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Authors
Jung, Su Yon
Ho, Gloria
Rohan, Thomas
et al.

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Interaction of insulin-like growth factor-I and insulin resistance-related genetic variants with lifestyle factors on postmenopausal breast cancer risk

Su Yon Jung[1] · Gloria Ho[2] · Thomas Rohan[3] · Howard Strickler[3] ·
Jennifer Bea[4] · Jeanette Papp[5] · Eric Sobel[5] · Zuo-Feng Zhang[6] · Carolyn Crandall[7]

Abstract

Purpose Genetic variants and traits in metabolic signaling pathways may interact with obesity, physical activity, and exogenous estrogen (E), influencing postmenopausal breast cancer risk, but these inter-related pathways are incompletely understood.

Methods We used 75 single-nucleotide polymorphisms (SNPs) in genes related to insulin-like growth factor-I (IGF-I)/insulin resistance (IR) traits and signaling pathways, and data from 1003 postmenopausal women in Women's Health Initiative Observation ancillary studies. Stratifying via obesity and lifestyle modifiers, we assessed the role of IGF-I/IR traits (fasting IGF-I, IGF-binding protein 3, insulin, glucose, and homeostatic model assessment-insulin resistance) in breast cancer risk as a mediator or influencing factor.

Results Seven SNPs in *IGF-I* and *INS* genes were associated with breast cancer risk. These associations differed between non-obese/active and obese/inactive women and between exogenous E non-users and users. The mediation effects of IGF-I/IR traits on the relationship between these SNPs and cancer differed between strata, but only roughly 35% of the cancer risk due to the SNPs was mediated by traits. Similarly, carriers of 20 SNPs in *PIK3R1*, *AKT1/2*, and *MAPK1* genes (signaling pathways–genetic variants) had different associations with breast cancer between strata, and the proportion of the SNP–cancer relationship explained by traits varied 45–50% between the strata.

Conclusions Our findings suggest that IGF-I/IR genetic variants interact with obesity and lifestyle factors, altering cancer risk partially through pathways other than IGF-I/IR traits. Unraveling gene–phenotype–lifestyle interactions will provide data on potential genetic targets in clinical trials for cancer prevention and intervention strategies to reduce breast cancer risk.

✉ Su Yon Jung
sjung@sonnet.ucla.edu

1 Translational Sciences Section, Jonsson Comprehensive Cancer Center, School of Nursing, University of California, Los Angeles, 700 Tiverton Ave, 3-264 Factor Building, Los Angeles, CA 90095, USA

2 Department of Occupational Medicine, Epidemiology and Prevention, Feinstein Institute for Medical Research, Hofstra Northwell School of Medicine, Great Neck, NY, USA

3 Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Bronx, NY, USA

4 Medicine & Nutritional Sciences, University of Arizona Cancer Center, Tucson, AZ, USA

5 Department of Human Genetics, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

6 Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles, Los Angeles, CA, USA

7 Division of General Internal Medicine, Department of Internal Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA

Introduction

Breast cancer is the most commonly occurring cancer in women and the second most common cause of cancer-related deaths in the United States [1]. The insulin-like growth factor-I (IGF-I)/insulin resistance (IR) axis demonstrates strong associations with breast cancer [2–5]. Total and/or free bioavailable IGF-I proteins are associated with higher breast cancer risk and worse cancer survival in premenopausal and postmenopausal women [6–8]. In postmenopausal women, high insulin levels have been associated with a twofold increase in breast cancer risk [9, 10], and homeostatic model assessment-insulin resistance (HOMA-IR), reflecting high blood levels of insulin and glucose, is positively associated with breast cancer in this population [11].

High IGF-I levels and IR (characterized by hyperinsulinemia and hyperglycemia) activate the IGF/insulin receptors, which are overexpressed in breast cancer. This overexpression results in the enhanced anabolic state necessary for cell proliferation, differentiation, and anti-apoptosis via deregulating or overactivating multiple downstream signaling pathways, including the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) and mitogen-activated protein kinase (MAPK) pathways [12–14]. Thus, high IGF-I levels and IR contribute to overexpression of relevant receptors and multiple abnormal cellular signaling cascades, and therefore may be associated with carcinogenesis.

Considering the relationships between IGF-I/IR traits and cancer risk, the IGF-I/IR-related genetic variants (e.g., single-nucleotide polymorphisms [SNPs]) that may influence high IGF-I, insulin, glucose, and HOMA-IR levels are possibly associated with increased risk of breast cancer. However, epidemiologic studies of these relationships among postmenopausal women are limited and have presented inconsistent findings [15–22], mainly due to different sets of covariates; lack of consideration of adjustment of relevant phenotypes and of interactions with lifestyle effect modifiers; and different races/ethnicities. In addition, the genetic alterations of IGF-I/IR signaling pathways lead to altered gene expression and protein function and are found in more than 70% of breast cancers [23]. Thus, the genetic variants that are related to IGF-I/IR signaling pathways are plausibly associated with increased risk of breast cancer, although population-based studies to examine these relationships have been limited [12, 24–27].

Breast cancer risk, particularly in postmenopausal women, is elevated among obese women [2, 28–30], and obesity status and obesity-related lifestyle factors such as inactivity are accompanied by elevated circulating IGF-I levels and IR traits [2, 5, 19, 20, 28, 29, 31–33]. Further, previous in vitro studies have revealed obesity–IGF-I/IR-related gene signature–breast cancer pathways, suggesting that genetic variants related to IGF-I/IR traits and their signaling pathways interact with obesity and jointly influence cancer susceptibility [3, 34].

Additionally, in postmenopausal women, endogenous estrogen (E) interacts with IGF-I/IR traits and their signaling pathways as well as their target genes in a synergistic cross-talk mechanism, resulting in the enhanced anabolic state necessary for tumor growth and development [2, 4, 28, 34, 35]. Likewise, oral exogenous E in this population has been postulated to interact with circulating levels of IGF-I/IR and their transduction pathways to affect cancer risk. However, unopposed E (i.e., E alone) has a different effect than opposed E (i.e., E + progestin [P]) has on IGF-I production. Because of the first-pass effect induced by oral E, resulting in suppressing hepatic production of IGF-I, E-only users have lower IGF-I levels, followed by increased IR [36, 37] and lower risk of breast cancer than non-users [38–40]. Owing to non-progesterone-like effects, E+P users (compared with E-only users) have different IGF-I levels, IR traits, and breast cancer risk [40–43], although the mechanisms are unclear.

In this case–cohort study among postmenopausal women, we evaluated the effect of IGF-I/IR traits (circulating levels of IGF-I, IGFBP3, insulin, glucose, and HOMA-IR) on cancer in two different roles: mediator (in relation to IGF-I/IR trait-related genetic variants) and influencing factor (in relation to IGF-I/IR signaling pathway-related genetic variants). We first focused on the mediation effects relating the genetic variants of IGF-I/IR traits (exposure) and breast cancer (outcome) and on the role of IGF-I/IR traits (mediator) in this association (Fig. 1). We first evaluated the magnitude of the total effect of the genetic variants of IGF-I/IR traits on breast cancer (i.e., the overall genetic effect, without considering the effect of IGF-I/IR traits). We then evaluated how this total effect is partitioned into indirect (through pathways mediated by IGF-I/IR traits) and direct effects (through pathways of other than IGF-I/IR traits). This approach allowed us to test the hypothesis that the genetic variants of IGF-I/IR traits are associated with increased risk of breast cancer and that the relationships depend on IGF-I/IR traits. Because IGF-I/IR traits are not mediators in the relationship between IGF-I/IR signaling pathway-relevant genetic variants and breast cancer, we examined the effect of IGF-I/IR traits as an influencing factor (Fig. 2).

Given that obesity status, physical activity (PA), and exogenous E influence both IGF-I/IR and their genetic factors and, through this complicated mechanism, are associated with breast cancer, we evaluated how the pathway of IGF-I/IR's genetic variants, IGF-I/IR traits, and breast cancer is influenced by obesity, PA, and exogenous E. Unraveling these complicated gene–phenotype–lifestyle
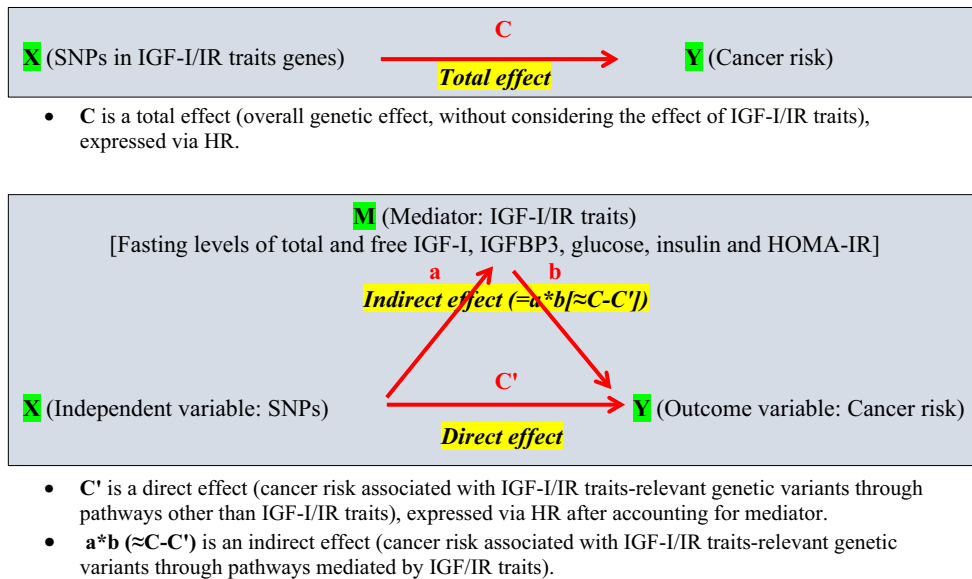
Fig. 1 Diagrams of total, direct, and indirect pathways of SNPs in IGF-I/IR *trait* genes, IGF-I/IR traits, and breast cancer risk. (*HOMA-IR* homeostatic model assessment-insulin resistance, *HR* hazard ratio, *IGF-I* insulin-like growth factor-I, *IGFBP3* IGF-binding protein 3, *IR* insulin resistance, *SNP* single-nucleotide polymorphism)

Proportion explained by IGF-IR traits of risk for breast cancer that are related to IGF-I/IR signaling pathway-related genetic variants was computed:

$$[(\text{HR}_{\text{without IGF-I/IR traits}} - \text{HR}_{\text{with IGF-I/IR traits}}) / (\text{HR}_{\text{without IGF-I/IR traits}} - 1.0)] \times 100$$

where $\text{HR}_{\text{with IGF-I/IR traits}}$ denotes the HR for the effect of SNPs on breast cancer after adjustment of IGF-I/IR traits.

Fig. 2 Pathways of SNPs in IGF-I/IR *signaling pathway*-related genes, IGF-I/IR traits, and breast cancer risk (*HOMA-IR* homeostatic model assessment-insulin resistance, *HR* hazard ratio, *IGF-I* insulin-like growth factor-I, *IGFBP3* IGF-binding protein 3, *IR* insulin resistance, *SNP* single-nucleotide polymorphism)
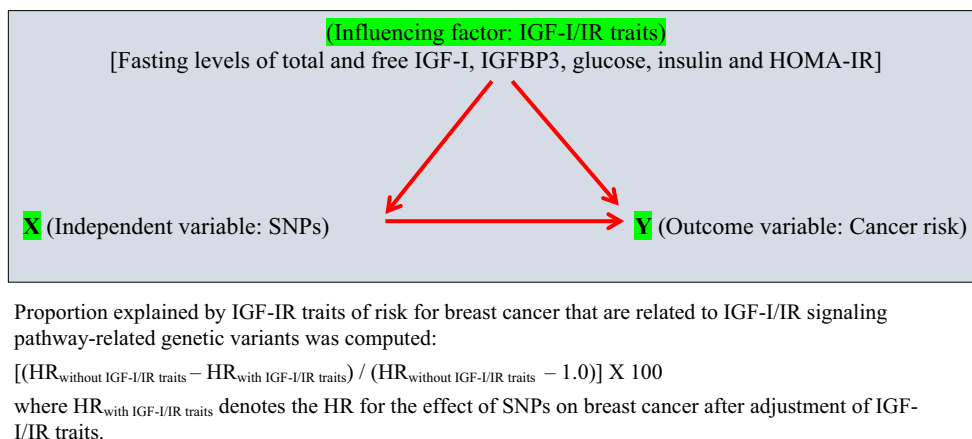
interactions will provide insights into the role of the IGF-I/IR axis in the development of breast cancer.

Materials and methods

Study population

This study included 1003 postmenopausal women enrolled in ancillary studies of the Women's Health Initiative

Observation Study (WHI-OS) between October 1, 1993 and December 31, 1998. Details of the WHI study's design and rationale have been described elsewhere [44]. Women were eligible for the WHI study if they were 50–79 years old, postmenopausal, planned to live in an area near the clinical centers for at least 3 years after study enrollment, and were able to provide written consent. The ancillary studies were designed for a nested case–cohort study within the WHI-OS. In this study design, non-cases were randomly selected from the parent cohort, forming a subcohort which was

representative of the underlying population. For the purposes of our study, we initially included 1491 women who reported their race or ethnicity as non-Hispanic white and were eligible for the breast case–cohort study (Figure S1). Of those 1491 women, we finally included a total of 1003 women (breast cancer cases = 539, controls = 464). As of February 29, 2004, the ancillary studies completed the selection of women with a mean follow-up of 77 months [10]. This study was approved by the institutional review boards of each participating clinical center of the WHI and the University of California, Los Angeles.

Data collection and cancer outcome variables

Standardized written protocols had been used to ensure uniform data collection. At baseline, self-administered questionnaires were completed by participants on demographic (age, education, family income, and family histories of DM or breast cancer) and lifestyle (PA, smoking status, and alcohol intake) factors and their medical (heart failure and hypercholesterolemia) and reproductive histories (oral contraceptive and exogenous E use [E-only and E+P users], history of hysterectomy or oophorectomy, ages at menarche and menopause, and history of pregnancy). Anthropometric measurements (height, weight, and waist and hip circumferences) were measured at baseline by trained staff. These variables were originally selected for this study on the basis of a literature review for their associations with IGF-I/IR and breast cancer; after multicollinearity testing and univariate and stepwise regression analyses, they were finally determined to be analyzed.

Cancer outcomes were determined using a centralized review of medical charts, and cancer cases were coded according to the National Cancer Institute's Surveillance, Epidemiology, and End Results guidelines [45]. The outcome variables were breast cancer and the time to development of breast cancer. The time from enrollment to breast cancer development, censoring, or study end-point was recorded as the number of days and then converted into years.

Genotyping

Ten genes (Tables S1.1–6) were selected based on the biological significance of their gene products, or whether epidemiologic and/or experimental data support an association between the gene and the levels of IGF and insulin or between the gene and risk of cancer [12, 15–22, 24–27]. For each gene, HTSNP2 (http://www-gene.cimr.cam.ac.uk/clayton/software/stata) searched all possible subsets of SNPs that best captured the full haplotype information. Specifically, the selected SNPs had a minimum allelic association of 0.8 with the unselected SNPs within an LD block. A total of 75 SNPs from 10 genes were identified.

The MassARRAY system (Sequenom, Inc., San Diego, CA), based on mass spectrometry, was used for genotyping. Quality assurance was conducted according to a standardized protocol, with a missing call rate of <1%, the number of discordant calls <3%, and Hardy–Weinberg Equilibrium of $p \geq 10^{-4}$.

Laboratory methods

Fasting blood samples had been collected from each participant at baseline by trained phlebotomists. Serum concentrations of glucose and insulin were measured by the Medical Research Laboratories (Highland Heights, KY) using assays with sensitivities of 0.5 mg/dL and 0.26 μIU/mL, average coefficients of variation (CVs) of 4.2 and 3.4%, and correlation coefficients of 0.95 and 0.98, respectively. The HOMA-IR was estimated as glucose (mg/dl) × insulin (μIU/ml)/405 [46]. Serum total and free IGF-I and IGFBP3 were measured using enzyme-linked immunosorbent assays (Diagnostic Systems Laboratories, Webster, TX) with sensitivities of 0.01, 0.015, and 0.04 ng/mL, average CVs of 8.2, 11.2, and 3.6%, and correlation coefficients of 0.96, 0.9, and 0.9, respectively.

Statistical analysis

Differences in baseline characteristics and allele frequencies, stratified by potential modifiers, were assessed using unpaired two-sample t tests for continuous variables and χ^2 tests for categorical variables. If continuous variables were skewed or had outliers, Wilcoxon rank-sum test was used. With the regression assumptions met, multiple linear regression was performed to estimate effect sizes and 95% confidence intervals (CIs) for the exposure (IGF-I/IR-related SNPs with additive, minor-allele dominant and recessive models) predicting the outcomes (IGF-I/IR traits: fasting total and free IGF-I, IGFBP3, glucose, insulin, and HOMA-IR levels) (Tables S2.1–2). The Cox proportional hazards regression model designed for case–cohort data (assumption test was done via a Schoenfeld residual plot and rho) was conducted to obtain hazard ratios (HRs) and 95% CIs for IGF-I/IR traits and IGF-I/IR-related SNPs in predicting breast cancer.

A total and direct effect size of IGF-I/IR trait-related SNP (exposure) on breast cancer (outcome) was produced from the HR for IGF-I/IR trait-related SNP on cancer in the Cox model that included all covariates, without (total) and with (direct) IGF-I/IR traits (mediator). The mediation effect size was produced via a traditional statistical method [47, 48]: the percentage change in the HRs by comparing a model that includes all covariates with a model that includes all covariates and the mediator. In addition, the effect of IGF-I/IR traits on risk for breast cancer that is related to IGF-I/IR signaling

pathway-relevant genetic variants was estimated using the same algorithm as that of mediator, but it was interpreted as an influential factor (Figure 2). To evaluate the role of obesity, PA, and exogenous E as effect modifiers on the IGF-I/IR genetic factor–IGF-I/IR trait–cancer relationship, we used a stratification approach. A two-tailed *p* value <0.05 was considered statistically significant. The R statistical package (v 2.15.1) was used.

Results

Participants' baseline characteristics and allele frequencies of 75 SNPs by obese status, level of PA, and exogenous E use are presented in Supplementary Tables S1.1–6 and S3.1–6. The participants had been followed up through February 29, 2004, resulting in 539 breast cancer cases (52% of non-obese and 56% of obese women).

Breast cancer risk associated with IGF-I/IR *trait*-related SNPs mediated through IGF-I/IR traits, stratified by obesity status (BMI, waist, and w/h), level of PA, and exogenous E use

We partitioned the total effect of IGF-I/IR trait-related SNPs on breast cancer risk into direct (not via IGF-I/IR traits) and indirect (via IGF-I/IR traits) effects. Each SNP in this analysis was mediated via fasting levels of total and free IGF-I, IGFBP3, glucose, insulin, and HOMA-IR. For each SNP with mediators, the IGF-I/IR trait–SNP–cancer association was evaluated, stratified by obesity status (BMI <30 vs. ≥30; waist ≤88 cm vs. >88 cm; and w/h ≤0.85 vs. >0.85), level of PA (MET ≥10 vs. <10) (Table 1), and by exogenous E use (non-users vs. E-only or E+P users) (Table 2).

Among 19 IGF-I/IR trait-related SNPs, 7 SNPs in *IGF-I* and *INS* genes were significantly associated with breast cancer risk (Tables 1, 2). Overall, the SNP–cancer associations differed between non-obese/active and obese/inactive women, and between exogenous E non-users and users. In both strata, the SNP–cancer risk effect was stronger in each SNP for a direct effect than an indirect (mediation) effect regardless of the mediator, but the mediation effects differed between non-obese/active and obese/inactive women and between exogenous E non-users and users.

Carriers of the *IGF-I* rs10860865 T and the *IGF-I* rs978458 T alleles had increased breast cancer risk among the non-obese (BMI <30, waist ≤88 cm) and inactive groups. The mediation (IGFBP3 level) effects on these SNP–cancer associations in the non-obese groups were minimal, compared with those (roughly 35%) in the obese groups. The mediation (insulin and HOMA-IR levels) effect in inactive women was also minimal, whereas in active women the mediation effect was moderate, roughly 45% (Table 1).

Carriers of the *IGF-I* rs5742671 A and the *IGF-I* rs1520220 G alleles had associations similar to those found in the inactive group of carriers of the *IGF-I* rs10860865 and the *IGF-I* rs978458, showing that they had an association with increased breast cancer risk in inactive women. Similarly, the mediation effects in those carriers on the SNP–cancer association were minimal in inactive women and smaller than those in the active women (roughly 40%).

In contrast, carriers of the *INS* rs689 T allele had decreased breast cancer risk in non-obese women (waist ≤88 cm), and the mediation effects of insulin and HOMA-IR in this group were negligible. Notably, these carriers among exogenous E non-users (Table 2) had an increased risk of breast cancer, with roughly 60% of cancer risk attributable to this variant that was mediated via HOMA-IR levels.

Breast cancer risk associated with IGF-I/IR *signaling pathway*-related SNPs and IGF-I/IR traits, stratified by obesity status (BMI, waist, and w/h), level of PA, and exogenous E use

Because IGF-I/IR traits are not mediators of the relationship between SNPs in IGF-I/IR signaling pathway genes (in this study, the *IRS1, TCF7L2, PIK3R1, AKT1/2,* and *MAPK1/3* genes) and breast cancer, instead of the mediation effect of the traits, we estimated a proportion explained by the traits as an influencing factor for the SNP–cancer relationship (Fig. 2). For each SNP, the proportion was estimated using the traits, stratified by obesity status, level of PA (Table 3), and exogenous E use (Table 4). Of the 56 IGF-I/IR signaling pathway-related SNPs, 20 SNPs were significantly associated with breast cancer risk. Overall, the association between the SNP and cancer differed by obesity, PA, and exogenous E use. Further, the proportion of the SNP–cancer association explained by traits differed between those strata.

In relation to the SNPs in the *PIK3R1* gene, carriers of 4 SNPs (rs12657050 T, rs171649 A, rs831123 G, and rs7707370 C alleles) had increased risk of breast cancer in obese women (BMI ≥30 kg/m^2), with roughly 40% of breast cancer risk owing to each genetic variant that was explained by traits (Table 3). In addition, carriers of another 4 SNPs in the *PIK3R1* gene (rs706711 A, rs1664577 G, rs3730089 A, and rs251404 T alleles) also had increased risk of breast cancer in obese women with w/h >0.85. About 50% of breast cancer risk associated with each genetic variant was dependent on traits (Table 3). Interestingly, carriers of the *PIK3R1* rs831123 G allele, in whom increased cancer risk was found in women with BMI ≥30, had an association with increased breast cancer risk in non-users of exogenous E (Table 4); however, the effects of total and free IGF-I on the SNP–cancer

Table 1 Mediation (indirect) effect of hormones on the relationship between IGF-1/IR-relevant SNPs and breast cancer risk, stratified by obesity status and physical activity level

SNP_[a]	Effect allele/ other allele	Non-obese/active group				Indirect effect[c]	
		Total effect[b] Breast cancer risk in relation to SNP		Direct effect[b] Breast cancer risk in relation to SNP through pathways other than hormone		Breast cancer risk in relation to SNP through hormone	
		HR	95% CI	HR	95% CI		%
		BMI <30 (n = 766)				IGFBP3 level	
IGF-IRS10860865_R	T/G	**2.05**	**(1.16–3.61)**	**2.03**	**(1.15–3.58)**		2.19
IGF-IRS6214_D	A/G	**1.42**	**(1.07–1.88)**	**1.41**	**(1.07–1.88)**		0.74
IGF-IRS978458_R	T/C	**2.03**	**(1.17–3.51)**	**2.02**	**(1.16–3.49)**		1.52
		Waist ≤88 cm (n = 662)[d]				Insulin level	
IGF-IRS7136446_D	C/T	**1.41**	**(1.03–1.94)**	**1.40**	**(1.02–1.92)**		2.58
INSRS689_D	T/A	**0.73**	**(0.55–0.98)**	**0.73**	**(0.55–0.97)**		0.21
						HOMA-IR level	
INSRS689_D	T/A	**0.73**	**(0.55–0.98)**	**0.73**	**(0.54–0.97)**		0.38
		w/h ratio ≤0.85 (n = 769)				IGFBP3 level	
IGF-IRS10860865_R	T/G	**1.83**	**(1.05–3.16)**	**1.80**	**(1.04–3.12)**		2.99
IGF-IRS978458_R	T/C	**1.86**	**(1.10–3.17)**	**1.84**	**(1.08–3.14)**		2.29
		Active group (MET ≥10, n = 545)[e]				IGFBP3 level	
IGF-IRS5742671_R	A/G	1.28	(0.56–2.92)	1.11	(0.48–2.58)	Total IGF-I level	61.07
IGF-IRS5742671_R	A/G	1.28	(0.56–2.93)	1.19	(0.52–2.75)		31.57
						Glucose level	
IGF-IRS10860865_D	T/G	**0.55**	**(0.40–0.77)**	**0.55**	**(0.40–0.77)**		0.04
IGF-IRS10860865_R	T/G	1.36	(0.73–2.54)	1.44	(0.76–2.70)		21.30
IGF-IRS1520220_R	G/C	1.35	(0.56–3.25)	1.47	(0.60–3.59)		35.69
IGF-IRS978458_D	T/C	**0.57**	**(0.41–0.80)**	**0.58**	**(0.41–0.81)**		0.43
IGF-IRS978458_R	T/C	1.30	(0.71–2.40)	1.43	(0.77–2.65)		42.26
		Active group (MET ≥10, n = 545)[e]				Insulin level	
IGF-IRS10860865_R	T/G	1.33	(0.71–2.50)	1.47	(0.78–2.77)		39.93
IGF-IRS5742671_R	A/G	1.24	(0.54–2.86)	1.34	(0.58–3.09)		41.99
IGF-IRS978458_R	T/C	1.26	(0.68–2.34)	1.40	(0.75–2.62)		51.26

Table 1 continued

SNP[a]	Effect allele/other allele	Non-obese/active group					
		Total effect[b] Breast cancer risk in relation to SNP		Direct effect[b] Breast cancer risk in relation to SNP through pathways other than hormone		Indirect effect[c] Breast cancer risk in relation to SNP through hormone	
		HR	95% CI	HR	95% CI	%	
						HOMA-IR level	
IGF-IRS10860865_R	T/G	1.33	(0.71–2.50)	1.50	(0.79–2.83)	49.01	
IGF-IRS5742671_R	A/G	1.24	(0.54–2.86)	1.35	(0.58–3.11)	46.40	
IGF-IRS978458_R	T/C	1.26	(0.68–2.34)	1.43	(0.76–2.69)	64.66	

SNP[a]	Effect allele/other allele	Obese/inactive group					
		Total effect[b] Breast cancer risk in relation to SNP		Direct effect[b] Breast cancer risk in relation to SNP through pathways other than hormone		Indirect effect[c] Breast cancer risk in relation to SNP through hormone	
		HR	95% CI	HR	95% CI	%	
BMI ≥30 (n = 236)						IGFBP3 level	
IGF-IRS10860865_R	T/G	0.49	(0.14–1.72)	0.31	(0.08–1.21)	37.02	
IGF-IRS6214_D	A/G	1.17	(0.65–2.12)	1.24	(0.68–2.25)	39.81	
IGF-IRS978458_R	T/C	0.50	(0.14–1.77)	0.32	(0.08–1.25)	36.87	
Waist >88 cm (n = 320)[d]						Insulin level	
IGF-IRS7136446_D	C/T	1.22	(0.76–1.93)	1.15	(0.72–1.84)	30.61	
INSRS689_D	T/A	1.13	(0.72–1.79)	1.09	(0.69–1.74)	29.83	
w/h ratio >0.85 (n = 233)						HOMA-IR level	
INSRS689_D	T/A	1.13	(0.72–1.79)	1.09	(0.69–1.73)	31.10	
						IGFBP3 level	
IGF-IRS10860865_R	T/G	0.51	(0.15–1.75)	0.35	(0.10–1.30)	30.60	
IGF-IRS978458_R	T/C	0.49	(0.14–1.69)	0.34	(0.09–1.24)	31.22	
Inactive group (MET <10, n = 457)[e]						IGFBP3 level	
IGF-IRS5742671_R	A/G	**3.13**	**(1.03–9.49)**	3.05	(0.99–9.37)	3.49	

Table 1 continued

SNP_[a]	Effect allele/ other allele	Obese/inactive group Total effect[b] Breast cancer risk in relation to SNP		Direct effect[b] Breast cancer risk in relation to SNP through pathways other than *hormone*		Indirect effect[c] Breast cancer risk in relation to SNP through *hormone*
		HR	95% CI	HR	95% CI	%
						Total IGF-I level
IGF-IRS5742671_R	A/G	**3.15**	**(1.04–9.56)**	3.11	(1.02–9.44)	1.96
						Glucose level
IGF-IRS10860865_D	T/G	1.10	(0.75–1.60)	1.07	(0.73–1.57)	30.07
IGF-IRS10860865_R	T/G	**2.94**	**(1.33–6.51)**	**3.55**	**(1.56–8.08)**	31.50
IGF-IRS1520220_R	G/C	2.66	(0.95–7.45)	2.92	(1.04–8.21)	15.99
IGF-IRS978458_D	T/C	1.10	(0.75–1.62)	1.07	(0.73–1.58)	30.96
IGF-IRS978458_R	T/C	**3.15**	**(1.43–6.93)**	**3.74**	**(1.65–8.44)**	27.39
		Inactive group (MET < 10, n = 457)[e]				
						Insulin level
IGF-IRS10860865_R	T/G	**2.96**	**(1.34–6.56)**	**3.01**	**(1.35–6.70)**	2.43
IGF-IRS5742671_R	A/G	**3.40**	**(1.10–10.50)**	**3.46**	**(1.12–10.72)**	2.45
IGF-IRS978458_R	T/C	**3.17**	**(1.44–6.98)**	**3.21**	**(1.45–7.11)**	2.04
						HOMA-IR level
IGF-IRS10860865_R	T/G	**2.96**	**(1.34–6.56)**	**3.13**	**(1.40–7.01)**	8.51
IGF-IRS5742671_R	A/G	**3.40**	**(1.10–10.50)**	**3.60**	**(1.16–11.18)**	8.04
IGF-IRS978458_R	T/C	**3.17**	**(1.44–6.98)**	**3.33**	**(1.49–7.41)**	7.36

Among SNPs having statistically significant association with cancer in either subgroup, the SNPs with ≥30% of indirect effect in this subgroup or its counterpart are included. Numbers in bold face are statistically significant

BMI body mass index, *CI* confidence interval, *HOMA-IR* homeostatic model assessment-insulin resistance, *HR* hazard ratio, *IGF-I* insulin-like growth factor-I, *IGFBP3* insulin-like growth factor-binding protein 3, *IR* insulin resistance, *MET* metabolic equivalent, *SNP* single-nucleotide polymorphism, *w/h ratio* waist-to-hip ratio

[a] Tag attached to the SNP name indicates a SNP analysis approach (CT: additive; D: minor-allele dominant; and R: minor-allele recessive)

[b] Multivariate regression was adjusted by covariates (age, education, family income, family histories of diabetes mellitus or breast cancer, heart failure ever, high cholesterol requiring pills ever, smoking status, alcohol intake, and reproductive history [oral contraceptive and history of hysterectomy or oophorectomy, ages at menarche and menopause, and history of pregnancy]); effect modifier variables (physical activity, BMI, and exogenous estrogen use), when not evaluated as effect modifier variables, were adjusted as a covariate; when stratified via waist circumference or w/h ratio, BMI was not adjusted

[c] Indirect effect estimated via the proportional change between the HRs without (total effect) and with (direct effect) accounting for hormone

[d] Participants with available insulin or HOMA-IR levels, stratified by waist circumference as non-obese (waist ≤88 cm; n = 662) or obese (waist >88 cm; n = 320)

[e] Participants with available IGFBP3 levels, stratified by physical activity (PA) level as active (MET ≥10; n = 544) or inactive (MET <10; n = 545) or inactive (MET ≥10; n = 457); participants with available total IGF-I levels, stratified by PA level as active (n = 544) or inactive (n = 458); participants with available glucose levels, stratified by PA level as active (n = 542) or inactive (n = 455); participants with available insulin levels, stratified by PA level as active (n = 534) or inactive (n = 448); participants with available HOMA-IR levels, stratified by PA level as active (n = 534) or inactive (n = 448)

Table 2 Mediation (indirect) effect of hormones on the relationship between IGF-1/IR-relevant SNPs and breast cancer risk, stratified by exogenous estrogen status (non-users vs. E-only users or E+P users)

SNP[a]	Effect allele/other allele	Non-user group					
		Total effect[b]		Direct effect[b]		Indirect effect[c]	
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP through pathways other than hormone		Breast cancer risk in relation to SNP through hormone	
		HR	95% CI	HR	95% CI	%	HOMA-IR level
		Non-users (n = 337)					
INSRS689_R	T/A	2.24	(0.81–6.17)	**2.96**	**(1.05–8.34)**	58.64	HOMA-IR level
		Non-users (n = 337)					
INSRS689_R	T/A	2.24	(0.81–6.17)	**2.96**	**(1.05–8.34)**	58.64	HOMA-IR level

SNP[a]	Effect allele/other allele	User group					
		Total effect[b]		Direct effect[b]		Indirect effect[c]	
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP through pathways other than hormone		Breast cancer risk in relation to SNP through hormone	
		HR	95% CI	HR	95% CI	%	HOMA-IR level
		E-only users (n = 289)					
INSRS689_R	T/A	0.57	(0.24–1.35)	0.58	(0.24–1.38)	1.92	HOMA-IR level
		E+P users (n = 281)					
INSRS689_R	T/A	0.65	(0.28–1.50)	0.61	(0.26–1.43)	6.34	HOMA-IR level

Note Among SNPs having statistically significant association with cancer in either subgroup, the SNPs with ≥30% of indirect effect in this subgroup or its counterpart are included. Numbers in bold face are statistically significant

CI confidence interval, *E* exogenous estrogen, *HOMA-IR* homeostatic model assessment-insulin resistance, *HR* hazard ratio, *IGF-1* insulin-like growth factor-I, *IGFBP3* insulin-like growth factor-binding protein 3, *IR* insulin resistance, *P* progestin, *SNP* single-nucleotide polymorphism

[a] Tag attached to the SNP name indicates a SNP analysis approach (CT: additive; D: minor-allele dominant; and R: minor-allele recessive)

[b] Multivariate regression was adjusted by covariates (age, education, family income, family histories of diabetes mellitus or breast cancer, heart failure ever, high cholesterol requiring pills ever, smoking status, alcohol intake, and reproductive history [oral contraceptive and history of hysterectomy or oophorectomy, ages at menarche and menopause, and history of pregnancy]); effect modifier variables (physical activity, BMI, and exogenous estrogen use), when not evaluated as effect modifier variables, were adjusted as a covariate; when stratified via waist circumference or w/h ratio, BMI was not adjusted

[c] Indirect effect estimated via the proportional change between the HRs without (total effect) and with (direct effect) accounting for hormone

Table 3 Effect of hormones on the relationship between IGF-I/IR signaling pathway-relevant SNPs and breast cancer risk, stratified by obesity status and physical activity level

SNP_[a]	Effect allele/other allele	Non-obese/active group					Proportion of relationship between breast cancer and SNP explained by *hormone** %
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP adjusted by *hormone*			
		HR[b]	95% CI	HR[b]	95% CI		
		BMI <30 (n = 766)[d]					
PIK3R/RS12657050_R	T/C	**0.41**	**(0.22–0.77)**[d]	**0.42**	**(0.22** – **0.80)**		IGFBP3 level
							3.26
PIK3R/RS171649_CT	A/G	1.05	(0.85 – 1.28)	1.05	(0.85 – 1.28)		0.66
PIK3R/RS171649_D	A/G	1.04	(0.79 – 1.37)	1.04	(0.79 – 1.38)		11.73
PIK3R/RS831123_D	G/C	1.05	(0.73 – 1.50)	1.04	(0.73 – 1.50)		Glucose level
							6.09
AKT/RS2494744_CT	T/C	**1.54**	**(1.06** – **2.22)**	**1.52**	**(1.05** – **2.20)**		Insulin level
							3.09
AKT/RS2494744_D	T/C	**1.57**	**(1.07** – **2.32)**	**1.55**	**(1.05** – **2.30)**		3.33
PIK3R/RS12657050_R	T/C	**0.41**	**(0.22** – **0.76)**	**0.41**	**(0.22** – **0.77)**		0.27
PIK3R/RS7707370_CT	C/T	0.90	(0.74 – 1.09)	0.90	(0.74 – 1.09)		0.42
PIK3R/RS7707370_R	C/T	0.87	(0.64 – 1.20)	0.88	(0.64 – 1.21)		0.92
AKT/RS2494744_CT	T/C	**1.54**	**(1.06** – **2.22)**	**1.52**	**(1.05** – **2.20)**		HOMA-IR level
							2.83
PIK3R/RS12657050_R	T/C	**0.41**	**(0.22** – **0.76)**	**0.41**	**(0.22** – **0.76)**		N/A

Table 3 continued

SNP_ [a]	Effect allele/other allele	Non-obese/active group — Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP adjusted by *hormone*		Proportion of relationship between breast cancer and SNP explained by *hormone**
		HR[b]	95% CI	HR[b]	95% CI	%
PIK3R/RS7707370_R	C/T	0.87	(0.64 – 1.20)	0.88	(0.64 – 1.21)	0.67
		Waist ≤88 cm (n=673)[e]				IGFBP3 level
AKT1/RS2494740_CT	T/A	1.08	(0.87 – 1.35)	1.11	(0.89 – 1.38)	32.57
AKT1/RS2494740_R	T/C	1.07	(0.66 – 1.73)	1.11	(0.68 – 1.80)	60.43
AKT1/RS2494744_CT	T/C	**1.57**	**(1.06 – 2.33)**	**1.58**	**(1.06 – 2.35)**	1.23
AKT1/RS2494744_D	T/C	**1.62**	**(1.07 – 2.46)**	**1.63**	**(1.08 – 2.47)**	1.12
						Insulin level
AKT1/RS2494744_D	T/C	**1.67**	**(1.10 – 2.54)**	**1.63**	**(1.07 – 2.49)**	5.62
						HOMA-IR level
AKT1/RS2494744_D	T/C	**1.67**	**(1.10 – 2.54)**	**1.63**	**(1.06 – 2.49)**	6.53
		w/h Ratio ≤ 0.85 (n=769)[f]				IGFBP3 level
AKT2/RS2304186_CT	A/C	1.04	(0.86 – 1.25)	1.03	(0.86 – 1.25)	9.60
AKT2/RS2304186_D	A/C	1.01	(0.76 – 1.34)	1.00	(0.75 – 1.33)	N/A
MAPK/RS2266966_R	G/A	1.20	(0.83 – 1.75)	1.22	(0.84 – 1.77)	7.37
MAPK/RS2283791_R	C/G	1.19	(0.82 – 1.73)	1.20	(0.83 – 1.75)	7.01

Table 3 continued

SNP_[a]	Effect allele/other allele	Non-obese/active group		Breast cancer risk in relation to SNP adjusted by hormone		Proportion of relationship between breast cancer and SNP explained by hormone*
		Breast cancer risk in relation to SNP				
		HR[b]	95% CI	HR[b]	95% CI	%
MAPK/RS2298432_CT	T/G	0.79	(0.64 – 0.97)	0.79	(0.64 – 0.97)	0.10
MAPK/RS9610505_R	A/T	1.16	(0.83 – 1.62)	1.18	(0.84 – 1.64)	11.03
PIK3R/RS706711_D	A/G	1.02	(0.77 – 1.36)	1.02	(0.76 – 1.35)	21.96
						Free IGF-I level
MAPK/RS2298432_CT	T/G	0.80	(0.65 – 0.98)	0.80	(0.65 – 0.98)	0.19
MAPK/RS2298432_R	T/G	0.54	(0.35 – 0.85)	0.55	(0.35 – 0.85)	0.62
PIK3R/RS1664577_R	G/T	0.58	(0.21 – 1.65)	0.58	(0.20 – 1.67)	0.29
						Insulin level
MAPK/RS2298432_CT	T/G	0.78	(0.63 – 0.96)	0.78	(0.63 – 0.96)	0.18
MAPK/RS2298432_R	T/G	0.55	(0.36 – 0.85)	0.55	(0.35 – 0.85)	0.69
PIK3R/RS3730089_CT	A/G	1.00	(0.77 – 1.30)	1.01	(0.78 – 1.32)	1.29
						HOMA-IR level
MAPK/RS2298432_CT	T/G	0.78	(0.63 – 0.96)	0.78	(0.63 – 0.96)	0.03
MAPK/RS2298432_R	T/G	0.55	(0.36 – 0.85)	0.55	(0.35 – 0.86)	0.04
PIK3R/RS1664577_R	G/T	0.54	(0.19 – 1.50)	0.55	(0.20 – 1.55)	2.05

Table 3 continued

SNP_[a]	Effect allele/other allele	Non-obese/active group				
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP adjusted by hormone		Proportion of relationship between breast cancer and SNP explained by hormone*
		HR[b]	95% CI	HR[b]	95% CI	%
PIK3R/RS251404_R	T/C	0.49	(0.20 – 1.20)	0.51	(0.21 – 1.26)	4.11 — Free IGF-I level
		Active group (MET ≥10, n = 520)[g]				
AKT/RS2494740_R	T/A	1.12	(0.64 – 1.97)	1.07	(0.60 – 1.88)	45.34
MAPK/RS5995550_R	G/C	1.33	(0.90 – 1.96)	**1.53**	**(1.02 – 2.27)**	59.07 — Insulin level
AKT/RS1130214_D	T/G	1.12	(0.80 – 1.55)	1.06	(0.76 – 1.47)	51.74
AKT/RS1130214_D	T/G	1.12	(0.80 – 1.55)	1.06	(0.76 – 1.48)	49.13 — HOMA-IR level

SNP_[a]	Effect allele/other allele	Obese/Inactive Group				
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP adjusted by hormone		Proportion of relationship between breast cancer and SNP explained by hormone*
		HR[b]	95% CI	HR[b]	95% CI	%
		BMI ≥30 (n = 236)[d]				
PIK3R/RS12657050_R	T/C	1.28	(0.42 – 3.85)	1.39	(0.45 – 4.24)	39.94 — IGFBP3 level
PIK3R/RS171649_CT	A/G	1.42	(0.93 – 2.16)	**1.72**	**(1.07 – 2.76)**	70.99
PIK3R/RS171649_D	A/G	1.85	(1.01 – 3.41)	**2.23**	**(1.14 – 4.37)**	44.56
PIK3R/RS831123_D	G/C	2.07	(1.00 – 4.26)	**2.39**	**(1.13 – 5.05)**	29.67 — Glucose level
AKT/RS2494744_CT	T/C	1.23	(0.55 – 2.72)	1.12	(0.50 – 2.53)	47.60 — Insulin level

Table 3 continued

| SNP_[a] | Effect allele/other allele | Obese/Inactive Group | | | | Proportion of relationship between breast cancer and SNP explained by hormone* |
| | | Breast cancer risk in relation to SNP | | Breast cancer risk in relation to SNP adjusted by hormone | | |
		HR[b]	95% CI	HR[b]	95% CI	%
AKT1RS2494744_D	T/C	1.48	(0.60 – 3.64)	1.32	(0.52 – 3.32)	33.78
PIK3R1RS12657050_R	T/C	1.43	(0.47 – 4.30)	1.56	(0.51 – 4.77)	31.30
PIK3R1RS7707370_CT	C/T	**1.79**	**(1.14 – 2.80)**	**2.05**	**(1.26 – 3.33)**	32.89
PIK3R1RS7707370_R	C/T	**2.44**	**(1.12 – 5.29)**	**3.21**	**(1.37 – 7.52)**	53.80
						HOMA-IR level
AKT1RS2494744_CT	T/C	1.23	(0.55 – 2.72)	1.14	(0.51 – 2.57)	36.96
PIK3R1RS12657050_R	T/C	1.43	(0.47 – 4.30)	1.55	(0.51 – 4.74)	28.46
PIK3R1RS7707370_R	C/T	**2.44**	**(1.12 – 5.29)**	**3.21**	**(1.35 – 7.60)**	53.20
Waist >88 cm (n = 329)[e]						IGFBP3 level
AKT1RS2494740_CT	T/A	**0.68**	**(0.48 – 0.97)**	0.70	(0.50 – 1.00)	3.85
AKT1RS2494740_R	T/C	**0.35**	**(0.16 – 0.76)**	**0.37**	**(0.17 – 0.81)**	6.25
AKT1RS2494744_CT	T/C	1.09	(0.61 – 1.97)	1.14	(0.64 – 2.03)	45.06
AKT1RS2494744_D	T/C	1.13	(0.59 – 2.17)	1.20	(0.63 – 2.29)	50.82
						Insulin level
AKT1RS2494744_D	T/C	1.14	(0.59 – 2.21)	1.08	(0.55 – 2.11)	40.63
						HOMA-IR level
AKT1RS2494744_D	T/C	1.14	(0.59 – 2.21)	1.10	(0.56 – 2.14)	29.18
w/h Ratio >0.85 (n = 233)[f]						IGFBP3 level
AKT2RS2304186_CT	A/C	1.41	(0.93 – 2.15)	**1.65**	**(1.04 – 2.63)**	58.74
AKT2RS2304186_D	A/C	1.76	(0.93 – 3.36)	**2.03**	**(1.02 – 4.03)**	34.65

Table 3 continued

SNP_ [a]	Effect allele/other allele	Obese/Inactive Group				Proportion of relationship between breast cancer and SNP explained by hormone*
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to hormone		
		HR[b]	95% CI	HR[b]	95% CI	%
MAPK/RS2266966_R	G/A	0.42	(0.19 – 0.92)	0.29	(0.12 – 0.74)	29.79
MAPK/RS2283791_R	C/G	0.39	(0.18 – 0.86)	0.27	(0.11 – 0.69)	30.47
MAPK/RS2298432_CT	T/G	1.36	(0.85 – 2.18)	1.49	(0.91 – 2.44)	36.03
MAPK/RS9610505_R	A/T	0.50	(0.24 – 1.06)	0.35	(0.14 – 0.83)	30.45
PIK3R/RS706711_D	A/G	1.77	(0.94 – 3.33)	2.13	(1.10 – 4.14)	46.62
						Free IGF-I level
MAPK/RS2298432_CT	T/G	1.28	(0.79 – 2.09)	1.38	(0.83 – 2.30)	34.05
MAPK/RS2298432_R	T/G	1.84	(0.71 – 4.76)	2.42	(0.85 – 6.87)	68.41
PIK3R/RS1664577_R	G/T	5.87	(0.76 – 45.31)	9.44	(1.15 – 77.33)	73.38
						Insulin level
MAPK/RS2298432_CT	T/G	1.30	(0.79 – 2.12)	1.46	(0.86 – 2.47)	52.88
MAPK/RS2298432_R	T/G	1.70	(0.67 – 4.30)	1.95	(0.74 – 5.16)	35.73
PIK3R/RS3730089_CT	A/G	1.75	(0.97 – 3.15)	2.04	(1.08 – 3.84)	39.13
						HOMA-IR level
MAPK/RS2298432_CT	T/G	1.30	(0.79 – 2.12)	1.42	(0.85 – 2.39)	41.65
MAPK/RS2298432_R	T/G	1.70	(0.67 – 4.30)	1.94	(0.74 – 5.08)	34.41
PIK3R/RS1664577_R	G/T	7.88	(0.96 – 64.93)	12.60	(1.30 – 122.30)	68.65
PIK3R/RS251404_R	T/C	8.38	(0.88 – 79.96)	13.73	(1.25 – 150.30)	72.42
Inactive group (MET <10, n = 438)[g]						
						Free IGF-I level
AKT/RS2494740_R	T/A	0.51	(0.27 – 0.97)	0.51	(0.27 – 0.97)	0.81

Table 3 continued

SNP_[a]	Effect allele/other allele	Obese/Inactive Group Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP adjusted by hormone		Proportion of relationship between breast cancer and SNP explained by hormone*
		HR[b]	95% CI	HR[b]	95% CI	%
MAPK/RS5999550_R	G/C	0.71	(0.45– 1.11)	0.67	(0.42– 1.06)	Insulin level 5.36
AKT/RS1130214_D	T/G	**0.55**	**(0.37– 0.82)**	**0.55**	**(0.37– 0.82)**	0.04
AKT/RS1130214_D	T/G	**0.55**	**(0.37– 0.82)**	**0.55**	**(0.37– 0.82)**	HOMA-IR level 0.09

Note Among SNPs having statistically significant association with cancer in either subgroup, the SNPs with ≥30% of indirect effect in this subgroup or its counterpart are included. Numbers in bold face are statistically significant

BMI body mass index, *CI* confidence interval, *HOMA-IR* homeostatic model assessment-insulin resistance, *HR* hazard ratio, *IGF-I* insulin-like growth factor-I, *IGFBP3* insulin-like growth factor-binding protein 3, *IR* insulin resistance, *MET* metabolic equivalent, *SNP* single-nucleotide polymorphism, *w/h ratio* waist-to-hip ratio

[a] Tag attached to the SNP name indicates a SNP analysis approach (CT: additive; D: minor-allele dominant; and R: minor-allele recessive)

[b] Multivariate regression was adjusted by covariates (age, education, family income, family histories of diabetes mellitus or breast cancer, heart failure ever, high cholesterol requiring pills ever, smoking status, alcohol intake, and reproductive history [oral contraceptive and history of hysterectomy or oophorectomy, ages at menarche and menopause, and history of pregnancy]); effect modifier variables (physical activity, BMI, and exogenous estrogen use), when not evaluated as effect modifier variables, were adjusted as a covariate; when stratified via waist circumference or w/h ratio, BMI was not adjusted

[c] Proportional change was estimated via difference in the HRs without (total effect) and with (direct effect) accounting for hormone NA due to either ≥ 50% difference between small effect sizes or ≥100% difference between two effect sizes

[d] Participants with available IGFBP3 levels, stratified by BMI levels, stratified by BMI as non-obese (BMI < 30, *n* = 766) or obese (BMI ≥ 30, *n* = 236; participants with available glucose levels, stratified by BMI as non-obese (*n* = 763) or obese (*n* = 234); participants with available insulin levels, stratified by BMI as non-obese (*n* = 750) or obese (*n* = 232); participants with available HOMA-IR levels, stratified by BMI as non-obese (*n* = 750) or obese (*n* = 232)

[e] Participants with available IGFBP3 levels, stratified by waist circumference as non-obese (waist ≤ 88 cm; *n* = 673) or obese (waist > 88 cm; *n* = 329); participants with available insulin levels, stratified by waist circumference as non-obese (*n* = 662) or obese (*n* = 320); participants with available HOMA-IR levels, stratified by waist circumference as non-obese (*n* = 662) or obese (*n* = 320)

[f] Participants with available IGFBP3 levels, stratified by w/h levels, stratified by w/h as non-obese (w/h ≤ 0.85; *n* = 769) or obese (w/h > 0.85; *n* = 233); participants with available free IGF-I levels, stratified by w/h as non-obese (*n* = 734) or obese (*n* = 224); participants with available insulin levels, stratified by w/h as non-obese (*n* = 756) or obese (*n* = 226); participants with available HOMA-IR levels, stratified by w/h as non-obese (*n* = 756) or obese (*n* = 226)

[g] Participants with available free IGF-I levels, stratified by physical activity (PA) level as active (MET ≥ 10; *n* = 520) or inactive (MET < 10; *n* = 438); participants with available insulin levels, stratified by PA level as active (*n* = 534) or inactive (*n* = 448); participants with available HOMA-IR levels, stratified by PA level as active (*n* = 534) or inactive (*n* = 448)

Table 4 Effect of hormones on the relationship between IGF-I/IR signaling pathway-relevant SNPs and breast cancer risk, stratified by exogenous estrogen use (non-users *vs.* E+P users)

SNP[a]	Effect allele/other allele	Non-user group (n = 340)[d]		Breast cancer risk in relation to SNP adjusted by *hormone*		Proportion of relationship between breast cancer and SNP explained by *hormone*[c] %
		Breast cancer risk in relation to SNP				
		HR[b]	95% CI	HR[b]	95% CI	
						IGFBP3 level
PIK3R1RS16897558_R	C/T	0.56	(0.22–1.47)	0.56	(0.21–1.46)	0.57
						Total IGF-I level
PIK3R1RS16897558_R	C/T	0.56	(0.22–1.47)	0.57	(0.22–1.50)	1.55
PIK3R1RS831123_CT	G/C	1.87	(1.07–3.25)	1.95	(1.11–3.42)	9.31
PIK3R1RS831123_D	G/C	1.94	(1.06–3.55)	2.04	(1.10–3.80)	11.13
						Free IGF-I level
AKT2RS4332845_D	A/T	0.60	(0.38–0.95)	0.60	(0.38–0.95)	0.20
PIK3R1RS831123_CT	G/C	1.81	(1.03–3.18)	1.83	(1.03–3.23)	1.96
						Insulin level
PIK3R1RS34306_R	A/G	0.35	(0.08–1.61)	0.38	(0.08–1.74)	7.94

SNP[a]	Effect allele/ other allele	E+P user group (n = 284)[d]		Breast cancer risk in relation to SNP adjusted by *hormone*		Proportion of relationship between breast cancer and SNP explained by *hormone*[c] %
		Breast cancer risk in relation to SNP				
		HR[b]	95% CI	HR[b]	95% CI	
						IGFBP3 level
PIK3R1RS16897558_R	C/T	3.36	(1.13–9.93)	4.18	(1.37–12.81)	35.16
						Total IGF-I level

Table 4 continued

SNP_ [a]	Effect allele/ other allele	E+P user group (n = 284) [d]				Proportion of relationship between breast cancer and SNP explained by hormone [c] %
		Breast cancer risk in relation to SNP		Breast cancer risk in relation to SNP adjusted by hormone		
		HR [b]	95% CI	HR [b]	95% CI	
PIK3R/RS16897558_R	C/T	**3.36**	**(1.13 – 9.93)**	**4.66**	**(1.52 – 14.25)**	55.25
PIK3R/RS831123_CT	G/C	1.18	(0.66 – 2.10)	1.24	(0.69 – 2.23)	38.11
PIK3R/RS831123_D	G/C	1.09	(0.57 – 2.07)	1.14	(0.60 – 2.16)	Free IGF-I level 55.57
AKT2RS4332845_D	A/T	1.10	(0.65 – 1.86)	1.14	(0.66 – 1.95)	39.24
PIK3R/RS831123_CT	G/C	1.08	(0.58 – 2.00)	1.12	(0.60 – 2.08)	45.48
PIK3R/RS34306_R	A/G	**55.05**	**(5.67 – 534.42)**	**71.46**	**(6.24 – 817.61)**	Insulin level 30.35

Note Among SNPs having statistically significant association with cancer in either subgroup, the SNPs with ≥30% of indirect effect in this subgroup or its counterpart are included. Numbers in bold face are statistically significant

CI confidence interval, *E* exogenous estrogen, *HR* hazard ratio, *IGF-I* insulin-like growth factor-I, *IGFBP3* insulin-like growth factor-binding protein 3, *IR* insulin resistance, *P* progestin, *SNP* single-nucleotide polymorphism

[a] Tag attached to the SNP name indicates a SNP analysis approach (CT: additive; D: minor-allele dominant; and R: minor-allele recessive)

[b] Multivariate regression was adjusted by covariates (age, education, family income, family histories of diabetes mellitus or breast cancer, heart failure ever, high cholesterol requiring pills ever, smoking status, alcohol intake, and reproductive history [oral contraceptive and history of hysterectomy or oophorectomy, ages at menarche and menopause, and history of pregnancy]); effect modifier variables (physical activity, BMI, and exogenous estrogen use), when not evaluated as effect modifier variables, were adjusted as a covariate; when stratified via waist circumference or w/h ratio, BMI was not adjusted

[c] Proportional change was estimated via difference in the HRs without (total effect) and with (direct effect) accounting for hormone

[d] Participants with available IGFBP3 levels, stratified by exogenous E use as non-users (n = 341) or E + P users (n = 284); participants with available total IGF-I levels, stratified by exogenous E use as non-users (n = 340) or E + P users (n = 284); participants with available free IGF-I levels, stratified by exogenous E use as non-users (n = 329) or E + P users (n = 266); participants with available insulin levels, stratified by exogenous E use as non-users (n = 337) or E + P users (n = 281)

relationship in this group were minimal. On the contrary, carriers of the *PIK3R1* rs16897558 C and the *PIK3R1* rs34306 A alleles had an association with increased breast cancer risk in E+P users, with roughly 35% of the SNP–cancer association explained by traits (Table 4).

A few SNPs in the *AKT1* gene were significantly associated with breast cancer (Tables 3, 4). Carriers of the *AKT1* rs2494744 T allele in non-obese women (BMI <30 and waist ≤88 cm) had increased risk of breast cancer, with minimal effects of insulin and HOMA-IR levels in those women (Table 3). Further, carriers of the *AKT1* rs2494740 T allele had decreased risk for breast cancer in viscerally obese (waist >88 cm) and inactive women, with minimal effects of the traits on the SNP–cancer association. Additionally, carriers of the *AKT1* rs4332845 A allele had an association with decreased risk of breast cancer in non-users of exogenous E; no effect of free IGF-I level on the SNP–cancer relationship was found in this group (Table 4).

Five SNPs in the *MAPK1* gene were associated with breast cancer risk (Table 3). While carriers of rs2266966 G, rs2283791 C, and rs9610505 A alleles had an association with decreased breast cancer risk in obese women with w/h >0.85, carriers of rs2298432 T allele had decreased cancer risk in non-obese women with w/h ≤0.85. Further, the former three carrier groups had a modest effect (30%) of IGFBP3 level on the SNP–cancer relationship, compared with the latter carrier group which had no trait effect.

Discussion

Population-based studies evaluating the association between the genetic variants of IGF-I/IR traits/signaling pathways and breast cancer are relatively few; information on the functionality of those SNPs associated with breast cancer is thus limited. To our knowledge, this is the first study to evaluate in postmenopausal women the association between these genetic variants and breast cancer by partitioning the genetic variant–cancer association into the pathway through IGF-I/IR traits and pathways through other than the traits. Additionally, we assessed the role of obesity with different adiposity measures (overall vs. visceral obesity), PA, and exogenous E use as the effect modifiers.

Among the 75 IGF-I/IR-related SNPs we evaluated, 7 (of the 19 related to IGF-I/IR traits) in *IGF-I* and *INS* genes and 20 (of the 56 related to IGF-I/IR signaling pathways) in *PIK3R1, AKT1/2,* and *MAPK1* genes were associated with breast cancer risk. These SNPs' associations with cancer risk differed between non-obese/active and obese/inactive carriers and between exogenous E non-users and users, indicating that the IGF-I/IR-related SNPs' interactions with obesity, PA, and exogenous E use influence cancer risk.

For seven SNPs in *IGF-I* and *INS* genes, the mediation effects of IGF-I/IR traits differed between the strata, but the direct effects on cancer risk in both groups accounted for a majority of the total effect: only roughly 35% of the cancer risk associated with those SNPs was mediated via IGF-I/IR traits. This suggests that the traits are not the main mediators through which IGF-I/insulin-related SNPs are associated with increased breast cancer risk; thus, further study is warranted.

In particular, carriers of genetic variants in the *IGFI* rs5742671 and the *IGFI* rs1520220 had increased breast cancer risk. The *IGFI* variants are related to glucose metabolism and are highly expressed in the liver, contributing to hepatic IR [49]. IGFI encodes IGF-I, which is well known to increase cancer risk [50, 51]. Previous studies of these genetic variants in relation to breast cancer are limited and showed inconsistent findings. For example, previous studies revealed no significant relationship of cancer risk and prognosis with the rs5742671 [52] and rs1520220 [5] variants; the rs1520220 variant had significant association with breast cancer risk [17] and breast tissue density [53]. In our study, these genetic variants' carriers had an association with breast cancer, but only among the inactive women, suggesting that obesity and the related lifestyle factors play a role in modulating the effect of these variants on carcinogenesis. Interestingly, the mediation effect of IGF-I/IR traits on the SNP–cancer risk association was negligible in this group, indicating that different pathways exist through which obesity and relevant lifestyles interact with these genetic variants and cancer.

The PI3K/Akt pathway leads to metabolic activity, including glucose uptake and decreased apoptosis, and the MAPK pathway leads to mitogenic activity [54]; both are main signaling cascades in controlling cellular process promoting carcinogenesis [55, 56]. *PIK3R1, AKT1/2,* and *MAPK1* genes are the key components of these pathways [26, 55, 57–59], but the studies of their genetic variants in relation to cancer have been limited [12, 24–27].

A previous study [26] showed an interaction between MAPK1 genetic variants and obesity and related lifestyle factors on breast cancer. Consistent with the results of that study, we found that carriers of the genetic variant in *MAPK1* rs2298432 had decreased breast cancer risk only in non-viscerally obese women, indicating that IGF/IR axis cellular pathway-related carcinogenesis in this variant intermingles with visceral adiposity. In addition, the carriers of several variants in *PIK3R1* and *AKT1* genes, when stratified by E+P use status, had decreased or increased cancer risk in non-users and increased risk in E+P users. This suggests that estrogen's cross-talk with target genes downstream of signaling pathways affects cancer risk [2, 4, 28, 34, 35] and that the extent of this influence may

be SNP specific and dependent on the type of estrogen usage [36–43], but the mechanism is unclear.

We did not conduct any subtype analyses of breast cancer cases due to insufficient statistical power. Also, because we conducted analyses to generate new hypotheses, we did not include any multiple testing adjustments in our analyses. We acknowledge that with many analyses, we might have a few false-positive results. Our findings from the mediation approach and proportion explained by traits of the SNP–cancer risk association should be interpreted statistically. Finally, because our study was conducted using data from only non-Hispanic white postmenopausal women, the generalizability of our results to other populations is limited. Despite these possible limitations, however, the potential impact of our findings clearly warrants further study.

In conclusion, our findings suggest that in postmenopausal women the IGF-I/IR axis has a potential role in the risk for breast cancer. Obesity, PA, and exogenous E modulate the IGF-I/IR genetic variant–cancer risk association through pathways other than IGF-I/IR traits. Further studies are needed to explore these complex mechanisms. Our results provide insight into gene–lifestyle interactions and suggest data on potential genetic targets for use in clinical trials for cancer prevention and intervention strategies to reduce breast cancer risk in postmenopausal women.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. American Cancer Society. Breast cancer facts & figures 2015–2016: American Cancer Society, Inc. 2015. http://www.cancer.org/acs/groups/content/@research/documents/document/acspc-046381.pdf
2. Rose DP et al (2012) The cellular and molecular mechanisms by which insulin influences breast cancer risk and progression. Endocr Relat Cancer 19(6):R225–R241
3. Weichhaus M et al (2012) A novel role for insulin resistance in the connection between obesity and postmenopausal breast cancer. Int J Oncol 41(2):745–752
4. De Marco P et al (2014) GPER1 is regulated by insulin in cancer cells and cancer-associated fibroblasts. Endocr Relat Cancer 21(5):739–753
5. Muendlein A et al (2013) Association of a common genetic variant of the IGF-1 gene with event-free survival in patients with HER2-positive breast cancer. J Cancer Res Clin Oncol 139(3):491–498
6. Agurs-Collins T et al (2000) Insulin-like growth factor-1 and breast cancer risk in postmenopausal African-American women. Cancer Detect Prev 24(3):199–206
7. Li BD et al (2001) Free insulin-like growth factor-I and breast cancer risk. Int J Cancer 91(5):736–739
8. Ferroni P et al (2016) Pretreatment insulin levels as a prognostic factor for breast cancer progression. Oncol 21:1041–1049
9. Kabat GC et al (2009) Repeated measures of serum glucose and insulin in relation to postmenopausal breast cancer. Int J Cancer 125(11):2704–2710
10. Gunter MJ et al (2009) Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 101(1):48–60
11. Sieri S et al (2012) Prospective study on the role of glucose metabolism in breast cancer occurrence. Int J Cancer 130(4):921–929
12. Parekh N et al (2015) Insulin receptor variants and obesity-related cancers in the Framingham heart study. Cancer Causes Control 26(8):1189–1195
13. Argiles JM et al (2001) Insulin and cancer (review). Int J Oncol 18(4):683–687
14. Arcidiacono B et al (2012) Insulin resistance and cancer risk: an overview of the pathogenetic mechanisms. Exp Diabet Res 2012:789174
15. Al-Ajmi K et al (2012) Insulin-like growth factor 1 gene polymorphism and breast cancer risk among Arab Omani women: a case-control study. Breast Cancer 6:103–112
16. DeLellis K et al (2003) IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. Br J Cancer 88(2):277–282
17. Al-Zahrani A et al (2006) IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. Hum Mol Genet 15(1):1–10
18. Slattery ML et al (2007) Genetic variation in IGF1, IGFBP3, IRS1, IRS2 and risk of breast cancer in women living in Southwestern United States. Breast Cancer Res Treat 104(2):197–209

19. Wang Q et al (2014) Genetic and dietary determinants of insulin-like growth factor (IGF)-1 and IGF binding protein (BP)-3 levels among Chinese women. PLoS One 9(10):e108934

20. Cleveland RJ et al (2006) IGF1 CA repeat polymorphisms, lifestyle factors and breast cancer risk in the Long Island Breast Cancer Study Project. Carcinogenesis 27(4):758–765

21. Quan H et al (2014) IGF1(CA)19 and IGFBP-3-202A/C gene polymorphism and cancer risk: a meta-analysis. Cell Biochem Biophys 69(1):169–178

22. Haiman CA et al (2013) Genome-wide testing of putative functional exonic variants in relationship with breast and prostate cancer risk in a multiethnic population. PLoS Genet 9(3):e1003419

23. Dillon LM et al (2015) P-REX1 creates a positive feedback loop to activate growth factor receptor, PI3K/AKT and MEK/ERK signaling in breast cancer. Oncogene 34(30):3968–3976

24. Wang Y et al (2007) Nested case-control study of energy regulation candidate gene single nucleotide polymorphisms and breast cancer. Anticancer Res 27(1B):589–593

25. Zhang H et al (2013) Association between insulin receptor substrate 1 Gly972Arg polymorphism and cancer risk. Tumour Biol 34(5):2929–2936

26. Slattery ML et al (2015) MAPK genes interact with diet and lifestyle factors to alter risk of breast cancer: the Breast Cancer Health Disparities Study. Nutr Cancer 67(2):292–304

27. Yarden RI et al (2010) Single-nucleotide polymorphisms in the p53 pathway genes modify cancer risk in BRCA1 and BRCA2 carriers of Jewish-Ashkenazi descent. Mol Carcinog 49(6):545–555

28. Iyengar NM et al. (2013) Obesity and inflammation: new insights into breast cancer development and progression. American Society of Clinical Oncology educational book/ASCO American Society of Clinical Oncology Meeting. pp. 46-51

29. Wasserman L et al (2004) Correlates of obesity in postmenopausal women with breast cancer: comparison of genetic, demographic, disease-related, life history and dietary factors. Int J Obes Relat Metab Disord 28(1):49–56

30. Werner H (2012) Tumor suppressors govern insulin-like growth factor signaling pathways: implications in metabolism and cancer. Oncogene 31(22):2703–2714

31. Booth FW et al (2012) Lack of exercise is a major cause of chronic diseases. Compr Physiol 2(2):1143–1211

32. Yaren A et al (2012) Insulin-like growth factor I (Igf-1) gene polymorphism in patients with non-metastatic breast cancer. Gene 503(2):244–247

33. Pendyala S et al (2011) Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis. Am J Clin Nutr 93(2):234–242

34. Creighton CJ et al (2012) A gene transcription signature of obesity in breast cancer. Breast Cancer Res Treat 132(3):993–1000

35. Franks SE et al (2012) Transgenic IGF-IR overexpression induces mammary tumors with basal-like characteristics, whereas IGF-IR-independent mammary tumors express a claudin-low gene signature. Oncogene 31(27):3298–3309

36. Clemmons DR (2007) Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. Nat Rev Drug Discov 6(10):821–833

37. Abbas A et al (2008) Role of IGF-1 in glucose regulation and cardiovascular disease. Expert Rev Cardiovasc Ther 6(8):1135–1149

38. Anderson GL et al (2004) Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA 291(14):1701–1712

39. Morimoto LM et al (2005) Insulin-like growth factor polymorphisms and colorectal cancer risk. Cancer Epidemiol Biomark Prev 14(5):1204–1211

40. Rudolph A et al (2013) Colorectal cancer risk associated with hormone use varies by expression of estrogen receptor-beta. Cancer Res 73(11):3306–3315

41. Campagnoli C et al (2005) Progestins and progesterone in hormone replacement therapy and the risk of breast cancer. J Steroid Biochem Mol Biol 96(2):95–108

42. Gunter MJ et al (2008) Insulin, insulin-like growth factor-I, endogenous estradiol, and risk of colorectal cancer in postmenopausal women. Cancer Res 68(1):329–337

43. Rossouw JE et al (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 288(3):321–333

44. The Women's Health Initiative Study Group (1998) Design of the Women's Health Initiative clinical trial and observational study. The Women's Health Initiative Study Group. Control Clin Trials 19(1):61–109

45. National Cancer Institute. SEER program: comparative staging guide for cancer June 1993

46. Matthews DR et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28(7):412–419

47. MacKinnon DP et al (2007) Mediation analysis. Annu Rev Psychol 58:593–614

48. Mackinnon DP et al (1995) A simulation study of mediated effect measures. Multivar Behav Res 30(1):41

49. Ingelsson E et al (2010) Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. Diabetes 59(5):1266–1275

50. Dupuis J et al (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42(2):105–116

51. Pechlivanis S et al (2007) Polymorphisms in the insulin like growth factor 1 and IGF binding protein 3 genes and risk of colorectal cancer. Cancer Detect Prev 31(5):408–416

52. Henningson M et al (2011) IGF1 htSNPs in relation to IGF-1 levels in young women from high-risk breast cancer families: implications for early-onset breast cancer. Fam Cancer 10(2):173–185

53. Tamimi RM et al (2007) Common genetic variation in IGF1, IGFBP-1, and IGFBP-3 in relation to mammographic density: a cross-sectional study. Breast cancer Res 9(1):R18

54. Bergman D et al (2013) Insulin-like growth factor 2 in development and disease: a mini-review. Gerontology 59(3):240–249

55. Boyault S et al (2012) Mutational characterization of individual breast tumors: tP53 and PI3 K pathway genes are frequently and distinctively mutated in different subtypes. Breast Cancer Res Treat 132(1):29–39

56. Li J et al (2015) Epidermal growth factor receptor and AKT1 gene copy numbers by multi-gene fluorescence in situ hybridization impact on prognosis in breast cancer. Cancer Sci 106(5):642–649

57. Wheler JJ et al (2015) Multiple gene aberrations and breast cancer: lessons from super-responders. BMC Cancer 15:442

58. Hardt O et al (2012) Highly sensitive profiling of CD44+/CD24− breast cancer stem cells by combining global mRNA amplification and next generation sequencing: evidence for a hyperactive PI3K pathway. Cancer Lett 325(2):165–174

59. Andrade VP et al (2015) Gene expression profiling of lobular carcinoma in situ reveals candidate precursor genes for invasion. Mol Oncol 9(4):772–782