}} = 0.05$; adjusted $F_{\text{Interaction}} = 0.20$); however, this was not discussed in the IGF-1 section because associations inside strata were not statistically significant.

Finally, regarding the CARTPT SNP rs3846659, in women with the GG genotype, compared to the lowest IGFBP-3 serum concentration tertile, women in the highest tertile showed an increased BC risk, but this effect was lost in the presence of the minor allele C. In a previous work, our group found that rs3846659 was associated with the risk of premenopausal BC. CARTPT encodes CART prepropeptide, which ends up generating multiple biologically active peptides that have a role in the regulation of appetite, energy balance, maintenance of body weight, reward and addiction, and the stress response. CART is involved in the pituitary hormone secretion. Intracerebroventricular or peripheral administration of CART increases the GH concentration in rats, suggesting that CARTPT variants may modify the synthesis of IGF-1 and IGFBP-3. In our study, we did not find an association between the CARTPT rs3846659 SNP and the serum concentration of IGFBP-3 (data not shown). CART is expressed in primary and metastatic breast cancer; mediates ligand-independent activation of ERα through the MAPK pathway; and is an independent poor prognostic factor in ER-positive, lymph node-negative BC tumors.

We were able to combine data from two population-based case–control studies conducted in the United States (4-Corner's Breast Cancer Study) and Mexico (Mexico Breast Cancer Study). Given that we used similar questionnaires for both studies, we were able to harmonize the lifestyle data. A tag SNP approach was used to characterize variation across candidate genes. This approach was implemented on a customized Illumina platform and included SNPs that were validated and considered to have a high probability of yielding results. We adjusted for multiple comparisons to identify SNPs with a relevant modifying effect, however spurious interactions cannot be excluded.

Regarding the limitations, only premenopausal women were included in this study. Previous large epidemiological studies have found statistically significant associations between IGF-1 and breast cancer only in premenopausal women; in postmenopausal women the associations were weaker and not statistically significant. The percentage of indigenous ancestry was higher for the MBCS women than for those included in the 4-CBCS study. Therefore, we adjusted for the effect of both the study and indigenous ancestry in all multiple models. IGF-1 and IGFBP-3 serum concentrations were measured in two different labs using two different assays. Some of the IGF-1 and IGFBP-3 serum concentration discrepancies (less IGF-1 and more IGFBP-3 in the 4-CBS than in the MBCS) between MBCS and 4-CBCS studies could be related to assay methodology or to differences in characteristics between women from both studies. More women in the 4-CBCS reported to consume alcohol than in the MBCS (controls = 41.8%, cases 47.5%; controls = 1.8%, cases = 3.9%, respectively). Alcohol consumption has shown to reduce IGF-I serum concentration and to increase IGFBP-3 serum concentration. Although the IGF-1/IGFBP-3 ratio may vary between studies (0.03 to 0.10); the ratio remained constant in both cases and controls. In our study, the ratio in the 4-CBCS sample was 0.03 for both cases and controls, while in the MBCS sample it was 0.05 in both cases and controls. Therefore, we consider that it is unlikely that these discrepancies influenced our results. Although evidence suggest that carrying a BRCA1 mutation could be a potential confounder of the association between IGF-1 serum concentration and BC risk, we did not exclude women with these mutations, because we have this information only for the MBCS women with breast cancer and not for controls. The prevalence of this mutation in the MBCS cases was 4.2%, therefore adjusting for women with this mutation, could have not significantly altered our results. Finally, there is no information about the functional effect on protein expression caused by several of the SNPs analyzed in this study, thus limiting the biological comprehension of the observed interactions.

In summary, our data show for the first time that variants of energy homeostasis genes modify the effect of IGFBP-3 serum concentration on BC risk in premenopausal women. We found that MBOAT4 and NPY SNPs modified the association between IGF-1 serum concentration and the risk of BC, and ADIPOQ, LEPR, and CARTPT SNPs modified the association between IGFBP-3 and the risk of BC. Our data suggests that these genes could act as modifiers through their influence on known signaling pathways involved in BC development. Our results should be replicated in longitudinal studies and the molecular mechanisms responsible for the effect modifications that we found need to be further investigated. This study provides data that supports and highlights the importance of analyzing the interplay between multiple risk factors to contribute to a better understanding of breast cancer etiology and, in the future, improving risk prediction.

Methods
Study population. As part of the Breast Cancer Health Disparities study, the study population included premenopausal women from the 4-Corner's Breast Cancer Study (4-CCBS) and the Mexico Breast Cancer Study (MBCS). The study population for the present study was restricted to premenopausal women because in longitudinal studies, the association between the serum concentration of IGF-1 and the risk of BC has been observed in pre- but not postmenopausal women. Briefly, the 4-CCBS included women living in Arizona, Colorado, New Mexico or Utah. Eligible cases were between 25 and 79 years old and were histologically confirmed between 1999 and 2004 (n = 2557), while controls (n = 2605) were selected from the target population and were frequency-matched to cases on the expected ethnicity and over a 5-year age range. Interviews were performed by certified personnel, blood was collected, and DNA was extracted for 79% of the participants. Height, weight and waist and hip circumferences were obtained at interview. For the present study, a subsample of 61 cases and 155 controls were included because they had fasting IGF-1 and IGFBP-3 measurements and were not receiving chemotherapy. The MBCS included 1000 cases and 1074 controls between 35 and 69 years of age living in Monterrey, Veracruz and Mexico City for the past 5 years. Eligible cases were women
with BC histologically confirmed between January 2004 and December 2007. Controls were randomly selected from the catchment area of the 12 participating hospitals using a probabilistic multistage design. Controls were frequency-matched to cases, according to 5-year age groups, membership to a health care institution and place of residence. Interviews were performed by standardized personnel using a questionnaire based on the 4-CBCS. Blood was collected, and DNA was extracted from 91% of women. MBCS anthropometric measurements were obtained by standardized nurses at the hospitals. For this study, a random subsample of 204 cases and 282 controls who had serum IGF-1 and IGFBP-3 measurements was included.

**Serum biomarkers.** For both studies, serum levels of IGF-1 and IGFBP-3 were measured in fasting blood samples\(^5, \)\(^6\). For the 4-CBCS, IGF-1 and IGFBP-3 serum concentrations were measured at the Maine Center for Osteoporosis Research as described elsewhere\(^59\). Briefly, serum IGF-1 serum concentrations were determined using the IGF-1 (IGFBP-3 blocked) radioimmunoassay (American Laboratory Products Company (ALPCO) Windham, NH), while IGFBP-3 serum concentrations were ascertained using the “Active” IGFBP-3 IRMA kit (Diagnostic Systems Laboratories, Inc., Webster, Texas). All samples were tested in replicate, and the coefficient of variation ranged from 0.06 to 6.5% for IGF-1 and 0.17 to 7.5% for IGFBP-3. For the MBCS, serum samples were frozen at temperatures between –20 and -70 °C and processed no later than 4 weeks after the blood was collected. The IGF-1 and IGFBP-3 serum concentrations were determined using radioimmunoanalysis at Laboratorios Clínicos de Puebla, México (ISO 9001:2008).

**Data harmonization.** Data were harmonized as described in Slattery (2012)\(^19\). Variables considered as potential confounders were age (years), genetic ancestry (% Indigenous), body mass index (BMI, kg/m\(^2\)), energy intake (kcal/day), height (cm), age at menarche (years), ever used oral contraceptives (no, yes), parity (nulliparous, 1 or 2, 3 or more), education (low, middle, high), study (4-CBCS, MBCS), first-degree family history of breast cancer (no, yes), ever consumed alcohol (no, yes), ever smoked cigarettes (no, yes) and IGFBP-3 serum concentration (ng/mL) when IGF-1 was the independent variable of interest, or IGF-1 serum concentration (ng/mL) when IGFBP-3 was the independent variable of interest. Weight used to calculate the BMI was measured at the time of interview for the MBCS and was either self-reported weight during the referent year or weight reported five years prior to diagnosis if the referent year weight was not available for the 4-CBCS.

**Genetic data.** DNA was derived from whole blood from both the 4-CBCS and the MBCS participants. Genotyping was conducted as part of the Breast Cancer Health Disparities Study\(^19\). European and Native American Ancestry were measured based on 104 ancestral informative markers. For this study, we evaluated 10 genes that regulate energy homeostasis: ADIPOQ (12 SNPs), CARTP (5 SNPs), CCK (4 SNPs), GHRL (8 SNPs), LEP (9 SNPs), LEPR (27 SNPs), MBOAT4 (1 SNP), MC4R (3 SNPs), NPY (4 SNPs), and POMC (5 SNPs). Supplementary Table S1 describes the 78 SNPs in detail.

**Statistical analysis.** A descriptive analysis of the study population was conducted. Means and standard deviations were used for continuous variables and percentages for categorical variables. Allele and genotype frequencies were obtained through direct counting. Hardy–Weinberg equilibrium (HWE) was tested by the exact test among control subjects. Comparison of genotype frequencies between cases and controls was adjusted for ancestry (% Indigenous) by means of logistic regressions; \(P\) values were obtained by using likelihood ratio tests. We used logistic regressions and likelihood ratio tests to compare ancestry adjusted genotype frequencies in control subjects from both populations. A \(P\) value < 0.05 was considered statistically significant. To evaluate the potential effect modification of the association between serum concentration of IGF-1 (tertiles) or IGFBP-3 (tertiles) and the risk of BC by energy homeostasis gene polymorphisms, multiple logistic regression models were performed. Associations with SNPs were assessed assuming a codominant model. Variables considered as potential confounders were described in the paragraph on data harmonization. Effect modification models are presented when \(P\) for the interaction was lower than 0.10. In addition, for multiple hypothesis testing correction Benjamini–Hochberg adjusted \(P\) values were calculated for the number of SNPs tested per gene\(^61, \)\(^62\); a threshold of 0.20 was considered significant\(^61, \)\(^62\). The association between SNPs and IGF-1 and IGFBP-3 serum concentrations were analyzed. The Benjamini–Hochberg adjusted \(P\) values were calculated for the number of SNPs tested per gene; a \(P\) value < 0.05 was considered statistically significant. Logistic regression analyses were performed using Stata12 (Stata Corporation, College Station, TX) and false discovery rate adjustments were performed using R version 3.6.2.

All participants signed informed written consent prior to participation; the study was approved by the Institutional Review Board for protection of human subjects at each institution.

**Ethics approval.** The study was approved by the Institutional Review Board for protection of human subjects at the National Institute of Public Health in Mexico and The University of Utah: “Comité de Ética en Investigación and Institutional Review Board, respectively” and was performed in accordance with the Declaration of Helsinki.

**Consent to participate.** Signed informed consent of all women participating in the study was obtained.

**Consent for publication.** All authors consent to the publication of this manuscript.
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Author contributions

G.T.-M., M.L.S. and A.A.L. conceived the study design. G.T.-M., M.L.S., E.Z., R.W., K.B.B. and L.H. conducted the data acquisition. G.T.-M., L.M.-M., L.G.-F.-R., R.R.-V., L.M.S.-Z., L.F.-I. and E.O.-P. prepared the data for analyses and carried out the analyses. G.T.-M. and R.R.-V. drafted the initial manuscript. M.L.S. and E.Z. undertook a thoroughly critical review of the manuscript. All authors revised and approved the final manuscript. None of the funding organizations had any role in the design and conduct of the study, or in the analysis of the data or in the writing of the manuscript.
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Competing interests
The authors declare no competing interests.

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