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ORIGINAL PAPER

Genetic variation in *IGF1*, *IGFBP3*, *IRS1*, *IRS2* and risk of breast cancer in women living in Southwestern United States

M. L. Slattery · C. Sweeney · R. Wolff · J. Herrick · K. Baumgartner · A. Giuliano · T. Byers

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Abstract
Background An insulin-related pathway to breast cancer has been hypothesized.
Methods We examine the 19 CA repeat of the *IGF1* gene, the -202 C > A *IGFBP3*, the G972R *IRS*, and the G1057D *IRS2* polymorphisms among 1,175 non-Hispanic white (NHW) and 576 Hispanic newly diagnosed breast cancer cases and 1,330 NHW and 727 Hispanic controls living in Arizona, Colorado, New Mexico, and Utah.
Results Among post-menopausal women not recently exposed to hormones, not having the 19 CA repeat of *IGF1* gene was associated with breast cancer among NHW women [odds ratio (OR) 2.14, 95% confidence interval (CI) 1.21–3.79] and having an R allele of G972R *IRS1* increased breast cancer risk among Hispanic women (OR 2.70, 95% CI 1.13–6.46). Among post-menopausal Hispanic women recently exposed to hormones the A allele of the -202 C > A *IGFBP3* polymorphism increased risk of breast cancer (OR 1.57, 95% CI 1.06–2.33). The *IGF1* 19 CA repeat polymorphism interacted with hormone replacement therapy (HRT) among NHW post-menopausal women; women who had the 19/19 *IGF1* genotype were at reduced risk of breast cancer (OR 0.64, 95% CI 0.47–0.88) if they did not use HRT. We also observed interaction between body mass index and *IGF1* 19 CA repeat (p=0.06) and between weight gain and the -202 C > A *IGFBP3* polymorphism (p=0.05) in NHW post-menopausal women not recently exposed to hormones.
Conclusions Our data suggest that associations between insulin-related genes and breast cancer risk among women living in the Southwestern United States may be dependent on estrogen exposure and may differ by ethnicity.

Keywords Breast cancer · Insulin · *IGF1* · *IGFBP3* · *IRS1* · *IRS2* · Obesity · HRT

M. L. Slattery (✉) · C. Sweeney · R. Wolff · J. Herrick
University of Utah, Salt Lake City, Utah 84108, USA
e-mail: mslatter@hrc.utah.edu

K. Baumgartner
Department of Internal Medicine, The Cancer Research and Treatment Center, Epidemiology and Cancer Prevention Program, University of New Mexico Health Science Center, Albuquerque, NM, USA

A. Giuliano
University of Arizona, Tucson, AZ, USA

T. Byers
University of Colorado School of Medicine, Denver, CO, USA

Present Address:
K. Baumgartner
University of Louisville, Louisville, KY, USA

Present Address:
A. Giuliano
Moffitt Cancer Center, Tampa, FL, USA

Introduction

Uncontrolled cell growth is central to the carcinogenic process. There is a growing body of evidence that suggests that insulin-like growth factors (IGF), insulin-like growth factor binding proteins (IGFBPs), especially *IGFBP3*, insulin, and insulin-receptor

substrate (IRS) play a significant role in the initiation of cell growth and proliferation of cancer [1–5]. Insulin has been regarded as primarily a metabolic signal while IGF-1 has been implicated as an important mitogen and cell differentiation factor [6, 7]. The IRS protein family contains several members, of which IRS-1 and IRS-2 are expressed in almost all cells and tissues [8–10]. In addition to their role in insulin signaling, IRS-1 and IRS-2 are also substrates for the IGF receptor and thus act in IGF signal transductions. While IRS-1 controls body growth and peripheral insulin action, IRS-2 regulates body weight control and glucose homeostasis [11]. Within tumors, IRS-1 may be a marker of an active IGF signal transduction pathway [4, 12]. Although IRS is involved in insulin and IGF signaling, it also appears to be important in regulating estrogen signaling [13].

Many factors are involved in the regulation of insulin and IGFs, including diet, lifestyle, hormonal, and genetic factors. Studies have shown that diet, physical activity, body size, and sex steroids are involved in the regulation of these hormones [14–21]; some data suggest that serum levels of IGF-1, IGFBP-3, and IRS also may be affected by polymorphisms in these genes [22–25]. We have previously published data on associations between IGF-1 and IGFBP-3 serum levels and genetic variants of these genes in non-Hispanic white (NHW) and Hispanic women living in the Southwest [14]. Data from that study suggest different allele frequencies between NHW women and Hispanic women for the *IGFBP3* polymorphism at nucleotide -202. In that study, we showed that serum levels of IGFBP-3 are correlated with genotype in a dose response fashion, i.e., AA > AC > CC. Furthermore, we observed that Hispanic women had higher IGFBP-3 serum levels even after adjusting for genotype. In a separate analysis [26], two associations were observed that were consistent in both Hispanics and NHW women: *IGF1* CA repeat alleles of length other than 19 were associated with higher mean waist-to-hip ratios (WHR), p=0.01, and women who carried an *IGFBP3* A allele, compared with women with the CC genotype, more often reported high birth weight [odds ratio (OR) 1.9, 95% confidence interval (CI) 1.1–3.2]. Additionally, we observed that having the A allele of *IGFBP3* was associated with height and that the R allele of *IRS1* G972R polymorphism was associated with smaller WHR. These observations add support for the functionality of these polymorphisms.

These genes also have been examined with cancer and other conditions thought to be important contributors to cancer risk. The Gly972Arg (G972R) polymorphism in *IRS1* has been associated with insulin resistance and type 2 diabetes; the *IRS1* R allele has been associated with increased risk of colon cancer [27]. The *IRS2 G1057D* polymorphism has been associated with obesity [28]. Variation in serum IGF-1 levels has been associated with the 19 CA repeat polymorphism in the *IGF1* gene 1 kb upstream of the transcription start site [28]. The most common allele containing 19 CA repeats is sometimes denoted ‘‘192’’ for the size of the PCR product [29–32]. This 19 CA repeat variant of the *IGF1* gene has been evaluated with prostate and colon cancer, where associations are generally more consistent for prostate cancer than for colon cancer [27, 29–32].

In this paper, we evaluated the associations of genetic polymorphisms in the *IGF1*, *IGFBP3*, *IRS1*, and *IRS2* genes with breast cancer. We evaluated interactions between insulin-related genes and hormone replacement therapy (HRT) as well as the body mass index (BMI) of kg/m^2, and weight gain. Since the association between obesity and breast cancer risk differs for pre- and post-menopausal women, we examined these groups separately. Associations were determined separately for NHW women and for Hispanic/American Indian (AI) women living in the Southwestern states of Arizona, Colorado, New Mexico, and Utah. Hispanics and AI have markedly higher prevalence of obesity, but lower breast cancer incidence, than NHWs, and the roles of insulin and IGF pathways in breast cancer in these ethnic groups may be different.

Methods

Study participants were women living in Cochise, Coconino, Maricopa, Pima, Pinal, Santa Cruz, and Yuma Counties in Arizona, or the states of Colorado, New Mexico, or Utah at the time of diagnosis or selection, excluding AI women living on reservations. Study hypotheses focused specifically on breast cancer in Hispanic women, therefore sampling was stratified on ethnicity to select Hispanic women in larger proportion than their representation in the population. All Hispanic women diagnosed with breast cancer during the study period were selected for the study. An age-matched sample of NHW women were randomly selected on a 1 to 1 ratio to the distribution of Hispanic cases in Arizona and Colorado; at a 4 to 1 ratio to the distribution of Hispanic cases in Utah; all Hispanic and non-Hispanic cases age 50 and under in New Mexico; and a 1 to 1 ratio for women over 50 in New Mexico. The GUESS program (Generally Useful Ethnic Search System) was used to identify women who were Hispanic [33].

Cases were histologically confirmed in situ and invasive breast cancer (ICDO sites C50.0–C50.6 and C50.8–C50.9) diagnosed between October 1999 and May 2004. State tumor registries were used to initially identify and subsequently confirm case eligibility. An electronic rapid case ascertainment system was used in Utah to identify cases while in the other states cases were identified through normal registry operations. The Utah and New Mexico state cancer registries are NCI funded Surveillance Epidemiology and End Results registries; Arizona and Colorado registries are part of the Center for Disease Control National Program of Cancer Registries. Cases were identified as Hispanic or Native American from registry abstract data where available.

Controls were selected from the target populations to match ethnicity and 5-year age distribution of cases. In Arizona and Colorado, participants under 65 were randomly selected from a commercial mailing list; in New Mexico and Utah controls less than 65 years were randomly selected from driver's license lists. In all states, women 65 years and older were randomly selected from Center for Medicare Services lists.

All women identified were screened for eligibility prior to study enrollment. As part of the screening, women were asked to self-identify their race and ethnicity. Women who reported their race as only African American or Asian were excluded from the study. Women initially identified as being Hispanic by the GUESS program who were determined not to be Hispanic or AI were ineligible for the study. All participants signed informed written consent prior to participation; the study was approved by the Institutional Review Board for Human Subjects at each institution.

Diet and lifestyle data were collected by trained and certified interviewers using an interviewer-administered computerized questionnaire. These methods have been described in detail [34, 35]. The questionnaire was translated into Spanish by two individuals with an arbitrator resolving differences in translation between the two original translators. The referent period was the year prior to diagnosis for cases or selection for controls. Respondents were given the option of having the interview administered in either English or Spanish.

Respondents were asked to self-identify their ethnicity and race as part of the study questionnaire. If a respondent described herself as belonging to more than one race or ethnic group, both were recorded. The questionnaire included information about medical history, reproductive history, family history, diet, physical activity, use of tobacco, medication use, diabetes history, and weight history, birth weight, and weight at ages 15, 30, 50 and during the referent year. Women were asked to ''best describe your menstrual status on (referent date)'' by selecting response from a card; this information was used to define individual menopausal status. Weight was measured at the time of interview to the nearest 0.50 lb and height was measured to the nearest 0.25 in. BMI was calculated using the formula of weight in kilograms (kg)/height in meters (m^2). Recalled weight at ages 15, 30, 50 (if over 50 years of age), and referent year were used to calculate BMI for each age and the referent year. We evaluated adult BMI using international cutpoints of <25 as normal weight, 25–29.9 as overweight, and 30+ as obese. Weight gain was calculated as difference in weight between recalled weight at age 15 and weight recalled during the referent year.

Dietary intake data were collected using an extensive diet history questionnaire that was modified to incorporate foods commonly eaten in the Southwestern United States [36]. An extensive physical activity questionnaire that was modified from the Cross Cultural Activity Participation Survey [37] and was used to collect information on activities performed at home, at work, and during leisure and included intensity of the activity and frequency at which activities were performed during the referent year, at ages 15, 30, and 50. Total metabolic equivalents or MET hours of activity during the referent year was calculated based on the compendium of MET values for physical activities [38].

Sixty-eight percent (68%) of women contacted participated in the study. Of these cases, 798 Hispanic and AI, and 1,527 NHW women were diagnosed with first primary breast cancer and are included in these analyses. Of controls identified, 945 Hispanic and AI and 1,671 NHW women participated (42% of participants contacted). Blood was collected and DNA extracted for 76.6% of participants in Arizona, 74.8% of participants in Colorado, 75% of participants in New Mexico, and 93.6% of participants in Utah.

Genotyping

IRS1

The *G972R* polymorphism was detected using PCR amplification with primers 5′-CTT CTG TCA GGT GTC CAT CC and 5′-TGG CGA GGT GTC CAC GTA GC. PCR cycling consisted of an initial denaturation at 94°C for 2 min, 10 cycles at 94°C 10 s, 60°C 10 s, and 72°C 10 s followed by 30 cycles at 94°C 10 s, 55°C 10 s, and 72°C 10 s. BstNI was used to digest the PCR products following manufacturer's instructions.

Alleles were scored as either G for glycine or R for arginine (absence or presence of the restriction site, respectively).

IRS2

The G1057D polymorphism was detected using a TaqMan assay. Primer sequences were IRS2-F 5′-GGA GCT GTA CCG CCT GCC and IRS2-R 5′-ACC AAA AGC CAT CTC GGT GT. The probes were 5′-FAM-CCG *G*GC GCC GCC TCA T-Tamra and 5′-VIC-CGG *A*CG CCG CCT CAT CGT T-Tamra [39]. Each 17 μl PCR reaction contained 20 ng genomic DNA, 900 nM of each primer, 130 nM of each TaqMan probe, and 8.5 μl TaqMan Universal PCR Master Mix (contains AmpErase UNG and AmpliTaq Gold enzymes, dNTPs, and reaction buffer). PCR was carried out under the following conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 62°C for 1 min using the BIO-RAD IQ detection system. The fluorescence of each sample was collected and analyzed version 3.0 of the iCycler IQ Real-Time detection software.

IGF1

IGF1 CA repeat genotypes were determined by PCR amplification using primers IGF1-F 5′-FAM-GCT AGC CAG CTG GTG TTA TT-3′ and IGF1-R 5′-ACC ACT CTG GGC GAA GGG TA-3′. PCR conditions consisted of a 2-min denaturation at 94°C followed by 30 cycles at 94°C 10 s, 57°C 10 s, and 72°C 15 s. The products were then sized using an ABI 3700 fluorescent sequencer. Alleles were assigned by the size of the fragment in base pairs and classified as ''192'' or not ''192.'' ''192'' is the PCR product size of the most common allele which contains 19 CA repeats.

IGFBP3

The -202 A > C polymorphism was amplified using primers F 5′-CCA CGA GGT ACA CAC GAA TG and R3 5′-TGA GCA GCC GGG GCC GAG. A 0.5 units of Amplitaq gold and 5% DMSO were used to increase efficiency of amplification. PCR conditions were 9 min initial denaturation at 95°C followed by 40 cycles at 95°C 10 s, and 66°C 20 s. The resulting PCR product was digested with 4 units of Alw21I at 37°C overnight. Digested products were separated on a 2% Nusieve gel stained with ethidium-bromide and visualized with ultraviolet light. Alleles were scored as either A or C (presence or absence of the restriction site, respectively).

Statistical methods

SAS statistical package, version 9 was used to conduct the analyses. *IRS1* genotypes were *GG*, *GR*, and *RR*, with the R allele being less common. Because of the rarity of the RR genotype (Table 1), associations with *IRS1* were only conducted on the dominant model. *IRS2* genotypes were *GG*, *GD*, and *DD*, with the *GG* genotype being most common. *IGF1* genotypes analyzed were 19/19 CA repeats, heterozygous, or no 19 CA repeat alleles; the absence of the 19 CA repeat allele was less common than the presence of the 19 CA repeat allele. *IGFBP3* genotypes were *CC*, *CA*, and *AA*. The dominant model was used to assess interaction.

Analyses included evaluating the distribution of the genotypes in the population, the independent associations of genetic polymorphisms with breast cancer risk, and the joint effect of genotypes and HRT and body size on breast cancer risk. Multivariable logistic regression models were used to estimate relative risk. Adjustment variables in these models included age, center, race/ethnicity (if not stratified analysis), parity, BMI, long-term physical activity, and energy intake. Center was used as an adjustment variable to help control for different proportion of cases and controls interviewed at each center. Data were analyzed by ethnicity and by menopausal status. Additionally, among post-menopausal women we evaluated differences in association between those women who had become post-menopausal within the past 2 years or were using HRT and those who have not been exposed to hormones during the past 2 years. Statistical interaction between polymorphisms and HRT, BMI, and weight change were assessed using multiplicative interaction models.

Results

IRS1, *IRS2*, *IGF1*, and *IGFBP3* were in HWE for both NHW women and Hispanic/AI women. Hispanic/AI women were significantly less likely to have an R allele of the G972R *IRS1* polymorphism than NHW women, and less likely to have an A allele of the -202 A > C *IGFBP3* polymorphism (Table 1). There were no significant differences in genotype frequency between Hispanic/AI and NHW women for either the *G1057D IRS2* polymorphism or the number of 19 CA repeats in *IGF1*. The majority of women were post-menopausal and had used HRT.

There were few significant associations in the polymorphisms assessed among NHW and Hispanic/AI women for pre-menopausal women or for post-menopausal

Table 1 Description of study population

	NHW				Hispanic/AI				*p*-Value[a]
	Case		Control		Case		Control		
	N	%	*N*	%	*N*	%	*N*	%	
Center									
AZ	164	14.0	263	19.8	116	20.1	164	22.6	
CO	237	20.2	218	16.4	112	19.4	132	18.2	
NM	465	39.6	496	37.3	252	43.8	250	34.4	
UT	309	26.3	353	26.5	96	16.7	181	24.9	
HRT use									
Ever	579	76.7	698	76.5	195	58.4	285	62.2	
Never	176	23.3	215	23.5	139	41.6	173	37.8	
Menopause status									
Pre/peri	415	35.4	414	31.2	238	41.5	265	36.6	
Post	757	64.6	915	68.8	336	58.5	460	63.4	
IRS1									
GG	1,017	87.1	1,154	87.0	516	89.9	665	91.6	0.006
GR	146	12.5	169	12.7	56	9.8	59	8.1	
RR	5	0.4	4	0.3	2	0.3	2	0.3	
IRS2									
GG	497	42.4	544	41.0	212	36.9	262	36.1	0.09
GD	546	46.5	594	44.7	264	45.9	347	47.8	
DD	130	11.1	190	14.3	99	17.2	117	16.1	
IGF1									
19/19	482	41.8	575	43.6	225	39.4	297	41.4	0.17
19/non19	526	45.6	594	45.0	267	46.8	319	44.4	
non19/non19	146	12.7	150	11.4	79	13.8	102	14.2	
IGFBP3									
CC	332	28.5	384	29.0	215	37.5	316	43.8	<0.001
CA	573	49.2	658	49.6	290	50.5	318	44.0	
AA	260	22.3	284	21.4	69	12.0	88	12.2	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Age (years)	55.2	0.32	56.2	0.33	52.4	0.46	54.0	0.44	
Referent year BMI (kg/m^2)	26.8	0.17	27.0	0.17	28.1	0.25	29.0	0.23	
Weight gain since age 15 (kg)	19.1	0.45	18.7	0.42	20.3	0.63	21.8	0.59	

[a] *p*-Value for the different allele frequencies between NHW and Hispanic controls

women overall (Table 2). However, among post-menopausal women who had not recently been exposed to hormones (Table 3), we observed a significant increased risk of breast cancer among NHW women without the more common 19 CA repeat allele of the *IGF1* gene. Having an R allele of the G972R *IRS1* polymorphism significantly increased risk of breast cancer among post-menopausal Hispanic/AI women who had not recently been exposed to hormones (OR 2.70, 95% CI 1.13–6.46). Having an A allele of the *IGFBP3* polymorphism was associated with increased breast cancer risk among Hispanic/AI women. Although the AA genotype was not statistically significantly associated, there were few Hispanic women with that genotype. Using the dominant model to assess the association between *IGFBP3* C > A polymorphism and breast cancer risk resulted in a significant increased risk associated (OR 1.57, 95% CI 1.06–2.33 for CA/AA genotypes relative to CC).

Interactions between the *IGF1* 19 CA repeat, the -202 C > A *IGFBP3* polymorphism and recent HRT use, BMI, and weight gain since age 15 are presented in Table 4 for NHW women and in Table 5 for Hispanic/AI women. There were no significant interactions between pre-menopausal breast cancer and *IGF1* and *IGFBP3* polymorphisms assessed in either Hispanic or NHW women. Having the 19/19 genotype of the *IGF1* gene resulted in reduced breast cancer risk among NHW women not recently using HRT (p interaction <0.01). Because of this significant interaction with HRT, subsequent interactions were presented stratified by recent hormone exposure. Among women not recently exposed to hormones, there was a significant interaction between *IGF1* CA repeat and referent year BMI that was borderline significant for both NHW and Hispanic/AI women (p=0.06). The greatest risk associated with obesity was among women who did not have the *IGF1* 19/19 CA repeat genotype. The *IGFBP3* polymorphism

Table 2 Breast cancer risk associated with *IGF1*, *IGFBP3*, *IRS1*, and *IRS2*

	NHW				Hispanic			
	Controls		Cases		Controls		Cases	
	N	*N*	OR	95% CI	*N*	*N*	OR	95% CI
Pre-menopausal								
IGF1								
19/19	177	169	1.00		108	103	1.00	
19/non19	192	186	1.00	(0.74, 1.35)	118	104	0.93	(0.62, 1.38)
non19/non19	41	50	1.31	(0.81, 2.14)	38	29	0.85	(0.48, 1.53)
19/non19 and non19/non19	233	236	1.05	(0.79, 1.40)	156	133	0.91	(0.63, 1.33)
p trend[a]			0.42				0.57	
IGFBP3								
CC	112	105	1.00		125	97	1.00	
CA	198	199	1.12	(0.79, 1.57)	111	116	1.32	(0.90, 1.95)
AA	102	103	1.04	(0.70, 1.54)	29	24	1.14	(0.60, 2.14)
CA/AA	300	302	1.09	(0.79, 1.50)	140	140	1.29	(0.89, 1.87)
p trend			0.83				0.32	
IRS1								
GG	360	359	1.00		244	214	1.00	
GR	54	51	1.09	(0.71, 1.67)	21	23	1.33	(0.70, 2.53)
RR	0	2	Undefined		0	1	Undefined	
GR/RR	54	53	1.11	(0.73, 1.70)	21	24	1.40	(0.74, 2.65)
p trend[a]			0.55				0.23	
IRS2								
GG	165	182	1.00		87	82	1.00	
GD	186	185	0.94	(0.69, 1.27)	132	117	0.98	(0.65, 1.48)
DD	62	47	0.73	(0.47, 1.14)	46	39	0.99	(0.57, 1.70)
GD/DD	248	232	0.88	(0.66, 1.18)	178	156	0.98	(0.66, 1.45)
p trend			0.21				0.94	
Post-menopausal								
IGF1								
19/19	397	310	1.00		188	119	1.00	
19/non19	400	339	1.07	(0.87, 1.33)	199	162	1.27	(0.92, 1.74)
non19/non19	108	96	1.13	(0.83, 1.56)	63	49	1.23	(0.78, 1.94)
19/non19 and non19/non19	508	435	1.09	(0.89, 1.33)	262	211	1.26	(0.93, 1.71)
p trend[a]			0.38				0.21	
IGFBP3								
CC	271	225	1.00		189	116	1.00	
CA	457	373	0.99	(0.78, 1.24)	205	171	1.40	(1.02, 1.94)
AA	182	156	1.03	(0.78, 1.37)	59	45	1.20	(0.75, 1.92)
CA/AA	639	529	1.00	(0.80, 1.24)	264	216	1.36	(1.00, 1.84)
p trend[a]			0.87				0.16	
IRS1								
GG	792	655	1.00		417	299	1.00	
GR	113	94	0.95	(0.70, 1.28)	38	31	1.26	(0.75, 2.12)
RR	4	3	0.89	(0.20, 4.09)	2	1	0.69	(0.06, 8.02)
GR/RR	117	97	0.95	(0.70, 1.27)	40	32	1.23	(0.74, 2.05)
p trend[a]			0.71				0.49	
IRS2								
GG	376	313	1.00		174	129	1.00	
GD	407	359	1.06	(0.86, 1.31)	213	143	0.96	(0.69, 1.32)
DD	128	83	0.77	(0.56, 1.06)	70	60	1.23	(0.80, 1.88)
GD/DD	535	442	0.99	(0.81, 1.21)	283	203	1.02	(0.76, 1.38)
p trend[a]			0.30				0.48	

[a] *p* trend based on three genotype categories

Table 3 Breast cancer risk associated with *IGF1*, *IGFBP3*, *IRS1*, and *IRS2* among post-menopausal women by hormone exposure

	NHW				Hispanic			
	Controls		Cases		Controls		Cases	
	N	*N*	OR[a]	95% CI	*N*	*N*	OR[a]	95% CI
Post-menopausal, no recent hormone exposure								
IGF1								
19/19	152	82	1.00		72	44	1.00	
19/non19	126	96	1.42	(0.96, 2.10)	81	57	1.05	(0.60, 1.82)
non19/non19	31	36	2.14	(1.21, 3.79)	25	18	1.28	(0.59, 2.81)
19/non19 and non19/non19	157	132	1.56	(1.08, 2.25)	106	75	1.10	(0.65, 1.85)
p trend[b]			<0.01				0.58	
IGFBP3								
CC	92	56	1.00		75	48	1.00	
CA	152	115	1.17	(0.77, 1.79)	84	53	1.05	(0.61, 1.82)
AA	66	45	1.05	(0.62, 1.76)	21	18	1.15	(0.51, 2.57)
CA/AA	218	160	1.14	(0.76, 1.70)	105	71	1.07	(0.64, 1.81)
p trend[b]			0.80				0.73	
IRS1								
GG	273	189	1.00		167	104	1.00	
GR	36	26	0.98	(0.56, 1.71)	13	15	2.70	(1.13, 6.46)
RR	0	1	Undefined		0	0	Undefined	
GR/RR	36	27	1.02	(0.59, 1.77)	13	15	2.70	(1.13, 6.46)
p trend[b]			0.82				0.03	
IRS2								
GG	141	94	1.00		63	45	1.00	
GD	130	99	1.09	(0.74, 1.60)	91	55	0.82	(0.48, 1.42)
DD	38	24	0.94	(0.52, 1.70)	26	19	0.99	(0.46, 2.13)
GD/DD	168	123	1.06	(0.73, 1.52)	117	74	0.86	(0.51, 1.44)
p trend[b]			0.97				0.80	
Post-menopausal, recent hormone exposure								
IGF1								
19/19	242	225	1.00		114	74	1.00	
19/non19	272	241	0.94	(0.72, 1.21)	116	102	1.35	(0.89, 2.04)
non19/non19	76	58	0.81	(0.55, 1.20)	38	31	1.21	(0.67, 2.15)
19/non19 and non19/non19	348	299	0.91	(0.71, 1.16)	154	133	1.31	(0.89, 1.94)
p trend[b]			0.30				0.32	
IGFBP3								
CC	178	167	1.00		112	66	1.00	
CA	300	256	0.93	(0.71, 1.23)	119	117	1.71	(1.13, 2.58)
AA	116	108	1.01	(0.71, 1.42)	38	26	1.15	(0.62, 2.11)
CA/AA	416	364	0.95	(0.74, 1.24)	157	143	1.57	(1.06, 2.33)
p trend[b]			0.96				0.19	
IRS1								
GG	514	461	1.00		247	191	1.00	
GR	76	66	0.89	(0.62, 1.28)	24	16	0.96	(0.48, 1.90)
RR	4	2	0.55	(0.10, 3.11)	2	1	0.76	(0.06, 8.98)
GR/RR	80	68	0.87	(0.61, 1.25)	26	17	0.95	(0.49, 1.84)
p trend[b]			0.41				0.84	
IRS2								
GG	232	217	1.00		111	83	1.00	
GD	276	256	1.00	(0.77, 1.29)	118	87	1.05	(0.70, 1.59)
DD	88	58	0.69	(0.47, 1.01)	44	39	1.23	(0.72, 2.09)
GD/DD	364	314	0.92	(0.72, 1.18)	162	126	1.10	(0.75, 1.61)
p trend[b]			0.13				0.48	

[a] OR and 95% CI adjusted for age, center, parity, energy intake, physical activity, and genetic admixture. [b] *p* trend based on three genotype categories

Table 4 Breast cancer risk and the interaction of referent year BMI, weight gain, and HRT with *IGF1* and *IGFBP3* in NHW women

	IGF1						*IGFBP3*									
	Ctrl	Case	19/19		Ctrl	Case	19/non19 and non19/non19		Ctrl	Case	CC		Ctrl	Case	CA/AA	
	N	*N*	OR (95% CI)[a]		*N*	*N*	OR (95% CI)[a]		*N*	*N*	OR (95% CI)[a]		*N*	*N*	OR (95% CI)[a]	
Pre-menopause																
Referent BMI (kg/m^2)																
<25	97	94	1.00		126	143	1.26	(0.86, 1.86)	60	56	1.00		164	182	1.23	(0.80, 1.90)
25–29.9	51	50	1.09	(0.66, 1.81)	56	47	0.87	(0.53, 1.44)	33	29	0.97	(0.51, 1.82)	75	67	0.99	(0.59, 1.63)
≥30	29	24	0.93	(0.49, 1.75)	50	45	0.83	(0.49, 1.41)	19	20	1.12	(0.52, 2.39)	60	51	0.86	(0.50, 1.49)
p interaction								0.38								0.57
Weight gain since age 15 (kg)																
≤10	61	54	1.00		76	90	1.52	(0.92, 2.49)	40	31	1.00		99	112	1.53	(0.87, 2.68)
10.1–20.0	53	55	1.30	(0.75, 2.25)	79	61	0.95	(0.56, 1.59)	34	31	1.18	(0.59, 2.38)	99	87	1.20	(0.68, 2.13)
>20.0	58	53	1.13	(0.66, 1.96)	71	78	1.17	(0.70, 1.96)	36	40	1.46	(0.75, 2.88)	92	92	1.24	(0.70, 2.20)
p interaction								0.14								0.33
Post-menopause																
Recent HRT use																
Yes	202	194	1.00		306	250	0.85	(0.66, 1.11)	159	139	1.00		352	312	1.04	(0.79, 1.37)
No	195	116	0.64	(0.47, 0.88)	202	185	0.98	(0.73, 1.30)	112	86	0.94	(0.65, 1.37)	287	217	0.90	(0.67, 1.21)
p interaction								<0.01								0.69
Post-menopause not recently exposed to hormones																
Referent BMI (kg/m^2)																
<25	48	21	1.00		63	43	1.72	(0.87, 3.37)	37	15	1.00		74	49	1.63	(0.79, 3.33)
25–29.9	51	39	1.94	(0.97, 3.87)	51	37	1.81	(0.91, 3.60)	34	21	1.77	(0.77, 4.08)	69	56	1.93	(0.94, 3.93)
≥30	52	22	1.06	(0.50, 2.22)	40	51	2.98	(1.50, 5.91)	20	20	2.40	(0.99, 5.84)	72	54	1.84	(0.90, 3.76)
p interaction								0.06								0.35
Weight gain since age 15 (kg)																
≤10	29	21	1.00		46	30	0.95	(0.45, 2.04)	26	10	1.00		50	41	2.30	(0.97, 5.46)
10.1–20.0	41	13	0.47	(0.20, 1.13)	44	31	1.20	(0.56, 2.58)	21	15	2.61	(0.93, 7.29)	64	29	1.32	(0.55, 3.18)
>20.0	68	47	1.11	(0.54, 2.28)	57	62	1.58	(0.78, 3.18)	37	31	2.57	(1.03, 6.37)	88	80	2.59	(1.15, 5.87)
p interaction								0.21								0.05
Post-menopause recently exposed to hormones																
Referent BMI (kg/m^2)																
<25	107	106	1.00		154	135	0.88	(0.61, 1.26)	83	73	1.00		177	171	1.11	(0.75, 1.63)
25–29.9	63	73	1.16	(0.75, 1.80)	101	90	0.88	(0.59, 1.32)	48	50	1.13	(0.68, 1.90)	116	114	1.13	(0.74, 1.71)
≥30	71	46	0.65	(0.40, 1.04)	93	74	0.76	(0.50, 1.15)	47	44	1.04	(0.61, 1.77)	122	79	0.72	(0.46, 1.12)
p interaction								0.45								0.35
Weight gain since age 15 (kg)																
≤10	64	55	1.00		99	84	0.99	(0.62, 1.59)	54	38	1.00		109	102	1.50	(0.90, 2.49)
10.1–20.0	73	74	1.17	(0.72, 1.92)	88	84	1.06	(0.66, 1.71)	50	53	1.63	(0.91, 2.91)	110	108	1.45	(0.87, 2.40)
>20.0	92	85	1.03	(0.64, 1.66)	149	119	0.88	(0.57, 1.38)	68	68	1.45	(0.84, 2.51)	178	138	1.15	(0.71, 1.87)
p interaction								0.90								0.15

[a] OR and 95% CI adjusted for age, center, parity, energy intake, physical activity, and genetic admixture

interacted with weight gain since age 15 among NHW post-menopausal women recently exposed to hormones. Women with the least weight gain since age 15 were at increased risk when they also had an A allele of the –202 *IGFBP3* polymorphism, however, gaining weight increased risk only among women with the CC genotype. This association was stronger among NHW women. There were no significant interactions between *IRS1* and *IRS2* polymorphisms and BMI, weight gain, and recent use of HRT in either Hispanic or NHW women (data not shown in table).

Discussion

Different allele frequencies for both the G972R *IRS1* and –202 *IGFBP3* polymorphisms were observed between Hispanic/AI and NHW women living in the Southwestern United States. The R allele of the G972R *IRS1* polymorphism was less common among Hispanic/AI women and also was associated with increased risk of breast cancer among Hispanic/AI women not recently exposed to hormones. Likewise, the A allele of the –202 *IGFBP3* polymorphism was less common in the His-

Table 5 Breast cancer risk and the interaction of referent year BMI, weight gain, and HRT with *IGF1 and IGFBP3* in Hispanic/AI women

	IGF1						*IGFBP3*								
	Ctrl	Case	19/19		Ctrl	Case	19/non19 and non19/non19		Ctrl	Case	CC		Ctrl	Case	CA/AA	
	N	*N*	OR (95% CI)[a]		*N*	*N*	OR (95% CI)[a]		*N*	*N*	OR (95% CI)[a]		*N*	*N*	OR (95% CI)[a]	
Pre-menopause																
Referent BMI (kg/m^2)																
<25	37	39	1.00		57	49	0.83	(0.45, 1.54)	47	33	1.00		47	54	1.66	(0.89, 3.09)
25–29.9	36	32	0.84	(0.42, 1.68)	54	47	0.78	(0.42, 1.48)	35	36	1.38	(0.70, 2.73)	55	43	1.10	(0.58, 2.07)
≥30	33	32	0.90	(0.44, 1.82)	45	36	0.81	(0.41, 1.57)	42	27	0.96	(0.48, 1.93)	37	43	1.61	(0.83, 3.13)
p interaction							0.97								0.18	
Weight gain since age 15 (kg)																
≤10	25	23	1.00		38	43	1.18	(0.56, 2.49)	33	29	1.00		30	37	1.45	(0.70, 3.00)
10.1–20.0	29	30	1.05	(0.47, 2.33)	48	32	0.65	(0.31, 1.39)	34	23	0.77	(0.36, 1.65)	44	39	0.93	(0.46, 1.85)
>20.0	49	45	0.85	(0.41, 1.78)	64	53	0.84	(0.41, 1.72)	55	42	0.82	(0.42, 1.61)	58	57	1.07	(0.55, 2.08)
p interaction							0.43								0.94	
Post-menopause																
Recent HRT use																
Yes	83	50	1.00		126	95	1.21	(0.77, 1.90)	86	46	1.00		124	101	1.60	(1.01, 2.54)
No	105	69	1.14	(0.70, 1.86)	136	116	1.50	(0.96, 2.35)	103	70	1.42	(0.87, 2.33)	140	115	1.72	(1.08, 2.72)
p interaction							0.78								0.36	
Post-menopause not recently exposed to hormones																
Referent BMI (kg/m^2)																
<25	17	11	1.00		17	15	0.98	(0.31, 3.06)	10	6	1.00		24	20	1.94	(0.54, 6.93)
25–29.9	19	20	1.30	(0.45, 3.74)	40	22	0.67	(0.25, 1.83)	26	16	1.23	(0.35, 4.40)	34	26	1.52	(0.45, 5.06)
≥30	36	13	0.48	(0.16, 1.43)	48	37	1.06	(0.41, 2.77)	38	25	1.54	(0.46, 5.19)	47	25	1.20	(0.37, 3.91)
p interaction							0.06								0.44	
Weight gain since age 15 (kg)																
≤10	13	10	1.00		13	11	0.68	(0.19, 2.49)	13	8	1.00		13	13	1.86	(0.52, 6.63)
10.1–20.0	14	11	0.77	(0.22, 2.68)	33	16	0.41	(0.13, 1.30)	15	6	0.58	(0.14, 2.44)	33	22	1.04	(0.34, 3.22)
>20.0	39	22	0.51	(0.17, 1.54)	50	40	0.86	(0.31, 2.40)	41	29	1.24	(0.41, 3.70)	49	32	1.13	(0.39, 3.30)
p interaction							0.19								0.47	
Post-menopause recently exposed to hormones																
Referent BMI (kg/m^2)																
<25	29	25	1.00		45	44	1.14	(0.56, 2.31)	29	13	1.00		45	58	2.74	(1.24, 6.05)
25–29.9	37	23	0.70	(0.32, 1.52)	60	56	1.00	(0.50, 1.97)	42	34	1.56	(0.68, 3.58)	58	45	1.65	(0.75, 3.64)
≥30	48	26	0.57	(0.27, 1.19)	48	32	0.73	(0.35, 1.50)	40	19	0.98	(0.41, 2.39)	54	39	1.43	(0.64, 3.21)
p interaction							0.90								0.17	
Weight gain since age 15 (kg)																
≤10	20	21	1.00		24	22	0.87	(0.36, 2.12)	18	8	1.00		26	37	3.36	(1.21, 9.30)
10.1–20.0	28	20	0.66	(0.27, 1.59)	43	47	0.92	(0.42, 2.03)	26	21	1.75	(0.61, 5.03)	47	46	2.05	(0.78, 5.41)
>20.0	61	32	0.45	(0.20, 0.98)	79	62	0.68	(0.32, 1.42)	63	36	1.19	(0.45, 3.15)	76	58	1.61	(0.63, 4.12)
p interaction							0.58								0.23	

[a] OR and CI adjusted for age, center, parity, energy intake, physical activity, and genetic admixture

panic/AI women and was associated with increased risk of breast cancer among post-menopausal Hispanic/AI women. Our data suggest that the associations between insulin-related genes and breast cancer are influenced by hormone exposure and body size.

Estrogen has been shown to regulate IRS–1 expression [40]. IRS-1 has been shown to be the predominant signaling molecule activated by IGF-1 and insulin [41]. Thus our finding that exposure to hormones mediates the association with insulin-related

genes is reasonable given the close relationship of the estrogen and insulin pathways. Our data suggest that in the absence of estrogen, post-menopausal women not recently exposed to hormones, variants of genes associated with increased IGF-1 levels were associated with increased risk of breast cancer. These results reinforce the close relationship between the estrogen and insulin pathways and support their influence on each other in influencing breast cancer risk.

Studies evaluating associations with serum levels of IGF-1 and breast cancer specifically report mixed results, although some studies suggest stronger associations among pre-menopausal women [42–49]. Our studies of genetic variants that may influence serum levels is undertaken as a means to explore these associations. The *IGF1* 19 CA repeat has been the most commonly studied polymorphism. Although it was not associated with serum IGF-1 levels in postmenopausal women in the Multiethnic Cohort [31], as study of predominately African American women showed a direct association between plasma levels of IGF-1 and the 19 CA repeat polymorphism in the *IGF1* gene [50]. In the Nurses' Health Study, women without a 19 CA allele had lower IGF-1 levels [51]. A study of 807 breast cancer patients and 1,588 matched controls from the EPIC Study examined 23 common SNPS in *IGF1*, *IGFBP1*, *IGFBP3* and the IGF acid-labile subunit. While they observed associations between SNPs of these genes and respective serum levels, the associations between the SNPs and breast cancer were weaker and limited to younger women [52]. In an earlier report based on the same 4-Corners study population as presented here, we observed higher IGF-1 levels among NHW women without the 19 CA repeat in the *IGF1* gene, while among Hispanic women, those with the 19 CA repeat had the highest IGF-1 levels [14] One of the few studies that evaluated polymorphisms of the *IGF1* gene and breast cancer showed an association with the C allele of the *IGF1* maker rs1520220 (not examined in this study). In that study of 4,647 breast cancer cases and 4,564 controls conducted in East Anglian region of the United Kingdom an increased risk of breast cancer was associated with the C allele of the *IGF1* gene rs1520220 (OR 1.41, 95% CI 1.11–1.79).

IGFBP-3 binds IGF-1 and thus it has been hypothesized that higher IGFBP-3 would reduce risk of breast cancer [53]. Some studies show no association between breast cancer and IGFBP-3 serum levels [54] and others observe that higher IGFBP-3 levels lower breast cancer risk [55]. We observed significantly higher levels of serum IGFBP-3 among both Hispanic and NHW women who had the AA genotype, as has been reported by others [14, 56]. However, the literature is limited on the association between polymorphisms of the *IGFBP3* gene, IGFBP-3 serum levels, and breast cancer [54]. The -202 polymorphism of the *IGFBP3* gene was not associated with breast cancer in the EPIC study [52], nor was there an association with breast cancer in a study by Schernhammer et al. [56]. However, a modest inverse association with the A allele of the -202 polymorphism of *IGFBP3* (0.87, 95% CI 0.77–0.99) was reported in the UK study population. We find evidence of a slight increased risk of breast cancer associated with the A allele, this risk was strongest among Hispanic women, but only among post-menopausal women who had been recently exposed to hormones.

Only one study to our knowledge has examined polymorphisms of either *IRS1* or *IRS2* and breast cancer and did not observe a significant association between variants of either gene and breast cancer risk [57]. In this study having an R allele of the G972R *IRS1* polymorphism significantly increased risk of breast cancer among post-menopausal Hispanic women not recently exposed to hormones. *IRS1* is regulated by estrogen [13], and the increased risk is only observed in the absence of estrogen.

Among NHW post-menopausal women, the 19 CA repeat *IGF1* polymorphism interacted with BMI among women not recently exposed to hormones (p=0.06). Our data also suggest that *IGFPB3* may regulate the association with weight gain among these women. In our previous work [35], BMI increased risk of breast cancer among NHW post-menopausal women not recently exposed to hormones, but not among Hispanic women. Others have reported similar associations between breast cancer and BMI [58]. Weight change and weight fluctuation have been shown in other studies to be directly associated with breast cancer risk [59–61], with the greatest effect among post-menopausal women who did not use HRT [58, 62]. We believe that the differences in association with body size by genetic polymorphism may indicate that obesity and weight gain operate differently in their association with breast cancer and that underlying metabolic or unidentified factors. Obesity can be representing a lifetime condition, with obesity at an early age having a potentially different metabolic consequence than obesity when older. The metabolic effects of gaining weight may influence insulin levels differently depending on age at onset of obesity or other factors.

The study has limitations. Although the sample size of Hispanic/AI women was one of the largest reported to date, we were hampered by small numbers when looking at interaction between genetic polymorphisms

and HRT, BMI, or weight gain. Our participation was less than desired; however associations with BMI are similar to those reported in other prospective and retrospective studies [35]. Although our response differed by center we had the benefit of identical data collection methods and questionnaires to help assure consistency across centers. We had no reason to believe that allele frequencies of the genes studied would be affected by response rates. Additionally, we evaluated one polymorphism of each gene assessed. These polymorphisms were selected because the literature suggested functionality associated with these variants, although other polymorphisms may be important and should be evaluated in future studies.

Our data provide support for the association between insulin-related factors and breast cancer risk among women living in the Southwestern United States. *IGF1*, *IGFBP3*, and *IRS1* polymorphisms appear to be most importantly associated with breast cancer in subsets of breast cancer cases. Different associations were observed for Hispanic women, consistent with the idea that the relative importance of various metabolic pathways in influencing breast cancer differs between these ethnic groups. Associations may be dependent on estrogen exposure either through endogenous estrogen in pre-menopausal women, exogenous estrogen in post-menopausal women taking HRT, or through estrogen levels in adipose tissue.

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